

## ARTICLE

# Neurohistochemical Studies of Adolescent Rats' Prefrontal Cortex Exposed to Prenatal Nicotine

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## Abstract

**Background:** Exposure to tobacco has frequently been associated with adverse implications on many body organs and systems. Maternal smoking can influence fetal development, causing intrauterine growth restriction, preterm birth, or even fetal death and spontaneous abortion. **Objectives** We investigated the effects of prenatal exposure to nicotine on the prefrontal cortex in adolescent rats. **Materials and Methods:** Twenty-four mature female Wistar rats were time mated and grouped according to Trimester into Control and Treated groups. Nicotine was administered intra-peritoneally to pregnant Wistar rats in the treated groups, while normal saline was given to the control groups, at each of the three Trimesters. The animals were allowed to litter and the pups were allowed to grow till postnatal day 35, when they were sacrificed and the brain removed and weighed. The prefrontal cortex was excised and either fixed in 4% paraformaldehyde for tissue histology or homogenized in sucrose solution for enzyme studies

(alkaline phosphatase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase). **Results:** Enzyme studies showed derangement in biochemical status of the prefrontal cortex of all the nicotine-exposed animals compared with their respective controls, and corresponding morphological and histological alterations, especially in animals exposed to nicotine during their 2<sup>nd</sup> and 3<sup>rd</sup> weeks of fetal life. **Conclusions:** The morphohistological and biochemical derangements that occur during neurodevelopment of nicotine-exposed offspring persist into adolescent life, and could underlie the neurological dysfunctions associated with such individuals.

**Keywords:** Adolescents, Prenatal nicotine exposure, Prefrontal cortex, Enzymes, Histology

## Introduction

Tobacco is consumed in every part of the world, and the rate of consumption has increased in developing countries

(1). Exposure to tobacco, either from smoking, snuffing or chewing, has frequently been associated with adverse implications on many body organs and systems, including alteration in the normal functions of the brain and the nervous system (2, 3). Nicotine is medically administered to reduce withdrawal symptoms in people seeking to quit cigarette smoking (4, 5). The frontal lobe is involved in motor action, motivation, foresight planning, memory, mood, emotion, social judgment, and aggression (6). Frontostriatal brain regions are essential in executive functions, such as attention processing and inhibitory response control, as well as decision-making processes (7, 8). The human brain continues to develop until about age 20, with synaptic pruning and myelination of frontal cortical regions maturing in the later stages of adolescence (9). Because the adolescent brain is still plastic, exposure to addictive substances, such as nicotine inhaled from tobacco smoke may greatly impact the development of frontal cortical regions, leading to various alterations in brain function that persist into adulthood (10). In the prenatal period, maternal smoking can influence fetal development, causing intrauterine growth restriction, preterm birth, or even fetal death and spontaneous abortion (11-13). Similarly in the perinatal period, nicotine exposure is associated with respiratory disorders, asthma, and sudden infant death syndrome (14). In the current study, effects of prenatal nicotine on the development of the prefrontal cortex in each trimester were determined using histological and quantitative histochemical techniques.

**Materials and Methods**

**Protocol**

Twenty-four albino Wistar rats were time mated overnight, and pregnancy was confirmed the following morning by

the presence of spermatozoa in the vaginal smear (15), and this was taken as day 0 of gestation. They were grouped according to gestational period of drug administration (Table 1). The treated groups were given intra-peritoneal nicotine (BDH Chemical Ltd. Poole, England) 0.06 mg/kg in two divided doses for six (6) consecutive days from the beginning of each trimester. The animals were monitored till the end of gestation.

**Specimen Collection**

At delivery, the weights of the pups were taken. They were sacrificed by cervical dislocation at postnatal day 35 (PND 35). The brain was dissected out and weighed; the prefrontal cortex dissected out, and weighed too. Specimens for quantitative histochemistry were homogenized in 0.25 M sucrose solution and centrifuged (Model 90-1; Gallenkomp, England) at a very low temperature at 5000 rpm for 5 min, while tissue samples for histology were fixed in 4% paraformaldehyde solution.

