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Evaluation of Some Biochemical Markers among Long Term Herbal Snuff Users in Keffi Town

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Abstract: The use of Moringa Herbal snuff is growing rapidly in Nigeria. This research study appears to investigate the use of Moringa Herbal snuff and its potential effects on various biochemical markers among snuff users in Keffi town, Nigeria. The study was conducted in three stages, which included an interview survey, phytochemical screening of four claimed Moringa herbal snuff brands, and a study of the effects of snuff consumption on blood pressure, blood glucose levels, liver and kidney function, and hematological parameters. The survey found that a majority of snuff users reported using snuff for headache, vision, and sexual enhancement. Phytochemical screening of the four snuff brands revealed the presence of alkaloids, glycosides, steroids, and terpenoids. Gas Chromatography-Mass Spectrometry analysis of Special Moringa Sundu (SMS) revealed the presence of 27 bioactive compounds, including n- Hexadecanoic acid, nicotine, cotinine, trans-13-Octadecenoic acid, and oleic acid. The study found a significant increase in both systolic and diastolic blood pressure an hour after snuff consumption compared to blood pressure before consumption in the test groups and the control. Blood glucose levels decreased after snuff consumption compared to levels before consumption. The study also found normal liver function in both test and control groups, but a significant increase in urea in all snuff user groups and an increase in creatinine levels only in chronic users (group 4) compared to the control. The white blood cell level was significantly higher among snuff users than the control. Leucocytes counts were normal compared to the control. Overall, the study suggests that Moringa Herbal snuff may be addictive and that persistent consumption may lead to high blood pressure, type2 diabetes, and predisposition to kidney malfunction.

Keywords: Moringa Herbal Snuff, Nicotine, Blood Pressure, Type2 Diabetes, Kidney Malfunction

1. Introduction

Snuff is any product made from ground or pulverized tobacco leaves intended to be placed in the oral or nasal cavity [1]. This distributes rapid nicotine sensation with long-lasting fragrance and essence. Snuff could be placed between lips and gum (moist snuff), or sniffed through the nose (dried powdered tobacco product) [1].

Snuff use has been practiced by a large number of responsible adults and young person without any sign of decline in use by these groups, who are at risk of becoming addicts of these products [2]. Tobacco can be consumed variedly, some people chew it, and others dip it. Non-smoked tobacco is the type of tobacco that is consumed without smoke, or burnt in the process of consumption, that is taken orally or nasally. Majority of snuff users usually dip the substance in their mouth, and when the tobacco juice builds up, they spit out the juice [3]. These tobacco products are either packaged moist or dried; majority of snuffs that are produced in America are packaged in loose bags, dissolvable

lozenges in small pouches just as in tea bags [4].

The major component of the modern West African snuff is *Moringa oleifera* a plant considered as one of the most beneficial trees in the world, with several medicinal, nutritional and industrial applications [5]. Moringa is rich in vitamins, antioxidants, β-carotene, amino acids, phenolics, and flavonoids [1]. These various components of moringa make potent free radical scavengers, enzyme inhibitors, antioxidants, anti-bacterial, anti-tumor, cholesterol lowering, antipyretic, anti-inflammatory, anti-diabetics' anti-ulcer among others [6].

Snuffs claimed to be made from Moringa are now widely accepted and sold in Keffi and in some parts of Nigeria. Although they are a blend of chiefly Moringa leaves, they are not devoid of tobacco [7]. Moringa snuffs are affectionately consumed by car drivers, motorcyclists, menial laborers and even some members of the elite [7]. They are sold under brand names such as: Special moringa sundu, AK-47 boss, Hajiyah aisha man power, Sweet mother, Normal tobacco, lion brand, Hajiyah Bilkisu, Hajiyah Fatima, Hajiyah Bilkisu Ma`a shaa Allah, Shehu Barhama, and Hajiyah Hauwa [7].

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 [7]. The addiction to snuff is obvious, although the addicts claim that, it has various therapeutic benefits against different ailments [7]. 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, and myocardial infarction [8].

The aim of this research was to determine some biochemical Markers among herbal Snuff users in Keffi town.

2. Materials and Methods

2.1. Snuff Samples

Four herbal powders which are commonly used as snuffs namely, Special Moringa Sundu (SMS), Hajiya Aisha (H. A), Hajiya Salma (H. S) and Hajiya Bilkisu (H. B) were purchased from a herbal store in Keffi town market. The solvents and all other reagents used are analytical grades purchased from Merck (Germany).

2.2. Extraction

The sample (1g) each was collected from Special Moringa Sundu, Hajiya Aisha, Hajiya Salma and Hajiya Bilkisu and placed in a separate 100 mL conical flask. Methanol (50 mL) was added to each of the flasks and allowed to macerate for 72 hours. The samples were then filtered and evaporated to yield crude methanolic extracts.

Extraction of Moringa Sundu Oil

Moringa sundu oil was extracted by dissolve the crude methanolic extracts in chloroform. The soluble portion was then evaporated to yield a dark oily substance with strong pungent smell.

