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Research Article
Tobacco Snuff-Induced Hepatic Alterations

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Abstract

Background and Objective: Tobacco snuff in Nigeria is the blended dried tobacco left with potash. This substance has been proven to cause systemic distortions and its consumption is unhealthy, thus tobacco snuff-induced hepatic alterations were investigated. **Materials and Methods:** In this study (8 weeks), liver histological changes were investigated after tobacco snuff ingestion. Forty-two experimental animals (Wister rats) were involved and they were divided into groups A (control), group B (test group 1), C (test group 2) and D (test group 3). The test groups were further divided into four groups receiving 2.4, 4.8 and 7.2 g graded doses of tobacco snuff for the experimental duration of 8 weeks. The experimental duration was phased into 2 weeks and at the end of each 2 weeks, liver organs were harvested for analysis. **Results:** Hepatic tissue damage was observed through examination of the stained paraffin-embedded sections. Edematous changes with parenchymal erosion as well as cellular necrosis, vacuolations, hemorrhagic exudations, inflammatory cellular infiltration, severe cellular infiltration, cellular degeneration and distortions of parenchymal tissue architecture were observed among the experimental animals. **Conclusion:** The present results showed that tobacco snuff is capable of inducing severe liver damage and the observed damages were dose and duration-dependent.

Key words: Tobacco snuff, experimental animal, liver histology, alterations, nicotine, leukoplakia, natron

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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

World health organization estimated that tobacco caused 5.4 million deaths in 2004 and 100 million deaths in the 20th century as reported[1]. Tobacco use is the single most important preventable risk to human health in developed countries and an important cause of premature death globally[1]. Despite these estimations, in the world, the use of smokeless tobacco is quite popular in most countries, with a rising trend in the United States of America (USA)[2,3] and an uncontrolled consumption rate in Nigeria.

Moreover, the two different forms of smokeless tobacco are tobacco snuff and chewing tobacco[4], although the most popular brand in Nigeria is tobacco snuff. Tobacco snuff is the powdered form with potash as the main additive[5-7] and due to the form, it is seen as the replacement for nicotine as cigarette contains known hazardous elements[8] and many believed it is not dangerous. However, addiction to nicotine and leukoplakia has been induced by smokeless tobacco[9]. In addition, Nigerian tobacco snuff has been proven to induce priapism with consequential necrosis[10]. Also, local tobacco snuff has proven to be harmful to the lungs, kidneys and liver as reported[6,7,11-13].

The health consequences of tobacco snuff are due to the phytochemical constituents and additive (natron)[5,14-17] and its ability to act as harmful drugs[6]. In addition, smokeless tobacco has been proven to generate oxidative radicals which is a major precursor of systemic damage in many diseases.

Because smokeless tobacco induces wide systemic damage due to its ability to generate free radicals and causes disturbance in liver hepatocytes[6,18,19], this study was designed to investigate the effect of tobacco snuff on the liver histology of Wistar rats.

MATERIALS AND METHODS

Experimental animals: Experimental animals (42) were purchased from the animal farm of Anthonio Research Center, Ekpoma, Edo State, Nigeria. After this was transferred to the experimental site and allowed 2 weeks of acclimatization. Wooden wire mesh cages were used to house the experimental animals under standard laboratory procedures[6]. The animal acclimatization, substance procurement (tobacco leaves and potash), actual animal experiment and evaluation of results, lasted from October, 2012 to January, 2013. However, the actual administration of tobacco snuff to the experimental animal lasted for 8 weeks.

Ethics on the use of animals in experimental studies: As contained in the US guidelines, the protocols employed were according to our Institutional guidelines as well as internationally accepted practices for the use and care of laboratory animals[6,20].

Substance of the study: Botanically identified tobacco leave (dried) with potash was purchased from a recommended market (Ogbete Main Market, Enugu State, Nigeria). The botanical identification of the tobacco leaves was carried out by a botanist in the Department of Botany at Ambrose Alli University, Ekpoma, Edo State, Nigeria[6].

