INHALATION OF SNIPER AND PASSIVE SMOKING DISRUPTS MOTOR ACTIVITY AND SPATIAL MEMORY IN FEMALE WISTAR RATS

Abstract.

Twenty-eight female Wistar rats were divided into four groups to study the effects of sniper and cigarette smoke on motor activity and spatial memory. Group A served as the control group, receiving only rat meal and water ad libitum. Group B was designated as a sniper-only group, and was given 100ml of sniper. Group C served as a cigarette-only group, and received two sticks of cigarette smoke. Group D was designated as the sniper + cigarette group, and they were given 100ml of sniper and two sticks of cigarette smoke at the same time. The experiment lasted 14 days, with each exposure lasting 10 minutes per day. Finally, each group's cerebellum, and hippocampus were removed for histological analysis. The results showed that the mean of the hanging wire test in the study animal decreased significantly ($P<0.05$), although the Morris water maze test had significantly ($P<0.05$) greater frequency. In all treatment groups, histological examination revealed symptoms of moderate neuronal degeneration in group D and a hypochromic appearance of the Nissl material. Finally, the results of this study uncovered that sniper and involuntary cigarette smoking has the power to impair females' long-term spatial memory and motor abilities of Wistar rats.

Keywords: Sniper, Cigarette smoke, Spatial memory, Motor functions

Introduction

The global smoking prevalence by gender revealed that African women smoke at a lower rate than other continents, while males smoke at a higher rate. As a result of inhaling cigarette smoke from their spouse and relatives, African women are involuntary or passive smokers.

Sniper and tobacco smoke include a variety of substances that are toxic to smokers and nonsmokers alike. Inhaling even a small amount of tobacco or sniper smoke might be dangerous (1). At least 250 of the over 7,000 compounds included in tobacco smoke are known to be toxic, such as hydrogen cyanide, carbon monoxide, and ammonia. At least 69 of the 250 known toxic compounds in tobacco smoke can cause cancer. In this country, smoking is the largest cause of preventable mortality. In Nigeria, cigarette smoking and sniper exposure cause roughly 480,000 early deaths each year. About 36% of individuals who die prematurely do so due to cancer, 39% due to heart disease and stroke, and 24% due to lung illness (2). Smokers have a mortality rate that is almost three times that of nonsmokers.

Exposure to sniper and cigarette smoke damages practically every internal organ and organ system, as well as a person's overall health. They cause malignancies of the lung, esophagus, larynx, mouth, and neck, as well as tumors of the liver, pancreas, stomach, cervix, colon, and rectum.

They also increase asthma symptoms in adults and cause heart disease, stroke, aortic aneurysm, chronic obstructive pulmonary disease (COPD), diabetes, osteoporosis, rheumatoid arthritis, age-related macular degeneration, and cataracts. Pneumonia, TB, and other airway infections are more common in smokers. Furthermore, smoking causes inflammation and weakens the immune system (3). Smoking makes it more difficult for a woman to conceive. A pregnant smoker is more likely to have a miscarriage, an ectopic pregnancy, a baby born too soon and with an abnormally low birth weight, and a kid born with a cleft lip and/or palate. Smoking during or during pregnancy raises the risk of Sudden Infant Death Syndrome (SIDS) in the baby (SIDS). Erectile dysfunction is more common among men who smoke (4).

The longer a person is exposed to cigarette smoke and sniper, the more likely they are to be harmed, including dying sooner. Secondhand smoke is a mixture of "sidestream" smoke (the smoke given off by a burning tobacco product) and "mainstream" smoke (the smoke inhaled by a smoker) that has been recognized as a known human carcinogen (cancer-causing substance) (5). Nonsmoking adults who inhale secondhand smoke develop lung cancer. According to studies, living with a smoker increases a nonsmoker's risk of lung cancer by 20 to 30 percent.

Pregnant women who are exposed to secondhand smoke have a higher risk of having a kid that is born with a low birth weight (1). There is no safe level of sniper or cigarette smoke exposure. Even one cigarette per day or a few minutes of sniper exposure over a lifetime can result in smoking/sniper-related diseases and premature death (6).

Although there have been reports on the effects of direct cigarette smoking on the cardio-pulmonary system, reproductive system, and hematological system, there is a paucity of literatures studying the effects of simultaneous exposure to sniper and cigarette smoke on the neurological system. As a result, employing Wistar rats as models, it's vital to investigate the underlying efficacy of sniper and passive smoking on motor functions, learning, and memory in females. In light of this, the current investigation is justified.

Materials and Methods

Preparation of Sniper and Cigarette

Sniper was measured in a 100 mil glass cylinder, and the chemicals used were not utilized a second time. Each group of cigarettes was given two sticks of the Rothmans brand.

Experimental design

The rats used in the study were 28 female Wistar rats weighing between 150 and 200 grams. The rats were kept in wire gauze cages with four compartments, each holding seven rodents. Before being exposed, the rats were permitted to acclimate for two weeks in the animal home

of Abia State University's Department of Anatomy. Throughout the trial, they were given unlimited amounts of rat feed and water.