Figure 1. *Herbal Snuff Sample*

2.3. Phytochemical Screening

The crude extract was subjected to preliminary phytochemical screening to determine the presences of different classes of secondary metabolites present.

2.3.1. Detection of Saponins

Froth Test: Crude extract 5cm^3 was mixed with 5cm^3 of distilled water in a test tube and was shaken vigorously. The formation of stable foam indicates presence of saponins [9].

2.3.2. Detection of Terpenoids

Salkowski's test: To 1cm^3 of the plant extract 2cm^3 of chloroform was added, and then 1.5cm^3 concentrated Sulphuric acid was added carefully. Formation of reddish brown colour at the interface indicates the presence of Terpenoids [9].

2.3.3. Detection of Steroids

Libermann Burchard's test: Extract 0.5cm^3 was dissolved in 2cm^3 of chloroform and filtered, the filtrate were treated with few drops of acetic anhydride boiled and cooled. 2cm^3 of sulphuric acid was carefully added to form lower layer. A reddish brown colour at the interface indicates the presence of a steroidal ring [9].

2.3.4. Detection of Alkaloids

Wagner's and Mayer's Test: The extract 2cm^3 was stirred with 2cm^3 of 10% aqueous hydrochloric acid. 1cm^3 was treated with a few drops of Wagner's reagent and second 1cm^3 portion was treated similarly with Mayer's reagents. Turbidity or precipitation with either of these reagents was taken as a preliminary evidence for the presence of alkaloid [9].

2.3.5. Detection of Phenols

Ferric Chloride Test: Extracts 2cm^3 were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols [9].

2.3.6. Detection of Tannins

Ferric Chloride Test: Ferric chloride solution 5% was

added drop by drop to 2cm^3 of the extract and the colour produced was noted. Condensed tannins usually give a dark green colour, and hydrosalable tannins give blue black colour [9].

2.3.7. Detection of Flavonoids

Alkaline Reagent Test: Ammonia solution 5cm^3 of 10% was added to a portion of the crude extract followed by addition of concentrated sulphuric acid. A yellow colouration observed in the extract indicates the presence of flavonoids. The yellow colouration disappeared on standing [9].

2.3.8. Detection of Glycosides

Keller-killiani"s test: To 1cm^3 of extract 2cm^3 of 0.5% ferric chloride solution was added and allowed for 1 minute. A reddish brown ring at the interface indicates the presence of cardiac glycoside [10].

2.3.9. Test for Anthraquinones

Borntrager's test: Powdered sample 0.5g was weighed and inserted in a test tube, 10cm^3 chloroform was added and shaken for 5minutes, then filtered and shaken with 10% ammonia for 5minutes. Formation of a bright pink at the upper layer indicates the presence of anthraquinones [10].

2.4. Gas Chromatography Mass Spectrometry (GC-MS) Analysis of Moringa Sundu Oil

The GC-MS analysis was carried out using Agilent 5977C GCMSD system equipped with Agilent 19001s-433ui HP-5ms ultra inert capillary column (30 m X 250µm X 0.25µm). Helium was used as carrier gas. The sample was injected in a split less mode at a volume of 2µL and the injector temperature was maintained at 250°C. The oven temperature was programmed from 70°C with an increase of 5°C/min to 250°C, then 30°C/min to 300°C. The mass spectra were obtained at 70eV and fragment from 50 to 650Da. The interpretation of the mass spectra was carried out using the database of National Institute Standard and Technology, version 2014.

2.5. Collection of Blood Sample

Blood samples were collected directly from the participants within Keffi town in different location by using sterile containers.

2.6. Research Design

The study was carried out in three stages: the first stage involved the use of questionnaire to obtain relevant information from snuff users. Inclusion criteria include healthy adult males and females from 18 years and above without diabetes, hypertension or currently undergoing treatment for such diseases. The exclusion criteria include the sick, elderly, smokers, children under 18 years and those who did not give consent. The second stage involved the phytochemical screening of the crude methanol extract of S. M. S, H. A, H. S and H. B followed by GC-MS of S. M. S. The final stage was the analysis of subjects' blood samples.

The subjects were divided into four groups based on their period of exposure to snuff:

Group 1: consist of 100 non-smokers, non-users of snuff (Control).

Group 2: consist of 100 users exposed to snuff between 1 to 3 years.

Group 3: consist of 100 users exposed to snuff between 3 to 5 years.

Group 4: consist of 100 users of exposed to snuff between 5 years and above.

The snuff consumers were tested for blood pressure and sugar levels before they were fed with snuff and an hour after. The controls were also checked for sugar levels and blood pressure. The results were recorded and the blood samples were taken to the laboratory for liver function, kidney function and hematological parameters tests.

2.7. Sample Size Determination

The sample size 400 was determined using the sample size determination Z-score table which was calculated using 95% confidence level, 0.5 standard deviation and ±5% confidence interval.

2.8. Ethical Clearance

Ethical approval was obtained from ethical committee, Nasarawa State University Keffi Medical Centre.

