International Journal of Clinical Biochemistry and Research 2023;10(2):171–175

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International Journal of Clinical Biochemistry and Research

Journal homepage: https://www.ijcbr.in/

Original Research Article

# Evaluation of c-reactive protein level, antioxidant and acetylcholinesterase enzyme activities among snuff users

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ARTICLE INFO

*Article history:*
Received 02-06-2023
Accepted 15-06-2023
Available online 14-07-2023

*Keywords:*
Acetylcholinesterase
Neurodegeneration
Nicotine
Snuff
Tobacco

ABSTRACT

The use of snuff in Nigeria especially in the North has increased alarmingly. It is no longer considered a health risk nor is it seen as morally questionable. This research was designed to investigate the effect of snuff on antioxidant activities and cognitive function on human participants. A total of 200 volunteers were divided into 4 groups based on their periods of exposure to snuff; the Control. Group 1 (1-3 years), Group 2 (3-5 years) and Group 3 (5 years and above). All these groups consist of 50 participants each. The groups were all tested for BP and Glucose levels using standard procedure before and after they were administered snuff. Blood samples were then collected. Assessments of SOD, GPx, CAT, MDA, AChE and C-reactive protein were carried out using standard methods. The result revealed a significant decrease in SOD, GPx and CAT activities and a decrease in MDA level among snuff users compared to the control. An increase in AChE activity and in C-reactive protein level were also recorded. These findings suggest that nicotine in snuff may induce oxidative stress and inflammation, and rapid degradation of acetylcholine.



## 1. Introduction

Snuff which is also sometimes called smokeless tobacco is widely consumed in many parts of Africa (Muhammad et al., 2021).[1] It is mostly made of ground tobacco that is consumed by sniffing. Most of the smokeless tobacco is consumed in countries like Pakistan, Iran and many parts of Africa (Warigon, 2019).[2] In Nigeria alone, it is believed that over 16,000 people are affected and whose lives have been irrevocably damaged due to excessive consumption of snuff (Warigon, 2019).[2] Consumption of snuff has many effects on the body. Although the smokeless nature of snuff reduces the risk of direct exposure to the lungs, there are other dangerous side effects caused by the intake of snuff some of which include nicotine exposure and the effects of tobacco-specific nitrosamines on the body (Muhammad et al., 2021).[1]

Snuff is grounded, cut or powdered tobacco that is consumed orally or via the nasal cavity/inhalation. This product, although may not contain smoke, contains nicotine just like regular tobacco. Snuff consumers believe it is a healthy alternative to regular tobacco because they believe that the absence of smoke makes it safer (Thomson et al., 2014).[3] Believing that they are not exposed to the toxic combustion from smoke that usually endangers and could potentially damage the lungs, snuff users consume snuff with no knowledge of how damaging it can be as well (Thomson et al., 2014).[3] The presence of nicotine and tobacco-specific nitrosamines makes snuff as dangerous as many other nicotine-containing products (Thomson et al., 2014).[3]

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https://doi.org/10.18231/j.ijcbr.2023.029

Tobacco is prepared by drying the leaves under the sun, and ground to fine powder, the powder is then mixed with calcium oxide and calcium carbonate primarily gotten from wood ash. They are also often mixed with flavours such as menthol and cardamom before being packed in polythene bags as tiny as tea bags. Snuff is sold in local stores under different trade names depending on the flavour or the mix. The grounded tobacco is inhaled while the chewable tobacco is placed under the tongue or between the gingival cavities. It is then spat out after it loses its flavour; this usually takes about 45 minutes (Welch, 2008).[4] The use of snuff in Nigeria especially in the North has increased alarmingly. It is no longer considered a health risk nor is it seen as morally questionable. The aim of this research is to evaluate the level of c-reactive protein, antioxidants and acetylcholinesterase enzyme among snuff users in Keffi metropolis, Nasarawa state, Nigeria.

## 2. Materials and Methods

### *2.1. Snuff samples, composition and preparation*

Snuff samples were purchased from Keffi Market, packaged and ready for use. According to the manufacturers, the snuff is composed of moringa, menthol and tobacco.