The rats were randomly grouped as follows;

Group A (Control) - was given only rat meal and water ad libitum.

Group B (Sniper only group) - were exposed to 100ml of sniper for 10mins

Group C (Cigarette only group) - were exposed to two sticks of cigarette smoke for 10mins

Group D (Sniper + Cigarette group) - were exposed to 100ml of sniper and two sticks of cigarette smoke concurrently for 10mins respectively.

The duration of exposure lasted for 14 days.

The animals were returned to their cages after being exposed. Chemicals were not re-used in any way. Inhalation chambers were installed in the Anatomy Department's postgraduate research laboratory.

Figure 1: A picture showing a session of exposure

Neurobehavioral Studies

During the research, two neurobehavioral experiments were carried out. The first test was performed a day before the chemicals were exposed to their respective groups. The following neurobehavioral experiments were undertaken during the second test, which lasted for 7 days after treatment and continued until the final week of exposure, before they were sacrificed:

Morris water maze

The Morris water maze (MWM) is a spatial learning test for rats in which they must navigate around the perimeter of an open swimming arena using distal cues to locate a submerged escape platform. The animal must learn to use distal signals to take a direct path to the concealed platform when it is thrown from various, random locations around the outside of the tank.

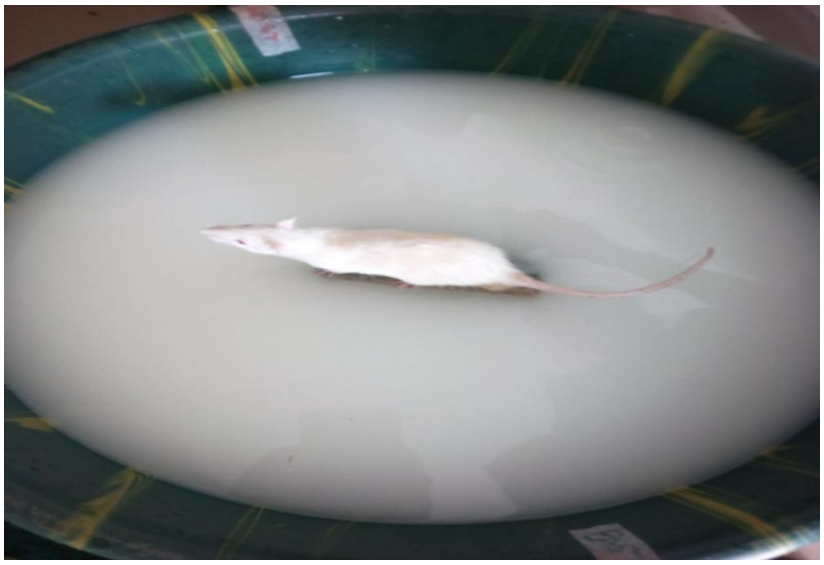


Figure2: Picture Showing An Expirimental Condition Of Water Maze Test.

Hanging Wire Test:

This test is designed to assess muscle strength and adaptation. Rats are dropped from a height and instructed to use their forelimbs to pierce an iron rod pole. They are then allowed to suspend at that range until they either gain stability with both limbs or suspend for a long time before falling.

Figure 3: Picture Showing An Expirimental Condition Of Hanging Wire Text

Histological Examination

The hippocampus and cerebellum were processed in the histology lab for histological examinations. For histological research, these tissues went through the standard stages of tissue processing.

Statistical Analysis of Results

The results were given as M ± SEM of triplicate measurements. The Statistical Package for Social Sciences (SPSS) version 23 was used to examine all of the data (IBM Corp., Armonk, New York). Hypotheses were tested by looking for significant differences at the $P < 0.05$ significance level.

Results

Table 1: Values of hanging wire test in study animals.

Groups	Day 0 (sec)	7 days (sec)	14 days (sec)
A (CG)	125.46 ± 4.88	129.60 ±41.45	137.94 ± 5.37
B (SOG)	120.55± 60.32	125.62 ± 11.85	118.47± 8.32[a]
C (COG)	119.51 ± 42.61	123.39 ± 9.96	121.14 ± 78.50[a]
D (SCG)	122.86 ± 54.52	124.40 ± 36.39	107.72 ± 26.02[a]

Key: CG: control group, SOG: sniper only group, COG: cigarette only group, SCG: sniper + cigarette group. Results are considered ([a]) significant in contrast to control.

At day 14 of the experiment, the test groups spent less time in the hanging wire test when compared to the control group.

