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Nasal Snuff (Nigeria Local Smokeless Tobacco) Administration Raises Hepatic Enzyme Plasma Levels in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author JSA conceived and designed the study, contributed to the interpretation of the results and critically revised the manuscript. Author TCU managed the literature searches, conducted the experiments, collected and analyzed the data, and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Smokeless tobacco (ST) is commonly used in many countries and is considered as alternative to tobacco smoke (TS) in view of the increasing awareness of the hazardous effects of TS. This study investigated the effects of 30 days oral administration of Nasal Snuff, a local ST in Nigeria (≈ 0.5 or 1 mg/kg nicotine) on liver function in prepubertal and adult rats, in comparison with nicotine.
Place and Duration Study: This study was conducted at the Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria; between February and April, 2014.
Methodology: Rats were divided into 10 groups (5 groups for prepubertal and 5 groups for adult), and each group contained six animals. Prepubertal and adult rats were given Nasal Snuff, a local

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ST product (≈ 0.5 or 1 mg/kg nicotine, p.o.), nicotine (0.5 or 1 mg/kg, s.c.) or distilled water (control). At the end of 30 days of daily treatment, the rats were sacrificed and plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and acid phosphatase (ACP) were measured. Histological analysis of the livers of animals was also done.

Results: ST had no effect on ALP, but caused elevations in plasma levels of AST, ALT and ACP in prepubertal rats, producing 126.8, 602.9 and 193.2% increases, respectively. ST also increased AST (122.7%) and ALT (169%) in the adult rats. Nicotine did not cause changes in ALP and ACP levels, but increased AST and ALT in prepubertal (159.4 and 275.6%, respectively) and adult rats (137.1 and 496.7%, respectively). ST and nicotine caused comparable cytoplasmic degeneration of liver microstructure in prepubertal and adult rats.

Conclusion: ST, particularly at high dose levels, poses potential danger of hepatotoxicity in young and adult animals.

Keywords: Hepatotoxicity; nicotine; phosphatase enzyme; smokeless tobacco; snuff; transaminase.

1. INTRODUCTION

Tobacco is known to cause several negative health consequences and most of its effects are attributed to the principal constituent, nicotine [1,2]. Generally, human exposure to tobacco occurs either through inhalation of tobacco smoke (e.g. cigarette, cigars, pipes etc.) or ingestion of smokeless tobacco (e.g. snus, moist snuff etc.). In Nigeria, the most popular tobacco smoke and smokeless tobacco products consumed are cigarette and Nasal Snuff, respectively. Cigarette smoking does not only cause cancer, but has also been associated with increased incidence of respiratory tract diseases [3,4], coronary heart disease [5,6], and reproductive toxicity [7-10]. The many documented health consequences of the use of tobacco products have been identified to increase mortality and morbidity in both developed and developing countries.

Apart from nicotine, tobacco products contain other components that are potentially toxic, carcinogenic, mutagenic, growth retardative and immunosuppressive. Such compounds include, polycyclic aromatic hydrocarbons like benzo[a]pyrene, cyanide, carbon-monoxide, lead, cadmium, nitric oxide, nitric dioxide, tobacco-specific N-nitrosamine (TSNA), N-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3- pyridyl)-1-butanone (NNK), ammonia, and flavours [11-14]. The level of exposure or absorption of these substances from tobacco products depends among other factors on the type of tobacco used, this being thought to be higher in smokeless tobacco users than cigarette smokers [8,11]. Unfortunately, most studies on tobacco use are focused on cigarette smoking and there is limited data on the toxicological profile of smokeless tobacco. This becomes a serious concern because of the current perception in some areas that smokeless tobacco products are less harmful than those that are smoked. Control measures by regulatory bodies and increased awareness of the risks of tobacco smoke may have helped in the reduction of cigarette smoking, but smokeless tobacco consumption appears to be on the rise. It has been reported that smokeless tobacco is consumed in many rural and urban areas, including Nigeria in unspecified quantities for various purposes, including medicinal purposes [8].

The aim of this study is to investigate the effect of subacute exposure of a local smokeless tobacco product in Nigeria (Nasal Snuff), which is commonly used as insufflation, on hepatic status in prepubertal and adult rats.