Inclusion criteria includes healthy adult male between the ages of 18-40, without high blood sugar, high blood pressure or currently undergoing treatment for such diseases. The exclusion criteria include the sick, elderly, non-smokers, women, children under 18 and those who did not give consent.

### *2.2. Experimental design*

The experimental design was in two phases: The first phase involved the use of questionnaires to obtain relevant information from the participants. Inclusion criteria includes healthy adult male between the ages of 18-40, without high blood sugar, high blood pressure or currently undergoing treatment for such diseases. The exclusion criteria include the sick, elderly, non-smokers, women, children under 18 and those who did not give consent.

The second phase of this research was approved by the Ethical Committee, Nasarawa State University Keffi, South Atlantic Petroleum Medical Centre on the $9^{th}$ of February, 2022. 200 participants were enrolled in the study after signing an informed consent form.

The participants were divided into 4 groups:

Group 1(control) consists of 90 non-smokers, non-users of snuff.

Group 2 consists of 90 users of snuff with a usage duration of 1 to 3 years.

Group 3 consists of 90 snuff users with a usage duration of 3 to 5 years.

Group 4 consists of 90 users of snuff with a usage duration of 5 years and above.

The participants' blood glucose levels, blood pressure and pulse were taken before the experiment's commencement. Afterward, participants were given snuff samples to sniff. After 30 minutes, the tests for the level of glucose, blood pressure and pulse were repeated and recorded.

### *2.3. Sample size determination*

The sample size was determined using the sample size determination formula. The sample size defines the number of individuals included in a research study to represent a population included in a study often categorized into age, gender and location. Sample size was calculated using confidence level, standard deviation and confidence interval. The confidence level was 95% which was converted to a z-score of 1.96. The standard deviation or deviation from the mean was 0.5 and the confidence interval chosen was ±5%.

Sample size = z-score^2 x Standard deviation x 1-Standard deviation/Confidence interval

Sample size = 1.96^2 x 0.5 x 1-0.5/±5 = 384.16.

### *2.4. Blood sample collection*

Blood samples were obtained from the participants in a plain bottle, allowed to coagulate and centrifuged at 1200 rpm for 15 minutes. The supernatant was stored at -4°C before analysis.

### *2.5. Biochemical analysis*

The following parameters were assayed these include Superoxide dismutase, SOD, Glutathione peroxidease, GPx, Catalase, CAT, Malondealdehyde, MDA Acetylcholinesterase, AChE and C-Reactive protein.

## 3. Result

The Table 1 describes the levels of bp and glucose 30 minutes before and after the consumption of snuff. The result shows a significant increase in snuff users' blood pressure compared with the control.

Table 2 describes lipid peroxidation and the levels of antioxidant enzymes after snuff consumption. The table shows a decrease in the MDA level of snuff users when compared to the control. A significant decrease in the activities of antioxidant enzymes compared to the control was also observed.

The Table 3 describes the levels of AChE and C-reactive protein in snuff users. The result shows a significant increase in AChE level when compared to the control as well as a significant increase in C-reactive protein level when compared to the control.

**Table 1:** BP and glucose levels among snuff users

| | Blood Pressure (Systolic) (mm/hg) | | Blood Sugar (mmol/L) | |
|---|---|---|---|---|
| Groups | BP (Before) | BP (After) | Glu (Before) | Glu (After) |
| Control | $108.43 \pm 4.11^{b}$ | $108.43 \pm 4.11^{b}$ | $4.87 \pm 0.49^{c}$ | $4.87 \pm 0.49^{c}$ |
| 1-3 years | $107.37 \pm 2.25^{a}$ | $118.23 \pm 3.87^{a}$ | $4.88 \pm 0.50^{b}$ | $4.20 \pm 0.50^{a}$ |
| 3-5 years | $114.58 \pm 4.38^{a}$ | $121.18 \pm 5.65^{b}$ | $4.99 \pm 0.55^{b}$ | $4.49 \pm 0.54^{b}$ |
| Above 5 years | $116.27 \pm 4.72^{b}$ | $125.79 \pm 4.21^{a}$ | $5.04 \pm 0.42^{a}$ | $4.49 \pm 0.40^{a}$ |

Results are expressed as Mean ± SD and are significantly different at $p < 0.05$. Keys: concentration of BP = blood pressure and Glu = Glucose. Group 1 (Control) is 0-1 years. Group 2 – 1-3 years Group 3 – 3-5 years Group 4 – Above 5 years. The superscripts a, b and c indicate significant difference in the values.

