Supported By
tetfund
TERTIARY EDUCATION TRUST FUND

# EFFECT OF SPECIAL MORINGA SUNDU AND TOBACCO SNUFF ON HEMATOLOGICAL PARAMETERS IN WISTER RATS

**Bawa Y. Muhammad [1] Sulaiman A. Barau [2*] Moses Z. Zaruwa [1] R. Maikefi[3]**
[1]Department of Biochemistry and Molecular Biology, Faculty of Natural and Applied Sciences, Nasarawa State University Keffi, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Jos, Jos Nigeria
[3]Department of Political Science, Faculty of Social Sciences, Nasarawa State University Keffi, Nigeria.
*Corresponding author: barausulaimana@gmail.com Mobile: (+234) 7069658575

**Received: December 13, 2021 Accepted: February 20, 2022**

**ABSTRACT** Besides smoking, tobacco and other products derived from it are widely consumed orally and intranasally without burning in form of different kinds of smokeless tobacco as alternative for nicotine delivery. This research was aimed at investigating the effect of two brands of snuff on hematological parameters in Wister rats. 30 rats were randomly divided into 5 groups. Group1 (control); received only distilled water. Group 2 and 3 (received 4mg and 2mg/kg b.w.t of tobacco, TBC, respectively). Group 4 and 5 (received 5mg and 2.5mg/kg b.w.t special Moringa sundu, SMS, respectively). After two months of treatment, the rats were anesthetized and blood sample collected via heart puncture. Result revealed significant decrease ($p<0.05$) in WBC count, PCV and platelet among group 2, 4 and 5 in relation to the control with a significant increase ($p<0.05$) in group 3. Hb (g/dL) level significantly decreases between group 2, 4 and 5 while it increases significantly in group 3 compared to the control. Lymphocyte and monocyte level increases significantly across all the groups compared to control. Group 2 demonstrate no significant increase ($p>0.05$) in level of eosinophil while group 3 and 5 indicate no significant decrease compared to control. There was significant elevation ($p<0.05$) in level of neutrophil in group 2, 3 and 4 compared to control with no significant reduction in group 5. Anemia, bone marrow suppression and hematophagocytosis are likely the influence of the two snuff brands on hematological parameters. This can adversely affect its users by inducing acute and chronic inflammation.

Keywords: hematological parameters, smokeless tobacco, snuff, tobacco, Moringa

## Introduction

Besides smoking, tobacco and other products derived from it are widely consumed orally and intranasally without burning in form of different kinds of smokeless tobacco (Klus *et al.*, 2009). Smokeless tobaccos do not generate combustion and pyrolysis products; such as polycyclic aromatic hydrocarbons (PAHs), carbon monoxide and nitric oxide produced by tobacco burning during puffing (Klus *et al.*, 2009). This could be the reason adduced for its popularity as alternative means of nicotine delivery to the system. There exist different kinds of smokeless tobacco, ranging from products for sniffing; which includes U.S dry snuff and European snuff; products for sucking; such as U.S moist snuff and Swedish moist snuff; and product for chewing; such as plug, twist, loose leaf and roll (Klus *et al.*, 2009; NCI and CDC, 2014).

In addition, another form of snuff purportedly made from Moringa plant and perceived to possess medicinal potentials such as relief of headache, toothache, sinus problem, nasal congestion and aphrodisiac effect among male users is now widely consumed in Nigeria by people aged 15 years and older. Like other forms of smokeless tobacco, Hajiya Aisha special Moringa sundu, Hajiya Aisha manpower, AK47, Burutai, Tobacco snuff, and many others, are other variants of snuff that is gaining popularity among youth as well as aged men and commonly sniffed and dipped typically for its purported medicinal activity which includes treatment of erectile dysfunction, enhancement of sexual performance and vision, penile enlargement, relieving tiredness, fever and back pain. One of the reason adduced for its popularity is the purported claim by its users and vendors that it is made from moringa (Muhammad *et al.*, 2021).

Snuff consumption have posed a great deal of danger and widespread challenge to public health and has gained limited attention from researchers and policymakers with limited data on its use, specifically in Sub-Saharan Africa, with focus largely being on cigarettes (Desalu *et al.*, 2010; NCI and CDC, 2014).

A review by International Agency for Research on Cancer (IARC) 2004 reveals based on epidemiological

and laboratory studies, that smokeless tobacco causes oral cancer, esophageal cancer, and pancreatic cancer in humans (NCI, 1993; NCI and CDC, 2014). Over 30 smokeless tobacco carcinogens have been identified, including tobacco-specific nitrosamines (TSNAs), which cause tumors affecting the nasal cavity, lung, trachea, pancreas, liver, and esophagus in animal models; in addition to causing oral adverse effects such as oral mucosal lesions, leukoplakia, and periodontal disease (NCI, 1993; WHO, 2009; World Health Organization, 2009; International Institute for Population Sciences, 2010).

