

Ameliorative effects of palm oil on sniper induced toxicity on the cerebral cortex of adult male Wistar rats

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Abstract

Introduction: Sniper insecticide is a highly toxic, water soluble, synthetic organophosphate belonging to the DDVP (2, 2 — dichlorovinyl dimethylphosphate) compound family of chemicals. It's wide spread indiscriminate indoor usage and more recently, an effective instrument for suicide related mortality which has been a source of great concern. Red palm oil, on the other hand, is an edible vegetable oil from the fleshy pulp of the oil palm that contains antioxidants; carotenoids that is responsible for its color (pigmentation) and Vitamin E that support brain health. This palm oil is consumed widely, used as an antidote in Nigeria and so is used for various ingested poisons.

Aim: This study is aimed at investigating the ameliorative effects of palm oil on sniper induced toxicity on the cerebral cortex.

Materials and methods: Sixteen adult male Wistar rats were randomly assigned to 4 groups (n = 4 per group). Group A was the Control, while Group B was administered just sniper (5 mg/kg), Group C was administered 2 ml of Red oil and Group D received 5 mg/kg of sniper + 2 ml of red oil. At the end of the experiment, the rats were sacrificed, their brains extracted and fixed in 10% formal saline for routine histological analysis.

Results: There was significant increase in the body weight of Groups A, B, C and D. The relative organ weight of Wistar rats showed an insignificant decrease in the relative brain weight of Group B compared to the Control. Groups C and D also had a relative decrease in comparison to the control. The cerebral cortex reveals neuronal damage and proliferation of glial cells in the sniper-induced group and the mild recovery of neuronal cells in the rats treated with sniper and palm oil.

Conclusion: The results from this study shows that there was a significant reduction in organ weight in Group B [Sniper only] when compared to the control group. When palm oil was administered, there was mild recovery of neuronal cells. This shows the ameliorative effects/ impact of Palm Oil in Neurotoxicity.

Keywords: Sniper insecticide; Neurotoxicity; Palm oil; Cerebral cortex; Antioxidant

1. Introduction

An insecticide is any toxic substance that is used to kill insects. Such substances are used primarily to control pests that infest cultivated plants or to eliminate disease-carrying insects in specific areas [1]. A large number of humans ingest insecticides whilst carrying out normal daily activities like eating foods processed or protected by insecticides, by breathing in insecticide polluted air or by working/living in areas where these chemicals are produced. The

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consequences of these ingestions are not farfetched ranging from irritation, nausea, dizziness and diarrhea in short term. Chronic health effects include brain and nervous system damage, reproductive issues and various cancers [2]. Palm oil (*Elaeis guineensis*) is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palms [3]. The oil palm starts fruiting two or three years after planting and bears fruit continuously throughout its life of around 25 years. Palm oil is mainly made up of palmitic and oleic fatty acids which tend to be semi-solid fat at room temperature [4]. Fresh palm oil is the most common antidote for ingested poisons amongst indigenous people of south-south Nigeria. It is said to be effective only before the poison is absorbed into systemic circulation. Palm oil is the most popular cooking ingredient in this area. Hence, it becomes the first aid in case of poisoning. Children are forced to drink large quantity of the oil to either regurgitate the poison or neutralize its effect [5]. This research investigated the ameliorative role of Palm Oil on Sniper Induced Toxicity on the cerebral cortex of male Wistar rats.

2. Material and methods

2.1. Place of Study

This research was carried out in the Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

2.2. Materials

Dissecting kit, Mettler Toledo weighing machine (Monobloc weighing technology 1118380934, 2012), Electronic weighing balance (Leica CT 250 1101735428, 2012), Absorbent cotton wool, 10% formal saline, Haematoxylin and Eosin (H and E) x 100, Dissecting pins/optical pins normal, Dash board, 20 Adult Wistar rats, Perplex cages, Drinkers (plastic), Pyrex Glass Beaker (100ml, 250ml and 500ml capacity), Measuring cylinder (100ml capacity), Distilled water, Plates (for feeding) and Saw dust/wood shavings.

2.3. Experimental Procedure:

2.3.1. Substance of Study

Sniper insecticide (DDVP, Loveland Products) was gotten and Red palm oil were gotten from Dubem Chemical store at head bridge market, Onitsha.