2. MATERIALS AND METHODS

2.1 Animals

Thirty male prepubertal (aged 5-6 weeks) and adult Wistar albino rats (aged 12 weeks) were used for the study. The animals were obtained from the Animal House of the University of Port-Harcourt, Nigeria. They were maintained in a well-ventilated room with a 12 h light/dark cycle at room temperature. They were fed with standard rat chow and allowed access to tap water *ad libitum*. All animal experiments were carried out in accordance with the ethical regulations of the National and Institutional Guidelines for the Protection of Animals Welfare [15].

2.2 Experimental Design

Prepubertal and adult rats were divided into 10 groups (5 groups each), n=6 animals per group. The prepubertal and adult animal groups labeled as Groups I, II, III, IV and V were given Nasal Snuff, a local smokeless tobacco product (≈ 0.5 or 1 mg/kg nicotine) once daily, nicotine (0.5 or 1 mg/kg) once daily, or distilled water (control). Nasal Snuff powder was dissolved in distilled water and given by oral gavage, while nicotine was injected subcutaneously. At the end of 30 days of treatment, the rats were anesthetized with diethyl ether and sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture into lithium heparinized bottles and centrifuged at 3000 rpm for 10 minutes and plasma was separated and stored at -80°C. Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and acid phosphatase (ACP) were measured afterwards, using a Mindray autoanalyzer (Model: BS 800M). Animal livers were removed and fixed in buffered formaldehyde. Tissues were later processed routinely, sectioned (5-7 µm thickness) and stained with haematoxylin and eosin (H & E). The slides were then viewed under a light microscope and all alterations of structures from the control groups were noted and photographed.

2.3 Statistical Analysis

The results are presented as mean ± SEM for each group. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnette's multiple comparisons test. Data were analyzed using GraphPad Prism Version 5 and p values < 0.05 were taken to be significant.

3. RESULTS AND DISCUSSION

Cigarette smoking constitutes a great challenge to public health globally due to its associated harmful effects [1,2,6,16]. With the increasing awareness of the harmful effects of cigarette smoking, the use of smokeless tobacco (ST) has become more popular as an alternative. The present study evaluated the effect of subacute administration of a widely consumed local smokeless tobacco product in Nigeria (Nasal Snuff) on plasma levels of liver enzymes to assess liver function in prepubertal and adult rats.

In smokeless tobacco (ST) administered prepubertal rats, AST, ALT and ACP levels increased dose-dependently, but only the values obtained in the 1 mg/kg administered group were significant, $p < 0.05$ when compared to control rats (Table 1). The values corresponded to 126.8, 602.9 and 193.6% increases, respectively. ALP was also elevated by ST, but the values obtained were not significantly different from control (Table 1). In addition, plasma levels of AST and ALT in nicotine treated prepubertal rats were significantly and dose-dependently increased (Table 1), while ALP and ACP were not affected (Table 1). The nicotine induced AST and ALT levels corresponded to 159.4 and 275.6% elevations, respectively. Intragroup comparison showed that the nicotine induced AST and ALT levels were significantly different from the ST induced values at 0.5 mg/kg (Table 1).

In the adult rats, AST and ALT levels were increased dose-dependently by ST treatment, but only the values in the group that received 1 mg/kg ST were significantly different from control, which corresponded to 122.7 and 179.1%, increases respectively (Table 2). ALP and ACP levels were not altered in all ST treated groups (Table 2). Furthermore, plasma levels of AST and ALT in 1 mg/kg nicotine treated adult rats were elevated (137.1 and 496.7% increases, respectively), while their values obtained in 0.5 mg/kg nicotine treated rats were not significantly different, compared to control group (Table 2). Similar to the result obtained in ST treated rats, plasma levels of ALP and ACP in nicotine administered adult rats were not altered, compared to control rats (Table 2). When compared, only the ALT levels that were obtained in the 0.5 mg/kg treated rats were significantly different (Table 2).

Livers of prepubertal rats that received low dose of Nasal Snuff (0.5 mg/kg) did not show any obvious microcellular change (Fig. 1C), but the rats that received 1 mg/kg showed moderate cytoplasmic degeneration (Fig. 1B) compared to control (Fig. 1A). Livers of all nicotine exposed rats showed varying levels of cytoplasmic degeneration (Figs. 1D and 1E), compared to the normal histology of control rats (Fig. 1A).

