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Research Article

The Pharmaceutical Properties, Microbial Quality, *In-vivo* Aphrodisiac Effect and Safety of Some Herbal Bitters Sold in Southwest Nigeria

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ABSTRACT

Aphrodisiac drugs are used to enhance sexual activity and rectify erectile dysfunction especially among older men. In Nigeria, herbal medicinal products formulated as herbal drinks/bitters are prevalent and sold in various locations including motor parks, store and markets. Despite their wide use, the potency and safety of the herbal drinks have not been ascertained. Thus, pharmaceutical and aphrodisiac properties of five randomly selected herbal drinks with aphrodisiac claim have been evaluated. Pharmaceutical and microbial qualities were evaluated using standard procedures and the *in vivo* aphrodisiac activities were evaluated in male Wistar rats. The effects of chronic consumption of the bitters on the biochemical and tissue histology were assessed. The herbal bitters exhibited low viscosity (< 10 cP), high alcohol content (30 – 52 %), acidic pH (3.33 - 5.40), and low density (0.942-1.070 g/ml). The phytoconstituents include alkaloids, flavonoids, saponins, cardenolides, tannins and anthraquinone. Microbial contaminations were with the limits for oral preparations. The bitters exhibited significant ($p < 0.001$) aphrodisiac effect but had no significant effect on fertility and hematological parameters. Chronic consumption of the herbal drinks at 250mg/kg following was hepatotoxic while two brands were cardiotoxic and nephrotoxic. There is therefore the need to monitor and control the quality and use of herbal bitters/drinks sold in the Nigerian market to safeguard public health.

Keywords: *Herbal Bitters, Pharmaceutical and microbial quality, aphrodisiac properties, safety*

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INTRODUCTION

Sexual function is an important component of quality of life and subjective well-being in humans. Sexual problems are widespread and adversely affect mood and interpersonal relationships (Sadovsky and Nusbaam, 2006). Erectile dysfunction (ED) is one of the major sexual problems causing significant distress in men. It ranges from partial decrease in penile rigidity to complete erectile failure (Rowland and Tai, 2003). ED is a hidden condition in which patients rarely volunteer information due to a variety of factors including embarrassment and a feeling that little can be done (Ariba *et al*., 2007). It is associated with significant morbidity and can impair the patient's quality of life. It may also be associated with depression, increased anxiety and poor self-esteem (NIH, 1993a). Erectile dysfunction is a serious health problem that has received public attention in recent times. The prevalence of ED has been estimated worldwide as 2 % in men who are younger than 40 years and as high as 86 % in men who are 80 years and above (NIH, 1993 b). Recent studies in sub-saharan Africa and specifically in Nigeria has reported the prevalence of ED as 34.6 -61.7 % (Ugwumba *et al*., 2018; Agaba *et al*., 2017; Yovwin *et al*., 2015). This situation highlights the need to pay more attention to this silent public health problem.

Studies have shown that the standard of living and current trend in healthcare have made man to opt for alternative medicine especially in developing countries (Oyelade *et al*., 2017). According to the World Health Organization (WHO), 80 % of the world's population depends on herbal medicine for treatment of various ailments (WHO, 1998). Despite availability of conventional medicines, plant-derived herbal remedies are most preferred by the populace because of their

perceived safety, effectiveness and affordability. The use of herbal medicinal products (HMPs) cuts across social class and this has led to the formulation of herbal drugs into conventional dosage forms such as solutions, suspensions, capsules, tablets, ointment, etc.

Traditionally, aphrodisiac drugs are used for the treatments of erectile dysfunction in older men. Recent incidence of prolific sexuality due to unfriendly socio-economic environment in Nigeria, characterized by poverty, work pressures and stress, has encouraged the use of sex enhancing drugs in such a way that it cut across ages (Bodeker and Kronenberg, 2002). Several plants have been claimed by herbal practitioners across the world to have aphrodisiac potentials for the treatment of sexual dysfunctions (Ajala and Omobowale, 2013). In Nigeria, there are several marketed herbal drinks with aphrodisiac claims sold in retail shops, markets and motor garages to enhance sexual performance. Utilization of these herbal medicinal products formulated as herbal bitters as sex enhancers is more prevalent in urban locations, where population and mass hysteric of sexuality are prevalent (Bodeker and Kronenberg, 2002). Among the Yoruba of southwestern Nigeria, gender space and sex culture now parade a number of locally formulated herbal drinks such as *Paraga, Alomo (Kasapreko*), *Alomo (Casaman),Osomo etc.*, for the enhancement of sexual performance (Ajala and Omobowale, 2013). These herbal bitter drinks are alleged to enhance sexual pleasure especially in individuals with low libido and other sexual dysfunctions that impaired sexual satisfaction or drive. This situation has become a threat to public health since men have been observed to be irrational as some take a bottle of about 100 - 200 mL content and just gulp it down the throat with the aim of enhancing their sexual performance. However, the potency, efficacy, toxicity profile and pharmacological properties of these preparations has not been fully substantiated nor documented. Thus, in the present study, the pharmaceutical quality, *in vivo* aphrodisiac activity and toxicity of five most common brands of herbal bitters used with claims of improvement of sexual performance among men are evaluated.

MATERIALS AND METHODS

Materials

Herbal drinks: Five brands of herbal drinks with aphrodisiac claim were procured from retail outlets in Ibadan metropolis, south west Nigeria, while Viagra® (sildenafil citrate) was obtained from Pfizer Pharmaceuticals (Paris, France). The details of the herbal drinks are presented in Table1.

Laboratory animals; Wistar rats: 110 male weighing 40-150 g and seven females weighing 60 -70 g) were obtained from the Animal House of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. The animals were housed in clean cages, placed in well ventilated environment and allowed free access to standard rat pellets and fresh water *ad libitum*. They were allowed to acclimatize to laboratory conditions for two weeks before commencement of the experiments. The protocols for the experiments were approved by the Animal Care and Use for Research Ethics Committee of the University of Ibadan with approval code UI-ACUREC/App/2016/014**.** All reagents used were of analytical grade.

