Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.3, No.3, 2013

The Anxiolytic Effects of Smokeless Tobacco in Mice

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Abstract
This study assessed the effect of smokeless tobacco leaf on anxiety in mice. Twenty male albino mice, aged 60-120 days were separated into 2 groups of ten each. Animals in the test group were fed on 0.1g of tobacco mixed with 10g of chow everyday for 14 days. While animals in the control group were given only 10g of chow for the same duration. The mice were then allowed 5 minutes each to explore the excavated plus maze and the light/dark transition box and behaviours were scored. Results in the elevated plus maze revealed that the duration in the open arms was significantly different ($p<0.05$) between the two groups with the test group (49.6 ± 11.9 sec/5 mins) being lower than the control group (89.1 ± 22.1 sec/5min). The frequency of stretch attend postures was significantly lowered ($p<0.05$) in the test group (8.8 ± 0.6) compared to the control (11.5 ± 1.8). There was no significant difference in the other parameters in the elevated plus maze.
The frequency of line crosses in the light box was significantly higher ($p<0.01$) for the test group (74.3 ± 9.8) than the control (47.4 ± 63). The frequency of stretch attends posture and rearing in the dark box was significantly higher ($p<0.05$) in the test group then the control group (180.5 ± 18.9). These results suggest that tobacco may have decreased anxiety in the test group.
Keyword: Anxiety, postures, elevated plus maze.

Introduction

Tobacco belongs to the nightshade family *solanaceae*, therefore related to the tomato and potato. Tobacco belongs to the genus *Nicotiana*, which is named from Jean Nicot, the then French ambassador to Portugal (Macon 1994). There are many species of tobacco but tabacum and rustica are the two common one (Hecht, 1989). Tobacco is used in two major forms: the smokers and the smokeless. The smokeless tobacco has different nature names according to Thomsen (2008), these are: Ntsu in South Africa, Toombak in Sudan, Shammah in South Arabia, plug chew in the United States. In Nigeria, the common names are Anwuru in Igbo, Taba in Yoruba and Hausa languages. Anxiety is the normal emotional and physiological response to feeling threatened. Different people behave differently when threatened. They may either run or fight however there are some threats that beforehand determine whether it will be flight or fight (Jorge, 2000). 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 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). In view of the numerous usage of tobacco, this paper reported the possible effects of smokeless tobacco on anxiety in mice.

Materials and methods

Twenty male albino mice between 60-120 days old were procured from the animal house, Department of physiology, University of Calabar, Calabar, Nigeria. The mice were kept and maintained under standard laboratory conditions of temp, humidity and light for a period of two weeks in the animal holdings of the Department of human anatomy, University of Calabar, Calabar before the commencement of the experiment. During this experiment the mice freely fed on chow and were given distilled water ad libitum.

Experimental protocol

In this study, a total of 20 mice were used for the experiments. Ten mice served as control while the other ten served as tests. Each of these mice were marked with either blue or red dye for identification after each experiment in order to prevent distraction due to the presence of dye mark on their body parts.
In this experiment, the apparatus known as the elevated plus maze was used. The various maze experiment were carried out in the neuro-behaviour laboratory, Department of Physiology: University of Calabar. True neurobehavioral experiments were carried out for the control mice and were repeated for the test mice and in all these experiments certain exhibited behaviours of the mice were recorded.

Results

The duration in plus maze open arms was 89.1±22.1 secs/5mins. In the control group while it was 49.6±11.9 secs/5 mins in the test group. The duration plus maze open arms was significantly higher in the control group compared to the test group ($p<0.05$). The frequency of stretch attend was 11.5±1.2 in the control group while it was 8.8±0.6 in the test group. The frequency of stretch attend posture in the plus maze was significantly higher in the control compared to the test group ($p<0.05$). The frequency of the plus maze arms entry was higher in the control group in both arms but was not significant.

