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Postnatal Neurodevelopment: Implication of Lactational Nicotine on Oxidative Status and Neurocortical Architecture in Juvenile Rats

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ABSTRACT

Despite numerous researches demonstrating detrimental effect of nicotine on various body systems, a large percentage of women population still use tobacco products or nicotine replacement therapy, and a substantial proportion fall back to smoking during breastfeeding. This study aimed at demonstrating the effects of lactational nicotine exposure on the development of frontal cortex in young rats.

Thirty female Wistar rats weighing 160 g averagely were mated based on their estrous cycle. After littering, the rats were grouped based on their lactating periods into 3; Group A (control group) received 0.1 ml normal saline from postnatal day (PD) 1-14; Group B received 0.05 ml of nicotine from PD 1-7; while Group C received 0.05 ml nicotine from PD 1-14. Administration was intraperitoneal. The weights of the pups were observed throughout the experiment. On the 15th day of lactation, the pups were euthanized. The brain was weighed and the frontal cortex excised. The frontal cortex was then processed for quantitative analysis of glutathione peroxidase (GPx), malondialdehyde (MDA) and tissue histology.

Significant weight reduction was observed in the treatment groups compared to control. The levels of oxidative enzymes (MDA and GPx) increased in the nicotine exposed groups with variations in increment in relation to duration of postnatal exposure. The frontal cortex of longer nicotine-exposed animals was markedly disrupted.

Use of nicotine during breastfeeding period is detrimental to the development of the baby, and could result in neuromorphological disturbances.

Keywords: frontal cortex, postnatal nicotine, rat weight, GPx, MDA, histology

INTRODUCTION

The frontal cortex is the centre of man's personality, and lesions of this part of the brain results in a variety of symptoms¹. The frontal cortex is very delicate and in mild to moderate traumatic brain injury, it has proven to be the most common region of injury². Breast milk provides an infant with nutrients it needs to grow³. Aside this, breastfeeding is about the most important route through which an infant receives all it needs to grow both physically and mentally.

Nicotine reduces the production of milk, by lowering the serum prolactin which must be present for milk synthesis to occur⁴. The primary alkaloid of tobacco is nicotine as most alkaloids found in tobacco share structures related to it⁵. Nicotine acts through various mechanisms. First, it mimics acetylcholine thereby activating acetylcholine receptors. Through this mechanism, it initiates increases in catecholamines such as adrenaline which in turn underlies fat burning (implicated in loss of weight) and potential addition.

Moreover, nicotine is pro-oxidative in nature⁶, as it induces oxidative stress both *in vivo* and *in vitro*⁷. Oxidative stress which occurs when there is an excess

production of free radicals followed by a low antioxidant defense, results in chemical alterations of biomolecules which in turn causes modifications in structures and functions⁸. Although the mechanisms by which nicotine generates free radicals are unclear, it has been observed that nicotine causes a disruption in the mitochondrial respiratory chain leading to an increase in production of superoxide anions and hydrogen peroxide⁹. Nicotine enhances lipid peroxidation and depletion of antioxidants¹⁰. Nicotine has anti-inflammatory properties which it exerts using its actions as a cholinergic agonist¹¹.

Nicotine accumulates in bodily fluid, especially saliva and gastric juices, also in breast milk^{12, 13}. Nicotine is rapidly absorbed in the lungs (mostly after smoking) and can be detected in the brain tissue about 10-20 seconds following a puff of cigarette¹⁴.

MATERIALS AND METHODS

Thirty (30) adult female Wistar rats of average weight 160 g were obtained and kept in the Animal House of the College of Health Sciences, University of Ilorin following ethical considerations from Ethical Review

Committee. The rats were fed with pelletized grower marsh which was bought from Ogo-Oluwa Livestock Enterprises, Ilorin, Kwara State. Nicotine (0.25 mg/ml) was obtained from BDH Chemical Ltd. Poole, England.

