

Full Length Research Paper

The Cytoarchitectural alterations in the neocortex of Wistar rats: Effects of aqueous tobacco (*Nicotiana tabacum*) leaves extract exposure

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This study investigated the effects of corresponding 11.7 mg/kg body weight and 5.8 mg/kg body weight/day of the tobacco leaves aqueous extract for a period of 20 days on the functions of rats' brain after extract administration. *Nicotiana tabacum* is the scientific name of the tobacco plant grown in several countries of the world. It has been used in variables ways such as smoking, snuffing, even chewing, etc. 24 young rats of both sexes were used. They were divided into 3 groups, A, B, C of 8 rats each (Female (n = 4) and male (n = 4)). Group A were given 11.7 mg of the extract per kg body weight, group B 5.8 mg of the extract per kg body weight in 0.5 ml of distilled water per day throughout the 20 experimental days while group C were given equal volume (0.5 ml) of distilled water as well. The rats were sacrificed at day 21 by cervical dislocation and the brains were excised and fixed in formol calcium for 4 days and processed using Haematoxylin and Eosin staining method and Cresyl Fast Violet (CFV) staining technique. There was a statistical significant decrease in the body weight, brain weight and relative brain weight between groups A and B compared to the control group ($p < 0.05$). There was enlargement of the somata in the group A administered with 11.7 mg/kg B.Wt per day of the extract while those in group B appeared more spindle compared to those cells in group C. Also, the non-homogenous appearance of myelinated neocortex of the neuropil appear clearly in the treated groups compared to control (Figure 3). Therefore, the results suggested that the consumption of the aqueous extract of *N. tabacum* leaves may alter the normal functions of the brain which may lead to brain dysfunction, despite its "pleasant" effects and also help in reduction in weight gain.

Key words: *Nicotiana tabacum*, cerebrum, frontal lobe, cytoarchitecture, neocortex.

INTRODUCTION

The key organ of the nervous system is the brain which is covered with a tough membrane called meninges. The brain floats within the skull in a liquid known as a cerebrospinal fluid (CSF), and it is responsible for co-ordination of body systems. The frontal lobe is chiefly concerned with voluntary motor functions, motivation, foresight planning, memory, mood, emotion, social judgement, and aggression (Salardin, 2001; James, 2002; Stranding et al., 2005). Therefore, because of this important

fact, a structural change that may occur within the frontal lobe (cerebrum) is vital to our health state. Tobacco is consumed in every parts of the world, most especially in the developing countries (Odebode, 2008; Aghaji, 2008; Uwakwe and Modebe, 2008). The nicotine is the highest and most toxic compound of aqueous extract of tobacco leave and of neurotoxic important (Sas, 1990; Philip, 2002; Penton and Lester, 2009).

Exposure to tobacco nicotine, either from smoking, snuffing or chewing, has been frequently associated with alteration in the normal functions of the brain and the whole nervous system (Stephen, 1999; Charles and Carroll, 2000; NIH, 2009). Nicotine, in medical product, is used to aid in smoking sensation and other nicotine addiction. Using a

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controlled amount of nicotine helps to reduce nicotine withdrawal symptoms when one quit from the use of tobacco products (Charles and Carroll, 2000; Penton and Lester, 2009; NIH, 2009). The World Health Organization has urged government across the world to ban tobacco advertisement, promotion and sponsorship, as part of measures to protect the world's 1.8 billion young people (Odebode, 2008; NIH, 2009).

According to data accrued from the World Health organization (WHO), there are about 2.4 billion people in the world today that consume the tobacco products either in form of snuff, chewing, smoking or snuff dipping. This representing almost one third of the world population; about 50 - 55% of men and less than 20% of women are estimated to be smoking globally, while 50% of men and less than 25% of women are estimated to be using smokeless tobacco globally. Also, an annual 5 million deaths is attributed to tobacco smoking; it is the second leading cause of mortality among adults worldwide (Aghaji, 2008; Uwakwe and Modebe, 2008; NIH, 2009). This frightening data attests to the death of about three million people in the year 2007 alone by WHO estimates (WHO Resolution, 1993; World Health Statistics, 2007; NIH, 2009). These findings and reports suggest the need for further experimental and clinical studies of the importance of tobacco intake on the brain functions. To the best of our knowledge, no histological and/or morphometrical studies of the effects of tobacco leaves aqueous extract on the cerebrum (frontal lobe) had been reported. Thus, we conducted some histological and morphological (morphometric) analysis of the cerebrum of young rats exposed to aqueous extract of tobacco leaves, with the aim of assessing the effects of the tobacco on the cerebrum cytoarchitecture and morphology.

