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Cerebral cytoarchitectural and biochemical alterations in mice: Effects of *Nicotiana tabacum* leaves smoke exposure

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ABSTRACT: The effects of tobacco use on one's health are well known, and are documented in detailed and reliable scientific reports. In 2001, Health Canada estimated that 21% of deaths in Canada were attributed to smoking. On the other hand, estimates for the total cost of smoking incurred by the whole of society, vary considerably. The arm of this study is to investigate the some effects of smoke tobacco on the neocortex of juvenile mice. This study investigated the some of the effects of corresponding 10.72 mg/kg body weight and 5.36 mg/kg body weight/ day of the tobacco leaves ethanolic extract and smoke for a period of 21 days on the frontal lobe in juvenile mice. The presumably healthy animals were randomly divided into 4 groups, A, B, C and D of 5 mice each. Group A 10.72 mg of the tobacco smoke exposure for 3 minutes, B 5.36 mg of the tobacco smoke exposure for 3 minutes, C were given 0.2 ml of normal saline and D were expose to equal weight (0.02 g) of cotton wool of 3 minutes for 21 experimental days. The mice were sacrificed, 4 hours after the last administration, by cervical dislocation and the brains excised, blotted, weight and some were fixed in formol calcium for neurohistological analysis, using Haematoxylin and Eosin, and Cresyl Fast Violet (CFV) while others were quickly homogenized in 0.5M sucrose solution for biochemical assay. There was a statistical significant decrease in the body weight, brain weight and relative brain weight between experimental groups compared to the control group ($p<0.05$). The results suggested that the consumption of *N. tabacum* leaves smoke may lead to some level of neurocellular degeneration, carbohydrate metabolism and also help in reduction in weight gain.

Keywords: *Nicotiana tabacum*, Frontal lobe, Nicotine, Neurobehavioural.

Introduction

The brain is a vital organ, if not the most vital organ in the body of any living organism and especially Man. In Man, its weight is 1500 g and the ratio of brain to body weight is 0.02 (Standring *et al.*, 2005). 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 (Stephen, 1999; Charles, 2000; Anthony, 2002; NIDA 2009A). Nicotine has been reported to be the highest and most toxic compound of tobacco leaves smoke (Sas, 1990; Leroy, 1999; Philip, 2002). Nicotine is used to aid smoking cessation and other nicotine addictions (Charles, 2000; NIDA, 2009A). Using a controlled amount of nicotine helps to reduce nicotine withdrawal symptoms when one attempts to quit the use of tobacco products (Charles, 2000; Adeniyi, 2007; NIDA, 2009A). The World Health Organization has urged governments across the world to ban tobacco advertisement, promotion and sponsorship, as part of measures to protect the world's 1.8 billon young people (Odebode, 2008).

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According to a 2007 National Survey on Drug Use and Health, an estimated 70.9 million Americans aged 12 or older use tobacco – 60.1 million (24.2% of the population) were current cigarette smokers, 13.3 million (5.4%) smoked cigars, 8.1 million (3.2%) used smokeless tobacco and thus makes to be listed as tobacco one of the most widely abused substances in the United Sates (NIDA Research Report Series, 2009). Also from the data accrued from the World Health Organization (2009), there was about 2.4billion people in the world today that consume tobacco products either in form of snuff, chewing or smoking or snuff dipping. This represents almost one third of the world population; about 50-55% of men and less than 20% of women are estimated to smoke globally, while 50% of men and less than 25% of women are estimated to use smokeless tobacco globally. This frightening data attests to the death of about three million people in the year 2007 alone (WHO, Resolution, 1993; World Health Statistics, 2007), these findings and reports suggest the need for further experimental and clinical studies of the role of tobacco intake on the body systems, most especially the brain in particular and the arm of this study is to investigate the some effects of smoke tobacco on the neocortex of juvenile 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 (1985) and that of University of Ilorin Animal Right Ethical Committee.