The duration in the light box in the control group was 180.5± 18.9secs/5min while the test group recorded 146.3±8.2secs/5mins. The duration in the light box was significantly higher in the control group when compared to the test group ($p<0.05$). The duration in the dark box recorded and significant difference in both groups (fig3). The frequency of the crosses in the control group was 47.4±6.2 and was significantly lower than the test group in the light box ($p<0.01$) which recorded 74.3±9.9. The dark box did not show any remarkable differences between both groups

The frequency of rear in the control group was 18.0±38 while the test group was 25.4±2.0. The frequency of rear in the control group was significantly lower when compared to the test group in the dark box ($p<0.05$)

In the frequency of stretch attended posture for the control group was 12.3±2.5 while 20.1±2.5 was recorded in the test group. The frequency of stretch attend posture in the control group was significantly lower when compared to the test group in the dark box ($p<0.05$)

Discussion

Tobacco whether in smoke or smokeless forms contains nicotine and over 19 known cancer-causing chemicals and more than over 4000 other chemicals which includes acetone, ammonia, carbon monoxide, cyanide, methane propane and butane (Downs, 2008). It has been documented that nicotine crosses biological membranes and the blood brain barrier easily (yildiz 2003).

The results of the study shows the stretch attends posture frequency of the elevated plus maze was significantly higher ($p<0.05$) in the control group (11.5±1.2) then in the test group (8.8+0.6). This is an indication of the fact that there was a higher level of anxiety among the mice in the control group than those in the test group. The control group seemed to exhibit a greater level of emotionality. This is in agreement with the report by Lister (1990) who reported that stretch attends posture is one of the behaviours that represent anxiety.

The open arm duration of the control group was 89.1±22.1 secs/5mins while the test group recorded 49.6±11.9secs/5mins. This gives a measure of anxiety and confirms that animals' displaying reduced open arm activity shows greater level of anxiety (Espejo, 1997).

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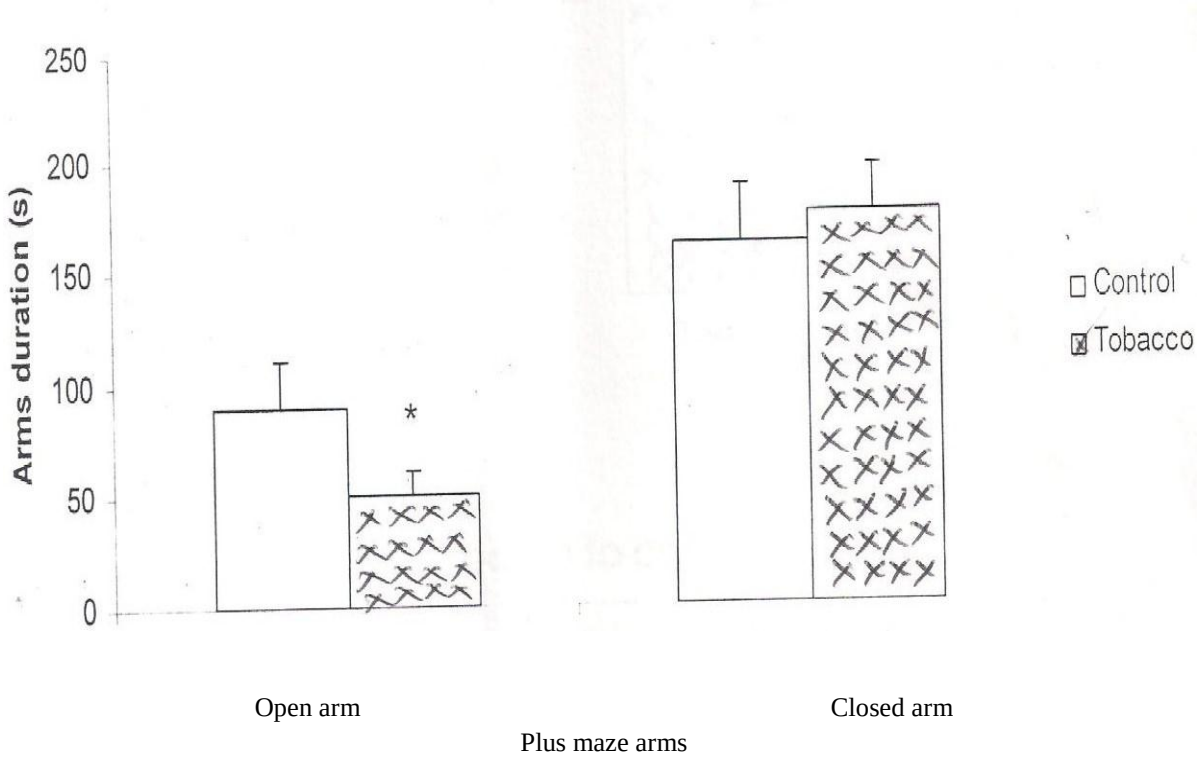
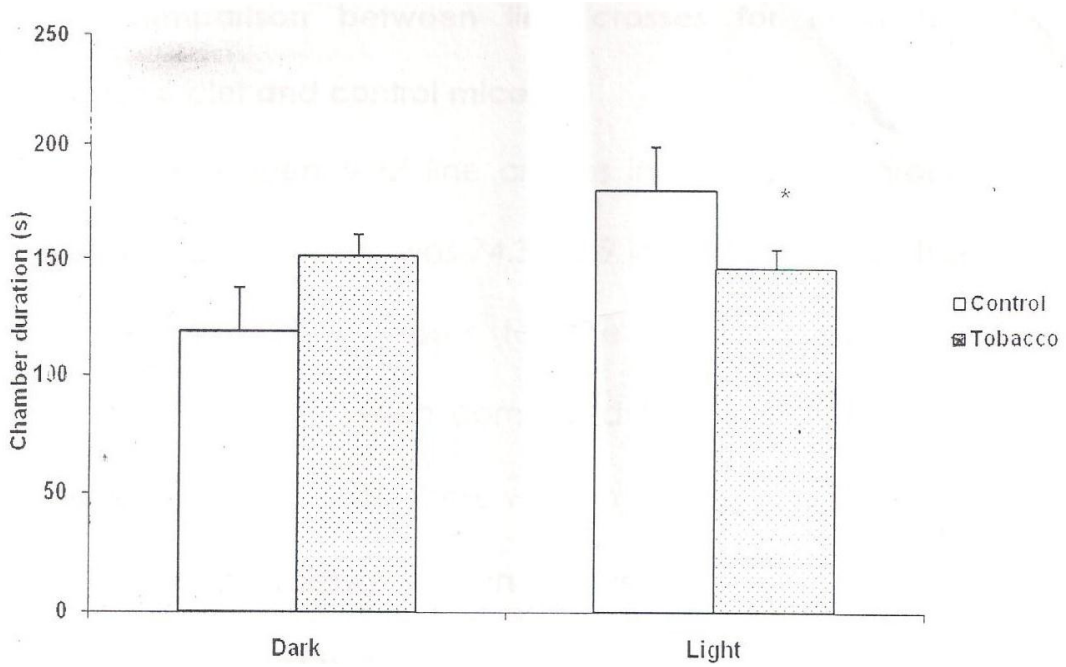
Figure 1: Comparison between duration in plus maze arms for mice fed tobacco diet and control mice.