**Table 2:** Level of antioxidant enzymes and MDA among snuff users

| Groups | MDA | CAT | SOD | GPx |
|---|---|---|---|---|
| Control | $11.25 \pm 0.98^{b}$ | $10.96 \pm 1.29^{b}$ | $40.10 \pm 4.18^{c}$ | $24.76 \pm 2.96^{c}$ |
| 1-3 years | $10.95 \pm 0.57^{a}$ | $10.02 \pm 0.95^{a}$ | $37.05 \pm 5.47^{b}$ | $21.86 \pm 2.78^{a}$ |
| 3-5 years | $10.74 \pm 0.78^{a}$ | $10.11 \pm 1.06^{a}$ | $37.50 \pm 5.17^{b}$ | $22.76 \pm 3.31^{b}$ |
| Above 5 years | $11.08 \pm 0.96^{b}$ | $9.99 \pm 1.08^{a}$ | $36.24 \pm 6.80^{a}$ | $21.60 \pm 1.96^{a}$ |

Results are expressed as Mean ± SD and are significantly different at $p < 0.05$. **Keys:** concentration of MDA = malondealdehyde, CAT = catalase, SOD = superoxide dismutase and GPx = glutathione peroxidase (reduced glutathione). Group 1 (Control) is 0-1 years. Group 2 – 1-3 years Group 3 – 3-5 years Group 4 – Above 5 years. The superscripts a, b and c indicate significant difference in the values

**Table 3:** Level of acetylcholinesterase enzyme and C-reactive protein among snuff users

| Groups | AChE (mmol/L) | C-Reactive proteins (mg/dL) |
|---|---|---|
| Control | $1.39 \pm 0.47^{a}$ | $28.13 \pm 5.14^{a}$ |
| 1-3 years | $1.81 \pm 0.46^{b}$ | $32.52 \pm 3.65^{b}$ |
| 3-5 years | $1.77 \pm 0.38^{b}$ | $33.64 \pm 4.56^{c}$ |
| Above 5 years | $1.76 \pm 0.45^{b}$ | $32.52 \pm 3.66^{b}$ |

Results are expressed as Mean ± SD and are significantly different at $p < 0.05$. **Keys:** concentration of ACHE = acetylcholinesterase enzyme and C-reactive protein. Group 1 (Control) is 0-1 years. Group 2 – 1-3 years Group 3 – 3-5 years Group 4 – Above 5 years. The superscripts a, b and c indicate significant difference in the values

## 4. Discussion

From the result, the BP level showed a significant increase in relation to the control. This suggests that the snuff consumed by the participant possesses some compounds that can increase BP levels. Among the ingredients used in producing snuff is tobacco powder which is rich in nicotine. Nicotine can act as an adrenergic agonist by mediating the release of catecholamine, angiotensinogen (and angiotensin in its active form) and vasopressin (and aldosterone) leading to vasoconstriction and thus increase blood pressure (Page et al., 2019).[5] As a consequence, an increase in catecholamine may lead to cardiac dysfunction (Page et al., 2019).[5]

This result tallies with the research conducted by Hergens et al., (2008)[6] who discovered that there is an increased risk of hypertension among Swedish male snuff users (Hergens et al., 2008).[6]

The result of the Random Sugar Test revealed a significant decrease in blood glucose level 30 minutes after consumption of snuff. The decrease in the glucose level could be attributed to nicotine content in snuff. Nicotine has been found to stimulate the cyclic AMP (cAMP) → protein kinase A (pkA). Cyclic AMP suppresses the inhibition of the glycolytic process thus resulting in decrease in blood glucose level (Wang et al., 2012).[7] Glycolysis or glucose metabolism promotes the formation of cyclic AMP either by increasing the availability of ATP or decreasing the concentration of inhibitory AMP thus stimulating the activity of adenylyl cyclase (Shuai et al., 2021).[8]

