Morphological and neurohistological changes in adolescent rats administered with nicotine during intrauterine life

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Summary: Tobacco smoking has been linked to many preventable diseases affecting various organs and systems of the body, including the brain. The current study was conducted to demonstrate the histological changes observable in the cerebral cortex of young Wistar rats exposed to nicotine during gestation. Vaginal smearing was conducted for the female Wistar rats to determine their oestrous cycle, after which they were exposed to male rats overnight, for mating. Pregnancy was confirmed and the pregnant rats were divided into 3 groups based on the 3 trimesters (A, B, C), with each group having a control and a treated subgroup. The Control Groups (A_1, B_1, C_1) were given 0.1 ml of normal saline i. p., while the Treated Groups (A_2, B_2, C_2) received 0.06 mg/kg/0.1 ml of nicotine intra-peritoneally. Treatment was for a period of 6 days only within each trimester for all subgroups. The pregnant animals were allowed to litter, and at postnatal day 35 they were sacrificed. The skull was dissected to expose and remove the brain; the temporal and parietal cortices were excised and fixed in 4% paraformaldehyde for histological tissue preparation, using cresyl fast violet staining techniques. Exposure of the developing brain to nicotine during gestation resulted in various degrees of abnormalities in the cytoarchitecture of the parietal and temporal cortices of young rats. The gestational period of nicotine exposure and specific cortical affectation are important factors to consider while investigating neurological abnormalities in offspring of tobacco smokers.

Keywords*: Cortex, Histology, Prenatal nicotine, Adolescent rats, Neurological abnormalities

INTRODUCTION

Nicotine, the addictive chemical in tobacco, is a pharmacotherapy for smoking cessation, and a useful probe drug for phenotyping cytochrome P450 2A6 (Hukkanem *et al*., 2005). It is used as an insecticide and fumigant, and forms salts with most acids (Hukkanen *et al.,* 2005).Use of tobacco products has been linked to abnormalities in brain morphology, neurochemistry, cerebral blood flow as well as neurocognition (Brody *et al.,* 2004). However, nicotine affects most organ systems in the body (Benowitz, 1996). Chronic smokers compared to non-smokers demonstrate lower cortical gray matter volumes and densities in the prefrontal cortex, smaller left anterior cingulated volume and lower gray matter densities in the right cerebellum (Brody *et al.,* 2004). Meanwhile, a study by Tanabe *et al*. (2005) revealed that nicotine may improve smooth pursuit eye movement performance in people with schizophrenia through cholinergic stimulation of the hippocampus and cingulated gyrus.

A link exists between nicotine administration and brain circuitry that mediates visuospatial attentional processing and withdrawal symptoms (Brody, 2006). Prenatal nicotine exposure elevates risk of cognitive and auditory processing deficit and of smoking in offspring (Jacobsen *et al.*, 2007). According to Thakur and co-workers (2013), maternal smoking during pregnancy is associated with a more severe form of attention deficit hyperactive disorder characterised by more severe clinical manifestations and poorer neuropsychological performance. When used at low doses, there is significant deregulation of transcription in placental and foetal cells (Votavova *et al.,* 2012).

Nicotine administration has been suggested to have beneficial effects in patients with hypoinsulinism (Hosseini, 2011). Although cigarette smoking has been associated with increased insulin resistance, this effect is not likely to be seen in healthy subjects (Xu *et al*., 2012). Studies by Liu *et al*. (2003) observed that long-term oral nicotine administration reduces insulin resistance in obese rats. The current study examined the effect of prenatal nicotine on the morphology and the histology of the cerebral cortex of adolescent Wistar rats.

MATERIALS AND METHODS

Twenty-four (24) adult female Wistar rats (*Rattus norvegicus*) with mean weight 215.83 ± 6.71 g were used. They were housed in wire-gauzed cages in the Animal House of the College of Health Sciences, University of Ilorin, at room temperature. Good ventilation, feeds and water were provided for the rats throughout the period of the experiment.

Vaginal smearing was done as earlier performed (Marcondes *et al*, 2002) and the oestrous phase of each female Wistar rat was determined.The rats were grouped according to their oestrous phases, and male rats were introduced to the female rats, overnight, in their proestrous phase. Pregnancy was confirmed through vaginal smears early hours of the following morning after perceived mating, as indicated by the presence of mucous plug and sperm cells in the vaginal smear.