Table 1:
Details of the herbal bitters used for the study

Product code	NAFDAC registration number	Contents as stated on product
VA	04-1509	Sildenafil citrate
OR	0000572	Neutral spint, sugar, citric acid, trisodium citrate, caramel extract (naartjie, chamomile, thyme, cinnamon and orange), Alcohol (30 %)
AL	A1-8029	Ethylalcohol, *Khaya ivorensis*, *Capparis erythrocarpus*, *Lecaniodiscus cupanoides*, *Dialium guineense*, *Treculia africana* and flavours. Alcohol (42 %)
OE	08-1509L	Ethyl Alcohol, Water, Bitter Extract, *Moringa oleifera*, Vanilla Flavour and Caramel. Alcohol (33 %)
BO	B1-4103L	Caramel Herbal flavour, Angela root, Cassia senna leaf, Rhubarb root & Aloe. Alcohol (42 %)
OS	A7-1298L	*Callichila bateri, Pachylotus edulos, Lecaniodiscus, Allium Sativum, Zingiba Officinale, Monodora Speciesa, Khaya Ivorensis, Pipernigrum, Eugenia Caryophyll*, Alcohol (15 %)

Pharmaceutical properties

The organoleptic properties of the herbal drinks were determined by transferring 5 mL of the sample into a clean test tube and viewed to determine the clarity and colour. The presence of particles was determined by centrifuging each herbal drink at 3000 rpm while pH was measured using a pH meter (Jenway, Model 3520, Essex, UK). The viscosity was determined using a Brookfield viscometer (VT 181, Karlsruhe, Germany) at 29.6 ± 2 ^{o}C with spindle number 3 and rotational speed of 100 rpm. Density of each HMP was done by determining the weight of 20 mL of the HMP and density was calculated using equation 1.

$$Density = Weight\ (g)/Volume\ (mL) \qquad (1)$$

Alcohol content was determined by gas liquid chromatography using US Pharmacopeia method II (USP 29-NF24) with slight modifications. Gas liquid chromatography consisted of a gas chromatograph equipped with a flame ionization detector and a 4 mm x 1.8 m glass column packed with 100 to 120 mesh chromatographic column packing No. S3, having nitrogen as a carrier. Chromatographic column was maintained at 120 ^{o}C, the injection port and detector were upheld at 210 ^{o}C while carrier flow and temperature were adjusted to that of acetonitrile; the elution was done between for 5 to 10 min. For analyses, 5 µL of each of test stock preparation and standard preparation were injected in

duplicates into the gas chromatograph and chromatograms were recorded and the peak response ratios determined. The alcohol content (v/v) was calculated and expressed in percentage.

Phytochemical tests for flavonoids, tannins, saponins, alkaloids, cardenolides and anthraquinones were performed on the HMPs using standard procedures (Watcho *et al*., 2007).

Microbial quality

Microbial quality of the herbal formulations was assessed by diluting 1 mL of each HMP was diluted with 9 mL of sterile distilled water. Serial dilutions were carried out and viability assessed using pour plate method. The Plates were incubated at 37 ^{o}C for 24 h. The media utilized were Nutrient agar, Cetrimide Nutrient agar, MacConkey agar, and Mannitol Salt agar. Plates were then placed on a colony counter and number of colony-forming units determined.

For detection of fungal growth, Sabouraud dextrose agar was poured into a plate and allowed to set, then 1mL aliquot of each sample was spread on the surface and plates were incubated at 27 ^{o}C for 72 h.

In-vivo aphrodisiac effect

The aphrodisiac properties of the herbal drinks in male rats were evaluated for 30 days using Viagra at 50 mg/kg and distilled water as positive and negative controls, respectively. HMPs were administered at various doses to different groups after which rats were randomly selected from the sub-group on days 10, 12, 15, 17, and 20 and put inside different cages. Female rats were introduced into the cages and observation of sexual behavior was done under dimmed light. Parameters evaluated include anogenital sniffing, mount latency and mount frequency. In addition, semen volume, sperm motility, sperm livability, nitric oxide assay and hormonal assay were determined as described below:

Determination of semen volume: Semen volume was measured by aspirating semen ejaculated at the corner of the glass dish into a pre-warmed tuberculin syringe graduated up to an accuracy of 0.01 mL (WHO, 2010).

Determination of semen motility: Sperm motility was determined by diluting half drop of semen on a pre-warmed clean glass slide with a drop of warm 2.9 % Sodium citrate, covered with a slip and examined at X100 magnification under a light microscope (Olympus CX21, Japan). Mass activity was recorded on a scale of five under light microscope using X40 magnification. Individual sperm motility was assessed by the modified method described by Wheeler and Andrews (Cyriac *et al*., 2013) and results were expressed in percentages.

Determination of semen livability: Sperm livability was determined using 1 % Eosin and 5 % Nigrosin in 2.9 % sodium citrate dehydrate solution according to the method described by Wheeler and Andrews (1943). The spermatozoa were counted by hemocytometer using improved Neubauer chamber (Deep 1/10 mm, Labart, Germany). Number of spermatozoa per ejaculate was calculated by multiplying concentration of the semen with semen volume (Jequier, 2010; Oyeyemi and Babalola, 2006).

Hormonal assay: Hormonal assay was carried out using 4-5 mL of blood collected into heparinized and plain sample bottles from inner canthus of the eye of each rat using a capillary tube. Serum was obtained after centrifugation of the blood at 2500 rpm for 10 min and appropriate serum samples (50 µl) were pipetted into assigned wells. Hormone – enzyme reagent solution (100 µl) was added into the entire wells. The microplate was swirled gently for 20 – 30 secs, covered and then incubated at room temperature for 60 min. Contents of the microplate were then discarded, 350 µl of wash buffer was added and aspirated and this was repeated twice for a total of three washes. Working substrate solution (100 µl) was added to all the wells and incubated at room temperature for 15 min. Stop solution (50 µl) was added to each well and mixed for 15 – 20 secs. Absorbance of each well was read at 450 nm in a micrometer reader within 30 min of adding the stop solution. Assay was done in duplicate.

Nitric Oxide determination: Nitric oxide assay was done by adding serum (100 µl) to microliter plates and then Griess reagent (100 µl) was added. The mixture was incubated for at least 20 min and the absorbance was read at 540 nm.

