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Histological changes in the cerebelli of adult wistar rats exposed to cigarette smoke

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Summary: The different constituents of tobacco smoke have been linked to different diseased conditions. In this work, the histological effects of cigarette smoke on the cerebellum of adult male Wistar rats were studied. Sixteen Wistar rats with mean weight of 153.24 ± 4.12 g were grouped equally into four. The Control Group A was exposed to fresh air, while Groups B, C and D animals were each exposed to smoke from one, two and three sticks of cigarette respectively. Each stick of cigarette was completely consumed within an average duration of 11 minutes. Improvised smoking chambers were constructed and used for the exposure daily, while treatment lasted for 28 days. The animals were thereafter sacrificed by cervical dislocation, the cranium was exposed and the brain gently removed and weighed; the cerebellum was excised, weighed, and fixed in formol calcium, and subsequently processed for histological observation using the Haematoxylin and Eosin staining principle. Loss of weight and reduction in weight gain were noticed in the treatment groups, with corresponding reduction in cerebellar weights, in a dose-dependent pattern. Histology also revealed loss of white matter, reduction in thickness of cell layers and their cellular components. Increasing dosage of cigarette smoke could predispose to progressive compromise in the structural integrity and composition of the cerebellum, and this might result in cerebellar dysfunction.

Keywords*:* Cerebellar histology, cerebellar dysfunction, cigarette smoke, rats.

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INTRODUCTION

The smoke of cigarette contains several chemicals that are potentially toxic and carcinogenic to the human body, and many of these constituents have been linked to different diseased states. Cigarette smoking is a known risk factor of many clinical conditions, and can also exacerbate some conditions (Henderson, 2008; Jill *et al*., 2006; Tatum and Shapiro, 2005). Since many of these constituents, like nicotine, cross the blood-brain barrier, the brain has become a target also of the toxic effects associated with tobacco smoke.

Studies on long-term neurotoxicity have observed increased formation of free radicals and oxidative stress in animals exposed to tobacco and alcohol *in utero* (Li and Wang, 2004). Carbon monoxide, a component of smoke, contributes to the hypoxic state of the brain. Prenatal exposure to carbon monoxide causes reduced birth weight and decreased weight gain in offspring of smokers, as well as lower behavioural activity levels, altered central catecholamine activity, and reduction in total brain protein at birth (Fechter and Annau, 1977). These go on through childhood to cause some forms of learning and memory deficits (Mactutus and Fechter, 1984). The high concentration of reactive oxygen species (ROS) in smoke is probably one of the major factors contributing to a high incidence of many clinical conditions linked with cigarette smoking (Palozza *et al*., 2006).

Chronic nicotine treatment induces CYP2E1 expression in the cortical pyramidal neurons and cerebellar Purkinje cells, and increased CYP2E1 in the brain may contribute to oxidative stress and alter localized metabolism (Joshi and Tyndale, 2006). Furthermore, long term nicotine administration is capable of causing a significant loss of white core of cerebellum (Tewari *et al*., 2010), and this probably could result in some forms of derangement in cerebellar functions.

Use of tobacco products during pregnancy has been related to some common neurobehavioral and cognitive outcomes in the offspring. Such features include, increased externalizing behaviour, decreased general cognitive function, learning and memory deficits, among others (Huizink and Mulder, 2006). The current study aimed at determining the effects of increasing doses of cigarette smoke on cerebellar cyto-architecture of adult wistar rats.

MATERIALS AND METHODS

The research work was conducted in the Animal House of the College of Health Sciences, University of Ilorin following basic ethical considerations.

Breeding of Experimental Animals

The rats were purchased from an area in Ilorin, Nigeria. They were housed in cages with adequate space to encourage free movement. They were housed under natural light and dark cycles (12hr light and 12hr dark) at room temperature and were given standard rat pellets and water *ad libitum*, and were allowed to acclimatize for two weeks.