Graph 1: Hanging wire test in study animals

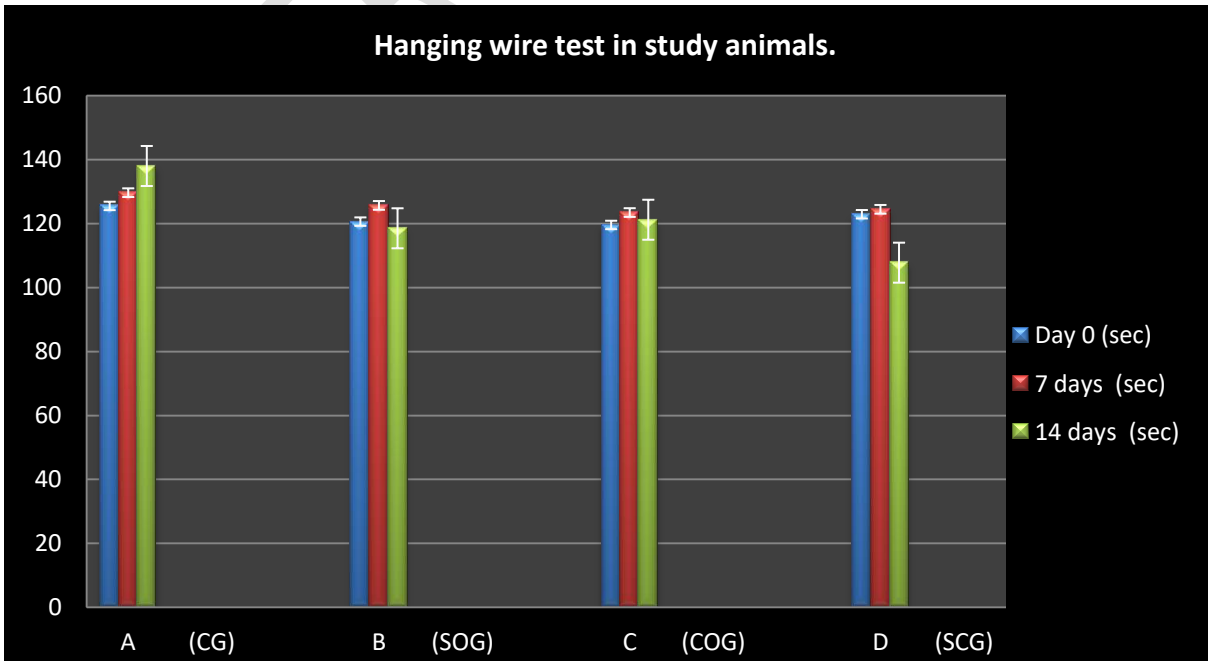


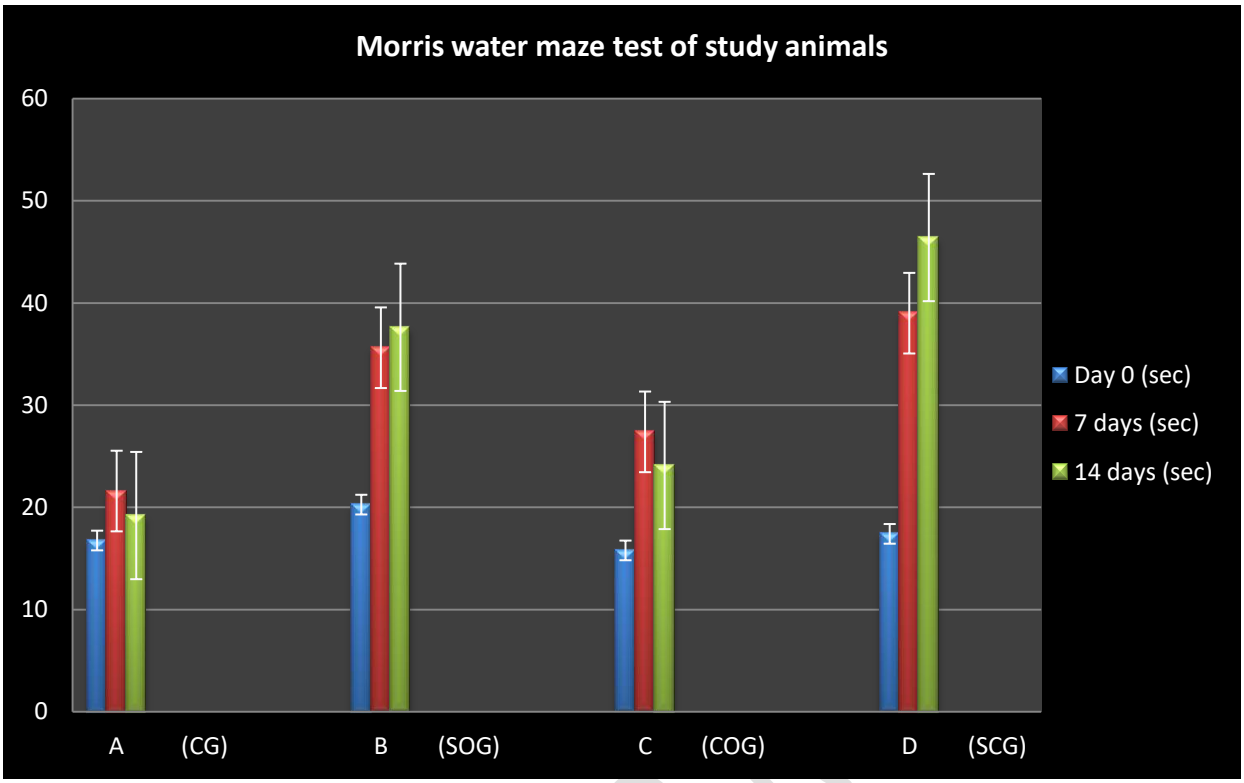
Table 2: Values of Morris water maze test of study animals.

