Original Research Article

Effect of cigarette smoke Inhalation on the Hippocampus of Adult Female Wistar Rat

Abstract.

Female exposure to cigarette smoke has been a challenge to the society due to the fact that female mental health is a major prerequisite for a healthier society and future. This work was carried out to examine the effect of cigarette smoke inhalation on the hippocampus of female Wistar rats. Fourteen Female Wistar rats weighing 150-200g were divided into two groups of 7 rats each. Group A was the control group and Group B was the experimental group which was exposed to (2 stick) of cigarette smoke daily in an inhalation chamber for 14 days. Neurobehavioral study was done before and after exposure and the rats were sacrificed. Two rat brains were fixed in Bouin's fluid for histological studies and the other five for antioxidant studies. Results were analyzed using SPSS and values were significant at $P \leq 0.05$. Results showed that animals in group B had a reduction in weight after the first week of exposure to the cigarette smoke. After a fourteen day period of the experiment, group B animals had an increase in weight compared to the control. The neurobehavioral studies showed that group B animals took more time to discover the escape platform in a Morris water maze test compared to the control. Histological study showed darkly stained medium-sized pyramidal cells in the hippocampus proper (conus ammonis)of group B rats. We therefore conclude cigarette smoke inhalation has neurodegenerative effects on the hippocampus of female Wistar rats.

Key words: inhalation, cigarette smoke, neurobehavioral, hippocampus

INTRODUCTION

Background

Over the last few decades, prevalence of smoking among women of reproductive age has increased, in the other hand, Life expectancy is at the high side of depreciation and female folks are often not considered to some of these risks factor such as environmental pollution, exposure to second hand smoke which has a range of adverse health effects, including cancer, respiratory infections, and asthma (American Lung Association. June 2007).

Cigarette use by pregnant women has been shown to cause birth defects, including low birth weight, fetal abnormalities, and premature birth. These effects are seen in pregnant women who smoke. Second-hand smoke also causes many of the same health problems as smoking, including cancer, increase heart disease risk by 25-30%, lungs cancer risk by 20-30%, second hand smoke has been estimated to cause 38,000 deaths per year, of which 3400 are deaths from lungs cancer in nonsmokers (Tobacco-Free Florida., 2013) (Board, California Environmental Protection Agency: Air Resources 2005). This has led to legislation and policy that prohibit smoking in many workplaces and public areas. Scientific evidence shows that no level of exposure to second- hand smoke is safe (National Cancer Institute 2005). Cigarette smoke contains over 7,000 chemical compounds, including arsenic, formaldehyde, cyanide, lead, nicotine, carbon monoxide, acrolein, and other poisonous substances. Over 70 of these are carcinogenic (Csordas *et al*,. 2013).

The hippocampus is associated with the formation and retention of memory. The hippocampus is a bilateral structure, located beneath the neocortex, on the basal medial surface of the temporal lobes. It extends from the amygdala to the septum along the temporal lobes (Mai *et al.*, 2003; Huang, 2011). The axis from the amygdala to the septum, along the temporal lobe defines the septotemporal axis of the hippocampus (Kuhn *et al.*, 2015). It receives its main afferents from the entorhinal cortex and sends efferents to other areas of the limbic and extra-limbic systems like the fornix and temporal neocortex. The hippocampus and entorhinal cortex represent an important memory center of the brain (Kilbum *et al.*, 1989). Tobacco contains nicotine. Smoking cigarette can lead to nicotine addiction, the addition begins when nicotine acts on nicotinic actylcholic receptors to release neurotransmitters such as dopamine, glutamate, and gamma-aminobutyric acid, which withdrawing from smoking leads to the symptoms such as anxiety and irritability (Benowitz et al,. 2010). The number of nicotine receptors in the brain returns to the level of a nonsmoker between 6-12 weeks after quitting. (Benowitz et al,. 2010)

METHODOLOGY:

Fourteen (14) female Wistar rats weighing between 150-200g were obtained from Abia State University Animal house, Uturu, Abia State Nigeria. The rats were housed in Wire gauze cages and allowed to acclimatize for one week before exposure. The rats were feed with rat chow and were provided with water throughout the duration of the experiment ad libitum. Rats were handled according to global best practices.

The weight of each rat was taken before the commencement of exposure using a sensitive digital weighing balance and was repeated seven days into the exposure period and the last day of exposure. The body weight for each group was determined, analyzed and compared using the student's T-test. Data were expressed as Mean ± SD. Difference were considered significant at $P \leq 0.05$. After acclimatization, all the rats were exposed to Morris water maze test for memory (initial test). This was done on day 8 following acclimatization.

Cigarette smoke was obtained from Rothmans brand of cigarette, of which 2 sticks were used per day which amount to 50mg of content. Group A rats (control) were not exposed to cigarette smoke. Group B rats were exposed to 2 sticks of cigarette in a glass inhalation chamber for 10 minutes daily for 14 days. After exposure to cigarette smoke, the animals (group B) were transferred back to their cages. On day 15, the day after the last exposure, the rats were subjected to Morris water maze test (final test).

