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## RESEARCH PAPER

### EFFECT OF CHRONIC CONSUMPTION OF POWDERED TOBACCO (SNUFF) ON ANXIETY, FEAR AND SOCIAL BEHAVIOURS

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## ABSTRACT

The effect of chronic consumption of tobacco powder on anxiety, fear and social behavior was studied using two groups of Swiss mice (control and test) weighing 18g-28g (n=15 each). The control received 100g of normal rodent chow, while the test received 1g of tobacco powder in 99g of rodent chow per day. Water was given *ad libitum* while daily food and water intake, as well as body weight changes, were monitored during the 31-day study. The elevated plus maze (EPM) and light/dark transition box (LDTB) were used to access anxiety and fear, while nest building was used to assess social behaviour. The results showed a significantly higher body weight ( $P<0.05$ ), as well as food and water intake ( $P<0.01$ ) in the test group. In EPM, the duration in the open arm frequency and head dips was significantly higher ( $P<0.01$ ) in the test group, while the frequency of stretch attend posture and defecation was lower in the test group ( $P<0.001$  and  $P<0.05$  respectively); signifying a decrease in anxiety and fear, which was also observed in LDTB, but no significant difference in nest building between both groups. Thus, chronic consumption of powdered tobacco may decrease anxiety/fear but has no effect on social behaviour.

**Keywords:** Powdered tobacco, Snuff, Anxiety, Fear, Social behaviour, Mice.

## INTRODUCTION

Man's quest for survival has led to the discovery of many plants that are of medicinal value even before the advent of orthodox medicine. One of such plants is tobacco *nicotiana* commonly called tobacco. Tobacco is a native medicinal plant of the nightshade family that is cultivated for their leaves which when cured is smoked as cigar or cigarettes, chewed as chow and snuffed as snuff. Tobacco is hallucinogenic in high doses and a stimulant in low doses (Breen, 1985; Terfz, 1990).

As an agricultural product, tobacco is processed from fresh leaves of genus *nicotiana*. Tobacco has long been grown in America, Africa, Europe and Asia about 6,000 Bc. Tobacco is known and used throughout all quarters of the globe in two major forms: the smoked and the smokeless. The smokeless tobacco has different native names in Nigeria and these include; Anwuru (in Igbo), Taba (in Hausa) and Uwong (in Efik).

Smokeless or spit tobacco comes in two different forms, that is snuff and chewing tobacco. Snuff is a fine grain tobacco that comes in cans or pouches. Chewing tobacco comes in pouches and in the forms of long strands of tobacco that are commonly called "plugs" or "chew". Many people believe that using smokeless tobacco is safer than smoking it. This however, is not true because smokeless tobacco can have health effects amongst which are addiction to nicotine and leukoplakia (Dempsey, 2001).

Generally, tobacco (smoked or smokeless type) contains nicotine and this accounts for its anxiogenic effects. In addition to nicotine, tobacco is said to contain over nineteen known cancer causing chemicals (Downs, 2008). Available literature suggest also that tobacco is a violent absolute poison which when introduced in a moderate quantity into the body system, can produce harmful effects. In fact, a small quantity of tobacco in the stomach can excites violent vomiting with other alarming symptoms like headache and nausea (Koop, 1986).

Considering the fact that the nervous system controls rapid activities of the body including muscle movement, changing visceral events, anxiety/fear, pain, emotion, behaviour, and even the rate of secretion of some endocrine glands (Guyton and Hall, 2006), this study investigates the effect of chronic consumption of tobacco on anxiety, fear and social behaviour.

## MATERIALS AND METHODS

**Experimental animal/grouping:** Thirty Swiss white mice of both sexes weighing between (18g-28g) and bred at the animal room of the department of human physiology, University of Nigeria, Nsukka, were used for this study after approval by the college ethical committee of Abia State University. The animals were transported to the animal room of the department of Physiology, Abia State University, Uturu, Abia State, Nigeria, where they were acclimatized under standard laboratory conditions and given free access to normal feed and clean tap water.

