Status of Some Biochemical Markers among Nasal Tobacco Snuffing Addicts in Yenagoa Bayelsa State Nigeria

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ABSTRACT

Heavy metals and poisons found in snuff may be harmful to human health. This study aimed on the status of a few biochemical markers among nasal tobacco snuffing addicts in Yenagoa, Bayelsa State, Nigeria. The 5 ml of blood sample used for this study were withdrawn from each of the non-tobacco snuffers (control group, n=53) and nasal tobacco addicted snuffers for 5-10 years (experimental group, n=53) respectively. All the volunteers were between the ages of 18 and 52. Each of these samples was introduced into a lithium heparin anti-coagulated bottle, which was then spun at 2,500 revolutions per minute to obtain plasma which was subsequently used to measure the following biochemical markers: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine, urea, lead, cadmium, malondialdehyde, glutathione peroxidase, 2-hydroxy-deoxyguanosine, troponin-1, creatinekinase-MB, C-reactive protein and interleukin-6 using SPSS 23.0 version for statistical analysis with data illustrated as means and standard deviation. The mean values of alanine aminotransferase (38.27 ± 2.17) U/I, aspartate aminotransferase (27.11 ± 1.91) U/I. alkaline phosphatase (49.85 ± 3.65) U/I, Creatinine (97.42 ± 2.96) µmol/L, urea (11.20 ± 1.80) mmol/L, lead (7.80 ± 1.42) × 10^{-2} ppm, cadmium (6.52 ± 1.21) × 10^{-2} ppm, malondialdehyde (15.27 ± 1.81) µmol/L, glutathione peroxidase (12. 72 ± 2.10) µmol/L, C-reactive protein (27.65± 2.72) mg/L, and interleukin-6 (29.71 ± 2.10) pg/ml were significantly elevated as compared to the control group alanine aminotransferase (4.02 ± 0.15) U/I, aspartate aminotransferase (3.91 ± 0.12) U/I, alkaline phosphatase (17.81 ± 1.15) U/I, creatinine (60.12 ± 3.17) µmol/L, urea (3.78 ± 1.14) mmol/L, lead (0.002 ± 0.01) × 10^{-2} ppm, cadmium (0.03 ± 0.01) ×10^{-2} ppm, malondialdehyde (2.10 ± 0.12) µmol/L, glutathione peroxidase (2.71 ± 0.02) µmol/L, C-reactive protein (2.40 ± 0.15) mg/L and interleukin-6 (11.80 ± 1.46) pg/ml respectively. However, the mean values of 2-hydroxy-deoxyguanosine (2.27 ± 0.74) ng/ml, troponin-1 (1.25 ± 0.28) ×10^{-2} IU/L and creatinekinase-MB (5.74 ± 1.05) IU/L were not significantly altered in the experimental group compared to the control group 2-hydroxy-deoxyguanosine (2.25 ± 0.71) ng/ml, troponin-1 (1.22 ± 0.27) × 10^{-2} IU/L and creatinekinase-MB (5.71 ± 1.02) IU/L. Nasal tobacco snuffing addicts between 5-10 years may be at risk of hepatic and toxico-inflammatory disorders.

KEYWORDS: *Nasal tobacco snuffing, Addicts, Status, Biochemical markers, Yenagoa, Bayelsa State, Nigeria*

1. INTRODUCTION

Tobacco that has been ground into fine grains and stored in cans or pouches is known as snuff (Adias *et al.*, 2014). The *Nicotiana tobacuum* plant, which is widely cultivated and commercially grown in many nations throughout the world, produces tobacco in the form of dried and processed plant leaves. Most commonly, it is used for smoking, chewing, snuffing, or dipping tobacco. Close to 1.1 billion people use it, and up to one-third of them fall inside the adult population (Gilman and Xun, 2004).

Snuff use is just as harmful as cigarette smoking. Due to the impact of its multiple chemical components, it is now regarded as a substantial cause of disease and mortality (Musa *et al.*, 2022). According to the World Health Organization, 5.4 million fatalities were attributed to tobacco use in 2004 (Mesembe *et al.*, 2008). The United States Center for Disease Control and Prevention describes tobacco use as the single biggest risk to human health that may be avoided in developed nations and a major factor in premature death worldwide (Villegier *et al.*, 2013).

The addictive substance nicotine is present in all tobacco products, including smokeless tobacco. The frequency and rate of tobacco use, as well as its effects on the body are directly correlated with nicotine (Ukoha *et al.*, 2014). Comparable amounts of nicotine are present in the blood of smokers of cigarettes and smokeless tobacco. Nicotine is directly absorbed into the bloodstream and then transported to the brain in smokers of smokeless tobacco. Nicotine is still being reabsorbed into the bloodstream after the tobacco has been taken out of the mouth. Furthermore, compared to smokers, smokeless tobacco users' blood levels of nicotine last longer (Omotoso *et al.*, 2013).