Furthermore, no obvious change was observed in the liver histology of adult rats that received 0.5 mg/kg Nasal Snuff (Fig. 2B), whereas the

rats that received 1 mg/kg showed moderate cytoplasmic degeneration (Fig. 2C) compared to control (Fig. 2A). Livers of nicotine exposed rats showed dose-dependent cytoplasmic degeneration (Figs. 2D and 2E), when compared with the control rats (Fig. 2A).

Table 1. Effects of smokeless tobacco (ST) on plasma levels of phosphatase and transaminase enzymes in prepubertal male Wistar albino rats

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	ACP (IU/L)
Control (Distilled water)	216.30±46.47	53.25±5.68	123.50±15.70	6.53±0.90
ST (0.5 mg/kg)	272.30±41.95	59.67±7.27	153.70±8.99	8.60±0.49
ST (1 mg/kg)	490.50±87.50[a]	374.30±98.98[b]	177.50±22.94	19.17±1.08[b]
Nicotine (0.5 mg/kg)	454.30±89.67[bc]	167.80±22.49[bc]	170.00±14.34	10.50±0.00
Nicotine (1 mg/kg)	561.00±45.48[b]	200.00±18.00[b]	178.80±13.45	11.65±1.15

Data are expressed as mean ± SEM, n = 6 animals per group. Data analyzed by one way ANOVA.
[a] Significantly different compared to control at $p < 0.05$; [b] Significantly different compared to control at $p < 0.001$;
[c] Significantly different compared to ST (0.5 mg/kg) at $p < 0.05$.
AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, ACP: Acid phosphatase