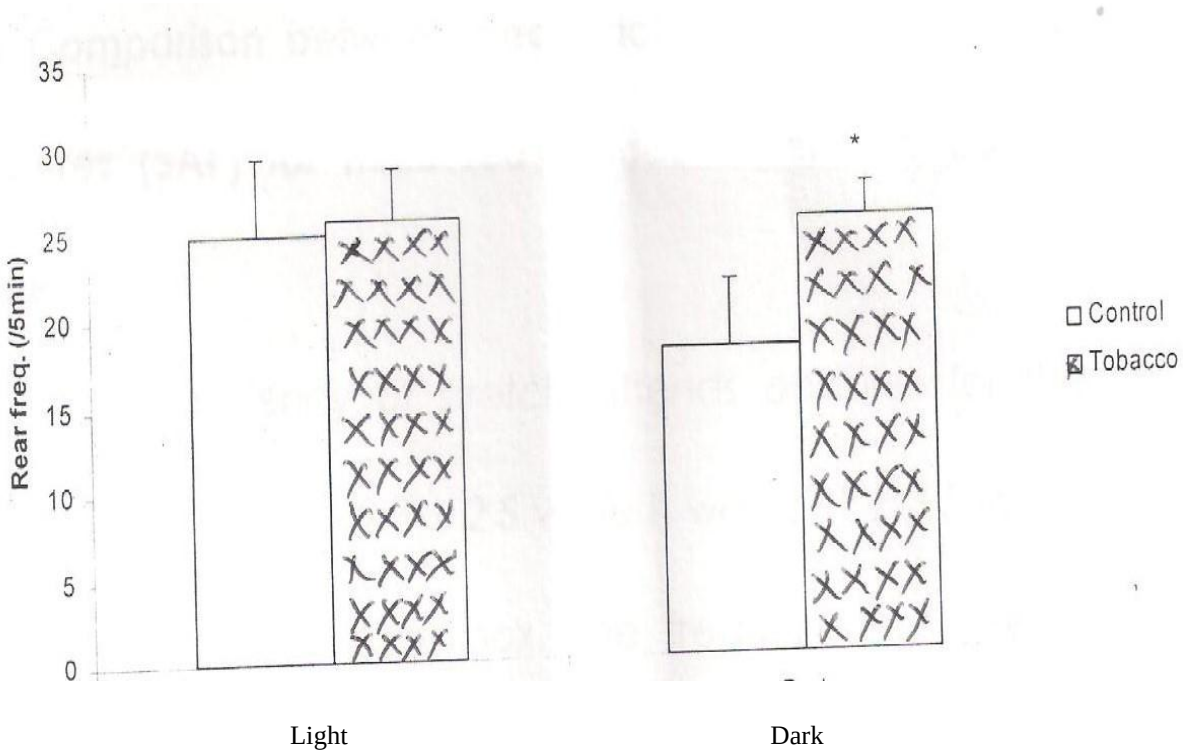
*- Significant at P<0.05 compared to control