Substance preparation: Mortar and iron pestle were used to blend dried tobacco leaves and potash into powder and were stored before the study. To obtain the graded doses of tobacco snuff used in the study, an electronic balance (Denver Company, USA, 200398.IREV. CXP-3000) was employed and feed pellets were prepared as described by Ugbor *et al.*[6] and Nwaopara *et al.*[21].

Animal grouping: The experiment was divided into four phases of 2 weeks each: 1st phase was for 2 weeks, the second phase was for 4 weeks, the third phase was for 6 weeks and the fourth phase was for 8 weeks. Each test group was divided into four phases representing 2, 4, 6 and 8 weeks duration. The experimental animals were fed with graded doses of tobacco snuff and at the end of each 2, 4, 6 and 8 weeks, respectively, the animals were sacrificed for organ collection[6].

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 experimental animals lasted for 8 weeks (2, 4, 6 and 8 weeks)[6].

Substance administration: In the first phase (2 weeks), group A (control) received 100 g of feed and distilled water only whereas, test groups B, C and D received, 97.6 g of feed+2.4 g of tobacco snuff, 95.2 g of feed+4.8 g of tobacco snuff and 92.8 g of feed+7.2 g of tobacco snuff, respectively. Each test group received distilled water given *ad libitum*.

In the second phase (4 weeks), group A (control) received 75 g of feed and distilled water only, whereas test groups B, C and D received, 72.84 g of feed+2.16 g of tobacco snuff, 70.68 g of feed+4.32 g of tobacco snuff and 68.52 g of feed+6.48 g of tobacco snuff, respectively.

Fig. 9(a-d): Test group C (2 weeks) sections (Liver, H&E×400) showing cellular necrosis, vacuolations and severe haemorrhage

Fig. 10(a-d): Test group C (4 weeks) sections (Liver, H&E×400) showing cellular necrosis, vacuolations and severe cellular infiltration with haemorrhage

inflammatory and necrotic cells with vacuolations and eosinophilic cells-indicative of cellular degeneration were observed in group B, C and D which shows hepatic alterations Fig. 2, 3, 8 and 9 . These hepatic changes are usually seen in hepatitis (chronic active hepatitis)[23], alcoholic hepatitis, liver cancer, cigarette smoking, drug and herbal abuse and other liver diseases and these indicate potential tobacco snuff-induced hepatitis. Smokeless tobacco-induced inflammation of the liver hepatocytes and causes cellular degeneration and blockage of liver sinusoids[1,18,24]. Interestingly, Ugbor *et al.*[6] reported tobacco snuff-induced severe acute and chronic hepatic profile alterations that are dosage and duration-dependent. Considering the subtle membrane changes that are usually induced by smokeless tobacco due to its ability to generate free radicals which have been implicated with hepatic injuries