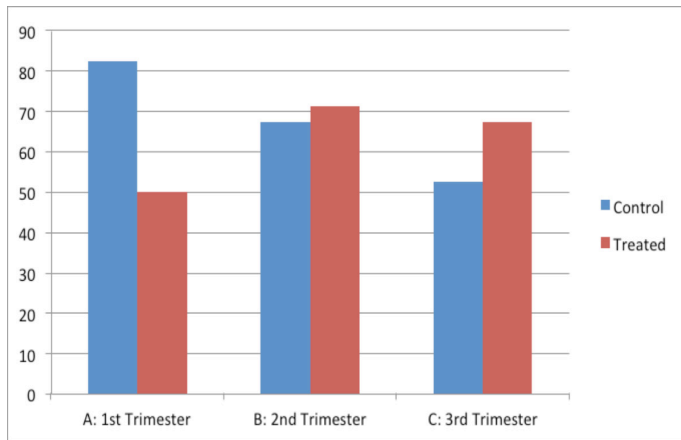
**Laboratory Studies**

The supernatant from the centrifuged samples was used in assessing the activities of alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH), using appropriate biochemical kits from Randox Laboratory, UK. Specimens of the prefrontal cortex for histological studies were fixed in 4% paraformaldehyde before processing using the routine Haematoxylin and Eosin stains.

**Data analysis**

Data from the enzyme study were analysed using statistical software (SPSS version 15.0) and presented as Mean ±SEM with a confidence limit of 95%.

Table 1. The groups of mice according to gestation period of drug administration			
Group	Subgroup	Treatment	Gestation Stage and Duration
Group A <sub>1</sub>	Control	0.1 ml normal saline	1 <sup>st</sup> week of gestation (days 1-6)
Group A <sub>2</sub>	Active	0.06 mg/0.1 ml nicotine	
Group B <sub>1</sub>	Control	0.1 ml normal saline	2 <sup>nd</sup> week of gestation (days 8-13)
Group B <sub>2</sub>	Active	0.06 mg/0.1 ml nicotine	
Group C <sub>1</sub>	Control	0.1 ml normal saline	3 <sup>rd</sup> week of gestation (days 15-20)
Group C <sub>2</sub>	Active	0.06 mg/0.1 ml nicotine	



**Figure 1.** Weight changes (in grammes) in adolescent Wistar rats at postnatal day 35.

Group	Brain weight (g)	Brain weight Diff in each Trimester (g)	PFC weight (g)	PFC-Brain weight ratio
A <sub>1</sub> : 1 <sup>st</sup> Trimester Control	1.6541 ± 0.0459	-	0.1243 ± 0.0073	0.0751
A <sub>2</sub> : 1 <sup>st</sup> Trimester Treated	1.3264 ± 0.0732	-0.3277	0.1119 ± 0.0043	0.0844
B <sub>1</sub> : 2 <sup>nd</sup> Trimester Control	1.4718 ± 0.0267	-	0.1217 ± 0.0025	0.0827
B <sub>2</sub> : 2 <sup>nd</sup> Trimester Treated	1.4884 ± 0.0194	0.0166	0.1410 ± 0.0128	0.0947
C <sub>1</sub> : 3 <sup>rd</sup> Trimester Control	1.4343 ± 0.0786	-	0.0867 ± 0.0165	0.0604
C <sub>2</sub> : 3 <sup>rd</sup> Trimester Treated	1.2702 ± 0.0237	-0.1641	0.0903 ± 0.0025	0.0711

## Results

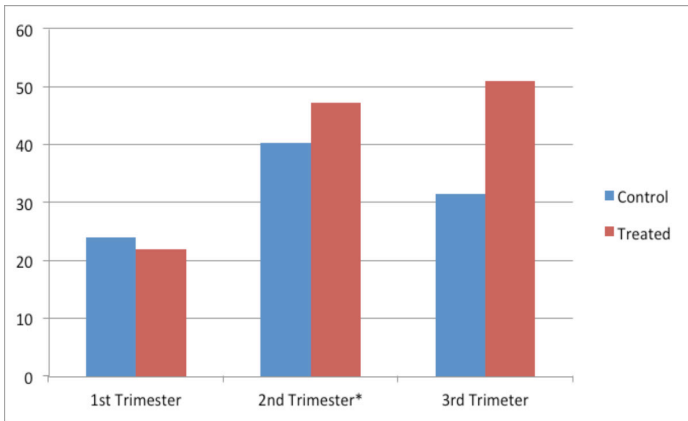
### *Weight Changes*

The weight of the animals on PND 35 was markedly reduced in the 1<sup>st</sup> trimester treated group, whereas in the subsequent trimesters, there was an increase in the weights of the treated groups, compared with their respective controls (Figure 1). Brain weight on PND 35 reduced in both 1<sup>st</sup> and 3<sup>rd</sup> trimesters; and the decrease was marked in the 1<sup>st</sup> trimester. However, a slight increase was noticed in the 2<sup>nd</sup> trimester group (Table 2).