2.3.2. Experimental Animals

A total of 24 adult male Wistar rats were obtained for the experiment. They were obtained from the Department of Physiology, Nnamdi Azikiwe University, Nnewi Campus and was housed in the Central Animal House, Nnamdi Azikiwe University College of Health Sciences Nnewi Campus. The animals weighed from 103 g-186 g. The rats were kept in perplex cages at optimum temperature, 12hrs light/dark cycle and fed with commercial grower mash and water *ad libitum*. The animals were fed with poultry feed known as Top Feed which was manufactured by premier feed mills company limited (a subsidiary of Flour Mills Nigeria plc) in Sapele Delta State and purchased from Nkwo Market, Nnewi Anambra State. The animals were fed with only the feed without being mixed with test substance during the two weeks of acclimatization. The animals were fed using plastic two plates in each of the groups. The rats were properly handled with the use of hand gloves. Each of the rats was identified using non-invasive method, that is, the use of permanent markers of different colors. The experiment was carried out in accordance with current rules and guidelines established for care of laboratory animals.

2.3.3. Experimental Design

Animals were randomly grouped as follows;

- Group A; Served as control that received normal feed and water daily throughout the period of experiment
- Group B; Received 5 mg/kg of body weight sniper
- Group C; Received 2 ml palm oil
- Group D; Co-administered 5 mg/kg of sniper + 2 ml of red palm oil

At the end of the experimental period, rats in each group were sacrificed by cervical dislocation. The brain of each rat was weighed, rinsed in saline solution and immediately fixed in a labeled container specific for each rat.

2.4. Histological Evaluation Sample Collection

Brain tissue samples were collected from all the animal groups. They were fixed using the standard fixative of 10% formal saline and were kept in a container until processing of the tissues.

2.4.1. Dehydration of Tissues

The tissues were first immersed into a bath containing 70% alcohol and left overnight followed by transferring tissues into different baths containing 90%, 95%, absolute I, II and III for 2 hours each and then absolute IV overnight.

2.4.2. Clearing of the Tissues

On removal of the tissues from absolute alcohol, the tissues were passed through 3 changes of xylene (I, II, III) for 1 hour 30 minutes each.

2.4.3. Impregnation of Tissue

On removal of the tissue from the clearing agent, they were immersed in a wax bath I for 2 hours and wax bath II in a hot air oven for one hour.

2.4.4. Embedding of Tissue

Infiltration was done in molten paraffin wax at a temperature of 60°C for two hours each in two changes.

2.4.5. Sectioning

The embedded tissues were sectioned with a rotary microtome.

2.4.6. Staining

Haematoxylin and Eosin method was used for staining after sectioning.

2.4.7. Microscopy/Cell Identification

The tissue slides were examined using a light microscope and the photomicrographs were taken.

2.5. Statistical Analysis

The result of the data was statistically analysed using SPSS windows version 21.0 software. Results are presented as mean and standard error of mean (SEM), analysis of variance (ANOVA) was used in comparing difference within groups where F-ratio and proportionality of significance were gotten. P-values less than 0.05 (< 0.05) at 95% confidence interval was considered as significant.

3. Results and discussion

Table 1 Effect of Red palm oil on body weight following sniper-induced toxicity

		Mean	±SEM	BWD (g)	P-value	T-value
Group A (Positive control)	Initial weight (g)	103.33	±0.88	54.00	0.03 ^a	-5.81
	Final weight (g)	157.33	±8.82			
Group B (5 mg/kg of Sniper only)	Initial weight (g)	145.00	±4.06	21.50	0.01 ^a	-7.01
	Final weight (g)	166.50	±2.02			
Group C (2 ml of Red oil)	Initial weight (g)	119.00	±4.00	47.67	0.03 ^a	-5.32
	Final weight (g)	166.67	±8.82			
Group D (5 mg/kg of Sniper + 2 ml of Red oil)	Initial weight (g)	141.33	±6.36	44.33	0.02 ^a	-6.63
	Final weight (g)	185.67	±3.17			

Data was analyzed using T-test and values were significant at $p < 0.05$; SEM: Standard error of mean, BWD: Body weight difference, ^a (significant), ^b (not significant).