People in numerous areas and nations, including North America, North Europe, India, other Asian countries, and portions of Africa, have used smokeless tobacco for a longer period of time. Tobacco snuff is used for cultural and traditional purposes, especially in Nigeria. It is either administered tropically or inhaled through the nose (Sinha, 2018). According to epidemiological research, smokeless tobacco products significantly contribute to malignancies, strokes, nervous system illnesses, and reproductive problems (Borgerding *et al.*, 2012).

Tobacco alkaloids, tobacco-specific nitrosamines, volatile N-nitrosamines, N-nitrosamino acids, polyaromatic hydrocarbons, radionucleotides, metals (such as chromium, arsenic, cadmium, and lead), pesticide residues, humectants, alfatoxins, and mycotoxins are some of the chemical substances that have been found in smokeless tobacco products so far (McAdam *et al.*, 2017). The presence of heavy metals in this smokeless tobacco could lead to oxidative stress that can eventually cause tissue damage (Kiline *et al.*, 2004).

Despite the numerous negative impacts on human health associated with tobacco snuffing that have been documented by a few number of researchers, its use is nevertheless on the rise. In order to shed more light on its threat to health, particularly when taken for a protracted period of 5–10 years, this study, which examines the status of some biochemical markers among nasal tobacco snuffing addicts in Yenagoa, Bayelsa State, Nigeria, was initiated and carried out.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out at the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

2.2 Ethical Approval

The College Health Research Ethical Committee gave their approval for this study, which adhered to the 2008 revision of the 1975 Helsinki Declaration's principles. Besides, all the volunteers gave their verbal agreement.

2.3 Study Design

2.3.1 Inclusion Criteria

The inclusion criteria were apparently healthy volunteers between the ages of 18 and 52 who gave their agreement and who did not use drugs, smoke cigarettes, or misuse alcohol before and during the course of this study.

2.3.2 Exclusion Criteria

Addicts to drugs, alcohol, and cigarettes were not included in this study.

One hundred and six volunteers who appeared to be in good health were chosen at random to participate in this study and were divided into the following categories:

2.3.4 Control Group

Fifty three volunteers who do not smoke cigarettes or use drugs or alcohol and who do not snuff tobacco through the nose made up this group.

2.3.5 Experimental Group

Fifty three volunteers who have used nasal tobacco snuff for five to ten years made up this group.

2.4 Sample Collection

Each volunteer in the control and experimental groups had their blood drawn using the venepuncture procedure, yielding a sample of 5 ml, which was then placed into the appropriate lithium heparin anti-coagulated bottles.

To prevent clotting, each sample was thoroughly blended and spun at 2,500 revolutions/minute to obtain the plasma used to measure the levels of the following biochemical markers: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine, urea, lead, cadmium, malondialdehyde, glutathione peroxidase, 2-hydroxy-deoxyguanosine, troponin-1, creatinekinase-MB, C-reactive protein and interleukin-6

2.5 Laboratory Analysis

2.5.1 Measurement of Alanine Aminotransferase

The United Kingdom-based Randox Laboratories Limited colorimetric method, as modified by Egoro *et al.* (2022) was used.

2.5.2 Aspartate Aminotransferase Concentration Measurement

The Randox Laboratories Limited United Kingdom stated colorimetric technique as adopted by Ilegbedion and Egoro (2020) was used.

2.5.3 Alkaline Phosphatase Measurement

The colorimetric technique described by Randox Laboratory Limited, United Kingdom and subsequently modified by Egoro (2020) was used.

2.5.4 Creatinine Measurement

The described version of Randox Laboratories Limited's Jaffe reaction method modified by Tonbra *et al.* (2021) was used.

2.5.6 Measurement of Urea

The Urease Berthelot method was adopted by Emmanuel *et al.* (2021) after being originally described by Randox Laboratories, United Kingdom.

2.5.7 Measurement of Lead

The solar thermo-elemental model SE-71906's described atomic absorption spectroscopy technique and modified by Emmanuel *et al*. (2023) was utilized.

2.5.8 Measurement of Cadmium

The solar thermo-elemental model SE-71906's atomic absorption spectroscopy approach as modified by Emmanuel *et al.* (2023) was employed.

