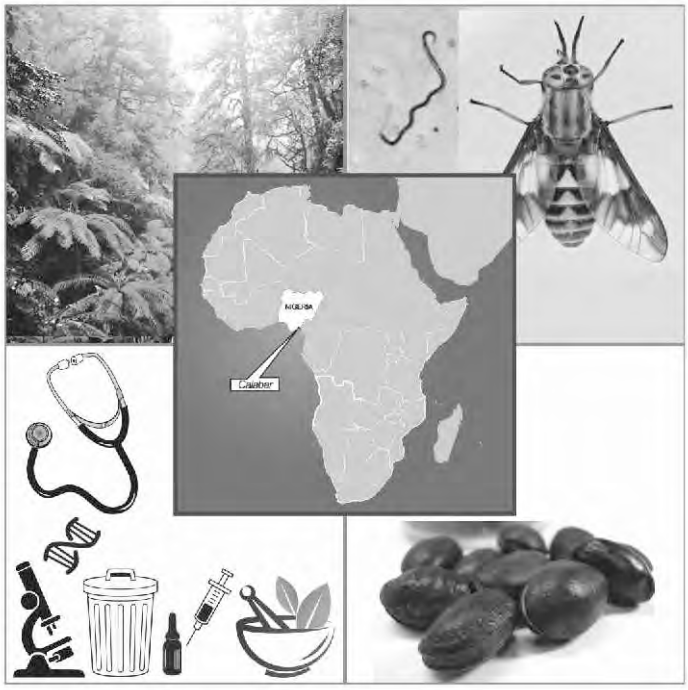
ISSN 2636-4786

CALABAR JOURNAL OF HEALTH SCIENCES

AN OFFICIAL PUBLICATION OF THE COLLEGE OF MEDICAL SCIENCES,

UNIVERSITY OF CALABAR

Published quarterly by the University of Calabar Press



VOLUME 2, NUMBER 1, 2018.

Published by
University of Calabar Press
P.M.B. 1115
Calabar – Nigeria.

COVER LOGO

Central block: Calabar is the home of the University of Calabar (founded in 1975) and the capital of Cross River State. The metropolis is situated between Latitudes 4˚30'N and 5˚15'N and Longitudes 7˚30'E and 8˚15'E along the banks of the Calabar River and about 50 nautical miles north of the Cross River estuary. The city which was the seat of the Niger Coast, Oil River and Southern Protectorates has a rich history steeped in colonialism that reflects the long-standing contact with Europe from early 15th century. The Calabar Slave History Museum is replete with relics of the trade in oil palm products and slaves. Today, the state and city are a burgeoning tourist haven in the West African sub-region.

Top left: The state is the location of a rich medley of tropical flora, typified by the tropical rain forest vegetation, so expansive and lush, that it generates a climate of its own. It is a virgin habitat for a variety of monkeys, flocks of gaudy plumaged canopy birds, vast underbrush communities of rodents and a wealth of insect lives.

Top right: The state is in a region endemic for loaiasis, a lymphatic and blood filariasis transmitted by the bite of the high canopy blood –sucking deer fly (mango fly) of the genus *Chrysops.* In infected persons, the adult worm often migrates beneath the conjunctiva (eye worm) or in the subcutaneous tissue, causing itchy fugitive swellings called "calabar-swellings"

Bottom right: The 'calabar beans,' (esere, in Efik), named for the place where the plant was first described, are shelled from pods of a herbaceous legume, *Physostigma venenosum.* The seeds have a high content of the alkaloid 'physostigmine' (eserine), the "ordeal poison". In the local folk lore, the seeds were used in the trials of suspects of grave social crimes and witch craft. Each suspect was made to chew and swallow a mouthful of the beans or drink a cup of the elixir. Guilt was usually established by prompt death from poisoning. Today, eserine as physostigmine salicylate has found its niche in clinical pharmacology as an antidote for reversible inhibition of acetyl cholinesterase activity, reversal of atropine activity and inhibition of anticholinergic drug toxicity of the central nervous system.

Bottom left: The iconic symbols represent the vast range and usage scopes of the disciplines in the health sciences.

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CALABAR

AN OFFICIAL PUBLICATION OF THE COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF CALABAR, CALABAR, NIGERIA

INSTRUCTIONS TO AUTHORS

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Calabar Journal of Health Sciences (CJHS), a quarterly publication of the College of Medical Sciences of the University of Calabar is published by the University of Calabar Press. The focus of CJHS is on the publication of new information on the clinical, physiological and sociological aspects of human health and disease. Commensurate emphasis is on disease etiologies, morbidity, mortality and the impact of new insights and advancing technologies on diagnosis, treatment, control and prevention.

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Efficacy of Selected Anthelminthic Drugs on Intestinal Nematodes Amongst School Children in Ugep, Cross River State, Nigeria.

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Abstract

The efficacy of selected anthelminthic drugs on intestinal worms given to primary school children in Ugep, Cross River State, Nigeria was evaluated between May and November, 2014. A cross sectional study design, simple cluster sampling method was adopted in the recruitment of 800 pupils aged 2-13 years drawn from primary 1-6 of four selected primary schools in the study area. Questionnaires were administered to obtain demographic information, knowledge and factors related to soil-transmitted helminthes (*Ascaris lumbricoides, Trichuris trichiura,* and Hookworm).Freshly passed stool samples were collected from each participant and examined within 24 hours of collection using direct smear, brine flotation and formol-ether concentration techniques to determine infection status. Screening and quantification of worm load was determined using Stoll's technique for egg count.A total of 533 infected pupils with one worm type or the other were treated with one of the three anthelminths (Albendazole, Pyrantel pamoate, or Levamisole).A post stool re-examination was carried out after two weeks interval to elucidate the efficacy of the anthelminths on specific intestinal nematodes. From the result, perception and response of pupils to infection was poor. Only 3.1% of the subjects were aware of intestinal nematode infection, 57.6% use either rivers/streams or water vendors as source of drinking water, 58.3% use open fields as toilet facility, and 34.9% wash hands after using the toilet and before food. The result of the analysis revealed a prevalence rate of 69.4% of intestinal nematode infections amongst the pupils. *Ascaris lumbricoides* was the most prevalent, with an infection rate of 38.5%, against infection rates of *Trichuris trichiura*(25.5%) and hookworm (24.3%) respectively. The effect of drugs on morbidity indicators for *Ascaris lumbricoides, Trichuris trichiura*, and hookworm was statistically significant, with albendazole having a percentage egg reduction rates of 98.1%, 85.0%, and 93.9% respectively, Pyrantel pamoate had, 95.8%, 58.3%, and 83.3% respectively while Levamisole gave 60.7%, 45.2%, and 65.6% respectively.Placebo had no effect on the controlled group. However, Albendazole was administered to the subjects on placebo at the end of the research. In conclusion, the three common anthelminths Albendazole, Pyrantel pamoate, and Levamisole are still efficacious, but levamisole efficacy makes it unpopular for use where helminth control is advocated. Albendazole with the highest impact on the helminths is recommended for the managemnt and control of helminth infections.

Key Words: Antheminthic drugs, intestinal nematodes, school children.

Introduction

Infections with the soil-transmitted helminthes are the most common infections of humans causing an estimated global burden of 39 million disability-adjusted life years lost (1). In spite of the current level of medical advancement and improvement in healthcare and personal environmental hygiene, the problem of helminthiasis still exist (2).

Griffen (3, 4) described helminthiasis as any macro-parasitic disease of humans and animals in which a part of the body is infected with parasitic worms called helminthes. This disease is seen to have infected more than a billion people, rivalling HIV/AIDS (5). Children and pregnant women are particularly vulnerable to this infection to the extent that affected children suffer serious illnesses, nutritional defects, cognitive impairments and occasional death (6).

The Nigerian environment in which most of the children live is poor in hygiene and conducive for the development of these parasites (7). Helminthiasis is treatable and controllable and since chemotherapy constitutes the mainstay of control of intestinal parasitic infections, it is important to assess the efficacy of the available chemotherapeutic agents in

current use from time to time (8). The selected anthelminthic drugs have been in use for decades and their efficacy need to be monitored.

This study therefore sought to determine the prevalence and intensity of the common intestinal nematodes in the study area, evaluate the influence of age and gender on their prevalence. The work also assessed the efficacy of anthelminthic drugs like Albendazole, Pyrantel pamoate, and Levamisole against multiple infections and determined the comparative efficacy of the selected drugs against them.

Materials and Methods

Study location

This study was carried out between May and November, 2014 in Ugep community, Yakurr Local Government Area of Cross River State, Nigeria. The community has a projected population of about 300,000 people with children under 13 years old accounting for over 150,000 (9). As in any such community, level of personal hygiene and environmental sanitation is low. There is also poor waste management of sewage disposal, lack of toilet facilities, poor portable water supply, and dispersal of ova and cysts of parasites by flood occasioned by heavy rains and warm weather which ensures the viability of ova and cysts. These risk factors are webbed into a vicious circle perpetuating continuous worm infestations and endemicity (5, 10, 11).

Ethical consideration / study population

Ethical approval with serial number CRS/MH/CGS/E-H018/VOL.11077 was obtained from the Cross River State Health Research Ethics Committee (CRS- HREC). Informed consents of parents, guardians of participating pupils were also obtained. The study subjects were primary school age children.

Inclusion criteria

Subjects aged between 2 and 13 years, resident within the community for up to 6 months and above, evidence of intestinal helminthiasis by stool microscopic examination, ability to ingest whole tablet or crushed, and informed consent of parents/ guardians were the inclusion criteria.

Exclusion criteria

Age < 2 years and >13 years, less than 6 months residence in the community, no evidence of intestinal helminthiasis by stool microscopic examination, inability to ingest tablets whole or crushed, and non-correspondence of parents/guardians formed the exclusion criteria

Methodology

Four public primary schools in the community were used as study sites.A total of 800 pupils were randomly selected and registered for the study, representing 200 pupils from each school.

The sample size was derived using the formular as prescribed by WHO [12], by substitution:

$$N = (Z^2 \times P \times Q)/E^2$$

Where: N= minimum sample size

Z= confidence limit at 95% (1.96)

P= expected prevalence ratio (46.5%)

Q= 100- P

E= marginal error (5%)

These selected pupils were registered with their names, age and gender. Their weight and height were also measured and recorded. The procedure for the exercise was explained to the pupils with the help of their teachers. Universal laboratory specimen bottles labelled with coded numbers were given to registered pupils who were of age while the teachers or parents assisted by receiving the bottles on behalf of the younger children for the purpose of collection of stool samples the following day. Consent forms were given to each pupil so that their parents or guardians could sign and return with the sample.

Processing of stool samples

Direct smear, brine floatation and formol-ether concentration techniques were used in the detection of intestinal nematodes (5, 13, 14).

The stool samples collected were examined on the same day for intestinal nematode. Any stool sample that was not examined was preserved in Merthiolate, Iodine Formalin (MIF) fixative (15). Stoll's technique for egg count was employed to determine the worm burden, which gives the intensity of infestation of human intestinal helminthes and to

elucidate the effect of the selected anthelminthics (8, 16).

Treatment

A randomized controlled Trial (RCT)/Cohort pattern of treatment was explored. A total number of 533 infected pupils who met the inclusion criteria were enlisted into one of the three groups of anthelminthic drug (Albendazole, Pyrantel pamoate, and Levamisole) treatments. A post treatment assessment was carried out two weeks after the pre-treatment to determine the efficacy of the drugs. To assess the efficacy of the study drugs on intestinal nematodes, a controlled group was placed on Placebo (vitamin A). Albendazole is available as tablets and each contains 400mg of the drug, single dose oral administration is usually required for all ages. Pyrantel pamoate was used at the usual dosage of 10mg/kg for children. The recommended dosage for levamisole for adults and children is 2.5mg/kg single dose.

Data analysis

The data obtained in this study were analyzed using the Predictive Analytical Software (PASW) 18.0. Chi-square was obtained for the study variables in order to determine the level of significance of intestinal nematodes infections by schools, age groups and gender.

P-values of less than 0.05 (<0.05), were considered statistically significant.

Results

Figure 1 is a Venn diagram showing the distribution of intestinal nematode parasites in the study area. The study registered 800 Pupils, but 768 Pupils returned bottles with samples and out of these, 445 were males and 323 females. Five hundred and thirty-three (64.9%) had parasites in their stool samples. Besides the intestinal parasites considered in the study, other parasites were also seen in pupils' stool samples namely, *Strongyloides stercoralis* (3.9%), *Giardia lamblia* (2.8%), *Entamoeba histolytica* (1.6%), *Taenia species* (1.1%), and *Hymenolepis* species (1.1%). Of the 768 pupils studied, 174 (22.65%) were infected with *Ascaris lumbricoides* alone, 120 (15.6%) had hookworm alone, and 98 (12.76%) had *Trichuris trichiura* alone. Forty-three (5.6%) pupils had both *Ascaris*

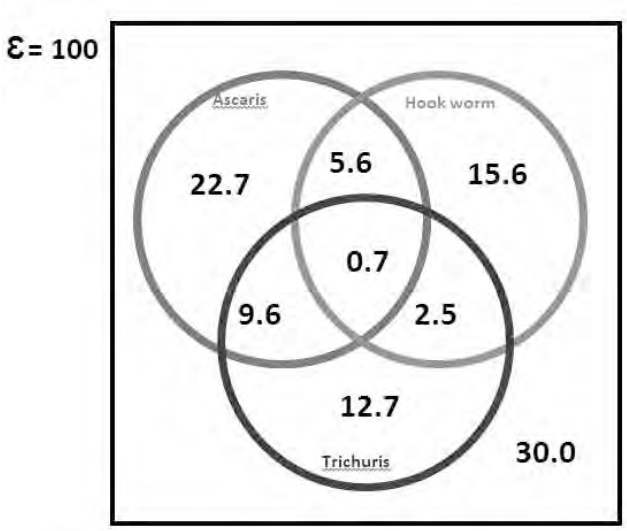

Fig.1: Venn diagram (%) of intestinal nematode infections in the study area

Age (years)	No. of pupils Examined	No. (%) of pupils Infected
2 – 5	394	272 (69.0)
6 – 9	108	77 (71.3)
10 -13	266	184 (69.2)
Total	768	533 (69.4)
Gender Males	445	310 (69.7)
Females	323	223 (69.0)
Total	768	533 (69.4)

Table 1: Prevalence of intestinal nematode infection by age and gender

lumbricoides and Hookworm, 74 (9.63%) had *Ascaris lumbricoides* and *Trichuris trichiura* while 19 (2.47%) had Hookworm and *Trichuris trichiura* and 5 (0.65%) pupils showed polyparasitism of *Ascaris lumbricoides,* Hookworm and *Trichuris trichiura*.

Figure 2 shows the cases of single, double and triple intestinal nematode infections among pupils by school. Out of 192 pupils screened in Apostolic Primary School, 47.4% had single infections, 16.1% had double infections, while 0.5% had triple infections. In Presbyterian primary school, 191 pupils were screened of which 52.9%, 19.9%, and 1.0% had single, double and triple intestinal infections respectively. Out of the 210 pupils screened at St. Mary's Primary School, infection status was 52.4%, 17.6%, for single and double infections respectively. Poly-parasitism was not

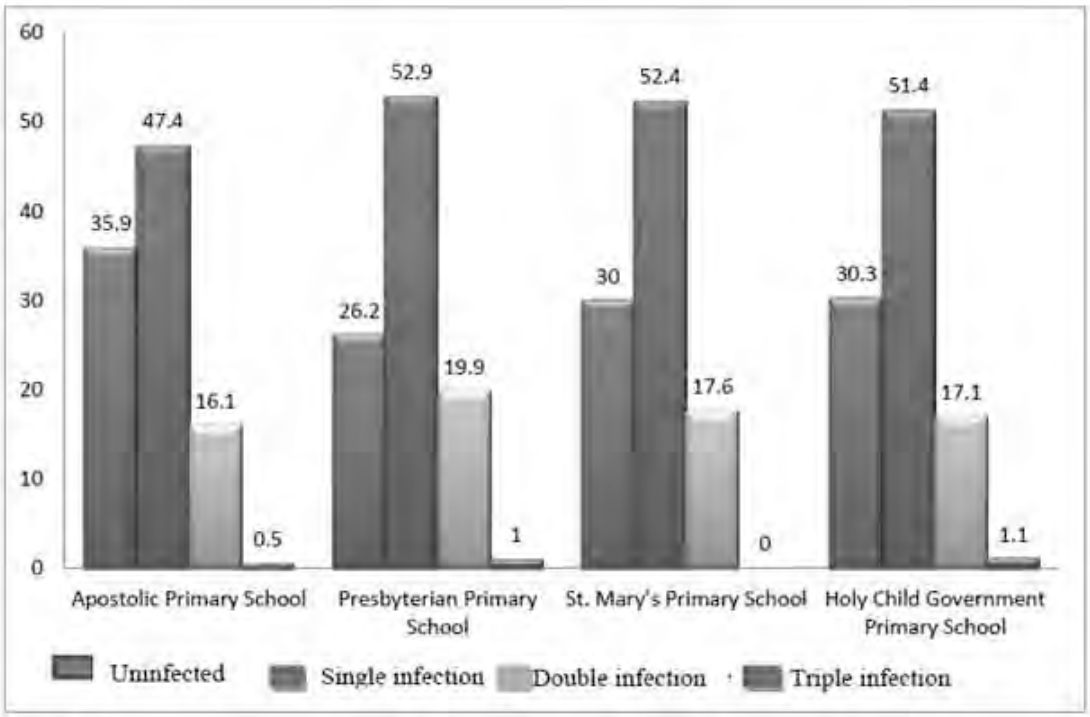

Fig. 2: Single and mixed intestinal nematode infections by schools

evident. In Holy Child Convent Primary School, 175 pupils were screened in which 51.4%, 17.1%, and 1.1% had single, double, and triple intestinal infections respectively.

Table1shows the prevalence of intestinal nematode infections according to age and gender of the pupils examined. The highest rate of infection occurred among the 6-9 years age group with 71.3% while the least rate of infection occurred in the age range 2-5 years with 69.0% ($\chi 2 = 0.214$, df = 2, P = 0.899). Out of 445 male samples examined, 310 (69.7%) were infected, while 223 (69.0%) were infected from 323 female samples. The males had a slightly higher prevalence rate of intestinal nematode infection than the females ($\chi 2 = 0.034$, df = 1, P = 0.457).However, there was no statistical significant difference in the prevalence of intestinal nematode infections by age of pupils and gender.

Table 2 shows the effect of drugs on morbidity indicators for *A. lumbricoides*. Ninety-five subjects who had ascariasis enrolled for Pyrantel pamoate treatment with an initial mean egg count of 2052.1 ± 996.4 and 91 (95.8%) were cured, 4 subjects had a mean egg count of 11.6 ± 5.5 after the treatment, giving a percentage reduction in egg count as 99.4%. For Albendazole treatment, the 104 subjects that were enrolled had mean egg count of 2090.3 ± 735.3 of which 102 (98.1%) were cured, 2 subjects had mean ova of 4.8 ± 2.1 after treatment giving a percentage reduction in egg count of 99.8%. With Levamisole treatment, the 61 subjects enrolled had mean egg count of 3038.7 ±723.1 out of which 37 were cured, 24 subjects had mean egg count of 126.2 ± 25.6 after treatment, giving a percentage egg count as 95.8%.

Table 3 shows the effect of drugs on morbidity indicators for Hookworm infection. Forty eight subjects infected with Hookworm enrolled for Pyrantel pamoate treatment with mean egg count of 1209.4 ± 1021.2 out of which 40 (83.3%) were cured, 8 subjects had mean egg count of 71.4 ± 33.8 after treatment, giving a percentage reduction in egg count of 99.4%. Forty- nine subjects enrolled for Albendazole treatment with mean egg count of 1066.7 ± 752.3 out of which 46 (93.9%) were cured , 3 subjects had 12.3 ± 10.3 after treatment, giving a percentage reduction in egg count as 98.9%. Thirty-two subjects enrolled for Levamisole treatment with mean egg count of 1096.3 ± 813.6 out of which 21 (65.6%) were cured, 11 subjects had mean egg count of345.5± 101.6 after treatment, giving a percentage

reduction in egg count as 68.5%.

Table 4 shows the effect of drugs on morbidity indicators for *T. trchiura.* Sixty subjects infected with *T. trichiura* enrolled for Pyrantel pamoate treatment with mean egg count of 480.5 ± 127.3 out of which 35 (58.3%) subjects were cured, 25 had mean egg count of 116.7 ± 23.0 after treatment, giving a percentage reduction in egg count as 75.7%. Fifty-three subjects enrolled for Albendazole treatment with mean egg count of 632.4 ± 99.4 out of which 45 (85.0%) were cured, 8 had mean egg count of 39.6 ± 99.4 after treatment, giving a percentage reduction in egg count as 93.7%. Thirty-one subjects enrolled for Levamisole treatment with mean egg count of 416.3 ± 116.1 out of which 14 (45.2%) were cured and 17 had mean egg count of 122.6 ± 69.1 after treatment, giving a percentage reduction in egg count as 70.5%.

The comparative efficacy of Pyrantel pamoate, Albendazole and Levamisole against *A. lumbricoides*, Hookworm and *T. trichiura* is presented on Table 5. Albendazole demonstrated the highest efficacy rate against *A. lumbricoides* (98.1 %), hookworm (93.9%), and *T. trichiura* (85.0%). Pyrantel pamoate had (95.8%), (83.3%) and (58.3%) respectively. Levamisole showed the least efficacy rate against intestinal nematodes (60.7 %), (65.6%), and (45.2%) respectively. The efficacy of Albendazole against intestinal nematode infections was statistically significant (P = 0.018). Placebo had no effect on the control group.

Table 6 shows on the perception of pupils/parents on infection with intestinal nematodes. Out of the 768 pupils sampled, 24 (3.1%) pupils were aware of intestinal nematode infection, 490 (63.8%) had a fair knowledge while 254 (33.1%) were ignorant of the infection. For possibility of water transmission, 72 (9.4%) pupils use pipe-borne water as source of drinking water, 254 (33.1%) use boreholes / wells, and 442 (57.6%) use either rivers / streams or water vendors. For possibility of toilet transmission, 148 (19.3%) pupils had flush toilet facilities, wash hands after using the toilet and before eating, 268 (34.9%) own pit toilets, occasionally wash hands after using the toilet and before eating while 448 (58.3%) use bush / open fields, seldom wash hands after using the toilet and before eating. In sampling for possibility of ground transmission, 496 (64.6%) pupils put on foot wears every time and also live in concrete buildings with cemented floors, 72 (9.4%) occasionally put on foot wears, live in concrete buildings with cemented floors, while 200 (26.0%) rarely put on foot wears and live in thatch houses with earthen floors, they also practice geophagy. For level of treatment, 196 (25.5%) pupils admitted to have taken anthelminths in the last 3-6 months, 142 (18.5%) had taken treatment but lack knowledge of what drug was given, while 430 (56.0%) never had treatment before. Infection status was obtained from the questionnaire as 490 (63.8%) pupils admitted to have passed out worms either in stool, vomit or sputum.

Discussion

A total of 800 pupils were registered for the study, eventually only 768 pupils could produce stool samples that were screened for intestinal nematode infections. Out of these, 533 (69.4%) pupils tested positive for the infections. The intestinal nematodes encountered and their prevalences in pupils of the four schools were *A. lumbricoides* (38.5%), *T. trichiura* (25.5%), and hookworm (24.3%). *A. lumbricoides* was the most prevalent and common among the pupils. The result obtained in the study showed moderate infections, but they corroborate the findings of previous researchers in terms of the species of intestinal nematodes encountered in school-age children and other age groups in Nigeria. The previous reports include those of (6, 10, 11, 17) in Katsina, Zaria, and Ibadan, who reported prevalences of 0.8 - 72.2% for *A. lumbricoides*, 13.5- 63% for hookworm and 0.3 - 74% for *T. trichiura*. The results showed that helminthiasis, specifically intestinal nematode infection is common to all age groups and to both sexes of pupils in the primary schools studied. Pupils were equally infected irrespective of their ages. The reasons being that, they share the same environmental conditions of inadequate sanitation, poor hygiene, nonchalant attitude toward foot wears, poor disposal of waste/sewage, lack of portable water, etc. Males were more infected than the females, probably because there were more males recruited in the study as class registers contained more of males than females.

The result of the comparative efficacy of these anthelminthes against the different intestinal

Table 2: Effect of drugs on morbidity indicators for *Ascaris lumbricoides*

Drugs	No. of Subjects treated	Pre – treatment mean egg count (mean±SD)	No. (%) cured	Post – treatment mean egg count (mean±SD)	% reduction in egg count	t-test	p.value
Pyrantel pamoate	95	2052.1±996.4	91 (95.8)	11.6±5.5	99.4	9.345	0.000
Albendazole	104	2090.3±735.3	102 (98.1)	4.8±2.1	99.8	8.986	0.000
Levamisole	61	3038.7±723.1	37 (60.7)	126.2±25.6	95.8	12.252	0.000

Table 3: Effect of drugs on morbidity indicator for Hookworm

Drugs	No. of subjects treated	Pre – treatment mean egg count (mean±SD)	No. (%) cured	Post – treatment mean egg count (mean±SD)	% reduction in egg count	t-test	p-value
Pyrantel pamoate	48	1209.4±1021.2	40 (83.3)	71.4±33.8	99.4	7.482	0.000
Albendazole	49	1066.7±752.3	46 (93.9)	12.3±10.3	98.9	5.371	0.000
Levamisole	32	1096.3±813.6	21 (65.6)	345.5±101.6	68.5	9.174	0.000

Table 4: Effect of drugs on morbidity indicators for *Trichuris trichiura*

Drugs	**No. of subjects treated**	**Pre – treatment mean egg count (mean±SD)**	**No. (%) cured**	**Post – treatment mean egg count (mean±SD)**	**% reduction in egg count**
Pyrantel pamoate	60	480.5±127.3	35 (58.3)	116.7±23.0	75.7
Albendazole	53	632.4±99.4	45 (85.0)	39.6±19.4	93.7
Levamisol	31	416.3±116.1	14 (45.2)	122.6±69.1	70.5

Table 5: Comparative efficacy of selected anthelminthic drugs used in this study

Drugs	No. treated for Asc	No. (%) Cured for Asc	No. treated for HW	No. (%) cured for HW	No. treated for Tt	No. (%) cured for Tt	
Pyrantel	95	91 (95.8)	48	40 (83.3)	60	35 (58.3)	
Albendazole	104	102 (98.1)	49	46 (93.9)	53	45 (85.0)	
Levamisol	61	37 (60.7)	32	21 (65.6)	31	14 (45.2)	

Asc: *Ascaris lumbricoides*; HW: Hookworm; Tt : *Trichuris trichiura*

Table 6: Perception of pupils on infection

Level of awareness of:				Total (%)
intestinal nematode infection	24 (3.1)	490 (63.8)	254 (33.1)	768 (100.0)
Possibility of water transmission	72 (9.4)	254 (33.1)	442 (57.6)	768 (100.0)
Possibility of toilet transmission	148 (19.3)	268 (34.9)	448 (58.3)	768 (100.0)
Possibility of ground transmission	496 (64.6)	72 (9.4)	200 (26.0)	768 (100.0)
Level of treatment	196 (25.5)	142 (18.5)	430 (56.0)	768 (100.0)
Infection status				
	Infected		Uninfected	
	490 (63.8)		278 (36.2)	768 (100.0)

nematodes showed a high activity of Albendazole across the three types of worms. Comparison of the effect of the three anthelminthic drugs against the worms showed that Albendazole and Pyrantel pamoate are still effective in the control and eradication of intestinal nematodes whereas Levamisole showed low efficacy against these worms, suggesting that the worms may have developed resistance possibly due to indiscriminate use of the drug over the past few decades. This proves to an extent the fear expressed by Benneth (18) about emergence of drug resistance of intestinal nematodes

against anthelminthes. In the case of *T. trichiura* control, the three drugs have demonstrated their current efficacy levels. The efficacy of Levamisole against *T. trichiura* was only 45.2%, that of Pyrantel pamoate was 58.3%, but Albendazole showed a higher efficacy of 85.0%. It is therefore obvious that a single dose of any of these drugs cannot achieve complete eradication of *T. trichiura*. This may equally be in agreement with literature report of Levecke (19) which eludes that more than one course of treatment may be required to eradicate *Trichuris trichiura*, especially in moderate and heavy infections.

Conclusion

The study has shown the prevalence of Ascaris lumbricoides to be 38.5%, Hookworm 24.3%, and *Trichuris trichiura* 25.5% with intensities of 3038.7±723.1, 1209.4±1021.2 and 632.4±99 respectively. Pupils were equally infected irrespective of their ages and gender. The comparative efficacy of the anthelminthic drugs used was established with Albendazole having the highest activity across the three intestinal nematodes, followed by Pyrantel pamoate while Levamisole had the lowest efficacy. Single-dose oral Albendazole, Pyrantel pamoate and Levamisole showed high cure rates against *A. lumbricoides*. For Hookworm infection, Albendazole was more efficacious than Levamisole and Pyrantel pamoate. Treatment of *T. trichiura* with single oral doses of current anthelminthics is unsatisfactory. New anthelminthics are urgently needed. No adverse reaction was encountered in the course of treatment with the study drugs. Placebo had no effect on the controlled group. However, the subjects were treated with Albendazole at the end of the study.

Recommendations

Mass Drug Administration should be accompanied with participatory hygiene and health education. More so, Control programs should focus more efforts on environmental and hygiene factors to reduce transmission and the cost of carrying out regular treatments since soil-transmitted helminths are readily available. The study suggests the need for choice of broad spectrum anthelminths with high sensitivity in management of suspected helminthiasis of unknown aetiology. Relatively short intervals between treatments (2-6 months) are required in areas with high environmental contamination of soil-transmitted helminths and multiple doses recommended against single dose therapy.

The study however highlighted the need to consider hygiene as an integral part of the health approach for the control of these infections while developing mass drug administration strategies.

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Evaluation of Serum α-Tocopherol Among Infertile Patients Attending Specialists Hospital, Sokoto, Nigeria

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Abstract

Infertility is the inability of a couple to achieve pregnancy over a period of one year despite adequate, regular and unprotected sexual intercourse. Avitaminosis E has been implicated in the development of infertility and hemolytic anemia in animals. There is however, little evidence that man is ever short in vitamin E. The aim of this study was to evaluate serum α-tocopherol levels and anemia among infertile patients attending Specialists Hospital, Sokoto. A total of fifty (50) infertile patients and fifty (50) apparently healthy fertile married men and women as control were recruited for this study. Blood samples were collected and analyzed for α-tocopherol using Hashim and Schuttringer (1996) method, and PCV. The data obtained were analyzed using independent students't-test. The p-value of less than or equal to 0.05 (≤0.05) are considered statistically significant. The results show that the level of serum α-tocopherol were significantly lower ($p<0.05$) in infertile patients (0.65±0.04µmol/L) compared to controls (1.38±0.02µmol/L). The result however show no statistically significant difference of PCV and BMI (37.58±0.4% and 21.14±0.34Kg/m^2 respectively) in infertile patients when compared to the controls (38.61±0.4% and 22.05±2.64Kg/m^2 respectively). A reduced serum α-tocopherol level among infertile patients was observed in this study. The α-tocopherol has been described to be a potent antisterility factor because sterility develops in its absence. We therefore, suggest the incorporation of α-tocopherol in both diagnosis and treatment of infertile patients attending Specialists Hospital Sokoto

Key Words: Infertility, α-tocopherol, anemia, Sokoto, Nigeria.

Introduction

Infertility is the inability of a couple to achieve pregnancy over an average period of one year (in a woman under 35 years of age) or 16 months (in a woman above 35 years of age) despite adequate, regular and unprotected sexual intercourse (1). According to American Pregnancy Association, infertility is defined as trying to get pregnant for at least a year without success (2). Conception is normally achieved within 12 months in 80 to 85% of couples using no contraceptive measures (3). In primary infertility the woman has never conceived despite exposure to sexual intercourse for at least two years, while in secondary infertility, the woman has previously conceived but is subsequently unable to conceive despite exposure to sexual intercourse for a period of two years (1). Tocopherol was derived from two Greek words: tokos (=offspring) and pherol (=to bear). Tocopherol therefore, literally means to bear children. This suggests the involvement of tocopherol in fertility (4). Alpha-tocopherol, a potent antioxidant vitamin that protects cells against damage from free radicals, was designated as antisterility factor on account of the development of sterility in its deficiency (5). The characteristic symptoms of experimentally-induced vitamin E deficiency vary from animal to animal. In mature female rats, sterility develops because of absorption of fetus after conception. While in males, the germinal epithelium of the testes degenerates and spermatozoa become non-motile. Avitaminosis E has also been implicated in the development of hemolytic anemia in monkeys (5).

Global estimates suggested that nearly 72.4 million couples experience fertility problems (6). World Health Organization estimated that between 8% and 12% of couples experienced some form of infertility during their reproductive lives. Thus, infertility affects 50 to 80 million people worldwide, out of which 20 to 35 million couples are in Africa (7). This can be extrapolated to 3 to 4 million Nigeria couples suffering from infertility (8). In Africa, the prevalence of infertility is higher particularly in Sub-

Sahara Africa ranging from 20% to 60% (9). An estimate of 19% infertile couples in Ile-Ife (10), and 15% from Usmanu Danfodiyo University Teaching Hospital, Sokoto have been reported (11). There is however, little evidence that man is ever deficient of the α-tocopherol. The aim of this study is to determine the serum levels of α-tocopherol and anemia among infertile patients attending Specialists Hospital, Sokoto.

Materials and Methods

Chemicals and Equipments

All chemicals used are of analytical grade. The chemicals used include xylene, ethanol, α-dipyridyl and ferric chloride purchased from Randox Company Ltd.

Study Population

A total of100 subjects were recruited for this study. They consisted of 50 apparently healthy married fertile men and women as control and 50 infertile patients attending Specialist Hospital, Sokoto.

Ethical Consideration and Clearance

The ethical approval for this study was sought and obtained from the Ethics and Research Committee of the Specialists Hospital, Sokoto prior to the commencement of the study.

Sampling Techniques

Arrangement was made with the clinicians for data collection from those that satisfy the study inclusion criteria. The nature and reasons for the study was explained fully to the subjects in appropriate language. Subjects consent was our priority and was obtained with their full compliance and co-operation.

Anthropometric Measurements

Standard procedure (12) was employed for anthropometric measurements. Body mass index (BMI) was determined by weight in kilogram (kg) divided by the square of the height in meters. The values of 20-25, <30 but >25, >30 and <20 were considered as normal, overweight, obese and underweight respectively.

Analytical Techniques

Serum α-tocopherol was estimated using the Hashim and Schuttringer method (13), while the pack cell volume (PCV) was read using microhematocrit centrifuge and reader (14).

Statistical/Data Analysis

The analysis of the data obtained was treated accordingly using Grap and Instat3 © (2008) Statistical package. The results were expressed as Mean ± SEM. Paired comparisons were carried out using independent students' t-test. A P-value ≤0.05 were considered statistically significant.

Results

The Socio-demographic characteristics of the study subjects are represented in Table1. They consisted of 50 infertile patients and 50 apparently healthy married fertile as controls. Table 2 shows serum α-tocopherol and BMI of patients and controls. The results show that the mean serum α-tocopherol of the patients was significantly lower (P<0.05) than the mean values for controls (1.38±0.02). There was no statistically significant difference (p>0.05) between the PCV of infertile patients and controls. The mean values of α-tocopherol and PCV of the study subjects are also presented in Table 2. The patients hard significantly lower α-tocopherol than control (p<0.05).

Table1: Demographic and clinical characteristics (Mean±SEM) of the study subjects

Subjects	N	Age(yrs)	BMI(kg/M^2)
Control	50	34.08±0.15	22.45±2.64
Male	30	34.30±0.36	21.54±0.60
Female	20	33.94±0.10	23.31±4.26
Patients	50	36.93±0.57	21.14±0.34
Male	22	35.75±1.21	20.47±0.64
Female	28	37.31±0.65	21.36±0.40

Mean±SEM.

Table 2: Serum α-tocopherol and BMI (Mean±SEM) of the study subjects

Subjects	N	Age(yrs)	α-tocopherol(µmol/L)	PCV(%)	BMI(kg/m^2)
Control	50	34.08±0.15	1.38±0.02	38.61±0.4	22.4±2.64
Patients	50	36.03±0.57	0.65±0.04	37.58±0.4	21.14±0.34
P-Value		>0.05	<0.05	>0.05	>0.05

Mean±SEM

Discussion

Many studies have demonstrated the damaging effects of elevated free radicals (reactive oxygen species) on sperm function. In the past decade, a number of studies were undertaken to ascertain whether such a proposal is truly helpful. Unfortunately, the few studies, the small sample size, and conflicting data have made it difficult for clinicians and researchers to agree on a recommendation. However, existing studies do seem to be encouraging (15, 16).

Oxidative stress, decreases antioxidant capacity and impaired sperm mitochondrial functions are the main factors contributing to infertility (16). In this study a significant lower level of serum α-tocopherol was observed in infertility compared to control. This is similar to the study conducted by Serena *et al,* (17), who showed a significant decrease in serum α-tocopherol level in infertile women. This is however, in contrast to the findings of Sasikumar *et al,* (18), who reported an increased level of serum α-tocopherol in the test group compared to the controls. Over the last decade, intensive research has been focused on various antioxidants and their optimal doses and combinations, for more effective and safe treatment of human fertility disturbances (15). Although, reactive oxygen species (ROS) have been shown to be required for sperm capacitation, hyperactivation, and sperm-oocyte fusion (19), excessive levels of ROS can negatively impact sperm quality (20). Improvement of sperm parameters after antioxidant therapy of infertility may result in higher pregnancy rate (21).

It has been evident that oral supplement of Vitamin E significantly improved sperm motility (22). There has also been existing evidence that suggests a relationship between daily antioxidant intake and better semen quality among healthy men (22). Semen analysis was performed on 97 healthy male volunteers and results were correlated with the results of a dietary assessment questionnaire (2). Higher levels of vitamin E intake were associated with higher levels of progressive sperm motility (2).

Conclusion

In conclusion, a reduced serum α-tocopherol in infertile subjects was observed in this study. The α-tocopherol has been described to be an antisterility factor on account of the development of sterility in its absence. We therefore, recommend the laboratory investigation of α-tocopherol and its inclusion in the treatment of infertility.

Authors' contributions

This work was carried out in collaboration between all the authors. Authors FUB designed the study. Author IZW did the literature searches. Authors DMK and MAS designed the protocol. Authors FUB and MIY carried out the samples collection and analysis. The first draft of the Manuscript was written by author FUB. All authors read, reviewed and approved the final manuscript.