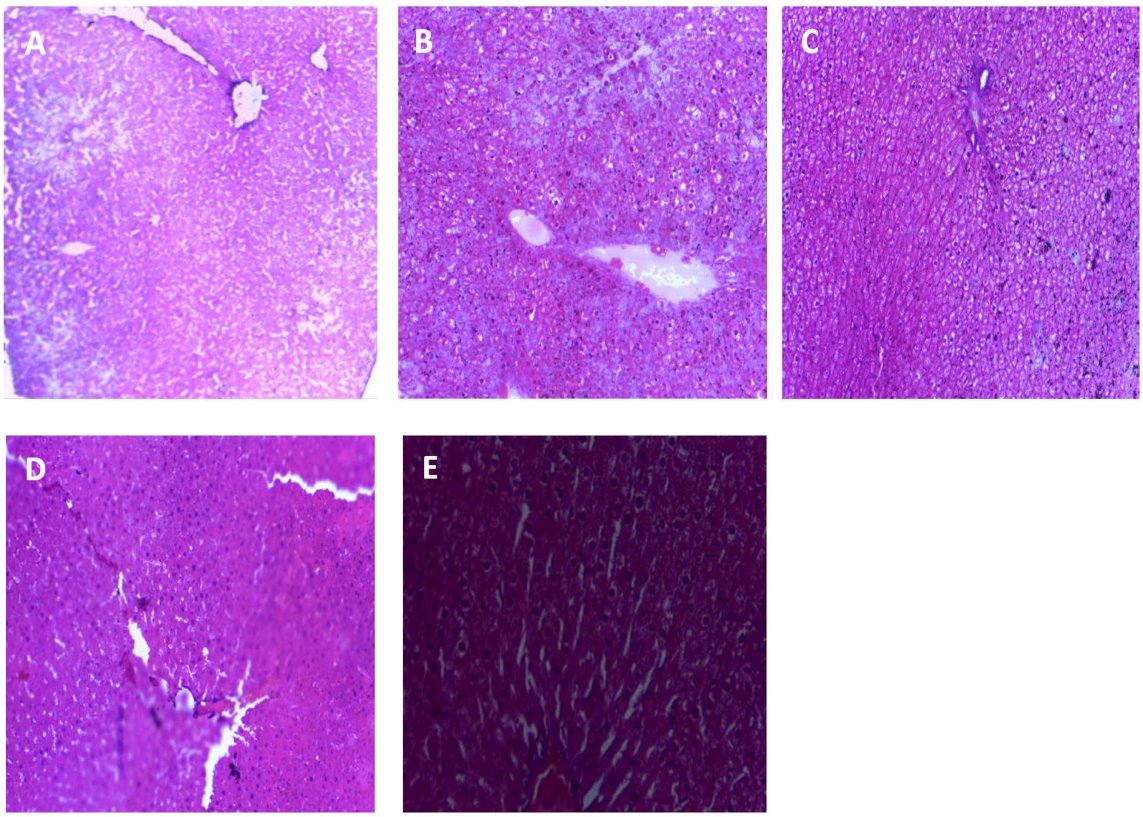

Fig. 1. Photomicrographs showing liver histology of prepubertal rats following 30 days administration of Nasal Snuff (smokeless tobacco, ST) and nicotine, stained with H&E (200x)
A- Control: Liver shows normal histology, with normal architecture and structure
B- ST (0.5 mg/kg): Liver shows no obvious histologic change
C- ST (1 mg/kg): Liver shows moderate cytoplasmic degeneration
D- Nicotine (0.5 mg/kg): Liver shows mild cytoplasmic degenerations
E- Nicotine (1 mg/kg): Liver shows varying levels of cytoplasmic degenerations

Table 2. Effects of smokeless tobacco (ST) on plasma levels of phosphatase and transaminase enzymes in adult male Wistar albino rats

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	ACP (IU/L)
Control (distilled water)	220.50±49.61	75.25±5.94	150.50±46.64	7.65±0.17
ST (0.5 mg/kg)	253.30±61.72	75.67±1.20	119.00±24.21	7.83±0.42
ST (1 mg/kg)	491.00±65.40[a]	210.00±57.65	192.50±27.21	7.95±0.21
Nicotine (0.5 mg/kg)	368.00±47.06	207.30±54.02[c]	217.00±10.02	8.35±0.92
Nicotine (1 mg/kg)	522.70±134.20[a]	449.00±156.60[b]	268.00±20.98	9.85±1.53

Data are expressed as mean ± SEM, n = 6 animals per group. Data analyzed by one way ANOVA.
[a] Significantly different compared to control at $p < 0.05$; [b] Significantly different compared to control at $p < 0.001$;
[c] Significantly different compared to ST (0.5 mg/kg) at $p < 0.05$.
AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, ACP: Acid phosphatase

