Neurobehavioural Activity In Albino Wistar Rats In The Open Field Maze Following Long Term Tobacco Diet Ingestion
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Abstract
Chemicals with behavioral activity are delivered to users when tobacco products are ingested. Nicotine in tobacco causes depression of locomotor activity in rats; but, tolerance to this activity develops following chronic administration. However there is a controversy in the way rodents differ in their sensitivity and direction of the effect (increased or decreased activity) of nicotine. This study aims to determine such a status in the albino Wistar rats. The open field maze (arena) was used to study locomotor, exploratory and anxiety related behaviors in 32 albino Wistar rats weighing between 180g – 230g. The rats were grouped into 4 consisting of 8 rats each. Group 1 animals which served as control had free access to normal rat chow and clean drinking water. Group 2 animals received 15% w/w tobacco diet; group 3 animals received 24% w/w tobacco diet; while group 4 animals received 30% w/w tobacco diet. Each animal was tested in the open maze for 5 minutes and behaviors scored. ANOVA and post-hoc t-test were employed for statistical analysis and p<.05, accepted as significant. Our results revealed changes in behaviour that may likely result from the total constituents of tobacco as against reported effects of nicotine alone.

INTRODUCTION
Smokeless or “Spit” are products of tobacco without combustion or pyrolysis at the time of use. One of the forms of smokeless tobacco, besides copart from chewing tobacco, is snuff. Snuff is a form of tobacco that is processed to fine grains and packaged either in cans or pouches. Its user takes a “pinch”, “dip”, or “quid” and places it between the lower lip or cheek and gum and suck on it 1. Another route for the use of snuff, though rare is by sniffing, i.e. nasal use. This route is common among Nigerian users.

Users of smokeless tobacco believe it is safer than smoking. However, it still has lots of effects. Addiction to nicotine is one of the side effects of its use 12. Another is cancer of the mouth and pharynx, for which there are inconclusive reports 13-15. Other side effect includes leukoplakia, gum recession, bone loss around the teeth, abrasion of teeth and bad breath 13-15.

One of the chemicals delivered to tobacco users in cigarette smoking or other forms of tobacco like snuff is nicotine. Tobacco contains an average of 1.5% nicotine by weight 16,17. This chemical affects behavioural and physiological activities, and has in fact, been implicated as being responsible for majority of the psychological actions of tobacco 8.

Research has shown that nicotine is very well absorbed from tobacco; it is very well distributed rapidly and in biologically active concentration to body organ especially the brain. Nicotine has also in many research works been implicated as it register the major cause of the predominant behavioural effects of tobacco and some of its physiologic consequences. It induces a dose-dependent increase in neuronal activity in a distributed system of brain regions, including the nucleus accumbens, amygdala, cingulate, and frontal lobes 9. It is known to produce a “biphasic” effect. At low doses, it cause ganglionic stimulation and in high doses produces blockage following brief stimulation 10-11. The nicotine at low doses directly stimulates the CNS especially the brainstem resulting in sympathetic neural discharge, which increases blood pressure and heart rate among other behavioural stimulations 10-12. It, at high doses, directly stimulates the peripheral nervous system producing ganglionic stimulation and the release of adrenal catecholamine. With very high dose administration of nicotine, hypotension and decreased heart rate result, mediated by peripheral vagal activation or by direct CNS depressor effects 13,14. Nicotine induced a
dose-dependent increase in several behavioral parameters, including feelings of “rush” and “high” and drug liking.

One of the effects of nicotine is development of tolerance to its own actions; a likely mechanism by which it produces addiction like other addictive drugs. After repeated use of nicotine, the responsiveness to the drug becomes decreased and increasingly larger doses will be required to produce the same effect. 1

Although nicotine is a major chemical constituent of tobacco, which affects neurobehavioural activity, other alkaloids are also present. These smaller quantities of chemicals although absorbed in small quantities may also affect behaviour and the effect of nicotine. The other alkaloids include; nornicotine, anabasine, myosmine, nicotyrine and anatabine. These make up 8 to 12 percent of the total alkaloid content of tobacco products. Nornicotine and anabasine have pharmacological activity qualitatively similar to nicotine, with potencies of 20 to 75 percent compared with that of nicotine, and depending on the test system and animal model. Some of the alkaloids apart from having a direct effect may influence the effect of nicotine e.g. nicotyrine inhibits metabolism of nicotine in animals thereby prolonging the effect.