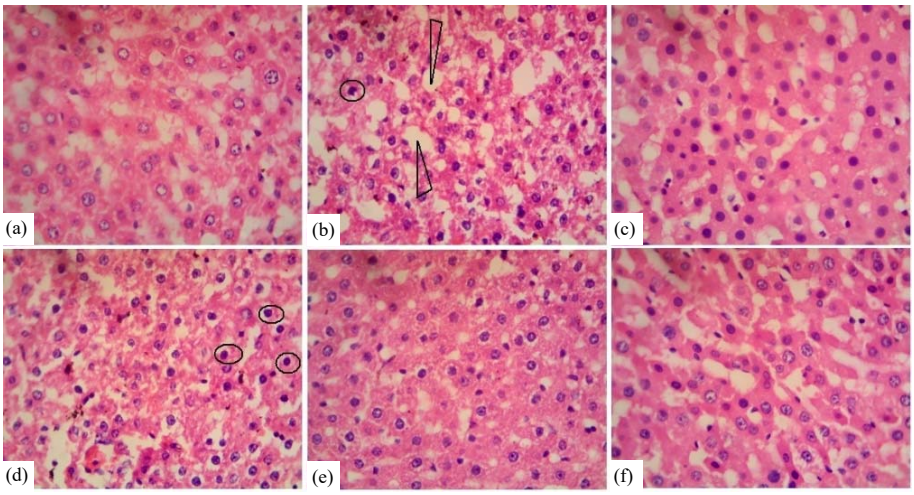

Fig. 11(a-f): Test group C (6 weeks) sections (Liver, H&E×400) showing cellular necrosis (encircled), vacuolations (triangular arrows) and cellular infiltrates

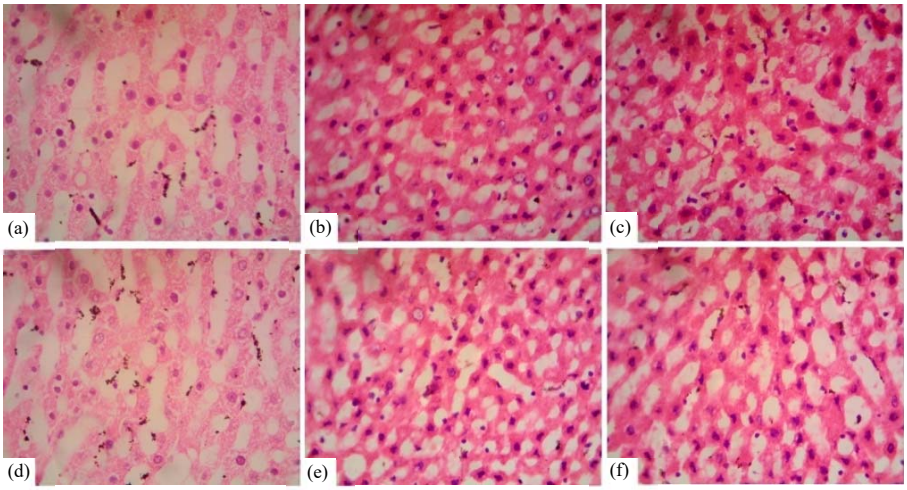

Fig. 12(a-f): Test group C (8 weeks) sections (Liver, H&E×400) showing distortions in parenchymal tissue architecture
Note the severe and generalized cellular necrosis with vacuolations

and which when persistent may cause liver cirrhosis, at 4 weeks stage of the study, groups B, C and D showed cellular necrosis, inflammatory cell infiltration, hemorrhagic exudations, degenerative fatty changes with vacuolations, severe cellular infiltration with hemorrhage and distortions in parenchymal tissue architecture with hemorrhagic exudations Fig. 5, 6, 10 and 15. These features agreed with the report of the Weissberg *et al.*[23] which stated that chronic active hepatitis reveals scar tissue and inflammatory cells, which disrupt the normal hepatic structure. In symptomatic cases, liver function tests are usually markedly abnormal, with evidence of hepatocellular injury (high transaminases) and diminished synthetic function (low albumin, prolonged prothrombin time), hence the report of Ugbor *et al.*[6,13] that tobacco snuff induced abnormally high liver function tests and inhibits the synthetic functions of the liver.

Illustratively, the scar tissue and inflammatory cells, which disrupts normal hepatic structure in chronic active hepatitis followed by abnormally high liver function tests indicate tobacco snuff potentials in causing tobacco-like hepatitis. Also, the degenerative fatty changes observed at this stage of the study indicate tobacco snuff-induced macrovesicular steatosis, a condition that describes abnormal retention of lipids within a cell. It also reflects an impairment of the normal processes of synthesis and elimination of triglyceride fat that is usually seen in cell toxins[25]. As for the 6th weeks stage of the