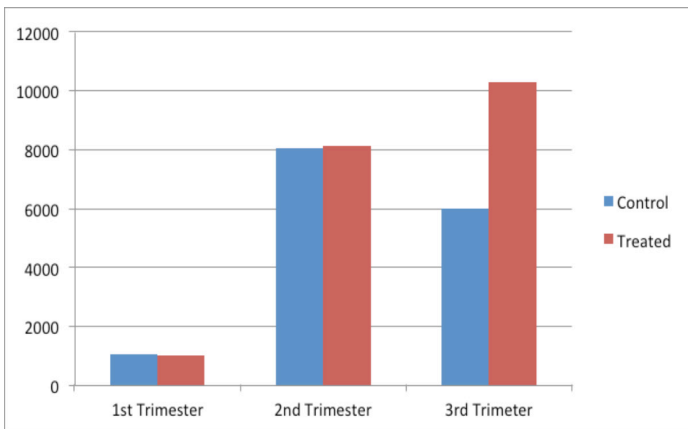
### *Quantitative Histochemical Studies*

The enzyme activity of ALP and LDH was more pronounced in the 2<sup>nd</sup> and 3<sup>rd</sup> Trimesters, compared with the 1<sup>st</sup> Trimester, while the activity of G-6-PDH was more in the 1<sup>st</sup> and 3<sup>rd</sup> Trimesters compared with the 2<sup>nd</sup> Trimester (Figures 2-4). The tissue activity of ALP was reduced in

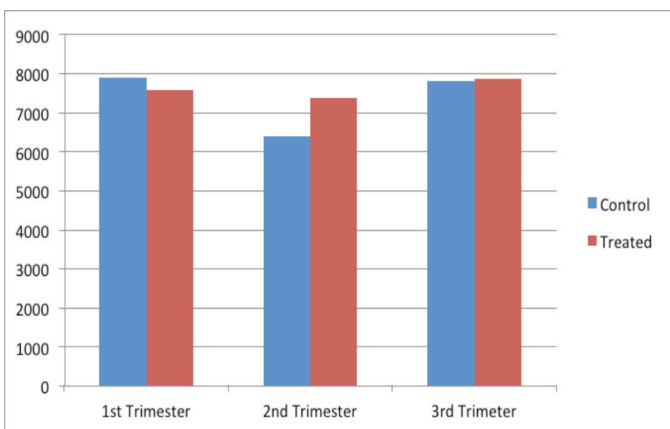
the 1<sup>st</sup> Trimester treated group ( $p > 0.05$ ), compared with the Control. However, in the 2<sup>nd</sup> Trimester, a statistically significant increase in ALP activity was observed in the treated group ( $p < 0.05$ ), and this activity further increased in the 3<sup>rd</sup> Trimester treated group. LDH activity decreased in the 1<sup>st</sup> Trimester treated group ( $p > 0.05$ ), but increased in 2<sup>nd</sup> and 3<sup>rd</sup> Trimesters, when compared with their respective Controls. The activity of LDH also increased markedly in the 3<sup>rd</sup> Trimester group treated with prenatal nicotine compared with the control (Figure 3). Changes in the activity of G-6-PDH were not as marked as those of other enzymes, except in the 2<sup>nd</sup> Trimester where the treated group increased markedly compared with the control; although the level increased also in the 3<sup>rd</sup> trimester, it was only minimal. In the 1<sup>st</sup> Trimester, however, G-6-PDH activity was reduced ( $p > 0.05$ ) compared with the Control (Fig. 4).



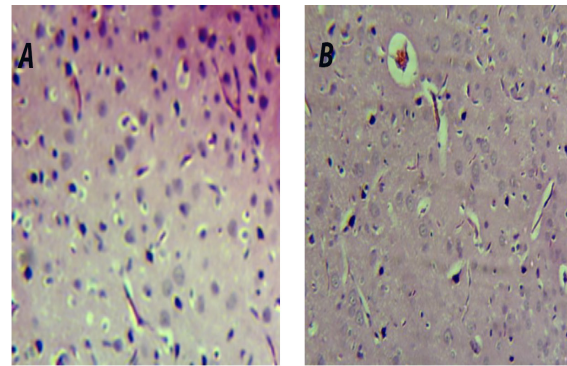
**Figure 2.** Activity of alkaline phosphatase enzyme (IU/L) in the PFC. Difference between the treated groups of 1st and 2nd Trimesters was statistically significant. \*Significant difference between control and treated groups of 2nd Trimester ( $p < 0.05$ ).



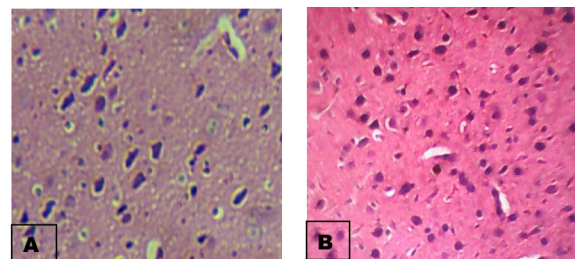
**Figure 3.** Activity of lactate dehydrogenase enzyme (U/L) in the PFC. Difference between the treated groups of 1st and 2nd Trimesters was statistically significant ( $p < 0.05$ ).