The animals were randomly assigned into two groups; a control and a test group. The animals in the control group received normal feed (rodent chow) only, while the test group received mixed feed of 1gram powdered tobacco per every 99g of rodent chow making 1% of tobacco diet for 31days. This is sequel to the fact that the determined LD50 for intra-peritoneal administration of powdered tobacco was 1316.44mg/kg.

**Experimental design:** Digital weighing balance was used to determine the body weight of the animals. The Elevated plus maze and the light/dark transition box was used to access anxiety and fear related behaviour.

The Elevated plus maze was used to assess anxiety and fear as designed by (Lister, 1990). It is made up of a closed and open arm. Each mouse was picked by the base of its tail and introduced into the maze at the center square between the close and open arm. The Mice were allowed to explore the maze for 5minutes and behaviors were scored within this period and then returned to its home cage. Behaviours scored were head dipping, open and close arm duration, stretch attend posture, frequency and duration of grooming. The apparatus was cleaned with 70% ethyl alcohol and then allowed to dry between tests to eliminate olfactory stimuli. This experiment lasted for a day.

Also, the Light/dark transition box is designed to test unconditioned anxiety and exploratory behaviours. It is based on the conflict between exploring a novel environment and avoidance of bright light (Brown and Hascoet, 2003). Each mouse was picked by the base of its tail and placed in the center of the light compartment facing the door and allowed to explore the apparatus for 5 minutes. The experiment lasted for one day and behaviours scored were transition, Light/dark box duration, stretch attend posture, frequency and duration of grooming.

**Statistical Analysis:** Data between the groups was analysed by one-way analysis of Variance (ANOVA) followed by post hoc student's t-test. Data were presented as Mean  $\pm$  SEM (Standard error of mean) p-value less than 0.05 was considered statistically significant.

## RESULTS

**Frequency of head dipping in the elevated plus maze (EPM) [Figure 1]:** The frequency of head dipping of both groups of mice in the Elevated plus maze is shown in figure 1. The frequency of head dips was significantly higher ( $p < 0.01$ ) for the tobacco group as compared to the control. Their mean head dips values were  $7.5 \pm 1.01/5\text{min}$  for control and  $12.43 \pm 2.16/5\text{min}$  for the tobacco group of mice.

**Frequency of stretch attend postures (SAP) in the elevated plus maze (EPM) [Figure 2]:** The frequency of stretch attend posture in the elevated plus maze for mice fed with tobacco diet and control diet is shown in figure 2. The bar chart shows a lower frequency of stretch attend posture (SAP) of the mice fed tobacco diet (significant at  $p < 0.01$ ) as compared to the control. The mean values were:  $8.57 \pm 1.38/5\text{min}$  for control and  $4.07 \pm 0.68/5\text{min}$  for mice fed with tobacco diet.

**Frequency of defecation in the elevated plus maze (EPM) [Figure 3]:** The frequency of defecation amongst the tobacco fed group was significantly lower ( $p < 0.05$ ) than the control as shown in figure 3. The mean number of fecal bole produced at the end of every five minutes spent in the EPM by the control group was  $3.16 \pm 0.37/5\text{min}$  and  $2.09 \pm 0.03/5\text{min}$  for mice fed tobacco.

**Frequency of transition in the Light/dark box [Figure 4]:** The frequency of transition that is, the number of times the mouse passes through the door linking the light box and the dark box to enter either the light area or dark area was not significantly different between that of the tobacco fed mice and the control. Their mean values were:  $12.57 \pm 1.28/5\text{min}$  for the control and  $13.60 \pm 0.89/5\text{min}$  for the tobacco diet fed mice.