2.9. Biochemical Assay

2.9.1. Serum Glucose Test

In the glucose oxidase/peroxidase method (GOD/POD) introduced here, glucose oxidase (GOD) is used to oxidize glucose by the oxygen in the air to gluconolactone.

$$C_6H_{12}O_6 + O_2 \rightarrow C_6H_{12}O_7 + H_2O_2$$

The glucose standard (10ul) and distilled water (10ul) were pipette into test tubes for standard, test and blank respectively. Working glucose reagent (1000ul) was pipetted into each of the tubes. The tubes were then incubated at room temperature for 10 minutes after which the absorbance of the sample (A sample) and standard (A standard) were read against reagent blank spectrophotometrically at 510nm.

2.9.2. Blood Pressure Measurement

The blood pressure was measured using digital blood pressure monitor. Digital blood pressure monitor was placed on the upper arm and blood pressure was read automatically in mmHg based on variations in the volume of blood in the arteries.

2.9.3. Determination of Alanine Aminotransferase (ALT)

The activity of ALT is determined using the Reitman and Frankel method as described by [11]. The absorbance was then read at 546nm.

2.9.4. Determination of Aspartate Aminotransferase (AST)

The activity of AST was determined using the Reitman

and Frankel method as described by [11] and the absorbance was read at 546nm.

2.9.5. Determination of Alkaline Phosphatase (ALP)

The activity of ALP was determined using the method of [12]. Kinetic determination of alkaline phosphate (ALP) based upon the reactions. Alkaline phosphatase, the absorbance was then read at 590nm and recorded.

2.9.6. Determination of Serum Urea

Urea concentration was determined using the method of [13] as described in Randox Kit. Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured spectrophotometrically. The absorbance of the sample (Asample) and standard (Astandard) against the blank was read at 546nm.

2.9.7. Determination of Serum Creatinine

The serum creatinine was determined using the method of [13] as outlined in Randox kit. Creatinine in an alkaline solution reacts with picric acid to form a colored complex. The amount of the coloured complex formed is directly proportional to the creatinine concentration.

$$C_4H_9N_3O_2 + C_6H_2N_3NaO_7 \xrightarrow{\text{Alkali}} C_{10}H_{10}N_6O_8$$

The absorbance A1 of the sample and standard will be taken at 492 nm. After 2 minutes, the absorbance A2 of the sample and standard were taken again.

2.9.8. Determination of Hematological Parameters

The whole blood collected into Ethylene Diamine Tetra-acetic Acid (EDTA) tubes was well mixed, the hematology analyzer machine was clean using the cleaning solution and the quantity control was run on the machine to determine its accuracy and efficiency.

The probe was opened and the sample was inserted, the blood is sucked up and the EDTA tube was removed and the probe was closed. The result displays on the screen of the machine showing the white blood cell count, red blood cell count, packed cell volume, platelet and hemoglobin.

2.9.9. Data Analysis

All data were presented as Mean ± S.E and analysis was done using Statistical Package for Social Sciences (SPSS) Version 19. Analysis of Variance (ANOVA) test was used where appropriate. Differences in mean was considered statistically significant at $P<0.05$.

3. Results and Discussion

3.1. Survey of Some Snuff Brands Commonly Consumed in Keffi Town

The result in table 1 revealed the response of 385 subjects. 92.2% of them were males and 7.8% females. 57.3%, claimed that snuff relieves headache, 38.3% improves vision, 86.1% dizziness at initiation, 62% enhanced sexual performance and 93% increase hunger. The relief from headache sequel to snuff intake may be as a result of the metabolite of tobacco (Cotinine) which has been established to have anti-depressant effect [14]. The dizziness and hunger experienced after herbal snuff consumption as observed during the survey maybe attributed to reduction in blood sugar level, as confirmed by rapid blood glucose Test (figure 4). The claim for improvement in sexual urge and performance by the respondents could be attributed to the presence of steroid in the snuff (table 2) which serves as precursors for sex hormones such as testosterone [15].

Table 1. *Survey of some Snuff Brands commonly consumed in Keffi.*

Parameter	Total number of respondents n= 385 (percentage of respondents)
1. Demographics of respondents	
a. Sex of respondents	
Males	355 (92.2%)
Females	30 (7.8%)
2. Snuff	
a. brand package consumed	
Moringa Sundu (16g)	191 (49.5%)
Hajiya Aisha (14g)	73 (19.0%)
Hajiya Bilkisu (15g)	32 (8.3%)
Hajiya Safiya (16g)	89 (23.2%)
b. frequency of use	Several times daily
c. Average consumption	
Finish a pack every week	218 (53.2%)
Finish a pack every two weeks	148 (36.1%)
Finish a pack every three weeks	33 (8%)
Finish a pack every four weeks	11 (2.7%)
Reported effects	
Relieves headache	220 (57.3%)
Improves vision	147 (38.3%)
Enhanced sexual performance	239 (62%)
Dizziness at initiation	331 (86.1%)
Diarrhoea Nausea and vomiting at initiation	100 (26.1%)
Increase in hunger	360 (93.4%)

The results in table 1 revealed about 385 people were interviewed through questionnaire, on the consumption rate, effects and experiences of the four snuff brands consumed in Keffi town.

