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Evaluation of the Effects of Yoyo Bitters on Albino Rats***Anionye JC¹, Onyeneke EC², Eze GI³, Edosa RO¹, Agu KC¹, Omorowa EF¹, Oghagbon ES¹.**¹Department of Medical Biochemistry, College of Medical Sciences, University of Benin, Benin City, Nigeria.²Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.³Department of Anatomy, College of Medical Sciences, University of Benin, Benin City, Nigeria.

ABSTRACT

This study evaluated the effects of Yoyo bitters on albino rats. Yoyo bitters is composed of five herbal constituents. Yoyo was administered using an oro-gastric gavage to different sets of male albino *Wistar* rats, after which biochemical and haematological indices and histological status of the bitters-fed rats, as well as the acute toxicity (LD₅₀) and subchronic toxicity of the Yoyo bitters on the rats were investigated using standard laboratory procedures. During the 28 days of feeding with the Yoyo bitters there was no sedation, no changes in nature of stool, urine and eye colour, no discharge from the eyes and ears, no haematuria, no diarrhoea and no uncoordinated muscle movements. The yoyo bitters were well tolerated as they improved rather than adversely affected the appetite of the rats. The minimal and non-significant differences (P>0.05) in all the indices used to assess, the liver, kidney and cardiac function statuses of the Yoyo bitters-fed rats compared to same in the control rats, was indicative that Yoyo bitters preserved the functions of these organs. The increase in the CD₄⁺-count and decrease in the level of the fasting blood glucose in the yoyo bitters-fed rats when compared to that of the control rats was significant (P<0.05). There was decrease in serum triacylglycerol, VLDL and LDL-cholesterol levels and an increase in HDL-cholesterol in the bitters fed rats, the decrease in serum triacylglycerol and VLDL being statistically significant (P<0.05). Yoyo bitters-fed rats had a significant decrease (P<0.05) in their serum malondialdehyde (MDA) levels and a significant increase (P<0.05) in their serum vitamin C and E levels and activities of antioxidant enzymes namely superoxide dismutase, catalase, and glutathione peroxidase, when compared with that of the control rats. The results from this study indicate that Yoyo bitters may be said to be hypolipidaemic, hypoglycaemic, immunity-boosting, choleric, hepatoprotective, antihypertensive, as well as having antioxidant properties and protective against heart and related vascular diseases.

Keywords: Yoyo bitters, haematology, serum, antioxidant, histopathology, rat.

INTRODUCTION

Herbal bitters are a combination of bitter herbs. Bitter herbs are fundamental to phytotherapy's contribution to holistic medicine. They are simply herbs that have a bitter taste, ranging from mildly bitter Yarrow to fiercely bitter Rue. The bitterness is often described as being due to a 'bitter principle', but this may be a volatile oil, an alkaloid or a sesquiterpene [1; 2]. In modern herbal medicine, "bitter principles" occupy a central place in herbal therapeutics, bearing the acrid constituents. Most people consuming herbal medicines complain about the bitterness of the medicines prescribed. This is the only defining attribute of herbal medicine and the only feature to set it apart from other therapies [2; 3]. The bitters are thought to stimulate a range of liver activities, especially increasing the production of bile, and the release of bile from the gallbladder. There is a very mild stimulation of endocrine activities, especially insulin and glucagon secretion by the Islets of Langerhans in the pancreas. Thus diabetics need to take care with bitters as they may change their blood sugar balance. In the hands of a skilled practitioner, however, such remedies may have a role to play in the treatment of non-insulin dependent diabetes [1; 3; 4]. The therapeutic implications of

bitters are impressive as the tonic effects go beyond the specifics of digestive hormone activity. As digestion and assimilation of food is fundamental to health, bitter stimulation may improve a condition that has nothing pathologically to do with the digestive process [2; 3]. There is much overlap in practice between the bitters and tonic remedies, the mechanism of action is not always clear, but it is evident that these herbs act to promote health - yet another wonderful gift of nature [1; 2]. Bitter stimulation effects are shared by any herb that can trigger the receptor sites on the tongue, but selection of appropriate bitter can be made by considering their inherent strength, other actions and specific indications [3; 4].