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Ascorbic Acid Level, Total Antioxidant Status and Cellular Immune Response Among Malaria Infected Children in Calabar Metropolis, Nigeria

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Abstract
Malaria infected children in Calabar metropolis were assessed for common anti-oxidative indicators alongside white blood cell counts as immunity platform against malaria infection. Participants were 10years and below comprising 122 children diagnosed for malaria infection and 60 apparently healthy children without malaria infection. The blood cell count was by automation using ADVIA 60, while, total antioxidant status and ascorbic acid were determined colorimetrically. Total leucocyte count (8.6±3.7x10^9/L) as well as the granulocyte (4.9±4.1 x10^9/L), lymphocyte and monocyte (3.7±1.7 x10^9/L & 0.6±0.6 x10^9/L respectively) subsets were significantly ($p<0.05$) raised when compared with the control group (5.4±1.5 x10^9/L, 2.6±1.2 x10^9/L, 2.5±0.7 x10^9/L and 0.4±0.1 x10^9/L respectively). There was significant depletion of both total antioxidant status (0.63±0.31mmol/L) and ascorbic acid level (38.6±18.8mmol/L) among malaria infected children compared to the control subjects (1.26±0.21mmol/L & 59.0±28.0mmol/L respectively) in the current study. Children with severe malaria infection showed raised parasite (8499.6±2556.3dL) and lymphocyte (4.2±1.8 x10^9/L) counts but reduced total antioxidant status (0.45±0.27mmol/L) as well as ascorbic acid level (36.6±20.9) when compared with their counterparts who had mild/ moderate malaria infection (3698.5±1210.8dL, 3.4±1.4 x10^9/L, 0.74±0.27mm0l/L & 39.7±17.0mmol/L). Those aged 6-10 years had significantly raised total antioxidant status (0.76±0.3mmol/L), reduced granulocyte (4.2±1.8 x10^9/L) and lymphocyte (3.0±1.4 x10^9/L) counts compared with the ones aged 5 years and below (0.59±0.3mmol/L, 4.5±3.0 x10^9/L & 4.0±1.5 x10^9/L respectively). Therefore, depletion of total antioxidant status and ascorbic acid level in malaria infection particularly in severe cases has shown the need for supplementation as a palliative in the management of malaria infection.

Key Words: Antioxidant status, Immune response, childhood malaria.

Introduction

The global efforts at combating malaria have undoubtedly yielded appreciable results. This is evidenced by the 21% and 29% reductions in incidence and mortality rates respectively between the period of 2010 and 2015 (1). These gains however have not diminished the perspective that malaria is a public health challenge. In 2015 alone, two hundred and twelve million clinical episodes and four hundred and twenty nine thousand deaths were recorded worldwide. There is a great need to tackle malaria in endemic areas including the present study setting as such areas apparently sustain the global spread. In fact, the relatively few occurrences in areas with history of successful eradication such as the United States have been largely linked to movement of travelers from endemic regions (2).

Currently the prevalence of malaria (by microscopic diagnosis) among children below five years of age in Calabar was reported as 40.1% (3). Such an alarming high prevalence calls for more investigative studies among this vulnerable group. It is also interesting to note that within endemic areas, malaria in children often co-exists with reduced levels of nutritional indicators that extend to general host immunity. The cycle continues with a net effect of increased morbidity and mortality (4,5). Acquired host immunity has been noted to determine the outcome of malaria; a reason for which children, particularly those below five years of age, are considered vulnerable (6). The cyclical fever episodes characteristic of malaria are thought to be induced through inflammatory mechanisms. While parasite clearance; a major possible positive outcome may be desirable, the risk of pathological disease progress is imminent in sustained host inflammatory response. Thus, clarity of the inflammation in malaria disease has continued to

attract research efforts. To this end, several pathways and mechanisms have been adduced including tissue cellular response and cytokine secretion. More recently, uric acid has been suggested as a target molecule for therapeutic arrest of inflammation in malaria (7).

Amidst the controversies surrounding the effect of ascorbic acid on uric acid levels, a meta-analysis of randomized controlled trials concluded that ascorbate supplementation significantly lowered serum uric acid levels (8). By extension, the recent implication of uric acid in the inflammatory response of malaria warrants the consideration for the effect of ascorbic acid on malaria parasitaemia. In view of this, total antioxidant status and ascorbic acid level noted as anti-oxidative indicators were measured alongside white blood cell counts as the immunity platform for assessment of malaria parasitaemia among the studied population.

Materials and Methods

This research work was carried out in Calabar, Nigeria. All participants were below 5years of age comprising 122 children microscopically diagnosed for malaria infection and 60 apparently healthy children without malaria. Ethical approval was granted and informed consent was obtained from the parents / guardians of the children. Two milliliters of venous blood was collected aseptically from each subject for direct thick and thin film making while the remain was transferred to into dipotassium ethylene diamine tetra-acetic acid ($EDTA\ K_2$) bottle for blood cell count by automation. using ADVIA 60 by Siemens Healthineers UK.

Total antioxidant status and ascorbic acid were determined by the methods of Miller *et al.* (9) and that of Roe and Kuether (10) respectively. SPSS 22.0 was used for the statistical analyses of data. Pearson's correlation coefficient (r) was used to express relationship between two variables. A two tailed P-value of <0.05 was considered indicative of a statistically significant difference.

Results

The total antioxidant status and ascorbic acid level of malaria-infected children were significantly lower compared to the control subjects. At the same time, total white cell, granulocyte, lymphocyte and monocyte counts of the former were significantly raised as compared to the later (Table 1). More of the affected children were within their first five years of age. This group had a much lower total antioxidant status but higher granulocyte and lymphocyte counts compared to the older ones as shown in Table 2.

Gender had no marked difference on both total antioxidant status and ascorbic acid level but indicated higher values of total white cell and monocytes counts among the malaria-infected male children compared to the females (Table 3).

Severity of the malaria disease as categorized by the observation of anaemia and/or convulsion was also considered. The severely affected children had much lower values for both total antioxidant status and ascorbic acid level but with increased lymphocyte

Table 1. Total antioxidant status, ascorbic acid level and white blood cell counts among malaria infected children in Calabar metropolis compared with control group

Parameters	Control subjects n=60	Malaria-infected children n=122	Significance
Total antioxidant status mmol/L	1.26±0.21	0.63±0.31	$p<0.05$
Ascorbic acid mmol/L	59.0±28.0	38.6±18.8	$p<0.05$
Total white cell count $\times10^9$/L	5.4±1.5	8.6±3.7	$p<0.05$
Granulocytes $\times10^9$/L	2.6±1.2	4.9±4.1	$p<0.05$
Lymphocytes $\times10^9$/L	2.5±0.7	3.7±1.7	$p<0.05$
Monocytes $\times10^9$/L	0.4±0.1	0.6±0.6	$p<0.05$
Parasite count dl	-	5391.3±2923.1	$p<0.05$

Table 2. Comparison of studied parameters based on age

Parameters	1-5 years n=93	6-10 years Significance n=29	Significance
Total antioxidant status mmol/L	0.59 ±0.3	0.76±0.3	p<0.05
Ascorbic acid mmol/L	37.9± 18.3	41.6 ±19.2	p>0.05
Total White cell count ×10^9/L	10.2 ±7.4	7.7± 2.1	p>0.05
Granulocytes ×10^9/L	4.5±3.0	4.2± 1.8	p<0.05
Lymphocytes ×10^9/L	4.0±1.5	3.0± 1.4	p<0.05
Monocytes ×10^9/L	0.6±0.5	0.5±0.4	p>0.05
Parasite count dl	3, 870±2,564.4	2,004.3±358.7	p>0.05

Table 3. Comparison of the studied parameters based on gender

Parameters	Females n=53	Males n=69	Significance
Total antioxidant status mmol/L	0.59 ±0.3	0.68±0.31	p>0.05
Ascrobic acid mmol/L	40.8± 19.2	36.9 ±18.3	p>0.05
Total White cell count ×10^9/L	7.9 ±2.8	9.3± 4.3	p<0.05
Granulocytes ×10^9/L	4.1±1.9	4.7± 3.2	p>0.05
Lymphocytes ×10^9/L	3.4±1.6	3.5± 1.5	p>0.05
Monocytes ×10^9/L	0.5±0.3	0.7±0.6	p<0.05
Parasite count dl	5,387.0±2,834.4	5,393.0±3002.1	p>0.05

count (Table 4). Both the total antioxidant status and ascorbic acid levels correlated negatively with the parasite count as shown on Figures 1and 2.

Discussion

Blood cell response to malaria parasitaemia as observed in the present study indicated high parasitaemia being associated with significant increases in total leucocyte count as well as the granulocyte and agranulocyte subsets. Previous reports confirm the commitment of granulocytes, especially neutrophils, in the immune response to malaria while the agranulocytes have been observed differently (11,12). Non-significant differences have also been reported earlier, although one such study had reported results in relative rather than absolute values (13). Ascorbic acid has long been identified as an enhancer of human immunity by mediating diverse cellular responses. It is actually considered essential for effective immune system function (14). Consequently, its depletion is commonly observed in inflammatory conditions including malaria as reported among children as well as pregnant women (15,16). The significantly increased values of total leucocyte count and the subsets coexisted with significant depletion of both total antioxidant status and ascorbic acid levels among malaria infected children compared to the control subjects in the current study. Active transport mechanism for ascorbic acid into leucocytes against a plasma concentration gradient

Table 4. Comparison of the studied parameters based on severity of infection

Parameters	Mild/Moderate n=73	Severe Disease n=49	Significance
Total antioxidant status mmol/L	0.74 ±0.27	0.45±0.27	p<0.05
Ascrobic acid mmol/L	39.7± 17.0	36.6 ±20.9	p<0.05
Total White cell count $\times 10^9$/L	8.3 ±3.7	9.1± 3.7	p>0.05
Granulocytes $\times 10^9$/L	4.4±2.8	4.4± 2.5	p>0.05
Lymphocytes $\times 10^9$/L	3.4±1.4	4.2± 1.8	p<0.05
Monocytes $\times 10^9$/L	0.6±0.5	0.6±0.5	p>0.05
Parasite count dl	3,698.5±1,210.8	8,499.6±2556.3	p<0.05

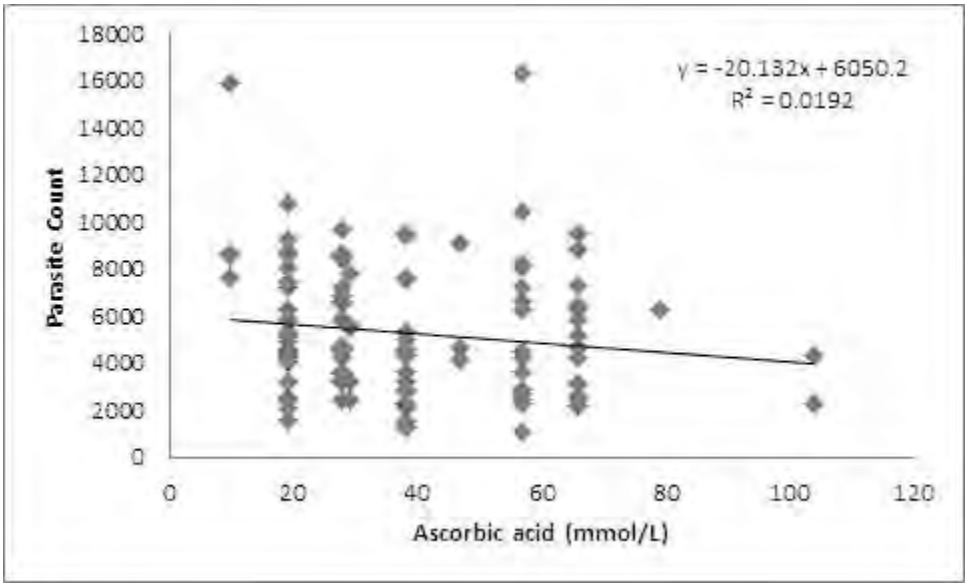

Fig.1 Scatter plot of parasite count against ascorbic acid

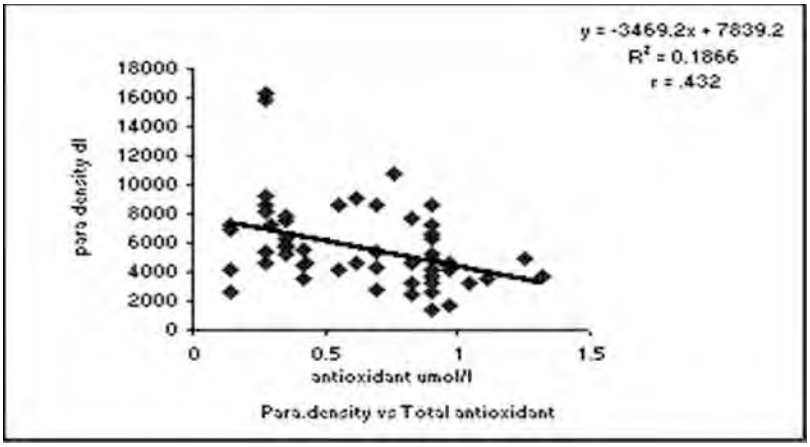

Fig.2 Scatter plot of parasite count against total antioxidant

of up to 80:1 has been highlighted as evidence to the importance of ascorbic acid in host immunity (14). Consequently, increase in leucocyte count as part of host immune response could expectedly be at the expense of plasma ascorbic acid concentration and by extension the total antioxidant status. Therefore, the negative correlations observed between the parasite count and both the total antioxidant status and ascorbic acid level seem to buttress the above assertion of increased consumption in the face of malaria infection. Those aged 5 years and below were more affected when compared to the older ones. This may be due to obvious lack of acquired immunity associated with that age group (17). However, male subjects generally had only higher monocyte and total white cell counts.

The children classified as having severe malaria were those who presented with the WHO (18,19) classification for severe malaria in children including anaemia and convulsion. Severity of malaria infection, apart from raised parasite count, occasioned a depletion of total antioxidant status and ascorbic acid as well as a raise in the lymphocyte count. This involvement of monocyte and lymphocyte subsets apparently signifies the adjustment towards a more adaptive response to malaria among the children. Acquired immunity to malaria, apart from the prospect of antibody secretion, is quite diverse with the advancement of certain subsets linked to increased immunity (20). Ascorbic acid level was particularly inversely related to the parasite count; apparently mediating host immunity of the infected children with a resultant low parasitaemia. For this vulnerable group who are still low on acquired immunity but living in malaria endemic regions, ascorbic acid supplementation could be a palliative to incessant bouts of malaria. Understanding the various facets to managing the malaria menace is obviously critical to the eventual eradication in the nearest future. This drive would undoubtedly require commitment from sub-Saharan Africa where almost a quarter of all child death has been attributed to malaria. Unfortunately, nutrition and immunity which influence outcome of the disease are still areas of considerable challenges in child health among resource poor regions (21).

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The Perception and Management of Work-related Stress among Nurses in Neuro-Psychiatric Hospital, Yaba, Lagos.

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Abstract

Stress is a condition or feeling experienced when a person perceives that the demands of a situation threaten to exceed the personal or social resources the individual is able to mobilize. Neuro-Psychiatric nurses in particular experience much job stress due to the unusual working environment where they are confronted with violent and aggressive patients. This study focused on investigating how nurses in the Neuro-Psychiatric Hospital, Yaba, Lagos manage occupation-related stress which appears not extensively studied in this institution. A descriptive survey design was used. Nurses were purposively selected for the study and proportionate stratified random sampling technique used to select 210 nurses; 187 nurses returned analyzable questionnaire (11% attrition). Self-developed validated questionnaire was used for data collection (r = 0.94). Percentages and mean were used for descriptive data and Pearson's correlation for analysis of hypotheses at 0.05 significance level. Findings were as follows: A positive and significant correlation between stress in workplace and job output among nurses (r = 0.947, p - value = 0.000 < 0.05); A positive and significant correlation between stress in workplace and nurses' input to their families (r = 0.782, p-value = 0.000 < 0.05) and a positive and significant correlation between stress in workplace and nurse well-being (r = 0.953, p-value = 0.000 < 0.05). Neuro-Psychiatric nurses are very susceptible to occupational stress which appears to impact on their job output, nurses' input to their families and physical wellbeing. This may also compromise productivity, performance and quality of patient care. Stress management and intervention programmes should be introduced in nursing institutions before the commencement of clinical nursing practice.

Key Words: Nurses, Psychiatry, Stress management, Stressors.

Introduction

Stress has been categorized as an antecedent or stimulus, as a consequence or response, and as an interaction. According to Jennings, (1) it has been studied from many different frameworks or perspectives. Lazarus (2) advocated a psychological view in which he stated that stress is "a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being". Stress is a condition or feeling experienced when a person perceives that the "demands of the situation threaten to exceed the personal or social resources the individual is able to mobilize." (3, 4). Nursing job involves shift work and long work hours. This causes both physical and psychological stress among nurses (5, 6). The nature of Nurse's environment include an enclosed atmosphere, time, pressures, excessive noise, sudden swings from intense to mundane tasks, no second chance, unpleasant sights and sounds and long standing hours (7). Psychiatric nursing is assumed to be a stressful area of nursing practice. However, evidence to support this claim is limited as research in this field is minimal. Psychiatric nurses experience much job stress due to the unusual working environment. For instance, they work in wards with locked entrances, and sometimes when there is confrontation and violence by aggressive patients who may attack the nurses while on duty and make them sustain injuries; the nurses do not have easy escape routes. Effective occupational stress management among nurses should be geared towards reducing and controlling nurses' occupational stress and improving coping at work.

According to Onasoga *et al*, (8) work-related stress significantly reduces brain functions such as memory, concentration and learning and impairs the immune system, all of which are central to effective performance at work. It may also be manifested as ineffective coping patterns, impaired

thought processes or disrupted relationships which renders the nurse incompetent and prone to errors in their clinical decision making and practice. Work related stress reduces productivity, increases management pressures and makes people ill in many ways, evidence of which is still increasing (9).

Stress as a global phenomenon interfaces with individual's work performance. Stress in an individual's job, family life and health leads to physical and emotional symptoms such as lack of concentration, frequent headaches, pain, poor judgments, depression or general unhappiness, social isolation and so on. Several studies have strong evidence for the intimate relationship between our psychosocial live and our physical health. (10, 11). This is because the mind and body are strongly linked. As mental health declines, physical health can wear down, and if physical health declines, it can make you feel mentally "down."

In view of the ongoing discussion, it is perceived that stress has negative effects on productivity of nurses working in Neuro-Psychiatric Hospital, Yaba, Lagos. It is towards this that this study focuses on finding out how nurses manage occupation-related stress. It also appears like the effect of stress on workers in this institution has not been extensively studied. This study therefore, investigated the perception and management of work-related stress among nurses in Neuro-Psychiatric Hospital, Yaba, Lagos in order to identify the prominent sources of job stress among nurses; determine the prominent effect of job stress among nurses; and explore the preferred coping mechanism to reduce job stress among nurses.

Materials and Methods

A descriptive survey design was used for this study. The population was made up of Nurses in Neuro-Psychiatry Hospital, Yaba, Lagos. Registered nurses from Nursing Officer II (NO II) to Chief Nursing Officers (CNO) were purposively selected. Sixty percent of this, totaling 210 were selected to participate in the study using proportionate stratified random sampling technique. However, 187 nurses returned analyzable questionnaire at the end of the study; making a total of 11% attrition.

Ethical Consideration

Ethical clearance was obtained from the Hospital Ethics Committee. Also, a written consent was obtained from participants selected for the study.

A validated Researcher-designed structured questionnaire, with reliability of 0.94 was used to collect data. The questionnaire had four sections. items in section A on socio-demographic characteristics of the respondents. Section B contained items regarding nursing stressors (work-related); section C with items on nurses' perception of their stress and section D on nurses' coping strategies/techniques. Descriptive and correlating data analyses were done with the aid of SPSS version 21.0 presented as means, percentages and correlation coefficients. Hypotheses testing were done at 0.05 significance level.

Results

Table 1 shows that for the gender distribution of the participants,137 (73.7%) are females and 50 (26.3%) of the participants are males. Age distribution of the participants shows that 113 (60.4%) are within the age range of 36-50years; therefore, they still have a minimum of 10 years in service. Only 11 people (5.9%) are very close to retirement. The marital status of the respondents shows that 33 (17.6%) are singles, 149 (79.7%) are married and 5 (2.7%) are widowed.

Educational qualification of the participants shows that they were Registered Nurses and Psychiatric Nurses (100%), while 199 (63,6%) were also Registered Midwives. The nurses with B.Sc. nursing degrees were 64 (34.2%), those without degrees were 102 (54.5%), and those with masters degrees were 21 (11.3%). This shows that a large number of nurses are yet to receive university education. The distribution of the nurses by year of employment shows that 31 (16.6%) have 1-10years experience, 87 (46.5%) have 11-20years experience, 59 (31.6%) have 21-30 years of experience while 10 (5.3%) have 31-35 years of experience. Majority of the respondents have spent between 11-20years in service and thus have adequate information about work-related stress.

Table 2 shows the various work-related stressors affecting nurses at work. The stressor which affects 89 (47.6%) of the nurses was job-related, while 63 (33.7%) of the nurses indicated that organizational system was the second leading stress factor. On the work interaction stressors affecting nurses, 52 (27.8%) indicated that

interpersonal conflict affects them, 52 (27.8%) indicated the lack of supportive interrelationship between colleagues at work, while 103 (55%) of the nurses indicated communication with colleagues as a major factor. Regarding job satisfaction stressors affecting nurses, 143 (76.5%) indicated that improper reward system affects them at work, 61 (32.6%) indicated that work place (environment) affects them, while 41 (21.9%) of the nurses indicated that team work affects them also. Regarding work-related stressors affecting nurses; 54 (28.9%) indicated that work instability (shift and rotation) affects them at work, 61 (32.6%) indicated that anxiety and fatigue of work affects them, while 71 (38%) of them indicated that financial difficulties affect them, only 1 (0.5%) of the nurses indicated that none of the stressors in the work-life balance affects her.

Table 3 shows that 13.4% of the respondents strongly agree, 53.5% agree, 16% disagree, while 17.1% strongly disagree that stress at work-place affects their general health. This means that majority of the respondents agree that work-related stress affects the general health.

On the negative effect of stress on output, 16% of

Table 1: Socio-demographical characteristics of participants (n= 187)

	Frequency	Percentage (%)
Gender		
Female	137	73.7
Male	50	26.3
Age		
25-30years	18	9.6
31-35years	23	12.3
36-40years	56	29.9
41-45years	29	15.5
46-50years	28	15.0
51-55years	22	11.8
56-60years	11	5.9
Marital Status		
Single	33	17.6
Married	149	79.7
Widow/Widower	5	2.7
Educational Qualification		
RN	187	100
RM	119	63.6
RPN	187	100
B. Sc Nursing	64	34.2
No degree	102	54.5
MPH	6	3.2
MPA	5	2.7
MBA	5	2.7
M. Sc	5	2.7
Years of employment		
1-10 years	31	16.6
11-20years	87	46.5
21-30years	59	31.6
31-35years	10	5.3

Source: Field Survey, 2016.

Table 2: Nursing Stressors

Items	Frequency	Percentage (%)
Work related Stressors		
Job stress	89	47.6
Organisation system	63	33.7
Lack of job autonomy	35	18.7
work interaction stressors		
Interpersonal conflict	52	27.8
Supportive interrelationships	52	27.8
Communication	103	55
Job satisfaction stressors		
Improper reward	143	76.5
Work place	61	32.6
Team work	41	21.9
work life balance stressors		
Work instability	54	28.9
Anxiety and fatigue	61	32.6
Financial difficulties	71	38
None	1	0.5

the respondents strongly agree, 67.4% agree, 11.2% disagree, while 5.3% of the respondents strongly disagree that stress in the work place negatively affects their work output. This means that majority of the respondents are negatively affected by work-related stress at the work place.

While 50.3% of the respondents agree, 33.7% disagree, 16% strongly disagree that stress affects their contribution to the success of the family. Majority of the respondents are of the perception that work-related stress affects their contribution to the success of the family.

Among the respondents, on the relationship between stress and work, 11.2% strongly agree that they experience work-related stress, 57.2% agree, while 26.2% disagree and 5.3% strongly disagree that the stress they experience is work-related. This implies that majority of the respondents attribute the stress they experience to their work place.

Of the respondents, 11.8% strongly agree, 38% of the respondents agree, 33.2% disagree, while 17.1% of the respondents strongly disagree that stress affects their work place relationship. This means that majority of the respondents agree that work-related stress affects work place relationship.

Table 4 shows the respondents' strategy or technique for managing work-related stress. For the debriefing/counselling, 55.6% of the respondents go for debriefing/counseling, 81.3% makes or listens to jokes, 68.4% use praying and meditating, while 6.4% use alcohol and drugs to cope with stress. Of the respondents, 68.4% sleep to counter work-related stress, while 38.5% engage in physical activities to counter stress, 29.9% avoid any stressful activity at the work place, 5.3% resort to transference, 10.6% of the respondents resort to dancing. A few (5.3%) of the respondents use listening to music to cope with/manage work-related stress. All these mean that majority of the respondents listen to jokes or make jokes to laugh and make themselves happy. Some of the nurses however, find comfort in religion by praying and meditating. Transference, dancing, listening to music and alcohol/drugs are the least used strategies.

Test of Hypotheses

Hypothesis 1. There is no significant relationship between stress in workplace and job output among nurses. **(Ho).**

This is a Bi-variate correlation; it is significant at 0.01 level (2- tailed)

From Table 5 above, it was observed that the returned Pearson's Correlation Coefficient (r) was calculated as +0.947, which indicates that the strength of association between the variables is very high ($r = 0.947$), and that the correlation coefficient is very highly significantly different from zero ($p < 0.001$). This indicates that there was a positive and significant correlation between stress in workplace and job output among nurses. Also, it can be inferred that 90% (0.947) of the variation in job output is explained by stress in workplace. Thus, the null hypothesis is rejected and alternative hypothesis

Table 3: Nurses' perception of their work-related stress

Items	SA	A	D	SD
Stress at the work place affects my general health.	25 13.4%	100 53.5%	30 16.0%	32 17.1%
Stress in the work place negatively affects my work output.	30 16.0%	126 67.4%	21 11.2%	10 5.3%
Stress affects my contribution to the success of my family.		94 50.3%	63 33.7%	30 16.0%
The stress I experience is usually work-related.	21 11.2%	107 57.2%	49 26.2%	10 5.3%
Stress affects my work place relationship with colleagues.	22 11.8%	71 38.0%	62 33.2%	32 17.1%

Source: Field Survey, 2016.

Table 4: Strategy/Techniques of coping with stress

Stress coping strategy/Technique practiced by Nurses	Frequency	Percentage (%)
Debriefing/counseling	104	55.6
Making jokes	152	81.3
Comfort in religion (praying and meditating)	128	68.4
Use of Alcohol/drugs	12	6.4
Sleeping	128	68.4
Physical activity for stress	72	38.5
Avoidance of stress	56	29.9
Transference	10	5.3
Dancing	20	10.6
Listen to Music	10	5.3

Source: Field Survey, 2016.

accepted because there is a significant relationship between stress in workplace and job output among nurses **(H_1).**

Hypothesis 2. There is no significant relationship between stress in workplace and nurses' input to their family. **(Ho).**

This is a Bi-variate correlation; it is significant at 0.01 level (2- tailed)
From Table 6, it was observed that the returned Pearson's Correlation Coefficient (r) was calculated as +0.782, which indicates that the strength of association between the variables is moderately high (r = 0.782), and that the correlation coefficient is moderately significantly different from zero ($p < 0.001$). This indicates that there was a positive and significant correlation between stress in workplace and Nurses' input to their family. Also, it can be inferred that 61% (0.782) of the variation in Nurses' input to their family is explained by stress in workplace. Thus, the null hypothesis is rejected and alternative hypothesis accepted because there is a significant relationship between stress in workplace and Nurses' input to their family **(H_1).**

Hypothesis 3. There is no significant relationship between stress in workplace and job physical well-being of nurses **(Ho).** This is a Bi-variate correlation; it is significant at 0.01 level (2- tailed)
From Table 7 it was observed that the returned Pearson's Correlation Coefficient (r) was calculated as +0.953, which indicates that the strength of association between the variables is very high (r = 0.953), and that the correlation coefficient is very highly significantly different from zero ($p < 0.001$). This indicates that there was a positive and significant correlation between stress in workplace and Nurses well-being. Also, it can be inferred that 91% (0.953) of the variation in Nurses well-being is explained by stress in workplace. Thus, the null hypothesis is rejected and alternative hypothesis accepted because there is a significant relationship between stress in workplace and Nurses well-being **(H_1).**

Discussion

From the biodata, most of the nurses are female, which is a main factor for stress, since women tend to carry pregnancies, rear children and keep the home. When this is combined with a job like nursing, it is usually very stressful, therefore, this study revealing that the female gender are stressed is not surprising with a female dominated profession and majority of them married. This finding supports the finding of the American Psychological Association (APA), (12, 13) where they revealed that women are more likely than men to report that their stress levels are on the rise, they are also much more likely than men to report physical and emotional symptoms of stress. However, one would have thought that since majority of the nurses were still relatively young (below 45 years of age), they would have demonstrated a better coping for stress since they are still physically strong. This finding

Table 5: Correlation between stress in workplace and job output among nurses.

		Stress in workplace	Job output among nurses.
Stress in workplace.	Pearson Correlation	1	.947**
	Sig. (2-tailed)		.000 (p- value)
	N	187	187
Job output among nurses.	Pearson Correlation	.947**	1
	Sig. (2-tailed)	.000 (p-value)	
	N	187	187

Source: Field Survey 2016

Table 6: Correlation between stress in workplace and nurses' input their family.

		Stress in workplace.	Nurse input to their family.
Stress in workplace.	Pearson Correlation	1	.782**
	Sig. (2-tailed)		.000 (p- value)
	N	187	187
Nurses input to their family.	Pearson Correlation	.782**	1
	Sig. (2-tailed)	.000 (p-value)	
	N	187	187

Source: Field Survey 2016

Table 7: Correlation between stress in workplace and physical wellbeing of nurses

		Stress in workplace.	Nurse well-being.
Stress in workplace.	Pearson Correlation	1	.953**
	Sig. (2-tailed)		.000 (p-value)
	N	187	187
Nurses well-being.	Pearson Correlation	.953**	1
	Sig. (2-tailed)	.000 (p-value)	
	N	187	187

Source: Field Survey 2016

showing a marked degree of stress among this age group points to the fact that stress is mainly a psychosocial phenomenon that affects all age groups. This finding also supports the finding of APA, (14, 15) which revealed that younger Americans reported experiencing the most stress and the least relief —they report higher stress levels than older generations and said they were not managing it well. The Biodata also revealed that all the nurses are psychiatric nurses which imply that they have the necessary professional qualification needed to cope with the job they are doing. Majority of them have also worked on this job for over a decade, which is another factor pointing to their being experienced enough on the job they do. This would have been thought to be a factor that would have helped them to reduce stress and increase coping, yet a very marked stress level is demonstrated by the nurses.

The various stressors indicated in this study are in line with factors revealed in previous studies and they are: work related, work interaction, job satisfaction, work life imbalance stressors affects the job performance of nurses in Federal Neuro-psychiatric Hospital, Yaba, and its Annex at Oshodi. (16) They were statistically tested and the result shows that there is significant relationship between stress in workplace and job output at P-value=0.000<0.05, r=0.947. This finding is similar to the finding revealed by the study of APA (17) and Fretwell (18) where stress related to staff issues and job satisfaction with communication were also found to be associated.

Other major issues in the workplace that may be accountable for stress in the workplace are: lack of job satisfaction, ineffective communication and the nature of the job itself. For example, in the area of job satisfaction and motivation, improper rewards and incentives make the nurse to carry out his/her duties ineffectively and inefficiently. Majority of the respondents are dissatisfied with the reward system in their organization. Also, not having proper tools to work with brings about a lot of dissatisfaction in the workplace.(19) Also, in the area of communication, a nurse may have to communicate the same message more than once in other for the receiver (nurses or patients) to understand and carry out the action as expected. According to Moola *et al*, (20) in order to reduce stress in the workplace, there has to be effective Communication which helps to reduce stress. Nursing as a profession is tedious and the nurses run shift duties which make the work more tedious and challenging to their families (21). Financial difficulties in the work life balance stressors affect nurses; this may be due to increase in the price level of fares, and other physiological needs. This in turn reduces the desire to work effectively and efficiently in order to increase output. Work interaction in form of interpersonal conflict in the workplace, lack of or low supportive interrelationships in the work place, poor communication resulting from poor communication skills affects the nurse job output. The consequences of the aforementioned are seen in the regular strike actions by health workers in the hospital.

Nurses' perception towards work related stress shows that Work place relationship affect nurses' job/work. Nurses are also of the perception that work related stress affects their general health (physical, emotional, psychological). It was found statistically that there is significant relationship between stress at workplace and physical wellbeing of the nurse at p-value=0.000<0.05, r=0.953. This finding is similar to the finding in the study conducted by Mojoyinola (22) where it was reported that there was a significant effect of job stress on physical and mental health of nurses in public hospital ($F = 2.736$, $df = 10/153$, $P > .05$). Statistically in this study, there is significant relationship between stress in workplace and nurse's input to his/her family at p-value=0.000<0.05 with r=0.782.

The finding in this study is also consistent with the previous study carried out by Loo-See and Leap-Han (23), where it was reported that respondents adopt more than one coping mechanisms to combat job stress based on scenarios, situations, and level of job stress. This finding was further supported by Lee (24) and Dawavandian *et al*, (25) when they also pointed out that coping strategy reduced stress level of nurses. In this study, some of the coping strategies put up by nurses were that majority of the nurses listened to jokes or make jokes to laugh and make them happy in order to reduce stress. Some of the nurses found comfort in religion by praying and meditating and sleeping. Nurses also use the following coping strategy/techniques due to the comfort and effectiveness derived from them. Transference, Dancing, listening to music are the most used coping strategy by nurses. In this study, the nurses agree that work-related stress do not have any one technique to manage it. Majority of the nurses make use of more than one coping technique to manage work-related stress.

Conclusion

This study investigated the perception and management of work-related stress among Nurses in Neuro-Psychiatric Hospital, Yaba, Lagos. Psychiatric nurses in this study are very much susceptible to occupational stress because of intense daily activity. Nurses perceived that they are experiening work-related stress which affects their health, work output, family and relationship with

colleagues and revealed that they practice coping strategies in varous ways which include physical activities, listening to music, religious activities among others. The study also further established that there is a positive correlation between stress in workplace and job output, nurses' input to their families and physical wellbeing.

Nurses are faced with a variety of work-related stressors and have been able to develop some coping skills but frantic efforts should be made by the management of the hospital to further increase work-based coping strategies for stress management among nurses.

Recommendations

The following recommendations were drawn from the research:

Nurses should be provided opportunities for learning a multitude of stress management strategies and self soothing techniques directly applicable to the nursing environment and easily utilizable on the job; this can be achieved by introducing into nursing colleges before the commencement of clinical nursing, how nurses can recognize impending stress and its management to prevent burnout and improve work effectiveness and effeciency.

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Nurses should be given incentives and there should be an increment in salary earned, this will greatly increase productivity, and ultimately decrease stress.

Effective communication systems should be enhanced among nurses and Nurses' executives should foster the building of relationships and other stressors elements within the workplace and create avenue for nurses to relate, talk about stressors, and commune with co-workers through mutual problem solving.

·Conducive environment and job security should be created for nurses in the hospital environment ; this fosters a team approach to completing tasks and determining system needs.

Establishing a mentoring program for new employees, creating a warm and inviting break room that is conducive to socializing is essential, and there should be professional respect among nurses.

Nurses' executives should provide ways for professional debriefing services / counseling of nurses in any of their weakness caused by work related stress, job dissatisfaction and poor general health among nurses. This should involve input from nurses as well as management in order to ensure collective commitment towards improving nurse and patient related outcomes.

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Assessment of the Impact of *Moringa oleifera* Leaf Extract on Altered Faecal Composition in High Salt Fed Rats

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Abstract

High salt intake has been linked to many deleterious effects on the body. This study was carried out to investigate the effect of aqueous leaf extract of *Moringa oleifera* on faecal composition of high salt loaded rats. The Wistar rats (twenty four in number) were randomly assigned into 4 main groups of 6 rats each. The rats were fed on either normal rat chow, high salt (8% NaCl diet + 1% NaCl drinking water) and/or *M. oleifera* extract (600mg/kg bw) for 6 weeks. Results obtained revealed that the extract had an LD_{50} value of 1,872.22mg/kg. The high salt fed rats showed a significant ($p<0.05$) reduction in body weight, which was reversed following extract treatment. The faecal compositions of the control group were sodium ion, 2.32 0.05 mmol/L; potassium ion, 7.47 ±0.24 mmol/L; chloride ion, 13.13 ±0.28 mmol/L; bicarbonate ion, 1.33 ±0.21 mmol/L; glucose, 0.08 ± 0.00 mmol/L; and protein, 12.50 ±0.43 mmoL/L. Faecal sodium, chloride, potassium, and bicarbonate ions, as well as faecal protein and glucose levels were significantly ($p<0.05$) increased in the high salt fed group compared to other groups. The findings revealed that, treatment with the plant extract reduced the rate of faecal bicarbonate, potassium, glucose and protein loss; suggesting the use of Moleifera extract to ameliorate increase faecal bicarbonate, potassium, protein or glucose concentrations in high salt fed animals.

Key Words: *Moringa oleifera,* faecal composition, high salt, rats.

Introduction

Most food consumed by modern man contains high amount of salt which leads to buildup of salt in the body due to excess consumption of dietary salt. The daily recommended intake of 1.5 - 2.0 g of salt (1,2,3). High salt diet is associated with increasing prevalence of obesity, hypertension, stroke and Type 2 diabetes mellitus (4,5). High salt loading in experimental animals causes myriad detrimental effects such as increased production of dopamine in jejunal epithelial cells which decreases jejunal sodium reabsorption in young rats, and impairs Na^+/K^+ ATPase activity (6). It also interferes with normal food digestion (especially protein) by reducing the production of pepsin that enhances protein digestion (5).

High salt loading could also destroy liver, pancreas and kidney cells in rats (7). Prolonged high salt intake increases the size of myenteric neurons in the wall of the intestine (7). High salt diet also enhances vasoconstriction of mesenteric arteries, contributing to elevated blood pressure in rats (3). It has also been shown to mediate the effect of insulin resistance and reduces the uptake of glucose by the cells rats, leading to the weight loss (8).

