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ERRATUM
Effects of Chronic Cigarette Smoke Exposure on the Histochemistry and Anti-oxidant Status of Wistar Rat's Cerebelli

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ABSTRACT

The health implications and complications of cigarette smoking are still prevalent worldwide. This study demonstrates the effects of chronic exposure to cigarette smoke on the Nissl bodies and the anti-oxidant status of the cerebelli of adult Wistar rats. Twelve male Wistar rats were divided into 4 groups: Control Group A was exposed to fresh air, while Treatment Groups B, C and D were exposed to smoke from one, two and three sticks of cigarette (Pall Mall®) respectively, daily, over a period of 28 days. Each stick contained an average of 0.8375 g of tobacco, completely burnt during exposure. At the end of exposure, the tissues were removed and weighed. Specimens for histological studies were fixed in formol calcium and processed using cresyl fast violet techniques, while tissues for anti-oxidant studies were homogenised using cold 0.25 M sucrose solution, centrifuged at 5000 rpm for 5 min, and put in freezer. Biochemical kit from Randox® was used to determine the tissue activity of glutathione peroxidase, as a marker for endogenous anti-oxidant status. Exposed animals showed reduction in body weights and cerebellar weights. Tissue glutathione peroxidase levels decreased. Histochemical changes include progressive loss of white matter, reduced cortical thickness, and Nissl granules distribution. Most of these effects were dose-dependent. Cigarette smoking produced deleterious changes in the normal histochemistry and the anti-oxidant status of the cerebellum, and could predispose to abnormal functioning of the cerebellum.

Keywords: cigarette smoke, glutathione peroxidase, Nissl granules

INTRODUCTION

Cigarette smoking is a risk factor of a variety of life-threatening conditions, such as cardiovascular, gastro-intestinal, metabolic, respiratory and reproductive disorders, including cancers[1,2]. Active or passive smoking of cigarette poses significant threats to man's health, considering the degree of morbidity and mortality arising from this. Although most people are aware of the danger of this lifestyle, it is often difficult to quit once an individual begins to smoke. Passive smokers, though not actively involved in smoking, inhale the side-stream smoke which contains many toxic constituents like the mainstream smoke [3].

The brain is the target of many of the toxic effects associated with tobacco smoke. When administered, nicotine crosses the blood-brain barrier, reaching the brain in few seconds, and thus stimulates the brain cells. The actions of nicotine on cerebellar function are poorly understood [4]. However, according to Joshi and Tyndale [5], **c**hronic 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**.** This could result in a significant loss of white core of cerebellum [6], thus making the derangement in cerebellar functions possible.

Cigarette smoke contains oxidants such as oxygen-free radicals and volatile aldehydes, including carbon monoxide, whose actions damage the cells [7]. When minimal, endogenous anti-oxidants are able to overcome the challenge of reactive oxygen species, until a breaking point is reached, when the presence of oxidants outweigh the normal cellular mechanisms meant to checkmate their activities, thereby leading to a state of oxidative stress, with its accompanying consequences.

This study was aimed at determining the effects of cigarette smoke exposure on the histochemistry and anti-oxidant status of the cerebellum of Wistar rats.

MATERIALS AND METHODS

Twelve male Wistar rats with average weight of 154.84 ± 4.75 g were used for the study. They were grouped into a Control Group A, exposed to fresh atmospheric air, and Treatment Groups B, C and D exposed to smoke from one, two and three sticks of cigarette (Pall Mall®) respectively.

Customized smoking chambers made of cylindrical plastic containers were constructed and used for the exposure procedure. The lid of the container has about 1 cm diameter hole, and was opened intermittently to

prevent suffocation. Each stick of cigarette contains an average of 0.8375 g of tobacco, and was allowed to completely burn during exposure. Exposure was once daily for each rat, and lasted for 28 days. While treatment group B received one stick of cigarette, groups C and D were exposed to smoke from two and three sticks of cigarette respectively. The weights of the animals were taken during the study. At the completion of exposure, the animals were sacrificed by cervical dislocation, and their cerebelli excised and weighed. Tissues for histological studies were fixed in formol calcium and processed using cresyl fast violet stains for demonstration of Nissl bodies, while tissues for anti-oxidant studies were homogenised in cold 0.25 M sucrose solution, centrifuged at 5000 rpm for 5 min, and put in freezer. Using a colorimetric method, appropriate biochemical kit (Randox®) was used to determine the tissue activities of glutathione peroxidase, as a marker for the endogenous anti-oxidant status. Data obtained was analyzed using the student's t-test, and the data presented as Mean ± SEM, with confidence interval at 95%.

RESULTS

Physical observation revealed reduced feeds intake in animals exposed to cigarette smoke compared with the Control group that was exposed to fresh air. Weight gain in the control animals was marked, while it was minimal in the treated animals (Table I). This was shown by the body weight difference, such that animals exposed to cigarette smoke had a progressive decline in body weight difference compared to the control (Table I).