The study was carried out using presumably healthy juvenile mice of both sexes weighing 18 – 25 g. The animals were kept under standard and good laboratory conditions (light, temperature, humidity and ventilation). They were given standard rat diet, purchased from the same company, Bethel Feeds, Ilorin, Nigeria.

Animal Grouping

A total of 40 healthy mice of both sexes, were used for this study. The animals were randomly divided in to five (5) groups, A, B, C and D, of ten (10) animals each. Group A 10.72 mg of the tobacco smoke exposure for 3 minutes, B 5.36 mg of the tobacco smoke exposure for 3 minutes, C were given 0.2 ml of normal saline and D were expose to equal weight (0.02 g) of cotton wool of 3 minutes for 21 experimental days.

Tobacco Leaves Preparation

The *N. tabacum* leaves pack was collected from Igboho, 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.

Conditioning of Animals

Animal were bred in the animal holdings unit of the Department of Anatomy of University of Ilorin, Nigeria to rule out the genetic effects on the investigation.

Animal Treatment

The animals were administered tobacco leaves smoke by exposing the animals to dried *N. tabacum* leaves wrapped with 0.02 g of cotton wool, to aid burning, for three (3) minutes (Burning time (BT); this was determined by allowing two or three of the *N. tabacum* leaves of known weight (10.72 mg and 5.39 mg) to burn (Chen *et al.*, 2002).

Animal Sacrifice

The administration was done for 21 days and 4 hours after which two (2) mice from each group were sacrificed for analysis of effects of the *N. tabacum* exposure on the animals. Four (4) Mice from each group were sacrificed by cervical dislocation at day 21 of the treatment while another four from each group were sacrificed at 7^{th} day later, for

withdrawal effects and their brains were excised, blotted with filter paper and the wet weights were taken and recorded, using Gallenkamp electric weighing balance (Model FA2104A) and thereafter two (2) brains were quickly transferred to a specimen bottle containing 10% formol calcium and fixed for 2 days and others were quickly transferred to a specimen bottle containing 0.25M sucrose solution for biochemical analysis. Thereafter, the frontal cortex was excised to process for further analysis and the wet weights of the brain and volume was recorded for analysis. The brain volume was determined by liquid (water) displacement method and recorded in millimeter (Ofusori *et al*., 2008A).

Neurohistology

The brains fixed in 10% formol calcium, the frontal cortex were excised and processed for Haematoxylin and Eosin and Cresyl Fast Violet (CFV) staining technique (Carleton, 1967; Bancroft, 1990). The slices of 5µ 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.

Biochemical Evaluation

The tissues for Biochemical assay were weighed using a sensitive balance and they were placed in 0.25M sucrose and homogenized in a cold mortar with pestle. The homogenate was poured into a test-tube and centrifuged at 5000rpm for 5 minutes using a centrifuge (Model 90-1). The supernatants were collected, using Pasteur pipettes, were immediately stored in the deep freezer (GC-B207WVQ) at -20ºC, and thereafter assayed. Using RANDOX Laboratories Ltd (UK) biochemical kits, the activities of Lactate dehydrogenase (LDH) in the homogenate and Glucose-6-dehydrogenase (G-6-PDH) enzyme in the homogenate were determined through spectrometry (colorimetric method) (Ofusori *et al*., 2008).

Statistical Analysis

The data were expressed as means ± Standard Error of Mean (SEM). Significance was determined using the student's t-test and ANOVA. A p-value less than 0.05 were considered statistically significant, using SPSS software version 14.0.

Results and Discussion

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 experimental appeared normal compared to the control group.

Animal Weight Changes

The average weight gain recorded for treatment group during the experimental period was reduced during the first 14 days in group A unlike those in B compare to C and D while those in group B remained relatively constant during the first 7 days and gained more weight till day 14 before losing weight in the next 7 days.

Brain weight (BWT)

The average brain weight recorded for treatment group during the experimental period reduced during the 21 days (see Table 2).