Momentary nicotine spike causes an increase in the activity of the sympathetic nervous system leading to a series of hormone and amino acid releases (such as L-arginine hormone, vasopressin and growth hormone). L-arginine stimulation also activates pkA (Fujiwara et al., 2014).[9] And in turn causes insulin release and thus a sudden crash in glucose level (Kaihara et al., 2015).[10] However, chronic and continuous consumption of snuff predisposes the user to Type 2 Diabetes by creating insulin resistance due to the nicotine content (D'Arrigo, 2021).[11] Nicotine alters the insulin transduction pathway by binding to nicotinic receptors causing activation of Mammalian Target of Rapamycin (MTOR) and inactivation of AMP-Activated Protein Kinase (AMPK). This will degrade Mitogen-Activated Protein Kinase resulting in the reduction of Insulin Receptor Substrate 1 (IRS-1) which causes insulin resistance in organs and cells. This report agrees with the findings of (Maddatu et al., 2018)[12] that cigarette smoking plays a major role in the development of Type 2 Diabetes.

The result of the antioxidant assay revealed a decrease in SOD, GPx and CAT as well as MDA. This scenario could be attributed to increased pro-oxidants in the blood that induce oxidative stress. This agrees to Animal studies reported by Muhammad et al., (2021)[1] on the effect of snuff on antioxidant enzymes. Furthermore, tobacco (nicotine) has been shown to decrease lipid profile in apparently healthy individuals. (Muhammad et al., 2021).[1] The decrease in MDA with corresponding decrease in antioxidant enzymes could be attributed to the decrease in lipid among snuff users. (Kamceva et al., 2016).[13]

Production of Angiotensin due to nicotine acting as an adrenergic agonist is followed by reactive oxygen species which then leads to vasoconstriction. The ROS released from this mechanism could have contributed to the decrease in antioxidant enzymes. (Aspera-Werz et al., 2018).[14]

This result tallies with the research conducted by (Naim et al., 2020)[15] who discovered that antioxidant activities decreased as prooxidant levels increased indicating that chronic snuff users are predisposed to diseases like diabetes, hypertension, schizophrenia and dementia caused by oxidative stress (Naim et al., 2020).[15]

The result shows a substantial increase in AChE activity in relation to the control. The decrease in AChE activity predisposes them to neurodegenerative diseases such as dementia and Alzheimer's disease (Ziani et al., 2018).[16] Acetylcholine can be found in the neuronal synapse and acetylcholinesterase hydrolyses acetylcholine to choline and acetic acid.

Acetate is converted to acetyl-coA by acyl-CoA short-chain synthetases (Moffett et al., 2020).[17] Elevated levels of AChE are seen in Alzheimer's patients. Once nicotine spike occurs and the nicotinic receptors are activated, dopamine is released which stimulates pleasure mood in snuff users (Ziani et al., 2018).[16] Nicotine in snuff binds to nicotinic ionotropic receptors which are ion-based ligands. This binding opens the ion-gated channels through the cell membrane which then leads to depolarization as a result of the influx of Calcium ion from the outer part of the cell-membrane to the inner part of the cell membrane. This depolarization results in a series of chemical and electrical signalling that triggers the release of dopamine and hence the feeling of pleasure experienced by users of snuff. (Wittenberg et al., 2020).[18] This result tallies with the studies conducted by Muhammad et al., (2021)[1] on rat brain which revealed that the activity of acetylcholinesterase increased in rats administered snuff orally (Muhammad et al., 2021).[1]

Similarly, the result of current research revealed a significant increase in the level of C-reactive protein in relation to the control. This will predispose snuff users to diseases caused by inflammation such as neurodegeneration (Sarika et al., 2016).[19] When nicotine binds to ligands and a downstream signalling occurs, macrophages are also activated. Nicotine stimulates the production of inflammatory mediators and Nuclear Factor Kapa B which are involved in the replication process of C Reactive Protein. (Ashworth and Sproston, 2018).[20]