Grouping of Animals and Administration

The rats were divided into 3 main groups (Groups A, B and C) with each group subdivided into 2 subgroups (1 and 2). The 3 major groups represented rats in the 3 trimesters of gestation period (each being a duration of 7 days, with a total of 21/22 days in Wistar rats) while the subgroups 1 and 2 represented the control and treated groups respectively. Treated animals received 0.06 mg/kg nicotine in 0.1 ml of vehicle in two divided doses, while the control animals received 0.1 ml normal saline intra-peritoneally, for six (6) consecutive days within each trimester.

Animal Sacrifice and Specimen Collection

The pregnant animals were allowed to litter, and at postnatal day (PND) 35, the pups were sacrificed by cervical dislocation. The skull of each pup was dissected to expose and remove the brain. The brain was weighed and the parietal and temporal cortices excised, and fixed in 4% paraformaldehyde to prevent autolysis and putrefaction. The fixed tissues were processed for histological examination using the cresyl fast violet staining technique, to demonstrate Nissl bodies in the neuronal cytoplasm and the general architecture of the parietal and temporal cortices, of both the control and nicotine-exposed offspring.

Data Analysis

Data obtained from the study were analysed with SPSS software version 16.0 using the student's t-test, and were presented as Mean ± SEM, with determination of level of significance at 95% confidence interval.

RESULTS

The weights of the offspring recorded at birth showed reduction in the birth weight of pups exposed to nicotine during the 1st and 2nd trimesters, compared with their respective Control; and this was significant ($p<0.05$) in the 2nd trimester group (Table 1). The birth weight of pups exposed to nicotine in the 3rd trimester was not taken. Weight of animals on postnatal day 35 increased in both the 2nd and 3rd trimester treated groups, compared with their respective controls. Although this weight change was not statistically significant in the 2nd trimester group ($p>0.05$), it was however significant in the 3rd trimester group ($p<0.05$). The body weight of nicotine-exposed rats in the 1st trimester significantly reduced when compared with the control group ($p<0.05$). Brain weight revealed a significant decrease in the 1st trimester group ($p<0.05$), a slight increase in the 2nd trimester ($p>0.05$), and a non-statistically significant reduction in the 3rd trimester ($p>0.05$). The brain weight difference between the nicotine-exposed groups of the 2nd and 3rd trimesters was significant ($p<0.05$) (Table 1).

Table 1: Weight of pups

Grp	Birth wt (g)	B.wt at PND 35 (g)	Br weight (g)	Br-B wt ratio
A_1:	8.50 ±0.47	82.50 ±5.95[‡]	1.65 ± 0.05[x]	0.02
A_2:	5.64 ±0.62	60.00 ±5.77[‡]	1.33 ± 0.07[x]	0.03
B_1:	6.58 ±0.08[φ]	68.75 ±2.39	1.47± 0.03	0.02
B_2:	5.61 ±0.18[φ]	71.25 ± 2.39	1.49 ± 0.02[y]	0.02
C_1: *	-	52.50 ±2.50[§]	1.40±0.07	0.03
C_2: *	-	67.50 ±1.44[§]	1.27±0.02[y]	0.02

A_1:1st Trimester Control, A_2: 1st Trimester Treated, B_1: 2nd Trimester Control, B_2: 2nd Trimester Treated C_1: 3rd Trimester Control, C_2: 3rd Trimester Treated, PND: postnatal day; *birth weights not obtained. Statistically significant difference between [φ]B_1 and B_2; [‡]A_1 and A_2; [§] C_1 and C_2; [x]A_1 and A_2; and between [y] B_2 and C_2 ($p<0.05$).
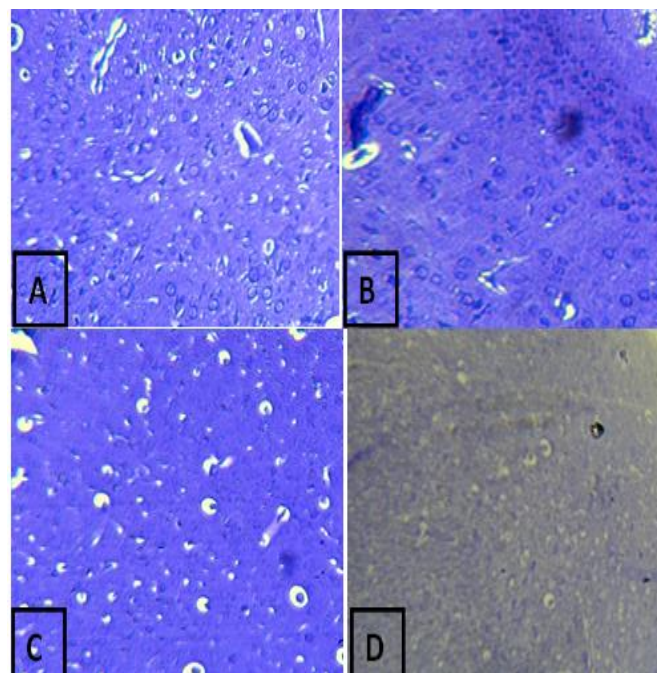