Table 1: Brain weight (g) and Relative Brain Weight (RBW) changes in animals during the experimental period (mean ± SEM)

GROUPS	DAY 21		DAY 28	
	BWT	RBW (10^{-2})	BWT	RBW (10^{-2})
A	0.5172±0.0112*	2.57	0.3786±0.0209*	1.89
B	0.3367±0.0123	1.70	0.3081±0.0343	1.47
C	0.3434±0.0122	1.61	0.3080±0.0066	1.28
D	0.03623±0.0212	1.52	0.3480 ±0.0370	1.42

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

Relative Brain Weight (RBW) changes

The RBW changed between the experimental groups was significantly different ($p<0.05$) at day 21 and as well as after 7 days of tobacco smoke withdrawal. Those in group A had the highest RBW, follow by B compared to C and D, and this is dose dependent. Although, the groups experience reduction in RBW, but that of group A (0.0068) is higher compared to others, B (0.0023), C (0.0033), and D (0.0010).

Brain volume (BRV) changes:

The volume of brain of the animals were relatively the same in both experimental and control groups, that is, no statistical different ($p>0.05$) between treated and control groups.

Cerebral Neurohistology

Cell body stain intensity: The cell bodies appeared more densely stained in the experimental groups in a dose dependent manner compared to the control groups.

Vaculations: There are more vaculations in the experimental groups, especially in group A that have vaculation in their stroma, compared to the control group C (see Plates 1 and 2).

Cell population: The population of the neural cells (pyramidal cells) appeared to be more in experimental compared to the control groups in dose dependent manner.

