

*Full Length Research Paper*

# Comparative effects of smoke and ethanolic extract of *Nicotiana tabacum* on hippocampus and neurobehaviour of mice

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The effects of tobacco use on health are well known, and are documented in reliable scientific reports. The aim of this study is to investigate some of the effects of both ethanolic and smoke tobacco on the hippocampus and behaviour of mice. The presumably healthy 32 mice were used for this study, the animals were randomly divided into four groups, A, B, C and D, of eight animals each. Group A were given 10.72 mg /kg body weight of the extract in 0.2 ml of normal saline, group B 10.72 mg /kg body weight of the tobacco smoke exposure for 3 min, group C were given 0.2 ml of normal saline and group D were exposed to the smoke of equal weight (0.02 g) of cotton wool for 3 min for 21 experimental days. The mice were sacrificed by cervical dislocation and the brains excised, blotted, weighed and fixed in formol calcium for neurohistological analysis, using Haematoxylin and Eosin and Cresyl Fast Violet. There were significant decreases in the body weight, brain weight and relative brain weight, pyramidal and granular cell layers and neurological scores between nicotine administered groups compared to the control group ( $p < 0.05$ ). The results suggested that consumption of *Nicotiana tabacum* leaves, either through smoking or chewing may lead to some level of neurohistoarchitectural alterations, brain weight changes and neurobehavioural disruption or also help in reduction in weight.

**Key words:** *Nicotiana tabacum*, hippocampus, nicotine, neurohistoarchitecture, neurobehaviour.

## INTRODUCTION

Smokeless tobacco products have been in existence for thousands of years among different populations. Over time, these products have gained popularity throughout the world (such as Tombak in Sudan, Snus in Sweden and Khaini in India) with mass marketing of new forms sold under different brand names (Kumar et al., 2006; Foulds et al., 2003; Idris et al., 1995). The term chewing tobacco is often associated with dipping tobacco (split tobacco, moist snuff) where users place a dip of tobacco between the lower or upper lip and the gum by resting the dip on the inside lining of the mouth. 'Maras powder' (MP), which is a kind of powder obtained from the shields of tobacco, is widely used in the south-east region of Turkey as smokeless tobacco and it is taken through

buccal mucosa or together with cigarette (Erenmemisoglu et al., 1999). The same research group has also found that Turkish smokeless tobacco MP prepared from *Nicotiana tabacum* L. leaves contain low amounts of nicotine (1.17%), nornicotine (0.04%), and 0.06% anabasine. International Agency for Research on Cancer (IARC) reported that moist snuff contains aliphatic and aromatic hydrocarbons, formaldehyde, ketones, alcohols, phenols, amines, amides, alkaloids, metals, radio elements such as polonium-210, uranium-235, 238. Carcinogens in tobacco, the most abundant and strongest being tobacco-specific N-nitrosamines (TSNA), such as N-nitrosornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are formed by N-nitrosation of nicotine (Kurucu et al., 1998). Tobacco consumption continues to grow all over the world. Inhalation of tobacco smoke, with its numerous toxic and mutagenic substances (for example, carbon monoxide, nicotine, polynuclear aromatic hydrocarbons,

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N-nitrosamines), may have toxic effects on brain function. Hippocampus is the structure that lies on the fringes of the medial aspect of each cerebral hemisphere (the limbic system) of the brain and it is involved in memory and learning. Exposure to tobacco nicotine either from cigarettes and other forms of tobacco including cigars, pipe tobacco, snuff, and chewing tobacco, has been reported to be associated with alteration in the normal functions of the brain and the whole nervous system (NIDA, 2009a; Charles, 2000; Katzung, 2005; and NIDA, 2009b). Nicotine has been reported to be the highest and most toxic compound of aqueous extract of tobacco leaves (Carla et al., 1997; Penton and lester, 2009; Grunberg, 1982). Nicotine is used to aid smoking cessation and other nicotine addictions (Charles, 2000; Katzung, 2005). Using a controlled amount of nicotine helps to reduce nicotine withdrawal symptoms when one attempts to quit the use of tobacco products (NIDA, 2009b; Charles, 2000; Adeniyi, 2007). Annually, about 5 million deaths are attributed to tobacco smoking contributing the second leading cause of mortality among adults worldwide (Aghaji, 2008; Uwakwe and Modebe, 2008). This frightening data attests to the death of about three million people in the year 2007 alone (NIH, 1993; Wilson and Philpot, 2002), these findings and reports suggest the need for thorough experimental and clinical studies of the effects of tobacco intake on the body systems, most especially the brain. The aim of this study therefore, was to investigate some of the effects of both ethanolic and smoke tobacco on the hippocampus of mice.