Grouping of Experimental Animals

A total of 16 male Wistar rats with an average weight of 153.24 ± 4.12 g were used for the experiment. They were grouped into four groups, each group with four rats. However, the grouping of rats was done taking into consideration their various weights.

Exposure of Animals to Cigarette Smoke

Each animal in the treated groups was exposed to cigarette smoke (Pall Mall®) daily for 28 days. Exposure time was 6.00 pm local time, and each stick of cigarette was completely burnt within an average period of 11 minutes. Four smoking chambers were constructed for the administration. They were made of plastic containers, with a hole of about 1 cm diameter created on the lid through which each cigarette was suspended with the aid of a thread. The lids were opened intermittently to prevent suffocation. Exposure of the animals was as previously reported (Omotoso *et al*., 2013), and also stated below:

Group A: Control, exposed to fresh air;

Group B: exposed to smoke from one (1) stick of cigarette;

Group C: exposed to smoke from two (2) sticks of cigarette; and,

Group D: exposed to smoke from three (3) sticks of cigarette.

Animal Sacrifice and Tissue Collection

All the Wistar rats were sacrificed 24 hours after the last exposure by cervical dislocation. The cranium of each animal was opened up using brain forceps and the whole brain was carefully removed and weighed; the cerebellum was then excised. Tissues were then fixed in formol calcium, and processed for histological observation using routine Haematoxylin and Eosin staining techniques (Bancroft and Cook, 1984).

The weights of the animals were taken at intervals during the experiment and prior to the time of sacrifice, and the cerebellar weight was also taken.

Statistical analysis:

Data were analysed using student's t-test, and presented as Mean ± SEM, with confidence interval at 95% and a P value less than 0.05 was considered statistically significant.

RESULTS

Physical Observation

The mean weight of the animals at the commencement of the experiment was 153.24 ±4.12 g. Changes in weight of animals were observed (Table 1) which showed marked reduction in body weights in all the groups exposed to cigarette when compared to the Control animals. The animals that received the highest dose of cigarette smoke (3 sticks of cigarette) had the lowest weight difference (that is, difference between the final and initial body weights), although this least growth rate was not statistically significant ($p>0.05$) when compared to the Control. However, the weight difference in animals exposed to 1 stick (Group B) and 2 sticks (Group C) of cigarette was statistically significant ($p<0.05$).

Cerebellar weights in all the treated groups decreased. While that of Group D that had the highest dose of cigarette smoke was lowest (0.2973±0.044; $p>0.05$) compared with the Control Group A (0.5287±0.057), weight reduction in other two exposed groups (Group B: 0.3623±0.007; Group C: 0.3124±0.023) was statistically significant ($p<0.05$).

The organ-body weight ratio also reduced markedly in all exposed groups in a dose- dependent pattern, with animals exposed to smoke from 3 sticks of cigarette having the least ratio compared to the Control animals.

Histological Observation

Histological sections of the cerebelli of control rats showed apparently normal architecture with distinct cortical layers: outer molecular, inner granular cell layer, between which is the single layer of large neurones called Purkinje cells; the central medullary region was also seen, made up of white matter (Figure 1). The granular cell layer was very populated with cells, unlike the molecular layer which had large numbers of unmyelinated fibres. Animals exposed to smoke from only one stick of cigarette (Group B) revealed an apparent increase in cerebellar medulla as well as decreased thickness of the middle layer of Purkinje cells (Figures 2).