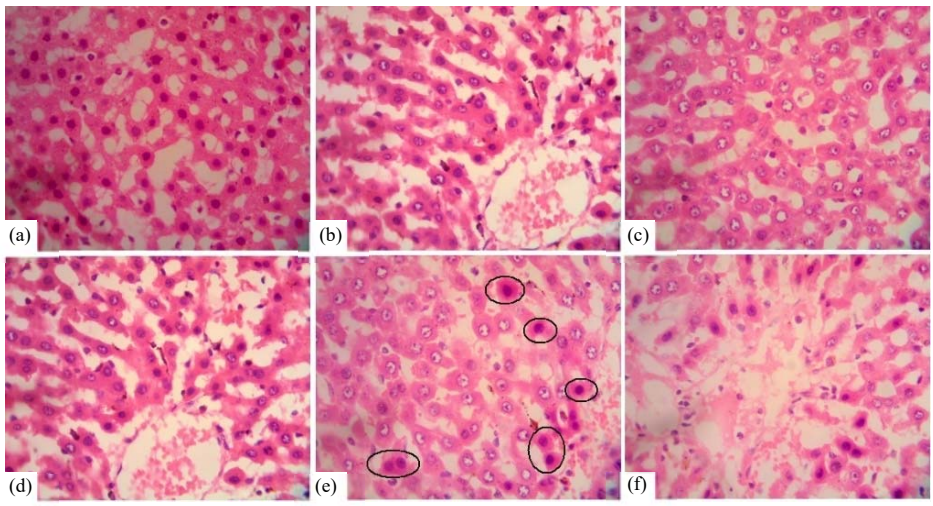

Fig. 13(a-f): Test group D (2 weeks) sections (Liver, H&E×400) showing distortions in parenchymal tissue architecture
Note the eosinophilic cells-indicative of cellular degeneration as encircled

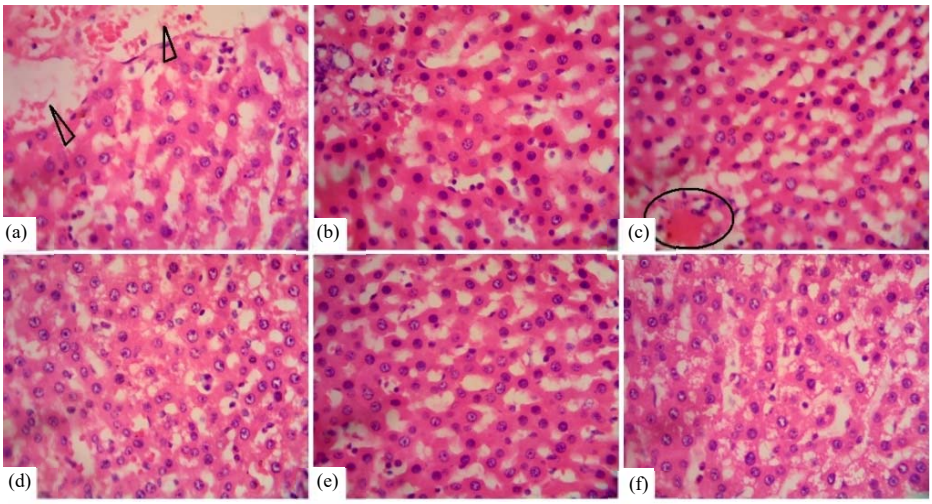

Fig. 14(a-c): Test group D (2 weeks) sections (Liver, H&E×400) showing distortions in parenchymal tissue architecture with edematous changes (triangular arrows)
Note the inflammatory and necrotic cells with vacuolations, as well as exudations as encircled (c)

study, groups B, C and D presented edematous changes with hemorrhagic signs, cellular necrosis with vacuolations, cellular infiltrates and distortions in parenchymal tissue architecture Fig. 6, 11 and 16. These pathologic changes are symptoms of hepatitis[26,27] and other hepatic diseases. The severity of these changes showed that tobacco snuff is capable of inducing dosage and duration-dependent progressive hepatic damage through a mechanism that may be associated with eosinophilic cell and inflammatory cell infiltration into the liver, which then may trigger hepatic fibrosis.