## MATERIALS AND METHODS

### Animal care

All experimental investigations were done in compliance with humane animal care standard outlined in the "Guide to the care and use of Animals in research and teaching", as approved by the Institute of Laboratory Animal Resource, National Research Council, DHHS, Pub. No NIH 86 – 23 (Anne, 2004). The study was carried out using presumably healthy 32 mice of both sexes (18 to 25 g) of three (3) months old. The animals were kept under standard and good laboratory conditions (12 h light and 12 h darkness, temperature (30°C ± 4.5°C), humidity and ventilation). They were given standard rat diet, purchased from the same company, Bethel Feeds, Ilorin, Nigeria.

### Extract preparation

The *N. tabacum* leaves were collected from Igboho, the northern part of Oyo State, Nigeria. Plant samples were authenticated at the Department of Plant Science, University of Ilorin, Nigeria. The leaves were air-dried at room temperature. Grinded leaves (50 g) were dissolved in 500 ml of 70% alcohol for 24 h at room temperature. The filtrate was thereafter obtained from the solution using Whatman's No 1 filter paper and evaporated to dryness in an air - dry oven at 40°C, the residue of the extract obtained in form of paste was stored in a capped bottle and kept in a desiccators (Obembe et al., 2010). The pH of the extract was determined before and after concentrating it, to be 4.19 and 5.72 respectively,

using pH meter (pH – 25 Model, Germany). The yield of the tobacco extract was determined to be 41.35% (Adeniyi, 2010).

### Animal treatment

The animals were given the *N. tabacum* are shown as follows:

**Tobacco extract:** This was given orally with the aid of an orogastric tube.

**Tobacco smoke:** It was administered by exposing each animal to dried *N. tabacum* leaves wrapped with 0.02 g of cotton wool in a burning chamber for 3 min (Burning time (BT); this was determined by allowing three of the *N. tabacum* leaves of known weight (equivalent of 10.72 mg/kg body weight) to burn and the average burning time was determined).

Administration was done for 21 days and 4 h after which mice from each group were sacrificed for analysis, while the rest were sacrificed by 7 days (a week) after the last administration, to study the withdrawal effects of the *N. tabacum* exposure on the animals.

### Experimental design

A total of 32 mice (16 males and females each), were used for this study. The animals were randomly divided into four (4) groups, A, B, C and D, of eight (8) animals each. Group A was given 10.72 mg /kg body weight of the extract in 0.2 ml of normal saline, group B 10.72 mg /kg body weight of the tobacco smoke exposure for 3 min, group C 0.2 ml of normal saline and D was exposed to smoke of equal weight (0.02 g) of cotton wool for 3 min for 21 experimental days.

### Neurobehavioural observations

The neurobehavioural analysis was done at 08:00 h of the day using elevated plus maze (EPM) to study the locomotion, exploration, and motor coordination in both the treated and control animals. The results are shown in Figure 3.