Groups	Day 0 (sec)	7 days (sec)	14 days (sec)
A (CG)	16.76 ± 3.46	21.60 ± 41.45	19.20 ± 4.53
B (SOG)	20.27 ± 1.25	35.62 ± 11.85[a]	37.62 ± 0.81[a]
C (COG)	15.79 ± 6.61	27.39 ± 9.96	24.10 ± 11.19
D (SCG)	17. 41 ± 3.84	39.00 ± 36.39[a]	46.40 ±2.55[a]

Key: CG: control group, SOG: sniper only group, COG: cigarette only group, SCG: sniper + cigarette group.
Results are considered ([a]) significant in contrast to control.

As shown in table 2, the test groups spent much more time in the Morris water maze at all levels which was significant in group b (sniper only group) and group d (sniper + cigarette group) When compared to the control group.

Graph 2: Morris water maze test of study animals



Histology of the cerebellum

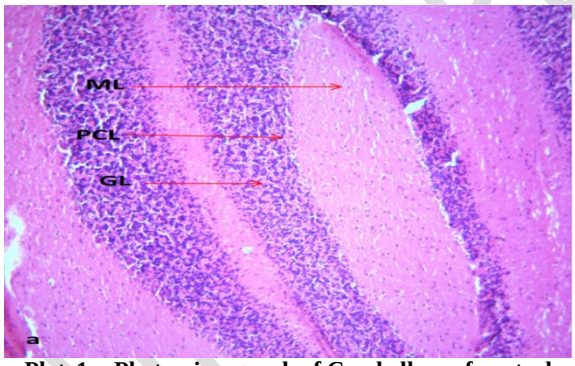


Plate1a: Photomicrograph of Cerebellum of control group (Mag.X125).

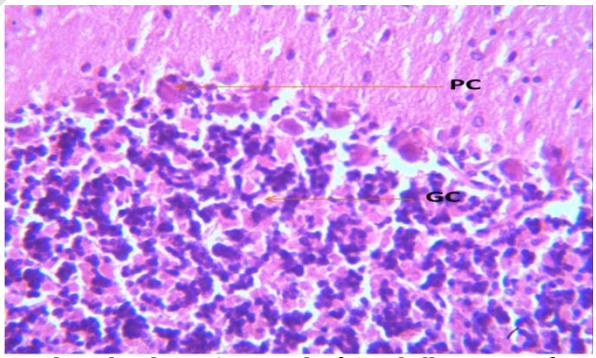


Plate 1b: Photomicrograph of Cerebellar cortex of control group (Mag.X500).

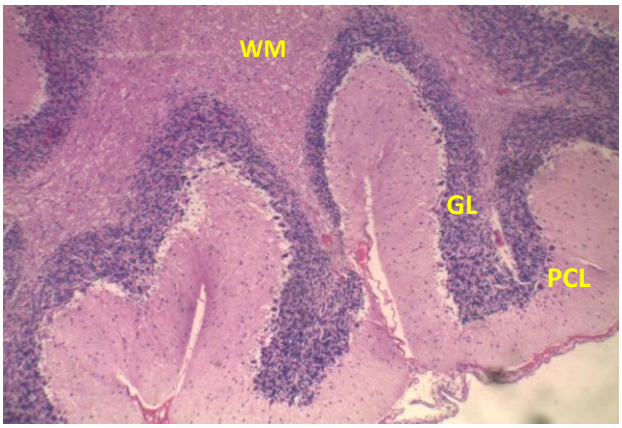


Plate 2a; Photomicrograph of Cerebellum of group B (Mag. X125)

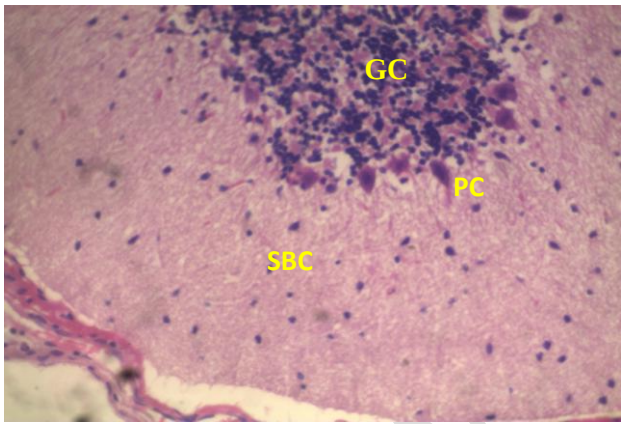


Plate 2b: Photomicrograph of Cerebellar cortex of group B (Mag. X500)