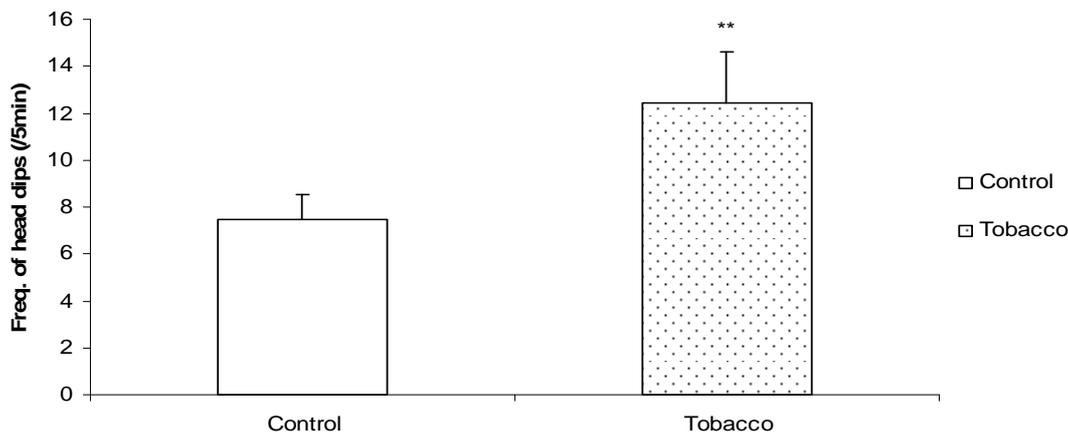
**Frequency of stretch attend posture (SAP) in the light/dark transition box [Figure 5]:** The frequency of the stretch attend posture in the mice fed with tobacco diet was significantly lower ( $p < 0.001$ ) than that of the control as shown in figure 5. Their mean values were:  $13.0 \pm 1.96/5\text{min}$  for control and  $5.73 \pm 0.86/5\text{min}$  for the tobacco test group.

**Chamber durations in the light/dark transition box [Figure 6]:** Both groups had higher preference for the dark chamber. However, the group of mice fed with tobacco diet spent significantly shorter time ( $p < 0.001$ ) in the dark than the control. The values were:  $234.07 \pm 8.1\text{sec}$  and  $173.4 \pm 13.11\text{sec}$  for both the control mice and the mice fed with tobacco diet respectively in the dark chamber. Also, the mice fed with tobacco diet spent significantly higher time ( $p < 0.001$ ) in the light chamber compared to the control. The values were:  $65.5 \pm 8.33\text{sec}$  (control) and  $124.0 \pm 14.09\text{sec}$  (test) in the light chamber.

**Nesting score, body weight changes, daily food and water intake:**

**Nesting score in the social behaviour test [Figure 7]:** The nesting score in the social behaviour test of nest building was  $3.85 \pm 0.35/5\text{min}$  for the control group of mice and  $4.2 \pm 0.23/5\text{min}$  for the mice group fed with tobacco diet. The score of the tobacco fed mice was not significantly different as compared to the control.

**Mean body weight change [Figure 8]:** The tobacco diet fed mice showed a significantly ( $p < 0.05$ ) higher body weight change when compared to those fed with control diet. The mean values were:  $8 \pm 0.4\text{gram}$  for the control and  $9.9 \pm 0.67\text{gram}$  for tobacco group.



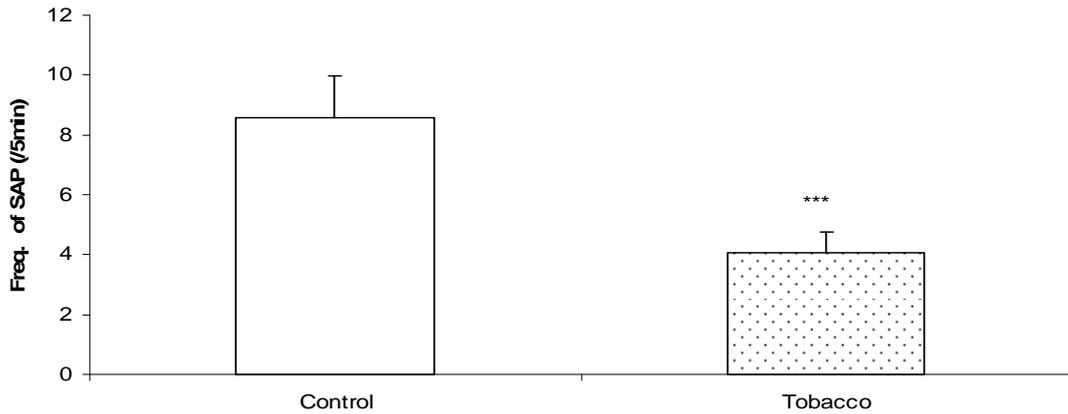
**Fig.1: Comparison between Frequency of Head dips in the elevated plus maze for mice fed tobacco diet and control.**

\*\* – Significant at  $p < 0.01$  compared to control.