The cytokines IL-1β and IL-6 are expressed in response to inflammation by macrophages. Macrophages are immune cells that originally function to kill foreign organisms by engulfing them. Their other functions include removal of dead cells and activation of other immune cells by presenting antigens to T cells as well as initiating inflammation by releasing cytokines. The cytokines released may be pro-inflammatory which worsens inflammation and/or anti-inflammatory which works to reduce/eliminate inflammation. IL-1β and IL-6 are proinflammatory cytokines released by macrophages. The release of these cytokines activates nuclear factor kappa B, NF-KB, which promotes transcription of DNA, cytokine production and cell survival. Over-expression of this NF-KB induces the release CRP thus, the mRNA of CRP is transcribed (Agrawal et al., 2003).[21] Increased transcription of CRP by NF-KB activates the β-site APP Cleaving Enzyme, BACE1 which cleaves APP to form Aβ plaques. These plaques subsequently result in cognitive deficits. In addition, increased levels of CRP can lead to an increase in the level of Tumor Necrosis Factor $\alpha$ (TNF-$\alpha$) which is linked to Aβ-plaques formation and thus, cognitive deficits. (Mao et al., 2012).[22]

This result agrees with the research conducted by Silvano et al., (2018) which revealed that CRP levels increases in tobacco users, but in periods of cessation, CRP levels decrease favourably (Silvano et al., 2018).

## 5. Conclusion

There was no increase in the MDA levels. However, there was a significant decrease in CRP levels which is also a biomarker for oxidative stress. Thus, snuff induces oxidative stress as nicotine in snuff inhibits the activities of antioxidant enzymes while increasing the activity of acetylcholinesterase thereby potentially predisposing chronic and continuous snuff users to such degenerative diseases like dementia, depression and Alzheimer's disease.

## 6. Source of Funding

None.

## 7. Conflict of Interest

No conflict of interest was declared by any of the authors.

## Acknowledgments

We acknowledge the support we received from the Department of Biochemistry and Molecular Biology, Nasarawa State University, Keffi, Nigeria.