### Animal sacrifice

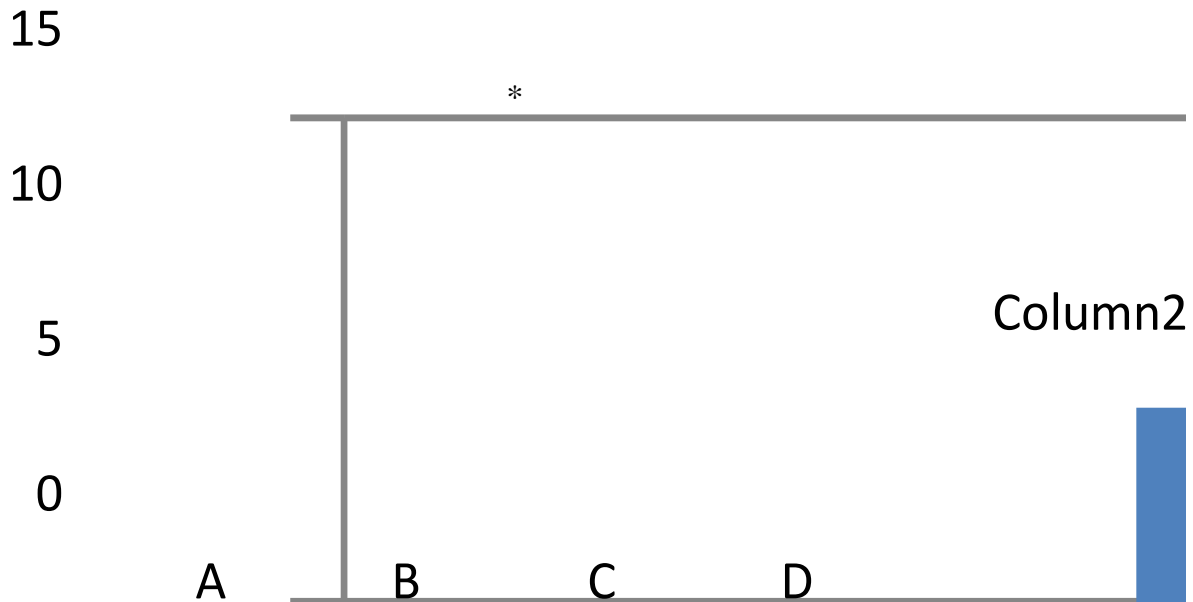
After administration, the mice four (4) from each group were sacrificed by cervical dislocation on day 21 and 28 of the treatment and their brains were excised, blotted with filter paper and the wet weights were taken and recorded and brains were quickly transferred to a specimen bottle containing 10% formol calcium and fixed for 2 days (Adeniyi et al., 2010a; Baron, 1986). Thereafter, the hippocampus was excised to process for histological analysis and the wet weights of the brain and volumes were recorded for analysis. The brain volume was determined by liquid (water) displacement method and recorded in millimeter (Ofusori et al., 2008).

Relative brain weight (RBW) changes: The RBW for each animal was calculated using the formula:

$$\text{RBW} = \frac{\text{Brain weight}}{\text{Body weight}} \times 100\%$$

Brain volume (BRV) changes: The BRV change for each group was calculated using the formula:

$$\text{Percentage BRV change} = \frac{\text{BRV at day 21} - \text{BRV at day 28}}{\text{BRV at day 21}} \times 100\%$$



**Figure 1.** Showing the head dipping (HD) of mice after 21 days of *N. tabacum* exposure. \* Statistical significant difference ( $p < 0.05$ ).

### Neurohistology

The brains are fixed in 10% formal calcium, hippocampus was excised and processed for Haematoxylin and Eosin (H & E) and Cresyl Fast Violet (CFV) staining techniques (Adeniyi et al., 2010a; Baron, 1986). The tissues were excised, imbedded in paraffin and processed for routine histologic studies. The slices of 5  $\mu$  were sectioned with the Letiz rotary microtome. The sections were mounted and examined with the light microscope and the photomicrography of each slide was recorded.

### Neurohistometry

The pyramidal (PCL) and granular (GCL) cell layers thickness was measured using the method of W.H.O and Ofusori et al. (2008) in which an oculometer was inserted into the microscope and focused through stained slides.

### Statistical analysis

The data were expressed as means  $\pm$  Standard Error of Mean (SEM). Significance was determined using the student's t-test. A p-value of less than 0.05 was considered statistically significant, using SPSS software version 16.0.

## RESULTS

### Gross observations

There were no significant changes in the skin colour and arrangement, the colour of their eyes was normal compared to the control groups. Also, the gross anatomy of the brain of the nicotine administered groups appeared

normal compared to the control groups.

### The animal weight changes

The average weight gain recorded for the treatment group during the experimental period was reduced during the first 14 days in group A and B compared to C and D. However, they all gained weight during the 7 withdrawal days.

### Animal behavior

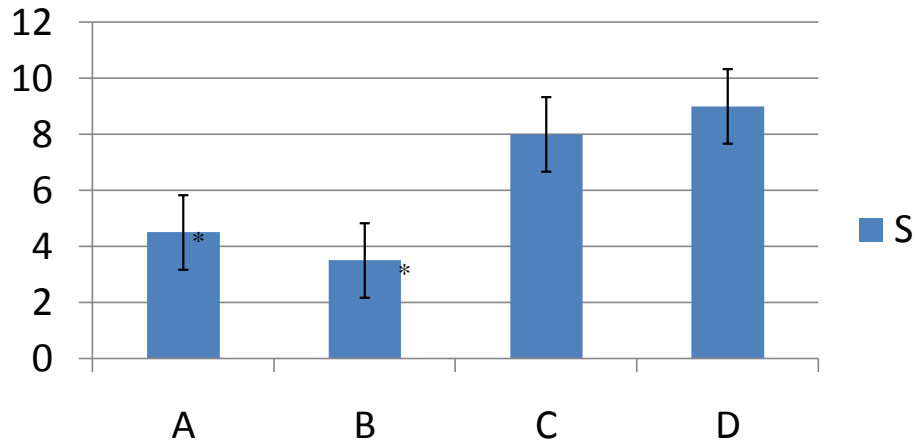
The general behaviour of the animals was comparatively normal. However, the rate of head deeding (HD), stretching (S), quadrate duration (QD) and transition (T) were significantly ( $p < 0.05$ ) different between nicotine administered groups and the control groups (Figures 1 to 4).