The resulting effects as reported lead us to investigate the behavioural pattern animal model will display in a novel environment after treatment with snuff (smokeless tobacco).

MATERIALS AND METHODS

The open field maze was used to study locomotor and exploratory behaviours as well as anxiety in albino Wistar rats fed different percentages of snuff diets. Thirty-two adult albino Wistar rats weighing between 180 - 230g were divided into four group each consisting of eight animals. Rats in group 1 served as control and were allowed free access to clean drinking water and normal rat chow. Rats in groups 2, 3 and 4 served as test groups and were fed 15%, 24% and 30% (w/w) respectively, snuff diets for a period of twenty-eight (28) days. The various percentages of snuff diets were formed by adding a known weight of snuff (finely process and ground tobacco leaves) to the powdered form of rat feed and thoroughly mixed at a ratio of 15:85w/w, 24:76w/w, 30:70w/w of snuff and rat chow respectively. The test animals also had free access to clean drinking water.

On the 29th day of experiments, each animal was tested individually in the open field maze. Each animal was allowed to explore the maze for 5 minutes and behaviours were scored. The behaviours scored included: line crossing, rearing, walling, centre square activity, urine puddles and number of faecal boli. Only complete naïve animals were used.

STATISTICAL ANALYSIS

Results were analysed using one-way analysis of variance (ANOVA) and post hoc students’ T-test. Results were expressed as mean ± standard error of mean (SEM). Values of probability level p< 0.05 were considered as significant.

RESULTS

EFFECT OF SNUFF ON LOCOMOTOR ACTIVITY IN ALBINO WISTAR RATS

Line crossing is a form of locomotor behaviour – horizontal locomotor activity. The number of line crosses by animals in the control group was 61.25 ± 7.5per 5minutes session. In the 15%w/w snuff diet fed animals, line crosses was 82.75 ± 9.2/5min, which did not differ significantly from control. The groups fed 24% w/w snuff diet however had a significantly (p<0.05) reduced line crosses (39.25 ± 3.62), when compared to the control, whereas the 30%w/w snuff diet fed group showed significantly (p<0.05) increased line crosses (83.5 ± 6.35/5min). See Figure 1.

Figure 1

Figure 1: Locomotor activity of control, and test albino Wistar rats fed 15%, 24% and 30% w/w snuff diets

EFFECT OF SNUFF ON REARING IN ALBINO WISTAR RATS

The frequency of rearing in the 30% w/w snuff diet group was 35.5 ± 3.8/5min and it was significantly higher (p<0.01) than the control, and in the 24% w/w snuff diet group...
(13.5 ± 1.53/5min), it was significantly lower (p< 0.05) compared to control which was 7.54 ± 3.04/5min. Rearing in the 15% w/w snuff diet group was 2.57 ± 3.24/5min.

Walling was significantly higher (p< 0.05 and 0.01 respectively) in the 15% and 30% w/w snuff diet fed groups, but significantly (p<0.05) lower in the 24% w/w snuff diet group compared to the control. Walling in the 15% snuff group was 23.75 ± 3.64/5min, 8.25 ± 1.63/5min in the 24% group and in the 30% snuff group it was 21.25 ± 2.05, while it was 9.5 ± 2.18/5min in the control.

