ACUTE EFFECT OF TOBACCO SNUFF CONSUMPTION ON PLASMA TOTAL PROTEIN, ALBUMIN, GLOBULIN AND FASTING BLOOD SUGAR LEVEL IN RATS

ABSRACT

This study was designed to investigate the effect of tobacco snuff consumption on total protein, albumin, globulin and fasting blood sugar level. Adult Wistar rats (12) weighing 150-300g were involved. They were divided into four groups; group A serving as control, while groups B, C and D served as the test groups. The rats were fed with varying doses of tobacco dust mixed with potash (tobacco snuff). At the end of the experiment, the animals were sacrificed and blood sample collected into lithium heparin container. Total protein (24.34 ± 4.10 g/dl), albumin (13.80 ± 4.66 g/dl), and fasting blood sugar were assayed and the results obtained showed statistically significant changes. However, fasting blood sugar (117.33 ± 38.50mg/dl) showed decrease that was not statistically significant in all the groups throughout the experiment. The results of this study suggest that tobacco snuff is toxic to the synthetic capacity of the liver and the observed changes were dose dependent.

Keywords: Tobacco snuff, Total protein, Albumin, Globulin, FBS, Rat

INTRODUCTION

While the prevalence of cigarette smoking is reducing in the developed world, the use of smokeless tobacco is on the increase (Siegel *et al.,* 1992). It has been suggested that this is as a result of smokers switching to the perceived less dangerous form of tobacco 'smokeless tobacco' (Omole and Ogunbanjo, 2009). Although this is not the same in developing countries where both cigarette smoking and smokeless tobacco use are on the increase and most smokers, also consume smokeless tobacco. Due to the ignorance of the harmful effect of smokeless tobacco in both developed and developing countries, Okonkwo *et al.,* (2013**a**) raised an alarm for more proactive measures in the regulation of smokeless tobacco, as not only smokers are liable to die young. Based on evidence, the recent increase in the consumption of smokeless tobacco products (snuff and chewing tobacco) has stimulated interest into the carcinogenic effects of these forms of tobacco (Bagchi *et al.*, 1995). In addition, Bagchi *et al.,* (1998) reported in a low dose subchronic administration of smokeless tobacco, an induced oxidative stress resulting in tissue damaging effects which may contribute to the toxicity and carcinogenicity of smokeless tobacco.

In fact, information has it that smokeless tobacco use can be addictive, cause oral leukoplakias (oral mucosal lesions) and gingival recession, and it may play a contributory role in the development of cardiovascular disease, peripheral vascular disease, hypertension, peptic ulcers, and fetal morbidity and mortality (Critchley and Unal, 2003). In addition, previous studies have demonstrated that aqueous and methanolic extracts of smokeless tobacco can inhibit collagen synthesis in osteoblast-like cells (Lenz *et al.*, 1990).

Smokeless tobacco is the form used without combustion eliminating the danger of direct exposure of toxic combustion compounds to the lung and other tissues of the user and of the people around (Rajani *et al.,* 2011). It is usually used intranasally or intraorally, mainly as snuff or chewed tobacco leaves and the types used around the world vary according to region, as do the health risks associated with them (Sapundzhiev and Werner, 2003 and Wyckham, 1999). Due to the progression in the scientific findings on the deleterious effects of smokeless

tobacco, this study therefore, investigates the acute effect of tobacco snuff consumption on plasma total protein, albumin, globulin and fasting blood sugar level in rats.

MATERIALS AND METHODS

Experimental Animals: Twelve adult Wistar rats of comparable size and weight (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. Overall, the animals were handled in accordance with the standard guide for the care and use of laboratory animals (Richard and Crawford, 2012).

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 groups of three rats each. Group A served as the control while groups B, C and D served as the test groups. The study lasted for 14 days (2weeks) and at the end all the animals were sacrificed for blood sample collection via cardiac puncture. ~~(The repetition has been corrected)~~

Study duration: The preliminary studies, animal acclimatization, substance procurement (tobacco leaves and potash), actual animal experiment and evaluation of results, lasted for five months. However, the actual administration of tobacco snuff to the test animals lasted for 14 days (2 weeks).

Substance administration: In substance administration, group A (control) received 25g of feed and distilled water only, whereas test group B, C and D 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 snuff 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**a**).

Sample collection and sample analysis: At the end all the animals were sacrificed and blood samples were obtained by cardiac puncture and placed in lithium heparin container. Total protein and albumin were analysed according to Falkner and Meites, (1982) and Doumas et al., (1971) whereas fasting blood sugar was measured routinely by using "One Touch Ultra Blood Glucose Meter" (AccuChekGluco Meter USA). The plasma samples obtained were stored at -70^{o}C before analysis.

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 showed the effect of tobacco snuff consumption on total protein and albumin levels of the experimental animals and control. Both total protein and albumin levels in the tests showed statistical difference ($P<0.05$) from the values of the control (24.34 ± 4.10; 13.80 ± 4.66 g/dl) throughout the experiment.

