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Herbal snuff (AK-47 and HAM) induce oxidative stress and increase acetylcholinesterase enzyme activity in rat brain

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Abstract

Snuff has resurfaced not only in western countries but in Africa including Nigeria. It is now almost generally acceptable, among young and old in Nigeria. This research was designed to investigate the Effect of Hajiya Aisha Manpower (HAM) and AK-47 on Antioxidant Status and Acetylcholinesterase enzyme activity (AChE) of Wister Albino Rats. Thirty (30) Wister rats (110-120 g) were arbitrarily divided into five groups. Group1 (control); received only distilled water. Groups 2 and 3 (received 6 mg and 3 mg/kg b.w.t of HAM respectively). Groups 4 and 5 (received 6 mg and 3 mg/kg b.w.t AK-47 of respectively). After two months of treatments, the rats were anesthetized, blood samples were taken through heart puncture and brains of all rats were isolated and homogenized. The Result revealed Non-significant decrease in Superoxide dismutase (SOD), glutathione peroxidase (GPx) activities and concomitant increase in GSH levels in treatment groups were observed in relation to the control. While a substantial increase ($p < 0.5$) in MDA was detected in treatment groups. Brain AChE activity increased significantly in all treatment groups in relation to the control. We conclude that Both AK-47 and HAM at high concentration induce oxidative stress, decreased antioxidant enzyme activities and promote degradation of acetylcholine in rat brain homogenate.

Keywords: Snuff; Brain Cognitive function; Antioxidants; Acetylcholinesterase enzyme; Smokeless tobacco

1. Introduction

Snuff is any product made from ground or pulverized tobacco leaves intended to be placed in the oral or nasal opening [1]. This distributes rapid nicotine sensation and long-lasting aroma and essence. Snuff could be Moist; that is mostly placed between lips and gum, or dried powdered tobacco usually sniffed through the nose. Example of these snuff include: "Naswar" a dipping smokeless tobacco commonly used in Pakistan, Afghanistan, Iran and South Africa [1]. Snuff became widespread in England throughout the seventeenth century, but powdered tobacco is recognized to have been used by native people of Brazil before the arrival of the Spaniards [2]. In the western world, Snuff reemerged as substitutes, for tobacco and became generally acceptable among younger generations; after the prohibition on smoking in numerous open places. The traditional snuff production involves the selection of varieties of tobacco leaves, sun-dried, then subjected to fermentation process which gives it characteristic scent, then converted to powder known as snuff blend and mixed with calcium oxide and wood ash (original fine snuff without addition of scents). However, most often varieties of spice, piquant, fruit, flora and menthol are added to the pure or blend snuff. Every snuff company usually, has unique formulae and composites for different individuals or customers. Common flavoring agents include: coffee, chocolates, honey, Vanilla, cherry, orange, apricot, plum, camphor, cinnamon, as cardamom rose and spearmint.

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Current essences include Bourbon, cola, and whisky. The widespread use of snuff is the fact that users think it is a substitute for tobacco smoking. The types of snuff include dry, wet, tumbling tobacco, munching tobacco, and creaming snuff [3].

Another form of snuff made from moringa is now widely accepted and sold in countries around West Africa including Nigeria. It is affectionately consumed by car drivers, *Okada riders*, menial laborers and even some members of the elite. They are sold under brand name such as: AK-47 boss, Hajiyah aisha man power, Sweet mother, Normal tobacco, lion brand, Hajiyah Bilkisu, Hajiyah Fatima, Special moringa sundu, Hajiyah Bilkisu Ma`a shaa Allah, Shehu Barhama, and Hajiyah Hauwa.

