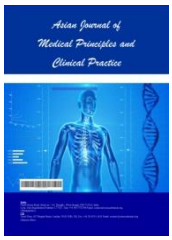

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# Effect of Cigarette Smoke Inhalation on the Hippocampus of Adult Female Wistar Rat

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The increasing female exposure to cigarette smoke is a public health challenge to the society as 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 two sticks of cigarette smoke daily in an inhalation chamber for 14 days. Neurobehavioral study (Morris water maze test) 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≤0.05. Results showed that animals in group B had a reduction in weight after the first week of exposure to the cigarette smoke compared to the initial weight. After a fourteen day period of the experiment, group B animals had significant increase in weight compared to the initial weight. The neurobehavioral studies showed that group B animals took more time to discover the escape platform in a Morris water maze test at the end of the experimental period compared to the initial values. Histological study showed darkly

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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.

*Keywords: Inhalation; cigarette smoke; neurobehavioral; hippocampus.*

## 1. INTRODUCTION

Over the last few decades, prevalence of smoking among women of reproductive age has increased significantly. A lot more reproductive age women are also constantly exposed to second hand smoke [1].

Cigarette use by pregnant women has been shown to cause birth defects, including low birth weight, fetal abnormalities, and premature birth. 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 (Board California Environmental Protection Agency: Air Resources 2005; Tobacco-Free Florida., 2013). 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).

A large percentage (30-35%) of men and women of reproductive age in the United States utilize nicotine via smoking cigarettes on a daily basis. An additional portion of nonsmokers, especially children, are also affected as “second hand smokers” by inhaling side stream smoke from burning cigarettes and exhaled smoke from smokers (U.S department of Health and Human Services) [2]. It can be said today that smoking has been established as the number one preventable cause of death and disease in the United States and in other countries worldwide.

Most lung cancer and emphysema, as well as a high percentage of heart attacks are caused by cigarette smoking. Recent scientific data reveal that the cancer risk from smoking is not limited to cancer of the lung. Many bladder, cervical, esophageal, and pancreatic cancers are also caused by smoking. In 1987, lung cancer replaced breast cancer as the number one cancer killer of women in America. It is now generally accepted that at least 434,000 Americans die each year of smoking related illnesses. Although 30 million Americans have given up smoking in the past 20 years, 50 million still smoke. These are not just loyal customers. They are, for the most part, addicted to cigarettes.

According to a current report [3], it is suggested that if current smoking rates continue in the world, more than one-fifth of the people alive in the developed world today will eventually die of smoking related causes, a far greater toll than previously thought. Cigarette smoke contains a large number of substances, including nicotine, carbon monoxide, and recognized carcinogens and mutagens, such as radioactive polonium, benzo(a)pyrine, dimethylbenz(a)anthracene, dimethylnitrosamine, naphthalene, and methylnapthalene [4]. Many of these constituents, however, have never been evaluated for their toxicological effects and their impact on the human body and general health and therefore the full ingredients of cigarettes and cigarette smoke remain unknown. Inhalation of cigarette smoke, whether through active or passive smoking, leads to absorption of these substances through the pulmonary vasculature and blood borne circulation throughout the body [5].

Because of the vast amounts of scientific and clinical data concerning smoking and health and because of the recent surge of interest in the effects of smoking on reproductive health, it is the intent of this review to limit the discussion to the effects of smoking on female and male reproductive health only [6]. Numerous other important aspects of smoking on general health care, public policy, contraceptives, and smoking prevention are beyond the scope of this review and will not be addressed.

Overall, when female fertility is not compromised, attempts to achieve conception typically involve having sex with a male and establishing the pregnancy without any medical assistance or interference.

Under normal circumstances, fertilization of an egg, subsequent attachment of the yielded embryo, and maintenance of pregnancy depend on a series of rather complex and somewhat interrelated events. Those events involve the presence of an intact, healthy female reproductive tract that can produce eggs, enable them to be fertilized in the Fallopian tubes and become embryos and then lead them into the uterus where they attach and grow and give rise to a healthy baby. Specifically, the female must have healthy ovaries that can produce eggs. Also, she must have adequate hormone levels that can properly stimulate the production of the eggs at the time of ovulation and to later support the attachment of the embryos to the uterine lining and maintenance of pregnancy. The whole process is rather complex, and cigarette smoking can interfere with almost every aspect of egg production, the fertilization process, embryo attachment, and the proper growth and development of the baby during the pregnancy. The mechanisms as to how smoking affects the above defined processes are not totally understood because of the complexity of the reproductive system and the process of fertilization, but the evidence and understanding of these modes are becoming clearer every day as more and more data become available. Data from several epidemiologic studies show that women “habitual” cigarette smokers had significantly increased frequency of infertility when compared to nonsmokers (21% versus 14%, respectively).

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 [7,8]. The axis from the amygdala to the septum, along the temporal lobe defines the septotemporal axis of the hippocampus [9]. 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 [10]. 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 [11]. The number of nicotine receptors in the brain returns to the level of a nonsmoker between 6-12 weeks after quitting [12].

## 2. 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 learning and memory (initial test). This was done on day 8 following acclimatization.