3.2. Phytochemical Screening of Aqueous Methanol Extract of Moring Snuff

Table 2. *Phytoconstituents of Aqueous Methanolic Extract of Herbal Snuff.*

Phytoconstituents	Result			
	SMS	HB	HS	HA
Carbohydrates	++	++	+++	+++
Saponins	-	-	+	-
Phenols	-	-	-	-
Flavonoids	-	-	-	-
Tannins	-	-	-	-
Alkaloids	++	++	++	++
Anthraquinones	-	-	-	-
Terpenoids	++	+	++	++
Steroids	++	++	++	+
111 Glycosides	++	+	+	++

"-", "+", "++" and "+++" indicate negative, positive (trace reaction), positive (moderate reaction) and positive (higher reaction) respectively. Key: SMS = Special Moringa Sundu, HB = Hajiya Bilkisu, HS = Hajiya Salma and HA = Hajiya Aisha.

Phytochemical screening of aqueous methanol fraction of Moringa Snuff revealed the presence of alkaloids, glycosides, steroids, terpenoids in Special Moringa Sundu (SMS), Hajiya Salma (HS), Hajiya Bilkisu, (HB) and Hajiya Aisha (HA) as shown in (table 2). Alkaloids are nitrogen-containing naturally occurring compound, commonly found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms [16]. This could be responsible for their much acclaimed medicinal values. Steroid's presence in the snuff is of great importance as they are of interest in pharmacy due to their relationship with such compounds as sex hormones. It was reported that phytochemicals such as alkaloids and terpenoids have been shown to have several biological properties which include antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, and anticancer activities [17].

3.3. Gas Chromatography-Mass Spectrometry Profiling of Aqueous Methanol Fraction of Moringa Snuff

A total of 27 compounds were identified from the GC-MS analysis of methanol fraction of Moringa snuff. Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)- (Nicotine) with area percentage of 5.07%, cotinine (2.05), Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, N-oxide, (2S)- (0.32%) and n-Hexadecanoic acid having the highest area percentage (42.29%) as indicated in (table 3). The presence of nicotine and its metabolites in this snuff could be as a result of tobacco in the moringa snuff. This agrees with work of (Muhammad *et al.,* 2021) [7] which stated that moringa snuff is not devoid of tobacco.

Table 3. *Phytochemical Components of aqueous methanol Extract of Snuff as Detected by GC-MS.*

PK	RT	Area Pct.(%)	Library id
1	5.1739	0.8622	2-Dodecenal, (E)-
2	6.7195	5.0701	Pyridine, 3-(1-methy l-2-pyrrolidinyl)-, (S)-
3	9.4037	0.3213	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, N-oxide, (2S)-
4	13.8981	2.0553	Cotinine
5	16.2469	0.4168	1H-Pyrazole-4-carbonitrile, 5-amino-3-cyanomethyl-1-(2 hydroxyethyl)-
6	19.7676	0.9765	Pentadecanoic acid, 14-methyl-, methyl ester
7	21.0438	42.292	n-Hexadecanoic acid
8	21.3212	3.6165	Heptadecyl heptafluorobutyrate
9	22.0647	3.374	2-Piperidinone, N-[4-bromo-n-butyl]-
10	22.7789	1.6366	Methyl 9-cis-11-trans-octadecadienoate
11	22.9683	1.6437	9-Octadecenoic acid (Z)-, methyl ester
12	23.5736	0.4447	Heptadecanoic acid, 16-methyl-, methyl ester
13	24.0371	9.8895	trans-13-Octadecenoic acid
14	24.5028	2.7184	Octadecanoic acid
15	26.3746	0.2624	9-Octadecynoic acid, methyl ester
16	26.6615	0.1929	13-Oxabicyclo[10.1.0]tridecane
17	26.8435	0.7695	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
18	27.188	2.9013	9-Tetradecenal, (Z)-
19	27.5935	0.3424	12-Methyl-E,E-2,13-octadecadien-1-ol
20	27.9775	1.3772	6-Nitroundec-5-ene
21	28.9435	0.6729	2-Methyl-Z,Z-3,13-octadecadienol
22	29.1017	0.6262	9,12-Octadecadienoyl chloride, (Z,Z)-
23	29.4438	1.0496	cis-13-Eicosenoic acid
24	29.625	2.3825	cis-13-Octadecenoic acid
25	29.9868	0.2653	cis-Vaccenic acid
26	30.0902	1.7408	Bis(2-ethylhexyl) phthalate
27	38.479	-0.1276	Oleic

Key: RT = Retention time, Area Pct. = Area Percentage and Library id = Library identity