**Daily food intake [Figure 9 and 10]:** The food intake curve for the tobacco diet fed mice showed no significant difference as compared to the control except on days 12 to 14 where tobacco diet fed mice ate significantly more

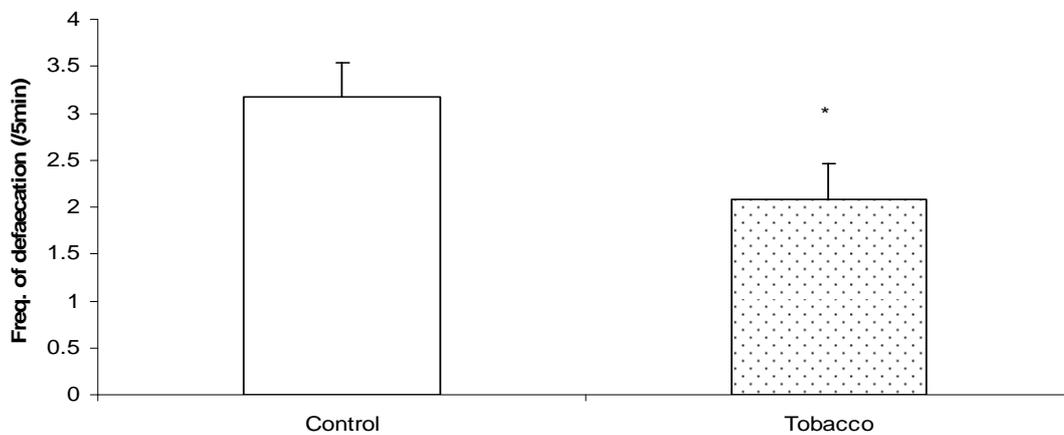
than the control group. However, when the mean daily food intake for the tobacco diet fed mice was compared with the control in 31 days of feeding, there was a significant difference ( $P < 0.01$ ). The mean food intake was  $7.27 \pm 0.059\text{g}$  and  $7.106 \pm 0.0427\text{g}$  in mice fed powdered tobacco and control diet respectively.

**Daily Water Intake [Figure 11]:** The statistical analysis of both groups shows that water intake by the tobacco fed mice was significantly higher compared to control except for days 27<sup>th</sup> to 30<sup>th</sup> day only. When mean daily intake of the tobacco diet fed mice was compared with their control for 31 days of the study, the mean daily water intake in the tobacco diet fed mice was significantly higher ( $p < 0.01$ ) than that of the control group. The mean daily water intake was  $7.967 \pm 0.0726\text{ml}$  and  $6.73 \pm 0.057\text{ml}$  respectively.



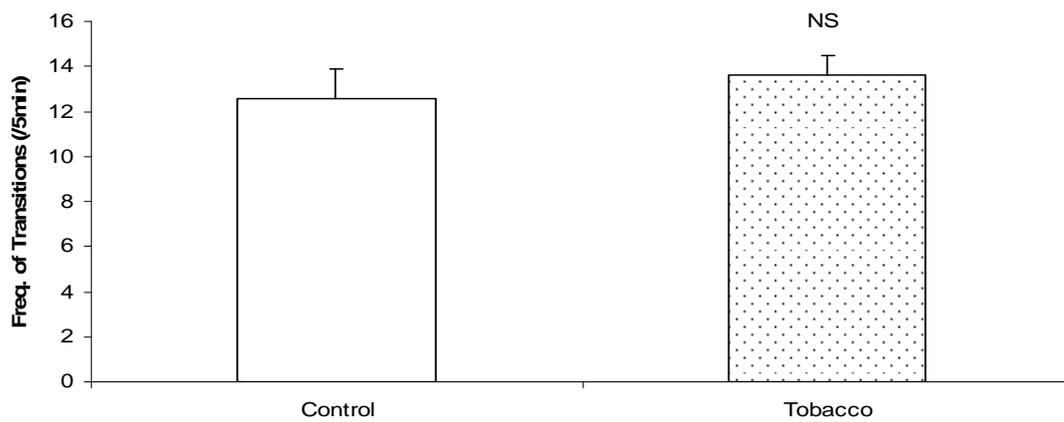
**Fig. 2: Comparison between frequency of stretch attend postures (SAP) in the elevated plus maze for mice fed tobacco diet and control.**

\*\*\* – Significant at  $p < 0.001$  compared to control.