Safety assessment

Safety of the HMPs was evaluated using blood pressure measurements, morphological abnormality of spermatozoa, haematological assessment, biochemical studies and histopathological evaluation of different tissues as follows:

Blood pressure measurement: The Channel High Throughput Non-Invasive Blood Pressure system (CODA) was used to measure the blood pressure of male Wistar rats. The CODA system includes a controller, laptop computer, software, cuffs, animal holders, infrared warming pads and thermometer. Each animal was gently placed into the rear of the holder and allowed to acclimatize to the holder for 5 min. Cuffs were accurately placed, the tail threaded through VPR cuff within 2 mm of occlusion and the tubing was secured in the notch. The cuffs were attached to the CODA controller and animal was thermoregulated for at least 5 min. The experiment continued for about 15 to 20 min. Data collection was sorted through Microsoft Excel and only the accepted cycles were taken. The mean and standard deviations were obtained and measurements having standard deviations greater than 30 were rejected.

Morphological abnormality: The morphological abnormality of the spermatozoa was determined from a minimum of 20 microscopic fields of smear prepared with warm Wells and Awa stains (0.2 g eosin and 0.6 g Fast green dissolved in distilled water and ethanol at the ratio of 2:1). In each microscopic field, abnormal spermatozoa were recorded. In total, a minimum number of fifteen microscopic fields were examined. Abnormal spermatozoa were classified with some modifications according established procedure (Nahak *et al*., 2015).

Haematological analysis: Haematological analysis was carried out using established procedure (Du Plessis and

Soley., 2011) while Red blood cell (RBC), white blood cell (WBC) and platelet counts were done using the Neubauer haemocytometer. Haematocrit or packed cell volume (PCV) and haemoglobin (Hb) concentration were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods, respectively.

Biochemical studies: For biochemical studies, serum was subjected to standard biochemical estimations for different parameters such as Alkaline Phosphatase (ALP), Alanine Transaminase (AST), Aspartate Amino Transferase (AAT/ASAT) and Total Protein (TP).

Histopathological studies: For histopathological studies, testes were collected and stored in Bouin solution, while the liver, kidney and heart were collected in formalin and the tissues were examined at the Chemical Pathology Laboratory of the University College Hospital, Ibadan. Tissues were fixed in 10 % buffered formalin of pH 7.2 and dehydrated through a series of ethanol solutions, embedded in paraffin and routinely processed for histological analysis. Sections of 2 µm thickness were cut and stained with haematoxylin – eosin for examination. The stained tissues were observed through an Olympus microscope (BX – 51) and photographed by a Chare – Couple Device (CCD) camera.

Statistical Analysis

Data were expressed as mean ± SEM (standard error of mean) and statistical significance of the effect of treatment was analysed using student's t-test statistics for two independent variables by comparing controls with groups treated using the HMPs. The probability limit was set at $p < 0.05$. Further statistical analysis was done with Graph-pad Prism version 5. The means across animal groups was compared using ANOVA.

RESULTS

Pharmaceutical and microbial qualities

The herbal drinks are all made in Nigeria with registration number from the National Agency for Food and Drug Administration and Control (NAFDAC) and had aphrodisiac claims (Table 1). The formulations are hydro-alcoholic solutions containing different plants with additives such as sugar. The organoleptic properties and phytochemical composition of the herbal drinks (Table 2) showed that colour ranged from light to dark-brown, the odours were characteristic garlic-like, alcohol or fruit-like. Three formulations had residues between 0.055-0.103 %w/v. Phytoconstituents present were alkaloids, flavonoids, saponins, cardenolides, tannin and anthraquinones. Physicochemical properties and microbial content of the herbal preparations indicated that pH was in the acidic range and density was close to that of water (Table 3). Viscosity was low, and alcohol content was higher than 30 % v/v, which deviated from labelled claims of alcohol content.

Bacterial contaminations varied considerably and fungal contaminations were observed in two herbal drinks. Microbial contaminants present in all the herbal drinks were aerobic microbes woth S*taphylococcus aureus* present in all products except OR. *Pseudomonas aeruginosa* was seen in AL, OS and OE. However, the limits of microbial contamination in oral formulations which is: total aerobic bacterial (10^5cfu/g), yeasts and moulds (10^3cfu/g), Enterobacteria and other Gram-negative organisms (10^3 cfu/g), *E. coli* and *Salmonella* should be absent (European Pharmacopoeia, 2007), were not exceeded.

Table 2:
Organoleptic properties and phytochemical composition of the HMPs

Parameters	OR	AL	OE	BO	OS
Colour	Light brown	Reddish brown	Dark brown	Dark brown	Brown
Odour	Fruity	Pungent	Garlic	Alcoholic and pungent	Pungent
Residues (%w/w)	0.000	0.000	0.055 ± 0.001	0.103 ± 0.010	0.055 ± 0.011
Alkaloid	+	+	+	+	+
Flavonoids	–	-	+	+	-
Saponins	+	+	-	+	+
Cardenolide	+	+	+	+	+
Tannin	–	+	-	+	+
Anthraquinones	+	+	+	+	+

Key: + Present, – Absent

Table 3:
Physicochemical properties and microbial content (Mean, n=4)

Parameters	**OR**	**AL**	**OE**	**BO**	**OS**
pH	3.88	5.40	4.10	4.97	4.06
Density (g/ml)	0.96	0.99	1.07	0.99	0.94
Viscosity (cp)	10.01	11.00	11.00	14.00	12.00
Alcohol cont. (% Label claim)	30.02	42.00	33.00	42.00	15.00
Alcohol cont. (% determined)	30.04	51.96	30.09	47.00	35.60
Bacteria (cfu/g)	1.25 x 10^3	3.35 x 10^4	3.14 x 10^4	3.42 x 10^4	2.93 x 10^4
Fungi (cfu/g)	-	-	5.02 x10^{-1}	5.01 x 10^{-1}	-

Aphrodisiac properties

The aphrodisiac properties of the formulations presented in Table 4 indicates that herbal bitters and Viagra elicited significantly higher ($p<0.05$) anogenital sniffing and mount frequency, and lower mount latency and intromission latency than the control group administered with water. However, aphrodisiac activity of Viagra (VA) was significantly higher ($p<0.05$) than those of the herbal bitters.

