Research Journal of Animal Sciences 4 (4): 99-102, 2010
ISSN: 1993-5269

The Neurobehavioural Effects of Smoke and Ethanolic Extract of *Nicotiana tabacum* Leaves Exposure in Mice

P.A.O. Adeniyi, O.K. Ghazal, A.A. Musa and E.A. Caxton-Martins
Department of Anatomy, College of Health Sciences,
University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

Abstract: The neurobehavioural effects of ethanolic leaf extract and smoke of *N. tabacum* was evaluated. About 24 presumably healthy animals were randomly divided into 6 groups, A-F of 4 mice each. Groups A and B were force fed with 10.72 and 5.36 mg of ethanolic extract per kg body weight, respectively groups C and D were exposed to 10. 72 and 5.36 mg of tobacco smoke for 3 min, respectively group E was exposed to equal weight (0.02 g) of cotton wool smoke for 3 min while group F was also given equal amount (0.2 mL) of normal saline for 21 days. The treated groups exhibited some behavioral changes such as restlessness, aggressiveness, agitation and disorientation. The neurobehavioural investigation was carried out using elevated plus maze. The results suggested that the consumption of *N. tabacum* leaves smoke and ethanolic extract can alter neurobehavioural activities.

Key words: *Nicotiana tabacum*, frontal lobe, nicotine, neurobehavioural, extract, behavioral changes

INTRODUCTION

The brain is the control center for movement, sleep, hunger, thirst and virtually every other vital activity necessary to survival. All human emotions including love, hate, fear, anger, elation and sadness are controlled by the brain. In Man, the ratio of brain to body weight is 0.02. Essentially, specialized brain cells called neurons receive and send information encoded in electrical impulses and neurochemicals. 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 (Carroll, 2000; NIDA, 2009a).

Nicotine has been reported to be the highest and most toxic compound of tobacco leaves smoke (Philip, 2007; Penton and Lester, 2009). Nicotine is used to aid smoking cessation and other nicotine addictions (Carroll, 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 (Carroll, 2000; Adeniyi *et al*., 2010; NIDA, 2009a). 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, 2009a). Also from the data accrued from the World Health Organization, there was about 2.4 billion 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 <20% of women are estimated to smoke globally while 50% of men and < 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, 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 and ethanolic extract of tobacco on the neurobehavioural activities in 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

Corresponding Author: P.A.O. Adeniyi, Department of Anatomy, College of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

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 (12 h light and 12 h darkness, temperature, humidity and ventilation). They were given standard rat diet, purchased from the same company, Bethel Feeds, Ilorin, Nigeria.

Extract preparation: The *N. tabacum* leaves pack was collected from Igboho, Oyo State, Nigeria in December 2009. Plant samples were authenticated at the Department of Plant Science, University of Ilorin, Nigeria. The leaves were air-dried at room temperature. About 50 g of the grinded leaves was 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 dessicator (Cerami *et al*., 1997; Adeniyi *et al*., 2010). The pH of the extract was determined, to be before (4.19) and after (5.72) concentrating it, using pH meter (pHs-25 Model). The yielding rate of the extract was determined to be 41.35%.

Animal treatment: The animals were given the *N. tabacum*.

The tobacco extract: This was given orally with the aid of an orogastric tube. The tobacco smoke was administered by exposing the animals 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 (10.72 mg and 5.39 mg)) to burn and their average burning time was determined. The administration was done for 21 days and 4 h after which 2 mice from each group were placed at the center of the maze to explore for 5 min.

Experimental design: About 24 presumably healthy animals were randomly divided into 6 groups, A-F of 4 mice each. Groups A and B were force fed with 10.72 and 5.36 mg of ethanolic extract per kg body weight, respectively, groups C and D were exposed to 10. 72 mg and 5.36 mg of tobacco smoke for 3 min, respectively group E was exposed to equal weight (0.02 g) of cotton wool smoke while group F was also given equal amount (0.2 mL) of normal saline for 21 days.

Neurobehavioural analysis: The neurobehavioural analysis was done to study the locomotion and exploration in both the treated and control animals. This was done using the elevated plus maze test method. The elevated plus plank (5 cm×0.5 m) with one closed arm (0.5 m×5 cm) was used and the following behavioural scores were carried out at day 21:

Head Dipping (HD): Frequency at which the animal dip its head to the ground (no unit).

Stretching: This is the risk attempt by the animal (no unit).

Close Arm Duration (CAD): The time spent in the close arm of the maze per trail (sec).

Transition (T): The frequencies which the animal cross from close arm to open arm (no unit).