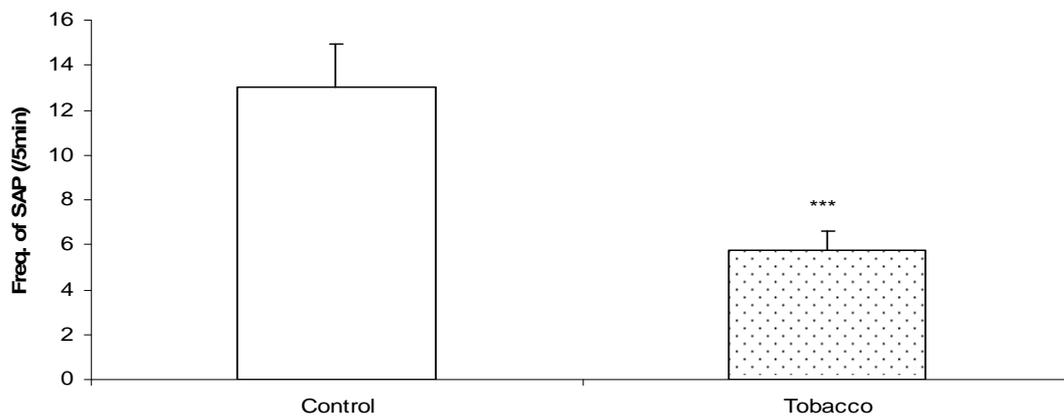
**Fig. 3: Comparison between frequency of defaecation in the elevated plus maze for mice fed tobacco diet and control.**

\* – Significant at  $p < 0.05$  compared to control



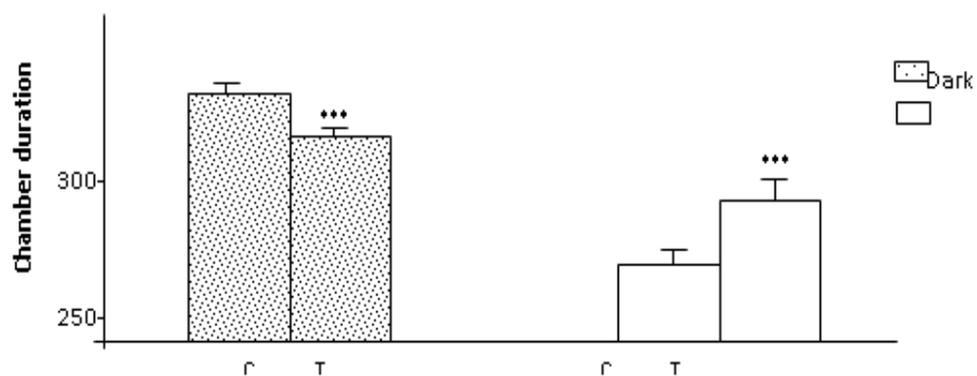
**Fig. 4: Comparison between frequency of transition in the light/dark box for mice fed tobacco diet and control.**