Table 4:
Aphrodisiac properties observed in the male Wistar rats (mean ± SD; n=5)

Product	Anogenital sniffing	Mount latency (min)	Mount frequency	Intromission latency (min)
OR	8.00 ± 4.94	3.00±1.85	6.00± 1.58	6.00 ± 1.58
AL	12.00±7.21	2.00±1.23	10.00±2.12	5.00 ± 1.87
OE	7.00 ± 1.22	2.00± 1.05	4.00 ± 1.58	9.00 ± 1.00
BO	10.00±1.58	4.00± 2.46	9.00 ± 1.87	5.00 ± 1.87
OS	9.00 ± 1.22	3.00± 1.85	7.00 ± 2.12	7.00 ±1.87
Viagra®	20.00±3.53	1.00± 0.62	15.00±1.58	4.00 ± 1.58
Distilled water	4.00 ± 3.53	6.00± 2.12	2.00 ± 1.22	15.00 ± 3.16

The results of the motility, viability, volume and count of spermatozoa in the experimental animals presented in Table 5 indicate that spermatozoa of animals treated with herbal bitters and Viagra showed slightly reduced motility, viability, and count of spermatozoa than those administered with water. However, the herbal drinks generally induced higher reductions in motility, viability (life/dead) and count of spermatozoa in a dose-dependent manner. The volume of semen generally ranged from 5.17 to 5.20 mL, which is within acceptable limits of 5.18 mL. This indicates that herbal bitters may not enhance the fertility of Wistar rats since they led to reduction in motility, viability, and count of the spermatozoa. On the other hand, Viagra induced a slight reduction in motility and count of the spermatozoa but there was little or no increase in livability of spermatozoa when compared with group administered with water.

Table 5:
Motility, Viability, Volume and Count of spermatozoa

Group	Dose (mg/kg)	Motility	Liva-bility (%)	Volume (mL)	Count
OR	150	64.00	94.20	5.18	106.60
	200	70.00	94.20	5.18	99.60
	250	73.33	96.50	5.18	104.67
AL	150	56.67	91.00	5.20	77.67
	200	64.00	94.20	5.18	85.40
	250	73.33	96.50	5.18	100.17
OE	150	60.00	94.33	5.17	83.50
	200	66.67	96.50	5.17	89.83
	250	72.00	96.80	5.18	99.80
BO	150	66.67	82.00	5.18	82.50
	200	73.33	96.50	5.17	99.67
	250	76.00	96.80	5.18	96.20
OS	150	70.00	95.20	5.18	96.00
	200	62.50	93.25	5.20	86.75
	250	76.67	96.50	5.18	105.17
Viagra®	50	89.29	97.14	5.19	128.00
Distilled water	-	93.33	97.00	5.18	136.83

The Serum hormone and nitric oxide levels of rats administered with herbal drinks are presented in Table 6. The results showed that herbal bitters induced an increase in serum hormones and nitric oxide levels in a dose-dependent manner with a ranking of AL < OR < OE < OS < BO. As the dose of the herbal drink increased, there was increase in testosterone indicating their ability to aid penile erection in the male rats. This supports the usage of herbal bitters as an aphrodisiac by males. Generally, the herbal bitters caused a statistically significant ($p<0.01$) increase in the serum hormonal levels in female rats when compared with Viagra and distilled water with a ranking of OR < AL < BO < OS < OE.

Table 6:
Serum hormone and nitric oxide levels of the rats administered with the HMPs (mean ± SD; n=5)

Products	Dose (mg/kg)	LH (IU/L)	FSH (IU/L)	T (IU/L)	Nitric oxide
OR	150	6.50 ± 0.50	4.50 ± 0.50	0.70 ± 0.00	6.15 ± 1.54
	200	10.00 ± 0.00	8.00 ± 0.00	1.05 ± 0.05	6.66 ± 0.93
	250	13.50 ± 0.50	11.50 ± 0.05	1.40 ± 0.00	8.32 ± 1.94
AL	150	7.00 ± 0.00	5.00 ± 0.00	0.60 ± 0.00	5.06 ± 1.47
	200	11.00 ± 0.00	9.00 ± 0.00	1.35 ± 0.50	5.09 ± 0.89
	250	15.00 ± 0.00	13.00 ± 0.00	1.80 ± 0.00	6.99 ±0.76
OE	150	8.50 ± 0.50	6.00 ± 0.00	0.55 ± 0.50	6.11 ± 0.90
	200	12.50 ± 0.50	9.00 ± 0.00	1.30 ± 0.00	6.68 ± 0.42
	250	15.00 ± 0.00	12.50 ± 0.50	1.95 ± 0.50	9.50 ± 1.78
BO	150	7.50 ± 0.00	5.50 ± 0.50	0.50 ± 0.00	3.24 ± 0.75
	200	11.00 ± 0.00	6.50 ± 0.50	1.50 ± 0.00	7.74 ± 1.30
	250	15.00 ± 0.00	11.50 ± 0.50	2.25 ± 0.50	9.73 ± 3.77
OS	150	8.50 ± 0.50	6.50 ± 0.50	0.70 ± 0.00	3.05 ± 0.51
	200	13.00 ± 0.00	10.00 ± 0.00	1.30 ± 0.00	5.23 ± 0.97
	250	14.50 ± 0.50	12.00 ± 0.00	1.80 ± 0.00	10.54 ± 2.08
Viagra®	50	5.50 ± 0.50	3.50 ± 0.50	0.55 ± 0.50	6.66 ± 0.53
Distilled water	-	5.50 ± 0.50	3.00 ± 0.00	0.50 ± 0.00	4.97 ± 1.56

LH – Leuitinising Hormone, FSH – Follicle Stimulating Hormone, T – Testosterone, IU/L – International Unit Per Litre

Safety assessments

The effect of the herbal drinks on blood pressure, mean arterial blood pressure and heart rate of Wistar rats are presented in Table 7. The result showed that herbal bitters and Viagra elicited a reduction in blood pressure and mean arterial blood pressure. In addition, the herbal drink elicited a reduction in heart rate while Viagra elicited an increase in heart rate. The effects of the preparations on total normal spermatozoa cells shown in Figure 1 indicate that the total normal cells in distilled water group is 88.57 % while Viagra group, had 87.37 %. However, percentage of total normal cells generally increased with increase in concentration of herbal drinks and ranged from 86.0 to 87.5%. Rodents are said to be fertile when total percentage of abnormal cells do not exceed 20 % or their

total normal cell is above 80 %. In this case, herbal drinks and Viagra did not seem to have any deleterious effect on percentage of normal spermatozoa cells.