Therefore, this study is aimed at investigating the effects of 2 brands of smokeless tobacco (special Moringa sundu and tobacco) on some hematological parameters in Wister rats.

**Materials and Methods**

**Smokeless Tobacco Samples and Preparation**

Special Moringa sundu and Tobacco snuff were obtained from a local vendor at Keffi main market, Nasarawa state, Nigeria. Snuff solutions were prepared by dissolving 1 g of snuff in 1000 mL distilled water.

**Experimental Design**

30 Wister albino rats (110-120 g) obtained from National Veterinary Research Institute (NVRI) VOM, Plateau state, were housed in cleaned plastic cages and bedded with clean rice husks. Animals were fed with grower's mesh (vital feed) and water for 2 weeks to acclimatize to the new laboratory condition. Afterward, they were weighed and divided randomly into 5 groups.

Group 1 (control) received only distilled water

Group 2 received 5 mg/kg b.w.t of special Moringa sundu (SMS)

Group 3 received 2.5 mg/kg b.w.t of SMS

Group 4 received 4 mg/kg b.w.t of tobacco snuff (TBC)

Group 5 received 2 mg/kg b.w.t of TBC.

The animals were treated with the snuff solutions by oral gavage for 2 months and allowed access to feed and water *ad libitium*.

**Blood Sample Collection**

At the end of the treatment period, the animals were weighed, anesthetized and blood sample collected via heart puncture in EDTA tubes. The blood samples were analyzed using Beckman Coulter DxH 900 hematology analyzer.

**Results**

Result shows significant decrease ($p<0.05$) in WBC count, PCV and platelets among group 2, 4 and 5 in relation to the control with a significant increase ($p<0.05$) in group 3 (Table 1). Hb (g/dL) level significantly decreases between group 2, 4 and 5, while it increases significantly in group 3 compared to the control (Table 1). Lymphocytes and monocytes level increases significantly across all the groups compared to control (Table 2). Group 2 demonstrates no significant increase ($p>0.05$) in level of eosinophils while group 3 and 5 indicate no significant decrease compared to control. There was significant elevation ($p<0.05$) in level of neutrophils in group 2, 3 and 4 compared to control with no significant reduction in group 5 (Table 5).

| Group | WBC x10$^3$/mm$^3$ | PCV % | PLT x10$^3$/mm$^3$ | Hb g/dL |
|---|---|---|---|---|
| 1 (Control) | 10.42±1.56 | 44.50±1.87 | 228.17±42.57 | 14.58±0.79 |
| 2 (TBCL) | 8.55±1.18* | 40.75±3.09* | 220.00±18.46* | 13.20±0.67* |
| 3 (TBCH.) | 13.17±0.57* | 48.67±5.51* | 296.00±9.85* | 15.87±1.70* |
| 4 (SMSL) | 7.65±0.39* | 44.34±3.78* | 220.17±42.43* | 14.24±1.01* |
| 5 SMSH | 3.90±1.66* | 37.25±2.22* | 216.25±11.30* | 11.73±0.75* |

**Table 1: Effects of TBC and SMS on some hematological parameters**

Results are expressed as Mean ± SD, values with asterisk (*) are significantly different from the control ($p<0.05$). Groups 1: control; 2, TBCL: Tobacco low dose; 3, TBCH: Tobacco high dose; 4, SMSL: special moringa sundu low dose; 5, SMSH: special moringa sundu high dose, WBC= white blood cells, PCV= Packed cell volume, PLT= platelets, Hb= hemoglobin.

**Table 2: Effects of TBC and SMS on white blood cells**

| Groups | LYMP% | MON% | E0S% | NEU% | BAS% |
|---|---|---|---|---|---|
| **1 Control** | 65.75±6.23 | 4.17±0.98 | 1.17±0.75 | 21.67±5.16 | 0.00±0.00 |
| **2 TBCL** | 67.33±4.73* | 5.00±3.56* | 1.75±0.50 | 27.50±9.11* | 0.00±0.00 |
| **3 TBCH** | 70.83±4.22* | 5.67±1.53* | 1.00±0.00 | 26.00±6.08* | 0.00±0.00 |
| **4 SMSL** | 68.00±5.09* | 6.33±2.50* | 1.17±0.75 | 26.67±5.50* | 0.00±0.00 |
| **5 SMSH** | 71.25±8.61* | 6.50±1.29* | 1.00±0.82 | 21.25±9.39 | 0.00±0.00 |

Results are expressed as Mean ± SD, values with asterisk (*) are significantly different from the control ($p<0.05$). Groups 1: control; 2, TBCL: Tobacco low dose; 3, TBCH: Tobacco high dose; 4, SMSL: special moringa sundu low dose; 5, SMSH: special moringa sundu high dose, LYMP= lymphocytes, MON= monocytes, EOS= eosinophils, NEU= neutrophils, BAS= basophils.