Table 1: The effect of tobacco snuff on serum total protien and albumin level in rats

Parameter	Duration	Control (A)	B	C	D
Total protein (g/dl)	2weeks	24.34 ± 4.10^{a}	5.82 ± 0.80^{ab}	4.71 ± 2.49^{b}	13.14 ± 1.25^{ab}
Albumin (g/dl)	2weeks	13.80 ± 4.66^{a}	3.44 ± 0.35^{b}	2.78 ± 0.44^{b}	3.86 ± 0.31^{b}

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

Table 2 below represents the effect of tobacco snuff consumption on plasma globulin and fasting blood sugar levels of the experimental animals and control. Plasma globulin level in the tests showed statistical difference ($P<0.05$) from the values of the control (10.74 ± 1.45 g/dl), except in group D that showed decrease but not statistical significant. However, irregular decrease that were not statistical significant was observed on the fasting blood sugar level throughout the treatment period.

Table 2: The effect of tobacco snuff on serum Globulin and fasting blood sugar (FBS) level in rats

Parameter	Duration	Control (A)	B	C	D
Globulin (g/dl)	2weeks	10.74 ± 1.45^{a}	2.38 ± 0.91^{b}	2.00 ± 1.92^{b}	9.28 ± 1.14^{a}
FBS (mg/dl)	2weeks	117.33 ± 38.50^{a}	87.33 ± 31.00^{a}	64.00 ± 48.00^{a}	82.00 ± 29.51^{a}

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

DISCUSSION

The results of this study demonstrated that tobacco snuff has deleterious effects on the parameters investigated. Interestingly, albumin is the most abundant serum protein representing 55-65% of the total protein. It is synthesised in the liver and its main biological

functions are to maintain the water balance in serum and plasma, bind and transport and store a wide variety of ligands such as metal ions, bilirubin, fatty acids, calcium, drugs and hormones like thyroxine. It also provides an endogenous source of amino acids (Grant *et al.,* 1987 and Guyton and Hall, 2006). Albumin level reflects the synthetic function of the liver hence, serum protein levels are regulated via synthesis in the liver (Rothschild *et al.,* 1972) and the significant reduction in serum proteins with tobacco snuff consumption is suggestive of the fact that tobacco snuff may have inhibited the synthetic function of the liver and as such hepatic impairment.

In a recent study, Raj Shrestha *et al.,* (2012) reported smokeless tobacco induced significant reduction in serum albumin, vitamin E and C and altered liver enzymes. This could be due to the damage and destruction of the liver tissue by smokeless tobacco components which has been proven to induce microsomal enzyme of liver cells (Burtis *et al.,* 2006 and Ugbor *et al.,* 2013**b**). Pramod *et al.,* (2006) and Gonzalez (1999) stated that aqueous extract of smokeless tobacco, impairs enzymatic antioxidant defence 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. So, the significantly reduced level of serum protein observed in this study showed the hepatotoxic potentials of tobacco snuff. Moreover, Ugbor *et al.,* (2013**b**) reported severe destructive effect of smokeless tobacco (tobacco snuff) on the liver hepatocyte's which according to Rajani *et al.,* (2011) revealed cirrhosis of the liver in a histopathological study. This deleterious effect possibly reduces the synthetic capacity of the liver, thus decreased serum protein.

According to Grant *et al.,* (1987), hypoalbuminaemia is associated with conditions such as impaired albumin synthesis in the liver, liver diseases, malnutrition or malabsorption, generalised shock and intestinal disease, the result of this showed possible tobacco snuff induced hypoalbuminaemia. Also, the fact that globulin is produced in the liver and contributes to immune system (Merleb and Hoehn, 2007), a significant decrease in globulin concentration indicates therefore, that if the liver is exposed to the active components of smokeless tobacco, the immune system may also be compromised. In fact, the cytotoxic activity of smokeless tobacco has been reported in *in-vitro* cells (Bagchi, 1996).

The observed reduced total serum protein noticed in this study could also be due to the adverse additive effect, as Oyeleke, (1988) reported that 'natron' the major additive causes severe growth retardation, skin changes and diarrhoea. Similarly, Soladoye and Oyeleke, (1989) showed that moderate intake of natron had adverse effects on growth rate and blood indices in rats even when diarrhoea was absent as earlier reported by Oyeleke, (1988). Due to the fact that albumin provides an endogenous source of amino acids (Grant *et al.,* 1987 and Guyton and Hall, 2006) and enhances growth, therefore, any substance that affects the site of protein synthesis, could possibly reduce serum protein and induce growth retardation. This agrees with the report of Ugbor *et al.,* (2013**b**) that the liver which produces the essential proteins for growth and the enzymes that catalyses these processes are adversely affected by tobacco snuff and its' additive 'natron'. In contrast to the observations of this study, the fasting blood glucose level showed decrease that was not statistically significant and this agrees with the work of Raj Shrestha *et al.,* (2012), however, the mechanism of the glucose reduction is unknown.

CONCLUSION

Judging by the findings of this study and by the report of Ugbor *et al.*, (2013**b**) that the deleterious effect of smokeless tobacco is dosage and duration dependent since it is a chronic habit, it is therefore imperative for an urgent intervention as life has no duplicate.

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