However, medicinal plants still remain the first line of medication in traditional health care system (9). The plant, *Moringa oleifera*, commonly called "Miracle Tree", is locally called 'Zogalegandi' by Hausas', 'Eweigbale' by Yourubas and 'Okweyibo by the Igbo. Its other names include horseradish tree, drumstick tree, benzolive, never die tree, moonga, mulangay, sajna or Ben oil tree, indicating the significance of the tree around the world. The trees originates from North Western region of India to South of the Himalayan mountain (10).

Moringa oleifera possess various pharmacological actions such as analgesic, anti-hypertensive activities, anti-inflammatory effects, hypo-cholesterolemic, and anti-diabetic (11,12). The leaves of *Moringa oleifera* is also used by traditional herbalist to manage hepatotoxicity, rheumatism, venomous bites, cardiac problems and wound healing (13).

Moringa oleifera extract enhances absorption and bioavailable of drugs and vitamins across the gastro-intestinal membrane (14). It also stimulates insulin release from the rodent pancreatic beta cells (15).

Fecal analysis has been a useful tool in the diagnosis and prognosis of certain disease conditions especially metabolic diseases like obesity, non-alcoholic fatty liver, hypercholesterolemia, which collectively cause

an undue burden on health care costs and significant morbidity and mortality. Nevertheless, it is not known if *M. oleifera* could ameliorate altered faecal composition induced by high salt loading in rats. This study was therefore designed to evaluate the effect of Moringa oleifera leave extract on faecal electrolytes, proteins and glucose concentrations in rats.

Materials and Methods

Experimental animals

Twenty four male Wistar rats weighing between 200 - 250g were obtained from the Animal House of the Department of Physiology, University of Calabar, Calabar Nigeria. The animals were acclimatized for one week, after which they were weighed and divided into four groups of six rats each. They were housed in cages, under controlled room temperature (25±2°C) and humidity (55±5%) under 12/12 hours light dark cycle and were fed on pelleted standard feed (Vital Feeds Ltd., Jos) and water ad libitum. The animals were handled in conformity with the international standards (Helsinki's Declaration of 2000).

Collection of plant material and extraction

Fresh leaves of *M. oleifera* were brought from the botanical garden Calabar, Cross River State, Nigeria. The leaves were identified and authenticated by Dr. Frank Adepoju of Botany Department, University of Calabar. The leaves were rinsed with distilled water, then dried for 3-4 weeks under room temperature at 28°C. The dried leaves were then ground into powder, using a locally made miller machine. The powder sample was macerated in distilled water for 18 hours. The resultant extract was filtered using Whatman no. 1 filter paper and evaporated by a rotary evaporator under reduced pressure and temperature at 40°C to give a semisolid residue (16). The semisolid extract obtained was stored in a refrigerator at -4°C for further use.

Preparation of high salt diet

High salt diet containing 8% of sodium chloride was prepared using a standard animal diet containing 0.3% sodium chloride following standard method (17).

Determination of LD_{50}

Eighteen albino mice were used in this study for the determination of LD_{50} following the method described by Lorke (18). White male albino mice (20-25g) were randomly assigned to six groups of three animals per group. Each group was injected intraperitoneally with one of the followings 0, 100, 200, 400, 800 and 1600mg/kg of the crude extract. The maximum volume injected was 0.5ml. The groups were returned to their home cages and given food and water ad libitum. The mortality in each cage was assessed 24hrs. after administration of the extract. The percentage mortality were converted to probits (a probability unit) and plotted against the Log_{10} of the dose of the extract. Regression lines were fitted by the method of least squares and confidence limit of the LD_{50} value calculated.

Experimental design

Twenty four male albino Wistar rats were randomly divided into four groups of six rats each. Group 1 (Control) received normal rat pellets with clean tap water. Group 2 (extract treated) was fed on normal diet with *M. oleifera* extract (600mg/kg bw orally once daily). Group 3 took high salt feed (8% NaCl) diet with 1% NaCl drinking water, while group 4 received same diet as group 3 with *M.* oleifera aqueous extract (600mg/kg bw orally, once daily). The feeding regimens lasted for 6 weeks.

Collection and preparation of faecal matter

One gram of faecal material was collected from the different groups at the end of the feeding period (6 weeks), and was placed in 15ml barrel. Fifteen mL of distilled water was then added and left to homogenize for 30 minutes. The homogenate was filtered using Whatman No.1 filter paper. The supernatant was then transferred into plastic containers and frozen at -15°C. The electrolytes Na+, K+, and Cl- were determined by the principle of ion selectivity.

Faecal Elemental Analysis

The elements, sodium and potassium were determined using a Flame Photometer (mode 1.410C, Petra Court Limited, England). The sample was sprayed into a non-luminous gas flame which became coloured by the characteristic emission of metallic ions. The wave lengths of metals, 598nm and 767nm for sodium and potassium respectively were selected by a light prism system and allowed to fall on a photosensitive detection system.

Determination of faecal bicarbonate:

Plasma carbon dioxide (CO_2) was measured by a modified method of Forrester (19). Phosphoenol

pyruvate carboxylase brings about the catalysis of the reaction between phosphoenol pyruvate and carbon dioxide to form oxaloacetate and phosphate ions and oxidation of an equimolar amount of reduced NADH (nicotinamide adenine dinucleotide) to NAD. The reaction is catalysed by malate dehydrogenase (MDH). This results in decrease absorption at 340nm that is directly proportional to CO_2 concentration in the sample.
The CO_2 in the sample is determine as follows:
Co_2 content: Absorbance of blank - Abs. of sample x Conc. of standard.
Absorbance of blank – Abs. of standard
The unit is Mmol/L

Determination of faecal chloride
A 2ml buffer solution was placed in a conical flask and 0.2ml of the solution of faecal sample was added and mixed. Four drops of diphenyl carbazene indicator were added and titrated with mercuric nitrate usinga 2ml micro pipette. The end point indication was violet colour. The standard solution (0.2ml) was added to a 2.0ml buffer solution together with indicator before titrated.
Faecal chloride concentration was obtained from the following calculation and expressed in units of meq/L
Calculation:
Faecal chloride= titrated values of text X 100 titrated value of standard

Faecal protein estimation
Faecal sample (0.1mL) was added into a test tube containing 1.9mL of 0.85 percent NaCl solution. The same solution was prepared using a standard sample. A blank standard was prepared using 2mL of NaCl solution in a test tube. To each of the test tubes was added Biuret solution and gently mixed by inverting the test tubes. The test tube were thereafter kept for 30 minutes, absorbance of each sample was read at 550nm.

Faecal glucose estimation
The method of God-Pap was employed (20).
In this method, glucose is oxidized by the specific enzyme glucose oxidase (GOD) in aqueous solution to gluconic acid and hydrogen peroxide.

Step 1: Glucose + O_2 + $2H_2O$ ----------> gluconic acid + $2H_2O_2$

Then, the hydrogen peroxide reacts in the presence of a peroxidase (POD) with phenol and 4-aminophenazone forming a red dye product:

Step 2: H_2O_2+ phenol + 4-aminophenazone ----------> red dye + $2H_2O$

The intensity of the colour formed is proportional to the glucose concentration and can be quantitated between 460 to 560 nm with a spectrophotometer.
Twenty µl of either serum or heparin plasma is used as sample. Samples not assayed within one hour of collection were frozen.

Statistical analysis
Research data were expressed as mean ±SEM, one-way analysis of variance (ANOVA) was used to analyze the data, significant ones were followed up with a post hoc test (LSD). Statistical significant were declared in cases where the probability level is less than 0.05.

Results
Acute toxicity test
As shown in Figure 1, the LD_{50} obtained for Moringa oleifera leaf extract from the present study was 1872.22mg/kg body weight. From this value a test dose of 600mg/kg (i.e. 1/3rd of the 50% lethal dose) was used derived and for the experiment.

Body weight change
The body weights of salt fed and salt treated groups were significantly ($p<0.05$) higher compared with the control and normal treated groups, (figures 2 and 3). After 42 days of feeding, the final body weight of salt fed, and salt treated group decreased significantly ($p<0.001$) compared with the control and normal treated groups. On the other hand, the final body weight of the salt treated group was significantly ($p<0.05$) higher compared to salt-untreated groups..
The growth rates of the different experimental groups were: 21.67 ±3.07g, 11.67 ±4.77g, -60.00 ±5.16g and -36.67 ±5.58g respectively for control, normal treated, salt fed and salt treated groups. The salt fed groups showed negative growth rate.

Faecal sodium ion concentration
The faecal Na^+ concentrations in the different experimental group were as follow: control, 2.32 ±0.05mmol/L; normal treated group, 2.02 ±0.03mmol/L; salt fed group, 13.33 ±0.84mmol/L and salt treated group, 17.67 ±0.71mmol/L, Table 1.
The faecal Na^+ level was significantly ($p<0.001$) higher in the high salt fed groups compared to control and normal treated groups. It was in turn significantly

Table 1: Comparison of faecal electrolyte, glucose and protein concentrations

Group	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	Glucose (mmol/L)	Protein (mmol/L)
Control	2.32 ±0.05	7.47 ±0.24	13.13 ±0.28	1.33 ±0.21	0.08 ±0.001	12.50 ±0.43
Extract treated	2.02 ±0.03	7.23 ±0.23	13.67 ±0.61	1.68 ±0.19	0.04 ±0.004***	11.33 ±0.33
Salt fed	13.33 ±0.84***,c	8.15 ±0.19*,a	31.70 ±1.58***,c	2.33 ±0.21**,a	0.10 ±0.006*,c	23.50 ±1.65***,c
Salt with extract	17.67 ±0.71***,c,z	7.20 ±0.37x	37.27 ±0.94***,c,z	2.00 ±0.37*	0.07 ±0.007c,y	15.50 ±0.43***,c,z

Values are presented as mean ±SEM, n = 6.
* = p<0.05, ** = p<0.01, *** = 0.001 compared with control
a = p<0.05, c = p<0.001 compared with extract treated
x = p<0.05, y = p<0.01, z = p<0.001 compared with salt fed

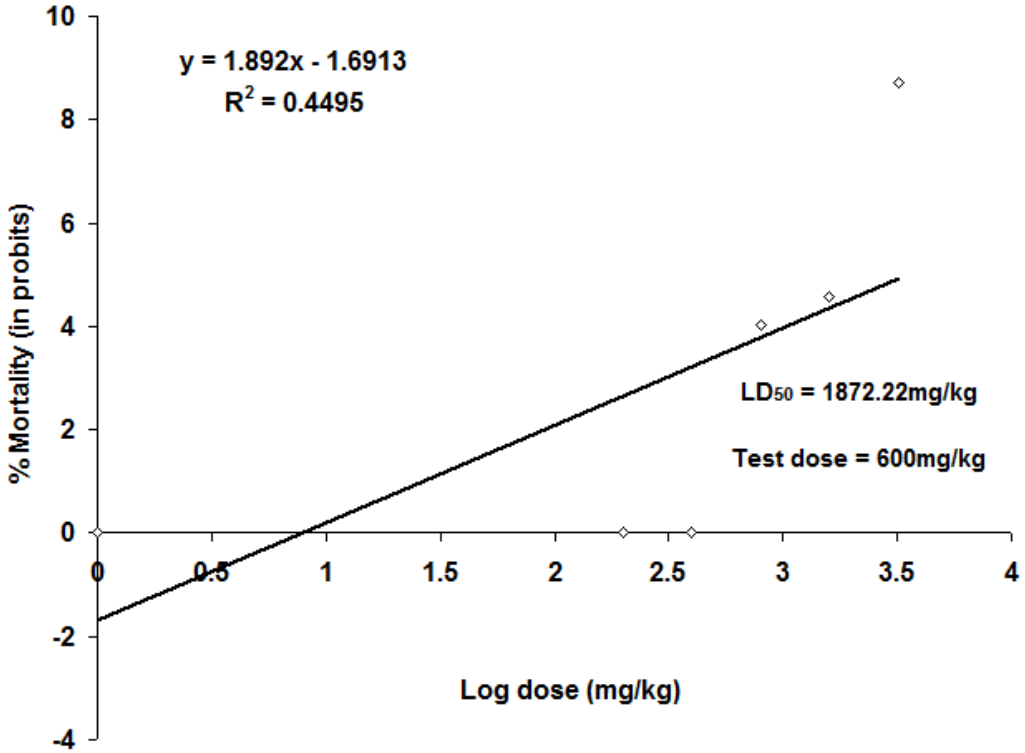

Fig. 1 Lethality studies showing the effects of administering graded doses (200 - 3200mg/kg i.p. mice) of *M. oleifera* extract against the percentage mortalities (converted to probits).

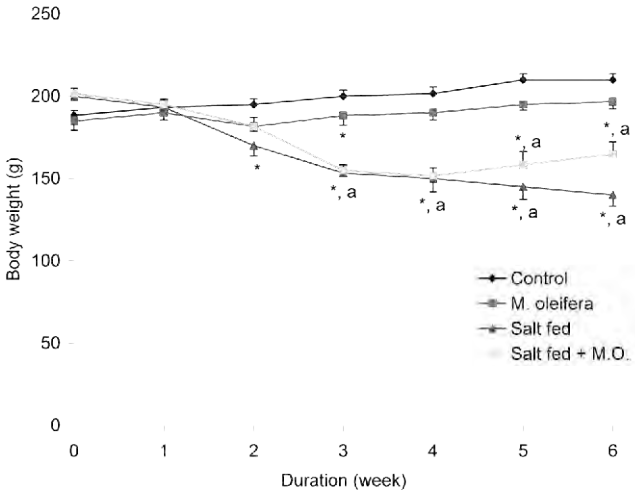

Fig. 2 Comparison of weekly body weights of the different experimental groups.

Values are expressed as mean ± SEM, n = 6.
* = p<0.05 vs control;
a = p<0.05 vs M. oleifera.

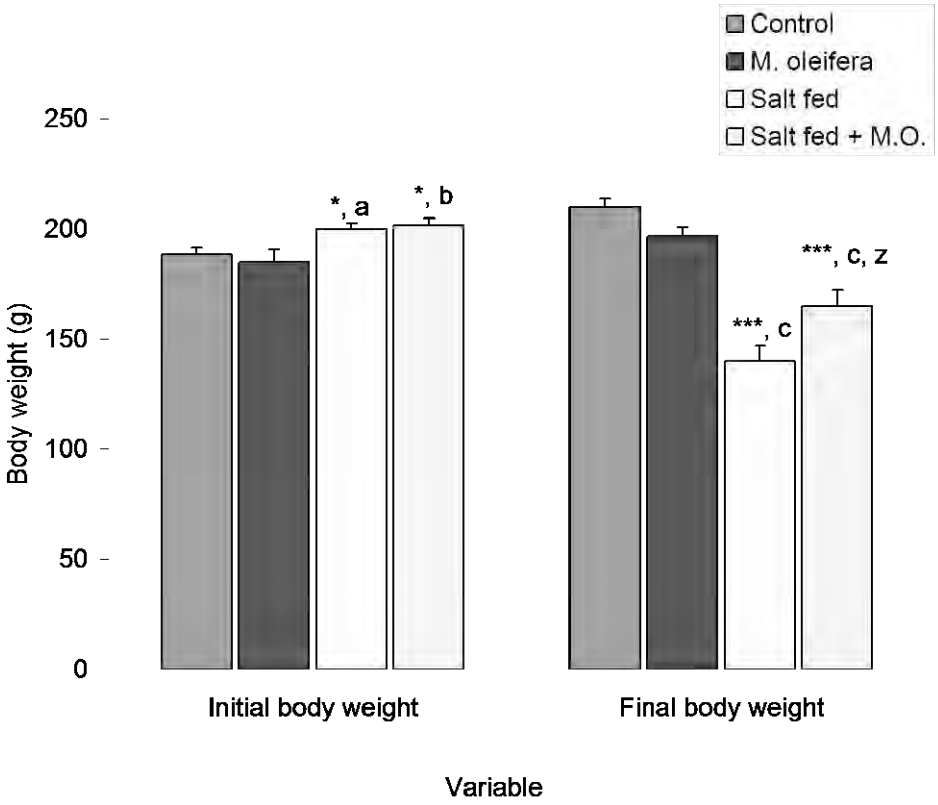

Fig. 3 Comparison of weekly body weights of the different experimental groups.

Values are expressed as mean ± SEM, n = 6.
* = p<0.05, *** = p<0.001 vs control;
a = p<0.05, b = p<0.01, c = p<0.001 vs M. oleifera.
z = p<0.001 vs salt fed.

($p<0.01$) higher in the salt treated group compared with the salt-untreated group, Table 1.

Faecal potassium ion concentration

The faecal K^+ concentrations in the different experimental groups were as follow: 7.47 ±0.24mmol/L in the control, 7.23 ±0.23mmol/L in group 2, 8.15 ±0.19mmol/L in group 3 and 7.20 ±0.37mmol/L in group 4. Showing a significant ($p<0.05$) increase in the faecal K^+ concentration in the salt fed group compared to control and normal treated groups. The faecal potassium ion concentration was in turn significantly ($p<0.05$) lower in the salt-treated group compared with the salt-untreated group, Table 1.

Faecal chloride ion concentration

The faecal Cl^- concentrations for the different experimental group were as follow: 13.13 ±0.28mmol/L, 13.67 ±0.61mmol/L, 31.70 ±1.58 mmol/L and 37.27 ±0.94 mmol/L respective for groups 1, 2, 3 and 4. Hence, there was a significant ($p<0.001$) increase in faecal Cl- concentration in the salt fed groups compared to control and normal treated groups. On the other hand, the salt-treated group had a significant ($p<0.001$) increase in faecal chloride ion concentrations compared with the salt-untreated group, Table 1.

Faecal bicarbonate ion concentration

The faecal HCO^-_3 concentrations for the different experimental group were as follow: 1.33 ±0.21mmol/L for control, 1.68 ±0.19 mmol/L for extract treated group, 2.33 ±0.21 mmol/L for salt fed and 2.00 ±0.37mmol/L for salt treated group. The results shows that there was a significant ($p<0.05$) increase in HCO^-_3 concentration in the salt fed groups compared to control and normal treated groups, Table

Faecal glucose concentration

The faecal glucose concentration (0.04±0.004mmol/L) was significantly ($p<0.001$) lower in the extract treated group compared to the control group which had mean glucose concentration of 0.08 ± 0.00 mmol/L. On the other hand, the high salt fed (0.10 ±0.006) had a significantly ($p<0.01$) higher faecal glucose level compared to control and extract treated groups, Table 1.

Faecal protein concentration

The faecal protein concentrations in the different experimental group were 12.50 ±0.43 mmoL/L, 11.33 ±0.33 mmol/L, 23.50 ±1.65, and 15.50 ±0.43 mmoL/L for control, normal treated, salt fed and salt treated groups: respectively. Showing significant ($p<0.001$) increase in faecal protein concentration of salt fed untreated group compared to control and extract treated groups, Table 1.

Discussion

The effect of aqueous extract of *M. oleifera* leaf on faecal Na^+, K^+, Cl^- and HCO_3^- composition, faecal glucose and protein concentration was assessed in high salt loaded rats. The results indicate that the aqueous leaf extract of *M. oleifera* leaf has a remarkable influence on gastrointestinal tract physiology. The high salt-dietary intake by the rats caused excessive urination and a significant reduction in the body weight. These observations were similar to the earlier claim (21) that salt loading increases glomerular filtration rate due to increase plasma volume, with a resultant pressure natriuresis.

High salt fed rat that were fed on *M. oleifera* leaf extract recorded higher body weight compared with their untreated counterpart. The increased body weight observed in the salt fed rats suggests the ability of the plant extract to reverse the blockade effect of high salt loading on tissue glucose uptake, which could result in the low plasma glucose level, *M. oleifera* leaf has earlier been reported to prevent destruction of beta cells with concomitant improvement in insulin uptake by tissue insulin receptors (22). Improvement in the absorptive potential of the small intestine by extract of *M. oleifera* leaf is another possible reason for the reversed increase body weight seen in the high salt fed rats treated with *M. oleifera* leaf extract. This is in line with an earlier observation (23) asserting that *M. oleifera* leaf extract improves absorption rate of the intestinal epithelium.

The elevated potassium and bicarbonate ions in the faeces of high salt fed rat also point to the deleterious effect of high salt intake on electrolyte balance. This alteration was reversed by *M. oleifera* extract treatment. The basic mechanism for ion exchange along the intestinal lumen is that bicarbonate ions are usually secreted into the lumen following

chloride reabsorption to neutralize the acidic lumen in the presence of high HCl in the lumen, while the H^+ are exchanged for K^+ ions (21). Therefore, the extract could promote HCO_3^- movement into the lumen rather than being excreted, a process that helps to neutralize excess HCl.

There was a remarkable loss of glucose and protein in the faeces of the salt loaded rats compared with other experimental groups. This is not surprising since high salt load has been implicated in insulin resistance and impaired glucose uptake by the tissues, which leads to massive loss of glucose in the faeces of high salt loaded rats (8). Defective protein reabsorption is a possible reason for the massive appearance of protein in the faeces of the high salt loaded rats (6). The ability of the extract to reverse this trend shows its potential in improving the absorptive ability of the villi and also prevents tissue wasting.

Conclusion

M. oleifera promotes faecal sodium and chloride ion loss in high salt fed rats, preventing accumulation of these ions in the body. It reverses the loss of faecal potassium and bicarbonate ions, as well as glucose and protein levels prompted by high salt loaded rats

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Sexual Dimorphism in Digit Ratio (2d:4d), Anthropometric and Serum Adiposity Measures of Hausas in Kano, Nigeria

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Abstract

Similar to second to fourth digit ratio (2D:4D), body adiposity measures demonstrate sexual dimorphism. The extent of this phenomenon shows population variation. The aim of this study was to investigate sexual dimorphism in digit ratio (2D:4D), anthropometric and serum adiposity measures of Hausas ethnic group in Kano, Nigeria. The study was a cross sectional study including 465 (266 males and 199 females) Hausas of Kano, with a mean age of 34.4 years and 32.0 years for males and females respectively. Systematic random sampling technique was employed for subject recruitment. Weight, Height, waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), body mass index (BMI), neck circumference (NC), body adiposity index (BAI), digit length and digit ratio were obtained using standard protocol. Overnight fasting blood sample was obtained for high density lipo-protein cholesterol (HDL), total cholesterol (TC), triglyceride (TG), low density lipo-protein cholesterol (LDL) estimation using standard laboratory protocols. Student's t test was used to compare between-group parameters of males and females, left and right hand. SPSS version 20 software was used for statistical analyses and $P < 0.05$ was set as level of significance. It was observed that 2D:4D, WHtR, HC and body adiposity index (BAI) were significantly higher in females. Sexual dimorphism in digit ratio was more pronounced in the R2D:4D. WHR and NC were higher in males. WC and BMI did not demonstrate sexual dimorphism. TC and HDL-C were higher in females while TG and LDL-C did not show significant sex difference. In conclusion Second to fourth digit ratio, serum and body adiposity measures are sexually dimorphic. Sexual dimorphism in digit ratio is more pronounced on the right side in the Hausa ethnic group of Kano:

Keywords: Sexual dimorphism, 2D:4D, Serum adiposity, body adiposity.

Introduction

The ratio of the second to fourth digit (2D:4D) is considered as a reliable retrospective biomarker of intrauterine testosterone exposure (1) Sexual dimorphism in this variable is well documented (2-9) and is widely attributed to the differential effects of estrogen and testosterone on the digits during embryogenesis in that; while testosterone preferentially stimulates the growth of the fourth digit (ring finger), estrogen stimulates growth of the index finger (10). This explains the sexually dimorphic nature of 2D:4D manifesting as higher digit ratio in females compared to males. Since genetic influences have also been identified to contribute to development of the digit ratio (1) it may seem that the ratio is a product of a complex interaction between genetic mechanisms and hormonal milieu in the developing embryo. The mean value of digit ratio is reported to be affected by population specific factors such as ethnicity (11, 12) and some attempts have even been made to establish normal reference values for some (Australian) populations (13). It is documented that the population variation in 2D:4D exceeds the sexual difference (11, 12).

Since 2D:4D is reported to correlate with many important biological traits (9, 14, 15). and some disease conditions like autism, depression and developmental psychopathology, congenital adrenal hyperplasia, polycystic ovarian syndrome (16, 17, 18, 19), it is important to determine the presence and extent of sexual dimorphism in this important anthropometric tool since sexual dimorphism may also indicate sex difference in those important body characteristics predicted by the ratio. Adiposity is

associated with adverse health consequences (20, 21, 22, 23). Assessment of body adiposity may be done through simple anthropometric measures, radiological techniques such as computer tomography scan and magnetic resonance imaging or directly by measurement of the concentrations of the different lipid components of serum.

There is sex difference in body adiposity reserve and pattern of adipose tissue distribution (24) which is documented to result from the fat distribution effect of sex hormone in that; while testosterone (male sex hormone) encourages fat deposition in the trunk, estrogen (female sex hormone) encourages fat accumulation in the hip and thigh region (24, 25). The different anatomical sites of fat aggregation and the individual constituents of serum lipids do not carry equal metabolic risk. However, identifying the extent of sexual difference in the serum and body adiposity measure may explain sex variation in the prevalence of adiposity related metabolic disorders. The difference in the cardio-metabolic consequences of different adipose tissue measures have been attributed to differences in physiological characteristics of various adipose tissues depot such as size, number of adipocyte, lipolytic responsiveness, lipid storage capacity, and inflammatory cytokine production (20, 26).

The aim of this study was to investigate the presence and extent sexual dimorphism in digit ratio (2D:4D), anthropometric and serum adiposity measures of Hausas ethnic group in Kano, Nigeria

Materials and Methods

Study location and population

Systematic random sampling technique was employed in selecting 465 original Hausas of Kano based on a history of at least two parental generation being Hausas from Kano. Participants were recruited from outpatient units of Murtala Muhammad specialist Hospital, Khadija Memorial Hospital, SU clinic, General Hospital Dawakin-Tofa and the old campus of Bayero University, Kano. The study included only subjects in the age range of 18 years to 68 years. Subjects with pregnancy, abdominal or pelvic space occupying lesions, congenital and/or acquired spinal or digit deformity, were however excluded. Subjects on statins or lipid lowering drugs were also excluded. Ethical approval was obtained from Kano state hospitals management board and written informed consent obtained from the subjects.

Anthropometry

Height was measured to the nearest 0.1cm as the vertical distance between the standing surface and the vertex of the head while the subject was standing erect in the frank forth plane and without shoes using a stadiometer. The weight was measured in kilograms using a digital weighing scale while the subject is in light clothes. The body mass index was calculated by first measuring the body weight which was then divided by the square of the height and the result was expressed in kg/m^2. Waist circumference was measured in centimeter with a non- stretchable plastic tape horizontally placed over the unclothed abdomen at the narrowest point between the lowest rib and the iliac crest. The hip circumference was measured while the subject was standing erect with the feet fairly close together; pockets emptied and the tape passed around the point with the maximum circumference over the bottom. WC was divided by the HC to obtain waist-hip ratio (WHR) and was divided by the height to obtain the waist-height ratio (WHtR). Neck circumference was measured in centimeter with a non- stretchable plastic tape horizontally placed over the unclothed neck at the level of the thyroid cartilage. Digit lengths was measured on the ventral surface of the hand from the basal crease of the digit to the tip of the finger using a digital sliding caliper (MicroMak, USA) measuring to 0.01mm and reported on questionnaire. This measurement has been reported to have high degree of repeatability (2, 27) Body adiposity index was obtained using the formula proposed by Bergman *et al.* (28). This formula has been shown to be a good measure of central adiposity in some (African-American) populations (28).

$$\text{Body Adiposity Index (BAI)} = \frac{\text{Hip Circumference (cm)} - 18}{\text{Height (m)}\ 1.5}$$

Serum lipid parameters

For the estimation of serum triglyceride and HDL-C, blood specimen was collected from 161 subjects after 10 to 12 hours of fasting via superficial veins of

the upper limb. From each selected subject, 5ml of venous blood sample was collected using a sterile 21G needle fitted with syringe. Blood collection was done during the morning hours to avoid the effect of diurnal variation or circadian rhythm in the blood parameters to be measured. Standard technique of venipuncture and universal safety precaution was employed. Blood sample was transferred into a plain blood specimen bottle and allowed to stand until it was properly clotted. The blood samples were preserved in an ice pack insulating container to preserve the temperature and then transported to the lab immediately after each exercise of sample collection. Sample was then centrifuged at 300rpm for 5 minutes after which serum was separated and immediately used for assaying Triglyceride and HDL-C. Serum HDL-C and TG concentrations were measured using enzymatic method by Wybenga*, et al.* (29). Into a clean test tube 0.5ml serum + 0.5 ml HDL reagent was mixed and allowed to stand for 10 minutes. It was then centrifuged for 20 minutes at 2000 rpm. The cholesterol reagent 1000µl was dispensed into three cleaned test tubes labeled blank, standard and sample. 50µl of supernatant was dispensed into tube sample, 50µl of standard was dispensed into standard tube and 50µl dispensed in to blank tube. All were mixed and incubated at 37^0C for 5 min and absorbance was read at 530 nm.
The results were calculated as

$$\text{Concentration of test} = \frac{\text{Absorbance of Test}}{\text{Absorbance of STD}} \times \text{Concentration of standard}$$

Where the concentration of the total cholesterol standard is 5.17 mmol/L and that of triglycerides standard is 2.28mmol/L. The data were expressed as mean ± standard deviations. To compare between-group parameters of males and females, left and right hand, Student's t test and one way ANOVA was used. SPSS version 20 (IBM Corporation, NY) soft ware was used for statistical analyses and $P < 0.05$ was set as level of significance.

Results

Table 1 shows sexual dimorphism in the anthropometric indices of adiposity of participants. There was no statistically significant difference in the mean value of the index of generalized adiposity (BMI) between the male and female subjects. Among the indices of centripetal adiposity, female subjects had significantly higher HC (88.96cm against 87.01cm, P< 0.017), WHtR (0.48 against 0.46 $p < 0.0006$). But the BAI and WC showed no statistically significant gender difference. Male subjects had significantly higher NC (34.9cm to 31.58cm) and WHR (0.89 against 0.85, $p < 0.0002$).

From Table 2, it was observed that male subjects had significantly ($P < 0.001$) higher digit lengths in both hands. However, the ratio of the second to fourth digit length was significantly ($P < 0.001$) higher in females in both hands. The sex difference in digit ratio was more pronounced on the right side ($t = -8.3$) compared to the left side ($t = -7.0$)
From Table 3 which shows gender comparison of the serum lipids of the study participants, it was observed that TC ($P < 0.0045$) and HDL-C ($P < 0.0016$) were

Table 1: Sex differences in the anthropometric indices of adiposity of participants

	Male (n=266)			**Female (n= 199)**				
Variables	Mean	SD	Min-max	Mean	SD	Min-max	T	P Value
Age	34.45	13.52	18-68	32.06	15.18	18-65	1.79	0.075
Height(cm)	169.15	6.27	142-182.3	158.53	6.83	136.9-175	17.39	<0.0001
Weight (Kg)	63.03	12.28	40.5-98.3	55.86	12.99	36-108.9	6.08	<0.0001
BMI	21.98	3.93	14.52-34.33	22.19	4.7	12.96-39.15	-0.52	0.602
WC (cm)	77.28	11.17	57-111	76.02	13	51-118.5	1.12	0.261
HC (cm)	87.01	7.8	72.1-109.9	88.96	9.86	65.6-136	-2.38	0.018
NC (cm)	34.99	2.29	30-42	31.58	2.46	26.5-39.5	15.38	<0.0001
W/H	0.89	0.08	0.71-1.11	0.85	0.11	0.65-1.25	3.69	0.00025
W/Ht	0.46	0.06	0.34-0.65	0.48	0.08	0.30-0.72	-3.42	0.00067
BAI	21.6	3.71	13.88-33.90	26.61	4.62	15.38-45.58	-12.95	<0.0001

BMI: body mass index, WC: waist circumference, HC: hip circumference, NC: neck circumference, W/H: waist-to-hip ratio, W/Ht: waist-to-height ratio, BAI: body adiposity index.

Table 2: Sexual dimorphism in the digit lengths and digit ratio of participants

	Male (n=266)			Female (n= 199)				
Variables	Mean	SD	Min-max	Mean	SD	Min-max	T	P Value
RI	74.22	5.45	61.17-90.46	67.97	5.02	53.06-79.06	12.64	<0.0001
RII	72.56	5.09	60.19-87.02	68.94	4.48	55.42-82.09	7.98	<0.0001
RIII	80.12	5.44	64.17-97.56	75.53	4.98	63.13-94.26	9.34	<0.0001
RIV	75.63	5.29	62.84-89.32	69.94	4.51	55.41-85.35	12.21	<0.0001
RV	62.11	5.31	47.17-85.87	57.6	4.26	44.97-67.32	9.83	<0.0001
R2D:4D	0.96	0.03	0.79-1.05	0.99	0.03	0.86-1.07	-8.39	<0.0001
LI	74.05	5.36	60.33-87.47	67.77	4.49	55.1-78.83	13.36	<0.0001
LII	73.32	4.85	60.04-85.81	69.08	4.4	57.19-80.44	9.7	<0.0001
LIII	80.5	5.61	66.12-96.55	76.23	5.56	50.09-98.92	8.15	<0.0001
LIV	76.03	4.91	62.92-87.81	70.1	4.71	57.45-82.26	13.11	<0.0001
LV	62.21	5.09	47.46-74.36	57.69	4.88	43.14-75.71	9.63	<0.0001
L2D:4D	0.96	0.03	0.85-1.10	0.99	0.03	0.92-1.09	-7	<0.0001

I: first digit, II: second digit, III: third digit, IV: fourth digit, V: fifth digit,
R: right hand, L: left hand, 2D:4D: second to fourth digit ratio

Table 3: Sexual dimorphism in the serum lipid measures of the study participants

	Male (n=120)			Female (n= 41)			t	
Variables	Mean	SD	Min-max	Mean	SD	Min-max		P value
TC	174.35	32.31	123.7-256.1	187.32	43.85	127.3-290.7	-2.02	0.045
HDL– c	44.1	6.32	28-54.1	47.83	6.71	38.9-60.6	-3.21	0.0016
TG	117.18	31.76	74.3-196.5	121.83	29.25	80.4-165	-0.83	0.41
LDL– c	106.81	32.44	58.14-192.82	115.12	44.05	54.36-214.46	-1.29	0.2

FBG: fasting blood glucose, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol,
TG: triglyceride, LDL-C: low density lipoprotein cholesterol, VAI: visceral adiposity index

significantly higher in female subjects. There was no significant sex difference in the mean values of TG and LDL-C

Discussion

The significantly higher 2D:4D ratio observed in the female subjects of this study is in keeping with previous studies (2-10). This sex difference is likely explainable by the differential effects of androgen and estrogen on the ring and index fingers during intrauterine development (2, 5, 12, 30). It is noteworthy from this study that even though the sexual dimorphism observed in 2D:4D agrees with previous studies, the observed mean value is higher than the suggested normal range of 0.947 ± 0.029 and 0.965 ± 0.026 in males and females respectively in a sample of Australian population (13).

It is documented that ethnicity (11, 31) and geographical location (11) significantly affect the digit ratio and that ethnic variation even exceeds the sexual differences (11, 31). This means that the difference in geographical location and ethnicity may partly explain the variation observed in this study. Considering that some of the subjects of this study were pooled from a clinic, a higher number of subjects compared to the general population may be harboring some metabolic syndrome (MetS) indices and excessive adipose tissue and since adiposity measures have been shown to be positive correlates of 2D:4D (7, 32), higher digit ratio may characterize some of the subjects of this study.

The observation from this study that HC and WHtR were significantly higher in female subjects is also in agreement with previous studies (33, 34). For the hip circumference, this difference is likely linked to the fat distribution effect of estrogen, in that; there is

preferential deposition of fats in the hip and thigh region in females (24, 25). WHtR is a fraction with body height as denominator and since studies revealed that the mean height of males is significantly higher than that of females (33, 35, 36). This may explain why the mean WHtR of females is higher than males as observed in this study. Although, the mean values of BMI and BAI of females is reported to be higher than in males while that of WC is higher in males (33, 34), In this study, there was no statistically significant difference in these indexes. The BMI as an index of body adiposity is reported to have limitations in certain individuals including younger and older people (37, 38) and since this study included some teenagers and older adults who represents such age group in whom BMI is reported to be less effective, this may explain why sexual dimorphism in BMI was not observed in this study. Moreover, body adiposity measures are tightly linked with metabolic parameters (33, 39, 40) especially visceral adiposity to which WC is a pointer (24, 41). Consequently, the presence and distribution of this metabolic parameters among the subjects of the study may affect sexual dimorphism in these adiposity measures.

Since the participants recruited for this study included subjects from outpatient departments of hospitals, some of whom were newly diagnosed hypertensive, diabetics or having hypertension – diabetes co–morbidity, this may affect the body adiposity trend and may be the reason why BMI and WC did not show sexual dimorphism in this study. Additionally, the age group of the women in this study included women of reproductive age, expectedly, many would be multi-parous and this could cause laxity of the anterior abdominal wall muscles leading to higher measurements of WC in females. The significantly higher NC and WHR observed for males of this study are in concordance with previous studies (42, 43, 44). This finding may not be unconnected with the fact that testosterone encourages fat deposition in the upper trunk (24) .The significantly higher mean serum concentration of TC observed in females of this study may be a pointer to the higher incidence of adverse metabolic profile in the females compared to males. Similar to previous studies (35, 36, 45) which reported a higher mean value for HDL–C in females when compared to males, this study has observed a higher mean serum HDL-C in females.

Conclusion

The study revealed that second to fourth digit ratio, anthropometric and serum indices of adiposity are sexually dimorphic in the Hausa ethnic group of Kano with 2D:4D, HC, WHtR, BAI, TC and HDL being higher in females than in males while NC, WHR are higher in males than in females. No significant sex difference was observed for WC, BMI, TG and LDL-C.

Note: LDL and LDL-C are used interchangeably to connote low-density lipo-protein cholesterol. In this write-up, we have adopted LDL-C which is a more complete description. Also to ensure uniformity in the entire article

Acknowledgements

We thank all those who volunteered to participate in this study. This work is an extract of a Ph.D. dissertation which was sponsored by Bayero University Research Grant Unit and Tertiary Education Trust Fund (TETfund) of Nigeria

Conflict of interest:

None was declared by authors

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Metastatic Tumors of the Jaws and Oral Soft Tissues: A Retrospective Analysis of Ten Cases.