**Figure 2**

Figure 2: Exploratory activity of control, and test albino Wistar rats fed 15%, 24% and 30% w/w snuff diets

- Animals fed 15% and 24% snuff diets explored the centre square less (p< 0.05) compared to the control. Frequency of entry was 3.0 ± 0.47 for 15% snuff diet group, 1.25 ± 0.55 for 24% snuff diet group, while that for control was 6.5 ± 0.47. For 30% snuff it was 6.25 ± 0.27/5min. The duration spent in the central square also followed the trend. Animals of the 15% snuff group spent 9.6 ± 2.58sec. Those of 24% snuff spent 3.48 ± 3.0sec., which was significantly lower (p< 0.01) compared to the duration of the control which was 21.1 ± 5.23sec. The animals of 30% snuff group spent 39.95 ± 4.08sec. See Figure 3.

**EFFECT OF SNUFF ON THE URINATION IN ALBINO WISTAR RATS**

There was no significant (p<0.05) difference between the snuff diet groups and the control. Except for the 24% w/w snuff diet group, others were higher than the control. Figure 4.
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**Figure 4**
Figure 4: Urination in control, and test albino Wistar rats fed 15%, 24% and 30% w/w snuff diets

**EFFECT OF SNUFF ON DEFECATION IN ALBINO WISTAR RATS**

The number of faecal boli in the 15% snuff fed group of animals was significantly lower (p< 0.05) compared to control. The number of facel boli was 4.5 ± 1.1 in control, 1.5±0.14 in 15% snuff group, 2.5 ± 0.7 in the 24% snuff group and 2.75 ± 0.12/5min in the 30% snuff group.

**Figure 5**
Figure 5: Number of faecal boli in control, and test albino Wistar rats fed 15%, 24% and 30% w/w snuff diet

**DISCUSSION**

Line crossing, rearing and walling are usually used as measures of locomotor activity as well as exploration and anxiety, with a higher frequency of these behaviours indicating increased locomotion and exploration, and low anxiety. In this study, the 15% and 30%w/w snuff diet groups had higher locomotor and exploration activities and low anxiety compared to the control, while the 24%w/w snuff diet group had lower frequencies of locomotion and exploration, with higher anxiety, as seen in line crosses, rearing and walling activities.

Su 13 reported stimulation of the central nervous system by low dose of nicotine, a substance contained in snuff, while Henningfield et al 14 reported that high dose nicotine stimulates the peripheral nervous system, which at a higher dose depresses the central nervous system. This is in variance with our results, as seen in higher locomotion and exploration in the 30%w/w snuff diet group.

Central square frequency and duration are measures of exploratory behaviour and anxiety with a higher frequency of these activities indicating higher exploratory behaviour and low anxiety 18 . In this study, the 15% and 24%w/w snuff diet groups had lower exploration and higher anxiety, while the 30%w/w snuff diet group showed a higher exploration and lower anxiety. This is in variance with a previous work 19 . It was reported that a low level of blood nicotine has stimulating effects, while a higher level of blood nicotine produces relaxation.

The number of urine puddles and fecal boli are usually represented as urination and defecation. They measure anxiety levels 17,18 . In this study, urination and defecation were lower in the test diet groups indicating low anxiety. Using these two behaviours as measures of anxiety has been severely criticized 20,21 .

Locomotion activation results from brain activation which manifest as excitation of the central neurons and an increase in cerebral metabolism. Brown et al 22 reported that natural occurring locomotor activation (like searching, grooming and rearing) and inhibition (like rest and sleep) may differ in its underlying mechanisms from similar behaviours induced by drugs.

Neurochemical mechanisms are involved in brain activation, with dopamine having a role to play 23,24 . Nicotine increases dopamine levels in the reward circuits of the brain 25 . Pharmacological increase in dopamine either by direct or indirect dopamine agonists result in hyper locomotion and stereotypy 26 . Increased dopamine may probably be a reason for brain hyperthermia and this has been known to correlate with increased locomotion and these effects being more complex depending on the drugs and experimental conditions 27,28,29 .

Our study revealed that snuff has different effects on rats depending on the dosage. This includes; increased
locomotion, exploration and anxiety, and these were dose dependent. These effects may be due to the total constituents of snuff.

References
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