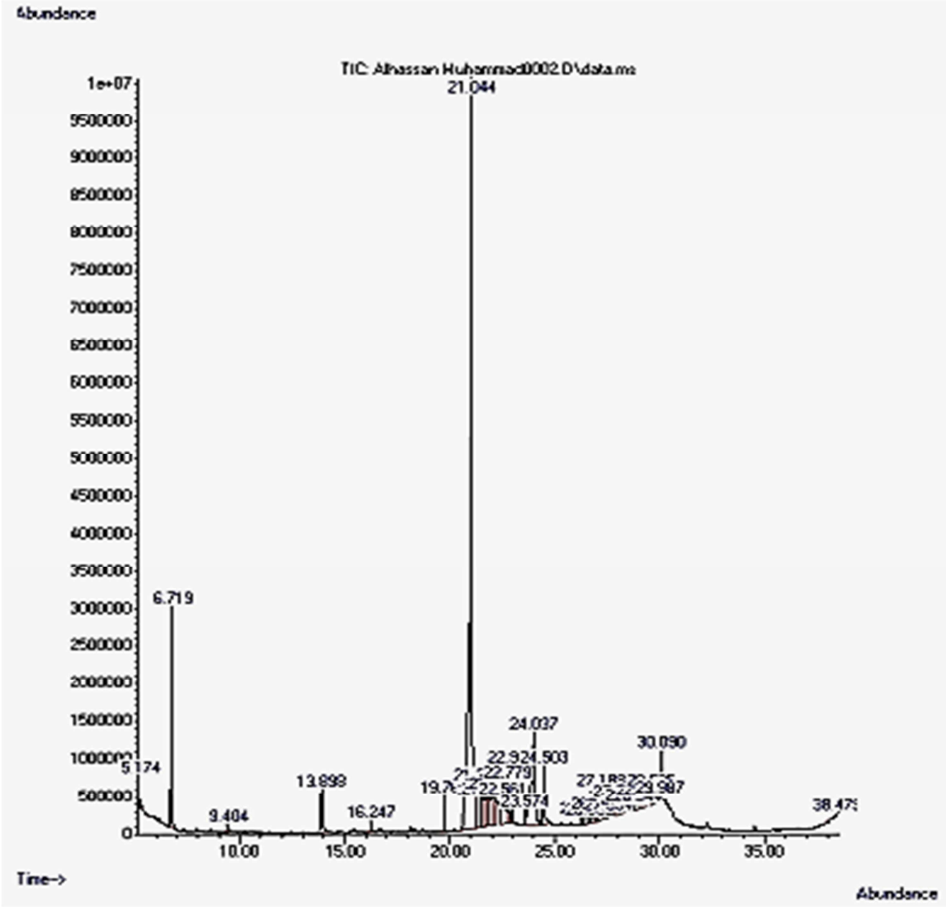
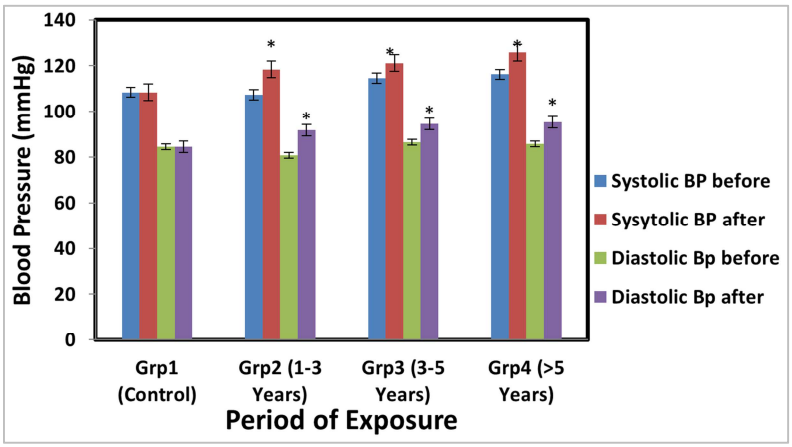


Figure 2. *GC-MS chromatogram of methanol extract of Special Moringa Sundu Oil.*

3.4. Effect of Snuff on Blood Pressure (Bp) Consumption

The significant increase (P<0.05) in blood pressure (BP) in figure 3 sequel to snuff consumption could be due to the presence of nicotine (Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-). Nicotine is the highly toxic and addictive alkaloid found in tobacco which binds to nicotinic acetylcholine receptors on the chromaffin cells in the adrenal medulla. Binding opens the ion channel allowing influx of sodium, causing depolarization of the cell, which activates voltage-gated calcium channels. Calcium triggers the release of epinephrine from intracellular vesicles into the bloodstream, which causes vasoconstriction and increased blood pressure. It was reported that in humans, nicotine can acutely increase BP [18]. Elevated BP is one of the best established risk factors contributing to clinical cardiovascular disease events such as acute myocardial infarction and mortality [18].



