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RESEARCH PAPER

TOBACCO INDUCED RENAL FUNCTION ALTERATIONS IN WISTAR RATS: AN 8 WEEKS STUDY

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ABSTRACT

This study investigates the effect of tobacco snuff on renal function using Wistar rat as a model. It involved adult rats (n=42) weighing 150-300g. They were assigned into a control group (A; n=6) and test groups B (n=12), C (n=12) and D (n=12). The groups were further divided into subgroups (1, 2, 3 and 4) representing durations of 2, 4, 6 and 8 weeks respectively. The test groups were fed varying doses of tobacco snuff (tobacco plus potash). At the end of every 2 weeks, three randomly selected rats were prepared for blood sample collection into lithium heparin containers for laboratory analysis of creatinine, urea and uric acid. Results showed that creatinine levels of the test-rats were higher than the control, but the recorded values were however, duration dependent. Interestingly, a similar but irregular pattern was observed for urea and uric acid levels. Over all, the significant increase ($P<0.05$) in renal function parameters of the test rats (as compared to the control values), suggests that the ingestion of tobacco snuff has harmful effects on kidney functions.

Keywords: Tobacco, Snuff, Kidney function, Nicotine substitute.

INTRODUCTION

In the world today, the use of smokeless tobacco is quite popular in the Far East, Middle East, and Europe (Bates *et al.*, 2003), with a rising trend in the United States of America (USA) (Changrani and Gany, 2005). In Nigeria however, the powdered form called 'tobacco snuff', has potash added to it to serve as additive. It is either inhaled (sniffed) through the nose or applied orally (Ureme *et al.,* 2007). According to Aduema *et al*. (2012), it comes in two different forms- 'Tobacco snuff' and 'Chewing tobacco'.

Despite the awareness, that absorption of tobacco snuff is sometimes considered inefficient to provide an adequate nicotine substitute (Armitage *et al.,* 1978; Turner *et al.,* 1985), some have advocated its use as nicotine substitute for cigarette, since it is supposedly devoid of hazardous elements like tar and carbon monoxide (Russel *et al.,* 1980). Unfortunately, chronic absorption of nicotine from smokeless tobacco results in nicotine addiction (Hatsukami et al., 2004; Hatsukami and Severson, 1999 and PHS, 1998).

Of greater concern however, is the fact that several scientific studies have reported that the phytochemical constituents of tobacco snuff is carcinogenic (IARC, 1985; Hecht *et al.,* 1986; PHS, 1986; Brunnemann and Hoffmann, 1992; NCI, 1992; Hoffmann and Djordjevic, 1997 and IARC, 2007). According to Jorenby *et al.* (1998), the precise health effects of smokeless tobacco are uncertain but are not necessarily limited to oral cancers. Generally, smokeless tobacco has been associated with periodontal disease (Ernster *et al.,* 1990; Fisher *et al.,* 2005), precancerous oral lesions (Mattson and Winn, 1989), oral cancer (Stockwell 1986), and cancer of the kidney

(Goodman *et al.,* 1986; Muscat *et al.,* 1995), as well as pancreas (Muscat *et al.,* 1997), and digestive system pathogenesis (Henley *et al.,* 2005).

In addition, the involvement of its highly reactive radicals in carcinogenesis, DNA damage, alteration of cellular antioxidant defense system and reduction in kidney glutathione (GSH), Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as the induction of lipid peroxidation in kidney (Pramod *et al.,* 2005), raises a lot of public health concern.

With focus on the rising trend in tobacco snuff addition therefore, this study investigates the effect of oral tobacco snuff consumption on renal function in adult Wistar rats.

MATERIALS AND METHODS

Experimental Animals: Forty two adult Wistar rats of comparable sizes and weighing (150-300g) 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 two weeks of acclimatization in a wooden wire mesh cages under standard laboratory procedure.

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.

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 Nwaopara *et al*., (2011).

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.

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 (2weeks, 4weeks, 6weeks and 8 weeks respectively).

Substance administration: In phase 1 (2 weeks), group A (control) received 100g of feed and distilled water only whereas test group B, C and D received 97.12g of feed, 2.4g of tobacco dust and 0.48g of potash**;** 94.24g of feed, 4.80g of tobacco dust and 0.96g of potash**;** and 91.36g of feed, 7.20g of tobacco dust and 1.44g of potash respectively. Each test group received distilled water *ad libitum*.