All over the world, the use of organic drugs is becoming increasingly popular. Even in the developed countries, the use of organic products in the therapy of certain diseases is becoming generally acceptable. Research has now proven that organic drugs are efficacious which allows for such products to be recognized and listed among registered drugs. In Nigeria, it is gradually becoming acceptable that organic drugs can be used side by side with orthodox medicine in the treatment of diseases. So far, all organic drugs in Nigeria are in a way recognized by the national regulatory body (i.e. National Agency for Food and Drugs Administration and Control - NAFDAC) Yoyo Cleanser bitters is an organic drug in the class of herbal bitters that was launched into the market in 2003 by the Company. Since its introduction into the Nigerian drug market it has received wide acceptance and usage by the general population. It will be an understatement to say that it has become a household name. Its acceptability may be attributed to the safety and efficacy in most cases as there has been minimal report of adverse reaction due to its administration. Yoyo Cleanser Bitters is a bitter in the class of the internationally acclaimed bitters. The Yoyo bitters are composed of five herbal constituents which include *Aloe vera* (True aloe, Lily of the forest), *Acinos Arvensis* (Basil thyme), *Citrus aurantifolia* (Bitter orange), *Chenopodium murale* (Nettleleaf goosefoot) and *Cinamomum aromaticum* (Cassia).

This Drug is formulated in such a way that the ingredients have a synergistic effect on the management of ailments of the digestive System: It decreases the stomach acidity in cases of ulcer, It diminishes the irregular production of gastric juice, It stimulate the liver to ensure proper and complete digestion, It helps to digest heavy and fatty foods, In the Circulatory System: It enhance blood circulation, Helps to facilitate reduction in blood pressure through arteries dilation, Assists in the elimination of cholesterol, sugar, triglycerides, creatinine and uric acid. In the Nervous System: it enhances effective function of the secretive glands, It is beneficial in the treatment of such disorders as insomnia, stress and depression. In the Urinary and Excretory Systems: It facilitates the process of blood purification by the kidneys, help to dissolve existing kidney stones and to prevent the formation of new ones, prevents kidney and bladder infections; help to normalize the operation of the intestine for excretion of faeces. In Ulcerations: It inhibits ulceration by eliminating any traces of stored toxins in the body system, and aid boosting body immunity. In Hardening of Tissues: It dissolves any encased toxic materials in the body, enhances cell formation and growth. In cases of Over Weight: It reduces excess body fat, and encourage healthy weight loss.

MATERIALS AND METHODS

Materials

Yoyo bitters was purchased from reputable pharmaceutical stores opposite the University of Benin Teaching Hospital (UBTH), Ugbowo Lagos Road, Benin City, Edo State, Nigeria. Yoyo bitters was bought as liquid formulations and stored at room temperature (30-36°C) throughout the period of the experiment.

Reagent kits and other reagents used were of standard quality and were purchased from qualified/accredited dealers/ suppliers or their manufacturers' representative in Nigeria.

All the experimental animals for all stages of this study were handled in strict compliance with international guidelines as prescribed by the Canadian Council on the Care and Use of Laboratory Animals in Biomedical Research, 1984 edition [5].

Male albino rats of the Wistar strain were obtained from the Anatomy Department, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. The rats were housed in a well ventilated room in the animal house of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, with the room temperature ranging from 30-36°C. They were allowed the diurnal cycle, which is the recommended 12-hr light and dark cycle. The rats were fed ad-libitum with standard pelleted mash and clean tap water for an acclimatization period of two weeks.

Acute Toxicity Study

The method of Miller and Tainter [6] as adapted by Randhawa [7] was used for the acute toxicity study. It was done in two phases.

Phase I Acute Toxicity Study: Experimental Design/Protocol

This was done using the "staircase method" for the determination of the lethal dose and dose range prior to the actual LD₅₀ determination. After 14 days of acclimatization, the 10 experimental animals for the determination were divided into 5 groups of two rats each with each set of 2 rats given a dose of the bitters higher than the precedings one to determine which dose will cause zero death and which one will cause 100% death after 72hrs of oral dose of the bitters using an oro-gastric gavage [6, 7, 8]. The Animals were observed for signs of toxicity and mortality. At the end of the 3 days, for each group, the dose(s) that caused no death and the 1st dose that caused the death of the 2 rats in each sub-group was noted; these doses were used to determine the range to be used in the LD₅₀ determination for the herbal bitters [6, 7, 8].

Phase II Acute Toxicity Study: Experimental Design/Protocol

This was for the determination of the LD₅₀. After 14 days of acclimatization, the 50 experimental animals for the determination of the LD₅₀, using the Miller and Tainter method [6], were weighed and divided into 5 groups of 10 rats each according to their weight range, making sure that the distribution was in such a way that the average weight per group was about 162g. Each group of 10 rats was given a dose of the bitters higher than the preceding one following the range as determined from pre-LD₅₀ determination study (phase I). This was to determine which dose will cause death ranging from 0 to 100%, after 72hrs of oral dose of the bitters [6, 7, 8]. The animals were observed for the first 2 hours, and then at the 6th, 24th, 36th, 48th, 60th and 72nd hours for any toxic symptoms. After 72hrs, the number of deceased rats were counted in each group and percentage of mortality calculated and tabulated. The percentage of dead rats for 0 and 100 was corrected before the determination of

probits as shown:

Corrected % Formula for 0 and 100%

Mortality [7]

For 0% dead = $100(0.25/n)$

For 100% dead = $100(n-0.25)/n$;

where $n = 10$, their values were 2.5 and 97.5 respectively.