NS – Not significant compared to control



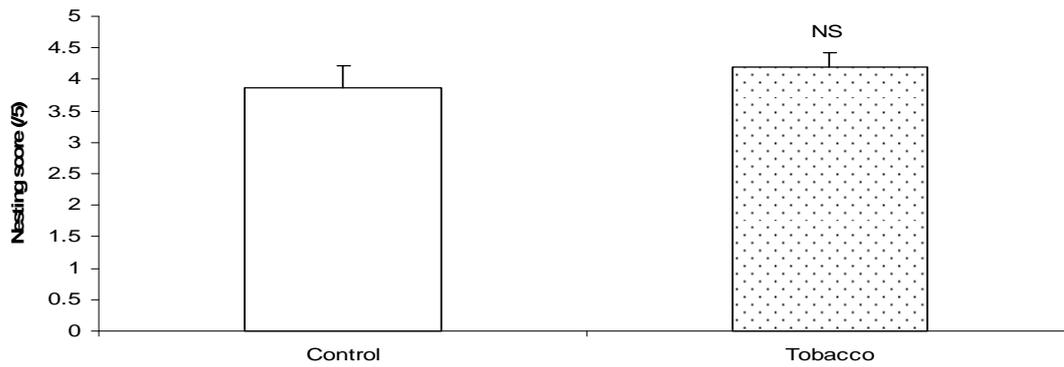
**Fig. 5: Comparison between frequency of stretch attend posture (SAP) in the light/dark box for mice fed tobacco diet and control.**

\*\*\* – Significant at  $p < 0.001$  compared to control



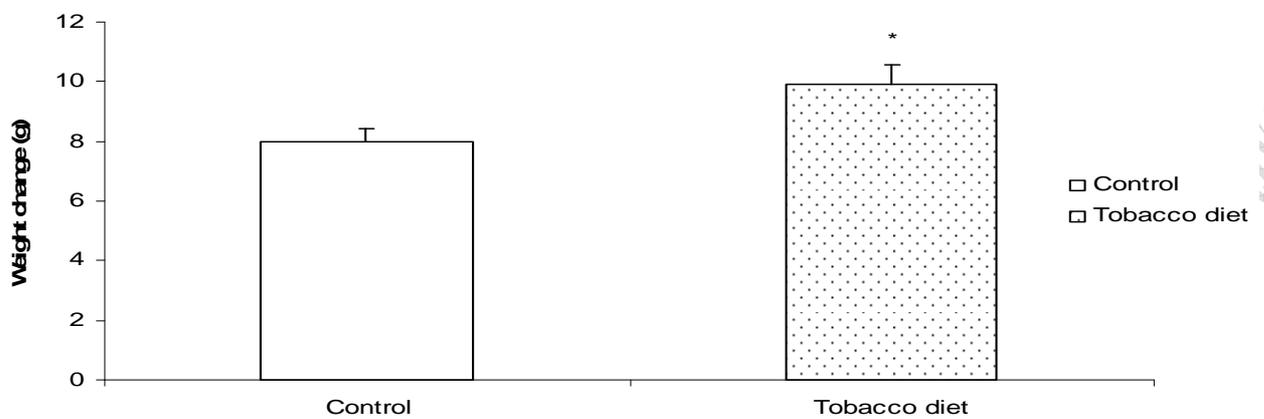
**Fig. 6: Comparison between chamber duration in the light/dark box for mice.**

\*\*\* - Significant at  $p < 0.01$  compared to control



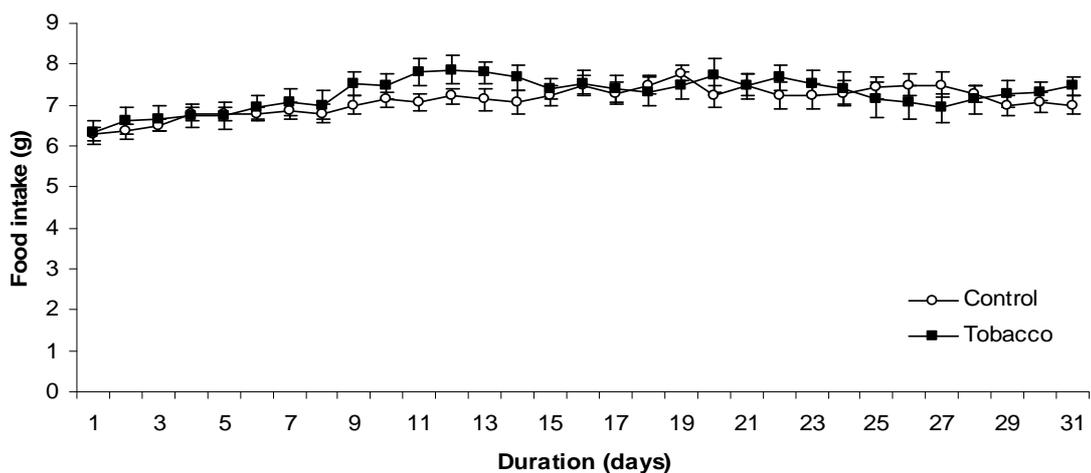
**Fig. 7: Comparison between the nesting score in the social behavior test of nest building by mice fed tobacco diet and control.**

NS – Not significant compared to control.