The effect of herbal drinks on heamoglobin (Hgb) and PCV of Wistar rats are presented in Figure 2. There were slight reductions in some of the hematological parameters which was not dose dependent. On the other hand, Viagra devoid of alcohol raised the Hgb levels but lowered platelets, WBC and lymph counts (data not shown) than the herbal drinks. Haematological disorders including anaemia are prevalent in alcoholics (Psaltopoulou *et al*., 2018).

The photomicrographs from histology of the testes of rats administered with 250 mg/kg are shown in Figure 3. Generally, the plates showed that there were no untoward histopathologic changes observed in the testes; spermatogenesis and seminiferous tubules were normal in all groups. However, administration of 250 mg/kg dose AL bitters (plate d) showed maturation arrest of the germ cells as evidenced in terminally differentiated spermatozoa cells and attached epididymis had scanty spermatozoa. This could mean that chronic and high dose intake of AL bitters may slow down the process of spermatogenesis.

The photomicrographs of transverse sections of the liver tissues of rats shown in Figure 4 (plates a-g) indicate disseminated moderate steatosis, focal area of tumour with increase nucleo-cytoplasmic ratio and pleiomorphism, congestion of vessels, mild peri-portal infiltration by inflammatory cells, inflammation in zone 2 and multi focal vacuolation/ballooning.

Table 7:
Blood Pressure (BP) of the Wistar Rats (mean ± SD; n=5)

Product	Dose (mg/kg)	Systolic BP (Mm/Hg)	Diastolic BP (Mm/Hg)	Mean Arterial BP (Mm/Hg)	Heart Rate
OR	150	157.67 ± 2.08	130.00 ± 3.46	139.00 ± 1.73	459.00 ± 7.78
	200	143.75 ± 4.86	117.75 ± 3.77	126.25 ± 2.50	475.25 ± 11.64
AL	150	148.00 ± 0.00	97.50 ± 10.61	114.00 ± 7.07	230.00 ± 0.00
	200	142.67 ± 1.53	114.33 ± 3.21	123.33 ± 1.53	367.00 ± 15.00
OE	150	146.20 ± 5.90	114.60 ± 3.51	124.80 ± 4.09	447.67 ± 15.55
	200	152.00 ± 7.55	122.33 ± 10.02	132.00 ± 8.66	403.00 ± 3.20
BO	150	148.17 ± 4.62	111.83 ± 3.92	123.33 ± 2.34	395.00 ± 8.20
	200	142.33 ± 2.33	106.00 ± 4.41	118.00 ± 3.35	340.22 ± 3.90
OS	150	148.40 ± 4.83	109.40 ± 1.95	122.00 ± 2.45	412.50 ± 2.10
	200	142.25 ± 6.95	112.75 ± 11.27	112.00 ± 9.09	437.00 ± 0.00
Viagra®	50	157.00 ± 8.89	136.83 ± 7.78	143.33 ± 8.04	507.00 ± 17.66
Distilled water		171.67 ± 1.53	134.33 ± 4.16	146.67 ± 3.06	460.33 ± 7.23

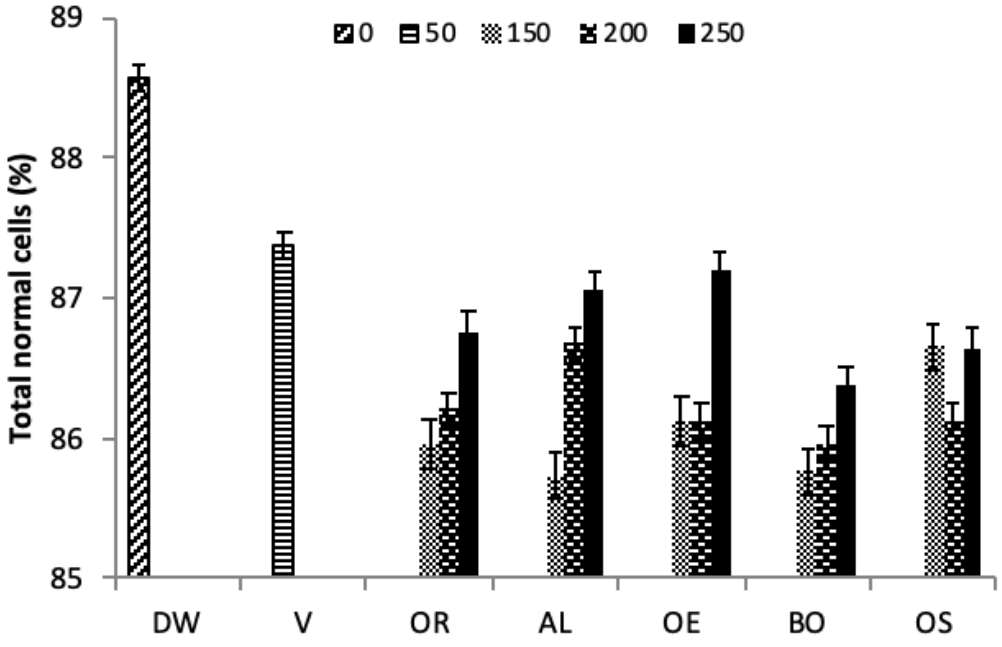

Figure 1:
Total normal spermatozoa cells present in the animals

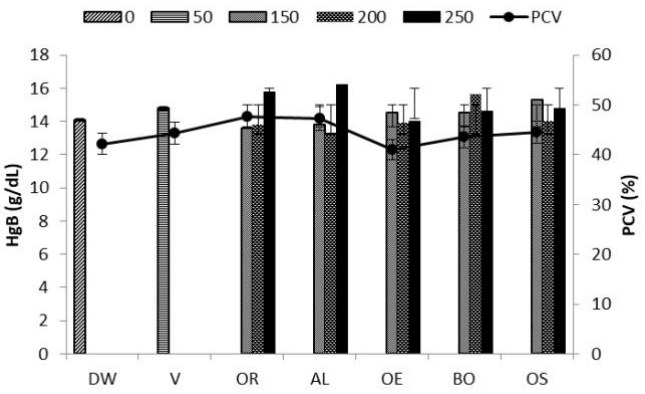

Figure 2:
The effect of the HMPs on the haemoglobin and PCV of the animals.