Results are expressed as Mean ± S.E, and are significantly different at p<0.05. Key: Bp = Blood Pressure, * = significant difference

Figure 3. *Effect of Snuff on Blood pressure (Bp).*

3.5. Effect of Snuff on Blood Glucose Levels

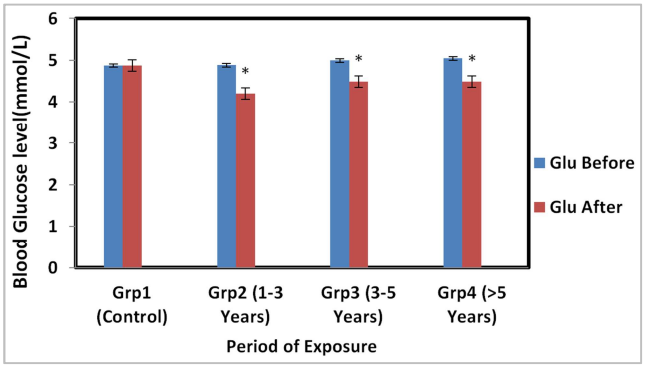


Figure 4. *Effect of Snuff on Blood Glucose Levels.*

Results are expressed as Mean ± S.E, and are significantly different at p<0.05. Key: Glu = Glucose, * = significant difference

The result in figure 4 revealed significant declines in sugar level in group 2 to 4 compared to the control. This could be due to the presence of nicotine. Momentary nicotine spike causes an increase in the activity of the sympathetic nervous system leading to series of hormone releases (such as L-arginine, vasopressin and growth hormone). L-arginine stimulates pkA and in turn insulin release therefore a crash in glucose level. However, chronic and continuous consumption of snuff predisposes the user to Type 2 Diabetes by creating insulin resistance due to the nicotine content [19].

3.6. Effects of Snuff on Liver Function

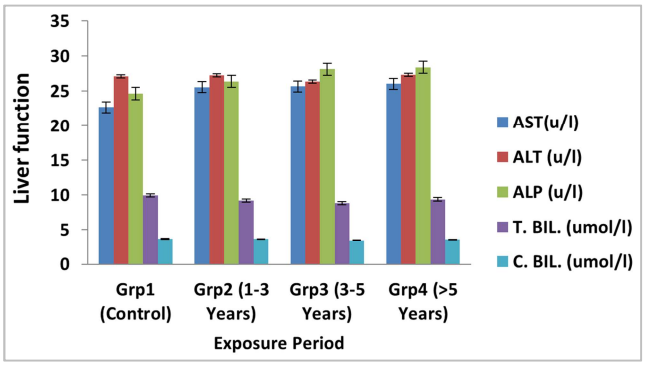


Figure 5. *Effects of snuff on liver function.*

Results are expressed as Mean ± S.E, and are significantly different at p<0.05. Key: AST = Aspartate amino transferase, ALT = Aanine amino transferase, ALP = Alkaline phosphate, T. Bil. = Total bilirubin, C. Bil. = Conjugated bilirubin.

The result in figure 5 showed no significant changes (P>0.05) in AST, ALT, ALP, T. Bil. and C. Bil. when compared to the control group. Liver is the major site of nicotine biotransformation, and nicotine exerts a number of adverse physiological effects on the liver ([20]. However, the normal liver functions observed in this study may be attributed to the presence of oleic acid. Carrillo *et al.,* (2012) [20] reported that oleic acid has anti-inflammatory properties which play role in the activation of different pathways of immune competent cells.

3.7. Effect of Snuff on Kidney Function (Urea and Creatinine)

Urea was observed to increase significantly (P<0.05) in group 2 to 4 while Creatinine increases significantly (P<0.05) only in group 4 compared to the control group (figure 6). The elevated values may be due to some toxic components that are nephrotoxic in the snuff such as nicotine, cotinine, and Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, N-oxide, (2S)-. The mechanism by which snuff induces renal damage may be through enhancing the synthesis of free radicals which according to Usunobun *et al.,* (2012) [21] lead to lipid and protein peroxidation, DNA damage and carcinogenesis. These may affect glomerular function leading to elevated serum markers of renal function. Progressive kidney failure can be associated with a gradual decrease of renal and non-renal elimination of nicotine and this potentiates nephrotoxicity [21].

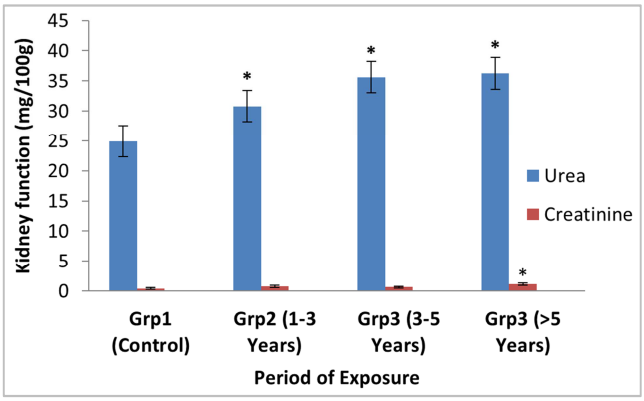


Figure 6. *Effect of Snuff on kidney Function (Urea and Creatinine).*

Results are expressed as Mean ± SE, and are significantly different at p<0.05. Key: * = significant difference, Grp = Group

3.8. Effect of Snuff on Hematological Indices

The result of hematological parameters indicated no significant changes (P>0.05) in RBC, PCV, Hb, PLT and significant increase (P<0.05) in white blood cell count (WBC) in group 2 to 4 compared to the control (figure 7). Similarly, group 2 increases significantly compared to group 3 and 4. The increase in WBC indicated inflammation which may be due to exposure to microbes or nicotine and its metabolites or both in the snuff. It was reported that nicotine causes the release of adrenalin and this increase WBC in the peripheral blood, bone marrow and spleen [22]. Similar report was made by Metin *et al.* (2004) [22] who observed that Maras powder consumers (Snuff) have increased WBC counts.