Finally, at 8 weeks the result showed degenerative fatty changes with cellular necrosis, inflammatory cell infiltration and vacuolations and distortions in parenchymal tissue architecture with severe and generalized cellular necrosis throughout the test groups B, C and D Fig. 7, 12 and 17. Imperatively, Ugbor *et al.*[6] reported the severity of tobacco snuff on hepatocytes due to the observed elevation of GGT in the previous study. These high levels of GGT were seen in patients with primary or secondary liver cancer and Rosalki *et al.*[28] also reported that liver necrosis and liver tumor are risk factors for elevated GGT. More

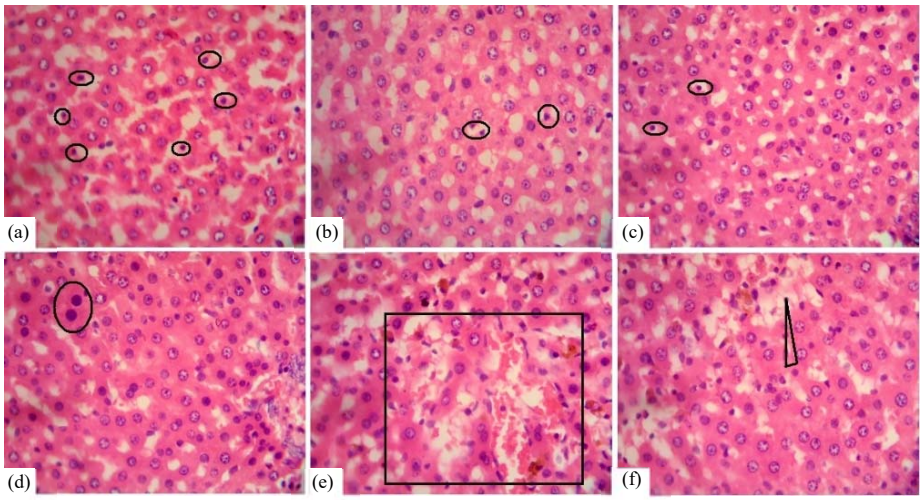

Fig. 15(a-f): Test group D (4 weeks) sections (Liver, H&E×400) showing distortions in parenchymal tissue architecture with hemorrhagic exudations (e) and degenerative fatty changes (triangular arrow, f)
Note also the inflammatory and necrotic cells with vacuolations as encircled (a, b, c and d)