The photomicrographs of histology of kidney tissues shown in Figure 5 indicated that while distilled water group showed no significant lesion, Viagra group had congestion of vessels and haemorrhagic lesion. The groups treated with herbal bitters also showed no significant lesion, except groups administered with 250 mg/kg dose of OE and BO. Group BO (Plates c) showed disseminated congestion of vessels, areas of tubular necrosis and renal casts while rats administered OE bitters (Plate d) showed marked disseminated congestion of vessels in renal cortex, focal area of extensive sub-capsular haemorrhagic lesion and focal area of fusion of glomeruli with bowman's capsule.

The Photomicrographs showing histology of heart tissue of rats administered with 250 mg/kg dose of herbal bitters presented in Figure 6 showed that there was no significant histopathological alteration observed in heart tissues of controls and most of treated groups. However, histology of heart tissues of rats from 200 mg/kg dose of AL bitters (Plate c) showed focal area of mild inflammation of the myocardium, mild hyperemia and mild haemorrhagic lesion heart tissues and congestion of vessels, focal area of myocardial infarction and hemorrhagic lesion increased in rats administered with 250 mg/kg of AL bitters (Plate d). In addition, rats given 250 mg/kg of BO also showed congestion of vessels and focal area of marked haemorrhagic lesion in the atrium (Plate e).

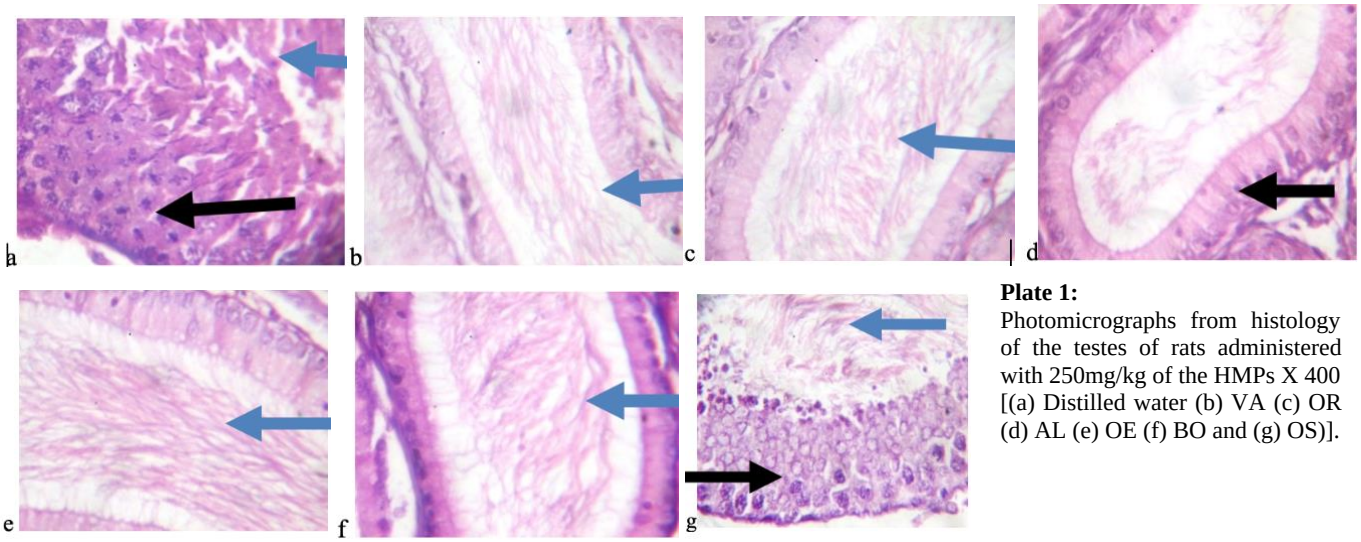

Plate 1:
Photomicrographs from histology of the testes of rats administered with 250mg/kg of the HMPs X 400 [(a) Distilled water (b) VA (c) OR (d) AL (e) OE (f) BO and (g) OS)].

N.B: Blue arrows show that the leydig cells are normal and the attached epididymis has scanty spermatozoa. Black arrows show that there is a focal area of sertoli cells undergoing mitosis

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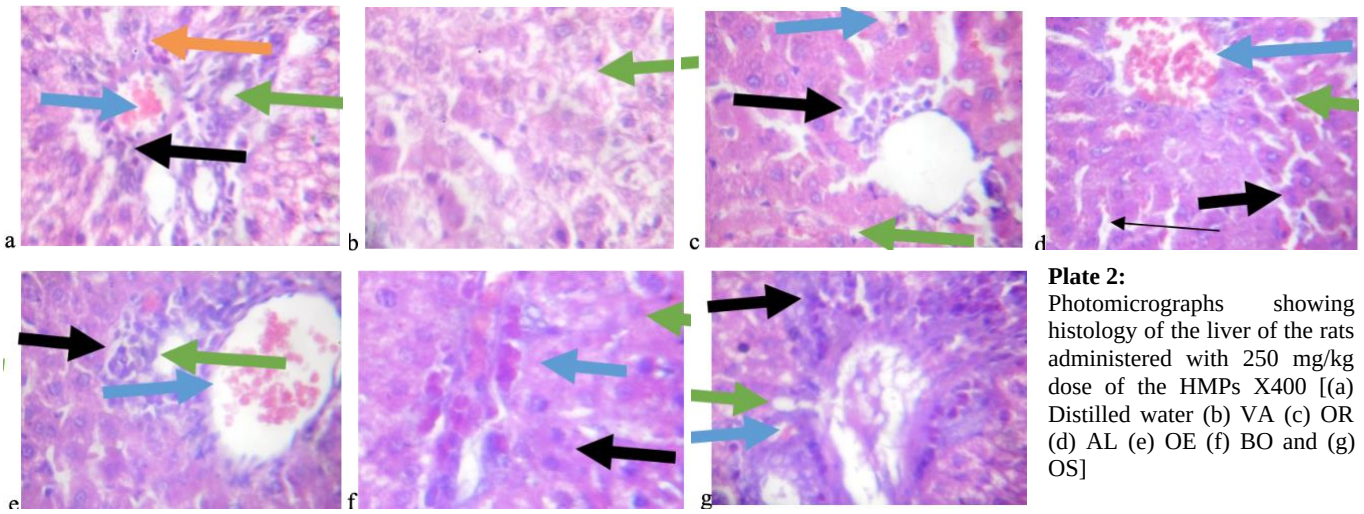