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Abstract

Metastatic tumours to the jaws and oral \soft tissue is rare, and primarily originates from the breast, lungs, and prostate. This study aimed to document the pattern of presentation, clinical, radiographic and course of metastatic lesions to the oral cavity in patients seen in our institution. The study was conducted in University of Calabar Teaching Hospital, Calabar, Nigeria, and the design was retrospective. All consecutive cases of metastatic lesions to the jaws and oral soft tissues that presented to the oral and maxillofacial unit of our institution over an 8 year period, from March 2008 to February 2016, were analyzed. Their ages, sex, primary and metastatic sites of tumours, clinical history, and histopathological features were obtained from the departmental records of Oral and Maxillofacial Surgery and Pathology. The data were analyzed using simple frequency and percentages for categorical variables, while continuous variables were analyzed by means and standard deviation. There was equal male and female representation, giving a gender ratio of 1:1. The age ranged from 45 to 79 years, with a mean (SD) of 63.8 (11.45) years. The primary malignant sites were the prostate 50% (5/10), breast 30% (3/10) stomach 10% (1/10), as well as uterus, 10% (1/10), and the mandible was most commonly affected 60% (6/10). Clinical presentations were jaw swellings (n=4, 40%), and exophytic soft tissue mass (n=3; 30%). Metastatic lesions to the oral region run a poor prognostic course with delayed clinical signs which often appear as common oral pathologies. Early detection by thorough clinical and radiologic examination, and management is necessary to improve prognosis.

Key Words: Metastatic Tumours, Jaws, Oral Tissues

Introduction

Metastatic tumors to the jaw and the oral region are rare; accounting for 1-3% of malignant jaw tumours (1). The origins of the primary source of these oral lesions vary and tends to reflect the relative incidence of cancer in a given community. Commonly reported primary sites are from the breast, lungs and kidneys (1). Gender has been reported to influence the sources of metastasis to the jaws. While malignancy involving the breast, ovary and thyroid commonly metastasizes to the jaws in females, metastatic jaw tumors in males frequently arises from prostate, lungs, kidney and bladder (2,3). In the jaw bones, the mandible is the most common location for secondary tumors, with the molar area being the most favored site (4).

The radiographic picture, vary from that of osteolysis for tumors arising from the breast, stomach or kidneys, to osteoblastic for prostate malignancy (5). Metastatic jaw tumors present with pain, swelling, parasthesia, ulceration and pathologic fractures in some cases. These tumors are of great clinical significance as their appearance may be the first indication of an undiagnosed malignancy at a distant primary site, or they may be the first evidence of spread of a known tumor from its primary site (1, 6).

Since Adebayo and Ajike (7) first reported 6 cases of metastatic jaw tumors in a review of 415 malignant oral and maxillofacial tumors over a 20-year period, there has been no other report from the Nigerian literature.

The aim of this study was to analyze the demographic and clinical characteristics of metastatic tumuors to the oral soft tissues and jaws seen over an 8-year period at the University of Calabar Teaching Hospital, Calabar, Nigeria.

Methods and Materials

This a retrospective analysis of all patients with metastatic jaw tumors seen over an 8 year period, from March 2008 to February 2016, at the Oral and Maxillofacial Surgery Unit of the University of Calabar Teaching Hospital, Calabar, Nigeria. All the patients seen in this study were referred to our unit, from other specialized clinics. The inclusion criteria were lesions whose oral histopathologic findings

concurred with the biopsy report of the extra gnathic primary tumours. This was necessary to avoid coincidental or co-existing but different pathological lesions in the supposed primary origin and the oro-facial region. Hematologic malignances with oral manifestations were excluded. Their demographic data, presenting complaints, primary tumor sites, clinical findings and histopathological reports of both primary lesions and the orofacial sites were analyzed. The data were analyzed using the Statistical Package for Social Sciences (SPSS), version 13, and the results presented as mean, and frequencies and percentages.

Results

Over the 8-year period of review 10 patients comprising equal number of both genders were seen, giving a male to female ratio of 1:1. The age ranged from 45 to 79 years, with a mean (SD) of 63.8 (11.45) years.

Table 1. Shows the clinical distribution of metastatic lesions to the oral soft and skeletal tissues. The primary malignant sites were the prostate 50% (5/10), breast 30% (3/10) stomach 10% (1/10), as well as uterus 10% (1/10), and the mandible was most commonly affected 60% (6/10), maxilla 10%(1/10) and the soft tissue, specifically the attached gingiva, 30% (3/10). Clinical presentations were jaw swellings, exophytic soft tissue mass, oro-antral opening in one case, bleeding from the gums and only two patients had lower lip parasthesia (Table 1). Although the distribution was not significant (Likelihood ratio, x^2=6.23; P=0.101), mandibular metastasis was twice as common in males as in females. All the gingival metastasis (2 mandibular and 1 maxillary) were observed in females (Table 1).

Discussion

Many authors agree that the incidence of malignant tumors to oral sites are rare and range from 1-3% (1,3,8). They have attributed this rarity to the ease of missing out these lesions at the stage of diagnosis and the fact that they often mimic benign oral conditions (7,9). In reality, the rarity of these lesions could be a result of the clinicians' initial low index of suspicion of the presence of a possible metastasis from a distant primary site to the oral tissues. This results in under reporting of cases. For instance a report of tumors and tumor-like lesions in 146 patients over 11years,(10) did not include any case of metastases from distant sites to the oral tissues but following the referral of a confirmed case with metastases to the jaws, ten patients were seen in eight years. Some authors believe that the actual incidence of these metastatic cancers to the oral cavity is unknown since their presence often a manifestation of an advanced lesion somewhere else (7, 9, 11). Also an unknown number of patients may have died without investigations for occult oral metastasis therefore giving an impression of rarity of occurrence (12).

The mouth and jaws are often excluded during autopsies due to family and socio cultural

Table 1 Clinical distribution of metastatic lesions to the oral region

Patient	Age	Gender	Metastatic Site	Primary Site	Clinical Appearance
1	79	M	Mandible	Prostate	Swelling
2	70	M	Mandible	Prostate	Swelling
3	68	M	Maxilla	Prostate	Oro-antral fistula
4	72	M	Mandible	Prostate	Swelling
5	50	M	Mandible	Prostate	Swelling; Pain
6	61	F	Mandibular Gingiva	Breast	Exophytic mass; gingival bleeding
7	45	F	Mandible	Breast	Parasthesia
8	62	F	Mandible	Breast	Exophytic mass
9	77	F	Maxillary Gingiva	Stomach	Parasthesia
10	54	F	Maxillary Gingiva	Uterus	Exophytic mass

prohibitions in this community (6, 7). In the present series the patients appear grossly defaced, pale, and weak with signs of possible multiple organ spread and obvious advanced stage of the primary lesion. This non-inclusion of oral tissues in autopsies could mask the true incidence of metastatic tumors to the jaws as observed by other authors (1,6,7).

The ages of the patients seen in this study were between 45 and 79 years with a mean age of 63.8 years. Majority, 8/10 (80%) of these patients were above 50 years. Most investigators believe that it is a disease of middle and old age (1, 7). The primary lesions are often seen and diagnosed about five years prior to the appearance of oral lesions when they were referred (13). Though early detection of oral metastases can be challenging (9), a five- year delay could be long enough for the lesion not to respond to contemporary forms of treatment. Shorter intervals between appearance and presentation reported (28.5 months) by some investigators responded to the various treatment procedures instituted which include chemotherapy in three cases, chemo-radiotherapy in two cases and surgery in one case (9). Late presentation precludes early commencement of interdisciplinary management which could prolong life or at least, improve its quality.

Contrary to the belief by some authors that the occurrence of prostate as primary fields of jaws metastasis is extremely rare (1, 14), half of the primary sources of metastatic tumors in the present survey, were of prostatic origin. Our findings also differ from those of an earlier study that was carried out in Northern Nigeria, about a decade ago, were no case of prostatic metastasis was observed among the six cases (2 males; 4 females) reported (7). In this series, all the male patients that presented with orofacial metastasis had a prostate primary. Oral metastasis from the breast and of gastric origin which occurred in females was deceptive, presenting as mere bleeding, with less pains and minimal disruption of function. Some authors had a similar experience where metastatic breast carcinoma presented as periodontal abscess (1), while others reported cases of breast cancer initially diagnosed as pulpal /periapical disease (9). Therefore, secondary oral deposits of distant malignancies should be considered in the differential diagnosis of common inflammatory and reactive lesions.

Jaw metastases can arise in primaries from any part of the body. No particular malignancies seem to specially favor the oral cavity. However some are found in the jaws more frequently than others. In most series reviewed, the mandible is more affected than the maxilla (1,3,7, 8). This was our experience with the posterior mandible being more favored. We observed that the secondaries in the metastatic jaw tumors of prostatic origin were lodged in the body and angle of the mandible producing pain, swelling and dysphagia. Bodner (9) reported more lung primary metastases to oral soft tissues while breast carcinomas were intra-bony in the oral region. The mode of spread to the jaw bones is believed to be haematogenous since the bones do not have a lymphatic drainage system. The preference for the mandible over the maxilla exists despite the fact that both bones have the same blood supply. Some authors (15,16) believe that the posterior mandible has richer blood supply and areas of active haemopoesis accounting for more secondary deposits. Other researchers had equal representation between anterior and posterior mandible (17).

A combination of the fact that tumor proliferation and survival of metastatic cells are angiogenesis driven and viable hoemopoietic center dependent, suggest that even at the late ages of occurrence of metastasis in oral tissues, these factors still interplay in accounting for the frequency of metastasis in the mandible in general and posterior part in particular. These 2 factors are age dependent; it is therefore probable that metastasis may have occurred to the oral region at a much younger age and manifest at advanced age as seen. This emphasizes the need for a thorough generalized radiological skeletal survey at the earliest suspicion of primary lesions to ensure that occult metastases are detected early. Obvious oral clinical signs of metastasis are considered a late presentation with multiple organ involvement (1). At these early stages, they may be asymptomatic, but growth can be rapid resulting in pain, chewing difficulties and dysphagia.

In this study, soft tissue metastasis presented as exophytic, multi-nodular, hemorrhagic and painful

swelling on the gingiva. There were super imposed infections resulting in oral fetor (characteristically fetid) and easily bleeding gums. Teeth present exhibited between first and second degree mobility. These were more seen amongst female subjects. Tenderness, swelling and third degree mobility of teeth present with occasional bleeding of overlying soft tissue characterized bony involvement of metastasis to the jaw. These clinical findings are similar to those seen by other authors. (1,2,7). Only two patients presented with chin numbness. This was similar to the results of Bodner et al, (9) who also observed 2 cases of chin numbness (the numb chin syndrome) in a series of 8 metastatic jaw tumors. Inferior dental or mental nerve anaesthesia should be considered an ominous sign for metastatic lesions to the mandible, in the absence of other obvious causes (12). In one of the cases, the palatal lesion was necrotic with slough formation, exposure of bone and fistula formation.

The routine use of x-rays in the diagnosis of metastatic lesions to the oral cavity presents its own challenges. It may be suggestive of the presence of an abnormal process in bone or the presence of radiographic changes confirms the suspicion of a possible primary site. In our series the patients were already known and being treated for a primary lesion so routine radiographic examination was considered superfluous. The diagnostic dilemmas posed by oral metastatic lesions are also reflected in the radiographic appearances. While some authors report radio-opacity associated with bone forming tumors such as prostate carcinomas and radiolucent secondaries in osteolytic tumors as in breast cancers (1, 3, 7), others reported radio-opacity for both breast and prostate carcinomas (18, 19). Due to the possible presence of occult early metastasis in bone, the lack of radiographic changes does not exclude the possible presence of small metastatic lesions in the jaw bones. Bodner (9), as a result, advocates use of bone scintigraphy for all suspected cases. However, this procedure cannot be done on all patients in every center, especially in this part of the globe owing to cost.

At the stage of presentation, palliative management was considered best to improve as much as practicable, the quality of patients' life. Non-invasive procedures such as antiseptic mouth rinses, regular professional oral toileting, analgesics and antibacterial medication were administered. Special high protein fluid diets and reassurances were carried out routinely in our center. In addition, the services of social health workers were employed. In spite of these, a maximum of three follow up clinical visit was achieved. All other forms of therapy had been attempted by various authors. These include supportive care surgery and radiotherapy surgery (1), radiotherapy palliative measure (7), surgery and chemo-radiotherapy and combination chemotherapy (19).

Prognosis for patients with metastatic tumours is generally poor, and some authors have cited 6 – 7 months and approximately 70% dying in 1 year. (1). All patients were lost to follow up within the first month of presentation to the maxillofacial clinic. In this part of the globe, attitudes of patients towards follow up is generally poor; and more so for advanced malignant conditions of this nature where only palliative treatment is possible. Patients often fall back to tradomedical care and home remedy until they passed on, and so making it difficult to estimate survival rate based on a specified time frame. This was further compounded by the relatively small sample size.

Conclusion

Metastatic tumours to the oral region run a poor prognostic course with delayed clinical signs which are often appearing as common oral pathologies. Thorough clinical and radiographic examination is inevitable to allow for early detection and institution of multidisciplinary management procedure.

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Effects of *Vernonia amygdalina* Supplementation on Pudding on Serum Glucose Level and Haematological Indices in Wistar Albino Rats

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Abstract

Effects of *Vernonia amygdalina* supplementation on *Vigna subterrenea* pudding on serum glucose level and haematological indices in Wistar albino rats was investigated in this study. Twenty five weanling rats were randomly selected into 5 groups of 5 rats each. Group 1(normal control) received normal rat pellet. Group 2 and 3 received 10% and 5% (w/w) non supplemented Bambara pudding respectively. Group 4 and 5 were fed with 10% and 5% (w/w) *Vernonia* leaf supplemented bambara pudding respectively for 21 days. The animals were sacrificed under anaesthesia at the end of the experiment and blood sample collected for serum glucose level and haematological indices determinations. The result of proximate analysis of supplemented and non-supplemented Bambara pudding showed a significant increase ($p<0.05$) in ash (6.20 ±0.00, 4.97 ± 0.03) and crude protein (25.07 ± 0.03, 22.40 ± 0.00) contents when compared to raw sample respectively. There was no significant difference in haematological indices of PCV, Hb, RBC and WBC in all experimental groups. Serum glucose level was significantly decreased in group 2 relative to control. There was a significant increase in relative body weight changes in the 5 grammes and 10 grammes *Vernononia* non supplemented groups. Data from this study indicated that consumption of Bambara groundnut pudding is probably safe and its fortification with green leafy vegetable may be useful in body weight control.

Key Words: Bambara groundnut, *Vernonia amygdalina***,** glucose, haematological indices and relative body weight.

Introduction

Bambara groundnut (*Vigna subterranean* L.*)* belongs to the family of *Leguminosae*. It is widely cultivated across some countries in Africa, Nigeria inclusive. In the continent of Africa, Bambara groundnut is ranked as the third most nutritive legume after groundnut and cowpea (1). There are two varieties of Bambara and the seeds possess four different colours which are black, red, brown and cream. The crop is essentially grown for human consumption; however, it is underutilized in Nigeria. According to (2), Bambara groundnut seed is a complete food, and a good supplement to cereal - based diets. Bambara groundnut is consumed in different forms and it contains about 63% carbohydrates, 19% protein, and 6.5% oil (3). The fresh seeds may be boiled and eaten as snacks whereas dry seeds of Bambara may be ground into flour, spiced and made into paste, then boiled as *moi-moi* or *okpa*. The paste may also be fried and eaten as *akara* (4; 5). Since Bambara groundnut is highly nutritious and of economic importance, it could be utilized to meet the burden of hunger and solve the devastating challenges of chronic diseases ravening a large population particularly in developing countries.

Vernonia amgydalina is a shrub extensively domesticated in some parts of West Africa, including Nigeria where it is popularly known as bitter leaf. Bitter leaf plays a significant role in human nutrition particularly in the preparation of bitter leaf soup. It possesses appreciable quantities of ascorbic acid, carotenoids, calcium, iron, potassium, phosphorus, manganese, copper and cobalt (6; 7). Apart from its nutritional benefits, several scientific studies has reported the antihyperglycemic, antilipidemic, hypoglycemic and hypolipidemic properties inherent in the leaves of the plants as demonstrated in animal models (8; 9).

Chronic diseases are long-term diseases that are not transmittable as well as largely preventable. They are the most common cause of death in the world and present a great burden for society, particularly diseases such as obesity, diabetes, cardio vascular disease, cancer, dental disease, and osteoporosis.

Making adjustment in terms of dietary lifestyle and physical activity can help reduce the risk of these chronic diseases (4). The emergence of these diseases results from the nutritional transition from the traditionally plant-based diets including legumes, cereals etc to a highly processed foods with increased sugar and animal fat contents. This transition in combination with a more sedentary life is the underlying risk factor of developing chronic diseases. Several studies have shown that a diet high in vegetables and fruits is recommended for good health. Therefore this study seeks to investigate the effect of *Vernonia amygdalina* supplementation on *Vigna subterrenea* (bambara groundnut) pudding on serum glucose level, heamatological indices and relative body weight changes in Wistar albino rats.

Materials and Methods

Sample collection

Dried cream coloured Bambara groundnut were purchased from a local market in Yala Local Government Area of Cross River State, Nigeria and conveyed to the laboratory of Department of Biochemistry, University of Calabar, Nigeria. Twenty five weanling albino Wistar rats of both sexes of ages four to six weeks old weighing between 50 – 53 g were purchased from Animal House of the Department of Biochemistry, University of Calabar. The rat feed was purchased from a Vital Feed dealer's outlet in Calabar Municipality, Cross River State, Nigeria.

The study samples (Bambara groundnut and *Vernonia amygdalina*) were identified and authenticated by a botanist, Mr. Frank Apejoyi of the Department of Botany, University of Calabar. Bambara groundnut was assigned voucher number (BOT/2014/032) while *Vernonia amgydalina* voucher number was assigned BOT/2014/011.

Sample preparation

Bambara seeds were sorted to remove extraneous materials and damaged seeds. Three hundred grammes of the sorted seeds was weighed and soaked in tap water for eight hours at room temperature for easy removal of the outer coat. After eight hours of soaking,the samples were washed, drained several times to dehull the coat from the cotyledon and then wet milled into paste using commercial milling machine. A portion from the raw paste was taken for proximate analysis. Cold water (200 mls) was added to the flour and mixed with recipes thoroughly. The mixture was used to prepare two kinds of diets. The first diet was prepared supplemented with fresh chopped *Vernonia amygdalina* leaves whereas the second diet was prepared without *Vernonia* leaves supplementation. A clean measuring cup of 20 ml size was used to dispense the mixed paste into a clean *Thalimanus danieli* (nkong) leaf (wrapping agent), 25 g of chopped fresh bitter leaves was added to each wrap in the *Vernonia* supplemented puddings. The same procedure was repeated in preparing the second type of diet but without addition of bitter leaves, this formed the non – supplemented puddings. Both diets were cooked for 45 minutes, after which they were oven dried at a temperature of 50 - 60ºc. The oven dried diets were separately blended into fine particles before incorporated into the rat feed.

Experimental protocol

The Wistar albino rats were randomly selected into their respective groups. Group 1(control) were fed with 100g rat pellet only, group 2 were fed with a mixture of (10g of *Vernonia* non-supplemented diet + 90g pellet), group 3 (5g of *Vernonia* non-supplemented diet + 95g pellet), group 4 (10g of *Vernonia* supplemented diet + 90g pellet), group 5 (5g of *Vernonia* supplemented diet + 95g pellet) for 21days. All the experimental groups were allowed access to fresh tap throughout the experimental period. The rats were weighed and the measurement recorded at four days intervals to monitor their relative body weight changes. Also, consumption pattern was monitored by taking record of the left over feed on daily basis; this was done using an electronic balance. The rats' beddings were changed at two days interval throughout the period of the experiment to avoid microbial growth and subsequent infection of the animals.

Collection and preparation of blood sample for analysis

The rats were fasted overnight prior to sacrifice, the rats were anaesthetized in chloroform enclosed chamber and then dissected using surgical scissors and forceps. Whole blood was collected by cardiac puncture using 5ml sterile syringe and needle into an EDTA bottles. The blood was allowed to clot for three hours after which it was centrifuged at 3000 revolutions per

minute for 10 minutes using a bench top centrifuge (Model SM 80 – 2 England) for easy collection of the serum. The sera from the different experimental groups were used for glucose assay and haematological indices estimations.

Determination of proximate compositions of cooked pudding and raw paste

After steaming for about 45 minutes the samples were allowed to cool, 5g of each sample was taken and coded for proximate composition analysis using the standard method (10).

Determination of moisture content

Moisture content was determined using the standard gravimetric method (11), 10 g of each sample were separately weighed and oven dried at 105º C for three hours. Then allowed to cool inside a desiccator and reweighed. The samples were returned to the oven for further drying. This process continues at hourly intervals before a constant weight was obtained.

Determination of ash content

Ash content determination was carried out using the standard gravimetric method (10, 11). The weight of sample measured into each porcelain crucible was 5g and then subjected to high temperature of 600º C in an electronic muffle furnace. The samples were allowed to burn until a grey ash colouration was seen. The grey ash content was carefully transferred to a desiccator and allowed to cool before reweighed. The weight of the inorganic matter was determined by difference as a percentage of the analyzed sample.

Determination of crude fibre

Crude fibre was determined using Weende method described (10). 5g of each sample was boiled in 200 ml of 1.26 % H_2S0_4 solution for 30 minutes. A muslin cloth was used to retain sample particles when washed in hot distilled water. The washed residue was transferred into the boiling flask with 200 ml of 1.25 % NaOH solution and boil for another 30 minutes. It was washed again and then transferred to a weighed porcelain crucible. The washed sample was oven dried for 1hour at 105º C and then left to cool in a desiccator before measurement was taken.

Determination of crude fat

Crude fat content of each sample was determined using method . 5g of each sample was wrapped in a weighed porous filter paper (Whatman No.1) and put in a soxhlet apparatus and mounted on an oil extraction chamber containing 200 ml of petroleum ether for 4hours. The amount of fat in each sample was calculated by dividing the weight of fat obtained by the weight of the sample multiply by 100.

Determination of crude protein

Crude protein content was determined by Kjeldahl method (11). This method was used to obtain total nitrogen content in each sample by the process of digestion, distillation and titration. The samples were digested with concentrated H_2SO_4 in the presence of Selenium catalyst. 40 % NaOH was added to the digest and the mixture was distilled in a macham distillation apparatus (Excello, England). The distillate was trapped in boric acid and then titrated using HCl to obtain a deep red end point.

Determination of carbohydrate content

Carbohydrate content in each sample was estimated by difference. The sum of other contents was subtracted from 100 to obtain the amount of carbohydrate present in the sample.

Determination of energy/caloric value

Energy value was determined by the summation of the crude values of fat, crude protein, and carbohydrate multiplied by the constant 9, 4, and 4 respectively.

Blood sampling and biochemical analysis

Estimation of haematological indices using automated haematology analyzer, KX-21 (non-cyanide haemoglobin analysis method) Packed cell counts, haemoglobin, red blood cells, white blood cells, platelet counts, differential white blood cell (lymphocytes and mixed), were analyzed using the Sysmsex® Automated Hematology Analyzer (KX-21N, Sysmex Corporation, Kobe-Japan).

Determination of glucose concentration was carried out by using glucometer (Accu-check Perfoma Roche Diagnostics, Manannheim, Germany).

Statistical Analysis

Values obtained were expressed as mean and standard deviations. Data were analyzed by one way ANOVA and significant differences between groups were determined by least significant difference (LSD). Statistical analyses were carried

out using SPSS, the statistical package for Windows, version 22.0 (SPSS Inc. Chicago, IL. USA). The acceptable level of significance was $p < 0.05$.

Results and Discussion

The result of proximate compositions of raw paste and cooked Bambara pudding is shown in Table 1; there was a significant increase ($p<0.05$) in moisture, crude ash and protein contents in both cooked samples (supplemented and non-supplemented puddings) when compared to the raw paste sample respectively. Also, crude fibre content significantly increased ($p<0.05$) in supplemented pudding when compared to the raw paste sample. Similarly, a marked increase at $p<0.05$ was observed in crude fat, carbohydrate and caloric contents in the supplemented and non-supplemented puddings when compared to raw sample.

The results of the raw paste proximate content were consistent with the values reported by (13). Also, values of the proximate composition of non supplemented pudding of cooked samples were comparable to report by (14). The low level of moisture content in bambara groundnut could be useful in the production of quality food products with improved shelf life, the seeds may be preserved for a long time without much deterioration. A significant increase in moisture content was observed in cooked pudding supplemented with *Vernonia amygdalina* when compared to cooked pudding non - supplemented with *Vernonia amygdalina*. This difference could be as a result of the moisture content from the *Vernonia* leaves. Incorporation of these leafy vegetables in foods could aid in peristalsis (15).

The crude ash content is indicative of some mineral levels present in the barabara groundnut especially calcium, magnesium and phosphorus. A higher ash content value was observed in this study as compared to values reported for other legumes like lima bean and ground nut (16, 17). The high amount of ash content value in the supplemented puddings as compared to the raw paste and non - supplemented pudding could have resulted from additional macro elements in *Vernonia amygdalina* leaves. This result agrees with several works that have reported the presence of macro elements in *Vernonia amygdalina* leaves (6, 7, 18).

Protein content in this study was similar to report of local cowpea in protein value (16; 17). Several reports have shown that increased protein intakes could be beneficial in preventing osteoporosis, reducing cardiovascular disease rate, maintaining healthy weight and muscle mass (19; 20). However, the high protein content here agrees with the report of (21) that Bambara groundnut could supplement diets lacking in animal protein. The crude fibre content from the present study was not consistent with other reports (14, 22). Fat content value of raw paste in this study was in agreement with the report by (14, 23). The increases in crude fat content in bambara groundnut do not necessarily pose health challenge.

The carbohydrate values have been reported in both raw (66.9 %) and processed (69.2%) local cowpeas (17). however, carbohydrate content value in the raw paste agreed with the report of (24) for the light brown/cream colour variety of Bambara groundnut.

Table I: Proximate compositions of raw paste and *Vernonia amygdalina* supplemented cooked bambara puddings

Group	Moisture	Crude ash	Crude protein	Crude fibre	Fat	CHO	Caloric value
RAW PASTE	10.2 + 0.06	4.52 + 0.04	19.30 + 0.00	2.51 + 0.00	5.25 + 0.00	57.24 + 0.00	353.44 + 0.03
SBD	12.35 ±0.00*	6.20 ± 0.00*	25.07 ± 0.03*	3.40 ± 0.00*	10.74 ±0.00*	42.27 ±0.03*	357.62 ± 4.17*
UFD	11.52 ± 0.01*,a	4.97 ± 0.03*,a	22.40 ± 0.00*,a	2.50 ± 0.00a	13.51 ± 0.01*,a	45.09 ± 0.01*,a	361.74 ± 4.17*,a

All values are in percentage (%); Values are expressed as mean ± SEM. n = 5; Values designated (*) vary significantly from RAW PASTE at $p<0.05$; (a) Vary significantly from FBD at $p<0.05$; SBD = supplemented Bambara pudding, UFD = non - supplemented Bambara pudding.

Table 2: Blood glucose level and haematological indices in albino rats fed with *Vernonia amygdalina* supplemented and non – supplemented bambara puddings for 21 days.

Group	Glucose	RBC	WBC	LYM	MONO	PCV	PLT	IIb
1	0.47 ± 0.03	5.48 ± 0.37	4.80 ± 0.32	4.13 ± 0.03	0.17 ± 0.09	46.83 ± 1.62	808.00 ± 181.43	12.03 ± 0.67
2	0.23 ± 0.03*	5.52 ± 0.53	4.33 ± 0.07	3.97 ± 0.15	0.10 ± 0.00	42.10 ± 4.29	1106.33 ± 105.59	12.57 ± 1.29
3	0.33 ± 0.03	5.76 ± 0.23	2.87 ± 0.41	2.50 ± 0.44	0.13 ± 0.03	43.80 ± 2.14	762.67 ± 142.92	12.57 ± 0.82
4	0.37 ± 0.03	5.83 ± 0.12	4.10 ± 0.20	3.80 ± 0.26	0.13 ± 0.03	44.60 ± 1.38	865.00 ± 91.66	12.93 ± 0.63
5	0.33 ± 0.03	4.20 ± 1.48	2.37 ± 0.70	2.03 ± 0.57	0.13 ± 0.09	32.73 ± 12.11	748.33 ± 185.61	8.93 ± 3.22

All values for glucose levels are in mmol/L; Values are expressed as mean ± SEM. n = 5; Values designated (*) vary significantly from group 1 at $p < 0.05$

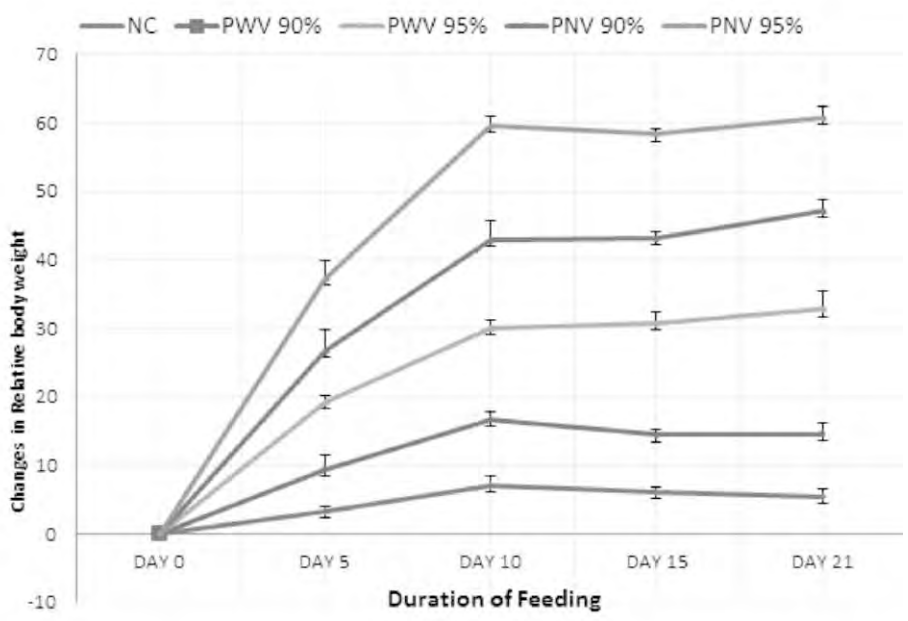

FIG. 1: Time course chart showing changes in Relative body weight taken at five days interval; Values are expressed as Mean ± SEM.

NC= normal control, PWV 90% =group 2 fed with 10% incorporation of supplemented diet, PWV 95% = group 3 fed with 5% incorporation of supplemented diet, PNV 90% = group 4 fed with 10% incorporation of non-supplemented diet, PNV 95% = group 5 fed with 5% incorporation of non-supplemented diet.

Serum Glucose Level and Haematological Indices

The results of serum glucose level and haematological indices obtained in this study are as presented in Table 2 below. There was a marked decrease at $p<0.05$ in group 2 (fed with 10% supplemented pudding) when compare to normal control. The fasting blood glucose levels in this study were relatively low in comparison with glucose level for normal healthy person (fasting blood glucose $\geq$ 7.0 mmol/l). However, this diet may be recommended for diabetic patients and those with increased blood sugar levels because of its postprandial glycemic response (13; 25).

The results for hematological indices showed no significant difference in all experimental groups. It may probably be that there was no inflammation or atherosclerotic disorder/haematotoxic effect from consumption of this diet. This report is similar to findings by (26) on hepatoprotective and non haematotoxic effect of *Vernonia* diet-induced

The Effect of V. *amygdalina* Supplementation on Pudding in Relation to Body Weight Changes is shown in Fig. I

The body weight changes showed increase in body weight for groups 2 and 3, whereas moderate body weight changes were observed in groups 4 and 5. The Normal control is used as an index of comparison hence it should be used when comparing other groups to it. The increase in body weight changes in groups 2 and 3 could be linked to increase in consumption pattern by the animals. Preliminary findings from this study could be exploited in diet fortification intended for body weight control.

Conclusion

Findings from this study have demonstrated that Bambara groundnut when supplemented with green leafy vegetables contributes to traditional food diversity and possible food security in rural communities, particularly in developing countries.

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Antibiogram of Bacteria Associated with Urinary Tract Infections in People Living with HIV/AIDS in Jalingo, Nigeria

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Abstract

Concurrent microbial infections have long been established as major causes of morbidity and mortality among people living with HIV/AIDS (PLWHA). Laboratory examination of their body fluids shows elevated load and a broad spectrum of pathogens. Studies have also shown diversities in the etiology of urinary tract infections (UTIs) in different geographical locations. This study was designed to identify the bacteria associated with UTIs in PLWHA and their antibiotic susceptibility patterns in Jalingo, Taraba State, Nigeria. Two hundred and twenty PLWHA and 180 non-HIV/AIDS patients (control subjects) visiting Government House Clinic, Federal Medical Centre and Specialist Hospital in Jalingo between February and July 2016 were recruited into the study. Urine samples were collected for quantitative microbiological analysis. Antibiotic susceptibility testing was performed on all isolates using modified Kirby-Bauer method and identification of *Enterobacteriaceae* using standard biochemical methods. Bacteriuria was detected in 143(65.0%) and 82(45.6%) of test and control subjects respectively. Of these, 62/143 (43.4%) had significant bacteriuria ($\geq 10^5$ CFU/mL) among test subjects and 25/82 (30.5%) among control subjects ($p=0.00009619$). A total of 173 bacterial isolates were obtained from test subjects, *Escherichia coli* 89(51.4%) was the most predominant uropathogen involved in infection, followed by *Staphylococcus aureus* 26(15.0%) and coagulase negative Staphylococci (CoNS) 18(10.4%). A similar pattern was observed in the control group ($p>0.05$). Bacteriuria was more common in female than male subjects at a ratio of 2.3:1. There was a general resistance of isolates to tested antibiotics with no significant difference between both groups ($p>0.05$). Antibiotic stewardship programmes should be established in our hospitals and clinics.

Key Words: Bacteria, Urinary tract infections, drug resistance, HIV/AIDS

Introduction

The etiologies of UTIs are well documented and vary within different geographical locations. Studies have revealed that almost (95%) of UTI cases are caused by bacteria that typically multiply at the opening of the urethra and travel up to the bladder, with the occasional haematogenous spread from the kidney. The bacteria most often associated with UTIs are of faecal origin, facultative anaerobes and members of the family *Enterobacteriaceae* (1,2,3).

The human immunodeficiency virus (HIV) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS) (4). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. HIV is a virus that attacks immune cells called CD-4 cells, which are a subset of T cells. The virus can be transmitted through contact with infected blood, semen or vaginal fluids. Within a few weeks of HIV infection, flu-like symptoms such as fever, sore throat and fatigue can occur. Then the disease is usually asymptomatic until it progresses to AIDS. AIDS symptoms include weight loss, fever or night sweats, fatigue and recurrent infections (5). No cure exists for AIDS but, strict adherence to antiretroviral regimens (ARVs) can dramatically slow the disease progress as well as prevent secondary infections and complications.

The ability of a bacterial strain to survive in the presence of higher antibiotic concentrations in comparison with phylogenetically related strains is termed resistance (6). Indeed, the rising trend of developing resistance to multiple antibiotics by microbes, leading to therapeutic failure, is a serious problem of global magnitude. Human development of antimicrobial drugs and their widespread clinical use has simply provided another selective pressure that promotes further evolution. Several important factors can accelerate the evolution of drug resistance. These include the overuse and misuse of antimicrobials,

inappropriate use of antimicrobials, subtherapeutic dosing, and patient noncompliance with the recommended course of treatment (7).

Although *E. coli* and *S. aureus* are the most frequently isolated uropathogens in both community-acquired and nosocomial UTI, they are however not the only microorganisms causing UTIs. Other organisms such as *Klebsiella spp., Enterobacter spp., Proteus spp., Enterococci spp., Pseudomonas spp.,* and coagulase negative staphylococci have also been isolated(1,3,8). Occasionally, *Mycoplasma*, *Ureaplasma,* and *Chlamydia,* have been recovered from patients with UTI(1,8,9,10). The cumulative incidence of UTI in children reaches 10% (1). In adulthood, almost half of all women will experience at least one episode of UTI during their life time. With these variations, the need to investigate the common etiologies of UTI and their antibiotic sensitivity pattern within the study area, specifically among HIV/AIDS sero-positive individuals, became apparent.

Materials and Methods

Study area

Patients attending the Government House Clinic (GHC), the Specialist Hospital (SH) and Federal Medical Center (FMC) all located within Jalingo Metropolis, Taraba State were recruited into the study. Taraba State is one of the states in the middle belt of Nigeria with a high prevalence of HIV infection. Recent studies by the National AIDS and Reproductive Health Survey (NARHS)(11) revealed a prevalence rate of (10.5%).

Study subjects

Subjects comprised 220 HIV seropositive individuals visiting GHC, SH and FMC for their routine CD4 cells count checks, while 180 non-HIV/AIDS patients visiting these facilities constituted the control group. Ethical approval for the study was obtained from the Research Ethics Committee, Taraba State Ministry of Health. Informed consent was given by all the subjects before collection of samples.

Sample collection

Five milliliters of early morning clean-catch mid-stream urine samples were collected into sterile screw-capped universal plastic bottles. To prevent the contamination of samples by normal vaginal, perianal and anterior urethral flora, subjects were instructed to wash hands before and after sample collection. All subjects were instructed further on the appropriate procedure for the collection of mid-stream urine samples into the sterile containers provided. Samples were collected between 8am and 9am as the patients came to clinic, labeled appropriately and processed within 30 minutes of collection.

Culture and identification

Using sterile micropipettes, 0.01mL of each urine sample was plated on Cysteine Lactose Electrolyte Deficient (CLED) agar and blood agar. Plates were incubated for 24hrs at 37^0C, before examining for bacterial growth. The number of colonies on the culture plate was determined and converted to count per millilitre. Bacterial isolates were preliminarily identified using colony appearance on culture plates, Gram reactions, cellular arrangements, biochemical tests including Kligler Iron Agar (KIA), Voges Proskauer, and oxidase test were used for identification of *Enterobacteriaceae.* Gram positive cocci were subjected to catalase and coagulase tests.

Antimicrobial susceptibility testing

Susceptibility testing was performed using the modified Kirby-Bauer disc diffusion technique on isolates from Mueller-Hinton agar. Ten commercially prepared antibiotics discs (Hardy Diagnostics, Santa Maria, USA) of Streptomycin (30mg), Gentamicin (10mg), Cotrimoxazole (30mg), Ciprofloxacin (10mg), Augmentin (30mg), Cefalexin (10mg), Ampicillin (30mg), Ofloxacin (10mg), Peflaxine (10mg) and Nalidixic Acid (30mg) on agar were incubated at 37^0C for 24 hours for sensitivity assay. Zones of inhibition were measured and classed as sensitive or resistant using standard interpretative chart (12, 13).

Results were analysed using SPSS data software version 18. The p-value obtained was considered statistically significant at $p<0.05$.