### Brain weight (BWT) changes

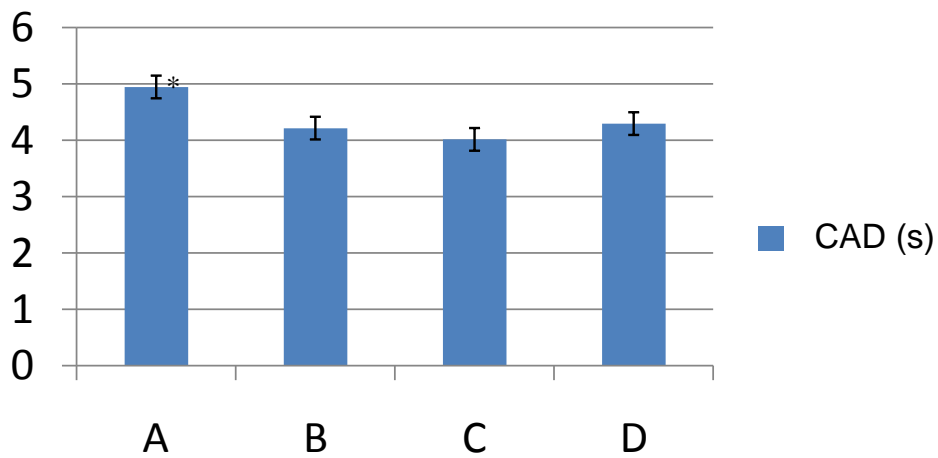
The average brain weight recorded for treatment group during the experimental period reduced during the 7 days of withdrawal (Table 1).

### Relative brain weight (RBW) changes

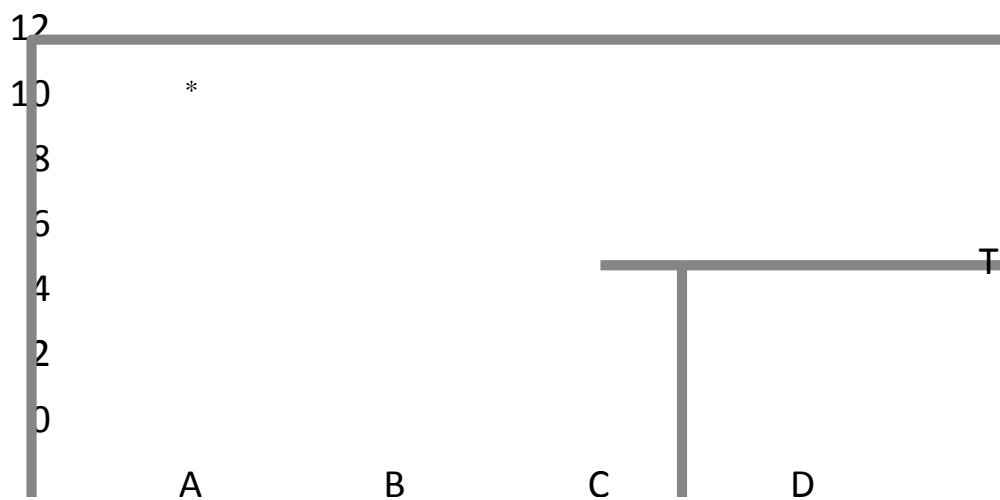
The RBW (Table 1) changed between the nicotine administered groups, group A had the highest RBW compared to C and as in group B compared to D after 21



**Figure 2.** Showing the stretching attempt (s) of mice after 21 days of *N. tabacum* exposure. \* Statistical significant difference ( $p < 0.05$ ).



**Figure 3.** Showing the close arm duration (CAD) in second of mice after 21 days of *N. tabacum* exposure. \* Statistical significant difference ( $p < 0.05$ ).