Statistical analysis: The data were expressed as means±Standard Error of Mean (SEM). Significance was determined using the student's t-test. A p-value <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. 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 (Katzung, 2005). The observed reduction in weight gain of the animals in the experiment may implicate nicotine in to tobacco plant use as reported by Grunberg (1982), Wilson and Philpot (2002) and Penton and Lester (2009).

The Head Dipping (HD) in group B that were exposure to 10.72 mg kg^{-1} body weight of ethanolic extract of *N. tabacum* were significantly ($p<0.05$) higher than that of other groups with those in group E lowers. HD is usually used as a measure of locomotor activitity but it also measures exploration.

High frequencies of these behaviours indicate increased locomotion and exploration (Walsh and Cummins, 1976; Ekong *et al*., 2008). As seen in this study (Fig. 1), the treated groups had higher locomotor and exploratory activities than the control groups. The increase activity may be due to the reaction to N. tabacum treatment. The Stretch-Attend (S) of the control was higher than the rest of the groups (Fig. 2). S postures are risk attempt behaviour which indicate that the animal is hesitant to move from its present location to the new position (Ekong *et al*., 2008) and thus high

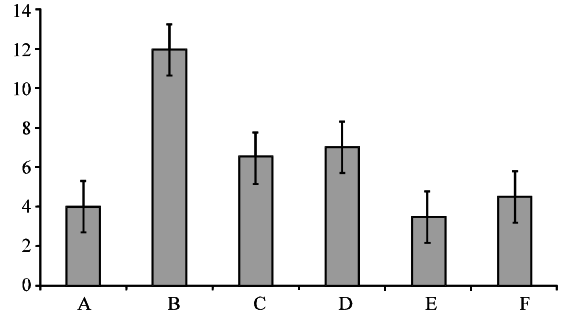

Fig. 1: Showing the Head Dipping (HD) of mice after 21 days of *N. tabacum* exposure

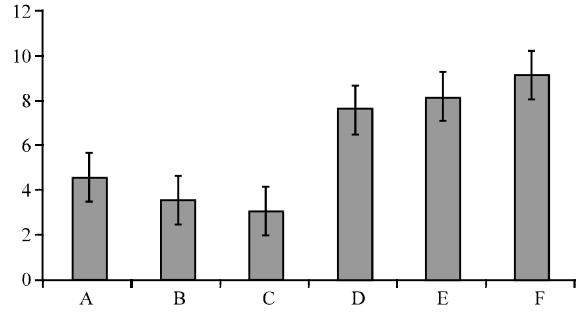

Fig. 2: Showing the Stretching attempt (S) of mice after 21 days of *N. tabacum* exposure

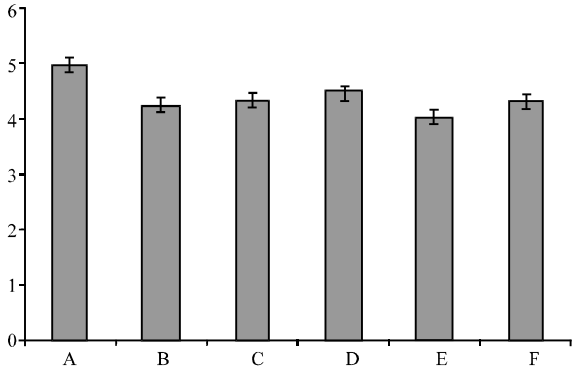

Fig. 3: Showing the Close Arm Duration (CAD) in sec of mice after 21 days of *N. tabacum* exposure

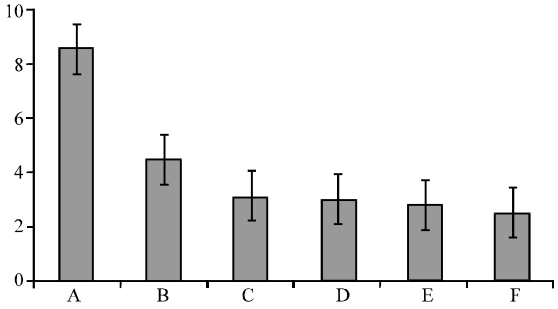

Fig. 4: Showing the Transition (T) of mice after 21 days of *N. tabacum* exposure

level of anxiety. This may be due to the adverse and stimulatory effects the *N. tabacum* might have had on the animals. Transition (T) in control groups were significantly ($p<0.05$) lower than the rest groups with that of group A been the highest but not Close Arm Duration (CAD) (Fig. 3). The frequency of transition (Fig. 4) and CAD are measures of exploration behaviour and anxiety (Schellinck *et al*., 2010). A higher frequency/duration of these beahioural parameters indicates high exploratory behaviour and low anxiety levels (Walsh and Cummins, 1976; Ekong *et al*., 2008). In this study, the group with higher dose treatment showed increase in T compared to the control groups. This may be due to adverse effect of the *N. tabacum* (Adeniyi *et al*., 2010).