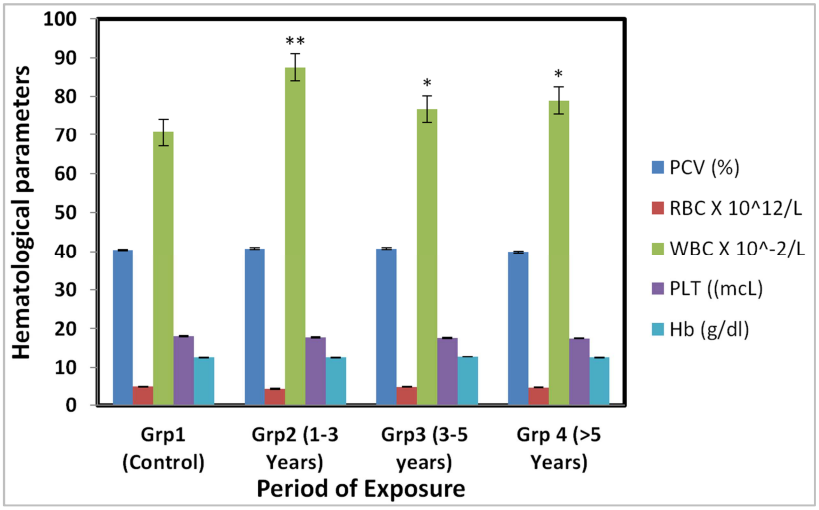


Figure 7. *Effect of snuff on hematological indices.*

Results are expressed as Mean ± S.E, and are significantly different at p<0.05. Key: * = significant difference, PCV = Packed cell volume, RBC = Red blood cell, WBC = White blood cell, PLT = Platelets and Hb = Hemoglobin

3.9. Effects of Snuff on Leukocytes Count

The percentage neutrophils increase in group 2 and 3 and lymphocyte increase in the test groups compared to the control could be indicative of inflammatory reactions, likely stimulated by microbe presence in the snuff (Figure 8). During fermentation (bacteria-mediated reactions), a portion of nitrate in fire-cured snuff is converted to nitrite, which then reacts with alkaloids to produce snuff specific nitrosamines (TSNAs) [23]. However, the GC-MS analysis of special moringa sundu revealed the presence of some antimicrobial substances which include 2-Dodecenal, (E)-, Pentadecanoic acid, 14-methyl-, methyl ester, 2-Piperidinone, N-[4-bromo-n-butyl]-, and Octadecanoic acid. These antimicrobial substances could reduce the effects of the microbes.

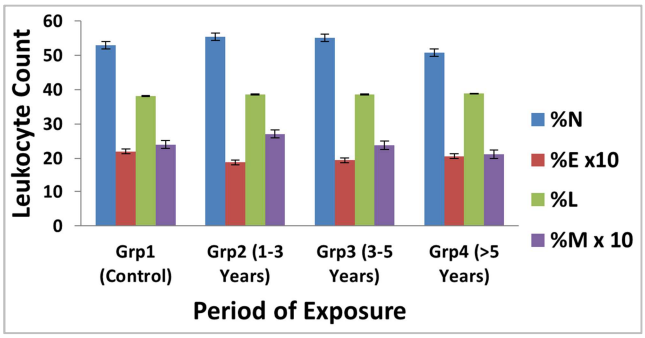


Figure 8. *Effects of snuff on Leukocyte count.*

Results are expressed as Mean ± S.E, and are significantly different at p<0.05. Key: %N = percentage Neutrophils, %E = percentage Eosinophils, %L = percentage Lymphocytes, %M = percentage Monocytes.

4. Conclusion

The findings of this study have identified addictive substances; nicotine and its metabolites in Special Moringa Sundu (SMS). Nicotine has been documented to affects many organs in human and animal studies. Its biological effects extend to all systems of the body. Based on this research continuous consumption of snuff can result to high blood pressure, type2 diabetes and kidney failure in chronic users.

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References

[1] Odangowei O., Tobia, P. E., and Adigwe, P. (2018). Microbial contamination of snuff sold in selected markets in Yenagoa, Bayelsa State, Nigeria. 3: 2455-4227.

[2] Bahl, V., Jacob, P., Havel, C., Schick, S. F. and Talbot, P., (2014). Third hand smoke: factors affecting exposure and remediation. e108258. doi: 10.1371/*journal.*pone.01082582.