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

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

In phase 4 (8 weeks), group A (control) received 25g of feed and distilled water only, whereas test group B4, C4 and D4 received 24.28g of feed, 0.6g of tobacco dust and 0.12g potash**;** 23.56g of feed, 1.2g of tobacco dust and 0.24g

of potash**;** and 22.84g of feed, 1.8g of tobacco dust and 0.36g 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 Bagchi *et al*. (1994) while that of potash was deduced from Ugbor *et al*. (2013).

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^{o} C prior to laboratory analysis.

Plasma creatinine, urea, and uric acid, were estimated by Jaffe's method (Fabiny and Ertingshauen, 1971), Urease-Berthelots colorimetric method (Sims, 2006) and enzymatic (uricase) colorimetric method (Fossati et al., 1980) respectively.

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 student's t-test and ANOVA (LSD) at 95% level of confidence.

RESULTS

Table 1 below represents the effect of tobacco snuff consumption on creatinine levels of the experimental animals and control. Creatinine levels in the tests showed no statistical difference (P>0.05) from the values of the control (0.76±0.12mg/dl) in the first 2 weeks, 6 weeks and 8 weeks treatment periods. However, for the 4-week period, there was a statistically significant increase (P< 0.05) in creatinine values in group C (1.40±0.32mg/dl) and D (1.31±0.34mg/dl) as compared with the control.

For urea, the results in table 2 showed that at the end of 2 weeks, there was no statistical difference (P>0.05) in the test groups when compared to control values (47.28±7.15mg/dl). In the 4-week ingestion period, it was observed that except for group C (115.08±96.60mg/dl), which showed a statistical increase in urea level, group B and D urea levels did not differ statistically from the control. In comparison with the control also, a statistically significant difference (P<0.05) was observed in group B (63.13±8.91mg/dl) and D (65.63±4.71mg/dl) at the end of 6 weeks, but only in group D (65.15±4.74mg/dl) at the end of 8 weeks.

Table 1: The effect of tobacco snuff on plasma Creatinine levels in rats

Parameters		Control Group A	Test groups		
			B	C	D
Creatinine (mg/dl)	2weeks	0.76±0.12^{a}	0.87±0.24^{a}	1.00±0.03^{a}	0.75±0.18^{a}
	4weeks	0.76±0.12^{a}	0.88 ± 0.16^{a}	1.40±0.32^{b}	1.31±0.34^{b}
	6weeks	0.76±0.12^{a}	0.72±0.32^{a}	0.85±0.07^{a}	0.83±0.07^{a}
	8weeks	0.76±0.12^{a}	0.79±0.16^{a}	0.75±0.18^{a}	0.97±0.05^{a}

Table 2: The effect of tobacco snuff on plasma Urea levels in rats

Parameters		Control Group A	Test groups		
			B	C	D
Urea (mg/dl)	2weeks	47.28±7.15^{a}	54.37±15.28^{a}	65.22±18.49^{b}	49.68±8.20^{a}
	4weeks	47.28±7.15^{a}	64.94±11.56^{a}	115.08±96.60^{b}	77.82±7.82^{a}
	6weeks	47.28±7.15^{a}	63.13±8.91^{b}	47.63±8.91^{a}	65.63±4.71^{b}
	8weeks	47.28±7.15^{a}	58.50±15.88^{a}	57.65±18.74^{a}	65.15±4.74^{b}

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

In table 3, uric acid showed no statistical difference in test group values when compared with the control values (7.13±0.47mg/dl) at the end of 2 weeks. But at the end of 4 weeks, a statistically significant increase was observed

in Group B (11.34±0.16mg/dl) and D (11.26±2.25mg/dl), while at the end of 6 weeks, only group B (12.11±1.22mg/dl) presented a statistically significant increase when compared with the control. In addition, group B (10.24±3.19mg/dl), C (13.40±1.97mg/dl) and D (10.71±0.45mg/dl) presented a significant increase in uric acid levels when compared to the control value, at the end of 8 weeks ingestion period.

In view of the observed changes in the parameters for assessing renal function herein investigated, our results did show that tobacco snuff consumption had an impact on renal function, and the observed changes were duration and dosage dependent.

