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RESEARCH PAPER

THE EFFECT OF TOBACCO SNUFF CONSUMPTION ON LIVER ENZYMES

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ABSTRACT

This study was designed to investigate the changes in liver biochemical profile following oral tobacco dust ingestion. Adult Wistar rats (42) 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 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 of 2, 4, 6 and 8 weeks respectively. The rats were fed with varying doses of tobacco dust mixed with potash. At the end of each phase, the animals were sacrificed and blood sample collected into lithium heparin and EDTA containers. Liver parameters (AST, ALT, ALP and GGT) were assayed and the results obtained showed statistically significant impairment of liver function. There was significant increase ($p<0.05$) in serum AST, ALT, and GGT levels in the different phases when the test groups were compared with the control. However, ALP shows no statistically significant increase in all the groups throughout the experiment. The results of this study suggest that potash-tobacco dust (local tobacco snuff) is toxic to the liver and the observed changes were dose and duration dependent.

Keywords: tobacco, potash, liver function, liver enzymes.

INTRODUCTION

For years man has used drugs for recreational purposes as long as history itself. Arabic traders smoked opium in the 3rd century BC, and the Aztecs enjoyed the effects of hallucinogenic mushrooms at a similar time. In the last 30 years, the number of people using recreational drugs and other addictives appears to have increased (Strang, 1995). By 1997, 25% of the population is reported using illicit drugs and other addictives at some point in their lives (Crowe *et al.,* 2000). Example of some of these recreational drugs includes heroin, cocaine, marijuana, cigarette, and tobacco snuff which are sniffed, smoked or even eaten with numerous health consequences. But of interest in this study is tobacco snuff (tobacco dust and potash).

Tobacco botanically known as *Nicotiana tabacum* is a perennial herbaceous plant and it is the most commonly grown of all plants in the Nicotiana genus. Its leaves are commercially grown in many countries and it grows to heights between 1 to 2 metre to be processed into tobacco products (Ren and Timko, 2001). Tobacco is known and used throughout all quarters of the globe in two major forms: the smoked and the smokeless.

Smokeless tobacco comes in two different forms, which are 'Tobacco snuff' and 'Chewing tobacco' (Aduema *et al*., 2012). Tobacco snuff is the powdered form blended with potash as the main additive in Nigeria (Ureme *et al.,* 2007) and has been recommended as a substitution for nicotine in cigarette since it is devoid of hazardous elements such as tar and carbon monoxide (Russel *et al,* 1980). For this reason, many people believe that using smokeless tobacco is

safer than smoking it. This however, is not true because smokeless tobacco can induce addiction to nicotine and leukoplakia (Dempsey, 2001).

Tobacco (smoked or smokeless) contains nicotine and other phytochemical constituents such as potent tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), 4-methyl-nitrosamino)-4-(3-pyridyl)-butanal (NNA), and N-nitrosonornicotine, heavy metals (Cadmium (Cd), mercury (Hg) etc) and 23 polycyclic aromatic hydrocarbons which has been implicated with tobacco associated cancers and diseases (Hecht *et al*., 1978; Hoffmann and Hecht, 1985; Hecht and Hoffmann, 1988; Chiba and Masironi, 1992; Hecht *et al.,* 2007; Addo *et al.*, 2008; Stepanov *et al*., 2010 and Addo *et al*., 2011).

Considering the report by Fernandez-checa and Kaplowitz, (2005) that every drug is associated with hepatotoxicity almost certainly due to its ability to generate free radicals and to cause disturbance in hepatocyte biochemistry, this study is designed to investigate the effect of oral tobacco dust (tobacco snuff) on liver enzymes in Wistar rats.

MATERIALS AND METHODS

Experimental Animals: Forty two adult Wistar rats of comparable sizes and weighing (150-300g) was 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 and were kept in a wooden wire mesh cages.

Substance of study: Dry leaves of tobacco and potash were purchased from Ogbete main market, Enugu state. 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 were 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 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 4weeks, stage 3 which lasted for a period of 6weeks and stage 4 which lasted for a period of 8weeks. They 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 of 2, 4, 6 and 8 weeks respectively. The rats were fed with varying doses of tobacco dust mixed with potash. At the end of each 2, 4, 6 and 8 weeks respectively, the animals were sacrificed for tissue and blood sample collection.

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).