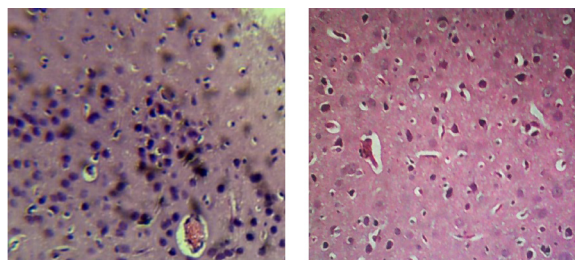
**Figure 4.** Activity of glucose-6-phosphate dehydrogenase (U/mL) in the PFC.



**Figure 5.** Photomicrograph of the prefrontal cortex of control (A) and nicotine-treated (B) groups in the 1st Trimester showing many well-stained neurons (white arrows) especially in the outer granular layer (OGL) of Control A; most neurons in Group B have reduced staining intensity of their cell bodies (yellow arrows) H&E x100.



**Figure 6.** Photomicrograph of the prefrontal cortex of the control (A) and nicotine-treated (B) groups in the 2nd Trimester showing slightly smaller-sized and more numerous neurons (B) compared with the Control (A) H&E x200.



**Figure 7.** Photomicrograph of the prefrontal cortex of the control (A) and nicotine-treated (B) groups in the 3rd Trimester showing many lightly stained neurons (green arrows) and more pronounced vacuolations (black arrows) in Group B compared to the control (A) H&E x100.

**Histological Observation**

The prefrontal cortex of the control groups showed apparently normal architecture. The treated group revealed poor staining intensity of the outer granular layer and most

cell bodies in the 1<sup>st</sup> Trimester (Fig. 5), slightly smaller-sized and more numerous neurons in the 2<sup>nd</sup> trimester group (Figure 6), and, lightly stained neurons with more vacuolations in the nicotine-treated group of the 3<sup>rd</sup> trimester (Figure 7).

### Discussion

Gestational nicotine has been associated with intrauterine growth restriction, low birth weight and reduced head circumference in prenatally exposed children (16). Findings from the current study revealed that the body weight and brain weight of animals exposed to nicotine during their 1<sup>st</sup> Trimester intra-uterine life remained low, even at PND 35, unlike in the other trimesters. Earlier studies have shown that prenatal nicotine exposure results in glucose intolerance and impaired brain response to insulin in the offspring (17). Some authors, however, observed that children who were prenatally exposed to nicotine experience a form of catch-up growth, with subsequent childhood obesity (18). This could be the reason for the increased weights seen at PND 35 in the animals that were exposed to nicotine in the 2<sup>nd</sup> and 3<sup>rd</sup> Trimesters of gestation.

Exposure to nicotine during gestation is detrimental to the developing brain. Prenatal nicotine administration is associated with morphological and biochemical changes in the cerebral cortex of the offspring, from birth to adulthood (19). Children exposed to nicotine during pregnancy are said to be at risk of becoming smokers themselves (20). The activity of alkaline phosphatase (ALP) enzyme was significantly raised in the latter part of the gestation in the current study. This could imply that ALP is more involved in fetal growth and development during this stage of gestation, especially the 3<sup>rd</sup> trimester. The vasoconstrictive effect of nicotine causes deprivation of oxygen and nutrients to the developing fetus, with the consequent stimulation of anaerobic glycolysis. Although this effect appeared to be very minimal in the first two trimesters in this study, a markedly raised level of lactate dehydrogenase was observed in the 3<sup>rd</sup> Trimester. This enzyme catalyses the conversion of lactate to pyruvate in the glycolytic pathway. Nicotine-induced cellular and membrane damage could lead to intracellular leakage of LDH, thereby presenting as elevated enzyme levels (21). Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme in the pentose phosphate pathway, which supplies reducing energy to cells by maintaining the level of the co-enzyme reduced nicotinamide adenine dinucleotide phosphate (NADPH). Although minimal changes in the activity of this enzyme

were observed, the level was especially raised in the 2<sup>nd</sup> Trimester nicotine –exposed animals. Aside from the production of NADPH, ribose is also produced, which is vital in RNA and DNA replication (22). The elevated level of this enzyme in the 2<sup>nd</sup> trimester could affect neurodevelopment, which begins in the early 2<sup>nd</sup> week of gestation in rats. The biochemical derangement observed in this study could underlie the structural alterations in brain histology resulting from nicotine-induced cell damage, especially at the beginning of neurodevelopment, and these effects could persist through childhood and adolescence with accompanying neurological dysfunctions.

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