Table 3: The effect of tobacco snuff on plasma Uric acid levels in rats

Parameters		Control Group A	Test groups		
			B	C	D
Uric acid (mg/dl)	2weeks	7.13±0.47[a]	8.96±6.76[a]	10.69±2.90[a]	7.89±1.55[a]
	4weeks	7.13±0.47[a]	11.34±0.16[b]	7.29±1.82[a]	11.26±2.25[b]
	6weeks	7.13±0.47[a]	12.11±1.22[b]	8.08±1.69[a]	8.07±1.49[a]
	8weeks	7.13±0.47[a]	10.24±3.19[b]	13.40±1.97[b]	10.71±0.45[b]

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

DISCUSSION

The observed alterations in renal function parameters are in line with the reports by Pramod *et al.,* (2006) and Gonzalez (1999), who stated that aqueous extract of smokeless tobacco, impairs enzymatic antioxidant defense system and induces oxidative stress/lipid peroxidation in liver, lung, and kidney. Already, this oxidative stress–induced lipid peroxidation, according to Gonzalez (1999) and Pramod *et al.* (2006), has been implicated in malignant transformation. However, our findings disagrees with the reports by Raj Shrestha *et al*. (2012), that Pan masala (a brand of smokeless tobacco) induces significant elevated levels of ALT and AST amongst consumers, but not serum creatinine, urea and uric acid.

Although high ratios of creatinine and urea is said to be a factor of either post-renal obstruction or pre-renal uremia superimposed on renal disease (Fossati *et al.*, 1994; Thoene, 1999; Newman and Price, 1998), it is known however, that elevated creatinine level is associated with abnormal renal function, especially glomerular function (Bishop *et al*., 2005; Usunobun *et al*., 2012). Interestingly, creatinine -being a definitive marker for kidney function, was observed to increase statistically in the 4th week of this study, but remained high thereafter; though not significant. Thus, mild and lethal doses of tobacco snuff may be renotoxic based on the results of this study.

As regards urea being an indicator for kidney disorder, the elevated values in the test groups, suggests that tobacco snuff may contain some toxic components that are nephrotoxic which, according to Varely *et al*. (1987), can be linked with the fact that the presence of toxic compounds increases blood urea and decreases plasma protein. The observed increase in uric acid amongst the test groups further points to the renotoxic potential of tobacco snuff. Moreover, there is evidence that an increase in renal retention of uric acid can occur in cases of acute or chronic renal disease/ failure (Newman and Price, 1998).

Nevertheless, the mechanism by which tobacco snuff induces renal damage may be through enhancing the synthesis of free radicals which, according to Danko and Chaschin (2005) and Usunobun *et al*. (2012), leads to lipid and protein peroxidation, DNA damage and carcinogenesis. These may affect glomerular function leading to elevated serum markers of renal function. Molander *et al*., (2000) and Ross, (2006) had earlier stated that a progressive kidney failure can be associated with a gradual decrease of renal and non-renal elimination of nicotine, and this potentiates nephrotoxicity. Also, the effects of heavy metals in tobacco and heavy metals like Cadmium (Cd), Mercury (Hg) and Lead (Pb), might be another possible mechanism for tobacco-induced renal damage (Addo *et al.*, 2008; Roszczenko *et al*., 2004; Satarug and Moore, 2004; Ross, 2006).

On the other hand, the observations of this study, may not be unrelated to the carcinogenic potentials of tobacco snuff as it has been previously reported that smokeless tobacco contains 4-(methylnitrosamino)-1-(3-pyridyl)-1-

butane (NNK) and *N*′-nitrosonornicotine (NNN) (Boffetta *et al*., 2008), which are known carcinogenic agents, and without doubt, have capacity to induce the elevation of markers and subsequently, kidney damage.

Judging by the findings of this study therefore, it is obvious that an excessive consumption of tobacco snuff may be toxic, which, in the case of the kidney, can alter renal function. As such, there is a need to draw the attention of consumers to the hazardous effects and subsequent health implications of excessive tobacco snuff consumption. Also, beyond the slogan by the Nigerian Federal Ministry of Health that "*tobacco smoking is dangerous to health, and that smokers are liable to die young*", there is still an urgent need for more proactive measures as "*a stitch in time, saves nine*.

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AUTHORS' CONTRIBUTIONS

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