Results

The demography and socioeconomic status of the study subjects showed that more females 154(70%) than males 66(30%) comprised PLWHA and the majority of these subjects 121(55%) had no formal

education (Table 1). At the time of sample collection, 79(36%) of test subjects were on Highy Active Antiretroviral Therapy (HAART), while 141(64%) were not on any antiviral drug. Seventy eight (55%) of those without treatment were not aware of their HIV status on time, whereas 63(45%) had started treatment but later stopped.

Although a greater proportion of these did not disclose their reasons for discontinuing treatment, others however claimed that the antiretroviral drugs induced infertility and comprised 54(44.6%) of those with no formal education and mainly farmers. Some of the test subjects exhibited common symptoms of UTIs, which included, burning sensation during urination, pain while passing out urine, urge to urinate frequently, feeling that the bladder is still full after urinating, blood in urine, pain around the pelvic region and fever.

Culture

One hundred and forty three of the 220 specimens from the test subjects yielded 173 bacterial isolates, comprising 118(68.2%) gram negative rods and 55(31.8%) gram positive cocci. From the control subjects, 82 of 180 specimens yielded a total of 87 bacterial isolates of which 71 (81.6%) were gram negative and 16(18.4 %) gram positive.

E. coli was the most frequently occurring bacteria among both male and female test subjects, accounting for 89(51.4%). It was followed by *S. aureus,* 26(15.0%) and coagulase negative staphylococci 18(10.4%). The frequency of occurrence of other isolates in the test subjects was in the following order; *Pseudomonas* spp. 12(6.9%), *Proteus* spp9(5.2%), *Klebsiella* spp 8(4.6%),

Table 1: Demographic and socioeconomic status of study subjects

		Test Subjects			Control Subjects		
Characteristics		Male	Female	Total	Male	Female	Total
Age	6 - 15	12	11	23	6	15	21
	16 - 25	6	42	48	31	40	71
	26 - 35	21	55	76	18	30	48
	35 - 45	14	26	40	9	11	20
	46 - 55	10	12	22	3	6	9
	>55	3	8	11	5	6	11
	Total	**66**	**154**	**220**	**72**	**108**	**180**
Education							
	Non formal			121			76
	Primary/Secondary			81			77
	Tertiary			18			27
	Total			**220**			**180**
Occupation							
	Farmers			98			55
	Civil servants			25			31
	Traders			36			32
	Total			**220**			**180**

Table 2: Distribution of bacterial isolates according to gender of subjects

Bacterial Isolates	Test Subjects			Control Subjects		
	Male	Female	Total (%)	Male	Female	Total (%)
Gram positive						
S. aureus	11	15	26(15)	8	4	12(13.8)
Streptococci	1	5	6(3.5)	0	0	0(0)
Enterococci	2	3	5(2.9)	0	0	0(0)
CoNS[a]	7	11	18(10.4)	3	1	4(4.4)
Total	21	34	55(31.8)	11	5	16(18.4)
Gram Negative						
E. coli	24	65	89(51.4)	20	28	48(55.2)
Pseudomonas spp.	4	8	12(6.9)	1	6	7(13.8)
Proteus spp.	2	7	9(5.2)	4	4	8(9.2)
Klebsiella spp.	2	6	8(4.6)	0	8	8(9.2)
Total	32	86	118(68.2)	25	46	71(81.6)

[a]CoNS – Coagulase negative staphylococci

Streptococcus spp 6(3.5%) and *Enterococcus* spp 5(2.9%). Similarly, *E. coli* was the most prevalent microorganism isolated from both male and female control subjects, with a total of 48(55.2%) isolates, *S. aureus* ranked second with 12(13.8%). *Proteus, Klebsiella,* and *Pseudomonas* made up 8(9.2%), 8(9.2%), and 7(8.1%) respectively (Table 2).

Gender analysis (Table 2) showed that bacteriuria was more prevalent in females than their male counterparts, with a ratio of 2.3:1 and 1.4:1 in test and control groups respectively. In both subject groups, isolates differed between genders, the prevalence of gram negative microorganisms was higher in both male and females, constituting 86 (71.7%) of the 120 isolates obtained from female test subjects and 46(90.2%) of 51 isolates from female control subjects.

In their male counterparts, gram negative bacteria constituted 32(60.4%) of the 53 bacterial isolates obtained from the 66 male test subjects, and 25(69.4%) in the control subjects. On the other hand, gram positive microorganisms had a prevalence of 21(39.6%) of 53 isolates from male test subjects and 34(28.3%) of the 120 isolates from the female test subjects, and *S. aureus* accounted for 26(15.0%) of the total isolates from test subjects (Table 2). Although the prevalence of bacteriuria was higher in the test subjects, the frequency and the type of bacterial isolates did not differ significantly between the two groups ($p = 0.09$).

Antibiotic Susceptibility Profile

The antibiotics tested exhibited low efficacy against bacterial isolates in this study. The susceptibility profile was determined using CLSI Antibiotics Susceptibility Interpretation chart (13). Of the antibiotics tested against 173 isolates from the test subjects, Gentamicin and Streptomycin demonstrated the greatest efficacy showing activity against 95(55%) and 90(52%) of the total isolates respectively. This was followed by Ofloxacin 76(44%) and Ciprofloxacin 74(43%). Higher resistance rates were recorded for Amoxicillin 141(82%) and Nalidixic acid 139(80%).

Analysis of the susceptibility profiles of individual isolates showed that *E. coli* exhibited greatest susceptibility with 39% of isolates being

Table 3: Antibiotics Susceptibility profile of Bacterial isolates from the Test subjects

Isolates	F	ST	CN	CTX	CPX	AUG	CEP	AMP	OFX	PEF	NA
	Antibiotic Sensitivity Profile (%)										
G- Negative											
E. *coli*	89	42(47)	55(62)	24(27)	41(46)	35(39)	27(30)	22(25)	41(46)	41(46)	21(24)
Proteus spp.	9	4(44)	4(44)	3(33)	3(33)	3(33)	2(22)	2(22)	4(44)	5(56)	3(33)
P. *aeroginosa*	12	3(25)	5(42)	3(25)	6(50)	0(0)	3(25)	0(0)	6(50)	2(17)	2(17)
Klebsiella spp.	8	3(38)	3(38)	2(25)	2(25)	1(12)	1(12)	2(25)	2(25)	2(25)	0(0)
Total	118	52(44)	67(57)	32(27)	52(44)	39(33)	33(28)	26(22)	53(45)	50(42)	26(22)
G- Positive											
S. *aureus*	26	19(73)	15(58)	7(27)	11(42)	8(31)	4(16)	3(12)	11(42)	8(31)	3(12)
Streptococci	6	4(66)	2(34)	1(17)	1(17)	1(17)	1(17)	0(0)	2(34)	2(34)	0(0)
CoNS	18	13(71)	10(55)	5(28)	8(45)	5(28)	2(12)	2(12)	8(45)	8(45)	3(17)
Enterococci	5	2(40)	1(20)	2(40)	2(40)	2(40)	1(20)	1(20)	2(40)	1(20)	2(40)
Total	55	38(69)	28(51)	15(27)	22(40)	16(29)	8(15)	6(11)	23(42)	19(35)	8(15)
AAS	173	90(52)	95(55)	47(27)	74(43)	55(32)	41(24)	32(18)	76(44)	69(40)	34(20)

KEY: F- Frequency, ST- Streptomycin, CN- Gentamycin, CTX_ Cotrimoxazole, CPX- Ciprofloxacin, AUG- Augmentin, CF- Cefalexin, AMP- Ampicillin, OFX- Ofloxacin, PEF- Peflaxine, NA- Nalidixic Acid, CoNS- Coagulase Negative Staphylococci. AAS- Average Antibiotic Sensitivity.

Table 4: Antibiotics Susceptibility Profile of Bacterial Isolates from the Control Subjects

Isolates	F	ST	CN	CTX	CPX	AUG	CEP	AMP	OFX	PEF	NA
	Antibiotic Sensitivity Profile (%)										
G- Negative											
E. *coli*	48	23(48)	28(58)	15(31)	23(48)	20(42)	17(35)	13(27)	22(46)	20(42)	12(26)
Proteus spp.	8	3(38)	4(50)	3(38)	2(25)	3(38)	2(25)	2(25)	4(50)	3(38)	3(38)
P. *aeruginosa*	7	2(29)	3(43)	2(29)	3(43)	1(14)	2(29)	1(14)	4(57)	1(14)	2(29)
Klebsiella spp.	8	4(50)	5(62)	2(25)	3(38)	0(0)	1(12)	2(25)	4(50)	2(25)	1(12)
Total	71	32(45)	40(56)	22(31)	31(44)	24(34)	22(31)	18(25)	34(48)	26(37)	18(25)
G- Positive											
S. *aureus*	12	9(75)	8(67)	4(33)	6(50)	4(33)	3(25)	2(17)	6(50)	2(17)	3(25)
Streptococci	0	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
CoNS	4	1(25)	2(50)	1(25)	2(50)	1(25)	0(0)	1(25)	2(50)	1(25)	1(25)
Enterococci	0	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Total	16	10(63)	10(63)	5(31)	8(50)	5(31)	3(19)	3(19)	8(50)	3(19)	4(25)
AAS	87	42(48)	50(57)	27(31)	39(45)	29(33)	25(29)	21(24)	42(48)	29(33)	22(25)

KEY: F-Frequency, S- Sensitive, ST- Streptomycin, CN- Gentamycin, CTX- Cotrimoxazole, CPX- Ciprofloxacin, AUG- Augmentin, CEF- Cefalexin, AMP- Ampicillin, OFX- Ofloxacin, PEF- Peflaxine, NA- Nalidixic Acid, CoNS- Coagulase Negative Staphylococci. AAS- Average Antibiotic Sensitivity.

susceptible to at least one of the antibiotics, followed by species of *Proteus* (37%), CoN Staphylococci (36%), *S. aureus (*34%) and Enterococci (32%). Streptococci*, Klebsiella and Pseudomonas* exhibited more resistance to the antibiotics with (77%, 77%, and 75%) of the isolates exhibiting resistance respectively. Sixty three percent of the isolates showed multiple antibiotics resistance (resistant to two or more antibiotics). The resistance pattern exhibited by isolates from the control subjects was similar to that of the test subjects; Ampicillin (76%), Nalidixic acid (75%), Cotrimoxazole (69%), Peflaxine (67%), Augmentin (67%), and Ciprofloxacin (55%). There was no significant observable difference in the susceptibility pattern of gram negative and gram positive bacteria isolated from the test and control groups (p=0.06). Thirty six percent of gram negative bacteria were susceptible to the antibiotics tested, while (33%) of gram positive bacteria isolated were susceptible.

Susceptibility patterns of the isolates were fairly distributed, though gram negative bacteria were more sensitive to Gentamicin 67(57%), Ofloxacin 53(45%), Streptomycin and Ciprofloxacin 52(44%) each, Peflaxine 50(42%), Augmentin 39(33%), Cefalexin 33(28%), and Cotrimoxazole 32(27%). Highest resistance was recorded against Ampicillin and Nalidixic acid 92(78%) each. Gram positive bacteria on the other hand, were more sensitive to Streptomycin 38(69%) followed by Gentamicin 28(51%), Ofloxacin 23(42%), and Ciprofloxacin 22(40%). Again highest resistance were recorded against Ampicillin 49(89%), Nalidixic acid 47(85%) and Cefalexin 47(85%).

Discussion

Data obtained from this study showed that *E. coli* was the isolated microorganism most frequently associated with bacteriuria in both HIV-positive test and HIV-negative control groups in Jalingo, having prevalence of (51.4%) and (55.2%) respectively. Other studies corroborate this finding. In their study of uropathogens isolated from HIV patients, Alemu and coworkers (14) in North-western Ethiopia reported that *E. coli* had a prevalence of (56.1%) among HIV/AIDS patients studied, while *S. aureus* and coagulase negative staphylococci (CoNS) had prevalence rates of (14.6%) and (9.8%) respectively. *E. coli* is the most frequently isolated uropathogen both in community-acquired and hospital-acquired UTI. It accounts for over 85% of UTI pathogens and is also associated with complicated cases(1,3,8). Asharam and coworkers(15) studied HIV and UTIs in 55 children in Durban, South Africa, and reported that *E. coli* was responsible for 48 (87.2%) of the infections in the study.

Bryan and coworkers(16) in a study of 219 HIV infected Jamaicans, found *E. coli* accounting for (53.8%) of the infections. In contrast, Inyang-Etoh and coworkers(17) in Calabar, Nigeria, reported *S. aureus* as the predominant organism causing UTIs, followed by *E. coli*. Wilmer and coworkers(18), studied the prevalence and risks of asymptomatic bacteriuria among HIV-positive pregnant women in Cape Town, South Africa; *E. coli* prevalence was (63% and 68%) in tests and control groups respectively. Furthermore, Olutosin and coworkers(19), in Ibadan Nigeria reported *E. coli* as the most frequently isolated microorganism with a prevalence of (48%) and *S. aureus* with a prevalence of (28%). Also, Akinbami and colleagues(20), reported a prevalence of (42.2%) for *E. coli*, which was the most often isolated microorganism. However, few studies have isolated *S. aureus* as the most common uropathogen in HIV seropositive individuals in Calabar, Nigeria(17) and in Bangalore, India(21).

In the present study, a wide range of other bacteria such as, Coagulase negative Staphylococci, *Pseudomonas*, *Proteus*, *Klebsiella*, Streptococci and Enterococci, were also isolated. CoNS are increasingly being implicated in UTI. The ratio of uropathogens in females to males in this study was higher, 2.3:1. This may be due to the nature of the anatomical structure of the female genitalia characterized by a short urethral length of 1.5 inches compared to 8 inches in males, making it easier for the microorganisms to reach the bladder and beyond. The genital orifice of females of child bearing age also provides an excellent environment for bacteria to flourish, due to the nourishment they derive from regular menstruation, as blood is a major source of iron for microbial biosynthesis of nucleic acids(22).

This ratio is also in line with observations from

studies that have shown that more women than men have UTIs(23,24). Antibiogram showed low efficacy of antibiotics against isolates, with greater number, 99(69%) of isolates in the test subjects and 54(62%) of isolates in the control subjects exhibiting resistance to the antibiotics tested. High level antibiotic resistance, as exhibited by uropathogens in this study, has also been observed by other workers(21,25, 26).

Although there exists no documented record of a similar study previously carried out within the study area, however, comparing the resistance profiles of the isolates in this study and those of previous studies in Adamawa, Cross River and Plateau States of Nigeria(27,28,29), it has been observed that Nigeria is not left out of the global increase in antibiotic resistance. The cause of this increase could be traced to over use and abuse of antibiotics including indiscriminate prescription of antibiotics by medical personnel and the hawking of antibiotics by vendors.

Most people, particularly those with no formal education and low socioeconomic status, patronize them without proper prescription and this trend has contributed to multiple antibiotic resistance. In this study, it was also observed that a good number of the study subjects were not placed on the relevant antiretroviral drugs. These were mainly among those with no formal education (55%); they believed that the drugs were a means of inducing infertility. This mindset is fast becoming a major public health challenge in the control of diseases, for example, most Northern Nigerians refused polio vaccines for their children due to the same reason(30).

Conclusion

This study has demonstrated that *E. coli* is the most prevalent organism associated with UTI in PLWHA in Jalingo. It also showed that more uropathogens were isolated from females than males. An alarming rate of antibiotic resistance was observed in the study area with the isolates showing multiple antibiotics resistance (resistance to two or more antibiotics), thus posing a serious threat to public health, especially in PLWHA. There is need for relevant government agencies and the Ministry of Health, Taraba State to enforce policies limiting the hawking of antibiotics and other practices that encourage emergence and spread of drug resistance. The need for more enlightenment campaigns for those who still do not know the importance of antiretroviral drugs cannot be over-emphasized.

Acknowledgement

The authors are grateful to the management and staff of the Government House Clinic, Jalingo, Taraba State, particularly Dr. Tukura, Mr. Ukwe, Mallam Isa and Aunty Patience for their assistance and technical support. The subjects involved in this study are also appreciated for accepting to be part of the study. We also disclose that there is no conflict of interest as the study did not receive funding from any organization.

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Radiation and Microbial Safety of Lead Aprons in Three Nigerian Cities.

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Abstract
This was a prospective study carried out to determine the protective integrity of lead aprons with respect to the structure of their internal lead and to know if they can be a reservoir to micro-organisms. Forty-four lead aprons used in Tertiary and Private Radiology units in three cities in South-South Nigeria were analysed based on their lead thickness, duration in use, storage conditions, cleanliness and hygiene practice. Swab samples were taken from some of the inspected lead aprons (those in Calabar specifically) for bacterial analysis in the laboratory. From evaluations made from the internal lead, 31 (70.45%) lead aprons were not defective while 13 (29.6%) were found to be defective. Cracks accounted for most of the defects (61.54%), the next being holes and ripped lead (15.38%) with a combination of cracks and holes being the least (7.69%). Thirty (65%) of the lead aprons have never been be inspected, 10 (26%) were inspected over a year ago and 4 (9%) were annually inspected. Twenty two (50%) were stored using the standard method of hangers and racks, while the other 22 (50%) were stored using other methods. Twenty one (48%) were clean, 14 (32%) were slightly clean and 9 (20%) were quite dirty. The laboratory results revealed the presence of micro-organisms, with Non-coagulase negative staphylococci being the most frequently isolated (64%), followed by *Staphylococcus aureus* (27%) and *Corynebacterium* spp. (9%). These results indicated a lack of compliance with routine inspection, use of inappropriate storage conditions, poor hygiene practice and that lead aprons pose an infection risk in the department.

Key Words: Lead apron, Bacteria, Radiation, Inspection, Handling

Introduction:

Ionizing radiation is any type of particle or electromagnetic wave that carries enough energy to ionize or remove electrons from an atom in the tissue it penetrates (1). The most important consequence of this displaced electron on human tissue is the potential damage it can inflict on DNA, which may occur directly or indirectly (2). The deleterious effect ionizing radiation has on human tissue are of two types, Deterministic (Non-stochastic) Effects and Stochastic Effects (2). The aim of protection against ionizing radiation is to prevent, or significantly reduce, the radiation damage in exposed individuals (3).

Shielding, as one of the three methods of ensuring basic radiation safety, is always used when the use of time and distance principles are not possible (4). Lead aprons are provided as valuable aids to keep radiation dose received by personnel under working conditions as low as reasonably achievable (ALARA). However, intensive use and poor handling can lead to age-related defects, often giving rise to multiple tears and defects. These expose its wearer to the risk of increased radiation absorption and the attendant harmful effects (5,6,7). Damaged lead aprons have been discovered to have lead dust particles on their surfaces. This can lead to lead poisoning (8).For this reason, annual inspection of lead aprons is important.

Owing to contact with patients, lead particles in the air, in the addition to the potential of x-ray equipment to harbor microorganisms from contacts with patients is believed to increase the risk of nosocomial infection (9). A study done in a Zimbabwean Hospital ranked lead aprons second in contamination after cassettes (10). These findings influenced the reason for conducting a bacteriological test on some of the lead aprons. This study is aimed at evaluating the safety of lead aprons from three cities in south- south Nigeria.

Materials and Methods

A total of 44 lead aprons were employed for this study. They were procured from tertiary and private Radiology units in Port Harcourt, Uyo and Calabar all in south- south Nigeria. The choice of these cities was due to the high congestion of Radiology units within them. All the aprons were identified and registered according to type, lead equivalent, duration of use storage condition, cleanliness/ cleaning method, physical appearance and defect type. Visual inspection, tactile inspection and

Radiologic inspection methods were used to evaluate the lead aprons (11).

The visual inspection was performed by laying the apron on a clean flat surface to check the surface covering for signs of deterioration such as tears, perforations or imperfections (e.g bumps). The apron closures (Velcro, buckles, etc) were checked to ensure they were in proper working condition.

The tactile inspection method involved slowly running both hands over the lead apron to feel for any creases or irregularities within the apron fabric.

The radiographic inspection method was carried out by taking radiographs of suspected areas of the aprons using X-ray units and films. The X-ray machines used for exposures in this study were Philips (Mobile Unit) with inherent filtration of 2.0mmAl, Philips (Floor Mounted) with filtration of 1.4mmAl, GE machine (Floor mounted) with inherent filtration of 2.5mmAl, GE Machine (Mobile unit) with an inherent filtration of 2.0mmAl and Minray machine with an inherent filtration of 2.0mmAl. The exposure factors of 70kV, 20mAs and 110cm focus-film distance (FFD) according to Nkubli et al (12) were used for this study. The choice of these exposure factors is in line with common exposure factors for routine x-ray examination. The images were processed and inspected for defects. The radiographs were evaluated in terms of holes, cracks and other defects, and measurement of the sizes of the defects done using rulers (13). Aprons were either accepted or rejected following standard

Rejection criterion	Maximum length of defect (cm)		
	0.25mm	0.35mm	0.50mm
Whole body	5.9	5.6	5.4
Reproductive region	1.9	1.8	1.7

Table 1: Maximum tolerable tear length (cm).

Lead aprons inspected in Calabar were subjected to bacterial analysis. This is due to ease of accessibility and availability to the researchers. Swab samples were taken from 10 lead aprons using sterile swab sticks and peptone water for a swab test. These samples were analyzed in the laboratory to identify the micro-organisms present. One of the lead aprons was swabbed, cleaned with disinfectant and swabbed again. This was to help evaluate the effect of disinfectants and appropriate cleaning on the growth of micro-organisms. Sample swabs were inoculated on blood agar, chocolate agar and McConkey agar media. Cultures were incubated for 24 hours, at 37^0C, after which the first plate readings were taken. The second plate readings were taken after 48 hours. Gram's stained smears were used to identify the organisms as either Gram-positive or Gram-negative, followed by further bacterial identification test (14). Bacterial counts were conducted for swabs used on disinfected and pre-disinfection aprons. The routine cleaning agents employed in this study were Dettol, Methylated Spirit, Soap and Water.

Results

The findings from this study as frequencies and percentages were generated and the results presented using tables and figures

Fig 1: Radiograph of Lead Apron without defects.

Figures (2-4) as shown from this study were different lead aprons with different anomalies discovered during their inspection.

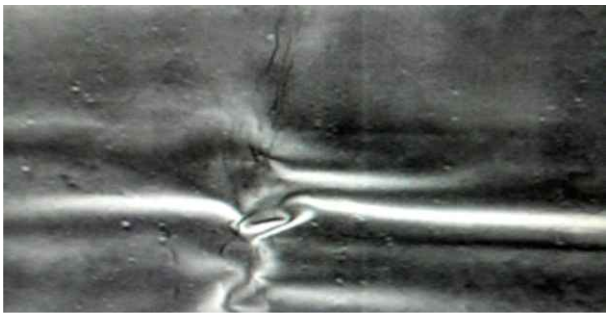

Fig 2: Lead Apron with perforations.

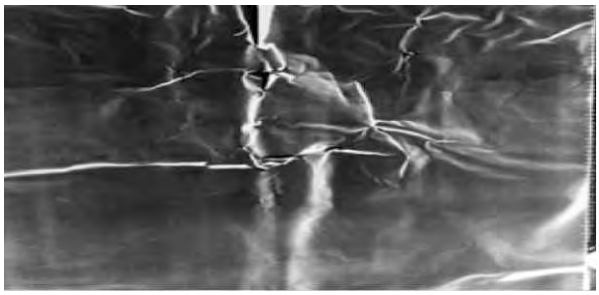

Fig 3: Lead Apron with cracks.

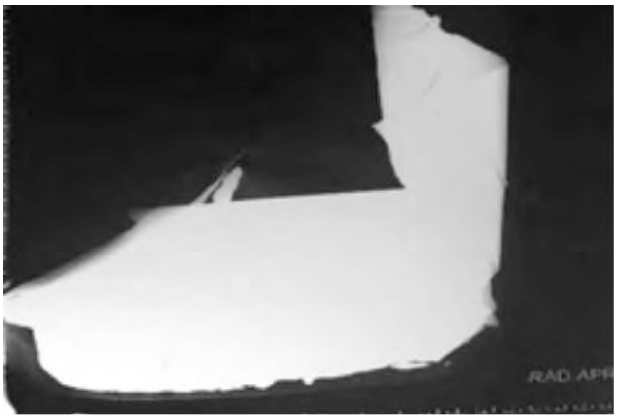

Fig 4: Lead Apron with torn lead sheets.

Table 2 shows the history of the lead aprons used in this study.

VARIABLES	No. (%)
AREA OF STUDY	
Port Harcourt	18 (41.0)
Uyo	14 (31.8)
Calabar	12 (27.3)
LEAD EQUIVALENT (mm)	
0.25	7 (15.9)
0.35	30 (68.2)
0.5	7 (15.9)
DURATION	
<1 Year	7(15.9)
1-2 Years	23 (52.3)
>2 Years	14 (31.8)

Table 3. General information on the inspected lead aprons; n=44.

ÍGËG ÈÎÎÎÍ STATUS	ÍÎB(%) OF LEAD APRON			TOTAL(%)	ÍÎÍÈÍ
	0.25mm	0.35mm	0.5mm		
GÑÑÑÒÑ	1 (2.3)	11 (25)	1 (2.3)	13 (29.6)	
Í ÖPÑÑÑÑP	6 (13.6)	19 (43.2)	6 (13.6)	31 (70.5)	
ÍÎÍÈÍ	**7 (15.9)**	**30 (68.2)**	**7 (15.9)**	**44 (100)**	

A total of 13 (29.6%) of the aprons were defective, while 31 (70.5%) were not defective.

Table 4. Defects in physical integrity of lead aprons

DEFECT TYPE	ACCEPTED(%)	REJECTED(%)	TOTAL(%)
Crack	3 (23.1)	5 (38.5)	8 (61.5)
Holes	1 (7.7)	1 (7.7)	2 (15.4)
Cracks/holes	0 (0)	1 (7.7)	1 (7.7)
Ripped/Broken lead	0 (0)	2 (15.4)	2 (15.4)
TOTAL	**4 (30.8)**	**9 (69.2)**	**13 (100)**

Table 4 shows the various defect types observed on the aprons in respect of acceptance or rejection criteria. Four (30%) of the aprons were accepted while 9 (69%) were rejected. Three (23.1%) of the accepted aprons had cracks, and 1 (7.7%) had holes. Cracks were found in 5 (38.5%). Two (15.4%) other rejected ones were ripped/broken.

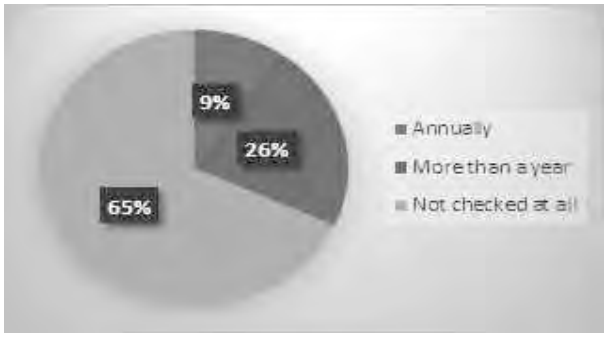

Fig 5: Schedule of periodic checking of Aprons

Figure 5 shows interval of periodic checks carried out on the lead aprons. Four (9%) of the aprons were inspected annually, 10 (26%) were inspected after more than a year, while 30 (65%) have never been inspected.

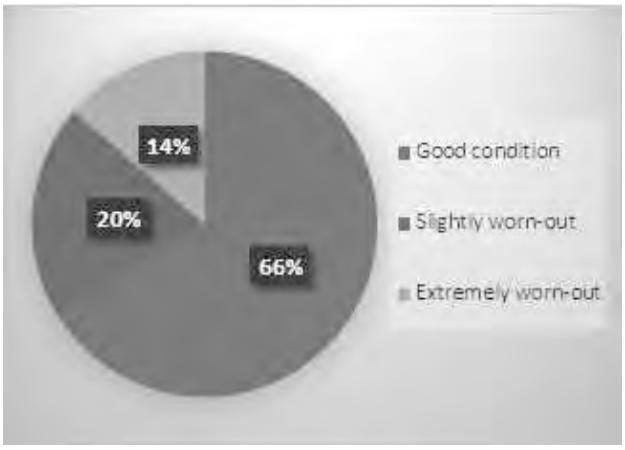

Fig 6: Overall Condition of the Lead Aprons

The above chart shows the overall condition of the lead aprons evaluated. 29 (66%) were in good condition, 9 (20%) were slightly worn-out while 6 (14%) were extremely worn-out.

Table 5: Storage practices of the Lead Aprons.

STORAGE CONDITION	No. (%)
Hangers/Racks	22 (50)
Hung on nails	8 (18.2)
Hung over boards/chairs	10 (22.7)
Folded	2 (4.6)
Spread on table	2 (4.6)
TOTAL	44 (100)

Table 5 describes the storage conditions of the lead aprons. 22 (50%)) were hung on hangers and racks, 8 (18.2%) were hung on nails, 10 (22.7%) were hung over boards and chairs, 2 (4.6%) were folded and 2 (4.6%) were spread out on a table.

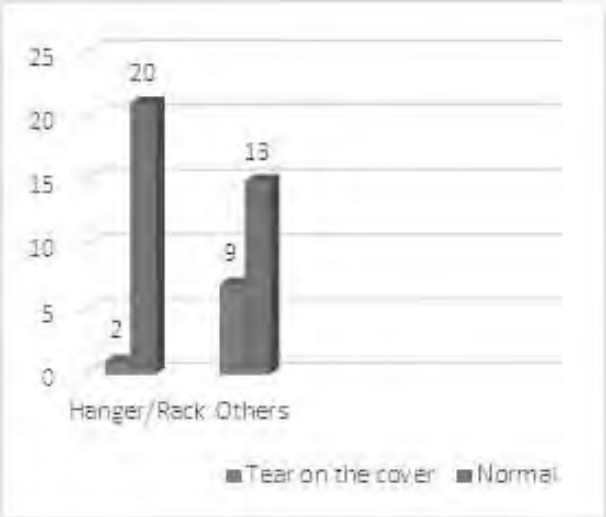

Fig 7: Chart Showing Storage Methods and the physical condition of the Lead Aprons.

The chart above shows that 2 (9.1%) of the lead aprons hung on hangers and racks had a physical tear, whereas 9 (40.9%) stored by other methods had physical defects.

Table 6: Cleaning Frequency of the Lead Aprons

CLEANING SCHEDULE	No. (%)
Weekly	13 (29.6)
Monthly	5 (11.4)
Once in 2 or 3 months	4 (9.1)
Once it is used	8 (18.2)
No routine	14 (31.8)
TOTAL	44 (100)

Table 6 provides information on the cleaning frequency of lead aprons inspected. Thirteen (29.6%) were cleaned weekly, 5 (11.4%) were cleaned monthly, 4 (9.1%) were cleaned once in 2 or 3 months, 8 (18.2%) were cleaned once it is used and 14 (31.8%) had no specific cleaning routine.

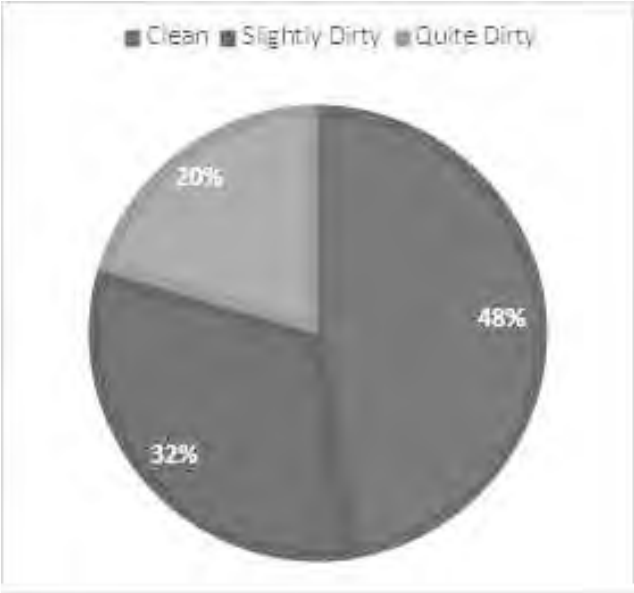

Fig 8: Pie chart showing hygiene condition of the Lead Aprons.

The above figure shows the conditions of the lead aprons in terms of cleanliness. 21 (48%) of the lead aprons were clean, 14 (32%) were slightly dirty and 9 (20%) were quite dirty

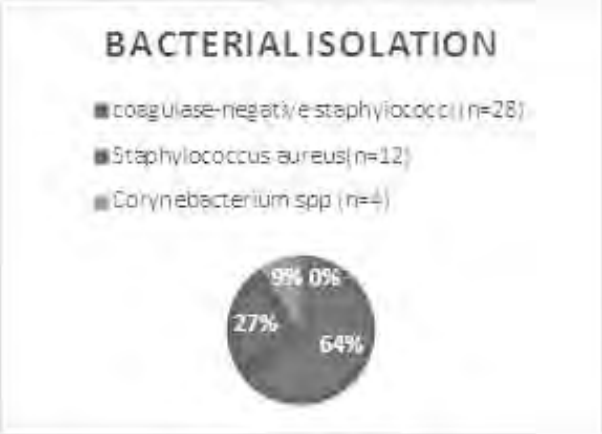

Fig 9: Bacterial isolates from lead aprons.

The most frequently isolated bacterial organism was coagulase-negative staphylococci with 28 (64%) isolates, next was *Staphylococcus aureus with 12* (27%) and the least was *Corynebacterium* spp. *with 4* (9%).

Discussion

This present study assessed the radiation and microbial safety of lead aprons as they constitute part of the protective garments worn to protect the body from the effect of ionizing radiation (15). To ensure these garments serve their purpose, some routine checks are required to be carried out on them (16).

The findings from this study are in tandem with previous reports (12, 13) though with some variations. The results from Table 3 show that 31 (70.5%) of the aprons had non-defective internal lead, with 13 (29.6%) showing defects. As seen in Table 4, 4 (30.8%) were accepted, while 9 (69.2%) fell into the criteria for rejection. This result is slightly different from an earlier observation (12), where 16 aprons were defective, with 9 being usable and 7 rejected. This could be because of the greater number of aprons (n=47) used as study size. When compared with the results from an earlier study (13), the percentage of defective lead aprons was higher (68.2%). This difference could be due to the advanced methods they employed in their evaluations.

According to Nkubli *et al* (12), cracks accounted for most of the defects (56.3%), with not much difference from hole defects (43.8%). Same was found in an earlier study (13), but the crack defects were approximately double the defects resulting from holes. The result from this study, cracks accounted for 61.5%, while holes and other defects accounted for 38.5%. This may be as a result of differences in handling and storage practices.

From this present study, poor compliance to regular inspection was a major factor as most of the lead aprons 65% have never been inspected, 26% were last inspected over a year ago and only 9% have annual inspection carried out on them. Similar attitude of lack of commitment to inspection was indicated in the study (12).

The overall conditions of the aprons are showed in those aprons in good condition made up 66%. Slightly worn-out aprons with ripped fabric, unstitched hem and old belt buckle accounted for (20%). Extremely worn-out aprons featured mostly with torn fabric, defects in their shape due to sags in the protective lead layer, wide tears and deformations around the armpit and changes in colour of the fabric (14%). From previous study (11), 52.9% of the aprons were in good condition, 30.6% were slightly worn-out and 16.5% were extremely worn-out.

It was observed that half of the aprons inspected were not stored according to the required standard of using hangers and racks. About 22 (50%) of the aprons were stored using racks/hangers, the other 22 were stored using other methods. The physical appearance of most of the aprons was influenced by their storage method, as aprons not hung on hangers/racks recorded the highest number of physical defects 40.9% such as ripped fabric and torn hem, as compared to 9.1% for hangers/racks. Similar findings have been reported (12). Inappropriate storage methods do not have effect on the physical conditions alone but also on the protective lead layer as it can cause defects to the internal lead.

The cleaning routine/ and the cleaning agent(s) employed influenced how clean the lead aprons were. where hygiene practice was fair, 21 (48%) of the aprons inspected were clean. The samples from the lead aprons that were cleaned with disinfectants showed no bacterial growth after 24 hours and then very little growth 48 hours later. This is evidence that regular cleaning of lead aprons with disinfectant can destroy micro-organisms thereby significantly reducing the risk of cross-contamination and infection.

The swab test revealed the presence of micro-organisms on the aprons. Coagulase-negative staphylococci were the most frequently isolated micro-organisms followed by *Staphylococcus aureus*. These organisms have been reported in various studies by other workers (10, 18). Although Coagulase Negative *Stapylococci* are commensal flora of the human skin and mucous membranes and rarely cause primary disease (20), they have been

identified as agents of clinically significant nosocomial bloodstream infections (19).. *Staphylococcus aureus* is a pyrogenic pathogen which causes boils, post-operative wound infection, septicaemia, osteomyelitis and pneumonia (20). The resistance of staphylococci to many antibiotics has been reported rendering them difficult to manage clinically (21). Most *corynebacterium* Spp are mostly known as commensale ditheroids and normal flora of the skin and upper respiratory tracts where they can be recovered readily from swab cultures (21).

Conclusion

From this study, it is obvious that regular inspection and care of the lead aprons used in Radio diagnostic centres are not maintained. Evaluations made from this study based on routine inspection, storage and hygiene practices leaves much to be desired on this all important personnel radiation protective wear.

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Molecular Detection of Vi Capsular Gene in *Salmonella enterica* Serovar *Typhi* Clinical Isolates Associated with Septicaemia among Children Aged 0-5 Years in Kano, Nigeria

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Abstract

Two strains of *Salmonella enterica Serovar Typhi*, the causative organism of human typhoid fever are known to be in circulation worldwide, *S. Typhi* that possess and express (Vi positive *S. Typhi*) and those that does not possess and express (Vi negative *S. Typhi*) the virulent capsular polysaccharides antigen known as Vi. Knowledge and accurate identification of circulating strain of *S. Typhi* is important in treatment and control of its infection. This study aimed at evaluating the molecular detection of *ViaB* gene in clinical isolates of *Salmonella Typhi* associated with septicemia among children aged 0-5 years. Ninety clinical isolates of *S. Typhi* obtained from the blood culture of children aged 0-5 years diagnosed with sepsis were randomly selected from 4 hospitals in Kano metropolis. The *S. Typhi* strains were screened for the presence of the *Via B* gene by polymerase chain reaction (PCR). 90 clinical isolates screened for the presence of the Vi antigen and was detected in 64 giving an overall prevalence of 71.1 %. The highest prevalence was found among subjects aged 19-48 months with 36 (40%) and the lowest in the subjects aged less than 1 month, 2(3%). This work confirms the circulation of Vi capsulated strain of *S. Typhi*, therefore it is advisable in line with WHO recommendation that Vi purified typhoid vaccine be included in the national immunization schedule to children aged 2 years and above in typhoid fever endemic areas.