After 3 weeks of acclimatization, the rats were exposed to males for mating. The female Wistar rats were then grouped based on their lactating periods into 3:

Group A (control group) received 0.1 ml normal saline from postnatal day (PD) 1-14;

Group B received 0.05 ml of nicotine from PD 1-7;

Group C received 0.05 ml nicotine from PD 1-14.

Administration was intraperitoneal using insulin syringe.

Sacrifice and Tissue Evaluation

On the 15th day of lactation, the pups were euthanised. The brain was weighed; the frontal cortex was excised and weighed too. The specimens for histological studies were fixed in 4% paraformaldehyde for further tissue processing. Histological sections were obtained using Haematoxylin and Eosin stain after the frontal cortex was dehydrated through grades of alcohol, cleared with xylene, infiltrated and embedded in paraffin and then sectioned using rotary microtome.

Biochemical analysis

The activities of glutathione peroxidase (GPx) and malondialdehyde (MDA) were assayed in the frontal cortex of the 15-day old pups. Homogenate of the

frontal cortex was made with ice cold 30% sucrose solution with the use of a Teflon homogenizer. The homogenate was then scooped and poured into a 5ml plain specimen bottle and then placed in the centrifuging tube containing ice. The homogenate was centrifuged for about 15 minutes at 3000 rpm. The supernatant of the homogenate was decanted into another plain specimen bottle and used to assess the levels of GPx and MDA.

Statistical analysis

Data collected were analyzed using Microsoft Excel and one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test (HSD) with the aid of SPSS version 20. Data were presented as means $\pm$ SEM (standard error of mean). P value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Weight changes

The rate of gain of weights differs in all the groups. It was least in the pups that received nicotine for 14 days postnatally, followed by those that received nicotine in the first 7 days of life, when compared with the Control. While minimal weight increase was recorded in the pups exposed to nicotine in the first 14 days of life, cessation of postnatal nicotine at PD 7 resulted in marked increase in weight gain for the next one week, though not as high as in the Control (Figure 1).