2.5.9 Measurement of Malondialdehyde

The described Thiobarbituric acid method as modified by Emmanuel *et al.* (2023) was utilized

2.5.10 Measurement of Glutathione Peroxidase

The Immuno-inhibitory approach as described by Atlas Medical unit, 4, William James House, Cowky Road, Cambridge CB40WX and further modified by Emmanuel *et al.* (2023) was used

2.5.11 Measurement of 2-Hydroxy-Deoxyguanosine

The enzyme linked immunesorbent assay (ELISA) method described by Randox Laboratories Limited, United Kingdom as modified by Catherine *et al.* (2016) was adopted

2.5.12 Measurement of Troponin-1

The dual vial liquid stable immunological turbidimetric method of the cardiac troponin-1 assay reagents described by Diazyme DZ 145A USA catalogue number and modified by Egoro (2022) was utilized.

2.5.13 Measurement of Creatinekinase-MB

The Atlas Medica immune-inhibito technique was used, with reagents produced by the Atlas Medical unit, 4, William James House, Cowky Road, Cambridge CB40WX, and modified by Emmanuel *et al.* (2023).

2.5.14 Measurement of C-Reactive Protein

A described version of the Spain-react Diagnostic Manual's latex turbidimetry method, as modified by Egoro (2023) was employed.

2.5.15 Measurement of Interleukin-6

The earlier described Elascience method with the catalog number E-EL- HO. 102 and modified subsequently by Emmanuel *et al.* (2023) was adopted.

2.6 Statistical Analysis

For the statistical analysis, data were divided into control and experimental groups and analysed using SPSS version 23.0. To evaluate the variations among the groups, an ANOVA was performed. The means and standard deviation of the data were then calculated, with a significance level of $p< 0.05$ being applied to mean differences across groups

2.7 RESULTS

In this study the mean values of the biochemical markers for hepato-renal, toxico-oxidative stress, and cardio-inflammatory disease were evaluated in 5–10 years old nasal tobacco snuffing addicts (the experimental group) in comparison to non-tobacco snuffers (the Control Group), as shown in Tables 1, 2, and 3, respectively.

Table 1: Results of mean ± SD of Hepato-Renal Biochemical Markers in Nasal Tobacco Snuffing Addicts (Experimental Group) Compared with Non-Tobacco Snuffers (Control Group)

Parameters	Control (n=53)	Experimental (n=53)	F-value	p-value	Remark
ALT (U/I)	4.02 ± 0.15	38.27 ±2.17	48.72	0.01	S
AST (U/I)	3.91 ±0.12	27.11 ±1.91	40.81	0.01	S
ALP (U/I)	17.81 ±1.15	49.85 ±3.65	38.02	0.01	S
Creat (µmol/L)	60.12 ±3.17	97.42 ±2.96	102.44	0.01	S
Urea (mmol/L)	3.78 ±1.14	11.20 ±1.80	47.32	0.01	S

KEYS: Values are in means ± Standard deviation (SD), ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, Creat = Creatinine, n = number of volunteers, S = statistically significant

Table 2: Results of mean ± SD of Toxico-Oxidative Stress Biochemical Markers in Nasal Tobacco Snuffing Addicts (Experimental Group) Compared with Non-Tobacco Snuffers (Control Group)

Parameters	Control (n=53)	Experimental (n=53)	F-value	p-value	Remark
Pb (x10-^{2}) ppm	0.02 ±0.01	7.80 ±1.42	107.21	0.02	S
Cd (x10-^{2}) ppm	0.03 ±0.01	6.52 ±1.21	110.51	0.02	S
MDA (µmol/L)	2.10 ±0.12	15.27 ±1.81	60.81	0.01	S
GPx (µmol/L)	2.71 ±0.20	12.72 ±2.10	50.73	0.01	S
2-OHdG (ng/ml)	2.25 ±0.71	2.27 ±0.74	0.18	0.82	NS

KEYS: Pb = Lead, Cd = Cadmium, MDA = Malondialdehyde, GPx t= Glutathione peroxidase, n = Number of volunteers, S = Statistically significant, NS = Not statistically significant

Table 3: Results of mean ± SD of Cardio-Inflammatory Biochemical Markers in Nasal Tobacco Snuffing Addicts (Experimental Group) Compared with Non-Tobacco Snuffers (Control Group)

Parameters	Control (n=53)	Experimental (n=53)	F-value	p-value	Remark
Troponin-1 (×10-$^{2)}$ IU/L	1.22±0.27	1.25 ±0.28	0.21	0.90	N S
CKMB (IU/L)	5.71±1.02	5.74 ±1.05	0.12	0.81	N S
CRP (mg/L)	2.40 ±0.15	27.65 ±2.72	78.35	0.02	S
IL-6 (pg/ml)	11.80 ±1.46	29.71 ±2.10	69.71	0.02	S