## References


1. Muhammad BY, Kingsley IU, Moses Z, Ruqaiyatu MA. Survey of Snuff Use and Preliminary Study of Effect of Two Brands, Brain Antioxidants and Acetylcholinesterase Enzyme of Wister Albino Rats. *Al-Azhar Int Med J*. 2021;2(3):60–6.
2. Warigon C. Tobacco use 'snuffs' out life of over 16,000 annually in Nigeria; 2019. Available from: https://www.afro.who.int/news/tobacco-use-snuffs-out-life-over-16000-annually-nigeria#:~:text=In%20Nigeria%2C%20tobacco%20use%20accounts,14%20years)%20using%20tobacco%20daily.
3. Thomson NC, Chaudhuri R, Spears M, Messow CM, Jelinsky S, Miele G, et al. Arachidonic acid metabolites and enzyme transcripts in asthma are altered by cigarette smoking. *Allergy*. 2014;69(4):527–36.
4. Welch W. Angiotensin II-dependent Superoxide: Effects on Hypertension and Vascular Dysfunction. *Hypertension*. 2008;52(1):51–6.
5. Page SJ, Zhu M, Appleyard SM. Effects of acute and chronic nicotine on catecholamine neurons of the nucleus of the solitary tract. *Am J Physiol Regul Integr Comp Physiol*. 2019;316(1):R38–49.
6. Hergens MP, Lambe M, Pershagen G, Ye W. Risk of Hypertension amongst Swedish male Snuff Users: a prospective study. *J Intern Med*. 2008;264(2):187–94.
7. Wang S, Zhang C, Liang B, Zhang M, Lee J, Zhu H, et al. Activation of AMP-activated protein kinase $\alpha 2$ by nicotine instigates formation of abdominal aortic aneurysms in mice in vivo. *Nat Med*. 2012;18(6):902–10.
8. Shuai H, Xu Y, Ahooghalandari P, Tengholm A. Glucose-induced cAMP elevation in β-cells involves amplification of constitutive and glycogen-activated GLP-1 receptor signaling. *Acta Physiol*. 2021;.
9. Fujiwara T, Kanzawa S, Ichibori R, Tanigawa T, Magome T, Miyata S, et al. L-arginine stimulates fibroblast proliferation through the GPRC6A-ERK1/2 and PI3K/Akt pathway. *PLoS One*. 2014;9(3):e92168.
10. Kaihara KA, Dickson LM, Ellenbroek JH, Orr CMD, Layden BT, Wicksteed B. PKA Enhances the Acute Insulin Response Leading to the Restoration of Glucose Control. *Diabetes*. 2015;64(5):1688–97.
11. D'arrigo T. How Does Nicotine Affect Blood Sugar?; 2021. Available from: https://www.webmd.com/diabetes/nicotine-blood-sugar#:~:text=Nicotine%20changes%20chemical%20processes%20in,blood%20sugar%20level%20goes%20up..
12. Maddatu J, Anderson-Baucum E, Evans-Molina C. Smoking and the Risk of type 2 Diabetes. *Transl Res*. 2017;184:101–7.
13. Kamceva G, Arsova-Sarafinovska Z, Ruskovska T, Zdravkovska M, Kamceva-Panova L, Stikova E. Cigarette Smoking and Oxidative Stress in Patients with Coronary Artery Disease. *Open Access Maced J Med Sci*. 2016;4(4):636–40.
14. Aspera-Werz RH, Ehnert S, Heid D, Zhu S, Chen T, Braun B, et al. Nicotine and Cotinine Inhibit Catalase and Glutathione Reductase Activity Contributing to the Impaired Osteogenesis of SCP-1 Cells Exposed to Cigarette Smoke. *Oxid Med Cell Longev*. 2018;6:3172480. doi:10.1155/2018/3172480.
15. Naim SA, Erfan D, Bahram N, Daem R, Mohammad JR, Hamid RA, et al. Effect of Chronic Nicotine Administration on the Pro-oxidant Antioxidant Balance of Mice serum: Role of Rosemarinic Acid. *Sci J Kurdistan Univ Med Sci*. 2020;25(2):54–60.
16. Ziani PR, Muller TE, Stefanello FV, Fontana BD, Duarte T, Canzian J, et al. Nicotine increases fear Responses and Acetylcholinesterase activity in a Context dependent Manner in Zebrafish. *Pharmacol Biochem Behav*. 2018;170:36–43.
17. Moffett JR, Puthillathu N, Vengilote R, Jaworski DM, Namboodiri AM. Acetate Revisited: A Key Biomolecule at the Nexus of Metabolism, Epigenetics, and Oncogenesis - Part 2: Acetate and ACSS2 in Health and Disease. *Front Physiol*. 2020;11:580171. doi:10.3389/fphys.2020.580171.
18. Wittenberg GM, Stylianou A, Zhang Y, Sun Y, Gupta A, Jagannatha PS, et al. Effects of Immunomodulatory Drugs on Depressive Symptoms: A Mega-analysis of Randomized, Placebo-controlled Clinical Trials in Inflammatory Disorders. *Mol Psychiatry*. 2020;25(6):1275–85.
19. Sarika VD, Nitin SN, Jagtap PE, Dhone SP, Belwalkar GJ. Tobacco Chewing and Smoking-Risk for Renal Disease. *Biomed Res*. 2016;27(3).
20. Ashworth JJ, Sproston NR. Roles of c-reactive protein at sites of inflammation and infection. *Front Immunol*. 2018;13:754.
21. Agrawal A, Cha-Molstad H, Samols D, Kushner I. Overexpressed nuclear factor-kappaB can participate in endogenous C-reactive protein induction, and enhances the effects of C/EBPbeta and signal transducer and activator of transcription-3. *Immunology*. 2003;108(4):539–47.
22. Mao J, Liu J, Pang X, Li M, Song J, Han C, et al. Nicotine Induces the Expression of C-reactive Protein via MAPK-Dependent Signal Pathway in U937 Macrophages. *Mol Cells*. 2012;34(5):457–61.


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**Cite this article:** Otuh MS, Muhammad BY, Abdullahi A, Lawal AH. Evaluation of c-reactive protein level, antioxidant and acetylcholinesterase enzyme activities among snuff users. *Int J Clin Biochem Res* 2023;10(2):171-175.