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**;** 97.6g of feed, 2.0g of tobacco dust and 0.4g of potash**;** 95.2g of feed, 4.0g of tobacco dust and 0.8g of potash**;** and 92.8g of feed, 6.0g of tobacco dust and 1.2g of potash respectively. Each test group received distilled water given *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.

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.

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.

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 the rats were sacrificed under chloroform anaesthesia. Blood samples were obtained by cardiac puncture and placed in lithium heparin and EDTA containers. The EDTA container is specific for Gamma-glutamyl transferase (GGT) analysis according to Szasz, (1969) as reported by Randox. The plasma samples obtained were stored at -70^{o} C before analysis.

Data analysis: All the data collected were then 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 shows the effect of tobacco snuff consumption on aspartate amino transferase (AST) activity. The result showed that AST activity at 2 weeks was not significantly different (P>0.05) when compared with the control, but a dose/duration dependent significant increase (P<0.05) was observed at 4weeks, 6weeks and 8weeks respectively.

Table 2 shows the effect of tobacco snuff consumption on alanine amino transferase (ALT) activity. At the end of the first 2 weeks, ALT showed a statistically significant increase (P<0.05) in group C when compared with the control and those of the other groups. Meanwhile, ALT values significantly increased (P<0.05) at 4weeks, 6weeks and 8weeks respectively, but the observed significant increase of ALT values (P<0.05) at 6weeks and 8weeks respectively, were dosage/duration dependent.

Table 3 shows the effect of tobacco snuff consumption on alkaline phosphatase (ALP) activity. At 2 weeks and 4 weeks respectively, ALT values showed no significant increase (P> 0.05) when compared with the control. Similar observation was made at the end of 6 weeks and 8weeks respectively.

Table 1: The effect of tobacco snuff on Aspartate amino transferase (AST) activity in rats

PARAMETERS		Control Group A	Test groups		
			B	C	D
AST(U/L)	2weeks	187.07±15.24^{a}	184.63±32.34^{a}	210.46±4.53^{a}	195.74±40.78^{a}
	4weeks	187.07±15.24^{a}	266.47±19.04^{b}	267.17±51.94^{b}	270.14±38.74^{b}
	6weeks	187.07±15.24^{a}	263.51±63.03^{b}	272.93±16.09^{b}	269.96±12.31^{b}
	8weeks	187.07±15.24^{a}	267.17±24.79^{b}	266.64±19.76^{b}	284.80±35.53^{b}

Table 2: The effect of tobacco snuff on Alanine amino transferase (ALT) activity in rats

PARAMETERS		Control Group A	Test groups		
			B	C	D
ALT(U/L)	2weeks	57.28±10.07^{a}	51.48±12.48^{a}	95.31±20.86^{b}	70.69±15.77^{a}
	4weeks	57.28±10.07^{a}	102.41±14.44^{b}	84.99±13.43^{b}	97.88±26.29^{b}
	6weeks	57.28±10.07^{a}	103.10±20.02^{b}	107.53±15.54^{b}	109.69±24.63^{b}
	8weeks	57.28±10.07^{a}	110.54±10.73^{b}	132.63±1.73^{b}	120.41±17.41^{b}

Table 4 shows the effect of tobacco snuff consumption on gamma-glutamyl transferase (GGT) activity. At 2 weeks, GGT activity showed a statistically significant increase (P<0.05) in test group D unlike in the other test groups

where no statistically significant increase (P> 0.05) was observed. Similarly, at 4weeks, GGT showed a statistically significant increase (P<0.05) in test group C only. Also, at 6 weeks, GGT values significantly increased in test group B only, but at 8-weeks, GGT showed a statistically significant increase (P<0.05) in all the test groups when compared with the control.

Table 3: The effect of tobacco snuff on Alkaline phosphatase (ALP) activity in rats

PARAMETERS		Control Group A	Test groups		
			B	C	D
ALP(U/L)	2weeks	19.32±10.20 [a]	32.14±25.80 [a]	27.29±13.31 [a]	21.76±5.41 [a]
	4weeks	19.32±10.20 [a]	18.39±7.29 [a]	31.73±18.97 [a]	18.40±11.31 [a]
	6weeks	19.32±10.20 [a]	8.59±5.07 [a]	15.03±16.49 [a]	40.48±53.38 [a]
	8weeks	19.32±10.20 [a]	23.12±13.45 [a]	10.35±2.93 [a]	14.26±5.85 [a]