Although the modern snuff is composed of chiefly *moringa* leaves, it is not devoid of tobacco just like the traditional tobacco. Recent studies suggested that long use of smokeless tobacco could predispose to free radical generation vis-à-vis oxidative stress [4,5]. Nicotine is a major bioactive constituent of tobacco. Previous investigations revealed that low doses of nicotine could improve memory performance and reduced plaque accumulation; thus could be a promising candidate for Alzheimer disease treatment. Also it could improve attention and performance in schizophrenia patients [6,7]. However, some studies have indicated that nicotine impairs cognition in human and animal subjects. These inconsistencies could be attributed to the doses. It seems high amount of nicotine could induce neurotoxicity [8] and stimulate oxidative stress, whereas low dose could improve cognitive performance [7]. On the other hand, the major component of the modern West African stuff is *Moringa oleifera*, a plant taken as one of the most valuable trees in the world, with several therapeutic, nutritious and industrial uses [9]. *Moringa* is opulent in vitamins, antioxidants, β -carotene, amino acids, phenolic, flavonoids [10]. These various components of *moringa* makes a potent free radical scavenger, enzyme inhibitors, antioxidants, anti-bacterial, anti-tumor, cholesterol lowering, antipyretic, anti-inflammatory, antidiabetics, anti-ulcer, among others [11].

The rate of *moringa* snuff consumption has rampantly increased in Nigeria particularly the Northern region; where in the past, snuffing was considered to be filthy. The addiction to snuff is obvious, although the addicts claim that it has various therapeutic consequences against different ailments. Unlike the “traditional snuff” (i.e. a blend of purely tobacco and flavouring agent) whose effect has been studied and documented to cause disorders such as cancer (of the mouth, lips, nasal cavities, oesophagus and gut), diabetes; hypercholesterolemia, myocardial infarction and death [4], modern snuff contains high percentage of *moringa* besides other adjuncts and was never studied. For this reason, the current study aimed to assess the effect of “*moringa*” snuff on antioxidant status and cognitive function of Wister albino rat.

2. Material and methods

2.1. Chemicals and kits

The chemicals used in this research include disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) sodium chloride (NaCl) and ethanol 95% and isoflurane were purchased from Fluka, Switzerland. All kits used were obtained from Solarbios Life Science Limited, Beijing China. These include Superoxide dismutase (SOD) Catalog Number: BC0175, Glutathione Peroxidase (GPX) Catalog Number: BC0174, Malondialdehyde (MDA) Catalog Number: BC002, reduced glutathione (GSH) Catalog Number: BC1175), Acetylcholinesterase (AChE) Assay Kit Catalog Number: BC2020

2.2. Snuff Samples, Composition and Preparation

The snuff samples (HAM and AK-47) were purchased from keffi market, Nasarawa State, Nigeria. They were both a mixture containing ground tobacco leaves, a little *moringa* powder and menthol. To prepare snuff solution; 1g of sample was dissolved in 1 liter of deionized water.

2.3. Experimental design

The research was ratified by the Nasarawa State University Animal Ethics Committee. Thirty (30) Wister albino rats (110-120g) 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 two (2) weeks to acclimatize to the new laboratory condition. Afterwards they were, weighed and divided randomly into five groups.

- Group1 (control): received only distilled water.
- Groups 2: received 6mg / kg b.w.t. of HAM.
- Group 3: received 3mg / kg b.w.t of HAM.

- Group 4: received 6mg / kg b.w.t of AK-47
- Group 5: received 3mg / kg b.w.t of AK-47

All animals were fed with growers mesh and allowed access to water throughout the treatment period (two month). Food gavage was used in the administration of the solution.

At the end of administering of snuff solution, rats were weighed, sedated using 5% V/v isoflurane and oxygen 1.5L / min flow, the skulls were dissected quickly and the brain tissues were rapidly removed and washed in phosphate buffer solution of pH 7.4. The brain tissues were instantly placed in ice and refrigerated at -20°C for 24 hrs.

2.4. Preparation of Homogenate

Brain tissues were cut into pieces, weighed and two (2g) grams was homogenized in 20 volumes of phosphate buffer saline pH 7.4 (0.1 M), at 700 g in ice for 10 min, then centrifuged for 15 min at 5000 rpm. The supernatant was then collected and stored at -20 °C until use [12].