Thereafter the rats were sacrificed by cervical dislocation and their brains harvested. Five brains from each group were introduced into phosphate buffer solutions and centrifuged at 10,000rpm to separate the supernatants from the residues. The supernatant was used to test for antioxidant parameters; malondialdehyde (MDA), superoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH). The other two brains from each group were collected and fixed in Bouins fluid for histological studies.

RESULTS

TABLE 1: Changes in body weight of Rats

Group	1st reading (initial)	2nd reading (7 days)	3rd reading (14 days)
A	66.67±3.33	76.00 ± 8.007*	80.33 ±4.24*
B	115.33± 2.91	110.00±5.77**	121.67±10.14*

Results were presented as Mean ± Standard deviation of 5 animals.

* Indicated statistical increase significance at 99% confidence level ($P \leq 0.01$).

** indicated statistical decrease significance at 99% confidence level ($P \leq 0.01$).

Animals in the control group showed significant weight increase throughout the period of the research. The group B had a reduction in body weight at day 7 of exposure, and at day 14 body weight increased significantly compared to the initial weight.

Table 2: Results of antioxidant studies

Group	MDA	SOD	CAT	GSH
A	0.68 ± 0.13	0.25 ± 0. 13	0.59 ± 0.11	0.63± 0.04
B	0.31 ± 0.01	0.84 ± .02*	1.91 ±0.01*	1.21± 0.02*

MDA level was lower in group B compared to control. Superoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH) levels were significantly higher in group B compared to the control.

Table 3: Result of Morris Water Maze for 14 days

Group	**1st reading (initial) (seconds ± SEM)**	**2nd reading (14 days) (second ± SEM)**
A	16.76 ± 3.46	14.20 ± 4.53
B	25.79 ± 6.61	25.10 ± 11.19

Table 3 reveals that it took lesser time for animals in group A (control) to locate the stage at the final test than it was at the onset and this is statistically significant. It took animals in the experimental group B more time to locate the stage at the end of the period of exposure than it took them before exposure.

Result of Histological Studies

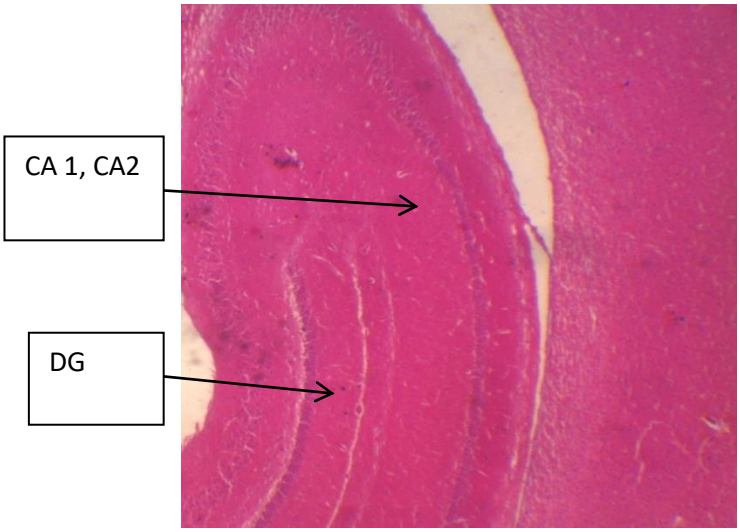

Plate 1A: Representative photomicrograph of hippocampus (Mag. X125) of control group show the conus ammonis (CA 1, CA2) and dentate gyrus (DG).

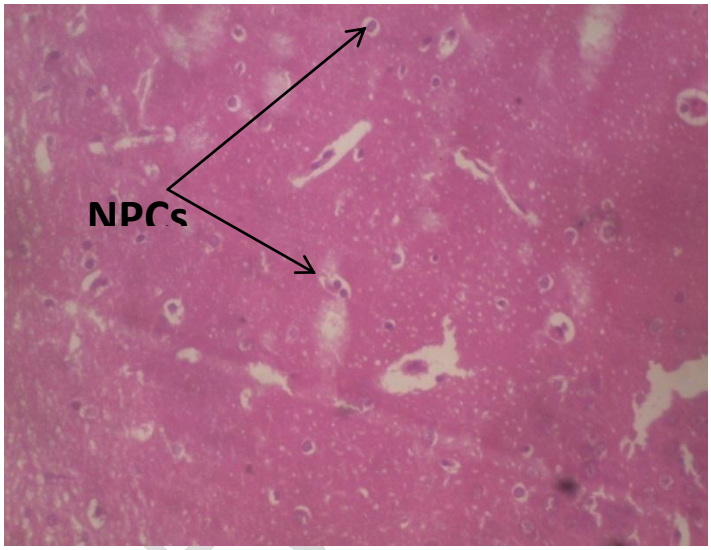

Plate 1B: Representative micrograph of hippocampus (Mag.X600) of control group show normal pyramidal cells (NPCs).