Key Words: *Salmonella Typhi*, septocaemia, capsular antigen, polymerase chain reaction.

Introduction

Salmonella Typhi is a Serovar of *S. enterica*. It is serologically made up of somatic antigen ("O antigen - a lipopolysaccharide) type 09 and 012; a flagellar antigen ("H - antigen) type-d; and a Virulent capsular polysaccharide (extracellular) antigen termed Vi antigen. The Vi antigen may or may not be present on *S. Typhi*. Humans are the only known natural host of *S. Typhi*, with the organism showing limited pathogenicity for most animals (1, 2, 3) this exquisite level of host restriction is reflected in the genome and population structure of the organism (2, 4). Two strains of *S. Typhi* the causative agent of typhoid fever are known to be in circulation worldwide; *S. Typhi* that possess and express the virulent capsular polysaccharide antigen known as Vi (Vi positive *S. Typhi*) and those that do not (Vi negative *S. Typhi*) (5, 6, 7).

The Vi capsular antigen is a linear polymer of: 1, 4(2-deoxy)-2-N-acetylgalacturonic acid variably O-acetylated at the C3 position (4, 9) that forms a coat on the external surface of the bacterial cell. This capsular polysaccharide is expressed in vitro in macrophages and during human infection. The Vi capsular antigen is a significant virulence factor for typhoid fever, as strains positive for Vi production have higher rate of infection and complication (10, 11). The production of Vi antigen is encoded by the *Via B gene* located in the DNA region, termed Salmonella pathogenicity island 7 (SPI7), (5, 9). In addition Vi antigen can elicit protection against typhoid fever following vaccination with the antigen (5, 8, 11). The Vi capsular antigen positive strain of *S Typhi* were capable of masking Pathogen-Associated Molecular Patterns (PAMPs), this enables the orgnism to avoid neutrophil-based inflammation (6, 11, 12), while the most common Paratyphi Serovar, Paratyphi *A*, does not. This partly explains the comparatively greater pathogenicity of *S. Typhi* (13). Additionally, it co-opt the macrophages' cellular machinery for their own reproduction after phagocytosis as they are carried

through the mesenteric lymph nodes to the lymphatics and finally the reticuloendothelial tissues (14). Once there, they pause and continue to multiply until some critical density is reached, afterward the bacteria induce macrophage apoptosis, breaking out into the bloodstream to invade the rest of the body (13).

A region within SPI7 contains genes for the expression of the Vi Capsular antigen. It is controlled by two chromosomal loci, the *viaA* and viaB. The *viaB* locus, contains genes coding for the biosynthesis and the export of the Vi capsular antigen (8, 15). The World Health Organization (WHO) recommended the use of Typhoid vaccines wherever there is an outbreak of the disease; where the disease is endemic and to persons travelling from non-endemic to endemic areas (7, 16), The two WHO approved Typhoid vaccines are: Ty21a and Purified Vi capsular polysaccharide (17). Thus, this work was undertaken to detect the presence of the *ViaB* gene which encodes the gene for production and expression of the Vi capsular antigen on the clinical isolates of *S. Typhi* associated with septicaemia by polymerase chain reaction (PCR), and also to determine the prevalence of the Vi positive strain among the research group.

Materials and Methods

Study Area

Blood culture of children aged 0-5 years diagnosed with sepsis were collected from four hospitals in Kano metropolis, Murtala Muhammad Specialist Hospital (MMSH), Hasiya Bayero Pediatric Hospital (HBPH), Aminu Kano Teaching Hospital (AKTH) and Muhammad Abdullahi Wase Specialist Hospital (MAWSH) Kano. An ethical clearance was also obtained from research and ethical committees of the selected hospitals while accent forms were completed by each consenting parent/guardian on behalf of the child.

Blood Culture

The etiology of septicemia was determined using an automated blood-culture system Bactec™ culture system (Bactec ™ 9050 and 9120, Becton Dickinson, Temse, Belgium). 1-3 ml of blood was aseptically collected. All positive vials were subcultured onto a set of MacConkey agar, sheep blood and chocolate agar plates. Direct gram stain of each positive vial was carried out at the time of subculture (18).

Identification

All Non lactose fermenting bacteria were identified by Analytical Profile Index (API) 20E test panel designed for the identification of Enterobacteriacea, (Biomerioux, France). Serological identification of *Salmonella specie* was also carried out by Slide agglutination with polyvalent and monovalent group-specific Salmonella antiserum (BD LLB, Belgium).

Primer Design

Various nucleotide sequences were obtined from NCBI for *S. Typhi Via B* gene. The primers were designed and standardized using Bioedit software (7,2,6,1) and Oligo-7 software. The forward and reverse primer were designed and sent to Inqaba biolabs for synthesis.

The following forward:
F:5'-AGAATTTGCGCTGAC and reverse primers R:5'-ATTCACGTATTTCTCGCTTTAATAATG-3': respectively were used, the size is 216bp.

Sample Preparation

DNA extraction was carried out using Inqaba ZR Bacterial /fungal DNA MiniPrep™ Catalog No. D6005 extraction kit. All procedures were conducted according to the manufacturer's protocol (ZR Biotec, Ibadan, Nigeria).

Preparation of Master Mix

50µL reactions was prepared containing the following: 5 µl of DNA template; 3µl of reaction buffer;2µl of dNTPs; 2µl of each forward and reverse primer (10 pmol),0.25µl of Enzyme mix; 3µl of MgCl to which 27.75µL of sterile RNase water was added to make it up to 50µl reaction.

Conventional PCR

The amplification was performed using Eppendorf Thermo Cycler with the following condition. The machine was allowed to heat up to 95°C for 30 seconds for the initial denaturation and then preceded as follows: denaturation: at 95°C for 15seconds; annealing: at 57°C for 60 seconds, and elongation: at 72°C for 60 seconds. A total of 35 cycles were carried out.

Agarose Gel Electrophoresis

The PCR product was then electrophoresed in a 2%

agarose gel. Tris-borate-ethylene diamine tetra acetic acid (TBE) buffer was prepared and used for the electrophoresis. Ethidium bromide was added at a concentration of (0.5 mg/ml) to TBE. The DNA amplicons along with DNA ladder of 100-1000bps were loaded in the wells after mixing with gel loading dye. Current was passed at 65 volts and 3amp for 35mins, and the gels were visualised under ultra violet transilluminator. ATCC *E. coli* 25922 and *Salmonella* group B were used as negative control (Figure 1).

Data Analysis
Data were presented using tables and analyzed using frequency and percentage.

Results
A total of 90 clinical isolates identified as S. *Typhi* were subjected to conventional PCR to determine the presence of Vi Capsular gene (*ViaB* gene).
Table 1 shows the frequency of *ViaB* gene among the screened clinical isolates of *S.Typhi.* Among the 90 clinical isolates of *S. Typhi* screened for the presence *Via B* gene, it was detected on 64 isolates, equivalent to an overall prevalence of 71.1%.
Table 2 shows the distribution of PCR result among clinical isolate of *S. Typhi* based on the 4 selected hospitals from which the samples were collected. A total of 56(62.2%) of the clinical isolates were obtained from HBPH; 25(27.8%) from MMSH; 9 (10.0%) from AKTH while 0(0.0%) from MWSH.

Table 3 shows the distribution of age group of research subjects and the presence *ViaB* gene among the *S. typhi* screened. The *ViaB* gene were found highest among subjects aged 18-49 months with a prevalence of 39(60.9%) and the least prevalence was detected among subjects aged < 1 Month 2(3.1%).

Table 1: Polymerase Chain Reaction (PCR) Result of S. Typhi Screened for ViaB gene.

TEST	ViaB gene status	Frequency	Percentage (%)
	ViaB Positive	64	71.1
PCR	*ViaB* Negative	26	28.9
	Total	90	100

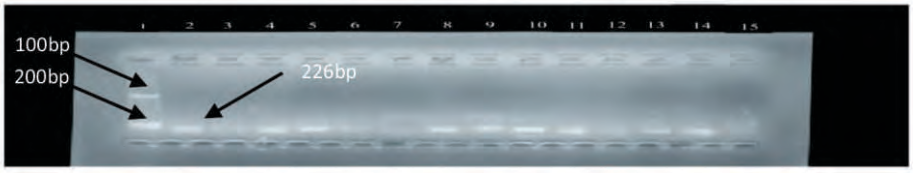

FIG. Agarose gel Electrophoresis for the detection of ViaB gene.
Key: 1= Ladder (100 - 1000bp); 2= Positive control; 3 and 15 = Negative Control; 4,5, 8,9,11,13 and 14= Positive; 6,7 and 12= Negative.

Table 2: Distribution of ViaB gene in Clinical Isolates of *S. Typhi* strains by hospital sources

SITE	*ViaB* Positive n (%)	*ViaB* Negative n (%)	Total n (%)
AKTH	8 (8.90)	1 (1.11)	9 (10.0)
MAWSH	0 (0.0)	0 (0.0)	0 (0.0)
MMSH	20 (22.22)	5 (5.60)	25 (27.8)
HBPH	36 (39.96)	20 (22.22)	56 (62.2)
Total	64 (71.1)	26 (28.9)	90 (100)

Key: AKTH - Aminu Kano Teaching Hospital; MAWSH - Muhammad Abdullahi Wase Specialist Hospital; MMSH - Murtala Muhammad Specialist hospital; HBPH - Hasiya Bayero Pediatric Hospital.

Table 3: Relationship Between Age Group of Patients and Presence of *ViaB* Gene Among *S. Typhi* Screened.

Age Group	Total n (%)	*ViaB* Positive n (%)
< 1 Month	2 (2.2)	2 (3.1)
1- 18 Month	17 (18.9)	14 (21.9)
19-48 Month	57 (63.3)	39 (60.9)
49-60 Month	14 (15.6)	9 (14.1)
Total (%)	90 (100.0)	64 (100.0)

Discussion

Molecular detection of a *Via B* - a region responsible for the synthesis of the Vi capsular antigen among phenotypically identified *S. Typhi* confirms the identification of the capsulated strain *Salmonella enterica Serovar* Typhi (8). Similarly Wain *et al.*(19) detect the presence of Vi capsular antigen in Pakistan from clinical isolates of *S. Typhi*, whereby both strains positive and negative for *ViaB* region were detected but contrary to this research less than 1% of isolates were confirmed Vi negative. In another study by Baker *et al.*(20) approximately 10% of the typhoid fever cases were observed due to Vi negative strains of serotype *Typhi.*

S. *Typhi* was found the commonest cause of childhood bacteraemia in North Western Nigeria including Kano, whereby it can account for not less than 24-60% of septiceamia among children aged 5 years and less (21). This study confirms that Vi positive *S. Typhi* is the most prevalent cause of septicaemia among children age 0-5 years in Kano, this in agreement with the findings that Vi positive *S. Typhi* were mostly associated with typhoid fever epidemics as reported in 2010 in India and Malaysia (7,18); and also associated with endemicity as reported in Pakistan (20). The prevalence of 71.1% in this research is significantly lower than is discovered in Asia where by less than 10% of Vi negative *S. Typhi*

were reportedly found to be in circulation although Asia was termed Typhoid endemic. Other relevant studies have attributed long term storage of isolates to the loss of *viaB* operon (10, 22). In addition, Vi positive strains were also associated with travelling which is a risk factor less likely to be found in infants and young children of the age group in this research. Hatta and Smits, (23) also employed the molecular detection of the Vi capsular antigen using PCR to identify Vi positive S. *Typhi* by a nested PCR, while Lim *et al.*(24) employed a Multiplex PCR.

Conclusion

Children aged 5 years and below were predominantly infected with the Vi positive serotype of *S. Typhi.* As a result children are largely at risk of developing complications from enteric fever. More studies are required to track prevalence and emergence of virulence factors in Salmonella isolates in Nigeria, while routine bacteriological culture and sensitivity test should include speciation of the aetiology in order to reduce morbidity and mortality.

Typhoid fever is completely preventable by good personal hygiene (which is cheaper and achievable). Provision of adequate health care, wholesome water for drinking and domestic use by the government is advised. The most infected age group was found to be 19- 48month. Regardless of the age group typhoid vaccines are not completely protective, but its introduction and availability in the country will help control the overall incidence of typhoid fever while its inclusion into the NPI will help minimize the mortality and morbidity of the infection in children less than years old

Acknowledgment

The authors are very grateful to Professor Stephen K. Obaro and the CAPBID team for providing clinical isolates used in this work.

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Effects of Physical Activity on Digit Ratio (2D:4D), Metabolic Syndrome Indices and Biomarkers among Hausa Ethnic Group in Kano, Nigeria

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Abstract

Globally there is growing body of literature on the subject of digit ratio as predictor and correlate of many important body traits. The aim of this study is to evaluate the effect of physical activity (PA) on the second to fourth digit ratio (2D:4D) and metabolic syndrome (MetS) indices. The design was a cross sectional study that included 465 (266 males and 199 females) Hausas of Kano, with a mean age of 34.4 years and 32.0 years for males and females respectively. Systematic random sampling technique was employed for subject recruitment. Height, weight, waist circumference (WC), body mass index (BMI) and digit lengths were obtained using standard protocol. Overnight fasting blood sample was obtained for fasting blood glucose (FBG), high-density lipo-protein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG), low-density lipo-protein cholesterol (LDL-C), Uric acid and adiponectin estimation using standard laboratory protocols. Blood pressure was measured following standard clinical procedure. Levels of physical activity were assessed using self-reported activity level in the last twelve months. To compare between-group parameters of males and females, left and right hand, groups of PA, Student's t test and one way ANOVA was used. SPSS version 20 software was used for statistical analyses and $P < 0.05$ was set as level of significance. Digit ratio (2D:4D), DBP, SBP, Serum TC, TG, LDL, FBG and uric acid all decreased significantly along PA levels. The concentration of Serum HDL-C and adiponectin increased along PA levels. Most of the measured indices did not however show significant change between moderate and optimal PA levels.

Key Words: Physical activity, 2D:4D, Metabolic syndrome indices, biomarkers, Hausas, Kano

Introduction

Epidemiological studies suggest that a significant part of the cardiovascular disease (CVD) epidemic is attributable to changes in lifestyle exemplified by reduction in physical activity (1). Previous studies have shown the inverse relationship between physical activity and adverse metabolic parameters, thus indicating its protective effect against MetS (2, 3, 4). Physical activity can have a profound effect on reducing body and visceral adiposity and therefore reduces metabolic syndrome risk (5, 6). Increased physical activity, especially that which is associated with reduced fat mass, corrects the dysfunction in adipokine and cytokine expression so that expression of adiponectin is increased in adipose tissue and production of inflammatory cytokines is reduced (7, 8). It is reported that the beneficial effect of exercise is partly mediated through changes in the adipokines profile, that is, by increasing anti-inflammatory cytokines and decreasing proinflammatory ones (9, 10).This effect has been described at the levels of gene expression, protein ligands, and receptor bindings (11). For instance, exercise increases insulin sensitivity through reduction of resting levels of TNF and augmentation of adiponectin levels (12).

However, quantification of the impact of the different levels of physical activity on each component of MetS has not been widely studied. Franks *et al*. (2) however indicated that the impact of physical activity on MetS may have a threshold. The study of Felix *et al.* (13) conducted on sub-Saharan African populace which used physical activity energy expenditure to stratify subject according to their level of activity into four categories and to determine the impact on metabolic health also showed an inverse trend. Since the cardio-metabolic benefits of physical activity depends on volume and dose of activity (14), and the different component of MetS do not carry the same magnitude of risk (15), it is important to quantify the

amount of physical activity that is needed to significantly impact on each metabolic parameter and also to investigate sex difference in the response of each parameter to PA.

2D:4D is a sexually dimorphic anatomical variable that is determined mainly by the concentration of intrauterine androgen exposure (16). Numerous evidences suggest its correlation with behavioral (17, 18) and physical body traits (19) and all body traits putatively linked to testosterone (20). The current study seeks to investigate the effect of PA on 2D:4D, indices and selected biomarkers (serum adiponectin and uric acid) of MetS. Such study which is scarce in the Nigerian literature and among the Hausa populace will help in identifying the amount of PA needed to combat each component of metabolic syndrome.

Materials and Methods

Systematic random sampling technique was employed in selecting 465 original Hausas of Kano based on a history of at least two parental generation being Hausas from Kano. Participants were recruited from outpatient units of Murtala Muhammad specialist Hospital, Khadija Memorial Hospital, SU clinic, General Hospital Dawakin-Tofa and the old campus of Bayero University, Kano. The study included only subjects in the age range of 18 years to 68 years. Subjects with pregnancy, abdominal or pelvic space occupying lesions, congenital and /or acquired spinal or digit deformity were however excluded. Also excluded were subjects who were on drugs that could affect components of the MetS. Ethical approval was obtained from Kano state hospitals management board and written informed consent obtained from the subjects.

Anthropometric and blood pressure measurement

Height was measured to the nearest 0.1cm as the vertical distance between the standing surface and the vertex of the head while the subject was standing erect in the frank forth plane and without shoes using a stadiometer. The weight was measured in kilograms using a digital weighing scale while the subject is in light clothes. BMI was calculated by dividing the weight in kilograms by the square of the height in meters and the result expressed in kg/m^2.

Finger Length Measurements:

Digit lengths was measured on the ventral surface of the hand from the basal crease of the digit to the tip of the finger using a digital sliding caliper (MicroMak, USA) measuring to 0.01mm and reported on questionnaire. This measurement has been reported to have high degree of repeatability (21).

Fig. 1: Measurement of digit length using digital caliper

A mercury sphygmomanometer was used for measuring blood pressure. Two measurements were taken, and at least 2 minutes was allowed between readings. While the diastolic reading was taken at the level when sounds disappear (Korotkoff phase V), the systolic was taken at the level when it appears (22). The brachial artery was the site of auscultation. Subjects were asked to refrain from smoking or ingesting caffeine for 30 minutes before measurement and the Measurement was taken after at least 5 minutes of rest (23).

Serum analyses

For the estimation of FBG, TG, HDL-C, LDL-C, TC, SUA and adiponectin, blood specimen was collected from 161 subjects after 10 to 12 hours of fasting via superficial veins of the upper limb. From each selected subject, 5ml of venous blood sample was collected using a sterile 21G needle fitted with syringe. Blood collection was done during the morning hours to avoid the effect of diurnal variation or circadian rhythm in the blood parameters to be measured. Standard technique of venipuncture and universal safety precaution was employed. Blood sample was transferred into a plain blood specimen bottle and allowed to stand until it was properly clotted. The blood samples were preserved in an ice pack insulating container to preserve the

temperature and then transported to the lab immediately after each exercise of sample collection. Sample was then centrifuged at 300rpm for 5 minutes after which serum was separated and immediately used for assaying the serum parameters. TC, TG and HDL-C concentrations were measured using enzymetic method by Wybenga, et al. (24); into a clean test tube 0.5ml serum + 0.5 ml HDL reagent was mixed and allowed to stand for 10 minutes. It was then centrifuged for 20 minutes at 2000rpm. The cholesterol reagent 1000µl was dispensed in to three cleaned test tubes labeled blank, standard and sample. 50µl of supernatant was dispensed in to tube sample, 50µl of standard was dispensed into standard tube and 50µl dispensed in to blank tube. All were mixed and incubated at 37^0C for 5 min and absorbance was read at 530 nm.
The results were calculated as

Conc. of test = Abs of Test_ x Conc. of standard /Abs of STD
Where the concentration of the total cholesterol standard is 5.17 mmol/L and that of triglycerides standard is 2.28mmol/L

LDL-cholesterol concentrations were calculated from measured values of TC, TG and HDL-C according to the Friedewald's Equation (25).

Friedewald Formula:
LDL-Cholesterol =TC - (HDL-C + Triglycerides/2.2) mmol/L.

Serum glucose was measured using enzymatic method of Trinder (26).
Into clean test tubes labeled, blank, standard and test, 1ml of glucose reagent was placed. Into the test tubes 10µl of distil water, standard solution and test serum was added to the test tubes respectively. These was then mixed and incubated at 37^0C for 10 minutes, after which the absorbance (Optical Density) of the test solution and standard was read at 505nm using the blank solution to zero the spectrophotometer.
The result was calculated as follows:

Conc of test = Abs of test x Conc of std/Abs of STD where the concentration of the glucose standard is 5.55 mmol/L

Serum uric acid concentrations were measured using method of Caraway (27);

Into a centrifuge tube, 4ml of water was dispensed followed by 0.5ml of serum, 0.25ml of sulphuric acid and 0.25ml of sodium tungstate. The solution was then mixed and allowed to stand for 5 minutes and then centrifuged. Three tubes were labeled as test, standard and blank and 1.5ml of sample was added to the tube marked- test and 1.5ml working standard into the tube marked- standard. Sodium carbonate, 0.5ml and 0.5ml phosphotungstic acid were added to all test tubes, mixed and allowed to stand for 15 minutes at room temperature and read at 710nm.

Uric acid concentration was then calculated as:
Conc of SUA = Abs of test x Conc of std/Abs of STD

Serum adiponectin concentrations were measured using Solid-Phase ELIZA method (28).
First, the sample was pretreated by adding 100µl of protease buffer and 400µl of sample pretreatment buffer to 10µl of sample and then stirred thoroughly. Dilution buffer, 1ml was added to 10µl of the pretreated sample and then stirred thoroughly under room temperature. Standard and diluted pretreated samples, 50µl each were added to the appropriate wells. The plates were covered with a plate sealer and incubated 1 hour at room temperature. The plates were decanted and stroked against an absorbent towel to remove excess liquid. Washing was done by adding 400µl of wash buffer to each well. The washed buffer was decanted and the plate was stroked against absorbent towel to remove residual liquid. This cycle was repeated for a total of three washes. Biotin labeled monoclonal antibody, 50µl was then added to each well. The plate was covered with a plate sealer and incubated for 1 hour at room temperature. At this stage, the washing process was repeated and 50µl of the enzyme streptavidin was added to each well and further incubated for 30 minutes. The third phase of the wash process was followed immediately. Then, 50µl of substrate solution was added to each well and incubated for 10 minutes. Finally, the absorbance was measured within 30 minutes using a microplate reader set at 492nm.

Assessment of levels of physical activity
The rapid assessment of physical activity (RAPA) questionnaire was administered to participants. Self-

reported level of physical activity in the last one year was used to group subjects into seven categories in ascending order of physical activity. Category I was scored as sedentary, II as under active, III as light physical activity, IV and V as regular but suboptimal activity, VI and VII as optimal level of physical activity. (29).

Student's t test and one way ANOVA were used to compare adiposity measures across physical activity levels and for multiple comparisons between subgroups. SPSS version 20 (IBM Corporation, NY) software was used for statistical analyses and $P < 0.05$ was set as level of significance.

Results

From Table 1 which shows the description of age, blood pressure, body and digit anthropometry of participants, a total of 465 subjects were studied, 266 males (57%) and 199 females (43%). The subjects had a mean age of 34.45 years and 32.06 years for males and females respectively. From Table 3 and 4 showing the overall effect of PA on BP and 2D:4D, there was significant overall effect of PA on digit ratio and BP in both sexes ($P < 0.01$). Also, multiple comparison of differences in the mean BP and digit ratio along the PA ladder shows that BP significantly decreased on moving from lower levels of physical activity through the higher levels but such difference was not observed for digit ratio. An exception to this trend was observed for SBP in females where no significant difference was observed between moderate and optimal levels of PA. The effect of PA levels on the biomarkers (Fig. 1 and 2) shows that while SUA levels decreased significantly and progressively along PA ladder, adiponectin levels increased from lower to higher PA levels. However, SUA and adiponectin did not show any significant change between inactive and mild PA, and between moderate and optimal PA levels, except in females where a significant rise in adiponectin was observed between moderate and optimal PA levels. It was also observed that the rate of change in the levels of SUA and adiponectin following PA is more drastic in females when compared to males.

Rapid assessment by physical activity questionnaire (29)

Questions	Response
I rarely or never do any physical activities	
I do some light or moderate physical activities, but not every week	
I do some light physical activities every week	
I do moderate physical activities every week but less than 30minutes a day or five days a week	
I vigorous physical activities every week but less than 20minutes a day or three days a week	
I do 30minutes or more a day of moderate physical activities five or more days a week	
I do 20minutes or more a day of vigorous physical activities three or more days a week	

Table 1: Description of age, blood pressure, body and digit anthropometry of participants

	Male (n=266)		Female (n= 199)			
Variables	Mean ± SD	Min-max	Mean ± SD	Min-max	t	P Value
Age	34.45± 13.52	18-68	32.06 ± 15.18	18-65	1.79	0.075
Height (cm)	169.15 ± 6.27	142-182.3	158.53 ± 6.83	136.9-175	17.39	<0.0001
Weight (Kg)	63.03 ±12.28	40.5-98.3	55.86 ± 12.99	36-108.9	6.08	<0.0001
BMI (kg/m^2)	21.98 ±3.93	14.52-34.33	22.19 ± 4.70	12.96-39.15	-0.52	0.602
DBP(mmHg)	82.59 ± 12.37	54-120	84.50 ± 12.99	60-120	-1.61	0.108
SBP(mmHg)	128.07 ± 20.09	90-200	130.66 ± 21.87	95-205	-1.33	0.185
RI (mm)	74.22 ± 5.45	61.17-90.46	67.97 ± 5.02	53.06-79.06	12.64	<0.0001
RII (mm)	72.56 ± 5.09	60.19-87.02	68.94 ± 4.48	55.42-82.09	7.98	<0.0001
RIII (mm)	80.12± 5.44	64.17-97.56	75.53 ± 4.98	63.13-94.26	9.34	<0.0001
RIV (mm)	75.63 ± 5.29	62.84-89.32	69.94 ± 4.51	55.41-85.35	12.21	<0.0001
RV (mm)	62.11 ± 5.31	47.17-85.87	57.60 ± 4.26	44.97-67.32	9.83	<0.0001
R2D:4D	0.96 ± 0.03	0.79-1.05	0.99 ± 0.03	0.86-1.07	-8.39	<0.0001
LI (mm)	74.05 ± 5.36	60.33-87.47	67.77 ± 4.49	55.1-78.83	13.36	<0.0001
LII (mm)	73.32 ± 4.85	60.04-85.81	69.08 ± 4.40	57.19-80.44	9.7	<0.0001
LIII (mm)	80.50 ± 5.61	66.12-96.55	76.23 ±5.56	50.09-98.92	8.15	<0.0001
LIV (mm)	76.03 ± 4.91	62.92-87.81	70.10 ± 4.71	57.45-82.26	13.11	<0.0001
LV (mm)	62.21 ± 5.09	47.46-74.36	57.69 ± 4.88	43.14-75.71	9.63	<0.0001
L2D:4D	0.96 ± 0.03	0.85-1.10	0.99 ± 0.03	0.92-1.09	-7.00	<0.0001

BMI: body mass index, I: first digit, II: second digit, III: third digit, IV: fourth digit, V: fifth digit, R: right hand, L: left hand, 2D:4D: second to fourth digit ratio.

Table 2: Description of Serum parameters and biomarkers of metabolic syndrome

	Male (n=120)			Female (n= 41)			t	
Variables	Mean	SD	Min-max	Mean	SD	Min-max		P value
Uric Acid	5.51	1.95	3.1-11.3	6.03	2.42	2.9-10.10	-1.38	0.17
Adiponectin	23.28	5.96	7.8-33.90	22.55	7.45	14.4-33.9	0.63	0.52
FBG	84.67	24.73	53.6-187.2	100.63	34.9	54.6-176.4	-3.19	0.0017
TC	174.35	32.31	123.7-256.1	187.32	43.85	127.3-290.7	-2.02	0.045
HDL–C	44.1	6.32	28-54.1	47.83	6.71	38.9-60.6	-3.21	0.0016
TG	117.18	31.76	74.3-196.5	121.83	29.25	80.4-165	-0.83	0.41
LDL–C	106.81	32.44	58.14-192.82	115.12	44.05	54.36-214.46	-1.29	0.2

FBG: fasting blood glucose, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, TG: triglyceride, LDL-C: low density lipoprotein cholesterol.

Table 3: Effect of Physical Activity on 2D:4D and blood pressure in male subjects

	Inactive (n=68)	Mild PA (n=40)	Moderate PA (n=86)	Optimal PA (n=72)		
Variables	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	F	P value
Height (cm)	169.97 ± 5.73	170.09 ± 6.37	169.28 ± 5.86	167.70 ± 7.01	2.01	0.11
Weight (Kg)	68.82 ± 14.24	66.90 ± 12.84	59.94 ± 10.02	59.12 ± 9.65	11.93	<0.001
DBP (mmHg)	94.38 ± 11.88 ^{a,b,c}	87.30 ± 11.15 ^{a,d,e}	79.48 ± 5.89 ^{b,d,f}	72.56 ± 8.31 ^{c,e,f}	72.65	<0.001
SBP (mmHg)	149.32 ±19.92 ^{a,b,c}	136.88 ±14.15 ^{a,d,e}	121.24 ± 9.71 ^{b,d,f}	111.26 ± 9.47 ^{c,e,f}	104.15	<0.001
RI (mm)	75.43 ± 5.80	75.32 ± 4.60	73.36 ± 5.27	73.49 ±5.55	2.85	0.038
RII (mm)	73.45 ± 4.85	73.54 ± 3.86	72.23 ± 5.05	71.57 ± 5.79	2.24	0.084
RIII (mm)	81.03 ± 5.02	80.72 ± 4.12	79.88 ± 5.50	79.21 ± 6.26	1.54	0.20
RIV (mm)	75.06 ± 5.40	75.77 ± 3.88	75.76 ± 5.30	75.93 ± 5.88	0.36	0.78
RV (mm)	62.96 ± 4.46	62.53 ± 6.24	62.31 ± 5.04	60.82 ± 5.66	2.16	0.092
R2D:4D	0.98 ± 0.03 ^{a,b}	0.97 ± 0.03 ^{d,e}	0.95 ± 0.03 ^{a,d}	0.94 ± 0.03 ^{b,e,}	18.82	<0.001
LI (mm)	75.67 ± 5.76	74.79 ± 4.66	73.49 ± 4.95	72.76 ± 5.47	4.17	0.007
LII (mm)	74.30 ± 4.21	74.84 ± 3.79	73.08 ± 5.01	71.85 ± 5.36	4.68	0.003
LIII (mm)	81.14 ± 5.26	81.09 ± 4.85	80.54 ± 5.53	79.53 ± 6.34	1.17	0.32
LIV (mm)	75.56± 4.66	77.14 ± 3.72	76.06 ± 5.11	75.83 ± 5.44	0.93	0.42
LV (mm)	62.86 ± 4.59	62.51 ± 4.72	62.56 ± 5.00	61.00 ± 5.70	1.94	0.12
L2D:4D	0.98 ± 0.03^{a,b}	0.97 ± 0.02^{c}	0.96 ± 0.04^{a}	0.95 ± 0.03^{b,c}	15.54	<0.001

I: first digit, II: second digit, III: third digit, IV: fourth digit, V: fifth digit, R: right hand, L: left hand, 2D:4D: second to fourth digit ratio,
DBP: diastolic blood pressure, SBP: systolic blood pressure.; P-value shows the overall effect of PA on the variables while similar superscript of variables indicates significant difference between the variables

Table 4: Effect of Physical Activity on 2D:4D and blood pressure in female subjects

	Inactive (n=65)	Mild (n=31)	Moderate (n=76)	Optimal (n=21)		
Variables	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	F	P value
Height (cm)	157.52 ± 7.37	158.54 ± 7.82	159.07 ± 5.19	159.41 ± 8.32	0.78	0.51
Weight (Kg)	64.66 ± 14.44	56.11 ± 11.11	51.06 ± 9.49	47.93 ± 6.56	22.14	<0.001
DBP (mmHg)	98.29 ± 9.32 ^{a,b,c}	84.58 ± 8.55 ^{a,d,e}	77.38 ± 6.55 ^{b,d,f}	71.22 ± 7.45 ^{e,c,f}	109.82	<0.001
SBP (mmHg)	154.34 ± 18.41 ^{a,b,c}	132.97 ± 11.29 ^{a,d,e}	116.95 ± 8.20 ^{b,d}	109.63 ± 7.10 ^{c,e}	128.19	<0.001
RI (mm)	66.65 ±5.46	69.84 ± 5.19	68.38 ± 3.98	67.87 ± 5.72	3.2	0.024
RII (mm)	67.98 ± 4.12	70.54 ± 5.51	69.28 ± 4.25	68.47 ± 4.22	2.62	0.052
RIII (mm)	74.15 ± 4.79	77.37 ± 6.64	75.77 ± 4.40	76.04 ± 4.03	3.34	0.02
RIV (mm)	68.58 ± 4.41	70.94 ± 5.25	70.36 ± 4.07	70.89 ± 4.45	3.22	0.024
RV (mm)	56.25 ± 4.29	59.59 ± 3.83	57.77 ± 4.30	58.10 ± 3.60	4.88	0.0027
R2D:4D	0.99 ± 0.02 ^{a}	0.99 ± 0.03^{b}	0.98 ± 0.03	0.97 ± 0.05^{a,b}	4.89	0.0027
LI (mm)	66.66 ± 4.72	69.94 ± 5.23	68.15 ± 3.42	66.89 ± 4.89	4.46	0.0047
LII (mm)	68.15 ± 4.18	70.74 ± 5.20	69.38 ± 4.27	68.60 ± 3.82	2.72	0.046
LIII (mm)	74.78 ± 5.57	77.36 ± 6.01	76.70 ± 4.95	77.11 ± 6.19	2.36	0.073
LIV (mm)	68.41 ± 4.57	71.63 ± 5.45	70.72 ± 4.34	70.69 ± 4.22	4.71	0.0034
LV (mm)	56.64 ± 5.95	58.49 ± 4.48	58.07 ± 4.13	58.24 ± 4.19	1.57	0.198
L2D:4D	1.00 ± 0.03 ^{a,b}	0.99 ± 0.03	0.98 ± 0.03 ^{a}	0.97 ± 0.03^{b}	6.75	0.0002

I: first digit, II: second digit, III: third digit, IV: fourth digit, V: fifth digit, R: right hand, L: left hand, 2D:4D: second to fourth digit ratio, DBP: diastolic blood pressure, SBP: systolic blood pressure.
P-value shows the overall effect of PA on the variables while similar superscript of variables indicates significant difference between the variables

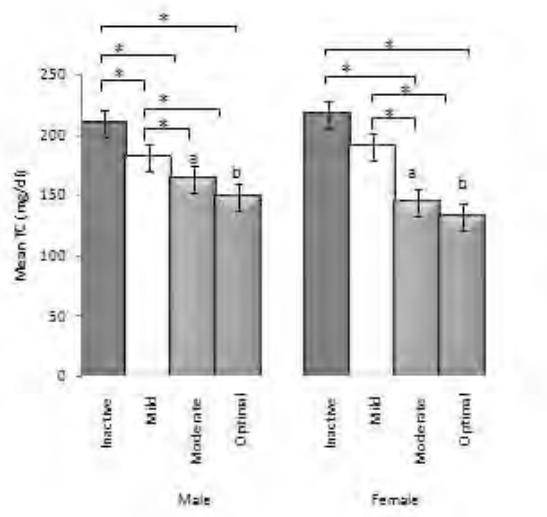

Fig 1: Effect of physical activity on serum uric acid level (* indicates P <0.05 between categories of PA within sexes & similar letter superscript indicate P < 0.05 between sexes for each category of PA).

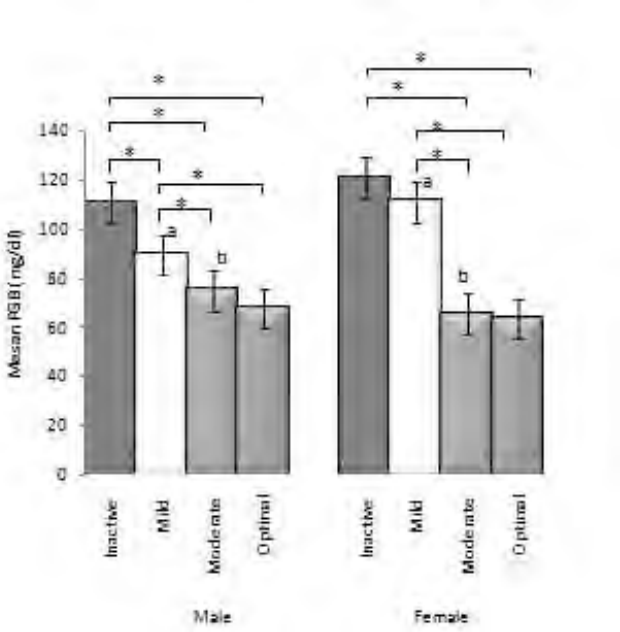

Fig 3: Effect of physical activity on serum glucose level (* indicates P <0.05 between categories of PA within sexes & similar letter superscript indicate P < 0.05 between sexes for each category of PA).

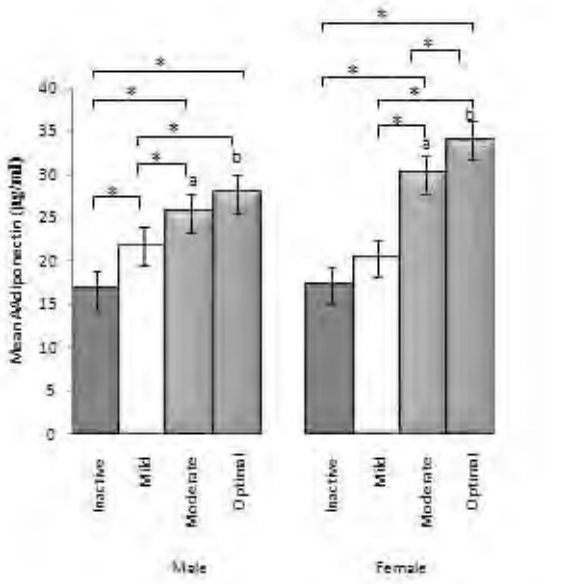

Fig 2: Effect of physical activity on serum adiponectin (* indicates P <0.05 between categories of PA within sexes & similar letter superscript indicate P < 0.05 between sexes for each category of PA)

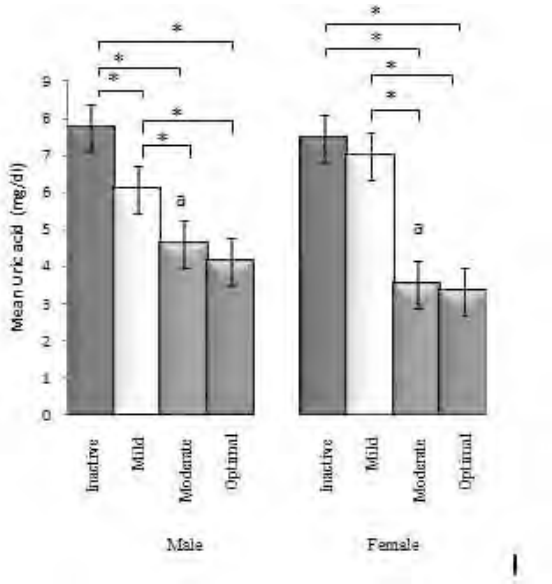

Fig 4: Effect of physical activity on serum TC level (* indicates P <0.05 between categories of PA within sexes & similar letter superscript indicate P < 0.05 between sexes for each category of PA).