Fig. 1: Photomicrographs of the parietal cortices of control rats (A) and those exposed to nicotine in the 1st (B), 2nd (C) and 3rd (D) trimesters, showing slight increase in Nissl staining in B and C, but reduced in D. Vacuolations were more in the treated groups especially in C.
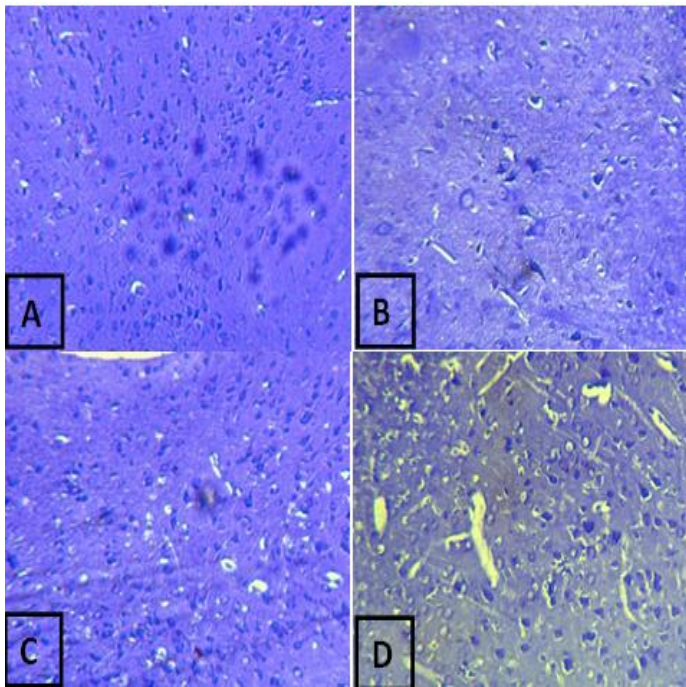
Fig. 2: Photomicrographs of the temporal cortices of control rats (A) and those exposed to nicotine in the 1st (B), 2nd (C) and 3rd (D) trimesters, with increased vacuolar spaces in nicotine-exposed groups. CFV (x100).

The histological sections of the parietal and temporal cortices of the treated groups were slightly more intensely stained than the Control, especially those exposed to nicotine in the 1st and 2nd trimesters, reflecting increased positivity for Nissl bodies (Figures 1 and 2). Variation was noticed in the 3 superficial layers of the cortex that were visible in the sections between the treated and control groups. Presence of vacuolar spaces was more in the parietal and temporal cortices of rats exposed to nicotine, especially in the 2nd and 3rd trimester, when compared with the Control.

DISCUSSION

The effects of nicotine are seen in every trimester of pregnancy, from increased spontaneous abortions in the first trimester, to increased premature delivery rates and decreased birth weights in the third trimester (Lambers and Clark, 1996). Birth weight is dependent on two factors: the gestational age of the foetus at the time of delivery and the rate of foetal growth. Nicotine affects both of these factors (Lambers and Clark, 1996). In the current work, intra-peritoneal administration of nicotine to pregnant rats caused significant reduction in the weight of the pups at birth. Gross examination of the brain, however, did not reveal any significant morphological changes in the appearance of both the control and treated groups.

The reduction in body weight of the pups in the treated group could be attributed to the loss of appetite experienced by the mothers. This corroborates some previous studies on the effect of nicotine on the satiety centre of the brain, and generally on food intake by nicotine treated animals (Chen *et al.*, 2012). It is well known, although not well understood, that smoking and eating just do not go together. Smoking is associated with decreased food intake and lower body weight. Nicotine, administered either by smoking or by smokeless routes is considered the major appetite-suppressing component of tobacco, and since it is the major addictive component, it reduces appetite and alters feeding patterns typically resulting in reduced body weight (Jo *et al.*, 2002). Nicotine may induce hyperglycaemia and hypercholesteremia (Effraim *et al.*, 2000). It produces profound central nervous system effects which manifest as decreased food intake, transient convulsion, artificial paralysis and loss of weight. These changes may be explained in the light of up-regulation of central nicotine receptor binding sites leading to initial stimulation and later diminished responsiveness or tolerance. According to previous studies, nicotine also indirectly affects the satiety centre (Effraim *et al.*, 2000). The ability of nicotine to regulate appetite and body weight is one of the factors cited by smokers that prevents them from quitting, and is the primary reason for smoking initiation in young people, especially teenage girls (Zoli and Picciotto, 2012). The slight increase in brain weight of pups exposed to nicotine in the second trimester might be due to the rapid brain growth that characterises the first few days of postnatal life, as low brain weight is characteristic of nicotine-exposed newborn (Santiago and Huffman, 2012).