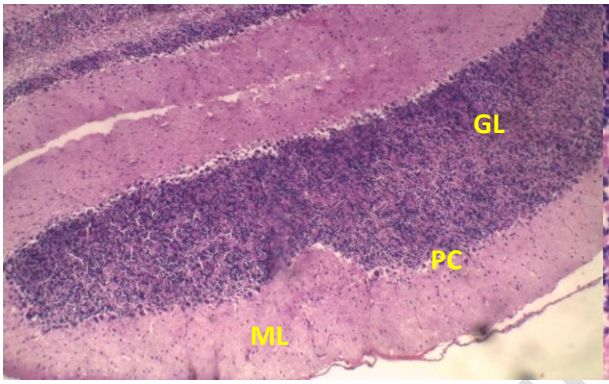


Plate 3a: Photomicrograph of Cerebellum (Mag.X125) of Group C

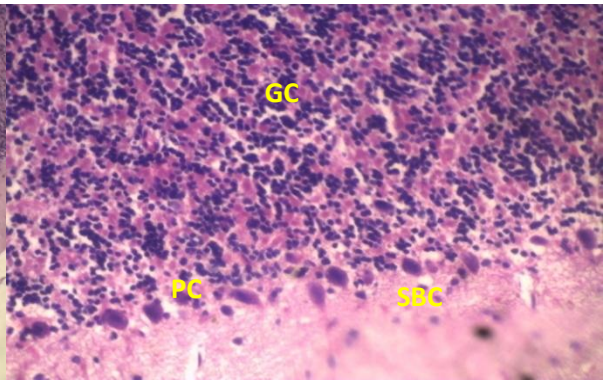


Plate 3b: Photomicrograph of Cerebellum (Mag.X500) of Group C.