Table 4: The effect of tobacco snuff on Gamma-glutamyl transferase (GGT) activity in rats

PARAMETERS		Control Group A	Test groups		
			B	C	D
GGT(U/L)	2weeks	2.41±0.76 [a]	5.79±1.16 [a]	5.46±0.62 [a]	9.45±4.92 [b]
	4weeks	2.41±0.76 [a]	17.62±20.34 [a]	43.03±37.73 [b]	20.84±21.61 [a]
	6weeks	2.41±0.76 [a]	28.07±25.77 [b]	6.94±1.16 [a]	4.39±0.15 [a]
	8weeks	2.41±0.76 [a]	34.51±6.44 [b]	8.32±0.24 [b]	27.02±2.18 [b]

Mean ± SD with different superscript in the same column are significantly different (P<0.05). Aspartate amino transferase (AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP) and Gamma-glutamyl transferase (GGT) activity

DISCUSSION

Despite the fact that several scientific literatures have speculated the medical uses of, *Nicotiana tabacum* others have also questioned its medicinal uses. Based on this, Tuner *et al*., (1985), stated that snuff has become quite a popular medication for long grief pain and aches. In the present study, the liver profile of wistar rats fed with tobacco dust was assessed and it was observed that the treatment with tobacco dust induced significant changes in the plasma levels of the liver parameters such as ALT, AST, and GGT in the study.

The observed statistically significant increase in ALT and AST agrees with the works of Bagchi *et al* (1995), Pramod *et al*., (2010), and Adekomi *et al.,* (2011) who reported that smokeless tobacco induces inflammation of the liver hepatocytes and blockage of liver sinusoids and this may due to its phytochemical constituents which may induce leakage of these enzymes into the blood. The elevated levels of these enzymes (ALT and AST) are due to the fact that they are present in the hepatocytes (liver parenchymal cells) and injury (inflammation) to these cells results in the plasma elevation of these enzymes due to leakage into the blood (Schmidt, 1993).

Also the observed statistically significant increase in plasma ALT and AST levels at early stage (2week and 4weeks) of the study is attributed to the fact that they rise dramatically in acute liver damage such as in paracetamol (acetaminophen) overdose (Schmidt, 1993) and are seen as the first markers of liver damage. Although elevated plasma AST level does not confirm directly liver damage due to its presence in red blood cells, cardiac and skeletal muscle, its plasma levels is based on ALT level which is a more specific indicator of liver inflammation than AST (Kondo *et al*., 1984 and Amna *et al*., 2011) and the ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage (Nyblom *et al*., 2004 and Nyblom *et al*., 2006). The above statements agree with the result of this work that presented elevated levels of ALT and AST in a dose and duration dependent fashion.

However, the presence of aminotransferases (ALT and AST) indicates increase in serum activity, which generally indicates that enzymes are leaking from the cytoplasm and mitochrondria as a result of tissue damage (Kuramitsu et al., 1985). The tissue damage may be due to the production of reactive oxygen species (oxygen free radicals) which was probably induced by smokeless tobacco and this agrees with the work of Bagchi et al., (1995) and Bagchi *et al.*, (1996) who reported that oral cells, peritoneal macrophages, and hepatic mitochondria and microsomes, produce reactive oxygen species (oxygen free radicals) following in vitro incubation with an aqueous extract of smokeless tobacco may causes most of the cellular degeneration in vivo. Therefore the observed plasma increase of aminotransferases (ALT and AST) seen in the present study suggests tissue damage.

Furthermore, Hoffmann *et al.,* (1977) discovered that serum ALP activity had an important role in characterizing bone and hepatic disorders, when obstruction of the duct system occurs at any level. Hepatic fibrosis also induces increased in serum activity of hepatic ALP, but the more common occurrence is its increase in association with hepatic lipidosis and severe starvation. Therefore the continues non-statistically significant increase on plasma ALP level in all the groups rules out the possibility of bone disease since liver and bone are the two main sources of ALP, although there were increase in ALP level at some stages when the test groups were compared with the control but are not statistically significant and this potentiates possible biliary duct problems.