Figure 2: Comparison between light/dark chamber durations for mice fed tobacco diet and control mice.



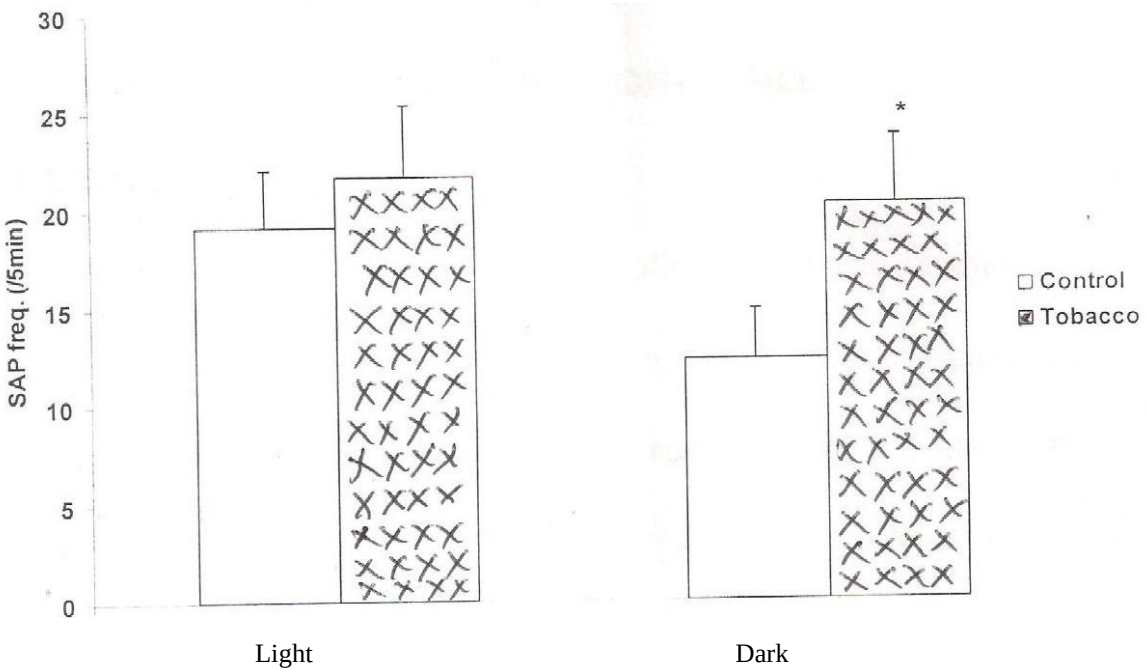
*- Significant at P<0,05 compared to control

Figure 3: Comparison between frequency of rear for mice fed tobacco diet and control mice



*- Significant at P<0.05 compared to control

Figure 4: Comparison between frequencies of stretch attends postures (SAP) for mice fed tobacco diet and control mice



*- Significant at P<0.05 compared to control

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