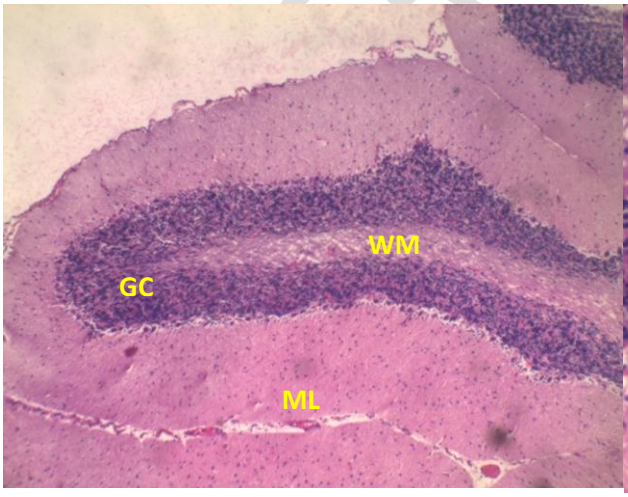


Plate 4a: Photomicrograph of Cerebellum (Mag.X125) of Group D,

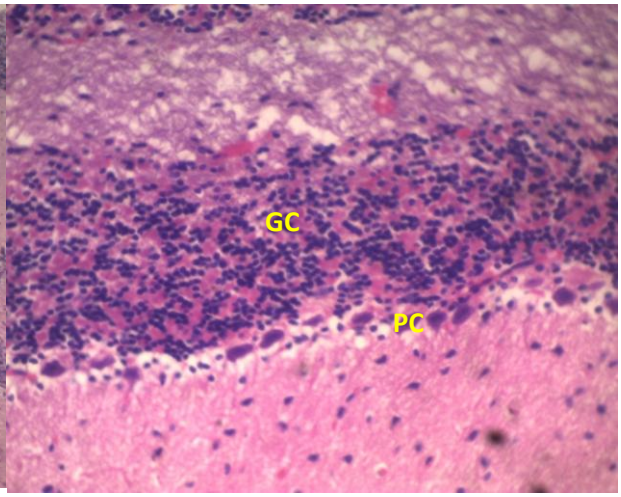


Plate 4b: Photomicrograph of Cerebellum (Mag.X500) of Group D

Histology of the hippocampus

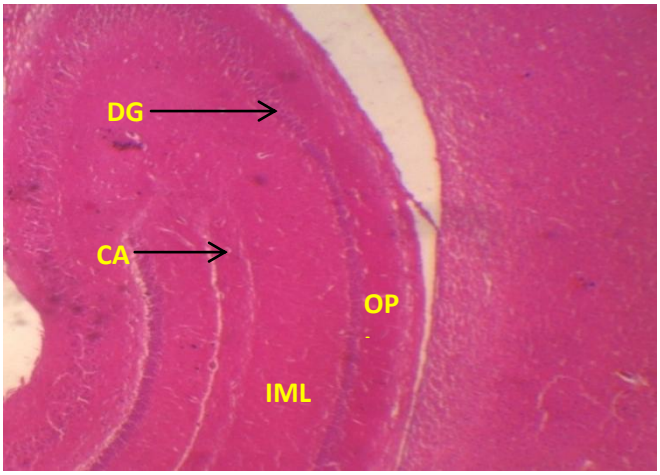


Plate 5a: Photomicrograph of hippocampus (Mag. X125) of control group

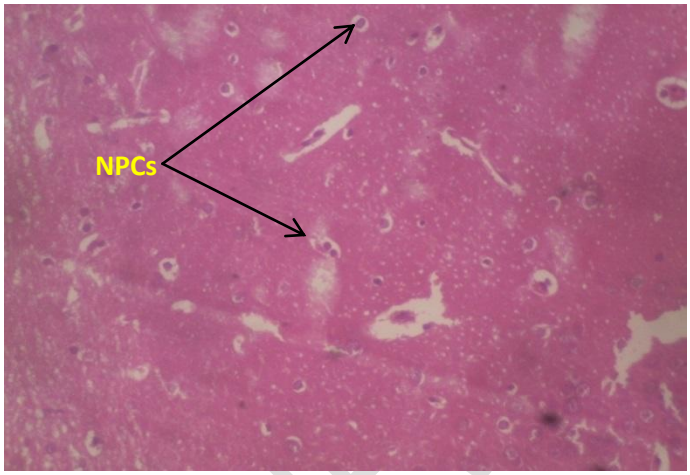


Plate 5b: Photomicrograph of hippocampus (Mag.X500) of control group

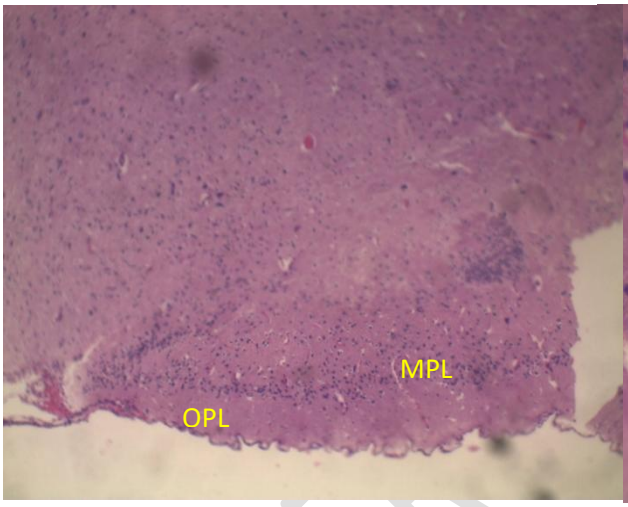


Plate 6a; Photomicrograph of hippocampus (Mag. X125) of Group B.