Determination of the LD₅₀:

The Probit values were plotted against log-doses and then the dose corresponding to probit 5, that is 50%, was extrapolated, the value identified and noted as the LD₅₀. Other calculations were made according to the method described by Miller and Tainter [6] and Randhawa [7].

Subchronic Toxicity Study

Animal for study: Sixteen (16) male albino rats of the wistar strain weighing between 110-210g, Average weight per group approximately 162g. Grouping of the animals: After 14 days acclimatization, the 16 animals were weighed and divided into two (2) groups A and B, of eight (8) rats each, making sure that the weights of those in a group were representative of the weight range of all the rats, such that the average weight of all the groups at the onset of the experimental period was 162g.

Feeding regime and care of the animals: The rats were fed ad-libitum on standard pelleted mash and clean tap-water during the entire course of the 28-day study and allowed the recommended 12-hr light and dark cycle. Care was taken to determine the quantity of feed consumed daily. The rats were housed in wooden cages with a tiny-wire meshed/iron gauze flooring to allow the rat-excreta to be collected into another steel tray receptacle below covered with a bedding material. The cages, their surroundings, the receptacle tray below with its bedding, were cleaned and disinfected daily.

Experimental procedure: In addition to the feed and clean pipe-borne water, the rats in group B were given orally, the yoyo bitters, using an oro-gastric gavage, according to the equivalent dose (to the weight of the rats for that week) of the effective dose already prescribed for man. An equivalent volume of distilled water was given to the control group which was group A. The animals were observed for signs of toxicity and mortality.

Dosage regimen: An adult man was expected to consume on the average 40ml of herbal bitters daily. Appropriate calculations were done to determine the initial equivalent doses of the bitters (distilled water in the case of the control group) in ml/g mean body weight of the rats to be given in each group. As the initial mean weights of rats in each group at the beginning of the study was 162g, the equivalent volume [in millilitres-(ml)] of the bitters/distilled water that was given to the rats was as calculated:

If 40ml was consumed by a 70,000g man (70kg)

How many ml was a 162g rat expected to

0.1ml for a 162g rat means a dose of 0.1ml/162g = approx. 6.2×10^{-4} ml/g of rat.

The rats were weighed weekly and the weight used to calculate the equivalent doses/volume to be administered for each group of rats for that week. The relationship between this weight and the quantity of feed consumed and appetite of the rats was also investigated.

Weekly Body Weight: The body weight of each rat was assessed using a sensitive balance during the acclimatization period, once before commencement of dosing (day 1), once weekly during the dosing period, (day 7, 14 and 21) and once on the day of sacrifice (day 29), [8].

Weekly Quantity of Feed Consumed: The quantity of feed given to each group of rats daily was determined by subtracting the quantity of feed left the next morning from that given the day earlier. From the results the average quantity consumed weekly by the rats was determined. This quantity of feed consumed by each rat was assessed using a sensitive balance from the commencement of dosing (day 1), until the day of sacrifice (day 29), [8].

Clinical Signs and Mortality: The animals were observed for signs of weakness, increased or decreased appetite, weight loss and other physiological changes including mortality. Clinical signs to be assessed before dosing, immediately and 4hrs after dosing, include level of sedation, restlessness, changes in nature of stool, urine and eye colour, excretion of worms, diarrhoea, haematuria, uncoordinated muscle movements, etc. The animals will be observed for toxic symptoms such as weakness or aggressiveness, food refusal, loss of weight, diarrhoea, discharges from the eyes and ears, noisy breathing and mortality, [8, 9, 10].

Blood Sample Collection and Preparation

Two specimen bottles were used for collection of blood from each animal. Anticoagulant bottles containing K_2 EDTA for haematological tests and lithium heparin bottles for assay of other parameters were used for initial collection of blood from all animals. The last dose of the bitters was administered on the morning of the 28th day. All meals were stopped by 7pm on the 28th day. After an overnight fast and following chloroform anaesthesia and opening up of the animals, blood samples were collected from the animals using syringes and needles via the inferior vena cava and

cardiac puncture, into already labelled K_2 EDTA and lithium heparin bottles without undue pressure to either the arm or the plunger of the syringe. The samples were then mixed by gentle inversion. The samples in the K_2 EDTA anticoagulant bottles were immediately sent for automated analysis for full/complete blood count and CD_{4+} T-Lymphocyte count. The samples in the lithium heparin bottles were centrifuged at 4000r/min for 10mins to obtain plasma. The plasma supernatants were then separated into sterile plain bottles and were used for assay of the required

parameters.

