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Research Article
Effect of Tobacco Snuff Consumption on Plasma Sodium and Potassium Levels in Rats

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Abstract

Background and Objective: Tobacco snuff is one form of smokeless tobacco usually blended with potash which is either sniffed or eaten and its long term use adversely affects the overall well being of users. Hence, this study was designed to investigate the effect of tobacco snuff consumption on plasma sodium and potassium levels. **Materials and Methods:** Forty two adult wistar rats weighing 150-300 g were involved in the experiment. The rats were divided into four experimental groups of A, B, C and D. Groups B, C and D represent the test groups while group A represents control group. The test groups were further divided into experimental phases/durations of 2 weeks (B1, C1 and D1), 4 weeks (B2, C2 and D2), 6 weeks (B3, C3 and D3) and 8 weeks (B4, C4 and D4) and they received varying doses of tobacco snuff. The control group (A) received feed pallets and water *ad libitum*. At the end of each experimental phase, blood samples were collected from randomly selected rats from each group. **Results:** Noticeable alterations in plasma sodium and potassium levels were observed across the test groups and the alterations increased with the dosage of tobacco snuff, though decrease with duration in some test groups. This no statistically significant alterations show imbalance in sodium and potassium level of the study. **Conclusions:** The current result of this study showed that tobacco snuff causes imbalance in sodium and potassium levels that are dose and duration dependent.

Key words: Tobacco snuff, consumption, plasma sodium and potassium level, imbalance, renal

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obviously, tobacco is known and used throughout all quarters of the globe in two major forms: The smoked and the smokeless[1]. Tobacco snuff is the powdered form of smokeless tobacco blended with potash as the main additive in Nigeria[2] and has been recommended to be safe not knowing that its long term use adversely affects the overall well being of users.

Widely used by many as recreational drug, tobacco snuff is either sniffed or eaten and several scientific studies have reported that the phytochemical constituents of tobacco snuff are carcinogenic and with other numerous health consequences[1-3]. The health consequences of tobacco snuff consumption could be directly or indirectly as its constituents have been implicated in several diseases such as cancer, oral lesion, leukoplakia, tissue and organ damage and nicotine addiction[4]. More so, tobacco snuff has been implicated with several systemic and organ damage in which[5] reported induced alterations in renal functions.

Emphatically, smokeless tobacco is a known potential generators of free radicals which are highly reactive radicals and Reactive Oxygen Species (ROS) act as initiators of carcinogenesis, cause DNA damage, activate pro-carcinogens and alter the cellular antioxidant defence system[6] and these facts contradicts the assertion that tobacco snuff is safe[7]. In a quantitative study showed that the level of carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is higher in smokeless tobacco users.

Systematically, sodium is the major cation of extracellular fluid and is responsible for almost one half of the osmotic strength of plasma, therefore it has a central function in maintaining the normal distribution of water and osmotic pressure in the extracellular fluid[8]. Potassium is the major intracellular cation, it is filtered through the glomeruli and almost completely reabsorbed in the proximal tubules[9] and is secreted in the distal tubules in exchange for sodium under the influence of aldosterone[10]. Due to electrolytes importance in maintaining the normal homeostatic mechanism and renal functions, this study was designed to investigate the effects of tobacco snuff consumption on plasma sodium and potassium as it has not been explored.

MATERIALS AND METHODS

Experimental animals: Forty two adult Wistar rats of comparable sizes and weighing (150-300 g) were purchased from the animal farm of Anthonio Research Center, Ekpoma, Edo state, Nigeria. They were transferred to the experimental site where they were allowed 2 weeks of acclimatization in a wooden wire mesh cages under standard laboratory procedure[1].

Ethics on the use of animals in experimental studies: The experimental protocols were according to our Institutional guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines[1,11].

Substance of study: Dry leaves of tobacco and potash were purchased from Ogbete main market, Enugu state, Nigeria. The tobacco leaves were authenticated by a botanist in the Department of Botany, Ambrose Alli University, Ekpoma, Edo State, Nigeria[1].

Substance preparation: The tobacco leaves and potash were blended into powder using a mortar and iron pestle and then stored prior to the study. The blended tobacco leaves with potash were weighed using an electronic balance (Denver Company, USA, 200398. IREV. CXP-3000) to obtain the various required doses. For the purpose of this study, feed pellets were prepared as described by[1,12].