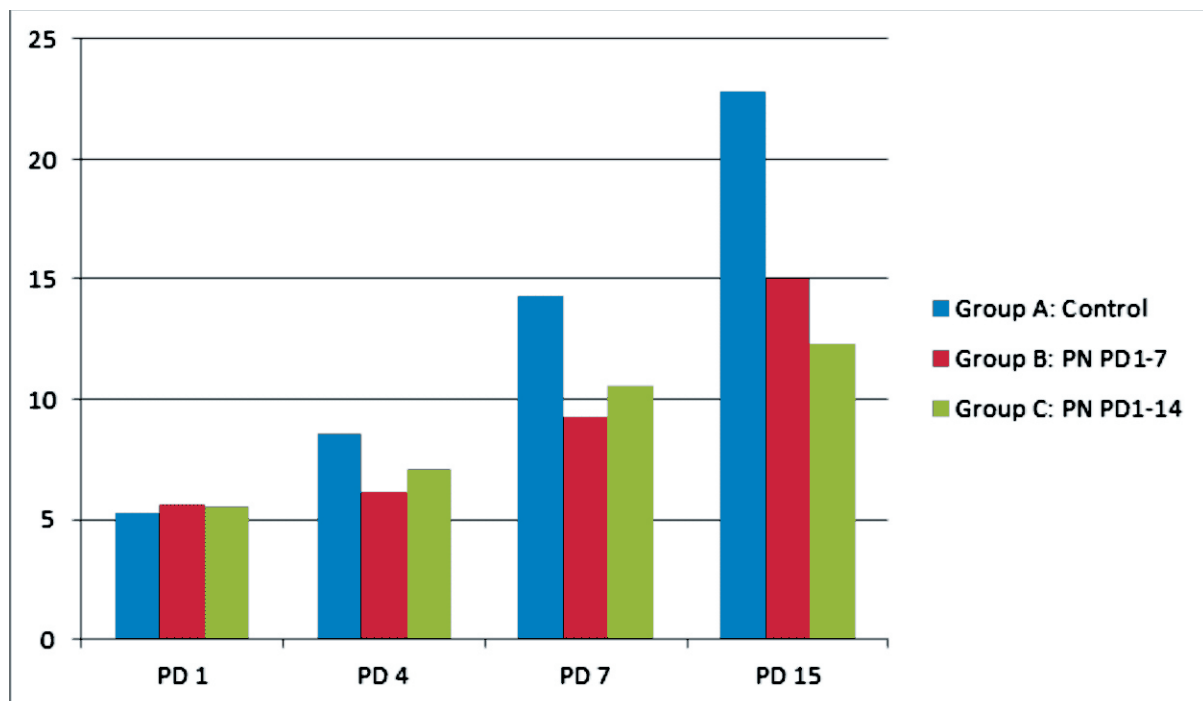


Figure 1: Weight of Pups; PN: postnatal nicotine, PD: postnatal day

Brain Weights

Table 1: Weights of the whole brain and frontal cortex

Groups	Brain (g)	Frontal Cortex (g)
Group A: Control	0.86±0.02	0.13±0.02
Group B:PN PD 1-7	0.99±0.03	0.11±0.01
Group C:PN PD 1-14	0.80±0.03 α	0.25±0.03 α

PN: postnatal nicotine, PD: postnatal day.

Brain weight

There was no significant change in the weight of the brain in the nicotine (groups B and D) treated animals compared to control (group A). However there is a significant reduction in the brain weight in animals exposed to nicotine for 14 days (group C) compared to animals exposed to nicotine for seven days (group B) (Table 1; Figure 2).

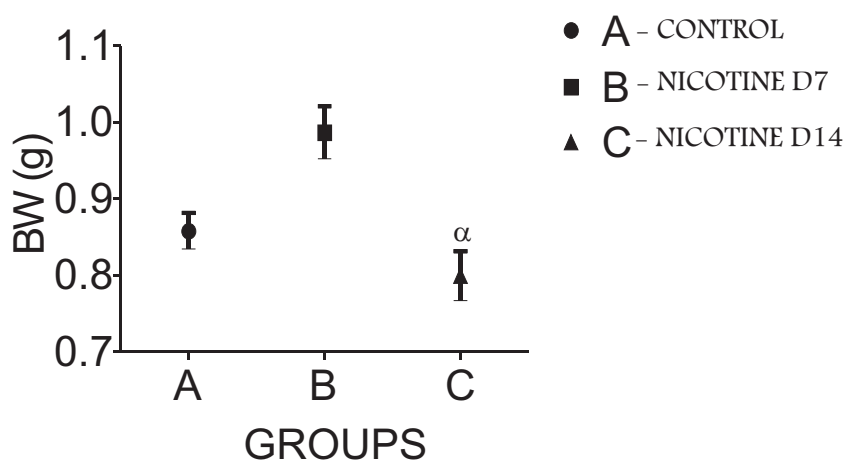


Figure 2: Graph showing the brain weights across all groups. α ($p < 0.05$) statistical significant difference compared to animals exposed to nicotine for seven days (group B).

Frontal cortex weight

There is no significant change in the weight of the frontal cortex in the nicotine (groups B and C) treated animals compared to control (group A). However there is a significant increase in the frontal cortex weight in animals exposed to nicotine for 14 days (group C) compared to animals exposed to nicotine for seven days (group B) (Table 1; Figure 3).

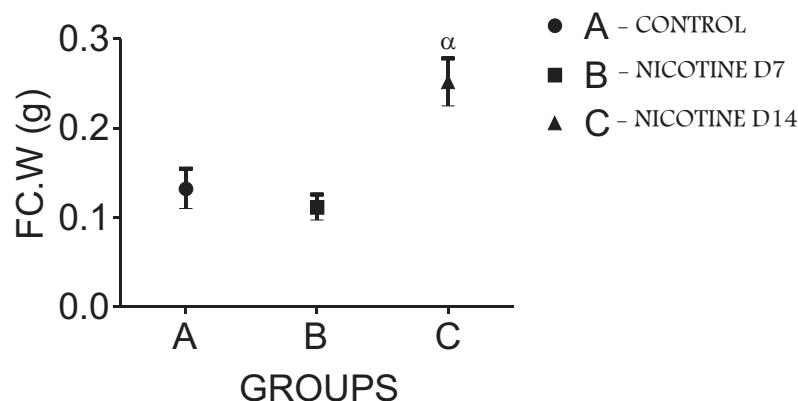


Figure 3: Graph showing frontocortical weights across all groups. α ($p < 0.05$) statistically significant difference compared to animals exposed to nicotine for seven days (group B).