**Figure 4.** Showing the transition (T) of mice after 21 days of *N. tabacum* exposure. \* Statistical significant difference ( $p < 0.05$ ).

**Table 1.** Brain weight (BWT) (g) and relative brain weight (RBW) changes in control animals and those exposed to tobacco smoke and extract for the experimental period (mean  $\pm$  SEM).

Groups	Day 21		Day 28	
	BWT	RBW (%)	BWT	RBW (%)
A	0.5172* $\pm$ 0.0112	2.57	0.3786* $\pm$ 0.0209	1.89
B	0.4121 $\pm$ 0.0121	1.93	0.3667 $\pm$ 0.0072	1.66
C	0.3434 $\pm$ 0.0122	1.61	0.3080 $\pm$ 0.0066	1.28
D	0.03623 $\pm$ 0.0212	1.52	0.3480 $\pm$ 0.0370	1.42

\*Significantly different from control mice (P<0.05).

**Table 2.** Brain volume (ml) changes in animals exposed to tobacco smoke and extract for the experimental period (mean  $\pm$  SEM).

Groups	Day 21	Day 28	Percentage brain volume changes (%)
A	4.0 $\pm$ 0.00	3.5 $\pm$ 0.50*	(12.50)
B	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	0.00
C	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	0.00
D	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	0.00

\*Significantly different from control mice (P<0.05).

days of treatment and this was dose dependent.

### Brain volume (BRV) changes

The volume of brain of the animals (Table 2) was relatively the same in nicotine administered and control groups. Although, there was slight (12.5%) increase in the brain volume in group A, the brain volume changes were relatively the same across the groups.

### Hippocampal neurohistology

**Cell body stain intensity:** The cell bodies were more densely stained in the nicotine administered groups in a dose dependent manner compared to the control groups but the architectural arrangement appeared normal Table 3.

**Vacuations:** There were more vacuulations in the nicotine administered groups compared to the control group C.

**Cell population:** The population of the neural cells (pyramidal cells) appeared to be more in the nicotine administered groups compared to the control groups in a dose dependent pattern.

**Pyramidal cell layer:** This appeared uniformly normal with cell bodies of nicotine administered groups densely stained compared to the control group.

**Granule cell layer:** Appeared uniformly normal with cell

bodies of nicotine administered groups densely stained compared to the control group (Figure 5).