Animal grouping: The experiment involved four stages: stage 1, which lasted for a period of 2 weeks, stage 2, which lasted for a period of 4 weeks, stage 3, which lasted for a period of 6 weeks and stage 4, which lasted for a period of 8 weeks. The rats were divided into four groups (A, B, C and D) with group A serving as control, while groups B, C and D served as the test groups. The test groups were further divided into four groups (B1, C1, D1, B2, C2, D2, B3, C3, D3 and B4, C4, D4) representing four experimental phases/duration (2, 4, 6 and 8 weeks) and varying doses of tobacco dust mixed with potash, respectively. At the end of 2, 4, 6 and 8 weeks respectively, 3 randomly selected rats from the groups were prepared for blood sample collection via cardiac puncture[1].

Study duration: The preliminary studies, animal acclimatization, substance procurement (tobacco leaves and potash), actual animal experiment and evaluation of results, lasted from September, 2012 to February, 2013. However, the actual administration of oral tobacco dust and potash to the test animals lasted for 8 weeks (2, 4, 6 and 8 weeks, respectively)[1].

Substance administration: In phase 1 (2 weeks), group A (control) received 100 g of feed and distilled water only,

whereas test group B, C and D received 97.12 g of feed, 2.4 g of tobacco dust and 0.4 8 g of potash, 94.24 g of feed, 4.80 g of tobacco dust and 0.96 g of potash and 91.36 g of feed, 7.20 g of tobacco dust and 1.44 g of potash, respectively. Each test group received distilled water *ad libitum*.

In phase 2 (4 weeks), group A (control) received 75 g of feed and distilled water only, whereas test roup B, C and D received 72.84 g of feed, 1.8g of tobacco dust and 0.36 g potash, 70.68 g of feed, 3.6 g of tobacco dust and 0.72 g of potash and 68.52 g of feed, 5.4 g of tobacco dust and 1.08 g of potash, respectively. Each test group received distilled water *ad libitum*.

In phase 3 (6 weeks), group A (control) received 50 g of feed and distilled water only, whereas test group B3, C3 and D3 received 48.56 g of feed, 1.2 g of tobacco dust and 0.24 g potash, 47.12 g of feed, 2.4 g of tobacco dust and 0.48 g of potash, and 45.68 g of feed, 3.6 g of tobacco dust and 0.72 g of potash, respectively. Each test group received distilled water *ad libitum*.

In phase 4 (8 weeks), group A (control) received 25 g of feed and distilled water only, whereas test group B4, C4 and D4 received 24.28 g of feed, 0.6 g of tobacco dust and 0.12 g potash, 23.56 g of feed, 1.2 g of tobacco dust and 0.24 g of potash and 22.84 g of feed, 1.8 g of tobacco dust and 0.36 g of potash, respectively. Each test group received distilled water *ad libitum*.

The concentrations of tobacco used in this study were deduced from the work of[13, 1] while that of potash was deduced from[14].

Sample collection and sample analysis: At the end of each stage of the experiment, blood samples were collected from the rats via cardiac puncture into a lithium heparin primed container. This was followed by centrifugation to obtain plasma samples which were then stored at -70°C prior to laboratory analysis. Plasma sodium and potassium were estimated using Flame Photometer (model- FP640 China) according to the standard procedures for determination of Sodium (Na+) and potassium (K+)[15] as carried out by Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria.

About 10 mL of deionized water in a sample tube was used to zero the flame photometer, 100 µL of commercial standard (Randox Bovine precision multi-sera level 2) in 10 mL of deionized water was used to standardize the flame photometer and the flame photometer was set at (140) for Na^+ and (4.3) for K^+. Equal volume of the test samples were analysed procedurally.

Data analysis: All the data collected were subjected to statistical analysis using SPSS (version 18). The test groups' values were compared with the control using ANOVA (LSD) at 95% level of confidence.

RESULTS

Table 1 below represents the effect of tobacco snuff consumption on sodium levels of the experimental animals and control. Sodium levels in the tests showed no statistical difference ($p>0.05$) from the values of the control (133.00 ± 20.50mmol L^{-1}) throughout the experimental period of 2, 4, 6 and 8 weeks. However, irregular increases and decreases that were not statistical significant was observed throughout the treatment period.

For potassium, the results in Table 2 showed that at the end of the experimental period, there was no statistical difference ($p>0.05$) in the test groups when compared to

Table 1: Effect of tobacco snuff on plasma sodium levels in rats

		Control			
Parameter	Duration (weeks)	A	B	C	D
Sodium (mmol L^{-1})	2	133.00 ± 20.50^a	142.33 ± 2.08^a	132.33 ± 21.73^a	132.67 ± 14.01^a
	4	133.00 ± 20.50^a	128.67 ± 26.72^a	152.33 ± 6.43^a	142.33 ± 16.04^a
	6	133.00 ± 20.50^a	150.00 ± 4.36^a	143.33 ± 8.08^a	135.33 ± 3.06^a
	8	133.00 ± 20.50^a	131.00 ± 18.03^a	141.50 ± 0.71^a	139.00 ± 1.41^a