Table I: Body and cerebellar weights of rats (g) in control and experimental groups of cigarette smoke

Groups	A: Control	B: 1 stick of cigarette	C: 2 sticks of cigarette	D: 3 sticks of cigarette
Final body weight	208.20±9.20	199.70±2.67	187.90±11.01	192.27±16.53
Initial body weight	136.70±8.17	163.20±4.15	155.80±1.23	163.67±5.43
Body weight difference	71.5	36.5*	32.1*	28.6
Cerebellar weight	0.5801±0.06	0.3563±0.01*	0.3298±0.02	0.2973±0.04

*Statistically significant difference compared with Control ($p<0.05$)

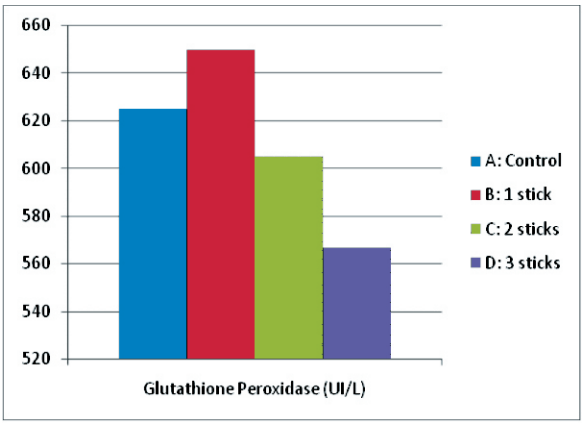

Figure 1: Tissue levels of glutathione peroxidase (GPx).