[3] Cattaruzza, M. S, and West, R., (2013). Why do doctors and medical students smoke when they must know how harmful it is? *European Journal of Public Health.* 2013; 32: 188–189. doi: 10.1093/eurpub/cktool.

[4] Rozi, S. and Akhtar, S. (2007). Prevalence and predictors of smokeless tobacco use among high school mates in Karachi, Pakistan. *EMHJ-Eastern Mediterranean Health Journal*, 13 (4), 916-924.

[5] Desale T. (2019). Nutritional and Medicinal Importance of *Moringa oliefera* 6: 1-5.

[6] Atta, A. H., Mouneir, S. M., Nasr, S. M. (2019). Phytochemical studies and anti-ulcerative colitis effect of *Moringa oleifera* seeds and Egyptian propolis methanol extracts in a rat model. *Asian Pacific Journal of Tropical Biomedicine*; 9: 98.

[7] Muhammad, B. Y., Moses, Z. Z., Ruqaiyatu, A. M., Mustapha, M. A. (2021). Survey of Snuff use and Preliminary Study of effect of Two Brands on Brain Antioxidants and Acetylcholinesteras Enzyme of Wister Albino Rats *Al-Azhar University, Faculty of Medicine, Cairo, Egypt*.

[8] Khan Z., Suliankatchi, R. A. and Heise, T. L. (2017). Naswar (Smokeless Tobacco) Use and the Risk of Oral Cancer in Pakistan: *A Systematic Review with Meta-Analysis*. Nicotine and Tobacco Research; 21: 32-40.

[9] Ukwubile C. A., Agu, M. O., Agabila, E. J. (2020). Phytochemical and acute toxicity studies of *Melastomastrum capitatum* (Vahl) A. Fern. and R. Fern. (Melastomataceae) leaf methanol extract, *American Journal of biological life science, 3 (5) (2015), pp. 151-154.*

[10] Tiwari, P., Kumar, B., Kaur, M., Kaur, G. Andkaur, H. (2011). Phytochemical screening and extraction: A review. *International pharmaceutical science,* 1: 98-106.

[11] Varley H, Gowenlock AH, Bell M. Practical clinical biochemistry. W. Heinemann, London. 1980; 1 (741-742): 892-908.

[12] Bowers G, McComb R. (975). Measurement of total alkaline phosphatase activity in human serum; 1. DOI: 10.21275/v5i4.nov162819.

[13] Bartels, H. and Bohmer, M. (1972). Quantitative determination of creatinine. *Clinical chimicaacta*, 37: 193.

[14] Grizzell, J. A., Mullins, M., Iarkov, A., Rohani, A., Charry, L. C., and Echeverria, V. (2014). Cotinine reduces depressive-like behavior and hippocampal vascular endothelial growth factor down regulation after forced swim stress in mice. Behavioral Neuroscience, 128 (6), 713–721. https://doi.org/10.1037/bne0000021.

[15] Eunice, I. B., Olayemi, F. F., Awagu F. (2011). Proximate and Phytochemical Composition of *Moringa oleifera* Leaves at Three Stages of Maturation. *Advance Journal of Food Science and Technology*. https://www.researchgate.net/publication/289715135.

[16] Kasolo, J. N., Gabriel, S., Bimenya, L. O. Joseph, O. and Ogwal-Okeng, J. W. (2010). Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities. *Journal Medicinal Plants Res.,* 4 (9): 753-757.

[17] Starlin T, Prabha PS, Thayakumar BKA, Gopalakrishnan VK (2019) Screening and GC MS profiling of ethanolic extract of Tylophora pauciflora. Biomed Inform 15 (6): 425 429.

[18] Pedrinelli, R., Ballo, P., Fiorentini C. (2012). Hypertension and acute myocardial infarction: an overview. *Journal of Cardiovascular Medicine. (Hangerstown),* 13, pp. 194-202.

[19] Arrigo, T. D. (2021). How Does Nicotine Affect Blood Sugar? *WEB MD*.

[20] Carrillo, C., M-delma C., Alonso-Torre S. (2012). Role of oleic acid in immune system; mechanism of action; a review. Nutr. Hosp. 27 (4): 978-90. Doi: 10.3305/nh.2012.27.4.5783.

[21] Usunobun, U., Adegbegi, J., Ademuyiwa, O., Okugbo, T. F., Evuen, U., Osibemhe, M. and Okolie, N. P. (2012): Nnitrosodimethylamine (NDMA), Liver Function Enzymes, Renal Function Parameters and Oxidative Stress Parameters: A Review. *British Journal. Pharmaceutical Toxicology.;* 3 (4): 165-176.

[22] Metin, K. Erdogar, O. Lihami, Y. Fatma, I. and Ergul, B. K. (2004). "The investigation of the effect of Maras's powder (smokeless tobacco) on hematological parameters," *Turkish Journal of Hematology*, vol. 21, no. 3, pp. 131–136, 2004.

[23] Greer, R. O. (2011). Oral manifestation of smokeless tobacco use. *Otolaryngol Clinical North American*. 44 (1): 31-56.