Cigarette smoke was obtained from Rothmans brand of cigarette, of which two 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.

## 3. RESULTS

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.

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

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.

**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 7 animals*
** Indicated statistical increase significance at 95% confidence level (P ≤ 0.05)*

## 3.1 Result of Histological Studies

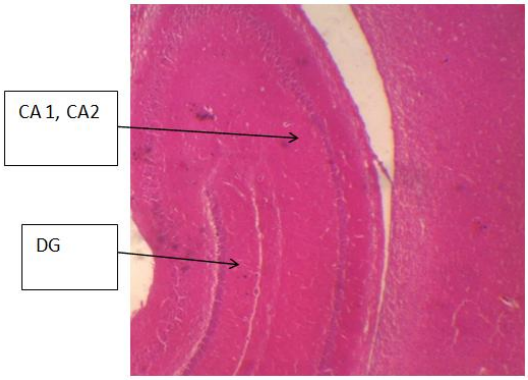


**Plate 1A. Representative photomicrograph of hippocampus (Mag. X125) of control group show the conus ammonis (CA 1, CA2) and dentate gyrus (DG)**

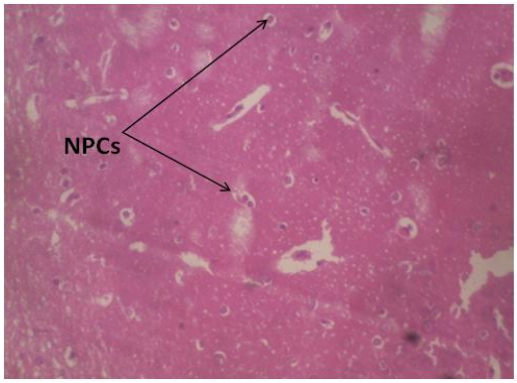


**Plate 1B. Representative micrograph of hippocampus (Mag.X600) of control group show normal pyramidal cells (NPCs).**

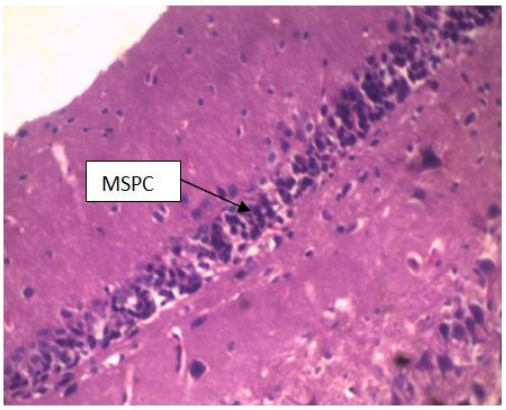


**Plate 2A. Representative photomicrograph of hippocampus (Mag. X125) of Group B showing conus ammonis (CA) and dentate gyrus(DG)**

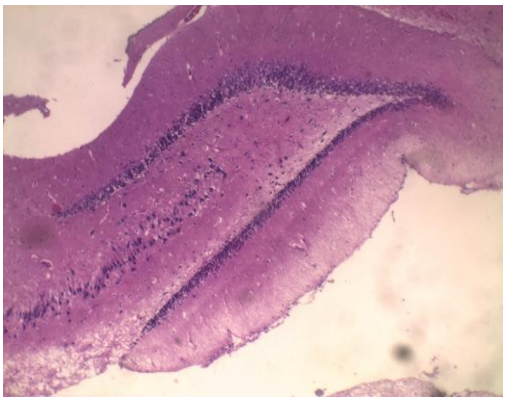

**Plate 2B. Representative photomicrograph of hippocampus (conus ammonis) (Mag. X125) of Group B showing darkly stainedmedium pyramidal cells**

**Table 2. Results of antioxidant studies**

| Group | MDA | SOD | CAT | GSH |
|---|---|---|---|---|
| A | 0.31 ± 0.01 | 0.84 ± .020 | 1.91 ±0.01 | 1.21± 0.02 |
| B | 0.68 ± 0.13* | 0.25 ± 0. 13* | 0.59 ± 0.11* | 0.63± 0.04* |

**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 |

## 4. 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 7 days caused significant weight decrease in rats. At day 14, there was a significant increase in the weight of the rats exposed to cigarette smoke when compared to the initial weight. This indicates adaptation to smoke-filled environment. Antioxidant studies reveals that the rats exposed to cigarette smoke were under oxidative stress. This was obvious as malondialdehyde (MDA) level which is an indicator of lipid peroxidation was significantly higher in the experimental group B compared to the control group A. Oxidative stress leads to a rise in MDA levels in rats [12, 13]. Furthermore, the antioxidant enzymes which are responsible for mopping up free radicals generated by lipid peroxidation (SOD, CAT and GSH) were all significantly lower 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 [14]. This study has shown that second hand smoke could predispose to 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.

Histological studies reveal that the conus ammonis of the test group showed darkly stained medium-size pyramidal cells. Several pyknotic and karyorhetic cells were equal present in the study. This, together with the results of the neurobehavioral studies, gives a trace of hippocampus impairment.

## 5. 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.

## DISCLAIMER

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.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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