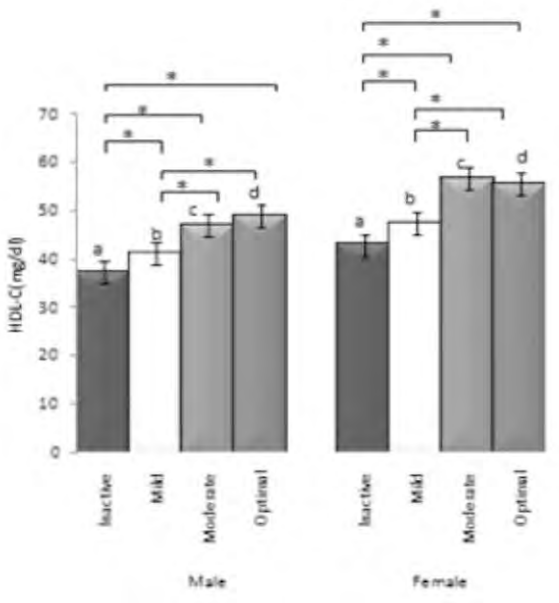

Fig 5: Effect of physical activity on serum HDL-C level (* indicates P <0.05 between categories of PA within sexes & similar letter superscript indicate P < 0.05 between sexes for each category of PA)

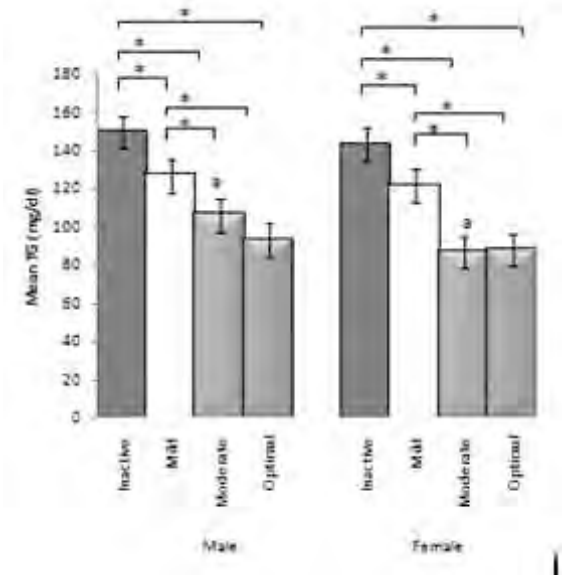

Fig 6: Effect of physical activity on serum TG level (* indicates P <0.05 between categories of PA within sexes & similar letter superscript indicate P < 0.05 between sexes for each category of PA)

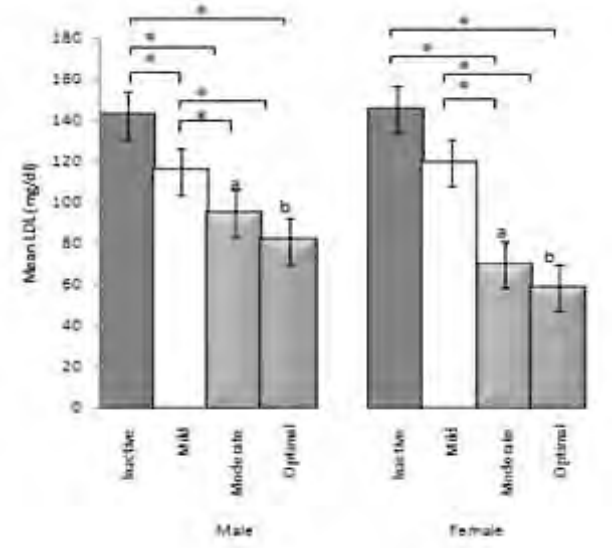

Fig 7: Effect of physical activity on serum LDL–C level (* indicates P <0.05 between categories of PA within sexes & similar letter superscript indicate P < 0.05 between sexes for each category of PA)

From Fig. 3 – 7 showing the effects of PA on FBG and lipid profile, even though significant reduction in FBG and lipids occurred with increase in PA, no significant reduction was observed between moderate and optimal PA in both sexes. Serum levels of HDL-C were observed to rise from lower levels of PA to higher levels but no significant change was observed between moderate and optimal PA.

Discussion

The significant effect of PA on MetS indices as demonstrated by significant reduction in FBG, LDL, TG, TC and blood pressure and increase in HDL with increase in PA level observed in this study indicates that PA confers protection against MetS. Also the pattern of change observed in the serum biomarkers also supports this notion in that while SUA decreased with increasing levels of PA, adiponectin increased. Also HDL–C, a component of lipid profile whose increased levels indicates a lower metabolic risk (30, 31) was also observed to rise with increasing PA. In keeping with this result, previous studies have shown the inverse relationship between physical activity and adverse metabolic parameters, thus indicating its protective effect against MetS (2,3,4). Further in support of the present study, it is documented that PA can have a profound effect on reducing body and visceral adiposity and therefore reduces metabolic syndrome risk (5, 6) and that PA, especially that which is associated with

reduced fat mass, corrects the dysfunction in adipokine and cytokine expression so that expression of adiponectin is increased in adipose tissue and production of inflammatory cytokines is reduced (8).

Some reports suggest that the beneficial effect of exercise as noted in this study is partly mediated through changes in the adipokines profile, that is, by increasing anti-inflammatory cytokines and decreasing proinflammatory ones (9, 10) This effect has been described at the levels of gene expression, protein ligands, and receptor bindings (11). For instance, exercise increases insulin sensitivity through reduction of resting levels of TNF- and augmentation of adiponectin levels (12). The reason why 2D:4D ratio was observed in this study to decrease with PA is not very clear. This is considering the fact that the ratio is a stable, hormonally and genetically determined congenital variable. However, since the ratio has been shown to be a marker of behavior (17). and has been associated with behavioral characteristics such as type of sexual behavior (17, 18) aggression (33, 34) and even sport performance (35), it is possible that the likelihood of an individual to adopt an active or sedentary life style is a biologic trait which similar to 2D:4D is also genetically and congenitally predetermined and manifests phenotypically in the digit ratio, behaviorally in the form of individual's desire for exercise and physiologically as threshold of exercise tolerance. This means that the digit ratio may provide a clue to an individual's exercise personality.

The observation from this study that FBG, TC, TG and LDL all decreased with increased activity level but did not show any significant decrease after moderate PA may suggest that, even though PA brings about reduction in measures of MetS indices, this effect probably has a threshold in that moderate PA may be enough to combat most adverse metabolic parameters and higher levels of PA may be supra threshold and thereby not conferring additional benefit.

Interestingly, the biomarkers (SUA and adiponectin) also demonstrated corresponding changes in that they remained relatively stable after moderate activity level. This finding is particularly important because Mets component have been shown to increase with age (36, 37, 38) making most victims to fall in the older age group. Since elderly people are also more likely to have other age related co –morbid conditions like ischemic heart disease and arthritic changes which may make strenuous PA unfit for them, this implies that exposure of this group of individuals to such high PA level in order to combat MetS may be unnecessary. Some serum parameters like TC and LDL in females did not show significant change after only mild PA indicating that very low levels of PA may be inadequate to significantly reduce these indices. These findings underscore the importance of regimenting exercise therapy based on its desired metabolic effect. The slight sex difference observed in the metabolic response to PA as noted for TC and LDL suggest that there is gender difference in the response of the metabolic parameters to PA and that such gender peculiarity should be considered in exercise therapy for MetS. Supporting this observation, some evidences exist to suggest that there is gender discrepancy in exercise induced insulin sensitivity, in that females respond better than males for age matched counterparts following the same dose and duration of physical activity (14).

Conclusion

The study revealed that, among the Hausa ethnic group in Kano, 2D:4D is lower in physically active individuals. While blood pressure, serum uric acid, TC, TG, LDL-C levels are lowered by physical activity, HDL and adiponectin are increased by physical activity. Moderate and optimal PA had similar effect on most metabolic indices. Slight sex difference was observed in the response of MetS indices to physical activity.

Acknowledgements

We thank all those who volunteered to participate in this study. Special thanks to Yusha'u Ma'ruf who assisted in mobilizing subjects that donated blood for biochemical analyses of blood. This work is an

extract of a Ph.D. dissertation which was sponsored by Bayero University Research Grant Unit and Tertiary Education Trust Fund (TETfund) of Nigeria

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Trichomonas vaginalis Infection among Pregnant Women with HIV in Sokoto Metropolis, Nigeria

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Abstract

This study investigated the association between *T.vaginalis* infection and the human mmunodeficiency virus (HIV) infection among some pregnant women attending ante natal clinic in some selected hospitals in Sokoto metropolis, Nigeria. A total of 286 high vaginal swabs and blood samples were collected from pregnant women aged between 15 and 44 years, who presented with vaginal discharges. Samples were examined by direct saline preparation, while their blood samples collected by venepuncture in sterile vacuutainers were screened for HIV using Determine and Stat-Pak in parallel to cross check discordant results. Seven (2.4%) of the patient with HIV were found to have been infected with *T. vaginalis*, 30 (10.5%) were having *T.vaginalis* infection without HIV and 14 (4.9%) were infected with HIV without *T.vaginalis* infection. The Chi square test result at 95% confidence interval indicated a strong association between *T.vaginalis* infection and HIV infection ($P < 0.5$) which indicated that sexually transmitted infections may be predisposing factors to the HIV infection, thus, for proper control of HIV in pregnant women it is advised that screening for *Trichomonas vaginalis* infection should be incorporated.

Key Words: *Trichomonas viginalis*; HIV; pregnancy

Introduction

Trichomoniasis caused by *Trichomonas vaginalis* is a sexually transmitted disease (STI) with important public health implications. It has been associated with vaginitis, cervicitis, urethritis, and pelvic inflammatory disease (PID). It also impacts upon birth outcomes and is a co-factor in human Immunodeficiency virus (HIV) transmission and acquisition (1).

The parasite is spread sexually by penis-to-vagina intercourse or vulva-to vulva (the genital area outside the vagina) contact with an infected partner. Women can get the disease from infected men or women, but men usually get it from infected women. For the parasite to establish itself in a host many mechanisms are thought to be involved including cell-to cell adhesion (2). Approximately 180 million women may be infected with *Trichomonas vaginalis* worldwide and prevalence estimates vary between populations studied ranging from 5-74% in women and 5-29% in men, the highest rates reported in either sex from STI clinics and in other high risk populations (2). Studies from Africa have suggested that *T. vaginalis* infection may increase the rate of HIV transmission by approximately two folds (3).

Materials and Methods

Study area

The area selected for this study was the metropolitan city of Sokoto state (seat of the caliphate), located at the extreme North West of Nigeria. The selected hospitals are Maryam Abacha Women and Children Hospital and Specialist Hospital Sokoto, North-Western Nigeria. Ethical permission was obtained from institutional Ethical Review Committees of the selected hospitals. Only individuals that gave informed oral consent were enrolled for the research.

FIG. Map of the Study Area, Source: Available from www.google.com (The gps coordinates.net/Nigeria/Sokoto. Accessed on 15.08.2016. 3:27).

Study population
This study involved pregnant women attending Antenatal Clinic (ANC) in Maryam Abacha Women and Children Hospital and Specialist Hospital Sokoto.

Study design
It is a prospective observational study aimed at determining the prevalence of *T. vaginalis* and HIV co-infection among pregnant women attending ANC in Maryam Abacha Women and Children Hospital and Specialist Hospitals in Sokoto.

Questionnaire administration
Socio-demographic information and obstetric history were obtained from participants by the use of a structured questionnaire.

Sample size
Two hundred and eighty six samples of blood and High vaginal swabs were randomly collected from pregnant women aged between 15 and 44 years who came for their antenatal check-up and who showed willingness to participate.

Sample collection
With the aid of vaginal disposable speculum (HVS) was collected by inserting speculum into the posterior fornix of the vagina while the patient was in the lithotomic position (under the supervision of a certified clinician) The swab was collected from each patient using sterile disposable swab stick. The samples were immediately examined microscopically within one hour of collection.

Collection of Blood sample
About 5ml of whole blood was collected by venopuncture, into a plain container; allowed to retract for 30 minutes and spun at 1500 rpm for 10 minutes. The serum obtained was used for the HIV screening.

HIV Screening Test.
Blood samples from each patient were screened using two kits in parallel. Determine and Stat-Pak Assay.

Determine Test Procedure.
The protective foil for each test was removed, this was labelled accordingly, 50µl of serum was placed on the sample pad and timed for 15 minutes, and result read within 20 minutes. Positive test appeared with a red line in the control window and test window, while a negative test appeared with only one red line in the control window of the strip. Where there was no red line in the test and the control windows the test was taken as invalid.

HIV Stat-Pak Assay procedure
The Chembio HIV Stat Pak (Chembio Diagnostic System. Inc.) was removed from its pouch and placed on a flat surface and labelled accordingly. The Pak was charged with 5.0µl of serum sample into the sample well using the loop provided by the manufacturer. Three drops of running buffer were added to each slowly and drop wise into the well. The test was read after 15 minutes. NO TEST WAS READ AFTER 20 MINUTES. Positive results appeared with two red lines that is control and test lines. Where there was no red line on both the control and test area, the result was regarded as invalid.

Direct saline wet preparation.
A drop of normal saline (0.5ml) was added to the swab container and shaken together with the swab stick so that the organism will be released into the normal saline. With the aid of the swab stick, the suspension was placed on a cleaned grease free glass slide and was carefully covered with a cleaned cover slip. The preparation was examined under the low power (10x) and high power (40x) magnifications for presence of motile trichomonads. Pear-shaped, motile flagellates were seen when *T. vaginalis* was present, moving with characteristic jerky, wobbling and rotating motions.

Data analysis
Data generated from the results were analyzed using chi square test to find the association between *T. vaginalis* and HIV in the age groups of pregnant women at 95% confidence interval.

Results
Of the 286 HVS and blood samples collected from

randomly selected pregnant women and screened, 30(10.5%) were positive for *T.vaginalis* as detected by wet preparation techniques, 14(4.9%) were positive for HIV infection as screened with Determine alongside Stak-Pak kits and 7(2.4%) were positive for both *T.vaginalis* and HIV

Age (Years)	No. of samples screened	No. (%) Positive for *T.vaginalis*	No. (%) Positive for HIV
15-20	74	7(9.5)	2(2.7)
21-24	58	8(13.8)	5(8.6))
25-29	52	5(9.6)	4(7.6)
30-34	34	4(11.8)	1(2.9)
35-39	27	3(11.1)	2(7.4)
40-44	41	3(7.3)	0(0)
Total	286	30(10.5)	14(4.9)

Table 1 shows the prevalence of *T.vaginalis* and HIV among pregnant women in relation to age. The highest prevalence of *T.vaginalis* infection was observed in the age group of 21-24 years 8(13.8%) and the least distribution was found in the age group 40-44 years 3(7.3%). Pregnant women aged (21-24) had the highest prevalence of HIV 5(8.6%) while the least distribution of HIV was observed in the age 15-20 years 2(2.7%), none was found in the age group 40-44 years (0%). No statistically significant difference was observed in the prevalence of *T.vaginalis* and HIV among pregnant women in relation to age (P>0.5).

Table 2: Prevalence of *T.vaginalis* in relation to gestational period.

Trimester (month)	No. of samples screened	No. (%) Positive for *T.vaginalis*	No. (%) Positive for HIV
First(1-3)	108	16(14.8)	6(5.6)
Second(4-6)	94	8(8.5)	4(4.3)
Third(7-9)	88	6(6.8)	4(4.5)
Total	286	30(10.5)	14(4.9)

The prevalence of *T.vaginalis* and HIV in pregnant women in relation to gestation period as presented in Table 2 showed that those in their first trimester (1-3month) had the highest prevalence of *T.vaginalis* 16 (14.8%) while the least distribution was observed in women during their third trimester (6-9month) 6(6.8%). The prevalence of HIV in pregnant women in relation to gestation period showed that those in their first trimester(1-3month) had the highest prevalence of HIV infection 6 (5.5%) while the least distribution of HIV infection was observed in women during their second trimester (6-9month) 4 (4.2%). No statistically significant difference was observed in the prevalence of *T.vaginalis* and HIV among pregnant women in relation to gestation period (P>0.5).

Table 3: Prevalence of *T.vaginalis* and HIV among pregnant women in relation to hospital

Hospital	No. of samples screened	No. (%) Positive for *T.vaginalis*	No. (%) Positive for HIV
Maryam Abacha Women & Children	198	18 (9.1)	9(4.5)
Specialist hospital	88	12(13.6)	5(5.7)
Total	286	30 (10.5)	14(4.9)

Table 3 shows the Prevalence of *T.vaginalis* and HIV among pregnant women in relation to hospital. The highest prevalence of *T.vaginalis* was observed in Specialist Hospital. 12(13.6%) while 18(9.1%) were positive for *T.vaginalis* infection in Maryam Abacha Women and Children. The prevalence of HIV infection in Specialist hospital was highest 5(5.7%) compared to 9(4.5%) HIV prevalence observed in Maryam Abacha Women and Children hospital.

Table 4: Prevalence of *T.vaginalis* and HIV infection based on socioeconomic status

Socioeconomic status	No. of samples screened	No. (%) Positive for *T.vaginalis*	No (%) Positive for HIV
Low	108	16(14.8)	9(8.3)
Medium	98	9(9.2)	3(3.1)
High	80	5(6.3)	2(2.5)
Total	286	30(10.5)	14(4.9)

Table 4 shows the prevalence of *T.vaginalis* and HIV infection based on socioeconomic status, patients of low socioeconomic status had the highest prevalence of *T.vaginalis* 16(14.8%) while the least prevalence was observed among pregnant women of high socioeconomic status 5(6.3%). However, in the case of HIV infection, the highest prevalence was observed in patients of low socioeconomic status 9(8.3%) while the least prevalence was in patients of high socioeconomic status 2 (2.5%). No statistically significant difference was observed in the prevalence of *T.vaginalis* and HIV among pregnant women in relation to socio economic status P>0.5

Table 5: *T.vaginalis* and HIV co-infection in the study population.

Age(years)	No. screened	No. (%) Co-infected
15-20	74	1(1.4)
21-24	58	3(5.2)
25-29	52	2(3.8)
30-34	34	0 (0)
35-39	27	1(3.7)
40-44	41	0(0)
Total	286	7(2.4)

Table 5 shows the prevalence of T.vaginalis and HIV Co-infection in the study population, women aged between 21-24 years had the highest prevalence of *T.vaginalis* and HIV Co-infection 3(5.2%) while the lowest prevalence 1(1.4%) was observed in pregnant women in the age range of 15-20 years and no positive result was observed in pregnant women in the age group 40-44 years. No statistically significant difference was observed in the prevalence of *T.vaginalis* and HIV Co-infection among pregnant women in relation to age P>0.5.

Discussion

Out of 286 pregnant women attending antenatal clinics in some selected hospitals in Sokoto metropolis, 12(13.6%) from Specialist Hospital were positive for *T.vaginalis* and 5 (5.7%) were positive for HIV infection while 18 (9.1%) from Maryam Abacha Women and Children Hospital were positive for *T.vaginalis* and 9 (4.5%) were positive for HIV infection respectively. The occurence of *T.vaginalis* in the study patients was 10.5% which is in conformity with the findings of Okonkwo et al. (4) who reported 12.3% prevalence of *T.vaginalis* in Ebonyi state, Nigeria. The distribution of this infection may be as a result of poor hygiene, low immunity, hormonal imbalance and polygamous life pattern that is being practiced in the Northern part of Nigeria.

The prevalence rate found in this study is slightly lower than 12.3% found in Abakiliki, Southern Nigeria, (4) but higher than other findings in Nigeria; 4.7% in Ilorin, (5), 5.2% in Calabar (6), and 2.8% in another study from South Eastern Nigeria (7). Research elsewhere reported higher prevalence; 17.7% in Uyo Nigeria (8), 18.66% in Zaria Nigeria (9), 24.1% in Jos Nigeria (10), 46.9% in New York (11) and 36.1% in Nebraska (12).

These differences in prevalence could be attributed to differences in social, cultural and environmental factors. The prevalence of T.vaginalis and HIV infection was much higher in Specialist Hospital compared to Maryam Abacha Women and Children Hospital. This may probably be because, most of the patients attending antenatal clinics at the Specialists hospital are less educated compared to those attending Maryam Abacha Women and Children Hospital as obtained from the Questionnaire (Demographic data). Specialist hospital is a primary health care centre that services the native communities of Sokoto metropolis, and is patronized by thousands of women who could not afford service-charges at the secondary and tertiary health facilities. Certain factors, common among such communities, include poor personal hygiene, low socio economic status and under development are also associated with high risk of infection.

In Africa, it is estimated that 2-50% of the populations carry the infection (13). The disease has important medical, social and economic implications. There is a higher prevalence of trichomoniasis among pregnant women than non-pregnant women. This might be due to the greater pelvic vascularity and oestrogenic activity on the vaginal epithelium which causes growth, maturation and exfoliation of the squamous cells and an increase in glycogen deposits in vaginal epithelial cells (14). *T.vaginalis* is reported to be associated with the alkaline vaginal environment that occurs during pregnancy due to changes in the pH of the

vaginal mucosa Women who are infected during pregnancy are predisposed to preterm rupture of the placental membrane, preterm labour, delivery of low birth weight infants and increased infant mortality among others.

Amongst the different age groups investigated, *T.vaginalis* infection distribution was highest in women aged 20 – 24 years. This study agrees with observed fact that the occurence of sexually transmitted diseases (STDs) including trichomoniasis, by the number of cases treated each year, is highest among the 15 - 30 year age.

Women in the first trimester of pregnancy were observed to have high rate of infection by *T.vaginalis* (14.4%) in contrast to pregnant women in the third trimesters that had a lower rate of infection (4.3%). Information retrieved from pregnant women revealed that the frequency of sexual intercourse decreases as pregnancy advances and this may likely be the reason for the low incidence of infection at second and third trimesters. The finding is consistent with previous report from Imo state, Nigeria, by Obiajuru and Ogbulie, (15) who reported that pregnant women in the first trimester of pregnancy had the highest prevalence of STDs.

Amongst the different age groups investigated. *T.vaginalis* infection rate was highest in women aged 20 – 24 years 8(13.8%). This study corroborate the findings by (9) in Zaria who reported a slightly high prevalence (18.66%). The findings in this study is also in agreement with observed fact that the incidence of sexually transmitted diseases (STDs) including trichomoniasis, from the number of cases treated each year, is highest among the 15 – 30 years age group.

In this study, pregnant women were significantly infected with *T.vaginalis* in their 1st and 3rd trimester in nearly equal prevalences. This agrees with the findings of Njoku et al. (16) that the prevalence is more in the first trimester, while another study found more in 3rd trimester (17).

The infection was more prevalent among low socioeconomic status individuals with 16(14.3%) followed by patients of moderate socio economic status 9(9.2%). However, (3), reported low prevalence in high class patients. The observed high prevalence in low income patients may be attributed to their active social living characterized by sexual exposure with little or no personal preventive measures. Sexual liberalism associated with poverty and ignorance, and lack of awareness of the public health repercussions may likely be the foremost in the list of risk factors.

Conclusion

The prevalence of *Trichomonas* infection of 10.5% and HIV in the studied patients is a public health risk, especially keeping in mind that HIV infection and other STIs can be enhanced with Trichomonas infection. Therefore, clinicians should routinely screen all pregnant women for the infection and provide early and appropriate treatment to prevent the spread of STIs and possible implications for the newborn baby. Emphasis should be placed on the youths especially those of low educational background as well as women of low socioeconomic status. Policy makers need to enlighten the community on girl child education, safe sex and good hygiene, and institute policies that will make health care services accessible, affordable and standard.

Recommendation

There is need for proper control of HIV infection in pregnant women and it is also advised that screening for *T.vaginalis* should be incorporated in the antenatal care for all pregnant women in all trimesters.

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Biochemical Effect of Ethanolic Root Extracts of *Uvaria charmae* on Liver Enzymes of Albino Wistar Rats Infected with *Staphylococcus aureus*.

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Abstract

The effect of ethanol extract of the root of *Uvaria charmae* on liver enzymes activity was investigated in albino wistar rats infected with Staphylococcus aureus. The liver enzymes assayed included aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Twenty five female adult albino Wistar rats weighing between 220-250g and divided into five groups were used. The control group (Group1) received feed and water, Group 2 was infected with *Staphylococcus aureus*, group 3 was infected with *Staphylococcus aureus* and treated with *Uvaria charmae* plant extract after 10 days post infection , group 4 infected with *S. aureus* was treated with Vancomycin after 10days post infection , group 5 received *Uvaria charmae* extract daily for 10 days . Blood samples were thereafter collected from the lateral tail vein of the albino rats and analyzed for liver enzymes (ALP, AST andALT). P-value < 0.05 was considered statistically significant. In the biochemical analysis, AST levels were elevated in group3 (170.25±12.764), group 4 (120.00±7.071) and group 5 (179.00±3.742) compared with the control group (46.00±5.657). These were statistically significant (P=0.001). Alanine aminotransferase (ALT) levels were elevated in group 3 (174.75±3.745), group 4 (104.50±3.775), and group5 (111.25±5.377) compared with the control group (36.50±2.121) and were statistically significant (P = 0.001). Alkaline phosphatase (ALP) was elevated in group 3 (303.75±5.315), group4 (263.50±39.854), and group 5 (325.25±8.139) compared to the control group (155.50±9.192). This was also statistically significant (P =0.001). The Tukey post hoc test showed that for AST levels, there was statistically significant difference between group 2 and group1 (P =0.001), group3 (P =0.0001) and group 4 (P = 0.015). For ALT levels, there was statistically significant difference between Group2 andGroup1 (P =0.001), group3 (P =0.001), group 4 (P =0.006) and group 5 (P = 0.001). For ALP levels, there were statistically significant differences between group2 and group1 (P =0.007), group5 and group4 (P =0.009). The significant elevation in ALP levels by *Uvarie charmae* aqueous extract shows that possible cholestasis occurred at the dose levels tested since a rise in plasma alkaline phosphatase (ALP) level is usually a characteristic finding in cholestatic liver disease. The exact cause of significant increase in ALP level at the doses is not known but it may be inferred that continuous usage of *Uvaria charmae* may be adversely affecting liver function owing to its associated pernicious anaemia. In conclusion, *Uvaria charmae* is harmful on continuous usage.

Key Words: Liver enzymes, *Staphylococcus aureus, Uvaria charmae*

Introduction

The plant *Uvaria charmae* is a small shrub growing up to 4mm tall. The plant is accessed locally and harvested and used as food and medicine in the South, East and Northern parts of Nigeria. It is known by various names such as “kaskaifi” or “atore” in Hausa, “eruju” or “okooja” in Yoruba, “ufuri-agu” in Igbo and “mboroineunikot” in Ibibio (1). The root of *Uvaria charmae* has been reported to possess bioactive components such as flavonoids, alkaloids, tannins, saponins and phenols in varied quantities (2). These bioactive compounds may be responsible for the medicinal use of *Uvaria charmae* in the treatment of bacterial infections, jaundice, yellow fever, sores, etc. It has also been reported to be good for treatment of acute stomach pain, cough, dysentery and liver conditions (3).

The bacterium, *Staphylococcus aureus* is an endogenous bacterial microorganism colonizing the nasal cavities, skin, gastrointestinal tract, anus and vaginal vaults of healthy women. *S.aureus* causes many opportunistic community acquired and

nosocomial infections of the musculoskeletal system as well as device-associated infections. *S.aureus* is generally spread by contaminated hands and can have access to underlying tissues or the blood stream at any point there is a broken and unprotected skin to cause infection. Immunocompromised persons are more vulnerable to the infection (4). Other than humans, *S.aureus* causes infection in animals such as pets (e.g. dogs) livestock (e.g. donkeys, rabbits) or wild animals (e.g. gorillas and bats) Although the zoonotic risk for humans has not yet been sufficiently studied, it is worthy of note that human associated *S.aureus* strain can cause both asymptomatic and fatal infection in animals such as goats and pigs (5).

The clinical manifestation of *S.aureus* infection occurs because it affects all organs of the body, though the liver and blood are the first organ and tissue to be affected. This multisystem scenario occurs because *S.aureus* is able to exhibit resistance against most commonly used antibiotics using the chromosomal MecA gene that specifies the production of an abnormal penicillin binding protein called $PBP2_a$ or $PBP2^1$ (Weems, 2001).The Penicillin binding proteins are membrane bound enzymes which target β-lactam antibiotics resulting in resistance to the penicillins and cephalosporins (6). The MecA gene chromosomal cassette has insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics.

The liver is the chemical factory of the body that carries out metabolic regulation of body internal environment, therefore any toxicant the body is exposed to is under regulation by the liver including infection by *S.aureus*. Liver injury is a common feature of bacterial toxemia. Once the body's natural barrier (the skin) has been compromised, *S.aureus* gains access into the internal organs of the body and spreads via the blood. *S.aureus* activates the host immune system by the peptidoglycan found on its cell wall causing the liver to release tumor necrosis factor TNF-α which causes excessive leukocyte recruitment, leading to hepatocyte necrosis and apoptosis,thus resulting in liver damage. As host immune system is triggered, *S.aureus* releases super antigens because of their capacity to induce intense T-cell activation and this super antigens bind to major histocompatibility complex encoded class II protein resulting in mass necrosis of liver tissues causing hepatotoxicity induced by *S.aureus* enterotoxins (7).

Over the years, accurate diagnosis, treatment and control of bacterial infections have been vital in the reduction of bacterial burden in most countries especially in the developed ones. In developing countries such as Nigeria, a recent effort to reduce this burden has been the approval of herbal preparations suggestive of antibacterial activity by the National Agency for Food and Drug Administration and Control (NAFDAC). However, this approval has been given without properly documented trials of such herbal products, especially as regards their hepatotoxicity and this forms the rationale for this study.This study will attempt to document if exposure to the plant *Uvaria charmae* could be hepatoprotective against *S.aureus* infection or hepatotoxic to the liver.

Materials and Methods

Animals and housing

Twenty five mature female albino Wistar rats weighing about 220-250g were obtained from the animal house of the University of Calabar, where they were born and bred and divided into five experimental groups. The animals were housed individually under standard laboratory conditions, with a 12h light/12h dark photoperiod. They were fed on rat chow pellets and received water *ad libitum.* The experimental protocol and procedures used in this study were approved by the Animal Ethics Committee of the College of Medical Sciences, University of Calabar, Nigeria and conformed to the *Guide of Care and Use of Animals in Research and Teaching* (published by ACURET).

Plant material

The roots of *Uvaria charmae* (bush banana) were collected for this work from Akpabuyo Local Government Area of Cross River State, Nigeria in July, 2016 and authenticated by Mr. Efa Andem in the Herbarium of the Botany Department of University Of Calabar,Calabar, Nigeria , where a voucher specimen registered under the number 397 is kept. The fresh roots of *Uvaria charmae* was thoroughly washed free of debris. They were cut

into pieces, air dried for few days and then grounded into fine powder. The roots were extracted exhaustively with 95% (v/v) ethanol for 48 hours. This was done by soaking the pulverized materials in 95% ethanol and shaking vigorously every 3 hours within the extraction period. After 48 hours, it was filtered using Whatman no. 3 filter paper and later condensed using a rotator evaporator (RotavourBiich R461, Switzerland) and given the code UC – *Uvaria charmae*. Stock solution of *Uvaria charmae* was prepared to cover four weeks of administration by dissolving 55.9 mg/g body weight in 20 ml of Dimethyl Sulphoxide (DMSO) diluted in 20 ml of distilled water.

Treatment

Twenty-five adult female albino Wistar rats were randomly separated into five groups (Group 1- 5). All the groups had equal number of rats i.e. five (5) in each group. Group 1 Animals received water as control group, group 2 animals received 0.5mg/g / kg of *S.aureus* isolates as standard whereas, group 3 animals received a single dose of *S.aureus* isolates 0.5mg/g/kg. After 10 days post treatment , 200mg/g/kg of *Uvaria charmae* extracts was given twice daily for 4weeks.Group 4 Animals received 0.5mg/g/kg/d of *S.aureus* as a single dose and after 10 days post treatment a 0.025mg/g/kg/d of Vancomycin was given twice daily for 4weeks**.** The animals in group 5 received of *Uvaria charmae* extract 200mg/g/kg/d for 4weeks.

Blood collection and preparation

The blood samples were obtained from the tails of rats into centrifuge tubes and allowed to clot for 30minutes. The clotted blood samples were centrifuged at 3000 revolution per minute (rpm) for 10minutes,Using table top centrifuge (Model 2420 KUBOTA) to separate the sera .The prepared serum samples were stored at 4^{o}C in a refrigerator (Thermo cool, Nigeria) for use in the study.

Enzyme analysis

The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were estimated following the method of Reitman and Frankel (8) and Babson (9). Alanine and aspartate aminotransferases were determined based on the colourimetric measurement of hydrazone formed with 2, 4 dinitrophenyl hydrazine (8), and alkaline phosphatase by the phenolphthalein monophosphate method (9).

Statistical analysis

Data obtained from this study were expressed as mean ± SEM. Analysis was performed by one-way analysis of variance followed by Tukey's post hoc test. A P - value < 0.05 was considered statistically significant.

Results

The *Staphylococcus aureus* infection in rats caused an increased serum levels of ALT, AST and ALP as compared to control(Table 1**).** The induced elevated serum levels were potentiated by the ethanol root extract of *Uvaria charmae* treatment (Table 1**) .** The potentiation of the serum enzymes levels were attenuated by the vancomycin antibiotic treatment (Table 1), and compared to *Staphylococcus aureus* induced enzyme levels. The data generated were statistically analyzed ($P<0.01$) using one way analysis of variance. The tukey post hoc test results showed comparative effect of *Uvaria charmae* treatments on *Staphylococcus aureus* induced liver damage.(Table 2).

Table 1: Effects of *U. charmae* treatment on *S. aureus* induced liver damage albino Wistar rats.

Analyst	Group	No.	Mean	SD	F	Df	P-value	Comment
AST	Control	2	46.00	5.657				
	S. aureus only	3	97.00	1.000				
	S.aureus and extract	4	170.25	12.764				
	S.aureus and vancomycin	4	120.00	7.071				
	Extract alone	4	179.00	3.742				
	Total	17	132.94	46.389	141.516	16	0.001	SIG.
ALT	Control	2	36.50	2.121				
	S.aureus only	3	82.00	1.000				
	S.aureus and extract	4	174.75	3.775				
	*S. aureus*and vancomycin	4	104.50	11.475				
	Extract alone	4	111.25	5.377				
	Total	17	110.65	43.716	169.756	16	0.001	SIG.
ALP	Control	2	155.50	9.192				
	S.aureus only	3	257.00	1.000				
	S.aureus and extract	4	303.75	5.315				
	S.aureus and vancomycin	4	263.50	39.854				
	Extract alone	4	325.25	8.139				
	Total	17	273.65	55.098	25.377	16	0.001	SIG.

Key: N-number of samples per group, SD-Standard deviation, F-F value or F Statistic, Df- degree of freedom , SIG-Significant

Table 2: Tukey Post hoc test of Comparative Effect of *U. charmae* treatment on *S.aureus* induced liver damage

		(I) Group	(J) Group	MD (I-J)	SE	P	Comment
		S.aureus only	Control	51.000*	7.045	.000	
AST			*S.aureus* and extract	-73.250*	5.894	.000	Sig
			S.aureus and vancomycin	-23.000*	5.894	.015	Sig
			Extract alone	-82.000*	5.894	.000	Sig
		S.aureus and extract	Control	124.250*	6.684	.000	Sig
			S.aureus only	73.250*	5.894	.000	Sig
			S.aureus and vancomycin	50.250*	5.457	.000	Sig
ALT		*S.aureus* only	Control	45.500*	6.072	.000	Sig
			S.aureus and extract	-92.750*	5.081	.000	Sig
			S.aureus and vancomycin	-22.500*	5.081	.006	Sig
			Extract alone	-29.250*	5.081	.001	Sig
ALP		*S. aureus* only	Control	101.500*	18.884	.001	Sig
			Extract alone	-68.250*	15.799	.007	Sig
		Extract alone	Control	169.750*	17.915	.000	Sig
			S.aureus only	68.250*	15.799	.007	Sig
			*S. aureus*and vancomycin	61.750*	14.627	.009	Sig

Key: MD: Mean deviation , SE-Standard Error, Sig-Significance, P- P Value

For AST levels, there was statistical difference between *S. aureus* only group with control at (P =0.001), with *S. aureus* and extract group (P =0.001) and *S. aureus* and vancomycin group (P = 0.015).

For ALT levels, There was statistically significant difference between *S. aureus* only and control (P =0.001), *S. aureus* and extract group (P =0.001), *S. aureus* and Vancomycin (P =0.006) and *Uvaria charmae* extract alone (P = 0.001).For ALP levels, there was statistically significant difference between *S. aureus* only and *Uvaria charmae* extract alone at (P =0.007) and *S. aureus* only and control group(P =0.001). Alongside this,*Uvaria charmae* extract alone and *S. aureus* and vancomycin at (P =0.009) were compared and observed to be statistically significant.

Discussion

In many regions of the world medicinal plants contribute immensely to the healthcare of the population. Many of our rural populations depend on a variety of herbs for their wellbeing (10). (M. Natural products, especially medicinal plants are increasingly used to cure various diseases such as infectious diseases, especially because of their multiplex properties and minimal adverse effects on the body. Alterations in the nutrient intake of living organisms could improve their metabolisms, leading to the protection against serious diseases (10). The plant, *Uvaria charmae* is one of such herbal remedies which have been widely used in herbal medicine in Nigeria . This study examined the impact of treatment of rats with a therapeutic dose of *Uvaria charmae* extract after infection by *S. aureus, s*erum and liver levels of AST, ALT and ALP activities, in an attempt to evaluate the hepatotoxic potential of this plant extract. One of the organs usually affected by ingestion of xenobiotics is the liver. Generally, hepatic injury is often associated with alterations in the serum and liver levels of some enzymes notably ALT, AST and ALP. Measurement of these diagnostic enzymes is useful for assessing the level of liver cytolysis and damage to the plasma membrane of the liver cells (11). Hyper-production of this enzyme could constitute a threat to the life of cells that are dependent on a variety of the phosphate esters for vital life processes (12). The significant increase in the liver ALP activity following administration of the *Uvaria charmae* extract may be due to increased functional activity of the liver. The kind of hyper production seen in this study may suggest that integrity of the liver cell plasma membrane was compromised following administration of the *Uvaria charmae* extract. This is in agreement with the findings of Yakubu *et al*. (13), who concluded that plants of the *Anonnaceae* family, of which *Uvaria charmae* is a member, can raise the AST and ALP levels of the liver. These patterns of increase in ALT, AST or ALP observed in this study as biochemical indicators of liver cytolysis suggest that the extracts may have adverse effect on the liver especially on continuous usage. The increases in AST and ALT serum levels suggest biochemical lesions of liver tissues (14), indicating that *Uvaria charmae* could be toxic .