This result revealed a significant ($p < 0.05$) increase in the body weight in groups A, B, C, and D when the initial weight was compared to the final weight.

Table 2 Effect of Red palm oil on the relative brain weight following sniper-induced brain toxicity

		MEAN	±SEM	P-value	F-value
Relative brain weight (g/%)	Group A (Control)	1.02	±0.04	0.25 ^b	
	Group B (5 mg/kg of Sniper only)	0.84	±0.13		1.86
	Group C (2 ml of Red oil)	0.82	±0.01	0.03 ^a	
	Group D (5 mg/kg of Sniper + 2 ml of Red oil)	0.83	±0.01	0.30 ^b	

Data was analysed using ANOVA followed by Post Hoc LSD comparison and values were considered significant at $p < 0.05$. SEM: Standard error of mean, ^a (significant), ^b (not significant)

This revealed an insignificant decrease in the relative brain weight in Group B compared to A ($p = 0.25$), Groups C and D had a decrease but was significant in Group C ($p = 0.03$) and insignificant Group D ($p = 0.30$) when compared to Group A.

4. Result

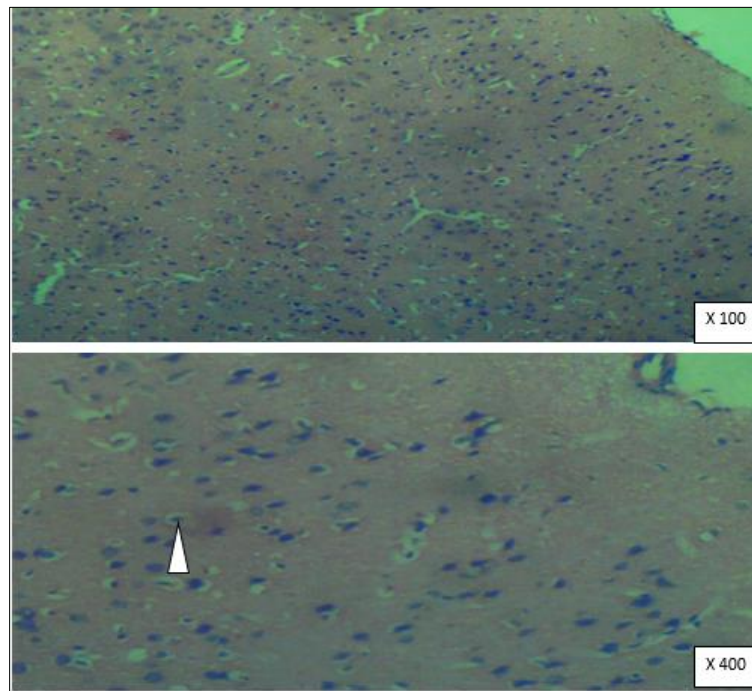


Figure 1 Group A (received feed and water): Photomicrograph of the brain section shows cerebral cortex with densely packed neurons and glial cells (arrowhead). The architectural morphology is normal and consistent with normal histology) (Stained by H & E, X 100; 400)