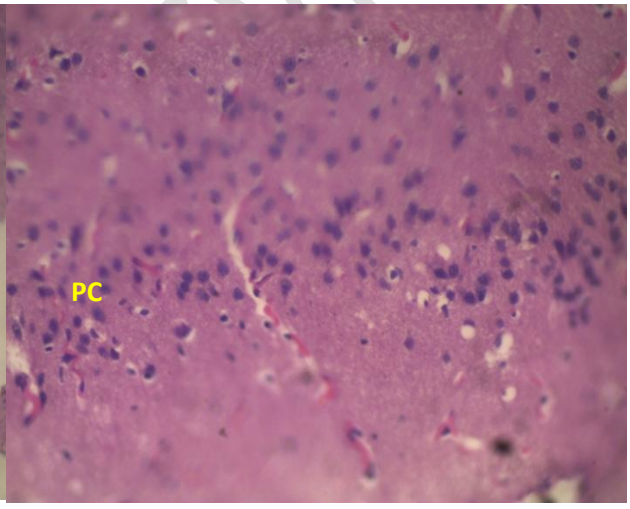


Plate 6b: Photomicrograph of Hippocampus (Mag. X500) OF GROUP B

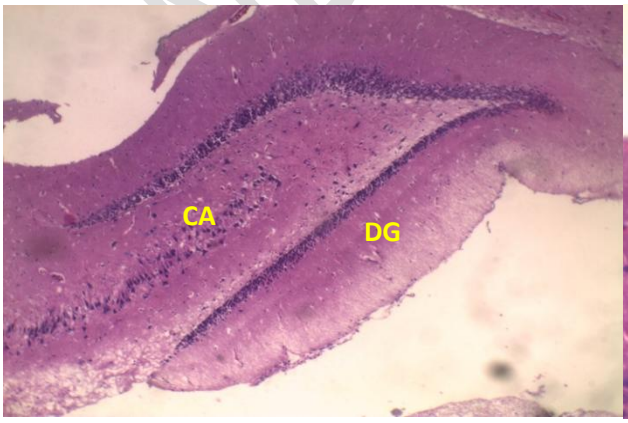


Plate 7a: Photomicrograph of hippocampus (Mag. X125) of Group C

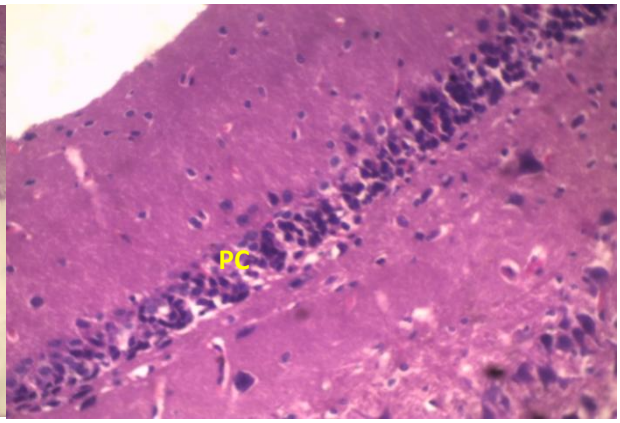


Plate 7b: Photomicrograph of hippocampus (Mag.X500) of Group C

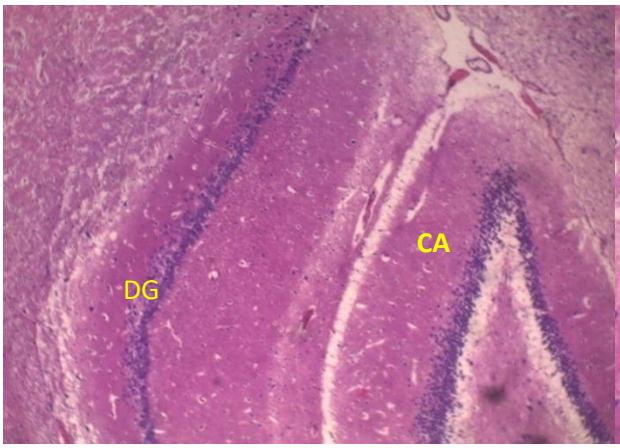


Plate 8a: Photomicrograph of hippocampus (Mag.X125) of Group D

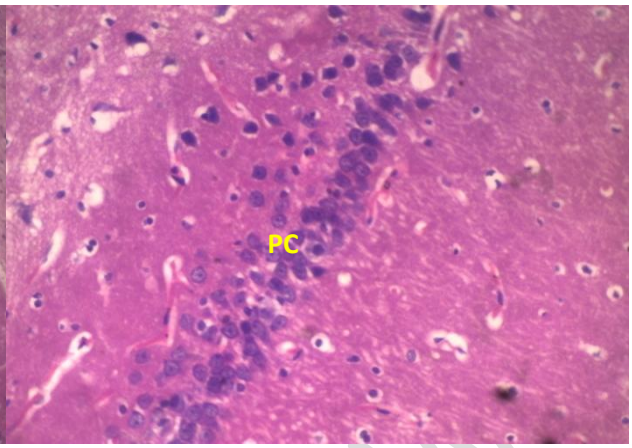


Plate 8b: Photomicrograph of hippocampus (Mag. X500) of Group D

DISCUSSION

The hanging wire test was used to assess muscular strength and, as a result, cerebellar function. In this study, the animals' forelimbs were used to suspend them from a hanging wire. The goal is to see how long they can hold their breath for, which is a good measure of muscle strength and motor activity.

In the control group A, we found a statistically significant difference between the initial and end results, as shown in table 1. This could indicate that after the initial experiment, the animals had already learned and acclimated to hanging on the wire.

However, there was a statistically significant reduction in the duration the animals could suspend on the hanging wire in group B after merely inhaling sniper. Because the animals could not support themselves on the hanging wire for long after being exposed to sniper inhalation, the results suggest that sniper inhalation can have a negative impact on muscle strength and motor coordination. This conclusion is confirmed by the findings of (7), who discovered a link between formalin and motor function abnormalities. This is comparable to what happens with Parkinson's disease sufferers, who lose their ability to move smoothly and controllably due to low dopamine levels.
When compared to the control group, the experimental group C, which was exposed to cigarette smoke, showed a decrease in time spent, however in group D, the reduction was statistically significant, signifying muscle dystrophy. This is in line with the findings of Aguwa (7).

The time spent in the Morris water maze rose in all test groups. The amount of time it took to find the departure platform rose dramatically. This could be attributed to the group's high level of discomfort. It shows that sniper and cigarette smoke can interfere with learning and memory.

The Morris water maze test showed that the rats in group A took longer to find the escape platform than the rats in the control group, but the difference was not statistically significant. The

rats in group B took longer to find the escape platform than the rats in the control group. This could be due to memory loss or the inability to form new memories. Damage to hippocampus cells is linked to problems with spatial learning and memory. In group C, the animal took nearly the same amount of time to discover the stage, but it took somewhat longer in the beginning than in the end, whereas in group D, the animal took significantly longer to locate the stage. Organophosphates such as triorthocresyl phosphate, mipafox, and trichlorfon compounds can be neurotoxic and are usually absorbed through the cutaneous and respiratory routes. Workers in factories that make these compounds, as well as agricultural workers who spray crops with them, are at a greater risk of becoming poisoned (8). This can also harm users who are always in close quarters.