**Discussion**

Our results demonstrated a significant decrease in hemoglobin and PCV level among group of rats treated with snuff in comparison with non-treated groups (control). Our findings are in conformity with those of Shaik and co-authors (2021), Shukla et al (2019), Rajasekhar et al, 2007 and Kılınç et al., (2004) (Kılınç *et al.*, 2004; Rajasekhar *et al.*, 2007; Shukla *et al.*, 2019; Shaik *et al.*, 2020) which revealed similar decrease in hemoglobin level of some smokeless tobacco users. Low PCV and hemoglobin levels may be suggestive of erythrocytes destruction, suppression of erythropoiesis or iron, folic acid and vitamin $B_{12}$ deficiency, which may arise when the vitamins are utilized as coenzymes or iron (as cofactor) in the metabolism of the snuffs. Iron, folic acid and vitamin $B_{12}$ play a critical role in erythropoiesis. Folic acid and vitamin $B_{12}$ are required by erythroblasts for their

proliferation during differentiation, and the deficiency of these vitamins results in inhibition of purine and thymidylate biosynthesis, impairment of DNA replication, erythroblast apoptosis, consequently, leading to anemia from ineffective erythropiesis (Koury and Ponka, 2004). Furthermore, large amount of iron, an essential component of hemoglobin, is required for hemoglobin biosynthesis by erythroblasts, the deficiency of which cause less hemoglobin formation in developing erythroid cells, leading to synthesis of microcytic erythrocytes that contain hypochromic hemoglobin (Koury and Ponka, 2004).

The concentration of platelet in rats treated with the snuff solution was significantly lowered when compared to the control. Similar result was reported by (Kılınç *et al.*, 2004; Shukla *et al.*, 2019). The observed lower level of platelets (thrombocytes) could indicate thrombocyte destruction and perhaps, suppression of bone marrow, thereby halting thrombopoiesis, which may be elicited by constituents of the snuff. Consequently, this may interfere with normal blood clotting processes and place the users of snuff at risk of excessing bleeding.

Our investigation revealed significantly higher concentration of white blood cell in treated groups in compared to control group, indicating an association between snuff (smokeless tobacco) use and decrease in white blood cells count. This is in agreement with the work of (Memon *et al.*, 2021). In contrast, Rajasekhar and co-authors and (Kılınç *et al.*, 2004) reported contrary findings.

The present study indicated significantly elevated level of lymphocytes, monocytes and neutrophils. Higher monocyte and neutrophil concentration could be indicative of inflammatory reactions, likely stimulated by microbial loads presence in the snuff which play a prominent role in fermentation and aging during the process of snuff production. During fermentation (bacteria-mediated reactions), a portion of nitrate in fire-cured tobacco is converted to nitrite, which then reacts with alkaloids to produce tobacco-specific nitrosamines (TSNAs) (Benowitz and Gourlay, 1997; IARC, 2007; Greer, 2011). Chemical markers indicative of bacterial and fungal growth have been identified in tobacco of various types and at various stages of production (Ayo-Yusuf and Omole, 2008; Pandey *et al.*, 2009). Various bacteria have been identified that are capable of converting nitrate to nitrite (nitrate reduction) in tobacco or tobacco products; these include: *Bacillus*, *Enterobacter*, *Staphylococcus*, *Corynebacterium*, *Clostridium*, *Serratia*, and *Escherichia* species (Benowitz and Gourlay, 1997; Gupta *et al.*, 2007; Yatsuya, Folsom and Investigators, 2010; Greer, 2011), as well as several genera of fungi, such as *Cladosporium*, *Alternaria*, *Candida*, *Fusarium*, *Aspergillus*, and *Acremonium* (Benowitz and Gourlay, 1997; Pandey *et al.*, 2009; Mushtaq *et al.*, 2010; Yatsuya, Folsom and Investigators, 2010). The combined presence of these pool of microbes could provoke inflammatory response, leading to concomitant elevation in level of monocytes and neutrophils. It could also be the result of tissue lesion caused by other constituents of the snuff.

**Conclusion**

The study revealed that anemia, bone marrow suppression and hematophagocytosis are likely the influence of the two snuff brands on hematological parameters, therefore, this can adversely affect its users by exposing them to acute and chronic inflammation.

**Acknowledgement**
We acknowledge the support given to us by the Department of Biochemistry and Molecular Biology Nasarawa State University Keffi, Nigeria.

**Conflict of Interest**
Authors declared no conflict of interest

**Disclosure**: The authors have no financial interest to declare in relation to the content of this article.

**Funding**
This work is part of the project financially supported by TETFUND through Institutional Based Research grant 2020.