### DISCUSSION

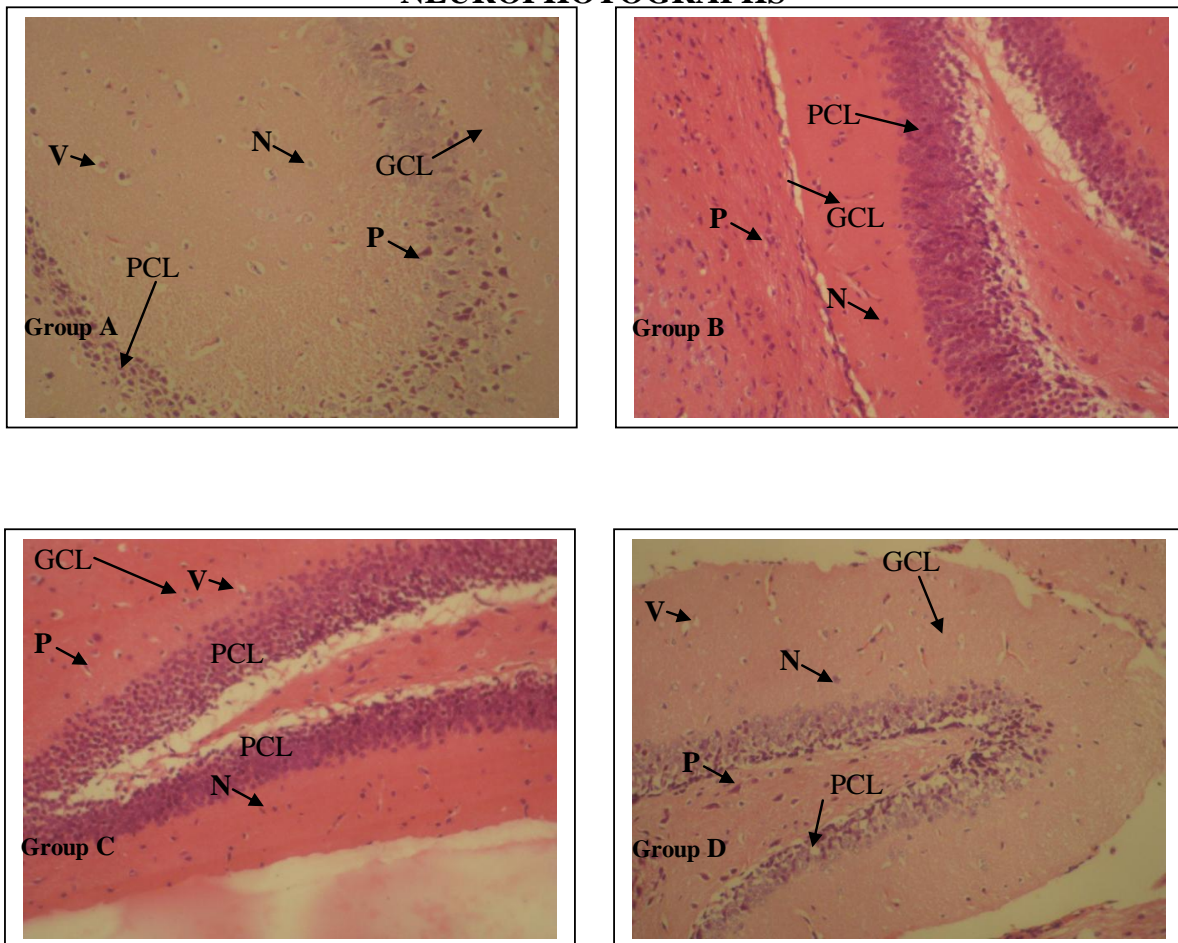
The observed reduction in weight of the animals in the experiment may implicate nicotine in the tobacco plant use as reported by Adeniyi et al. (2010a) and Chen et al., (2005) and this may be associated with reduction in food intake by the tobacco users. Also the brain weights after administration and withdrawal were significantly different from the mice in group A compared to those in groups C and D (p<0.05), but relative brain weight (RBW) of those in group A have the highest RBW compared to C (p<0.05) and as in group B compared to D (p<0.05) after 21 days of treatment and it was dose dependent. These results are related to our findings on the leaf extract of aqueous of *N. tabacum* in Wistar rats (Adeniyi et al., 2010b). The observed increase in locomotory activities of mice (Figures 1 to 4) in the treated groups compared to the control groups is in agreement with our earlier report (Adeniyi et al., 2010b), reflecting the possibility of tobacco increasing anxiotic characteristics in the treated groups. This may probably explain the reason for increased cell density observed in the treated groups compared with the control groups (Adeniyi et al., 2010a, b). The stretch – attempt (S) of the control was higher than the rest of the groups. S postures are ‘risk - assessment’ behaviour which indicates that the animal is resistant to move from its present location to the new position (Ekong et al., 2008; Blanchard et al., 2001), and thus high frequency of these activities indicates high level

**Table 3.** Hippocampal histometry analytic changes in animals on day 21 of tobacco exposure (mean  $\pm$  SEM).

Groups	PCL ( $\times 10^{-3}$ mm)	GCL ( $\times 10^{-3}$ mm)
A	5.2000 $\pm$ 0.6633	7.3333* $\pm$ 1.4530
B	6.8000 $\pm$ 0.9695	10.5000* $\pm$ 1.3229
C	6.0000 $\pm$ 0.4083	9.2500 $\pm$ 1.1087
D	5.4000 $\pm$ 0.4000	9.8000 $\pm$ 1.2806

\*Significantly different from control mice ( $P < 0.05$ ), PCL: Pyramidal cell layer, GCL: Granular cell layer.

### NEUROPHOTOGRAPHS



**Figure 5.** Hippocampus (H & E) day 21 *N. tabacum* exposures: Mg X 480: PCL: Pyramidal cell layer; GCL: Granular cell layer; P – pyramidal cell, N – neuroglia cell, and V – vacuole.

Note: The population of the neural cells (pyramidal and neuroglia cells) appeared to be more in the nicotine administered groups compared to the control groups in a dose dependent pattern and there were more vacuulations in the nicotine administered groups compared to the control group C.

of anxiety (Ekong et al., 2008).

### Conclusion

Tobacco has been reported to cause different neurological

effects in both human and experimental animal, from all the changes observed between the treated and control groups, it is worthy to conclude that the administration of tobacco leaves smoke and extract, as revealed from this study, can result in brain weight loss, distorted neurohistoarchitecture and alterations in locomotory activities.

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