Biochemical Studies

Nicotine at durations tested (groups B and C) caused a slight increase in the levels of Glutathione peroxidase (GPx) but not statistically significant compared to control groups (group A). Nicotine at durations tested (groups B and C) also caused a slight increase in the levels of malodialdehyde (MDA) but not statistically significant compared to control groups (Table 2).

Table 2: Table showing GPx and MDA levels across all experimental groups

Groups	GPx (IU/L)	MDA ($\mu\text{mol/L}$)
Group A: Control	2390 $\pm$ 25	10.5 $\pm$ 0.5
Group B:PN PD 1-7	2515 $\pm$ 110	13.5 $\pm$ 1.5
Group C:PN PD 1-14	2472 $\pm$ 104	14.5 $\pm$ 1.5

Histological observation

The histological sections revealed apparently normal architecture in the control, while the treated groups showed varying degrees of poor cortical delineation, reduced staining intensity, reduced cell population and alteration in cell shape especially in the frontal cortex of animals exposed to lactational nicotine for 14 days, where there were poorly stained ovoid shaped nuclei, reduced nuclear size and decrease in cell population.

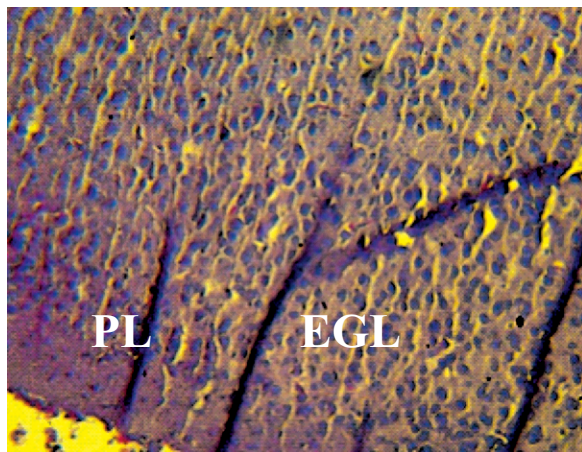


Figure 4: A Representative photomicrograph of the frontal cortex of 15 days old Wistar rats in the control group administered with normal saline in the first 14 days of postnatal life (PNL), showing apparently normal cortical architecture, numerous cells, and well-stained. The cortical layers were fairly well delineated H&E (x100) (PL-pyramidal layer EGL-external granular layer)