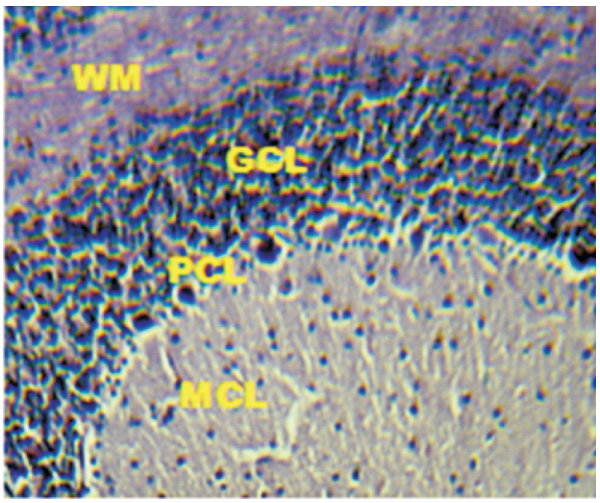

Figure 2: Photomicrograph of cerebellum of rat in control group A,

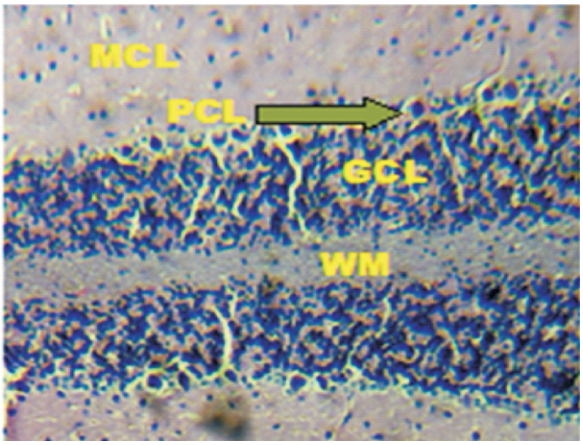

Figure 3: Photomicrograph of cerebellum of rat in group B

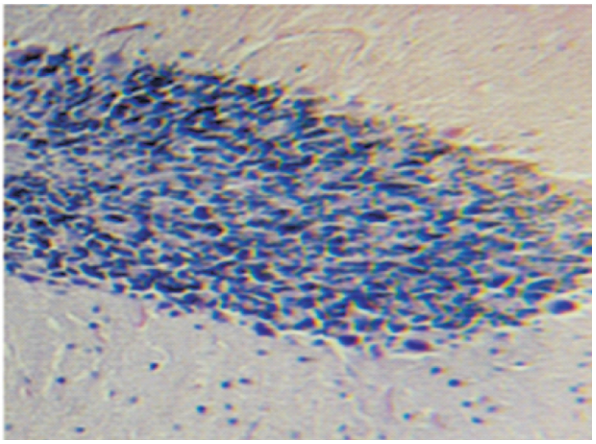

Figure 4: Photomicrograph of Group C

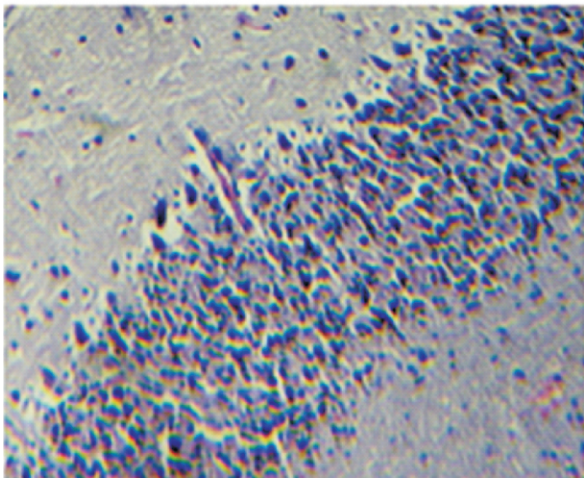

Figure 5: Photomicrograph of Group D

Figure Legends

Figure 2: Photomicrograph of cerebellum of rat in control group A, showing a normal purple dark coloration indicating positive Nissl staining, molecular layer (MCL), Purkinje cell layer with a layer of Purkinje cells (PCL), granular layer (GCL), and an area of white matter (WM). (CFV x100).

Figure 3: Photomicrograph of cerebellum of rat in group B exposed to 1 stick of cigarette smoke, showing slight reduction in color intensity for Nissl bodies, neurons and thickness of cortical and white matter. Arrow points to the single layer of Purkinje cells (CFV x100).

Figure 4: Photomicrograph of Group C showing further reduction in color intensity of Nissl staining, and reduced cell number in the cortical layers. There was significant reduction in thickness of the central white core (CFV x100).

Figure 5: Photomicrograph of Group D showing reduction in color intensity and neurons in the cortical layers, and absence of the white matter layer (CFV x100).

Tissue glutathione peroxidase (GPx) levels decreased, except in animals exposed to only one stick of cigarette that had an elevated cerebellar GPx level (Figure 1). Histological alterations involve a significant progressive loss of white matter in the cerebelli of exposed animals, reduction in cortical thickness and cell density, as well as a decreased staining intensity for Nissl granules (Figure 3-5), compared with animals exposed to fresh air (Figure 2).

DISCUSSION

Nicotine increases lipid consumption in the body, thereby leading to weight loss. This explains the reason for the low body weights observed in animals exposed to cigarette smoke, which also had a reduced growth rate compared with the control animals. Cigarette smoking is known to be associated with poor feeding rates or reduced appetite[8]. This appetite-suppressing effect is as a result of the induction of a temporary hyperglycaemic state, by cigarette smoke[8]. Also, smokers have decreased insulin sensitivity [9], which predisposes to depletion of the adipose store, since fat deposits are utilised as means of energy supply during negative energy balance [8].

Glutathione peroxidases (GPx) are the primary antioxidant enzymes that scavenge hydrogen peroxide and organic hydroperoxides[10]. As noted earlier, cigarette smoke alters the detoxification of hydrogen peroxide through a decrease of GPx activity[7]. In the current study, activity of GPx decreased especially in animals exposed to higher doses of cigarette smoke, with the highest dose resulting in the lowest tissue activity of GPx. Overproduction of hydrogen peroxide predisposes to oxidative stress involved in many disease conditions [7]

Endogenous anti-oxidants normally respond to any challenge posed by reactive oxygen species in the body system. Nicotine and the carbon monoxide component of cigarette smoke generate more free radicals and induce oxidative stress, out-weighing the capability of endogenous anti-oxidants; hence, the depletion of the endogenous anti-oxidant status, as seen in the present study. Furthermore, nicotine affects some cellular processes in the body, including modulation of enzyme activities [11].

Nissl bodies or substances are strongly basophilic inclusions, composing of endoplasmic reticulum and ribosomes, and present in the soma or cell body of neurons. They are crucial in neuronal protein synthesis, and important to the growth and development of every cell or tissue. In the present study, a gradual depletion in the concentration of Nissl bodies was noticed, as indicated by a progressive reduction in staining intensity of the cresyl fast violet stain. Hence, as the quantity of cigarette consumed increases, it could affect the store of Nissl substance, and associated protein metabolism or other processes dependent on Nissl granules in the neurons. However, further studies are essential in this respect. With a possible derangement in protein synthesis, other compensatory mechanisms, such as increased lipid consumption, set in.

In addition to the changes in the cell bodies, reduction in thickness of the cerebellar cortical layers and neuronal cells population were observed. Interaction of nicotine receptors and nicotine results in death of brain cells, through the process of apoptosis[12], leading to reduction in cell population in the cerebelli.

In conclusion, cigarette smoke is associated with gradual loss of cerebellar cells and tissues, and depletion of the cerebellar endogenous anti-oxidant status; these may impair normal physiology of the cerebellum.

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