NEUROPHOTOGRAPHS

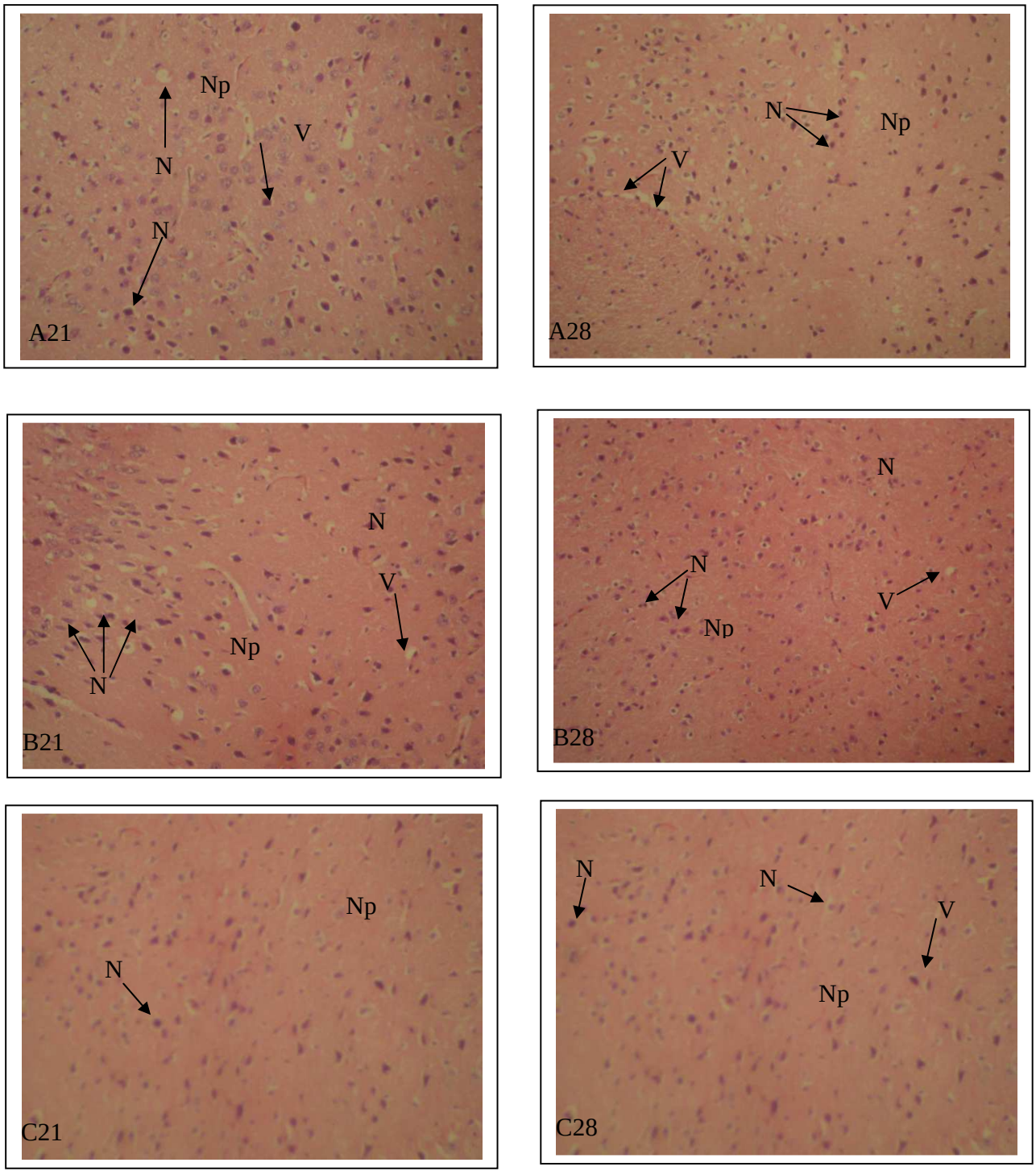


Plate 1: Frontal Cortex (H & E) at day 21 (D21) & 28 (D28): Mg X 480: A: Group A; B: Group B; C: Group C; Np: Neuropil, N: Neural cell; V: Vacuole; BV: Blood Vessel