2.5. Biochemical Analysis

2.5.1. Determination of Superoxide Dismutase (SOD) Activity

SOD activities were determined colorimetrically according to manufacturer's (Solarbios Life Science) instructions. SOD catalyzes the conversion of superoxide molecules ($O_2^{\cdot-}$) into either oxygen molecule (O_2) or hydrogen peroxide (H_2O_2). The $O_2^{\cdot-}$ reduces nitro-blue tetrazolium to form blue formazan, which absorbs at 560 nm. SOD reacts with $O_2^{\cdot-}$ and suppresses the blue reaction. The blue color generated is inversely proportional to the SOD activity [13].

2.5.2. Glutathione Peroxidase (GPx) Activity

Assay kit for Glutathione Peroxidase Activity was used to assay glutathione peroxidase in the tissue extracts according to manufacturer's protocol. The protocol harnesses the conversion of GSH to GSSG catalyzed by GPx in the presence of GSH, GSHR and NADPH GPx activity corresponds to oxidation of NADPH to NADP. Absorbance was measured at 340 nm [14].

2.5.3. Determination of total reduced glutathione (GSH)

Glutathione (GSH) is a natural three peptide containing sulfhydryl (SH). It is made up of glutamic acid, cysteine and glycine; Glutathione reacts with 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) to give a yellow product that absorbs maximum ally at 412 nm. The amount of GSH was determined as GSH (μg /g tissue) [15].

2.5.4. Determination of Malondialdehyde (MDA) level

Malondialdehyde (MDA) is one of the convenient markers of lipid peroxidation. At an acidic pH and high temperature, MDA and thiobarbituric acid (TBA) condenses to form brown red 3,5,5- three methyl sulfamethoxazole -2,4-two ketone with largest absorption wavelength of 532 nm. The level of MDA was calculated by the difference between the absorbance at 532 nm, 450 nm and 600 nm as instructed by the kit's manufacturer [16].

2.5.5. Determination of acetylcholinesterase (AChE) Activity

AChE catalyzes Ach hydrolysis to generate choline, and choline reacts with 5,5'-dithiobis(2-nitrobenzoic acid) to form 5-mercapto nitrobenzoic acid (TNB) which absorbs at 412 nm. AchE activity was expressed as U/g tissue as described by the kit manufacturers [17]

3. Results

3.1. LD₅₀ Determination for different snuff products.

The results of the acute toxicity studies for the two snuff products showed mild toxicity, such as difficulty in respiration, emotional instability. The LD₅₀ was estimated to be \pm 2500 mg / kg and 2400 mg / kg respectively per body weight for AK-47 and HAM respectively.

The results in groups 3, 4 and 5 showed a substantial ($p > 0.05$) decrease in SOD and GPx activities with concomitant increase in MDA level in relation to the control. However, the GSH level also increased significantly ($p < 0.05$) compared

to the control. Furthermore, the AchE activities of the treatment groups increased significantly ($p < 0.05$) when compared to the control.

Table 1 Effect of Snuff on Brain Levels of SOD, GSH, MDA and AchE Activity of Wister Albino Rats

Group	MDA (nmol/g tissue)	SOD (u/g tissue)	GPx (U/g tissue)	GSH (µg/g tissue)	AchE (U/g tissue)
1. (Control)	20.58 ± 7.54	70.25 ± 5.23	29.31±5.33	54.73 ± 6.22	27.63 ±5.99
2.(HAMH)	19.05 ±3.65	68.41 ± 16.09	26.30± 3.80	60.05 ±8.71*	51.45 ± 8.04*
3. (HAML)	10.77 ± 1.19*	59.77 ± 10.54	24.61± 3.32*	79.83 ± 7.69*	128.21 ± 28.15*
4. (AKH)	6.87 ± 1.56*	57.81 ±15.21*	20.16±2.16*	104.16 ± 14.65*	78.36 ± 11.38*
5. (AKL)	8.47 ±1.87*	45.01 ±16.33*	16.33±2.23*	80.12 ±10.66*	38.18 ± 23.34*

Results are expressed as Mean ± SD, n=6 values with asterisk are significantly different from the control at 0.05%. Groups 1: control; 2, HAMH; Hajiya Aisha Man power high dose HAML = Hajiya Aisha Man power low dose, AKH = Ak47 high dose AKL: AK47 low dose.