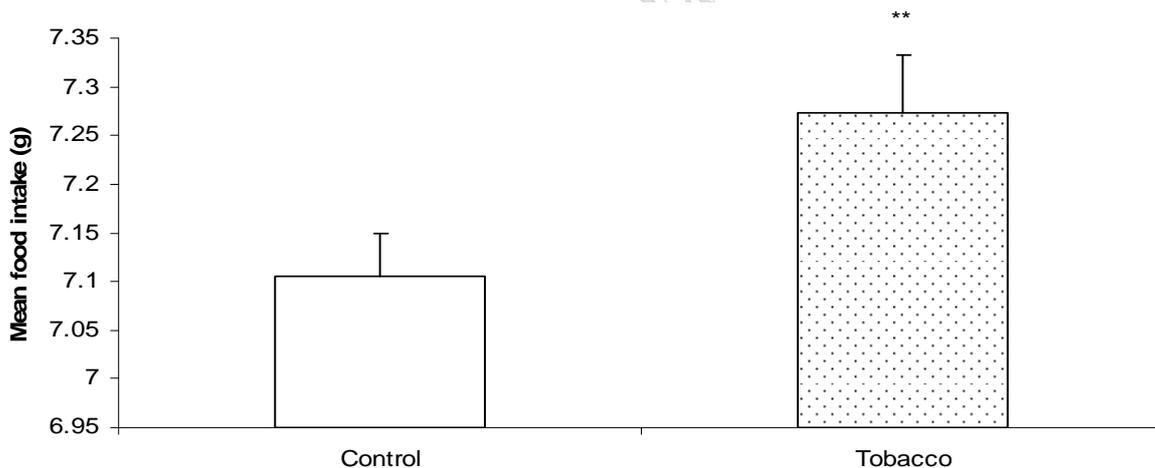


**Fig. 8: Comparison of mean body weight changes in mice following consumption of control diet and Tobacco diet.**

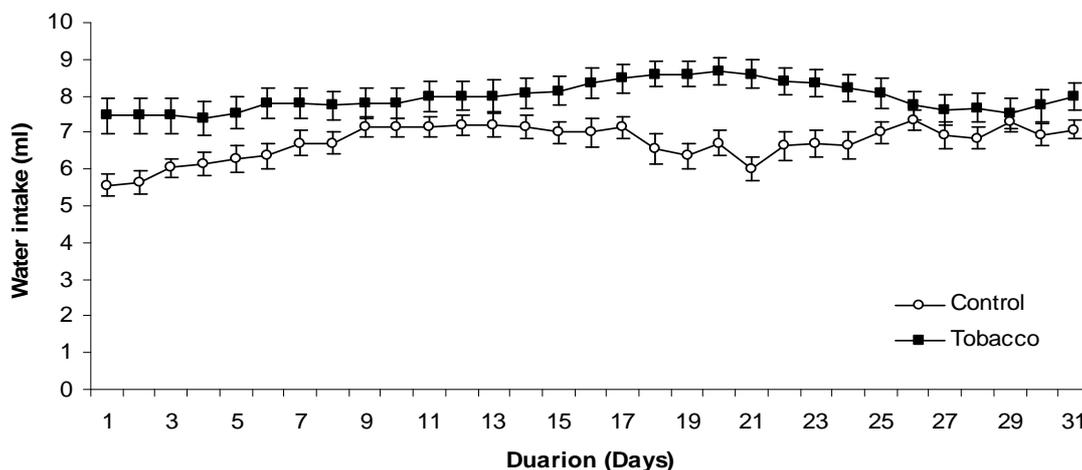
\* Significant at ( $P < 0.05$ ) compared to control