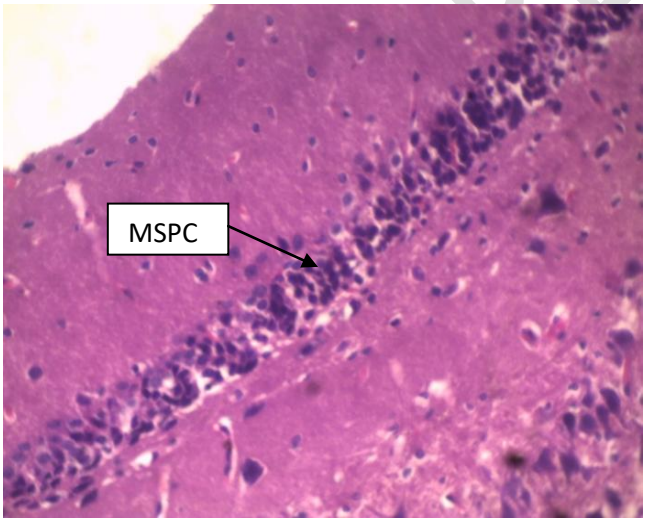

Plate 2A: Representative photomicrograph of hippocampus (Mag. X125) of Group B showing conus ammonis (CA) and dentate gyrus(DG).

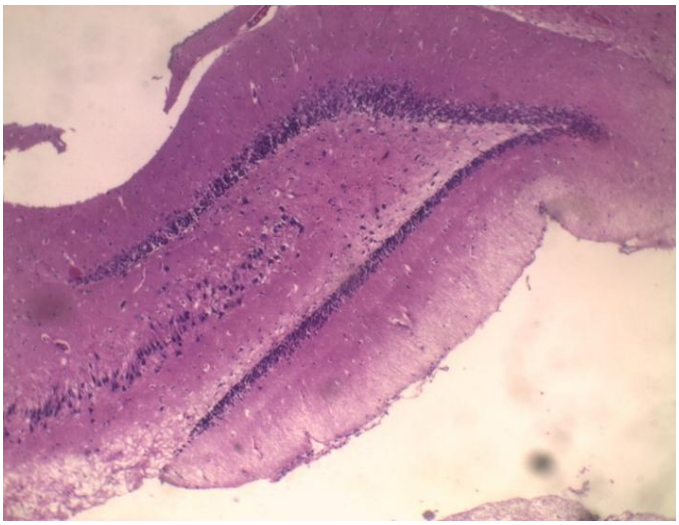

Plate 2B: Representative photomicrograph of hippocampus (conus ammonis) (Mag. X125) of Group B showing darkly stainedmedium pyramidal cells

DISCUSSION

Cigarette smoke contains over 7,000 chemical compounds, including arsenic, formaldehyde, cyanide, lead, nicotine, carbon monoxide, acrolein, and other poisonous substances. Over 70 of these are carcinogenic (Csordas et al,. 2013).

Nicotine present in the cigarettes travel to the brain within 8 seconds of the first inhalation. Since a nicotine molecule is shaped similar to a neurotransmitter, it gets lodged onto the brain receptors and activates areas of the brain involved in producing feelings of pleasure and reward.

Second-hand smoke causes many of the same health problems as smoking, including cancer (Board, California Environmental Protection Agency: Air Resources 2005).

In this study, it was observed that cigarette smoke exposure for 10 minutes each day for 14 days caused significant weight decrease at day 7 when compared to the initial body weight. At day 14, there was a significant increase in the weight of the rats exposed to cigarette smoke when compared to the control. This indicates adaptation to smoke-filled environment.

Antioxidant studies reveals that the rats exposed to cigarette smoke were not under oxidative stress. This was obvious as malondialdehyde (MDA) level which is an indicator of lipid peroxidation was highly significantly decreased in the experimental group B compared to the control group A. Oxidative stress leads to a rise in MDA levels in rats (Gornall et al, 1949;

Plate 2B: Representative

enzymes which are responsible for mopping up free radicals generated by lipid peroxidation (SOD, CAT and GSH) were all significantly increased in the experimental group compared to the control. Oxidative stress has been associated with many chronic diseases, some of which are incurable, including cancer, diabetes and high blood pressure (Tongnit et al., 2004).This study has shown that second hand smoke is not one of the major causes of any of this kind of disease.

The hippocampus belongs to the limbic system and is involved in emotion and memory. Any impairment on the hippocampus will negatively affect memory, ability to remember as

well as emotional stability of the affected animal. The Morris water maze test is a neurobehavioral study that tests for memory among other things. In this study, it took lesser time for animals in group A (control) to locate the stage at the final test than at the onset. Animals in the experimental group B took more time to locate the stage at the end of the period of exposure than it took them before exposure. This may be a sign of gradual but steady loss of cells involved in building and storing memory. The conus ammonis of the test group showed darkly stained medium-size pyramidal cells. This, together with the results of the neurobehavioral studies, give a trace of hippocampus impairment.

CONCLUSION

We therefore conclude from this work that exposure to second hand cigarette smoke for ten minutes daily for 14 days may lead to hippocampal impairments.

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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