Table 1: Weights of rats and cerebelli following exposure to different doses of cigarette smoke

Group	Initial weight (g)	Final weight (g)	Weight difference (g)	Cerebellar weight (g)	C-B.Wt ratio
A	131.25 ±8.17	214.70 ±9.20	83.45	0.5287 ±0.057	0.0025
B	165.48 ±4.15	198.23 ±2.67	32.75*	0.3623 ±0.007*	0.0018
C	154.80 ±1.23	196.82 ±11.01	42.02*	0.3124 ±0.023*	0.0016
D	163.67 ±5.43	192.27 ±16.53	28.6	0.2973 ±0.044	0.0015

*P<0.05, C-B.Wt= Cerebellar-Body weight

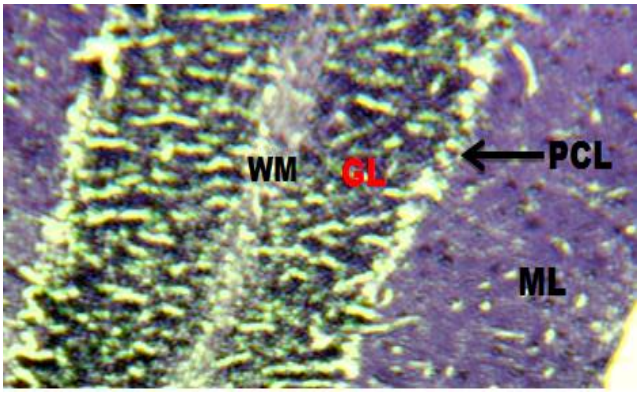

Figure 1: Photomicrograph of the cerebellum of control rat (Group A) showing apparently normal histology of highly cellular granular cell layer (GL), single Purkinje cell layer (PCL), molecular layer (ML) with fewer cells, and the central medulla of white matter (WM) (H &E x100).

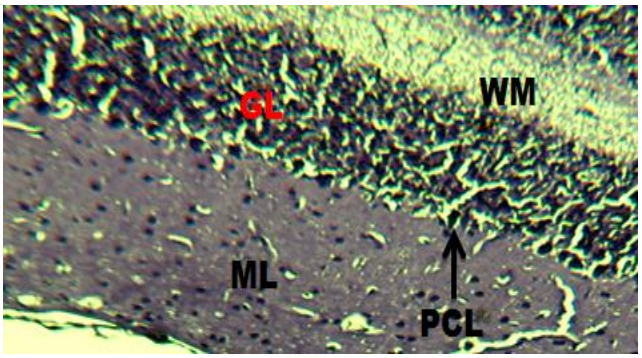

Figure 2: Photomicrograph of the cerebellum of rats exposed to one stick of cigarette (Group B) showing increased thickness of white matter (WM) compared with the Control, decreased thickness of Purkinje cell layer (PCL), with no distinctive alteration in the architecture of the granular cell layer (GL) and molecular layer (ML). (H &E x100).

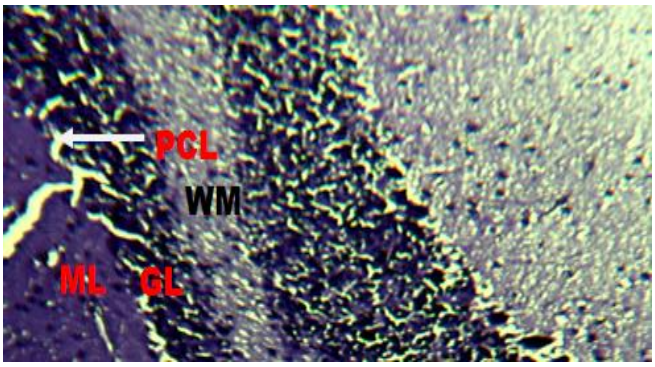

Figure 3: Photomicrograph of the cerebellum of animals exposed to two sticks of cigarette (Group C) showing a decrease in thickness of the granular cell layer (GL), Purkinje cell layer (PCL), molecular cell layer (ML) and a loss of white matter (WM) (H &E x100).

Photomicrograph of the cerebelli of animals exposed to two sticks of cigarette (Group C) showed a decrease in thickness of the cortical layers, with possible reduction in number of cells, and a loss of white matter in the medulla (Figures 3).