Assay of Haematological Indices

These were determined following the instructions of the manufacturers of the automated instrument: The full/complete blood count, was determined using a KX-21N, an automated blood cell count analyser [11], while for the CD₄₊ - T-Lymphocyte count, CYFLOW SL- GREEN, an automated portable flow cytometer for the enumeration of CD₄₊ - T-Lymphocyte cells in the whole blood was used [12, 13].

Assay of Fasting Blood Glucose:The blood glucose was assayed using the glucose-oxidase method (14), as outlined in the glucose kit by Randox lab. UK.

Assay of Serum Lipid Profile

The parameters assayed are total cholesterol, triacylglycerol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol using Randox kit (Randox lab. UK) and following the standard procedures as described by the manufacturers [15, 16].

Assessing the Liver Function Status

The parameters assayed are total protein, albumin, total bilirubin, conjugated bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase and gamma-glutamyl transferase, using Randox kit (Randox lab. UK) and following the standard procedures as described by the manufacturers [17, 18, 19, 20, 21].

Assessing the Kidney Function Status

The parameters assayed are the electrolytes-Na⁺, K⁺- using the Flame Photometer [22]; Cl⁻ - using the mercurimetric (titrimetric) method [23]; HCO₃⁻ - using the titrimetric method [24]; urea- using the Berthelots reaction method [25]; and creatinine- using the spectrophotometric method [26].

Assessing the Cardiac Function Status

The cardiac enzymes assessed are creatine kinase - using the UV method [27] and lactate dehydrogenase- using the UV method [28].

Assessing the Antioxidant Status and Lipid Peroxidation Effect

The parameters assessed in vivo and the methodology employed are-malondialdehyde (MDA) level [29]; vitamin E [30]; vitamin C [31, 32]; catalase (CAT) [33]; superoxide dismutase (SOD) [34]; glutathione peroxidase (GPx) [35].

STATISTICAL ANALYSIS

Data was subjected to appropriate statistical analysis using the students paired t-test from the computerized statistical package for the social sciences, edition 17 (SPSS 17). P<0.05 was considered significant. The results were expressed as Mean±SEM.

Subchronic Toxicity and Pharmacological/Biochemical Effects of Herbal Bitters on Rats

Table 1: Feed consumed by rats after administration of Yoyo bitters for four weeks

Groups	Week 1	Week 2	Week 3	Week 4
Control	19.34±1.49 ^a	17.76±0.87 ^a	16.92±0.87 ^a	19.38±2.10 ^a
Yoyo	19.50±0.89 ^a	20.03±1.05 ^a	20.37±1.34 ^a	15.75±1.93 ^b

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. Though the feed consumed by the bitters-fed rats were consistently higher, apart from the 4th week, the feed consumed were not significantly different (P>0.05) from that consumed by the control.

Acute toxicity: determination of the LD₅₀ of the various bitters

Table 2: The LD₅₀ of the various bitters

Bitters	Yoyo
LD ₅₀ (ml/kg)	164.00±63.00
	164.00±63.00
LD ₅₀ (mg/kg x10 ³)	
LD ₅₀ (g/kg)	164.00±63.00

The values were expressed as mean±S.E.M.

Groups	Leucocyte Count (x 10 ³ /μL)	Lymphocyte Count (%)	Monocyte Count (%)	Neutrophil Count (%)	CD ₄ Count (/μL)	Platelet Count (x 10 ³ / μL)
Control	5.79±1.22 ^a	65.60±2.19 ^a	11.39±1.34 ^a	23.43±1.90 ^a	186.00±7.63 ^a	72.63 ±11.58 ^a
Yoyo	4.16±0.45 ^a	64.95±3.59 ^a	10.98±1.13 ^a	24.08±3.81 ^a	203.50±9.04 ^b	75.00±5.98 ^a

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another.

Though the total white blood cell count, the lymphocyte in rats fed yoyo bitters were slightly decreased compared to that of the control, statistical evaluation show that there were no significant difference (P>0.05) between them. The CD₄ lymphocyte count and the Platelet count on the other hand were elevated, but only that of the CD₄⁺ was significant (P<0.05). The monocyte and neutrophil counts show a non-significant (P>0.05) decrease.