The facts are sufficiently manifold and exact to be able to affirm a harmful action of tobacco upon the brain. Binetnotes the problem that nicotine, once inside the person is not eliminated except very slowly. The smoke is also comprised of other chemicals, by-products of combustion and especially, carbon monoxide, prolonged tobacco use results in lesions in the cerebral cortex. Brain damage such as abnormally increased brain size involves a combination of factors, increased blood pressure, altered heart rate, etc., repeated fluctuations as smokers take doses of poison then it wears off, they take more, etc. Tobacco is also a brain poison. It injures the brain and weakens the nerves. When chronically used, it causes loss of memory. It makes many who use it peevish and dissatisfied when for any reason they are without it for a short time. Like the other narcotics, appetite for it grows stronger constantly and the more the appetite is satisfied the worse is the tobacco-user's condition. The persistent interaction between nicotine and nicotinic acetylcholine receptors must ultimately lead to downstream plasticity at the molecular, cellular and circuit levels that then result in the behavioural desire to continue to intake nicotine (Penton and Lester, 2009).

The tobacco plant, Nicotiana has probably been responsible for many more deaths than any other herb (NIDA, 2009a, b; Penton and Lester, 2009). At present, tobacco smoking is causing over 3 million deaths a year worldwide and if current smoking trends continue the annual mortality may exceed 10 million by around 2030. Add to this the mortality from cancers caused by oral uses and the death toll becomes still higher. Undoubtedly, tobacco is the most important avoidable cause of premature death and disease in the world (Charlton, 2004; NIDA, 2009b). In many cases, brain damage results in

fairly specific language and related sensorimotor functions. This may be the reason for the results obtained in this study.

CONCLUSION

Above all, from all these changes observed from neurobehavioural analyses between the experimental and control groups its safe to conclude that the administration of ethanolic extract of tobacco leaves and smoke resulted in alterations of exploratory activities in mice.

REFERENCES

Adeniyi, P.A.O., O.K. Ghazal, B.U. Enaibe, G.A. Adefolaju, A.O. Oyewopo and E.A. Caxton-Martins, 2010. The cytoarchitectural alterations in the neocortex of wistar rats: Effects of aqueous tobacco (*Nicotiana tabacum*) leaves extract exposure. Afr. J. Biotechnol., 9: 7539-7543.

Carroll, C.R., 2000. Drugs in Modern Society. 5th Edn., McGraw Hill Publication, New York, pp: 161-186.

Cerami, C., H. Founds, I. Nicholl, T. Mitshuhashi and D. Giordano *et al*., 1997. Tobacco smoke is a source of toxic reactive glycation products. Proc. Natl. Acad. Sci. USA., 94: 13915-13920.

Charlton, A., 2004. Medicinal uses of tobacco in history. J. R. Soc. Med., 97: 292-296.

Ekong, M.B, A.O. Igiri and O.E. Mesembe, 2008. The effects of administration of Amidiaquine on some parameters of neurobehaviuor of Wistar rats. Nag. J. Physiol. Sci., 23: 51-54.

Grunberg, N.E., 1982. The effects of nicotine and cigarette smoking on food consumption and taste preferences. Addictive Behav., 7: 317-331.

Katzung, B.G., 2005. Basic and Clinical Pharmacology. 9th Edn., McGraw-Hill Companies, New York, pp: 100-106.

NIDA, 2009a. Infofacts. Research Report Series, US Department of Health and Human Services, pp: 1-5.

NIDA, 2009b. Tobacco addiction. Research Report Series, US Department of Health and Human Services, pp: 1-11.

Penton, R.E. and R.A.J. Lester, 2009. Cellular events in nicotine addiction. Seminars Cell Dev. Bio., 20: 418-431.

Philip, A.A., 2007. Tobacco use and your health christ focused. News Lett. Atunbi Baptist Church, 3: 1-4.

Schellinck, H.M., D.P. Cyr and R.E. Brown, 2010. Advances in the Study of Behavior. Vol. 41, Academic Press, Burlington, VT USA., pp: 255-366.

Walsh, R.N. and R.A. Cummins, 1976. The open-field test: A critical review. Psycol. Bull., 83: 482-504.

Wilson, M.M.G. and C. Philpot, 2002. Cigarette smoking and weight loss in nursing home residents. Medicine on-line, http://priory.com/med/cigsmoking.htm.