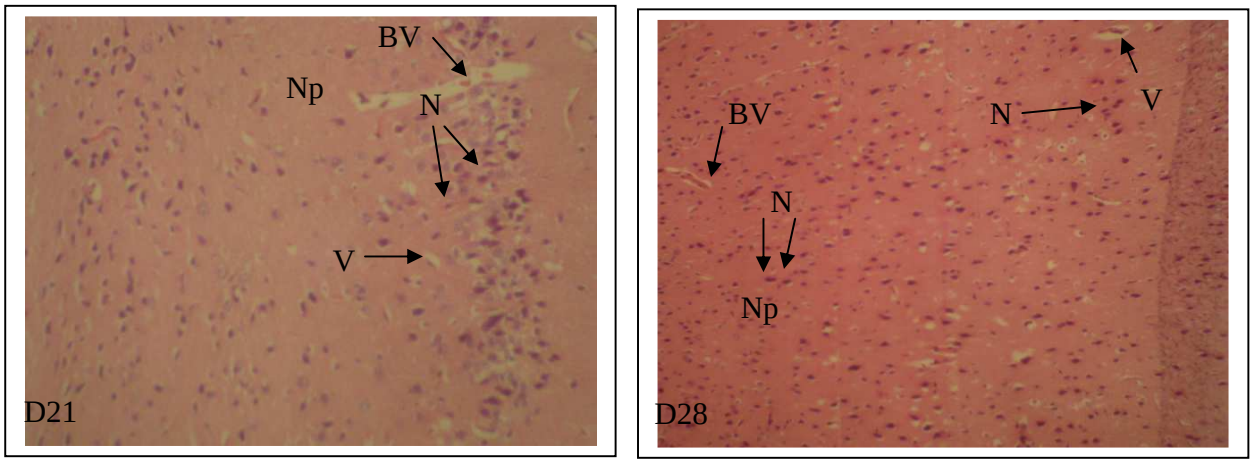
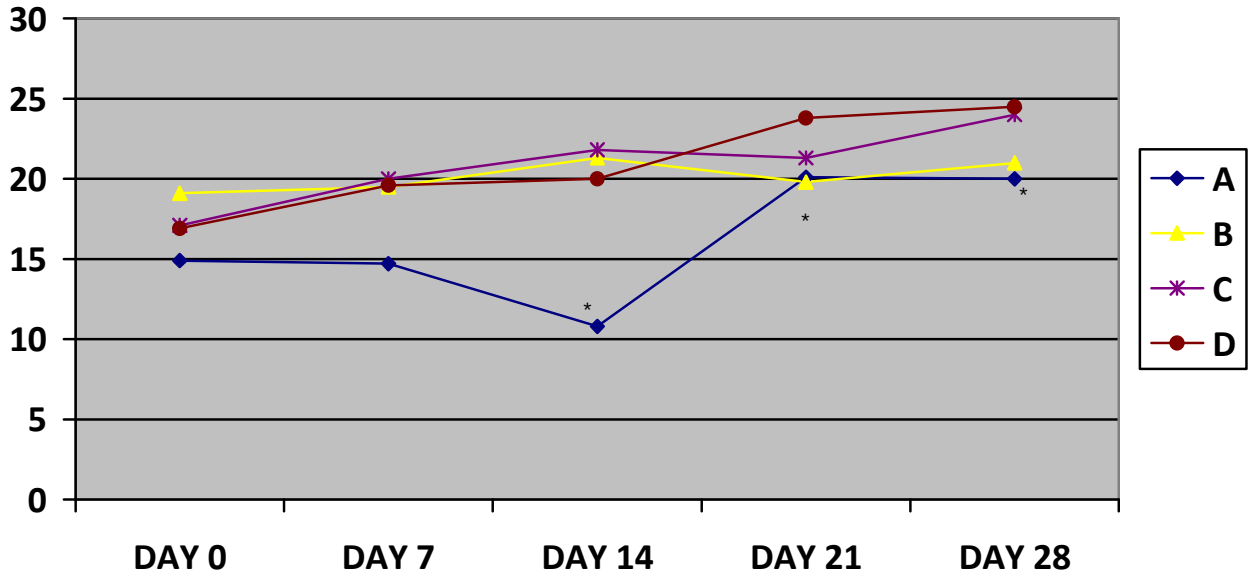


Plate 2: Frontal cortex (H & E) at day 21 (D21) & 28 (D28): Mg X 480: D: Group D

Np: Neuropil, N: Neural cell; V: Vacuole; BV: Blood Vessel



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

Figure 1: Body weight (g) changes in animals during the experimental period

Nicotine acts as a physiological neurotransmitter when present in the brain, it has been found to have both excitatory and inhibitory effect depending on the concentration and the sites at which it occurs (Purves *et al*., 1997; Batranm, 2005). And the results of our investigation revealed that smoke administration of tobacco leaves showed

histological derangement, degenerative changes and vacuolations both in the treated compared to the control sections (see Plates).

The observed reduction in weight gain of the animals in the experiment may implicate nicotine in to tobacco plant use as reported by Grunberg (2002), Russell (1985), Wilson & Philpot (2002) and Penton & Lester (2009), and this may associated for reduction in food intake by the tobacco users (Grunberg, 2002; Chen *et al*., 2004). Also the brain weight were relatively constant across the groups but relative brain weight those in group A and B have the higher ($p<0.05$) RBW compare to C and D. This may account for the shift the carbohydrate metabolic pathway due to stress in duce by the activities of nicotine in the brain of the animals as implicated in the LDH and G-6-PDH activities in this study (Tables 2 and 3).