MATERIALS AND METHODS

Animals care

Twenty four (24) rats of Wistar strain of both sexes (female, $n = 12$) and male ($n = 12$), weighing between 108 and 140 g were harvested from their mother and allowed to grow in the animal holdings unit of the Department of Anatomy of University of Ilorin, Nigeria. Ethical approval was sought and received from the University of Ilorin Ethical committees on the use of animals for experimental investigations on the need to observe completely the rules guiding the employment of animals for scientific findings. This study was carried out in accordance with the National Institute of Health guide for the care and use of laboratory animals. The rules guiding the good laboratory practices were also adhered to. The rats were kept, four in a cage (of the same sex) and fed with standard rat pellets, purchased from Bethel feed, Lagos road, Ilorin. All rats were fed with water *ad libitum*.

Preparation of tobacco leaves aqueous extract

After the collection of the fresh tobacco leaves pack from Igboho (northern part of Oyo State, Nigeria), the plant samples were authenticated by Prof F.A. Oladele and Dr O.T. Mustapha (H.O.D)

of Department of Plant Science at University of Ilorin, Nigeria. The leaves were oven-dried at 60°C. Thereafter, 50 g of the grinded leaves materials was kept in 500 ml of distilled water for 24 h at room temperature. The filtrate was thereafter obtained from the solution using the What-Man No 1 filter paper and evaporated to dryness in an oven at 60°C, the residue of the extract obtained in form of paste was stored in a capped bottle and kept in a desicator (Carla, 1997; Olatunji et al, 2005).

Animal treatment

Three (3) groups of animals (A, B and C) each comprising eight (8) rats of opposite sex, received 11.7 and 5.8 mg per kg body weight/day dissolved in 0.5 ml of distilled water and control group also given equal volume of distilled water (Bertram and Katzung, 2005). Administration was done orogastrically for 20 days. After administration the rats were sacrificed by cervical dislocation on day 21 and their brains were excised and fixed in formol calcium for 4 days.

Neurohistological analysis

After fixing the brain of both control and experimental rats, the frontal lobe were excised and processed for Haematoxylin (H) and Eosin (E) method and Cresyl Fast Violet (CFV) staining procedure for Nissl substance (Carleton, 1967; Bancroft and Stevens, 1999). The sections of 5 μ were produced with the Letiz rotary microtome. The sections were mounted and examined with the light microscope and the photomicrograph of each slide was taken for further analysis for any pathological changes.

Statistical analysis

Data collected were analysed by the student's *t* – test using the SPSS 13 statistical software package.

RESULTS

Gross observations

No gross changes were observed between the experimental groups A, B and control group till the completion of the experimental procedure. The brain (with its component parts) of rats in both the control and the experimental groups (A and B) appeared morphologically normal.

Body weight

The average body weight (BW) in each groups A, B and C were calculated on day 1, 6, 11, 16 and 21 of the experimental procedures (Figures 1 and 2). There was a significant decrease in the body weight ($P < 0.05$), between groups A (with average body weight gain of 5.05 g) and B (with average body weight gain of 5.21 g) compared to group C (with average body weight gain of 12.52 g). The observed retarded increase in BW in groups A and B could be due to the negative effects of the extracts on the normal processes of metabolism as