Plate 2:
Photomicrographs showing histology of the liver of the rats administered with 250 mg/kg dose of the HMPs X400 [(a) Distilled water (b) VA (c) OR (d) AL (e) OE (f) BO and (g) OS]

N.B: Green arrow shows disseminated moderate steatosis; Yellow arrow shows focal area of tumour with increased nucleo-cytoplasmic ratio and pleiomorphism; Blue arrows show congestion of vessels; Black arrow show mild periportal infiltration by inflammatory cells; Slender arrows show inflammation and multi focal

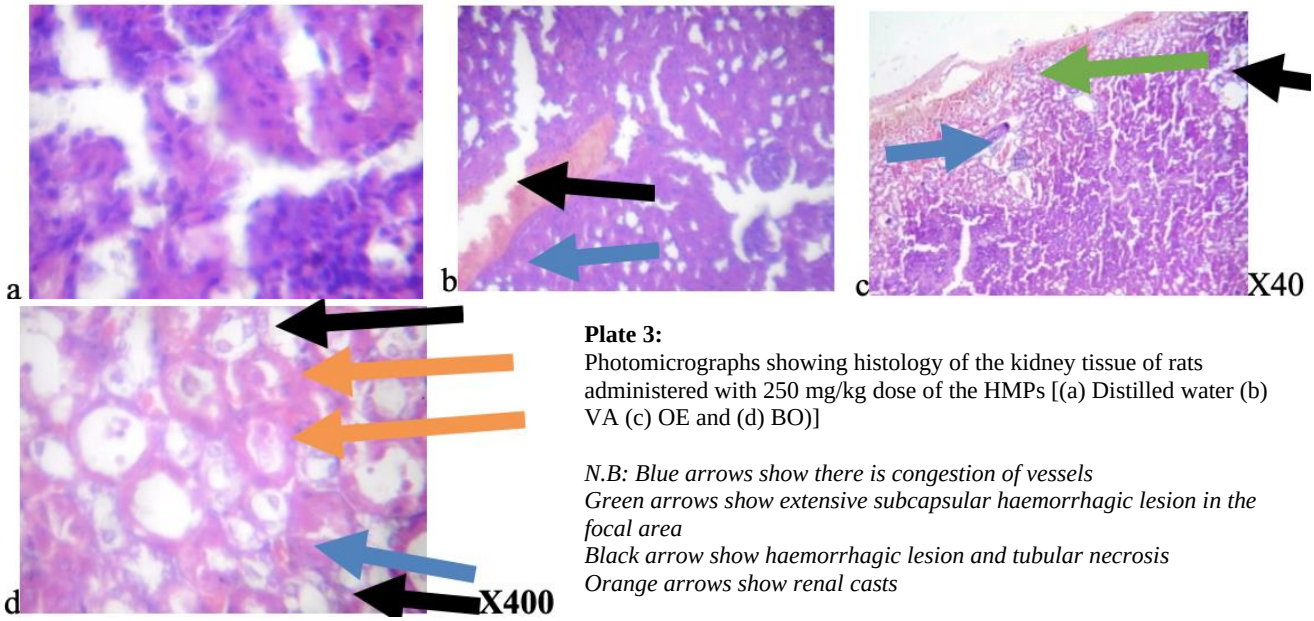

Plate 3:
Photomicrographs showing histology of the kidney tissue of rats administered with 250 mg/kg dose of the HMPs [(a) Distilled water (b) VA (c) OE and (d) BO)]

N.B: Blue arrows show there is congestion of vessels
Green arrows show extensive subcapsular haemorrhagic lesion in the focal area
Black arrow show haemorrhagic lesion and tubular necrosis
Orange arrows show renal casts