KEYS: CKMB = Creatinekinase-MB, CRP = C-reactive protein, IL-6 = Interleukin-6, n = number of volunteers, S = statistically significant, NS = not statistically significant

DISCUSSION

The results of the hepato-renal biochemical markers in Table 1, showed that the experimental group had significantly higher mean levels of alanine aminotransferase (p=0.01), aspartate aminotransferase (p=0.01), alkaline phosphatase (p=0.01), (hepatic biochemical markers), creatinine (p=0.01), and urea (p=0.01) (renal biochemical markers) than the volunteers in the control group. The considerable increases in the hepatic enzymes which may be suggestive of hepatic disorder due to the prolonged sniffing of toxic heavy metals such as lead and cadmium which are one of the compositions of snuff could have triggered liver injury, thus leading to the release of these enzymes from the liver into the plasma, these toxic heavy metals may also trigger kidney damage after being filtered in the glomerulus and subsequently reabsorbed by the proximal convoluted tubule cells where free radicals that initiate the apoptosis process of

the kidneys are produced. These findings are consistent with the earlier study by Musa *et al.* (2022).

The results in Table 2 showed that the mean levels of lead (p=0.02), cadmium (p=0.02), malondialdehyde (p=0.01), and glutathione peroxidase (p=0.01) were significantly higher in the experimental volunteers than they were in the control group. The significant increase in both lead and cadmium may be suggestive of the prolonged snuffing of the dry tobacco (snuff) by these addicts which may have led to the build-up and subsequent bioaccumulation of these toxic heavy metals (lead and cadmium) which are non-biodegradable in their body. These findings are in agreement with the past work of Owusu-Asante *et al.* (2022). The significant elevation of malondialdehyde may be suggestive of an increased production of free radicals as a result of the prolonged snuffing of dry tobacco (snuff) which contains some toxic substances such as lead, cadmium etc. The significant elevation of glutathione peroxidase in the snuffing addicts may be attributed to the first line defence action of this anti-oxidant in a bid to inhibit the excessive production of free radicals which may lead to gross oxidative damage due to their ability to subdue the body cell defence system. These large elevations in the mean values of both malondialdehyde and glutathione peroxidase correspond with earlier research by Kiline *et al.* (2004). However, when compared to the control group, the mean values of 2-hydroxy-deoxyguanosine in the experimental group were not statistically different (p=0.82). This was as found in this study.

In Table 3, the results revealed that C-reactive protein (p=0.02) and interleukin-6 (p=0.02) levels were found to be significantly elevated in the experimental group, indicating inflammation contrary to that of the control group. This large increase, which may be related to an inflammatory response of the lungs to toxins like lead and cadmium contained in dry tobacco, is consistent with earlier research by Sushobhan *et al.* (2016). However, in comparison of the mean values of the experimental group to the control group, the results of the cardiac biochemical markers showed no appreciable increases in troponin-1 (p= 0.90) and creatinekinase-MB (p= 0.81). This finding which may be suggestive of normal cardiac function is contrary to the previous work reported in www.mayoclinic.org art-20047428.

CONCLUSION

In conclusion, 5–10 years old nasal tobacco snuffing addicts, may develop hepato-renal, toxico-oxidative stress, and inflammatory problems

Competing Interest

No competing interest

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REFERENCES

Adias, T. C., Anslem, O. A., Tosan, A. E., Babatunde, I. A. and Celestine, C. A. (2014). Effect of sub-lethal doses of smokeless tobacco (snuff) on some haemato-rheological parameters using albino wistar rats. American Journal of Medical Sciences and Medicine. 2 (3), 54-57.

Borgerding, M.F., Bodnar, J.A. Curtin, G.M. and Swauger, J.E. (2012). The chemical composition of smokeless tobacco: A survey of products sold in the United States in 2006 and 2007. Regulatory Toxicology Pharmacology. 64, 367-368.

Catherine, N.B., Mariska, B., Peter, G.S. and Brenda, W.J.H.P. (2016). Sociodemographic and lifestyle determinants of plasma oxidative stress marker 8-OHdG. Oxidative Medicine and Cellular Longevity. 1-10

Egoro, E.T. (2020). Effects of indiscriminate consumption of paracetamol (acetaminophen) on selected biochemical parameters in Rattus norvegicus rats. GSC Biological and Pharmaceutical Science. 13(03), 054-063:

Egoro, E.T. (2022). Symptoms and some vital organs biochemical evaluation among sawmill operators in Amassoma Bayelsa State Nigeria. Nigerian Journal of Scientific Research. 21(2), 496-501.