Animals in Group D that were exposed to the highest dose of cigarette smoke had a dose-dependent reduction in the size of the cortical layers compared with other treated groups, and a significant decrease in the number of cell bodies, and reduced white matter in the medulla (Figure 4).

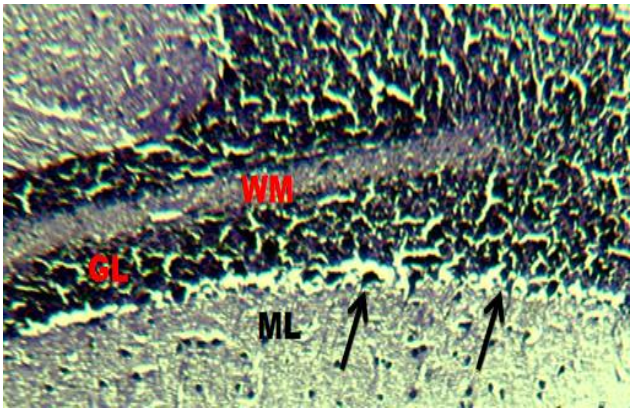

Figure 4: Photomicrograph of the cerebellum of rats exposed to three sticks of cigarette (Group D) showing further decrease in thickness of the granular cell layer (GL), decrease in the number of cell bodies in the molecular cell layer (ML), Purkinje cells (arrows), and loss of white matter (WM) (H &E x100).

DISCUSSION

Significant weight loss was observed in all the animals exposed to cigarette smoke when compared to animals in the control group, probably due to reduced food intake in the exposed groups. This was similar to previous studies on cigarette smoking which have linked weight reduction with this lifestyle both in man and experimental animals (Bishop *et al*., 2004; Bellinger *et al*., 2003; Albanes *et al*., 1987). Smoking is known to decrease insulin sensitivity (Gupta *et al*., 2006; Targher *et al*., 1997), and cause significant reduction in body weight of the animals with a significant reduction in adipose tissue (Chen *et al*., 2005), particularly white fat masses, as fat deposits might be used as an energy supply under these conditions of negative energy balance (Chen *et al*., 2005). Aside the changes in animal weight, the cerebelli of the animals in the exposed groups were also significantly reduced in weight in a dose-dependent fashion, with animals exposed to the highest dose of cigarette smoke having the lowest cerebellar weight and those exposed to the lowest dose of only one stick of cigarette having the highest cerebellar weight of the three exposed groups. In a similar pattern with the organ weights, the organ-body weight ratio dose-dependently decreased.

The development of different parts of the brain, including the cerebellum, is affected by nicotine exposure during prenatal and postnatal periods in rats (Dwyer *et al*., 2009). The adult cerebellum is also susceptible to the damaging effects of nicotine treatment by causing a significant depletion of the white core of cerebellum (Tewari *et al*., 2010). Through the process of apoptosis, interaction of nicotine receptors and nicotine results in cell death in brain tissue (Denissenko *et al*.,

1996). In the current work, both the cerebellar Purkinje cell and granular cell layers were noticed to exhibit some degree of shrinkage, or reduction in thickness, in animals exposed to cigarette smoke, and this was however more in those that received the higher doses, compared with the Control animals. Earlier studies with nicotine also revealed that administration of nicotine specifically inhibits cerebellar Purkinje cells (de la Garza *et al*., 1989), which could result in the reduction of Purkinje cells, and consequently, the middle Purkinje cell layer of the cerebellum. According to Opanashuk and colleagues (2001), activation of nicotinic acetylcholine receptors directly affect the development of primary cerebellar neuroblasts. With increasing dosage of cigarette smoke, a progressive loss of white matter was observed, which correlates with the work of Tewari *et al*. (2010) that long-term nicotine exposure during adulthood resulted in loss of white matter of the cerebellum in rat model system.

It could be concluded that increased exposure to cigarette smoke causes a progressive neurotoxicity on cerebellar cytoarchitecture, which could serve as basis for some cerebellar lesions in individuals who smoke.

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