Table 4: Red Blood Cell (RBC) Count, Haemoglobin Concentration, Packed Cell Volume (PCV) and Red Cell Indices of rats fed with yoyo bitters

Groups	Red Blood Cell, RBC Count (x10 ⁶)	Haemoglobin Concentration (g/dl)	Packed Cell Volume, PCV (%)	Mean Corpuscular Volume, MCV (fl)	Mean Corpuscular Haemoglobin, MCH (pg)	Mean Corpuscular Haemoglobin Concentration, MCHC (g/dl)
Control	6.39±0.64 ^a	14.88±1.19 ^a	41.56±3.80 ^a	48.98±0.99 ^a	19.48±0.45 ^a	36.79±0.70 ^a
Yoyo	6.03±0.61 ^a	17.66±0.59 ^a	53.13±1.80 ^b	49.53±0.93 ^a	8.99±0.73 ^a	36.59±0.65 ^a

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. Though there were differences in the RBC count, haemoglobin concentration, MCV, MCH and MCHC in rats fed with Yoyo bitters when compared to that of the control, statistical evaluation shows that these differences were not significant (P>0.05). The PCV however was significantly(P<0.05) elevated in the bitters fed rats.

Table 5: The effect of yoyo bitters on fasting blood glucose (FBG) level of rats.

Groups	FBG (mg/dl)
Control	91.63±8.57 ^a
Yoyo	55.13±6.25 ^{b,c}

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. Statistical evaluation indicates that the fasting blood glucose levels in rats fed with Yoyo bitters were significantly (P<0.05) reduced compared to that of the control.

Table 6: The effect of yoyo bitters on lipid profile of wistar rats

Research Groups	Cholesterol (mg/dL)	Triacylglycerol (mg/dL)	HDL-Chol. (mg/dL)	LDL-Chol. (mg/dL)	VLDL-Chol. (mg/dL)
Control	98.63±3.02 ^a	58.00±2.74 ^a	18.88±2.14 ^a	68.15±1.75 ^a	11.60±0.55 ^a
Yoyo	93.00±1.87 ^a	47.25±3.06 ^b	23.25±3.06 ^a	60.30±4.73 ^a	9.45±0.61 ^b

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another.

Yoyo bitters caused a reduction in rat blood cholesterol, triacylglycerol, LDL-cholesterol and VLDL-cholesterol levels and an increase in the HDL-cholesterol when compared to that of the control. The reduction in triacylglycerol and VLDL-cholesterol was statistically significant (P<0.05).

Table 7: liver function indices of rats administered with yoyo bitters

Analytes	Control	Yoyo
Total Bilirubin (mg/dl)	0.28±0.03 ^a	0.29±0.03 ^a
Conjugated Bilirubin (mg/dl)	0.16±0.02 ^a	0.16±0.03 ^a
Aspartate Transaminase (IU/L)	28.25±2.97 ^a	22.38±1.89 ^a
Alanine Transaminase (IU/L)	3.88±0.30 ^a	3.13±0.23 ^a
Alkaline Phosphatase (IU/L)	10.13±0.61 ^a	12.38±0.82 ^a
Total Protein (mg/dl)	4.93±0.10 ^a	4.98±0.17 ^a
Albumin (mg/dl)	3.11±0.10 ^a	3.08±0.11 ^a
γ-Glutamyl Transpeptidase (IU/L)	2.75±0.16 ^a	3.75±0.27 ^a

Values are expressed as Mean±SEM. Values in the same row with different superscript letters differ significantly (P<0.05) from one another.

Though there are differences in the liver function status indices of the control and yoyo bitters-fed rats, these differences are minimal and statistical evaluation shows that there are no significant difference (P>0.05) between them.

Table 8: Kidney Function Indices of rats administered Yoyo bitters

Analytes	Control	Yoyo
Na ⁺ (mmol/L)	137.13±2.43 ^a	131.88±1.91 ^a
K ⁺ (mmol/L)	14.05±1.08 ^a	14.13±1.45 ^a
Cl (mmol/L)	108.63±3.39 ^a	111.00±2.21 ^a
HCO ³⁻ (mmol/L)	5.13±0.97 ^a	4.00±0.63 ^a
Urea (mg/dL)	35.38±2.43 ^a	36.88±2.86 ^a
Creatinine (mg/dL)	1.15±0.12 ^a	0.85±0.08 ^a

Values are expressed as Mean±SEM. The values in the same row with different superscript letters differ significantly (P<0.05) from one another.

Though there are differences in the kidney function status indices of the control and yoyo bitters-fed rats, these differences are minimal and statistical evaluation shows that there are no significant differences (P>0.05) between them.

Table 9: Cardiac Function Enzymes of the Control and Test Groups

Analytes	Control	yoyo
Creatine Kinase (U/L)	40.89±5.98 ^a	38.28±2.44 ^a
Lactate Dehydrogenase (U/L)	125.50±0.82 ^a	124.50±2.12 ^a

Values are expressed as Mean±SEM. The values in the same row with different superscript letters differ significantly (P<0.05) from one another.

Though there are variations in the levels of the enzymes used to assess the cardiac function status of the control and Yoyo bitters-fed rats, these differences are minimal and statistical evaluation shows that there are no significant differences (P>0.05) between them.