Nicotine can be considered to be teratogenic, and could cause an increased risk of spontaneous abortion among smokers. In rats, increased embryonic resorptions and maturation delay occur with nicotine during pregnancy (Benowitz, 1998). Nicotine also delays the implantation of blastocyst (Card and Mitchell, 1979). This subsequently adversely affects other important intrauterine activities like neurulation and organogenesis. Although nicotine may not produce congenital malformations, it does affect foetal growth. Ovum entry into the uterus, blastocyst formation, shedding of zonapellucida and implantation are delayed by nicotine (Yoshinaga *et al.,* 1979). Delayed implantation is not due to a decreased secretion of oestrogen, but rather to a delay in the increase in progesterone secretion arising from hormonal imbalance in the hypothalamo-pituitary axis, in nicotine-exposed subjects (Yoshinaga *et al.,* 1979). An increased progesterone level is essential for endometrial preparation of the uterus for the implanting blastocyst.

Maternal smoking is a well-established cause of intrauterine growth restriction. Heavy cigarette

smokers are also more likely to have a premature delivery. Pregnant women who smoke have increased risks of preterm labour, premature rupture of membranes, and premature delivery (Narahara and Johnston, 1993). Nicotine can induce embryonic abnormalities before and during the early stages of organogenesis, in a concentration-dependent manner (Zhao and Reece, 2005), with exposed neonates having smaller head circumference, and by implication lower cerebral mass (Krol *et al.,* 2012). Similar to what obtains at birth, findings in the current work revealed increase in the whole brain weight at postnatal day 35 in animals that were exposed to nicotine in the 1st and 3rd trimesters.

There were alterations in the cytoarchitecture of the temporal and parietal lobes of the cerebral cortex of the rats in the treated group when compared with those in the control group. Nicotine induced a dose-dependent increase in several behavioural parameters, including feelings of "rush" and "high" and drug-liking. Nicotine also induces a dose-dependent increase in neuronal activity in different brain regions, including the nucleus accumbens, amygdala and the cingulate gyrus. Activation of these structures is consistent with nicotine's behaviour-arousing and behaviour-reinforcing properties in humans (Stein *et al.,* 1998). The increase in nicotinic receptors in the cerebral cortex and hippocampus of smokers may modify the central nervous system effects of nicotine and contribute to an altered response of smokers to nicotine (Perry *et al.,* 1999). Nicotine modulates a number of brain neurotransmitters and produces both direct and indirect effects on information processing (Rusted *et al.,* 1998). There is evidence that nicotine can improve short term memory and performance which could be as a result of stimulation and release of acetylcholine, the neurotransmitter long associated with processes of attention, learning and memory in both animals and humans (Rusted *et al.,* 1998). The effects of nicotine on memory in minimally-deprived smokers improved recall performance of semantically related items (Rusted *et al.,* 1998). There is evidence that maternal cigarette smoking during pregnancy poses a unique risk for neuro-developmental impairment among children and provides an additional reason for pregnant women not to smoke cigarettes (Olds *et al.,* 1994). The increased staining intensity of the cerebral cortex of nicotine-exposed rats points to the fact that there was possible proliferation of cells and therefore more synapses and release of neurotransmitters. This may be the basis of improved short term memory as seen in the study carried out by Rusted and co-workers (1998). These cytological alterations may affect to some extent the functions of the lobes in particular, and the brain in general, as previously suggested that morphological changes in the hippocampus following prenatal nicotine exposure may contribute to behavioural

abnormalities (Roy and Sabherwal, 1998). Of important note is the effect on long and short term memory, including verbal memory, hearing and auditory perception, manipulation of objects, and integration of sensory information from different modalities.

Prenatal administration of nicotine alters the microscopic appearance of the cerebral cortex, and could cause a derangement in normal brain functions, particularly those of the parietal and temporal cortices.

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