Table 2: Brain Volume (ml) changes in animals during the experimental period (mean ± SEM)

GROUPS	DAY 21	DAY 28	Percentage brain Volume changes (%)
A	4.0±0.00	3.5±0.50	(12.50) *
B	4.0±0.00	4.0±0.00	0.00
C	4.0±0.00	4.0±0.00	0.00
D	4.0±0.00	4.0±0.00	0.00

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

BIOCHEMICAL OBSERVATIONS

Table 3: Showing the LDH activities in the Mice after 21 days of tobacco exposure and after 7 days of withdrawer from tobacco treatment

GROUPS	LDH (U/L) at Day 21ST	LDH (U/L) at Day 28^{TH}	LDH(U/L) Level changes
A_1	**515***	**205***	**(310)**
B	**85***	**865***	**780***
C	**320**	**125**	**(195)**
D	**370**	**700**	**(330)**

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

Table 4: Showing the G-6-PDH activities in the Mice after 21 days of tobacco exposure and after 7 days of withdrawer from tobacco treatment

GROUPS	G6PDH(U/L) at Day 21^{ST}	G6PDH(U/L) at Day 28^{TH}	G6PDH(U/L) Level changes
A	**1180***	**1345***	**165***
B	**3365***	**675***	**(2690) ***
C	**1510**	**3200***	**1690**
D	**1310**	**140**	**(1170)**

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

The weight loss caused by tobacco smoke exposure in mice appears to be correlated to the effects observed in cigarette smoking in humans (Chen *et al*., 2002). Also the cell bodies of the experimental groups were appeared more densely stained which is a reflection of the height activity level in treated groups A and B exited by tobacco product administered (Russell, 1985; Katzung, 2005). In this study it is important to note the change in activity of the enzymes of carbohydrate metabolism over time (after 21st day of exposure and 7 days of withdrawer) (see tables 2, 3), Glucose system of the brain depends on the stage of development of the animal, in the embryonic brain the cells are characterized by anaerobic metabolism while the adult neurons are characterized by aerobic metabolism (Purves e*t al*., 1997; Standring *et al*., 2005).

In cases of excitotoxicity which is induced by nicotine, the glucose metabolic pathway is altered. This study of the enzymes LDH which catalyses the conversion of Lactate to pyruvate; which is an end product of Pentose phosphate pathway (glycolysis) serves a s a substrate which is then converted to Succinyl - Co.A, a starting enzyme in the tricarboxylic (Krebs Cycle), this however explains the anaerobic system of the neurons which is believed to partly occur in the astrocytes and the substrate is then supplied to the neurons, G-6-PDH in another case is an important although not the starting enzyme of the hexose monophosphate shunt (HMP) serves a s an indicator of glycolysis preferred to Hexokinase as an indicator (Katzung, 2005). Determination of LDH and G-6-PDH levels is to describe the shift model in carbohydrate metabolism in the study, otherwise energy production and consumption pattern adopted for each mode of treatment, concentration and duration and time.

After 7 days of withdrawal from tobacco smoke exposure, first defining the model on control groups (C and D) the data shows that the LDH:G-6-PDH is about 1:4, this implies that the normal metabolic activity in the mice brain depends 4 times more on oxygen based energy production of the HMP than the non-oxygen dependent energy production of the pentose phosphate pathway (PPP); using this as a standard, group A shows a LDH:G-6-PDH ratio of 1:2.5, this result shows that on administration of nicotine in group A experienced a partial shift from aerobic to anaerobic based energy production compared with C and D. Animal in group B shows a surge in glycolysis indicates that at a lower dose nicotine has an excitatory effect rather than an inhibitory effect as its been accounted for by 3 fold increase in aerobic glucose consumption and a drastic fall in anaerobic energy production such that the LDH:G-6-PDH ratio is 1:40 compared to group A and control groups. Withdrawal of these animals for the next 7 days shows a major reversal effect; animal in group B shows reduction in glucose metabolism but maintains a major dependence on aerobic metabolism in the PPP rather than HMP.

Conclusion

Above all, from all these changes observed from analyses between the experimental and control groups its save to conclude that the administration of tobacco leaves smoke resulted in general body weight loss, increase neuronal activities and alterations of enzymes of carbohydrate metabolism.

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