Table 10: The Effect on Lipid Peroxidation (MDA) and Antioxidant Status of rats administered with yoyo bitters

Group	Malondialdehyde (MDA) (U/mg protein x10 ⁻⁴)	Vitamin C (g/100 ml)	Vitamin E (mg)	Superoxide Dismutase (SOD) (U/mg protein x10 ⁻²)	Catalase (CAT) (U/mg protein)	Glutathione Peroxidase (GPx) (U/ml)
Control	2.98±0.29 ^a	0.87±0.07 ^a	0.79±0.05 ^a	3.90±0.52 ^a	0.35±0.08 ^a	0.53±0.08 ^a
Yoyo	1.19±0.14 ^b	1.92±0.11 ^b	0.83±0.12 ^a	7.11±0.48 ^b	1.28±0.23 ^b	3.55±0.58 ^b

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ significantly (P<0.05) from one another. The parameters used to measure the level of lipid peroxidation and antioxidant status show general statistically significant (P<0.05) increase in their level or activity in the Yoyo bitters-fed rats when compared with the control, with the exception of Vitamin E whose level was not significantly (P>0.05) different from that of the control rats.

DISCUSSION

is often summarized that bitters stimulate digestive secretions and the metabolism as a whole and in so doing increase appetite, relieve constipation, and generally ease the heavy glumness of sluggish digestion. But, this is really too simple and cursory a summation, and a deeper look into the actions of bitters is not only theoretically insightful but practically invaluable, especially as some plant products have been known to be toxic to the human system [1,3]. Their composition as they are presently constituted have never been ascertained neither has their numerous pharmacological claims being subjected to proper scientific scrutiny yet the use of bitters is getting popular.

The LD₅₀ of the bitters in this study is high, meaning that one may have to consume them more than 10 times their normal therapeutic dose before one gets a lethal/toxic effect. Research and endeavours are geared towards discovery of new therapeutic agents or newer and richer sources of known drugs of natural origin, and the basic goal of such drug discovery efforts always hinges on developing new products with enhanced therapeutic benefits, that is, higher efficacy and low toxicity profile [36, 37].

Even if the weight changes between the control and bitters fed groups was not statistically significant ($P>0.05$), all the groups progressively gained weight, though at different rates throughout the 4 weeks period of this study (result not shown).

The results of this study show that the feed consumed per day in the 4 weeks of this study is higher in rats fed Yoyo bitters when compared with that of the control; statistical evaluation however showed that there was no significant difference ($P>0.05$) in the mean of the feed consumed. These changes however gives the impression that even if Yoyo bitters were generally well tolerated and did not lead to a drastic reduction in food consumption, it may well be it causes an increase in appetite more

than other bitters, leading to more food being consumed and giving credence to the claim that some bitters increase appetite [1], with Yoyo bitters being the favoured products in this regard. The findings of this study is also in agreement with the findings of Aniagu *et al* [8] and as with Nature Cure bitters they worked with, we can say that the diets were well accepted by the bitters fed rats, suggesting that the bitters did not possibly cause any drastic alterations in the carbohydrate, protein or fat metabolism in these experimental animals in such a way as to prevent a weight gain expected of animals that are continually supplied with food and water *ad libitum* [8]. Some innate property in Yoyo bitters may have made rats fed with it to consume more food with consequent less weight change and less weight gain, but since their weight were not statistically different ($P>0.05$) from that of control, one cannot say definitively that they can be recommended as supplements for those desirous of not gaining too much weight. The weight gain/change seen in Yoyo bitters fed rats since they are not significantly different statistically ($P>0.05$) from the weight gain/change seen in the control rats can be said to be a healthy weight gain that is expected of animals that are continually supplied with food and water *ad libitum*.

If a herb or herbal tonic is toxic, this can be reflected in a reduction in some or all of the haematological parameters measured in a full/complete blood count because of direct toxicity to or lysis of the cells in the blood. If however it is non toxic or actually nourishing and immunity boosting, this will reflect in the maintenance or increase in levels of some of the haematological parameters and cells especially those implicated as imparting immunity, though this increase will not be as high as the increase seen in a pathological state. The cells implicated as contributing especially to natural immunity are maintained at normal levels or raised to normal levels or a little above normal levels by herbs. Herbs have been shown to be more involved in imparting natural immunity than acquired immunity, though it can enhance acquired immunity when necessary [38; 39; 9]. The results of this study indicates

that the Yoyo bitters did not exhibit any form of haematological toxicity, as statistical evaluation did not show any significant difference ($P>0.05$) between the values of the haematological parameters studied in the rats fed herbal bitters compared to the control. The significant ($P<0.05$) increase in the CD_4^+ Count in rats fed with Yoyo bitters could be an attestation to the claim that bitters improve body immunity as it may be arising from the fact that the bitters may contain biologically active principles that have the ability to boost the immune system through increasing the population of defensive white blood cells [9].