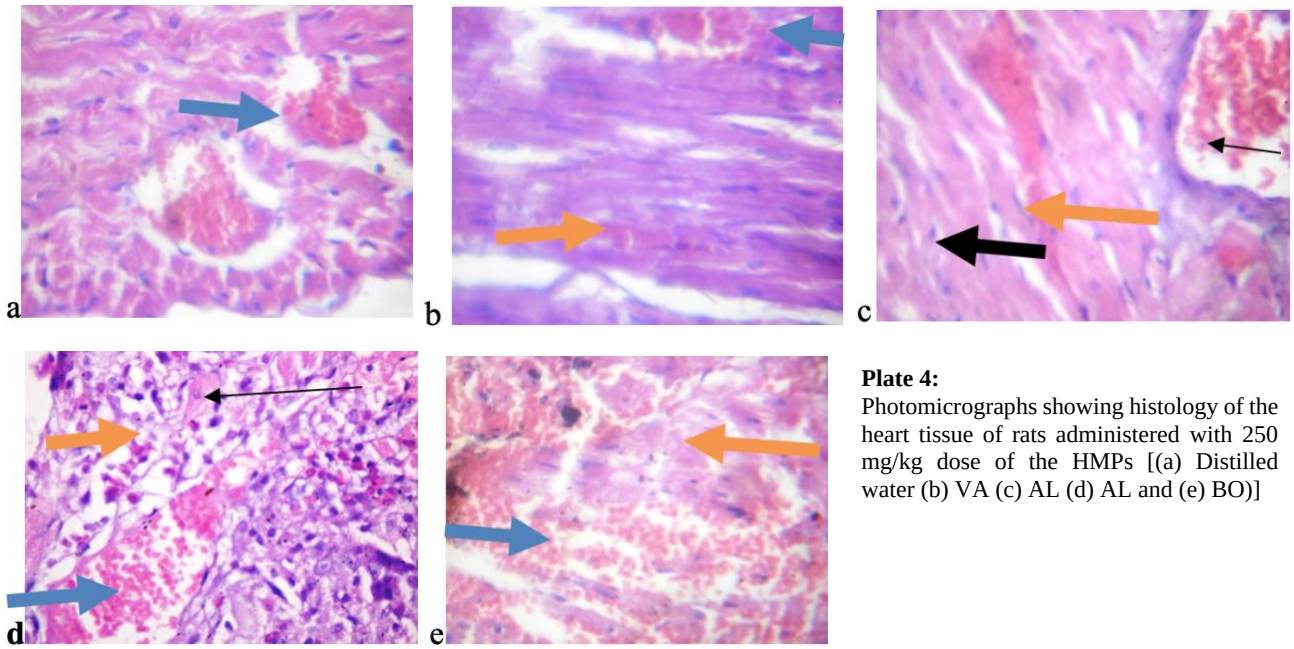

Plate 4:
Photomicrographs showing histology of the heart tissue of rats administered with 250 mg/kg dose of the HMPs [(a) Distilled water (b) VA (c) AL (d) AL and (e) BO)]

N.B: Blue arrows show focal area of mild congestion of focal area and vessels
Yellow arrows show mild focal haemorrhagic lesion
Black arrows show mild inflammation of the myocardium
Yellow arrows show mild haemorrhagic lesion and focal area of myocardial infarction
Slender black arrows show mild hyperemia

DISCUSSION

The herbal drinks were all hydro-alcoholic solutions containing different plant types and parts; they were listed by National Agency for Food and Drug Administration and Control (NAFDAC), which is the regulatory agency in charge of drug registration in Nigeria. All formulations except OR bitters, had alcohol contents higher than the labeled content while OE had lower value. The herbal bitters were contaminated to varying degrees with microorganisms which are within the accepted limits for oral formulations and pathogenic microorganisms were absent. Male rats which received the herbal bitters advanced towards the females immediately after their introduction into the cage and began pre-copulatory behaviors such as chasing and anogenital sniffing. The animals also exhibited moderate pre-sexual behaviors in a dose dependent manner while animals given Viagra® elicited advanced sexual behavioral response when exposed to female rats. Aphrodisiac parameters such as anogenital sniffing, mount latency, intromission time and serum nitric oxide were all enhanced by the herbal bitters showing improved sexual behaviour. In addition, the herbal

drinks reduced blood pressures of hypertensive rats more than Viagra®, there was increase in blood level of testosterone, a hormone which is necessary for sperm production. Herbal bitters had no effect on hematological parameters and blood proteins. Histological examination of the heart tissues indicates that chronic use of AL and BO bitters at 250 mg/kg dose were cardiotoxic. Generally, chronic intake of the herbal products at 250mg/kg dose was capable of inducing liver and kidney tissue damage.

The contamination observed in the formulations could be as a result of soil, harvesting, drying, storage conditions and improper handling which has been reported to influence the microbiological quality of herbal drugs (De Sousa Lima *et al*., 2020). To produce herbal medicines that are free of microbial contaminants, quality control of the raw materials including the solvents, machinery and production premises are important aspects. This indicates that there is the need for the manufacturers of the herbal bitters to carefully ensure that Good Manufacturing Practices are adhered to.

The improved sexual parameters observed in the animals given herbal bitters as compared to those given distilled water could be due to the presence of phytoconstituents in the formulations. The various herbal ingredients that are present in the formulations could elicit synergistic effect thus offering improved aphrodisiac properties. Generally, the positive control (Viagra®) showed superior aphrodisiac parameters compared to the herbal drinks and this compares favourably with other reports (Fauche *et al*., 2015).

Hepatotoxic, nephrotoxic and cardiotoxic outcomes of chronic administration of the herbal drinks could be due to the high percentage of alcohol in the formulations. Alcohol intake causes steatosis which is a defective acid metabolism due to mitochondrial damage by alcohol (Adams *et al*.,2009). Steatosis is an alcohol-related fatty liver disease which can progress to cirrhosis and hepatocellular carcinoma. Furthermore, alcohol is a direct hepatotoxic agent and could manifest in liver disease as fatty liver, alcoholic hepatitis, and alcoholic cirrhosis, tubular necrosis or nephrotoxic ATN (Shanley *et al*. 1986). The Viagra® administered group had a focal area of tumour with an increased nucleocytoplasmic ratio and this agrees with a previous study which showed obscure hepatotoxicity attributed to sildenafil (Wolfhagen *et al*., 2008).

The clinical outcome of the findings of this study is that the men taking these herbal drinks would somehow have improved sexual performance and reduced erectile dysfunction. On the other hand, the humanistic outcome of the findings is that these men lack understanding of the dangers inherent in taking these herbal drinks due to high alcohol content. This is due to the toxicity observed in the animal model used. There will be the need for intervention studies among men who take these drinks due to the potential health hazard. In addition, the regulatory agencies need to regulate the production of herbal drinks with high alcohol content.

The economic challenge created by the findings is embedded in the high probability of the users coming down with terminal illness due to toxicity. This challenge will far outweigh the clinical benefit in the long run. A lot of money, time and man-hours will be expended on any patient that comes down with kidney disease or the like. It is hereby recommended that the alcohol content of the formulation be reduced to tolerable levels.

In conclusion, The herbal bitters had acceptable pharmaceutical, phytochemical and microbial properties. There was increased vasodilation with increase in dose showing the ability of the formulations to aid penile erection. The herbal bitters would not enhance the fertility due to their ability to reduce motility, viability and count of spermatozoa. Furthermore, a pattern was seen in test groups where percentage of total normal cell increased as dose increased indicating that fertility was not hindered. While four herbal bitters caused no untoward histopathologic changes in rat testes, AL bitters caused a maturation arrest of the germ cells as evidenced in terminally differentiated spermatozoa cells and attached epididymis had scanty spermatozoa. This indicate that chronic and high dose intake of AL bitters may slow down spermatogenesis. AL and BO bitters caused more alterations in heart muscle and prolonged use at high dose could be cardiotoxic and nephrotoxic. The hepatological changes seen in the liver of all treated groups suggest a possible hepatotoxic effect after a chronic use of the herbal bitters. There is therefore the need to monitor and control the quality and use of herbal bitters/drinks sold in the Nigerian market to safeguard health.

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