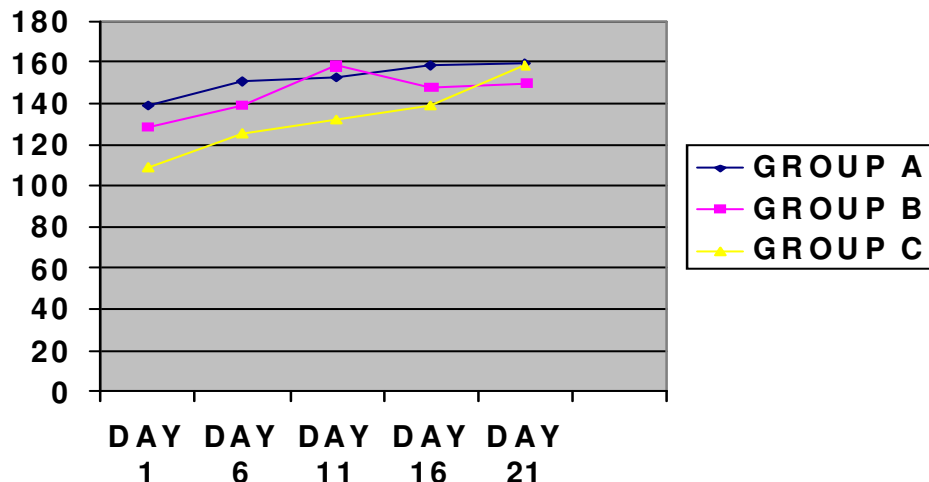


Figure 1. Body weight changes (g) of rats administered aqueous leaf extract of *Nicotiana tabacum*.

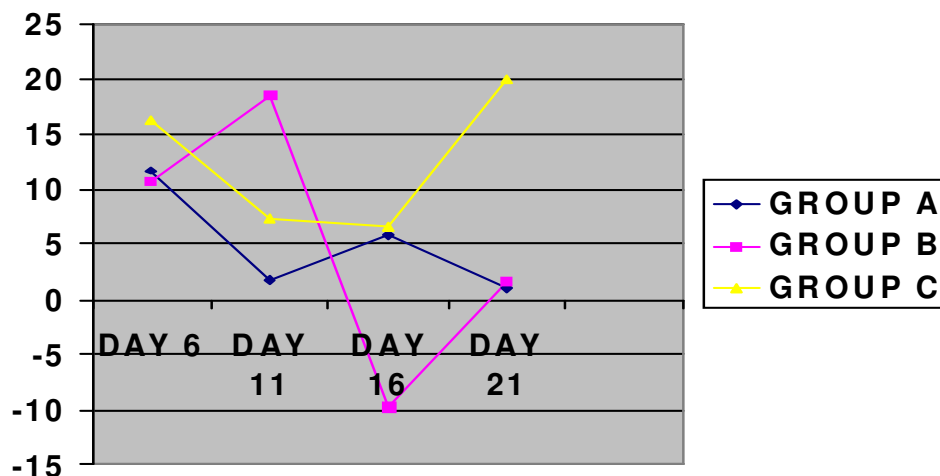


Figure 2. Body weight changes gain (g) of rats administered aqueous leaf extract of *Nicotiana tabacum*.

reported by West and Russell (1985).

Brain weight

There was significant ($P < 0.05$) decrease in the brain weight between day 1 and 21 in the experimental groups A (1.6053 g) and B (1.6526 g) compared to the control (1.7115 g) (Table 1).

Relative brain weight

There was significant decrease in the relative brain weight between day 1 and 21 in the experimental groups compared to the control (Table 1).