The manufacturers of the Yoyo bitters used in this study made claims that bitters help to increase the activity of the pancreas by bringing sugar level under control, assist in the elimination of sugar, to the assertion that it helps in the prevention of diabetes. The results of this study seem to provide some form of evidence to this claim as the fasting blood sugar in rats fed the bitters were all reduced compared to that of the control with the reduction in Yoyo bitters being statistically significant ($P<0.05$). The medicinal properties of plants used by traditional medical practitioners including their hypoglycaemic properties may be due to one or more of the many arrays of phytochemical constituents of these plants [40]. These phytochemicals include complex carbohydrates, alkaloids, flavonoids, tannins, glycopeptides, peptides and amines, terpenoids, cyanogens, steroids, lipids, coumarins, sulphur compounds and inorganic ions, just to mention but a few, most of which are contained in the bitters used for this research.

The results of the study of the lipid profile of rats fed with Yoyo bitters compared with that of the control reveal that generally, yoyo bitters relatively have hypo-cholesterolaemic and hypo-triacylglycerolaemic effects, while decreasing the LDL-cholesterol (bad cholesterol) and VLDL-cholesterol levels and increasing the HDL-cholesterol (good cholesterol) level. This result seems to give credence to the claim by bitters manufacturers that they have hypo-lipidaemic effect. Yoyo bitters act on

both the pancreas and liver/gall bladder, helping to promote the production and release of the pancreatic enzyme lipase and bile, which ensure good digestion of fats and oils and proper functioning of the excretory functions of the liver thereby conferring on it hypolipidaemic properties. It acts as a liver tonic, being hepatoprotective and enhancing its functions. A healthy flow of bile helps rid the liver of waste products, prevents the formation of gallstones, and emulsifies lipids, which the pancreatic enzymes then breakdown along with proteins and carbohydrates for absorption in the small intestine. The results of this research on the serum lipid profile give positive evidence that the herbal bitters have the potential of being a lipid-lowering supplement/drug in mixed hyperlipidaemic states. There is evidence that a salient relationship exists between high serum cholesterol levels and the incidence of atherosclerosis and cardiovascular disease [9], the observed hypocholesterolaemic effect of these herbal bitters is therefore a desired positive effect.

Liver cell damage is characterised by a rise in plasma enzymes (AST, ALT, LDH etc). From the results of this study AST concentrations were consistently higher than the ALT level, which is to be expected since body cells contain more AST than ALT, this is in agreement with the findings of previous research[9]. But since AST is more intracellular than ALT which is localised primarily in the cytosol of hepatocytes, ALT is a more sensitive marker of hepatocellular damage than AST. Thus the minimal and non-significant differences ($P>0.05$) in the AST and ALT levels in Yoyo bitters-fed rats compared to that of the control rats of this study is indicative that the bitters did not cause any hepatocellular damage to the liver of the rats [41].

The minimal and non-significant differences ($P>0.05$) in the ALP, total bilirubin and conjugated bilirubin levels in the Yoyo bitters-fed rats compared to that of the control of this study is indicative that Yoyo bitters did not cause any form of cholestasis, excessive haemolysis, nor did it impair the capacity of the liver to excrete bilirubin. Cholestatic

liver disease is characterised by an elevation in the plasma level of alkaline phosphatase (ALP), while hyperbilirubinaemia is seen in conditions causing excessive haemolysis and hepatic liver diseases that impair the excretion of bilirubin[41].

The minimal and non-significant differences ($P>0.05$) in the serum albumin and total protein levels in the bitters-fed rats compared to that of the control of this study is indicative that Yoyo bitters did not cause any dysfunction in the synthetic function of the liver [41]. Increased synthesis of Gamma-glutamyl transpeptidase in the liver resulting from microsomal enzyme induction by some drugs and alcohol (in chronic drinkers) produces increased plasma level [41]. The minimal increase seen in the level of Gamma-glutamyl transpeptidase in plasma of all the bitters fed rats of this study may well be as a result of their “high” alcohol content, this increase however did not result in a level of Gamma-glutamyl transpeptidase that is significantly different ($P>0.05$) from that of the control, so this increase is not associated with any hepatocellular damage [41].

The result of this study indicates that in some of the parameters used to assess the kidney function status of the control and bitters fed rats, there are differences which are minimal but statistical evaluation shows that there is no significant difference ($P>0.05$) between them. The reduced levels of sodium and creatinine probably indicate that the bitters did not interfere with the renal capacity to excrete these metabolites. The lack of significant difference between the metabolites of the control and bitters fed groups used in assessing the kidney function status may also be a reflection of the preserved renal integrity of the treated rats [8]. Hence the bitters can be said not to have a reno-toxic effect on the kidneys of the bitters fed rats as they preserved its renal integrity and did not affect its capacity to excrete metabolites.