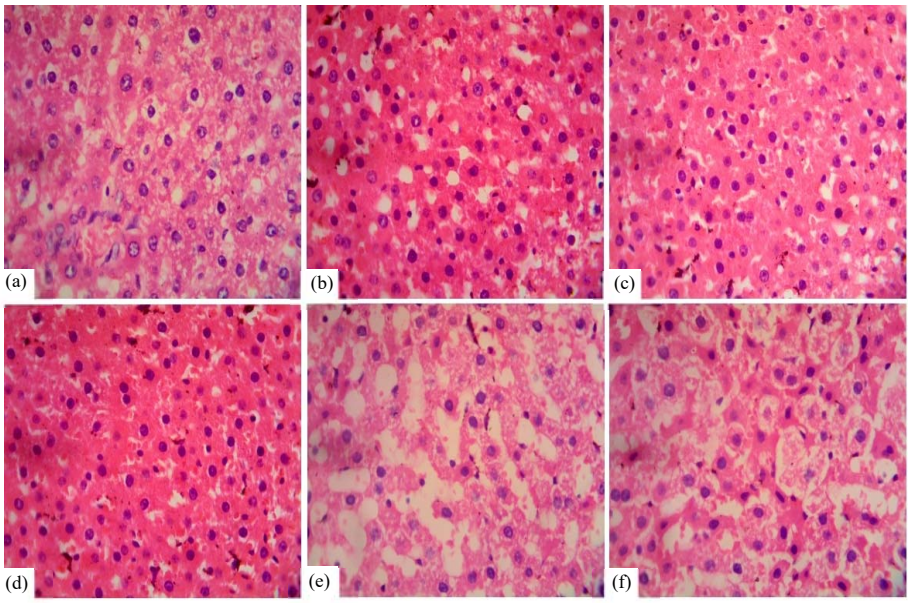

Fig. 16(a-f): Test group D (6 weeks) sections (Liver, H&E×400) showing distortions in parenchymal tissue architecture
Note the necrotic cells with vacuolations

and more, the Salunke *et al.*[29] revealed smokeless tobacco-induced cirrhosis of the liver and goblet cell hyperplasia of the small intestine.

Confirmative, the severe and generalized cellular necrosis observed implicates possible tobacco snuff-induced liver tumor. Meanwhile, severe cellular infiltration, vacuolations and distortions in parenchymal tissue architecture portray progressive hepatic destruction that is dosage and duration-dependent. It is worthy of note that tobacco snuff-induced hepatic damage is without doubt caused by its ability to generate free radicals, constitutive carcinogenic components, harmful heavy metals and 23 polycyclic aromatic hydrocarbons that are implicated in increased activity of serum hepatic parameters[6,7,30] and the progression of macrovesicular steatosis showed retrogression in the liver's metabolic functions. The alterations observed in this study indicate that tobacco snuff is harmful and not good for human consumption.

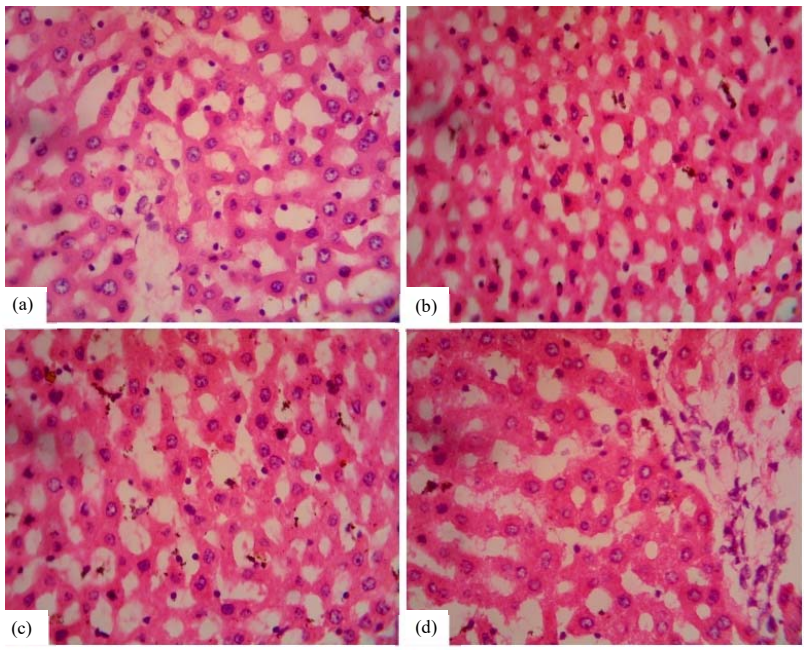

Fig. 17(a-d): Test group D (8 weeks) sections (Liver, H&E×400) showing distortions in parenchymal tissue architecture
Note the severe cellular necrosis with vacuolations

CONCLUSION

Tobacco snuff causes progressive hepatic destruction with associated systemic distortions. In addition, the pattern of hepatic destruction is dosage and duration dependent with severe retrogression in liver metabolic functions.

SIGNIFICANCE STATEMENT

Tobacco snuff is consequentially unsafe for health and it also showed the destructive nature of tobacco snuff and the new health condition 'Tobacco Snuff Hepatitis'. This study reveals the possible cause of most unknown hepatic disorders with devastating health consequences.

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