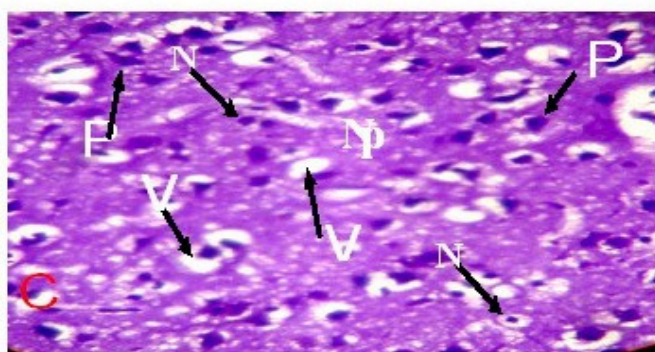
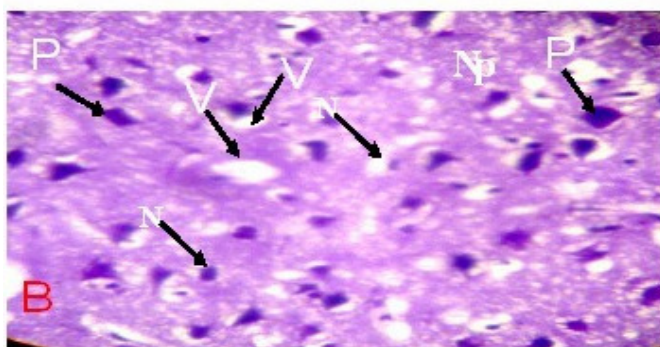
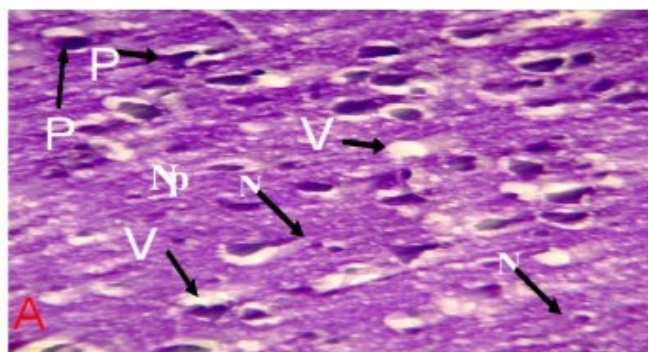
DISCUSSION

In this study, exposure of young Wistar rats to 11.7 and 5.8 mg/kg of *N. tabacum* aqueous extract per day resulted in significant decreases in the body weight, brain weight and relative brain weight. However, there was no significant gross change in the normal appearance of the brain of both experimental and the control group. The decrease in the body weight, brain weight and relative brain weight is an indication of general reduction in the body weight of the rats which may have been to reduce metabolic activity and reduction in appetite for food or reduction in fat deposition in the rat exposure to the extract.

Although, the effects of the extract on the metabolic activity and fatty metabolism as well as neurobehaviour

Table 1. Showing the brain weight, relative brain weight and percentage brain weight changes of the rats.

Groups	No. of rats	Brain weight	Relative brain weight	%BW
A	8	1.6053 ± 0.035	0.0102 ± 0.0009	1.02
B	8	1.6526 ± 0.050	0.0109 ± 0.0007	1.09
C	8	1.7115 ± 0.067	0.0136 ± 0.0037	1.36

**Figure 3.** Photographs of the neocortex of rats CFV strain (x1200) administered aqueous leaf extract of *Nicotiana tabacum*. Plate A & B (group A and B), non-homogenous myelinated neuritis (Np) unlike that in the plate C (Control group); P – pyramidal cell, N – neuroglia cell, and V – vacuole.

were not determined in the present study, however, the findings are comparable to the effects of nicotine in rats as reported by West and Russell (1985) and Penton and

Lester (2009).

Since the treated groups (A and B) of rats in the study were fed with diet containing equal percentage of minerals as in that of the controls (group C), the observed effects might not be due to mineral deficiency. However, the possibility that the extract impairs absorption of minerals may not be ruled out (Maisto et al., 1999).

The scarcity of documented data on the effects of the extract of *N. tabacum* on the cerebrum has hampered meaningful comparisons. More so, an enlargement of somata in the group A (administered with 11.8 mg/kg B.Wt/day) was observed. This may result from the higher tobacco extract administered which may be due to increase in the protein synthesis in the cells and/or chromatolysis that result from hyperactivity of the brain cells due to nicotine stimulation in brain as reported by Balfour (1982) and Penton and Lester (2009).

Conclusion

With the results of this present study, since all the rats used in this present study were subjected to the same environmental condition and same mode of liquid administration, we hereby concluded that the administration of the aqueous extract of *N. tabacum* in rats can cause neurotoxicity; which may alter normal brain functions, reduce weight gain and the effects are dose dependent.

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