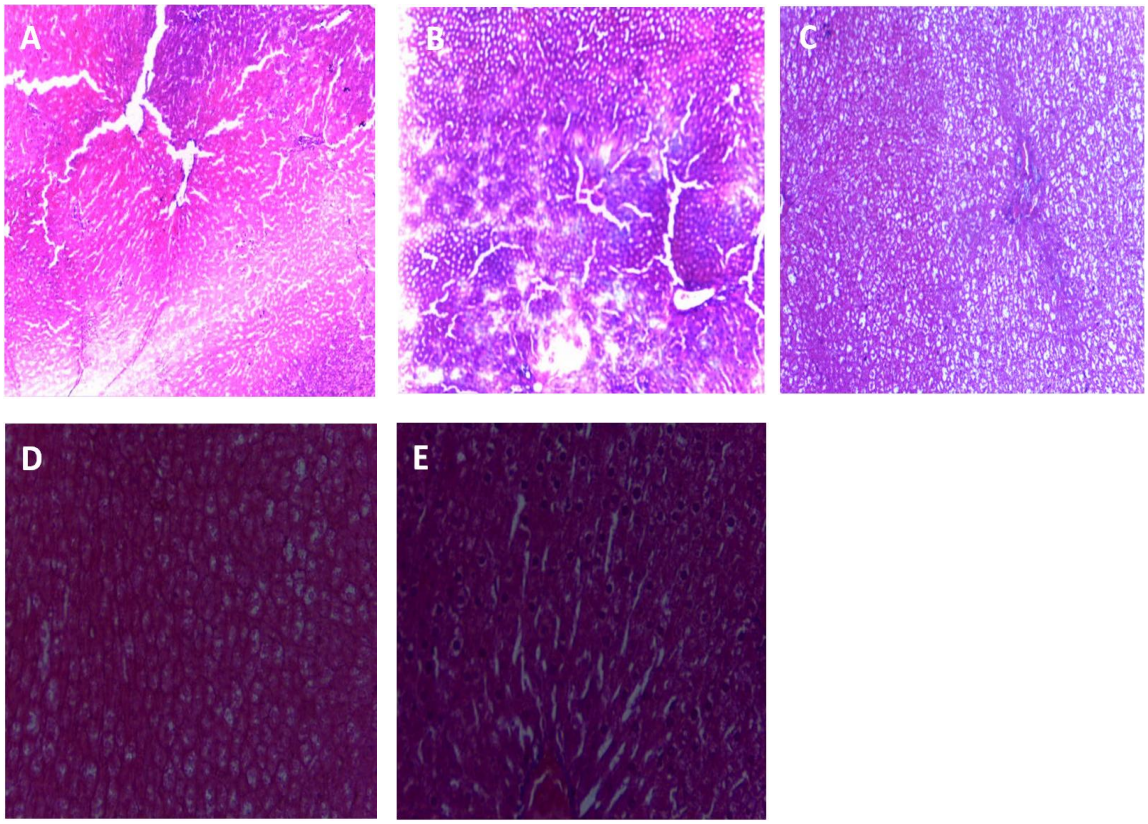

Fig. 2. Photomicrographs showing liver histology of adult rats following 30 days administration of Nasal Snuff (smokeless tobacco, ST) and nicotine, stained with H&E (200x)
A- Control: Liver shows normal architecture and cell structures
B- ST (0.5 mg/kg): Liver shows no obvious histologic change
C- ST (1 mg/kg): Liver shows moderate cytoplasmic degeneration
D- Nicotine (0.5 mg/kg): Liver shows mild cytoplasmic degeneration
E- Nicotine (1 mg/kg): Liver shows moderate cytoplasmic degeneration

Nicotine is a potent and powerful agonist of nicotinic receptors and has a high addictive potential. It is a major component of ST and cigarette smoke and is considered to be responsible for the addiction and most of the toxicological effects of tobacco products. We

herein report that subacute administration of smokeless tobacco (ST) leads to marked elevation in the plasma levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which is indicative of hepatocellular damage as reported previously [17,18]. This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation due to hepatic cellular damage. Expectedly, administration of equivalent dose levels of nicotine also caused elevation in the levels of the enzymes in this study. Elevation of ALT, which is a specific marker of hepatotoxicity [19] by both ST and nicotine strongly indicates that ST is a potential hepatotoxic compound. This was corroborated in the histological findings of cytoplasmic degeneration of liver cells by both ST and nicotine exposures. But as ST and nicotine did not alter ALP level, their hepatotoxicity effects may not involve cholestatic injury [18,20], but may more likely cause hepatocellular damage.

When the ST induced effects were compared with those caused by equivalent dose levels of nicotine, it revealed that nicotine may produce greater level of hepatic alteration than ST at the lower dose in both prepubertal and adult animals, while the effects were comparable at the higher dose. This observation was not different in the histopathological results, as the low dose treatment of ST did not cause alteration in the histology of liver in prepubertal and adult rats, but nicotine induced histological changes at all dose levels. It is important to note that ST contains many other potentially toxic components in addition to nicotine [13]. The results thus suggest that these other components may have no significant contribution to the observed hepatic toxicity potential of Nasal Snuff. Further, in both ST treated prepubertal and adult rats, the AST and ALT induced elevations were statistically significant only in the groups that received the higher dose (1 mg nicotine), which indicates that ST is more likely to affect hepatic function at high concentrations. Additionally, the effects may be more pronounced in the prepubertal animal as its effects on AST and ALT were higher in prepubertal rats (126.8, 602.9%, increases respectively), compared to the adult rats (122.7 and 179.1%, increases respectively).

4. CONCLUSION

Because tobacco has a high addictive potential, tobacco users are often compelled to use them over long periods of time, in some cases life-long. The present study shows that the use of Nasal Snuff (a local tobacco smoke in Nigeria) may pose potential danger of hepatotoxicity to users (young and adult) particularly at high dose levels. Thus, it may not be the ‘‘safe’’ alternative to cigarette smoking.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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