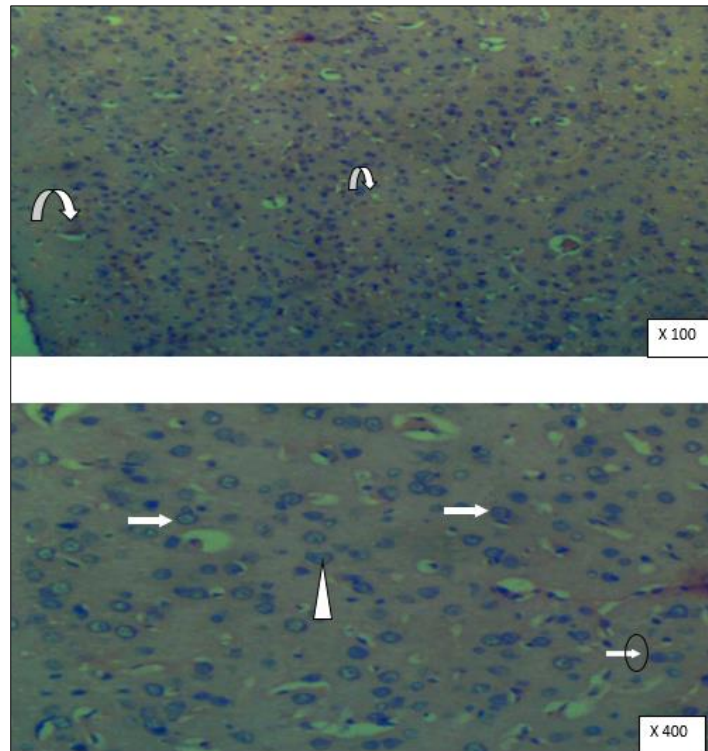


Figure 2 Group B (received 5 mg/kg of Sniper only): Photomicrograph of the brain section shows cerebral cortex with densely packed neurons and glial cells (arrowhead), hyperchromatic nuclei (arrows), mild inflammatory (curved arrows) and mild degeneration of neurons (arrows). The architectural morphology is normal and consistent with normal histology) (H&E)

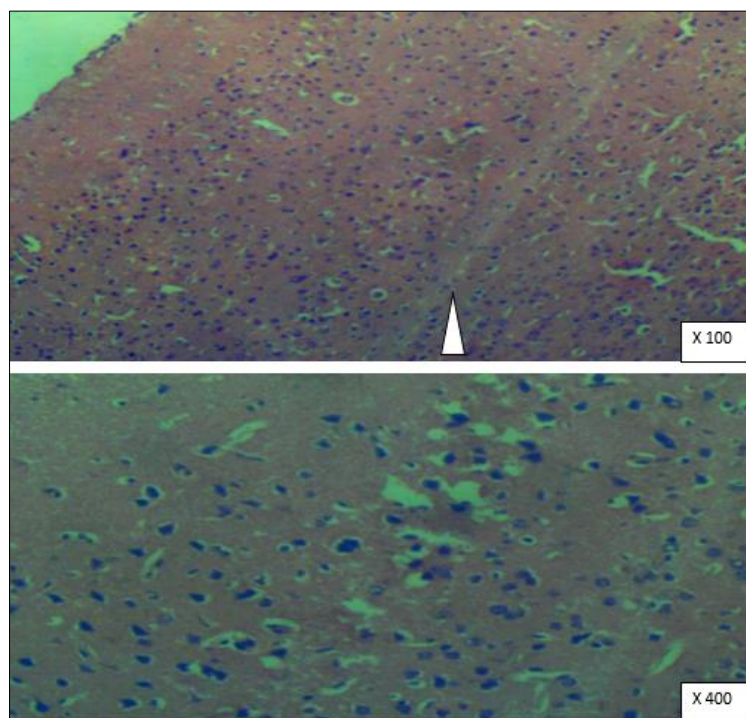


Figure 3 Group C received 2 ml of red palm oil: Photomicrograph of the brain section shows cerebral cortex with densely packed neurons and glial cells (arrowhead). The architectural morphology is normal and consistent with normal histology) (Stained by H&E, X100; 400)