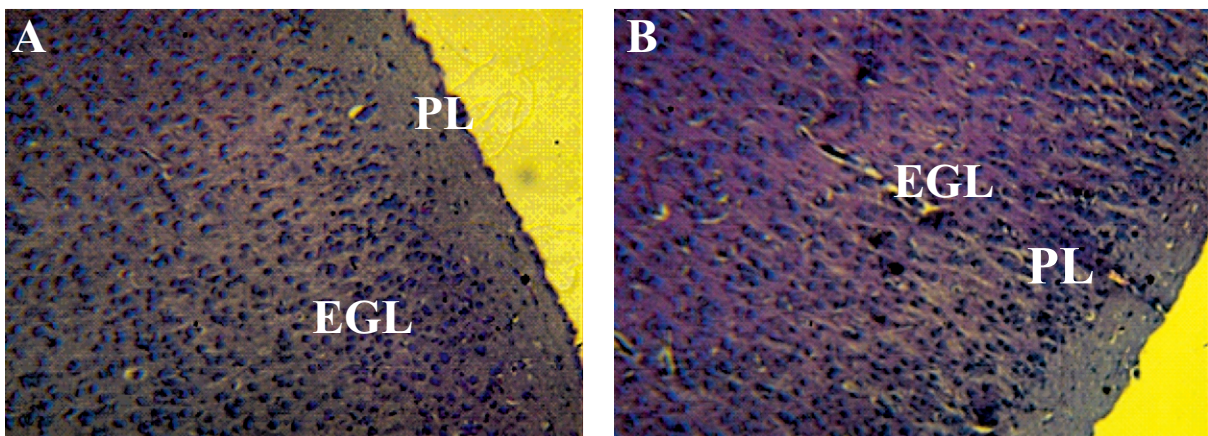


Figure 5 (a): A Representative photomicrograph of the frontal cortex of 15 days old Wistar rats treated with low dose lactational nicotine in the first 7 days of postnatal life, showing lightly stained tissue, reduced population of cells and cell size, the cortical layers were poorly well delineated compared with the control group. **(b):** A Representative photomicrograph of the frontal cortex of 15 days old Wistar rats treated with low dose lactational nicotine in the first 14 days of postnatal life, showing poor cortical architecture, ovoid shaped nuclei reduced nuclei size and numbers, not well- stained, the cortical layers are poorly delineated compared with the control group H&E (x100).

DISCUSSION

Nicotine is known for the role it plays in reduction of insulin sensitivity which in turn leads to reduction in body weight of animals¹⁵. This is in concordance with the result observed from the pattern of weight gain in the pups. At the 15th day of lactation, the weight of the pups in the treatment group was very low, compared to control. However, the pups that received low dose nicotine for only 7 days recorded a weight with high significance compared to pups that received the same dose for 14 days. This can probably be because, as previously noted¹⁶, smokers gained weight as they quitted smoking. Also, the weights of the pups that received high dose nicotine were significantly lower than those treated with low dose. A similar result was earlier reported¹⁷, in which the body-organ weight ratio decreased in a dose-dependent pattern. Also, Jo *et al.*,¹⁸ termed nicotine to be the major component of tobacco that suppresses appetite. Nicotine works by increasing the levels of hormones which are implicated in weight loss as a consequence of suppressed appetite¹⁹. Nicotine acts on nicotinic cholinergic receptors in the brain and autonomic ganglia²⁰. As nicotine binds to the nicotinic receptors, ion channels are opened, allowing entry of sodium and calcium, which, in turn, supplement the release of various neurotransmitters. This leads to release of dopamine, norepinephrine, serotonin, acetylcholine, glutamate, and other neurotransmitters.

Malondialdehyde is commonly used to monitor or determine the rate of lipid peroxidation and indirectly oxidative stress *in vitro* and *in vivo*²¹. The data from this experiment showed elevated MDA levels in rats administered with nicotine. This suggests that nicotine induces increased lipid peroxidation with its attendant pathologies. On the other hand the increased generation of reactive oxygen species may elicit cellular upregulation of glutathione peroxidase (GPx) to combat the increased generation of free radicals²² resulting in elevated GPx as reported in the current study at the the end of seven days of nicotine administration. However, there was a reduction in GPx level at the end of 14 days of nicotine administration, suggesting that GPx, an enzymatic antioxidant, might have been overwhelmed by the increased level of lipid peroxidation over a prolong duration resulting in oxidative stress.

The histology of the frontal cortex of the control group given normal saline presented with apparently normal cortical architecture, numerous cells, and well-stained. The cortical layers were fairly well-delineated. The histological evaluation of the treatment groups presented with lightly stained tissue, reduced population of cells and cell size, the cortical layers were poorly well delineated compared with the control group. This shows the effect of postnatal nicotine exposure on postnatal development of the frontal cortex. This is in line with earlier findings of Dwyer *et*

*al.*²³ which stated that the prenatal and postnatal development of different parts of the brain is affected by nicotine. We have earlier observed significant changes in the neocortex of adolescent rats exposed to nicotine during the prenatal life²⁴.

In conclusion, postnatal nicotine exposure is detrimental to the developing frontal cortex and its functions, and lactating women should avoid the exposure of their infants to nicotine products

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