Conclusion

Ethanolic extract of *Uvaria charmae* caused raised levels of AST, ALT and ALP in liver biochemical assay as compared with the control, indicating that the extract can cause liver cytolysis which can eventually result in liver damage. Increased public awareness of the risk of taking these herbal remedies will guard against the potential danger inherent in these products.

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Proximate Composition and Selected Mineral Profile of *Hyptis verticillata* Cultivated in Calabar, Cross River State, Nigeria

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Abstract
The present study was aimed at elucidating the proximate and selected mineral composition of the leaves of *Hyptis verticillata* cultivated in Calabar, Cross River State, Nigeria. The official methods of Analytical Chemist was used for the estimation of proximate content and mineral composition. Results of the proximate analysis indicated 70.59±0.19% carbohydrate while moisture, crude protein and crude ash contents were 12.58 ± 0.07%, 6.43 ± 0.14% and 8.36 ± 0.33% respectively, and the crude fat content was 2.07 ± 0.08%. The mineral content of *H. verticillata* leaves showed very high concentrations of calcium (2,640.26 ± 2.84mg/kg), potassium (2,423.09 ± 2.78mg/kg) and sodium (1,681.09 ± 12.50mg/kg) while the other minerals were present in low concentrations. We therefore suggest that the leaves of *H .verticillata* is rich in macrominerals and carbohydrate thus can serve as a potential source of supplements and energy when consumed as tea/decoction.

Key Words: Proximate composition, micro/macro nutrients, *Hyptis verticillata*.

Introduction

All cultures from the ancient times to the present have made use of some plants as a source of medicine. According to the World Health Organization (WHO), plants are relied on as an important element in primary health care systems by the World's population (1). Most people living in developing countries are almost entirely dependent on traditional medicinal plants for their primary healthcare needs and higher plants are known to be the main source for drug therapy in traditional medicine (2). *Hyptis verticillata* jacq, commonly called John Charles is a perennial plant that belongs to the family Lamiacae also known as mint family. It is a medicinal plant that originates from Central America and spreading to other parts of America and Caribbean countries including Nigeria (3). It is one amongst such herbal plants because it has a widespread traditional use, and pharmacological activities which have attracted very long and detailed research since the early 1970s. Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are very crucial for the physiological functions of human body. Nutrients and biomolecules like carbohydrates, fats and protein play essential role in meeting the human needs for energy and life processes (4).

Minerals are very important for the normal function of the body especially in maintaining electrolyte balance and the amount of minerals needed in the body is not directly proportional to their significance (5). The best way the body obtains its mineral requirement is via the consumption of a wide variety of foods. The bone, heart, brain and the muscles rely on macrominerals to carry out their activities while most of the microminerals are incorporated into enzymes and hormones necessary for the body's metabolism, with the exception of chromium which plays a role in the maintenance of blood glucose (6). Macrominerals play crucial roles as electrolyte, they help to maintain the acid-base balance and fluid/water balance, which when interrupted can lead to disorder. They also help in the regulation of nerve and muscle function (6). There is paucity of information on the proximate content and some mineral elements present in *H.verticillata* cultivated especially in the tropical environment like Nigeria. This present study was designed to evaluate the proximate and selected mineral profile of *H. verticillata* cultivated in Calabar, Cross River State, Nigeria.

Materials and Methods

Plant material and treatment

Hyptis verticillata was cultivated in a homestead garden in Calabar Municipality, Cross River State, Nigeria, from where the fresh leaves were harvested. Fresh leaves of the plant were authenticated by a Botanist, Mr Frank Apejoyi of the Department of Botany, University of Calabar, Nigeria, with voucher number of BOT/Hv/2015/001 deposited in the herbarium of the same Department. The leaves were washed and air dried at room temperature for two weeks. The dried leaves were blended to fine powder

and the powdered sample was subsequently extracted in varying reagents and used for the determination of moisture, ash, crude fat, crude protein, carbohydrate and selected minerals.

Chemicals and reagents
Petroleum Ether, HCL, H_2SO_4, K_2SO_4 and $CuSO_4$ were purchased from Sigma-Aldrich (St. Louis, Mo, USA). The reagents and chemicals were all of analytical grade.

Proximate analysis
Proximate composition was determined using the methods of AOAC (7) after the sample was brought to uniform size.

Determination of moisture
Oven drying method was employed in the determination of moisture. Well-mixed sample of 1.5g was weighed into a dry and clean crucible (W_1), after which the crucible (containing the sample) was put in an oven at a temperature of 102 C for 10 hours until a constant weight was achieved. The crucible was then put in a desiccator for 30 minutes to cool, after which the weight was taken again (W_2).
Note: moisture free sample was used for the rest of the analyses.
Percentage moisture was calculated with the formula:

$$\% \text{ Moisture} = \frac{W_1 - W_2 \times 100}{\text{Wt. of sample}}$$

Where,
W_1 = initial weight of crucible + sample
W_2 = final weight of crucible + sample

Determination of crude ash
An empty clean crucible was weighed (W_1) after it has been placed in a muffle furnace at 600°C for an hour and cooled in a desiccator. A quantity (1g) of the sample was put in the crucible (W_2) and the sample was placed over a burner and kindled till it was burnt. The crucible was then placed in muffle furnace at 550 C for two to four hours and the complete oxidation of all organic matter in the sample was indicated by the appearance of gray white ash. The furnace was switched off after ashing, and crucible was cooled and weighed (W_3).
Percentage ash was calculated with the formula:

$$\% \text{ Ash} = \frac{\text{Wt. of ash} \times 100}{\text{Wt. of sample}}$$

Wt. of ash = $W_3 - W_1$, Wt. of sample = W_2.

Determination of crude fat
Dry extraction method was employed in the determination of crude fat content, it involved the extraction of dry sample using an organic solvent since all the fat materials examples fats, phospholipids, sterols, fatty acids, carotenoids etc. are extracted together and the results are frequently called crude fat. Crude fat was determined by ether extract method using soxhlet apparatus. A quantity (1g) of sample was first wrapped in a filter paper, placed in a fat free thimble and then introduced into the extraction tube. Weighed, cleaned and dried receiving beaker was filled with petroleum ether and then fitted into the apparatus. Water and heater were then turned on to start extraction. Ether was allowed to evaporate, after four to six siphoning and the beaker was disconnected before the last siphoning, extract was then transferred into a clean glass dish, washed with ether and evaporated on water bath. The dish was then placed in an oven at 105 C for two hours and subsequently cooled in a desiccator.
The percentage crude fat was calculated using the formula:

$$\% \text{ Crude fat} = \frac{\text{Wt. of ether extract} \times 100}{\text{Wt. of sample}}$$

Determination of crude protein
Kjeldahl method was used in the determination of protein. Some (1g) of dried sample was taken in digestion flask and 15ml of concentrated H_2SO_4 and eight grams of digestion mixture was added (K_2SO_4 and $CuSO_4$). The flask was swirled so that the contents of the flask were mixed thoroughly, and then the flask was placed on a heater for digestion. After digestion the mixture became clear (blue-green colour). The digest was subjected to distillation using Markam Still distillation apparatus. An aliquot (10ml) of digest was introduced in the distillation tube and 10ml of 0.5N NaOH was gradually added via the same way. The distillation process continued for at least 10minutes and NH_3 that was produced was then collected as NH_4OH in a conical flask containing 20ml of 4percent boric acid solution with few drops of modified methyl red indicator. Yellowish colour appeared during distillation due to the presence of NH_4OH and the distillate was then titrated against standard 0.1N HCl solution till a pink colour appeared. A blank was also run through all steps as

above.
Percentage crude protein was calculated by the formula:

% Crude protein = 6.25* x %N (*Correction factor)
$$\%N = \frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Wt. of the sample} \times V}$$
Where, S= Sample titration reading, B= Blank titration reading, N = Normality of HCl, D = dilution factor of sample after digestion, V = Volume taken for distillation, 0.014 = Milli equivalent weight of Nitrogen.

Determination of carbohydrate (nitrogen free extract- NFE)
Difference method was used in the calculation. Carbohydrate content was gotten by subtracting the sum of moisture, protein, fat and ash from 100 percent.
Percentage carbohydrate (NFE) was calculated by the formula:
NFE = 100 – (% moisture + % crude protein + % crude fat + % ash).

Mineral profile
Determination of calcium, potassium and sodium
Calcium, potassium and sodium were determined using flame photometer as described by AOAC (8). Some (2g) of the sample was ashed using the muffle furnace. Then, 5ml of 2M HCl was used in digesting the ash of each sample in a crucible and then heated to dryness on a heating mantle. Another 5ml of 2M HCl was added, then heated to boil and Whatman No.1 filter paper was used to filter the mixture into a volumetric flask of 100ml capacity, after which the filtrate was made up to mark using distilled water, stoppered and made ready for the reading of the concentration of calcium, potassium and sodium on the flame photometer using the filter corresponding to each mineral element.

Determination of phosphorus by spectrophotometer
Phosphorus was determined by Vanado-Molybdate spectrophotometric method as described by AOAC (8). A quantity (2g) of the sample was ashed using muffle furnace. The ash of the sample was digested using 2M HCl as described for calcium above, after which 10ml of the filtrate solution was pipetted into a standard flask of 50ml and 10ml of vanadate yellow solution was added to the mixture. The flask was made up to mark using distilled water, stoppered and left for 10 minutes for the full yellow colour development. The absorbance of the yellow solution was read at a wavelength of 470nm on a spectronic 20 spectrophotometer.

Determination of selenium, magnesium, manganese, iron, zinc, copper, chromium and cobalt using BUCK 200 AAS.
Selenium, magnesium, manganese, iron, zinc, copper, chromium and cobalt were all determined using Buck 200 atomic absorption spectrophotometer (AAS) as described by AOAC (8). Some (2g) of the sample was ashed using muffle furnace. The ash of each sample was digested using 2M HCl as described above for calcium and the digest was washed with distilled water into a 100ml volumetric flask and was made up to mark using distilled water. This resulting diluent was aspirated into the Buck 200 Atomic Absorption Spectrophotometer (AAS) via the suction tube and each trace element was read at their respective wavelength with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

The meter reading for each of the mineral element was used in calculating their respective concentrations using the formula:
mg/kg (of any of the elements) = Meter reading x slope x dilution factor.
Note that the dilution factor for phosphorus is 2,500, 10,000 for magnesium and 100 for other minerals.

Results
The results of proximate composition and selected mineral composition of *Hyptis verticillata* leaves are presented below.

Table 1 below shows that the leaves of *H. verticillata* contain high concentration of carbohydrate (70.59±0.19%), appreciable concentration of moisture, crude ash and crude protein (12.58±0.07%, 8.36±0.33 % and 6.43±0.14%, respectively) and low concentration of crude fat (2.07±0.08%) while in Table 2, the result obtained indicates the presence of calcium, potassium and sodium with concentrations of (2,640.26±2.84mg/kg, 2,423.09±2.78 mg/kg and 1,681.10±12.50 mg/kg, respectively) while the other

minerals were present in low concentration ranging from copper ($0.02\pm2.0x10^{-3}$ mg/kg) to phosphorus (0.82±0.01 mg/kg) in *H. verticillata* leaves.

Table 1: Proximate composition of *H. verticillata* leaves

Parameter	Content (%)
Moisture	12.58 ± 0.07
Ash	8.36 ± 0.33
Lipid	2.07 ± 0.08
Protein	6.43 ± 0.14
Carbohydrate	70.59 ± 0.19

Values are expressed as mean ± standard deviation of triplicate determinations.

Table 2: Mineral composition of *H. verticillata* leaves

Parameter	Content (%)
Sodium	1,681.10 ± 12.50
Potassium	2,423.09 ± 2.78
Calcium	2,640.26 ± 2.84
Phosphorous	0.82 ± 0.01
Magnesium	0.23 ± 0.01
Manganese	$0.12 \pm 4.0 \times 10^{-3}$
Iron	0.19 ± 0.01
Copper	$0.02 \pm 2.0 \times 10^{-3}$
Zinc	$0.18 \pm 2.0 \times 10^{-3}$

Values are expressed as mean ± standard deviation of triplicate determinations

Discussion

The results obtained from the proximate analysis indicated high concentration of carbohydrate and low level of crude fat, protein and ash respectively in *H. verticillata* leaves (Table 1). This result was similar to the work carried out by Nnamani *et al*. (9) on three underutilized indigenous leafy vegetables of Ebonyi State, Nigeria. From the results obtained from this study, *H. verticillata* leaves could serve as a good source of carbohydrate. The low-fat content of *H. verticillata* leaves indicated low risk of lipid related complications on consumption of the plant as tea/decoction. The crude ash content which was simply the measure of the amount of minerals present was within the same range as reported by Nnamani *et al*. (9) and higher than those of *L. africana, H. crinita and G. latifolium* as reported by Alobi *et al*. (10). The crude protein value showed closeness to the report of Agbaire (11) on cassava leaf (*M. esculenta*) and also within the range of the five medicinal plants reported by Adnan *et al.* (4). The moisture content was not high comparable to the carbohydrate content of the plant, however, the level was in appreciable amounts which was higher than those of the five medicinal plants reported by Adnan *et al*.(4). Moisture content is used to measure the stability of food as it predisposes the food to microbial spoilage according to Osabor *et al*. (12). The carbohydrate content of the plant corresponds to the work of Adnan *et al.* (4) that investigated five medicinal plants of humid and sub-humid regions in the North-west Pakistan. Nnamani *et al*. (9) also had similar report on three indigenous leafy vegetables of Ebonyi State, Nigeria.

Minerals are very essential for the proper functioning of the body and the amount needed by the body is not directly proportional to its importance in the works of Romito and O'Brien (5). There was a decrease in all the minerals with the exception of calcium (Ca), potassium (K) and sodium (Na) (Table 2). It is a known fact that the sodium levels of plant food in Nigeria are far less than those of potassium as reported by Adesuyi *et al*.

(13) and this is in agreement with the present study. Potassium is the most abundant of all minerals present in agricultural product in Nigeria by Rimbach *et al.* (14) and the result obtained from this study indicated a similar pattern except the calcium level which had the highest concentration. Calcium, potassium, sodium and magnesium are macrominerals and they play important roles in maintaining body fluid balance, the normal functioning of bone, heart, brain and muscles are related to adequate amount of these macrominerals as reported by Larry (6). The high level of calcium indicates that the plant is a very good source of calcium. The value of calcium is slightly higher than that reported on *Garcinia kola* by Adesuyi *et al*. (13).

Microminerals which were present in very low concentration in *H. verticillata* leaves are essential in metabolic activities because they can be incorporated into enzymes or hormones necessary for the body's metabolism except for Cr which is required for blood glucose maintenance by Larry (6). Selenium and copper were of the lowest concentration in *H. verticillata* leaves. When selenium level exceeds its safe upper limit (400µg) it can lead to selenosis/ selenium poisoning but at low dose it is beneficial to human health and can serve as antioxidant, anticarcinogen and immunomodulator reported by Pham-Huy *et al*. (15). Copper is a component of many enzymes involved the generation of energy, formation of red blood cells, bone and connective tissue and for the metabolism of iron. It also has antioxidant activity and when its level exceeds the safe upper limit (10,000µg) it leads to copper toxicity (5, 6).

Conclusion

The present study investigated the proximate and selected mineral composition of the leaves of *H. verticillata* cultivated in Calabar, Cross River State, Nigeria. The proximate analysis of the leaves showed increased carbohydrate level making it a good source of fuel/energy with low crude lipid. We therefore suggest that consumption of *H.verticillata* as tea or decoction may meet the macromineral requirement especially calcium, potassium and sodium in the body.

Conflict of interests

The authors did not have any conflict of interest.

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The Effects of Nanosilver and Endothelial Modulators on Cardiovascular Indices in Salt-Induced Hypertensive Sprague- Dawley Rats

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Abstract

Silver Nanoparticles (AgNPs) have gained extensive biomedical application on its cellular functions, especially pro-oxidant/antioxidant and vascular endothelial activities. This present study examined the effects of nanosilver and vascular endothelial modulators on cardiovascular indices on salt-induced hypertensive Sprague Dawley rats (SHRs). Forty-two (42) rats (110-130g) were randomly separated into seven groups of six rats each. Group 1 [(Control rats (CR)] received normal rat chow containing 0.3% NaCl and given tap water to drink *ad libitum*. Group 2 received chow containing 8% NaCl (high salt diet-HSD) and tap water. Group 3 received normal rat chow and 50 mg/kgbw/day of 10 ppm nanosilver solution (NAg). The remaining experimental groups concomitantly received HSD + 50 mg/kgbw/day of NAg and/or 50 mg/kgbw/day of L-arginine, L-NAME and Vitamin C orally respectively for six weeks. Measurements of blood pressure (Bp) and heart rate (HR) were by pressure transducer via cannulation of the left common carotid artery following anaesthesia intraperitoneally by a mixture of 25% urethane: 5% chloralose at 5 ml/ kgbw. Fasting blood was collected for blood glucose determination spectrophotometrically. The results showed that Bp was significantly ($p<0.05$) elevated (181.27±4.92/116.39 ±6.23) mmHg in SHRs and remained fairly constant (114.73±11.33/69.07±6.78) mmHg in nanosilver treated normotensive rats (NTNRs) compared to the CR (127.92±5.37/76.00±6.31). Also, Bp was significantly elevated in the HSD-fed rats cotreated NAg, and/or with L-Arginine and particularly enhanced (204.58±8.12/154.17±3.63) mmHg in L-NAME cotreated group with cerebral accident and hemiplegic manifestations by half the number of rats. Vitamin C in combination with NAg resulted in attenuation of Bp particularly diastolic Bp. Elevation in Bp was greater in systolic than in diastolic blood pressure. NAg significantly ($p<0.05$) decreased fasting blood glucose level of normal and HSD-fed rats. Whereas NAg in combination with Vitamin C prevented a decrease in blood glucose level. Heart rate showed no significant difference in all the groups. In conclusion, these findings support the fact that high-salt diet induces hypertension in rats and blood pressure was elevated in HSD-fed rats cotreated nanosilver, and/or with L-Arginine and L-NAME but attenuated in cotreated Vitamin C in combination with NAg, particularly in diastolic and mean arterial pressure. However, bp remained normal in normotensive rats treated NAg.

Key words: Salt-induced hypertension, nanosilver, blood pressure, and blood glucose.

Introduction

Several epidemiological studies and experimental animal models have demonstrated the critical importance of high sodium salt intake in the development of hypertension (1-3). High sodium diets are commonly used to study diet-induced hypertension, since increasing levels of circulating sodium cause cells to release water, which elevates the pressure on blood vessel walls (4). Lewis Dahl developed, from selectively inbred Sprague Dawley rats, the Dahl salt-sensitive (Dahl SS) and Dahl salt-resistant (Dahl SR) rats based on their response to 8% NaCl diet (5-6). Experimental animal models have shown that Sprague-Dawley rats [(SDR, (inbred)] rapidly develop elevated blood pressure (>170 mm Hg) when fed 8% NaCl diet (7-9). The mechanisms linking high salt intake to hypertension appear to be complex and involve alterations in both reflex function and in the contractile properties of the vascular smooth muscle (10-11).
Furthermore, in various types of hypertension in the rat, conflicting reports exist on decreased or enhanced endothelium-dependent vasorelaxant effect in vitro (2).

The endothelium is a monolayer endothelial cells that lines the entire cardiovascular system and constitutes a barrier between blood and tissues and regulates the exchange of molecules between blood and tissues (12-13). Functionally the vascular endothelium secretes a wide variety of vasoactive substances and factors

including nitric oxide (NO), prostacyclin, kinins, endothelium-derived hyperpolarizing factors (EDHF) endotheline-1, prostagladin-2 and reactive oxygen species (ROS) in response to both humoral and mechanical stimuli, which are of importance in the mediation and modulation of vascular tone and reactivity, blood pressure, protection of the vasculature from inflammatory damage and provides a permeability barrier to regulate blood flow, volume and electrolyte content (13,14). NO is an important mediator of endothelial cell function and several factors are known to modulate its function. Endothelial dysfunction (EDF) reflects pathophysiological changes in the phenotype and functions of endothelial cells that result from and /or contribute to a plethora of cardiovascular diseases including atherosclerosis, hypertension and inflammatory syndrome (13,15). Specific endothelial modulators that promote and inhibit NO synthesis including L-Arginine and L-nitroargine methyl ester (L-NAME) have proved very useful in understanding effects of NO (16). It is now well established that endothelial factors are of importance in the mediation and modulation of both vasoconstriction and vasodilatation (17-19), thereby affecting blood pressure. It is not known, however, whether the actions mediated by EDNO or by EDHF are affected by the level of salt intake. Increasing evidence has accumulated in recent years to suggest that a high salt diet increases oxidative stress and endothelial factor, endotheline-1(Et-1) activates NADPH oxidase in isolated arterial tissue and increases the production of reactive oxygen species (ROS) (20). Under physiological conditions, a balance exits between levels of reactive oxygen species produced in normal cellular metabolism and the endogenous antioxidant defense. An imbalance between the antioxidant capacity and the production of ROS leads to oxidative stress. In recent times however, changes in lifestyle and dietary supplementation have been shown to be effective in the management and /or prevention of hypertension in combination with routine clinical therapies (21-23).

Nanoparticles are heterogeneous substances with a size range of between 1 - 100 nm in at least one dimension and characterized by a high surface area-to-mass ratios resulting in better activity (24-25). Nanotechnology has received much significance recently owing to its wide applications in industrial and biomedical sciences. Nanosilver in the form of colloidal silver, has been used for more than 150 years and has been registered as a biocidal material in the United States since 1954 (26). Silver nanoparticles (AgNPs) are one of the fastest-growing nanomaterial categories for consumer, industrial, and biomedical applications owing to their unique properties including high electrical and optical conductivity, chemical stability, catalytic activity, and particularly enhanced surface area ratios (27). Recent studies on use of silver in nanosize as an alternative to antibiotics and its probiotic properties with increasing immunity have led to use of this nanoparticles largely, especially in veterinary and dependent sciences (28). Experimental studies were not designed to probe the mechanism of vascular effects but rather an observation of abnormal vascular changes associated with nanoparticles studies conducted primarily for pharmacokinetic and biodistribution endpoints (29).

There are flash point indications that nanoparticles may be detrimental to arterial function, thereby compromising cardiovascular effectiveness in redistribution of blood (30-32). Furthermore, there is paucity of information about the effects of these nanoparticles on environmental impact, human health, particularly more specific in the cardiovascular system, since this system represents an important route of action in terms of distribution, bioaccumulation and bioavailability of the different circulating substances in the bloodstream. To our knowledge, literature is scarce on the effects of nanosilver particles and silver ion on blood pressure and vascular endothelial modulators. Therefore, present study was carried out to examine the effects of nanosilver on cardiovascular indices, blood glucose level and modulation by antioxidant (vitamin C), vascular endothelium nitric oxide (NO) donor (L-Arginine) and NO synthase inhibitor (L-NAME) on salt-induced hypertensive Sprague-Dawley rats.

Materials and Methods

Animals

This experimental study was performed with Forty-two (42) Sprague-Dawley rats (SD) (inbred) (110-130g) randomly separated into seven groups of six rats each. The experiments were done in line with guidelines on use of animals for experiments as issued by the Physiological Society of Nigeria and Physiological Society, London. Animals were maintained according to standard laboratory procedure and given free access to normal rat chow and tap water ad libitum. Group one, control rats (CR) received normal rat chow and water *ad libitum*. Group 2 received high salt diet (HSD) containing 8% NaCl as described by Sofola *et al* (5). Briefly, the high salt chow was prepared by mixing 76 g of NaCl with 924 g of chow.

The rats were fed on these diets for 6 weeks with tap water given *ad libitum*. Group 3 was treated with normal rat chow + 0.18 ml /kgbw/day of 10ppm 50mg nanosilver solution (NAg), Group 4 was treated with HSD + NAg, Group 5 was treated with HSD + NAg + L-arginine (100 mg/kgbw/day), Group 6 was treated with HSD + NAg + L-NAME (25 mg/kgbw/day) and Group 7 was treated with HSD and NAg + Vitamin C (50 mg /kgbw/day) orally for a period of 6 weeks. Measurements of blood pressure [(Bp) (mmHg)] and heart rate (HR) were by blood pressure transducer via cannulation of the left common carotid artery following anaesthesia as previously reported (33-34). Briefly the rats were anaesthetised by a mixture of 25% urethane: 5% chloralose given intraperitoneally at the dose of 5 ml/kg body weight. The trachea was exposed by blunt dissection and cannulated for smooth breathing. The left common carotid artery was similarly exposed and cannulated. The arterial cannula was then connected to a pressure transducer (Statham 23D) and coupled to a twin channel Ugo Basile (Gemini 7070) recorder which was previously calibrated with a mercury manometer for recording blood pressure and heart rate. 3 ml of fasted blood was taken for blood glucose analysis and determination spectrophotometrically.

Chemicals

The following chemical reagent and drugs were used: Sodium Chloride, L-Arginine, Vitamin C (analytical) and L-nitro-arginine methyl ester (L-NAME) (Sigma Chemical Company, USA). Nanosilver (Innovative biotech Inc., Abuja).

Statistical analysis

Graphs and statistical analyses were by means of OriginPro 8.0 software and Students t-test; followed by one-way analysis of variance (ANOVA). Data are presented as means ± SEM. P-values less than 0.05 was considered statistically significant; while n-values denote number of animals in each experimental group

Results

Changes in blood pressure following dietary supplementation

Modulation in blood pressure (Bp) (mmHg) following dietary supplementation of Nanosilver, L-Arginine, L-NAME and Vitamin C in salt-induced hypertensive Sprague-Dawley rats is shown in Fig.1A & B. Both systolic and diastolic blood pressure were significantly elevated (181.3±4.9/116.4±6.2) mmHg respectively in the salt-induced hypertensive rats (SHRs) compared to the control (127.9±5.4/76.0±6.3) mmHg. Blood pressure remained low (114.7±11.3 and 69.1±6.8) mmHg in nanosilver treated normotensive rats (NTNRs) whereas in the high-salt diet cotreated nanosilver, and/or with L-Arginine and L-NAME, nanosilver was ineffective in Bp attenuation. Vitamin C in combination with nanosilver resulted in attenuation in Bp particularly in diastolic Bp. N = 6, ($p<0.05$).

Mean arterial blood pressure (MAP) was significantly elevated in SHRs and in the HSD-fed rats (HSDRs) co-treated nanosilver, and/or with L-Arginine and L-NAME respectively compared to the control. In contrast, MAP significantly decreased in nanosilver treated normotensive rats (NTNRs) compared to the control and attenuated in HSDRs co-treated nanosilver in combination with Vitamin C compared to HSDRs cotreated nanosilver as shown in table 1. N=6, ($p<0.05$).

Table 1: Influence of administration of nanosilver and/or with L-Arginine, L-NAME and Vitamin C on SHRs on MAP.

GROUPS	MAP (mmHg)
Control	93.30 ± 5.35
Salt-induced hypertensive rats (SHRs)	*135.90 ± 6.32
NANOSILVER (NAg)	*84.42 ± 7.67
HSDRs + NAg	*167.35 ± 10.50
HSDRs + NAg + L-Arginine	*117.75 ± 13.99
HSDRs + NAg + L-NAME	*170.97 ± 4.42
HSDRs + NAg + Vitamin C	93.14 ± 5.83

* Significantly different values in means ± SEM compared to the control; $P<0.05$

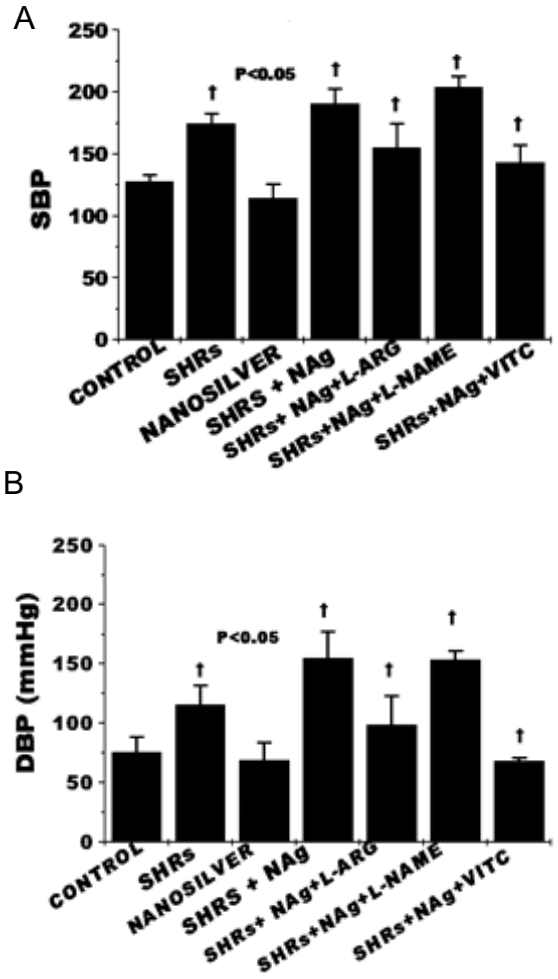

Fig.1: Systolic blood pressure (SBP) mmHg

Table 2: Influence of administration of Nanosilver and/or with L-Arginine, L-NAME and Vitamin C on pulse pressure (mmHg) in SHRs

GROUPS	MEAN
Control	51.91 ± 5.81
Salt-induced hypertensive rats (SHRs)	*58.55 ± 4.51
NANOSILVER (NAg)	**45.66 ± 8.00
HSDRs + NAg	**35.46 ± 4.70
HSDRs + NAg +L-ARGININE	*57.00 ± 10.00
HSDRs + NAg + L-NAME	50.41 ± 7.08
HSDRs +NAg+VITAMIN C*	*75.55 ± 11.87

* Significant increase. ** Significant decrease

Pulse pressure (PP) mmHg significantly ($p<0.05$) increased in SHRs and HSD-fed rats (HSDRs) cotreated NAg in combination with L-Arginine and/or Vitamin C; but decreased significantly in NTNRs and HSDRs cotreated with NAg compared to the control. N = 6.

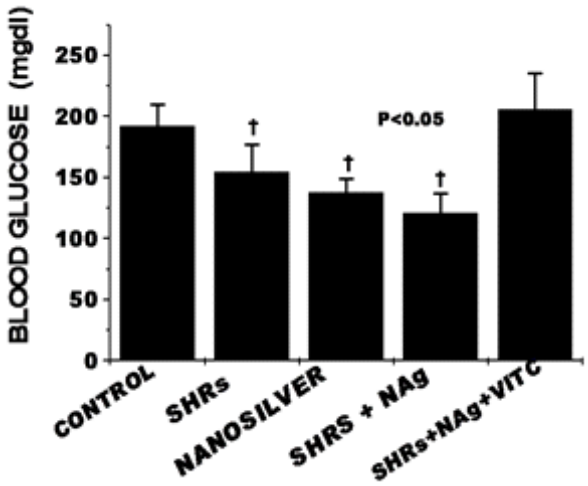

Fig.2: Changes in blood glucose level in test groups
Blood glucose level in test groups

In Figure 2, blood glucose level was significantly ($P<0.05$) decreased in SHRs and the decrease particularly marked in NTNRs as well as SHRs cotreated NAg compared to the control; whereas NAg in combination with Vitamin C prevented a decrease in blood glucose in SHRs. N = 6; means ± SEM.

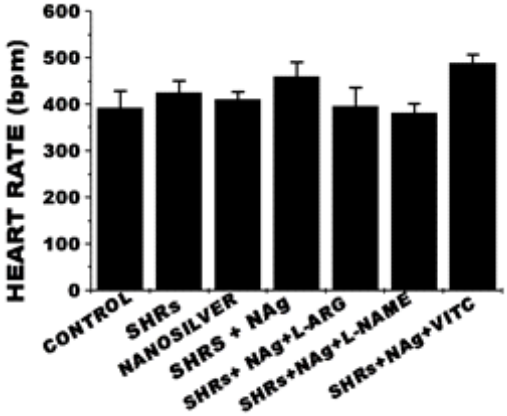

Fig.3: Changes in heart rate (bpm) in test groups

There was no significant change in heart rate in all the test groups compared to the control as shown in figure 3.

Discussion

The present study examined the effects of nanosilver on cardiovascular indices (blood pressure and heart rate), blood glucose level and modulation by vascular endothelium nitric oxide (NO)- donor (L-Arginine) and NO synthase inhibitor (L-NAME) as well as vitamin C (antioxidant), on salt-induced hypertensive Sprague- Dawley rats. The results from the present study has clearly demonstrated the significant elevation in systolic, diastolic and mean arterial blood pressure in Sprague-Dawley rats fed

with 8% NaCl for a period of six weeks. This, further confirmed previous studies that chronic administration of high salt diet (8% NaCl) resulted in elevated blood pressure in Sprague Dawley rats (5,35). Also, blood pressure remained fairly constant in nanosilver treated normotensive rats compared to control whereas blood pressure was significantly elevated in the high-salt diet cotreated nanosilver, and/or with L-Arginine and markedly enhanced (>200mmHg) in L-NAME with cerebral accident, death and hemiplegic manifestations by half the number of rats in this group.

With regards to mechanism of action, previous studies suggest that aside from inducing hypertension, high-salt diet induces oxidative stress and that hypertension is associated with higher than normal lipoperoxidation and imbalance in antioxidant status suggesting that increased vascular oxidative stress contributes to pathophysiology of endothelial dysfunction and hypertension (38,39). Also hypertension has been shown to be associated with impaired nitric oxide (NO) synthesis (18,40). In general, vascular endothelium is acknowledged to play a vital role in cardiovascular health owing to its production of nitric oxide which is important in maintaining vascular homeostasis. In another experiment, high-salt diet fed rats were co-treated with nanosilver and endothelial modulators-vascular endothelium nitric oxide (NO) donor (L-Arginine), NO synthase inhibitor (L-NAME) and antioxidant (vitamin C). L-arginine being the substrate for the enzyme nitric oxide synthase (NOS), which is responsible for the endothelial production of nitric oxide is thought to augment NO production via oral supplementation thereby improving vascular health (36). However, the data from this study showed that L-arginine was ineffective in ameliorating elevation in blood pressure in high-salt diet fed Sprague-Dawley rats. The observation in blood pressure elevation in high-salt diet fed rats co-treated nanosilver and L-arginine appears to corroborate with early report that chronic L-arginine supplementation causes endothelial dysfunction through up-regulation of Arg-II, an enzyme that metabolizes L-arginine and is predominantly involved in accelerating vascular endothelial cell senescence (37).

Additional observation is the greater blood pressure attenuation effect of vitamin C in salt-induced hypertension particularly in diastolic and mean arterial blood pressure compared to L-arginine an endothelium nitric oxide donor. Vascular endothelium derived nitric oxide (eNOS) plays a critical role in the regulation of vascular tone and reactivity and it appears that vitamin C may offer endothelium protection effect and probably a greater oxidative stress-related influence in salt-induced hypertension. Pulse pressure was also significantly decreased in HSDRs co-treated with nanosilver (35.46 ± 4.70 mmHg) compared to the control but elevated in HSDRs co-treated with nanosilver in combination with vitamin C (75.55 ± 11.87 mmHg). This could be attributed to the elevation in systolic blood pressure in HSDRs co-treated with nanosilver in combination with vitamin C and decreasing diastolic blood pressure (figure 1) thereby widening pulse pressure; which may be associated with atherosclerosis and stiffening of arterioles (41).

Previous experimental nanosilver animal models studies have reported associated increased oxidative stress with increased production of superoxide anion which can react with NO and may impair normal physiological cellular activities (42). Also experimental hypertension is associated with increased production of superoxide anion, products of lipid peroxidation may decrease NO and prostacyclin formation and exhibit direct vasoconstrictor properties (23,7,24). Similarly, there have been some reports that nitric oxide (NO) may play a role in mechanism of salt-induced hypertension. Chen and sanders(43) showed that intravenously given L-arginine lowered blood pressure in Dahl salt sensitive rats whereas Nitric oxide synthase (NOS)-inhibition raised blood pressure in dahl resistant. Luscher and coworkers (44) had earlier described endothelial dysfunction in high salt diet induced hypertension and proposed diminished EDHF-production (now identified as NO) as a causetive factor of elevated blood pressure.

Epidemiological studies have demonstrated that the dietary intake and plasma concentrations of ascorbic acid, a potent water-soluble antioxidant correlate inversely with hypertension and its clinical sequelae, namely, stroke and cardiovascular disease (18,39). Several previous studies have shown some beneficial effect of ascorbic acid treatment on endothelium-

dependent vasodilation in essential hypertension (23,40). The observation in the effect of Vitamin C in combination with nanosilver in ameliorating salt-induced hypertension in this study is in consonance with this report. There were no significant changes in heart rate in all the test groups which probably excludes autonomic nervous system inclusion in the modality of activities of the various vasoactive substances studied.

It was however observed that blood glucose level was significantly decreased in the salt-induced hypertensive rats (SHRs) and much more decrease in SHRs co-treated with nanosilver but reversed in the SHRs co-treated with Vitamin C. Previous study (45) reported that administration of nanosilver inhibits carbohydrate digestive enzymes (α-glucosidase and α-Amylase) which enhances glucose uptake rate. Thus the significant decrease in blood glucose concentration could be as a result of these carbohydrates enzymes that are inhibited by Nanosilver, thereby preventing glucose uptake and resulting in a decrease in blood glucose concentration.

Conclusion

In conclusion, the findings of the present study suggest that nanosilver and L-arginine were ineffective in modulation of blood pressure elevation in salt-induced hypertensive rats, however, blood pressure was attenuated particularly in diastolic and mean arterial pressure in high-salt diet-fed rats co-treated nanosilver in combination with Vitamin C. Blood pressure remained normal in normotensive rats treated with nanosilver while blood glucose level was decreased in all the test groups except in high-salt diet-fed rats co-treated nanosilver in combination with Vitamin C.

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