Of greater interest is the fact that GGT, a more specific and sensitive diagnostic marker for liver problems, presented statistically significant increase throughout the experiment at different stages. Elevated plasma GGT activity can be found in diseases of the liver and is similar to alkaline phosphatase (ALP) in detecting disease of the biliary tract (Betro et al., 1997). Also GGT is elevated by large quantities of alcohol ingestion and isolated elevation or disproportionate elevation compared to other liver enzymes (such as ALP or ALT) may indicate alcohol abuse or alcoholic liver disease (Kaplan et al., 1995). This may be due to the fact that alcohol increases GGT production by inducing hepatic microsomal release, or may cause the leakage of GGT from hepatocytes (Kaplan et al., 1995).

In view of this, tobacco dust may induce increase plasma GGT level by acting through the same mechanism with alcohol by producing reactive oxygen species which induces hepatic mitochondria and microsomes cellular degeneration because of subtle membrane changes that is sufficient to allow passage of intracellular enzymes to the extracellular space according to the report of Bagchi *et al.*, (1996) and Teitz, (2001). Indeed, the high-fold statistically significant increase of plasma GGT indicates the alcoholic like effect of tobacco dust consumption and these are in line with the report of Teitz, (2001) which states that GGT was found to be threefold higher than normal in individuals with alcoholic hepatitis and biliary obstruction.

It is worthy to note that, alcohol may cause swelling and inflammation (hepatities) in the liver and can also subsequently lead to scarring and then cirrhosis of the liver which is the final phase of the alcoholic liver disease (O'Shea *et al.,* 2010). This agrees with the work of Rajani et al., (2011) which stated that histopathological studies on the effect smokeless tobacco (tobacco snuff) to the livers revealed cirrhosis of the liver. Worman *et al*., (2002) stated that GGT are markedly increased and serves as a marker of alcohol liver disease and Berk and Korenblat, (2007) also stated that liver cirrhosis is a risk factor for elevated level of plasma GGT. Thus, elevated level of plasma GGT in the present study potentiates the possibility of tobacco liver disease and liver cirrhosis since tobacco snuff consumption is a chronic habit like alcohol. Also according to Berk and Korenblat, (2007) the use of hepatotoxic drugs induce elevated plasma GGT level and so, tobacco dust as drug may cause hepatotoxicity due to the elevated levels of GGT.

The elevated level of GGT may also be potentiate the possibility of lever cancer as tobacco has be identified to posses carcinogenic components (Mitchell et al., 1999; Garg et al., 2010). Although, Tuner *et al*., (1985), stated that tobacco snuff has become quite popular medication for long grief pain and aches. Teitz, (1987) stated that high levels of GGT are seen in patients with primary or secondary liver cancer and Berk and Korenblat, (2007) also reported that liver necrosis and liver tumor are risk factors for elevated GGT. In view of this, the elevated levels of plasma GGT level which was significant in the present study and some increased levels at some stages of the study that were not statistically significant may be potentiating the possibility of liver cancer. This may be as a result of potential carcinogenic component of smokeless tobacco and agrees with the work of Mitchell *et al*., (1999) which states that the carcinogens in smokeless tobacco includes polonium-210, N-nitrosamines (including tobacco-specific nitrosamines), volatile aldehydes and polycyclic aromatic hydrocarbons that are linked to increased risks of oral, cervical, prostatic and pancreatic cancers. Also Boffetta et al., (2008) stated that smokeless tobacco contains nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK) and *N*′-nitrosonornicotine (NNN) in relatively high concentrations. In fact, every gram of smokeless tobacco contains approximately 1 to 5 μg of tobacco-specific nitrosamines such as NNN and NNK an established carcinogens as reported by Boffetta et al.,

(2008). Also Garg et al., (2010) and Addo *et al.*, 2008 reported that tobacco snuff has been attributed to be carcinogenic as a result of heavy metals.

Finally, the effect of tobacco dust (tobacco snuff) on the liver cannot be complete without its major addictive – potash (natron) which has been reported by Oyeleke, (1988) to cause severe growth retardation, skin changes and diarrhoea. Also Soladoye and Oyeleke, (1989) has shown 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). Hence, the liver which produces the essential proteins for growth and the enzymes that catalysed these processes are likely affected by natron and this leads to alteration in amino acid synthesis which likely induces an increased level of plasma aminotransferases (AST and ALT). Therefore, the results on the present study suggest that tobacco snuff may likely cause several liver diseases since its consumption is a chronic habit

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AUTHORS' CONTRIBUTIONS

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