Based on the findings of neurobehavioral investigations, we may deduce that muscle retention associated with the cerebellar section of the brain suffered greater damage than the hippocampus, which serves as a memory text. Junquera's report was supported by other physical observations (9).

The H and E stain was used to stain the histopathological features of the cerebellum. Plates 1 and 2 (group a - CG) showed a normal cerebellum architecture, with a normal molecular layer (ML), purkinje cell layer (PCL), and granular layer (GL), as well as prominent Stellate and basket cells in the molecular layer, large Purkinje cells (PC), and numerous granule cells at a magnification of X 500. (GC).

Plates 3 and 4 (group b - SOG) exhibited a grey mater outer cortex encasing a white mater inner cortex (WM). In the cerebellar cortex, stellate and basket cells (SBC) in the molecular layer, very large Purkinje cells (PC), and numerous granule cells (GC) are seen as in the control group, and cell layers- outer molecular (ML), Purkinje cell layer (PCL), and granular layer (GL) are seen as in the control group, also in high magnification there were stellate and basket cells (SBC) in the molecular layer

This is consistent with the findings of Aguwa *et al.*, (2018) and Kim *et al.*, (2016), who discovered that exposure to the chemicals chloroform and formalin, as well as the poisonous agent arsenic, causes cell death. At a higher magnification, plate 6 shows three cortical cell layers: molecular (ML), Purkinje (PCL), and granular (GL) in the experimental group c. Purkinje cells in group b are similar in size to those in group b, but they are larger than those in the control group. As in group b, granule cells are many and unique.

The molecular layer's stellate and basket cells are more prevalent in group b than in the control. Plates 7 and 8 (group - SCG) show three cortical layers, which are the same as in the control and test groups. When comparing the control group and test groups b and c, white mater (WM) may be observed deep within the granular layer, with medium-sized Purkinje cells, distributed and smaller granule cells visible at a magnification of X 500.

Cell death in varying degrees and stages, deformation of the Purkinje cell layer, infiltration of cells in the granular cell layer, and general confusion of the architecture of the cerebellum are

histopathological symptoms. There was also a scant dispersion of white matter on the granular layer, particularly in group d.

The Purkinje cell is the cerebellar cortex's only motor output. A reduction in its quantity, size, or direction may cause problems with motor activities such as loss of fine movement, gripping, maintaining balance, and muscle tone regulation. Furthermore, neuronal degeneration in the cerebellum has been demonstrated to impact the level of copper concentration in the cerebellum, which can affect the action of the neurotransmitter dopamine, which is critical for motor function. This is due to the fact that nicotine acts as a modulator for the neurotransmitter dopamine, and a drop in tar levels may cause dopamine activity to decrease. The current study's findings of granular cell infiltration and other forms of cytoarchitectural degeneration in the granular layer back up Gageli *et al* (10) findings of abnormal cytoarchitecture of the brain in infants prenatally exposed to mercury. Mercury is known to bind to microsomal and mitochondrial enzymes, causing cell injury and death. This could explain the poor results obtained in the cerebellar function hanging wire test following exposure to sniper and cigarette smoke.

Plates 9 to 16 show the histology results on the hippocampus, which show negative effects on the cells and cytoarchitecture of the rat hippocampus in the test groups. Degenerated regions, necrotic cells, and deformation of cellular layers of the hippocampus were found as general degenerative changes in the hippocampus. The hippocampus' pyramidal cells were negatively impacted, displaying many symptoms and phases of necrosis, including pyknosis and karyorhesis.

The hippocampus' pyramidal cells showed evidence of cell degeneration and distributed big pyramidal cells, which can be related to sniper exposure because these manifestations were not seen in the control group. Also, the drop in size can be linked to cigarette smoke, as it differs from the control, in which the pyramidal cell was darker and smaller than normal when both substances were combined. This means that the hippocampus's involvement in memory formation and learning may be harmed, as well as the hippocampus's role in information storage and retrieval. The outcomes of this study are consistent with Wolf et alresearch. .'s (11).

Nicotine shortage, according to Benowitz *et al.* (12), can shift glutamate's modulatory role from excitatory to inhibitory, altering its function in learning and memory.

These findings back up our findings, which reveal that sniper and cigarette smoke impair neuromuscular function and memory in rats, resulting in cell death and tissue cytoarchitectural deformation. These toxicity was manifested in the rats' poor performance in neurobehavioral tests, despite the fact that they had done significantly better before being exposed to the sniper chemical and cigarette smoke.

5.2 Conclusion

Our research has demonstrated the effect of inhaling this overlooked substance such as sniper and cigarette smoke on the brain, indicating that there is a pressing need to educate the public

about this effect, particularly women who are first-hand users, and to provide additional advice on other protective measures of usage and lighter effect chemicals that may be helpful.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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