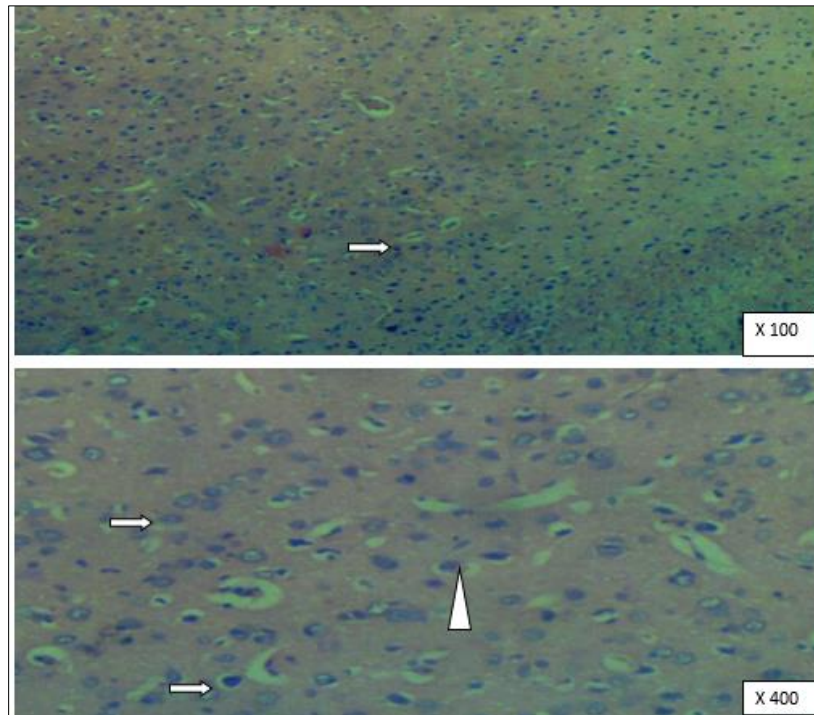


Figure 4 Group D received 5 mg/kg of Sniper and treated with 2 ml of red palm oil: Photomicrograph of the brain section shows cerebral cortex with densely packed neurons and glial cells (arrowhead), mild hyperchromatic nuclei (arrows) and mild recovery of neuronal cells. The architectural morphology is normal and consistent with normal histology) (Stained by H&E, X100, 400)

5. Discussion

Sniper insecticide is a common, synthetic organophosphorus, which belongs to the 2, 2-dichlorovinyl dimethylphosphate compound chemical family (DDVP). The active ingredient in this deadly chemical is binfenethrin which is classed among the pyrethroids and hence has been used in industrial pest control, insect control and also domestically. It has also been observed that sniper is ranked among the most available tools for suicide here in Nigeria [6]. This is obviously due to its proven toxicity on pests and insects and its high availability. The indiscriminate wide spread of this insecticide especially domestically and its availability in small sizes and at household stores has aided its more recent trend as a tool for suicide. Hence, sniper insecticide has been banned by the National Agency for Food, Drug, Administration and Control (NAFDAC) to reduce sniper enhanced mortality.

Palm oil (*Elaeis guineensis*) on the other hand is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palm [3]. Fresh palm oil is the most common antidote for ingested poisons amongst indigenous people of south-south Nigeria. Palm oil is the most popular cooking ingredient in this area. Hence, it becomes the first aid in case of poisoning [5]. Red palm oil is rich in Vitamin E (tocotrienol), Carotene and Lycopene, that improves brain function and blood pressure [7].

In this study, a significant increase in the body weight of animals in Group A (Control) was observed. This could be physiological as the only substances they were fed was water and feed. A significant body weight increase was also noted in the experimental groups B, C and D when compared to the control group A. This could be as a result of adequate feeding. This study agrees with Meggs [8] where it was documented that there is a significant increase in body weight of rats treated with organophosphate insecticide when compared to the control group. This study, however, shows a level of dissimilarity with Grewal [9] where it was opined that insecticides significantly reduced body weights of rat subject.

Findings from the study comparing organ weight reveals that there was a reduction in organ weight in Group B. This could be due to the toxicity of the sniper insecticide. This agrees with Grewal [9] where it was documented that there is a reduction in brain weight of rats when only sniper insecticide is administered compared to the brain weight of the control group.

Histopathological findings from Group A revealed no significant change in the histo architecture of the cerebral cortex. This could be physiological as they were just administered just feed and water as the control group. Histological findings from Group B [5mg/kg sniper] agrees with Gupta [10] and Grewal [9] in that there was marked increase in glial cells and marked neuronal damage as a result of administration of insecticides. This could be as a result of toxicity and neurodegenerative effects of sniper insecticide and other organophosphates. Histological findings also indicated densely packed neurons with no abnormality in the Group C that was administered 2ml of red palm oil. This could be as a result of the anti-oxidant properties of palm oil. This is in line with the work of Zaida [11] where he opined that there was no evidence of substantial toxicity at any dose of palm oil. Histological findings from Group D [palm oil 2ml plus sniper 5mg/kg] of this study shows mild recovery of neuronal cells which could be as a result of the oxidative properties of palm oil. This agrees with Putri [12] that palm oil has ameliorative effects on insecticide induced poisoning.

6. Conclusion

The results from this study shows that there was a significant reduction in organ weight in group B [sniper only] when compared to the control group. When palm oil was administered, there was mild recovery of neuronal cells. This shows the ameliorative effects/ impact of palm oil on neurotoxicity.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC).

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