**Fig. 9: Comparison of daily food intake for mice fed control diet and Tobacco diet.**



**Fig. 10: Comparison of mean food intake between powdered-tobacco fed mice and control.** \*\* - significant at  $p < 0.01$  compared to control



**Fig. 11: Comparison of daily water intake for mice fed control diet and Tobacco diet.**

## DISCUSSION

In this study, parameters such as anxiety, fear, social behaviours, body weight change, food intake, water intake were considered. The elevated plus maze (EPM) and the light dark transition box are tests of anxiety, fear and exploration in rats and mice. The principle is based on the conflict between exploring in a novel environment and avoidance of bright light (Bourin and Hascoet, 2003). The nesting test is a test of social behaviour in rodent. For example, mice testing to determine their ability to build nest as reported by Brown et al. (1999). In addition to these tests, body weight change was determined to find out if chronic consumption of powdered tobacco (snuff) diet affected the growth of the animals used for the study.

Considering the observations in the elevated plus maze experiment, tobacco may be said to have a role in controlling anxiety on the central nervous system. Similarly, the observations in the light/dark transition box, as in the case of SAP and duration of time spent, which are index behaviours for anxiety, tobacco may also be said to reduce the level of anxiety.

Fear and anxiety are basically controlled by neural circuitry involving the amygdala mostly, and the hippocampus. However, other areas of the brain that may be involved in the control of fear and feeling of terror in the animals are also documented (Osim, 2008). Powdered tobacco (snuff) is known to contain cardiac glycosides reduce and alkaloid such as nicotine as its constituents. Cardiac glycosides reduce heart contraction (Pierce, 1998), whereas the alkaloid, nicotine decrease tension and depressive feelings and promote the relaxation of skeletal muscle tone (Benowitz, 1998). Thus, it is possible that the presence of these compounds and other constituents in the powdered tobacco could be responsible for the anxiolytic property of powdered tobacco (snuff) which act by inhibiting the excitability of the amygdala through increase in the threshold response of the cells of these nuclei, thereby reducing fear related behaviour in the mice (Costal et al., 1989; Adolph et al, 2005). However, the mechanism by which tobacco causes decrease in anxiety and fear is not certain.

Food intake is known to be controlled by the lateral hypothalamic nucleus and ventromedial hypothalamic nucleus (Guyton and Hall, 2006). It was stated by Guyton and Hall (2006) that the lateral hypothalamus is the hunger centre and when stimulated, the animal eats and drinks voraciously while on the other hand, ventromedial hypothalamic nucleus is satiety centre and when stimulated the animal stops feeding. Considering therefore the observation on the mean food intake in the test group, it is possible therefore, that powdered tobacco diet may have a stimulatory effect on the lateral hypothalamic nucleus, thus, increasing food intake. Although, previous research have shown that pure nicotine inhibits hunger (Benowitz, 1998). It is however, not the only constituent of tobacco, as Kesangau (2007), Pierce (1998) and Hohmes (1960), have shown that cardiac glycosides, terpenoids and steroids in tobacco, increases food intake, body weight, enhance protein synthesis, as well as promote growth and other molecules in the body respectively.

Also, the observed significant water intake in the tobacco treated group can be explained by the associated control of water intake by osmoreceptors or “thirst receptors” in the hypothalamus. Osmoreceptors stimulates thirst when the blood concentration of electrolytes (osmolarity) is high (Guyton and Hall, 2006). Conversely, inhibition of this centre reduces thirst. It is likely therefore, that since it has been reported by Hohmes (1960) that steroids increase thirst. The presence of steroid in the powdered tobacco may be responsible for the stimulation of the thirst centre to increase water intake. The exact mechanism by which powdered tobacco increases food and water intake remain to be elucidated and by implication, a need for further studies in this regard.

Our findings therefore suggest that the chronic consumption of tobacco decreases anxiety and fear but induces no social behavioural changes.

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#### **AUTHOR(S) CONTRIBUTION**

All authors have contributed one way or the other to the success of this paper.