## References

Ayo-Yusuf, O. . and Omole, O. B. (2008) 'Snuff use and the risk for hypertension among black South African women', *S.A. Fam. Pract.*, 50(2), pp. 64–64.

Benowitz, N. and Gourlay, S. G. (1997) 'Cardiovascular toxicity of nicotine: implications for nicotine replacement therapy', *JACC*, 29(7), pp. 1422–31.

Desalu, O. . *et al.* (2010) 'Smokeless Tobacco Use in Adult Nigerian Population', *Nigerian Journal of Clinical Practice*, 13(4).

Greer, R. J. (2011) 'Oral Manifestations of Smokeless Tobacco Use', *Otolaryngol .Clin. North Amer.*, 41(1), pp. 31–56.

Gupta, B. K. *et al.* (2007) 'Cardiovascular risk factors in tobacco-chewers: a controlled study', *J. Assoc. Physicians India*, 55, pp. 27–31.

IARC (2007) 'Smokeless Tobacco and Some Tobacco-Specific N-Nitrosamines. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2007;89: 1–592', *IARC Monogr Eval Carcinog Risks Hum*, 83, pp. 1–592.

International Institute for Population Sciences, I. (2010) *Global Adult Tobacco Survey (GATS)*. New Delhi, India. Available at: http://www.searo.who.int/LinkFiles/Regional_Tobacco_Surveillance_System_GATS_India.pdf.

Kılınç, M. *et al.* (2004) 'The investigation of the effect of Marafl powder (smokeless tobacco) on hematological parameters.', *Turkish journal of haematology: official journal of Turkish Society of Haematology*, 21(3), pp. 131–136.

Klus, H. *et al.* (2009) 'Smokeless Tobacco – An Overview *', *Contributions to Tobacco Research*, 23(5), pp. 248–276. doi: 10.2478/cttr-2013-0865.

Koury, M. J. and Ponka, P. (2004) 'New insights into erythropoiesis: the roles of folate, vitamin B12, and iron', *Annu. Rev. Nutr.*, 24, pp. 105–131.

Memon, S. M. *et al.* (2021) 'Evaluation of C-reactive protein and hematological parameters in smokeless tobacco users: A comparative cross-sectional study', *Pakistan Journal of Medical Sciences*, 37(4).

Muhammad, B. Y. *et al.* (2021) 'Survey of Snuff Use and Preliminary Study of Effect of Two Brands on, Brain Antioxidants and Acetylcholinestrase Enzyme of Wister Albino Rats', *Al-Azhar International Medical Journal*, 2(3), pp. 60–66.

Mushtaq, N. *et al.* (2010) 'Smokeless tobacco and prevalence of cardiovascular disease', *J. Okla State Med. Assoc.*, 103(11–12), pp. 539–544.

NCI, N. C. I. (1993) *Smokeless Tobacco or Health: an International Perspective*. Smoking an. Washington, DC, USA: NIH publication no. 93-3461.

NCI, N. C. I. and CDC, C. for D. C. and P. (2014) *Smokeless Tobacco and Public Health: A Global Perspective*. Edited by S. A. Dorothy K. Hatsukami, Mitch Zeller, Prakash Gupta, Mark Parascandola.

Pandey, A. *et al.* (2009) 'Association of exclusive smokeless tobacco consumption with hypertension in an adult male rural population of India', *Tob. Induc. Dis.*, 5(15). doi: 10.1186/1617-9625-5-15.

Rajasekhar, G. *et al.* (2007) 'Some hematological and biochemical parameters in smokeless tobacco (Jharda) chewers', *African Journal of Biotechnology*, 6(1).

Shaik, F. B. *et al.* (2020) 'Correlation between smokeless tobacco (Gutkha) and biomarkers of oxidative stress in plasma with cardiovascular effects', *Heliyon*, 7(2). doi: https://doi.org/10.1016/j.heliyon.2020.e05487.

Shukla, A. K. *et al.* (2019) 'Smokeless Tobacco and Its Adverse Effects on Hematological Parameters: A Cross-Sectional Study', *Advances in preventive medicine*, 2019.

WHO, W. H. O. (2009) *Global Adult Tobacco Survey: Bangladesh Report*. Dhaka, Bangladesh. Available at: http://www.who.int/tobacco/surveillance/global_adult_tobacco_survey_bangladesh_report_2009.pd.

World Health Organization, W. (2009) *Noncommunicable disease Risk Factor Survey*. Myanmar, US.

Yatsuya, H., Folsom, A. R. and Investigators, A. (2010) 'Risk of incident cardiovascular disease among users of smokeless tobacco in the Atherosclerosis Risk in Communities (ARIC) study', *Am. J. Epidemiol.*, 172(5), pp. 600–605.