4. Discussion

The brain is an important portion of the biological system whose function helps to regulate other portions of the body. However, any damage or form of stress experienced in this component of the body may have serious influence on the entire organism. Numerous investigations revealed that, the mechanism of snuff action in animals is linked to production of ROS [5,18–20]

SOD detoxifies superoxide radicals to H_2O_2 , which can be further detoxified to water by GPx. In this investigation there is a significant increase in the levels of MDA after administration of HAML, AKH and AKL respectively ($p < 0.05$) with concomitant decreased in SOD and GPx level. This agrees to previous studies which revealed decrease in SOD, GPx and catalase in “Maras” powder (smokeless tobacco snuff) users [21].

It indicates that tobacco induces ROS generation and inhibits antioxidant enzymes activities at the same time. ROS can interact with cellular biomolecules, principally, lipids to generate MDA and other lipids peroxide and deplete GSH level. These lipid peroxides can interact with different proteins such as enzymes to interfere with their active site thus their activities [12]. The decrease in the activity of SOD and GPx may be owing to the consumption of these enzymes in converting O_2^- to H_2O_2 and to H_2O respectively.

Glutathione is an antioxidant produced in cells. It's contained basically three amino acids: glycine, glutamine, and cysteine. The quantity in the system may decline due to inadequate nourishment, toxins and stress. Decrease in GSH raises the tendencies of oxidative stress.

Malondialdehyde (MDA) is generally used as a biomarker for evaluating oxidative stress. Lipid peroxidation is a normal phenomenon that occurs continuously at low levels in every individual. Lipid peroxidation is an indicator of secondary brain damage due to trauma or otherwise, as in major neurosurgical procedures—*eventually* leading to brain oedema increase and promote brain damage [18]. MDA, a marker of lipid peroxidation and indicator of antioxidant status, can signify *primary* signs of this process inside the cerebrospinal fluid (CSF). In this research, Brain MDA level increased and concomitant decrease in antioxidant enzymes.

Acetylcholinesterase is an enzyme that hydrolyzes acetylcholine, an essential neurotransmitter, at neuronal synapses, and at neuromuscular junctions, during signaling process [22]. In certain neurological disorders such as Alzheimer's disease (AD), acetyl cholinesterase is over activated in the synapses so that levels of acetylcholine in the brains is significantly diminished, which leads to weakened neurotransmission and thereby memory loss and other adverse effects [22].

Significant increase is observed in the quantity of acetyl cholinesterase on the administration of high dosage of HAM and AK-47. This will have overall effects of decreasing acetylcholine levels; which in turn will contributes characteristic symptoms of Alzheimer's disease [23]. This could include dementia that produces loss of memory and problems of

thinking and behaviour. Severe case could affect lifelong hobbies or social life. Eventually, great percentage of the brain is entirely damaged by Alzheimer disease [23].

The AchE activities in all treatment groups increased significantly, which could have a consequential decrease in cognitive function [24]. Ach is among the central neurotransmitters, which play a significant role in memory, learning and cognition [25]. It relays signal from one neuron to another in the central nervous system and from neuron to muscle fiber in the peripheral nervous system. AchE is the enzyme that brakes acetylcholine to the choline and acetate [25]. Increase in AchE activities decreases the quantity of AchE at the synaptic junction and affect the signal [26]. This would eventually increase the amount of choline uptake and decreases the number of nicotinic receptors; which in turn will weakened memory, learning and cognition in AK47 and HAM treated rats [26].

5. Conclusion

This research indicates that Both AK47 and Hajiya Aisha man power at high concentration induces oxidative stress, decreased antioxidant enzyme activities and promotes hydrolysis of acetylcholine in rat brain homogenate. This will predispose to neurodegenerative disorder in chronic users.

Compliance with ethical standards

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Statement of ethical approval

The ethical approval for the use of rats was obtained from Ethical Committee on Animal Use and Care of Nasarawa State University, Keffi, Nigeria. All experiments were performed in compliance with the established guide for the care and use of laboratory animals.

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