N/B: All the values of the test groups with different subscript from the controls are significantly different at $p<0.05$

Table 2: Effect of tobacco snuff on plasma potassium levels in rats

		Control			
Parameter	Duration (weeks)	A	B	C	D
Potassium (mmol L^{-1})	2	9.82 ± 2.72^a	9.73 ± 3.84^a	10.47 ± 4.60^a	11.90 ± 3.77^a
	4	9.82 ± 2.72^a	12.23 ± 3.84^a	11.63 ± 0.68^a	12.53 ± 0.78^a
	6	9.82 ± 2.72^a	12.83 ± 2.31^a	11.40 ± 1.82^a	9.80 ± 0.44^a
	8	9.82 ± 2.72^a	10.90 ± 1.95^a	11.05 ± 0.78^a	9.25 ± 0.35^a

N/B: All the values of the test groups with different subscript from the controls are significantly different at $p<0.05$

control values (9.82±2.72 mmol L^{-1}). However, irregular increases and decreases that were not statistical significant was observed throughout the treatment period.

DISCUSSION

The non-significant increase in the level of sodium and potassium in the test groups at both high and low doses implies that tobacco snuff affect their normal physiology. Also the alterations in the level of sodium and potassium observed in accordance with the duration, indicates that the changes in the normal physiology is duration and dosage dependent. These alterations observed could be as a result of the adverse effect of tobacco snuff on the renal functions as reported by[5], moreover the non-significant decrease in the test groups compared with control could be as a result of homeostatic balancing from the adverse effect. It is without doubt that the results of the present study showed that tobacco snuff induces adverse health consequences which definitely affect the normal homeostatic mechanism and renal functions. The alterations observed in sodium and potassium levels of this study showed how unsafe tobacco snuff is to the intracellular and extracellular system. At 2 weeks, sodium showed acute increase in group B and mild decrease in groups C and D and at 4 weeks, it showed moderate decrease in group B with increase in groups C and D. At 6 and 8 weeks, respectively, sodium showed increase in groups B, C and D compared with the control. Furthermore, potassium levels showed increase at 2 and 4 weeks across the test groups. However, it maintained the increased levels at 6 and 8 weeks in groups B and C with decrease in group D. In line with these findings[6], reported that smokeless tobacco induces impairment in the hepatic and renal antioxidant defence system, causing reduction in renal functions. However, any deviation in the normal levels of electrolytes in blood, due to several factors, indicates renal impairment[16] and the result of this study, though showed differences that were not statistically significant among the investigated parameters, agrees with the report of [17]. It was important to remember that according to Oyeleke as reported by[1] that with chronic natron ingestion, there was severe growth retardation, skin changes and diarrhoea. This clarifies that tobacco snuff and its additive (natron) has the potentials to induce systemic changes that could affect electrolyte imbalance creating danger to health. To explain the clinical importance of electrolyte, increase in extracellular (plasma) potassium level indicates cellular damage and according to Soladoye and Oyeleke as reported by[1] showed that moderate intake of natron had adverse effects on blood indices in rats. This effect could be the reason behind the differences that were not statistically significant when sodium and potassium levels of the test groups were compared with the control. Despite this[18-19], revealed that high salt (sodium chloride) diets might have adverse effects on the kidneys and by implication distorts its physiological activities.

Interestingly, assumed by many as harmless, scientific literatures have it that smokeless tobacco contains glycyrrhizinic acid which has potent mineralocorticoid hormone activity that causes hypokalemia. It is also a known fact that longstanding intracellular potassium depletion causes extracellular alkalosis and prolonged potassium depletion impairs the renal concentrating mechanism and may cause polyuria with potassium depletion and of grate concern is the finding of [4], that tobacco snuff induced kidney organ shrinkage and this could be due to its degenerative effect as reported by[6,4]. The increased potassium level, though not statistically significant throughout the study, showed the possible tobacco snuff induced intracellular potassium alterations and tendency of renal impairment. Though[5], revealed tobacco snuff induced renal damage.

CONCLUSION

It was concluded from the present results that tobaccos snuff has the capacity to induce both extracellular and intracellular distortions. However, the increase and decrease levels of sodium and potassium in this study indicate changes in renal function and possible toxicity and also may lead to misinterpretation of electrolyte results.

SIGNIFICANCE STATEMENT

This study discovered that tobacco snuff which is assumed to be safe has adverse health consequences. It is the common slogan of the Nigerian ministry of health that tobacco smokers are liable to die young, not knowing that tobacco snuff with its additive natron is a hidden tsunami waiting to explode. This study will help the health practitioners to uncover the mysteries behind some illnesses and misinterpretation of electrolyte results since this area has not been explored.

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