There are minimal differences in the parameters used to assess the cardiac function status of the control and bitters fed rats but statistical

evaluation shows that there is no significant difference ($P>0.05$) between them. Though other tissue damage may lead to a rise in our metabolites of interest, cardiac cell/muscle damage is characterised by a combination of a rise in plasma enzymes (creatine kinase, LDH etc), from the results of this study, there was no significant increase ($P>0.05$) in either creatine kinase nor LDH, infact the creatine kinase level was consistently lower in all the bitters fed rats compared to the control suggesting some form of cardio-protectivity. Thus the minimal and non- significant differences ($P>0.05$) in the creatine kinase and LDH levels in the bitters-fed rats compared to that of the control of this study is not just a reflection of the preserved cardiac integrity of the treated rats but indicative that the bitters did not cause any cardio-cellular damage to the heart of the rats [41]. Hence the bitters can be said not to have a cardio-toxic effect on the heart of the rats as they preserved its cardiac integrity.

Oxidative Stress represents an imbalance in production and clearance of reactive oxygen species/free radicals in biological systems [42]. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including protein, lipid and DNA, hence in humans, oxidative stress has been identified as one of the causal factors in many diseases [43]. Reactive oxygen species may be beneficial as they are used by the immune system as a way to attract and kill pathogens [43]. Excessive oxidative stress particularly at unwanted places (e.g vascular lining, blood brain barrier) will damage the defence system. The result of this study indicate that the MDA levels in the Yoyo bitters-fed rats were reduced when compared to the MDA level in the control. Malondialdehyde (MDA) is a product of lipid peroxidation that can be easily measured and the results of this research shows the herbal bitters prevented the lipid peroxidation of the membranes of tissues and cells in the rats; this is an antioxidant effect, meaning the bitters have antioxidant constituents. This aligns with the known fact that antioxidant constituents can delay or inhibit the oxidation of lipids and other

compounds by inhibiting the propagation of oxidation chain reaction [44; 33].

The results of this study indicate that the Vitamin C levels of the Yoyo bitters-fed rats were increased significantly ($P < 0.05$) when compared to the control. As for vitamin E, its level in the bitters-fed rats were increased but not significantly ($P > 0.05$). This can be said to be as a result of the bitters adding to and preserving the immediate use of these vitamins in the rats as its inherent antioxidant capacity act as firstline antioxidants as well as protects the rats from excessive use of its indigenous antioxidants [45; 33]. Herbaceous plants and species like those used as constituents of bitters are harmless sources for obtaining natural antioxidants. Primarily, their antioxidant effect is due to phenolic compounds such as phenolic acid, flavonoids and phenolic diterpenes and their mode of action as antioxidant compounds is due to their redox reaction properties which can absorb and neutralize free radicals by quenching singlet and triplet oxygen [45; 33]. Increasing the vitamin level of the bitters fed rats means the rats will have the positive effects associated with vitamins. Vitamins are important and their deficiencies cause adverse effects on the metabolism of the human body and even in a trace amount, they are very essential for the body metabolism [33].

Generally the superoxide dismutase activity, the Catalase activity and Glutathione Peroxidase activity of the rats fed with yoyo bitters were significantly ($P < 0.05$) increased compared to the activities of these same enzymes in the control rats. This further confirms the antioxidant improving capacity of Yoyo bitters generally. The enzymes are all antioxidant enzymes that battle oxidants and free radicals implicated in causing many diseases especially cardiovascular diseases and cancer. The results of this study imply that these bitters contain the herbaceous plants and species that are harmless sources for obtaining the natural antioxidants that may not only be anticarcinogenic but may also protective against cardiovascular diseases.

The described changes in the histopathological studies (photomicrographs not shown) done on the tissues of the heart, kidneys, liver, pancreas, small intestine and colon of bitters- fed rats did not reveal adverse differences when compared to those of control organs.

CONCLUSION

The results of this study showed that the Yoyo bitters could be safe for consumption, as the acute toxicity (LD₅₀) of the bitters, indicated they have a relatively high LD₅₀ and so they will have a low-lethality at doses they were likely to be consumed. The biochemical and haematological assay results of this study revealed that Yoyo bitters could have the following pharmacological properties:- hypolipidaemic, hypoglycaemic, anti-anaemic and anti-inflammatory, stimulant and immunity-boosting, antihepatotoxic, *in vivo* and *in vitro* antioxidant capacity and by extension antineoplastic as well as antihypertensive properties and the ability to protect against/prevent coronary artery disease and cardiovascular diseases generally.

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