Egoro, E.T. (2023). Status of some biochemical markers in Sprague dowley rats following sub-chronic consumption of alum treated water. Sokoto Journal of Medical Laboratory Science. 8(1), 116-122.

Egoro, E.T., Ilegbedion, I.G. and Ogenerobo, E.V. (2022). Dexamethasone induces biochemical and histomorphological changes in hepato-renal system of Norway rats (*Rattus norvegicus*). Nigerian Journal of Scientific Research. 21(1), 291-298.

Emmanuel, T.E., Emmanuel, S.O. and Jonathan, E.L. (2021). Occupational effect of spray painting fumes on some biochemical parameters in automobile spray painters in Yenagoa Bayelsa State Nigeria. World Journal of Advanced Research and Reviews. 09(02), 038-044

Emmanuel, T. E., Godwin, I. I., Emmanuel, S. O. and Hope, C. (2023). Assessment of some toxico-inflammatory, hepato-renal and cardio-oxidative stress biomarkers among waste pickers in Ajegunle Lagos State, Nigeria, GSC Advanced Research and Reviews. 16(03), 111-119.

Gilman, S.L. and Xun, Z. (2004). Smoke-A Global History of Smoking (pp.). The University of Chicago Press, Cicago, IL, USA

Ilegbedion, I.G. and Egoro, E.T. (2020). Effects of Dissostis rotundofolia stem extract consumption on some Biochemical parameters and hepato-renal organs in Rattus norvegicus rats. Sokoto Journal of Medical Laboratory Science 5940, 35-40.

Kiline, M.M. Okur, E., Kurutas, E.B., Giler, F.I. and Yildirim, I. (2004). The effect of maras powder (smokeless tobacco) on oxidative stress in users. Cell Biochemistry Function. 22, 223-236.

McAdam, K. (2017). Comprehensive survey of radionuclides in contemporary smokeless tobacco products. Chemistry Central Journal. 11: 131.

Mesembe, O., Bisong S., Ekong,M. and Ekeema, A. (2008). Neurobehavioral activity in albino wistar rats. The open field maze following longterm tobacco diet ingestion. The Internet Journal of Neurology. 10(2), 345-363.

Musa, A. H., Abdulmajid, I. A., Haruna, B. A. and Ismaila, J. (2022). Effect of chronic consumption of smokeless tobacco (snuff) on liver enzymes of males in Maiduguri, Northeast Nigeria. Sokoto Journal of Medical Laboratory Science. 7(1), 107-114

Omotoso, G.O., Kadir, R.E., Alabi, J.T., Alabi, A.S. and Oyabambi, A.O. (2013). Exposure to cigarette smoke altered the cytoarchitecture and anti-oxidant activity of the frontal cortex in wistar rats. International Journal of Biology and Chemical Science. 7(4), 1595-1601.

Owusu-Asante, O.J., Mensah, P., Duah-Gyamfi, A., Owusu, J., Minkah, E., Wumbeidow, H., Ibrahim, S., Ofori, E. and Nyarko, D.E. (2022). Assessment of arsenic, cadmium and lead in snuff in Ashanti region of Ghana. African Journal of Chemical Education. 12(2), 41-59.

Sinha, D.N. (2018). Global burden of all cause and cause specific mortality due to smokeless tobacco use: Systematic review and meta-analysis. Tobacco Control. 27, 35-42.

Tonbra, E.E., Remi, R.A.R. and Godwin, II (2021). Comparative alteration of some biochemical parameters in Rattus norvegicus rats following flunitrazepam administration. International Journal of Medical Laboratory Research. 5(3), 12-18.

Ukoha, U., Uchechukwu, D. and Stephen, M. (2014). The effect of sub-lethal doses of smokeless tobacco (snuff) on certain haematological and haemostatic parameters in wistar rats. Journal of Experimental and Integrative Medicine. 2(3), 225-230.

Villegier, A.S., Blanc, G., Glownski, J. and Tassin, J.P. (2013). Transient behavioural sensitization to nicotine becomes long-lasting with monoamine oxidases inhibitors. Pharmacology Biochemistry Behaviour. 76(2), 267-274.

www.mayoclinic.org art-20047428

Sushobhan, B, Krishnendu,, M., Ujjal, D, Amitava, K., Anirban, P., Aaveri, S., Surajit, B., Saurabh, G. and Sanjit, D. (2016). Smokeless tobacco consumption impedes metabolic, cellular, apoptotic and systemic stress pattern: A study on Government employees in Kolkata India. Scientific Report. 5, 18284