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Physicochemical properties and biological evaluation of *Yoyo* bitters

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Abstract

Background: The current widespread distribution and use of *Yoyo* bitters; an herbal bitters made in Nigeria calls for an assessment of its content, efficacy and extent to which this product achieves the labelled claim of being an herbal cleanser.

Methods: The pH, analysis for trace metal and preliminary phytochemicals screening were assessed. In addition, the total phenolic acid content, antioxidant activity using DPPH inhibition and microbiological assay were evaluated using standard procedures. The biological effect of different doses on weight, blood glucose, haematological parameters, liver function and tissues pathology were investigated in healthy Wistar rats over a 28-day period.

Results: *Yoyo* bitters is a slightly acidic liquid (pH 5.46), containing 0.110 µg/L of zinc, little quantities of saponins, alkaloids, anthraquinones and cardenolides. Low total phenolic acid content (537.7±22.38 mgGAE/mL), poor radical scavenging activity; DPPH IC_{50} of 855.27±85.8 mg/mL compared with 1.27±0.03 and 1.24±0.02 mg/mL for gallic acid and ascorbic acid respectively. There was lack of antibacterial activity. The weight, blood glucose level and liver function were not affected, while only WBC and platelet levels were increased significantly ($p = 0.003$). Gut associated lymphoid tissues (GALT) was observed in the intestine as well as hepatic lesions with some of the treated groups.

Conclusion: Yoyo bitters has a weak antioxidant activity, thus may not possess significant effect on the enhancement of general body health. It has immune-potentiating effect with the risk of development of hepatic degeneration.

Keywords: *Yoyo bitters, antioxidant activity, microbiological assay, rat histopathological evaluation, immune-potentiating effect,*

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Résumé

Contexte: La courante distribution rependue et utilisation des Yoyo amers; un herbier amer produit au Nigeria appelle à l'évaluation de son contenu, efficacité et mesure dans laquelle ce produit atteint la réclamation marqué d'être un nettoyant herbier.

Méthodes: Le pH, analyse de trace métal et dépistage préliminaire des composés phytochimiques ont été évalués. En outre, la teneur totale en acide phénolique, activité antioxydant utilisant inhibition DPPH et dosage microbiologique ont été évaluées en utilisant des procédures standard. L'effet biologique des doses différentes sur le poids, la glycémie, les paramètres hématologiques, la fonction du foie et la pathologie des tissus ont été étudiés chez des rats Wistar sain sur une période de 28 jours.

Résultats: Yoyo amers est un liquide légèrement acidique (pH 5,46) contenant 0,110 µg/L de zinc, petites quantités de saponines, alcaloïdes, anthraquinones et cardénolides. Faible teneur totale en acide phénolique (537,7 ± 22,38 mg GAE / mL), faible activité des radicaux de balayage; DPPH CI_{50}de 855,27 ± 85,8 mg / mL comparé à 1,27 ± 0,03 et 1,24 ± 0,02 mg / ml pour acide gallique et acide ascorbique, respectivement. Il y avait un manque d'activité antibactérienne. Le poids, niveau glycémique et fonction hépatique n'ont pas été affectés, tandis que seuls les niveaux de WBC et des plaquettes ont significativement augmenté ($p = 0,003$). Tissus lymphoïdes associés au Gut (TLAG) a été observée dans l'intestin, ainsi que des lésions hépatiques avec certains des groupes traités.

Conclusion: Yoyo amers a une activité antioxydant faible, donc ne peut pas avoir d'effet significatif sur l'amélioration de la santé générale du corps. Il a un effet immune-potentialisant avec le risque du développement de dégénérescence hépatique.

Mots-clés: *Yoyo amers, activité antioxydant, analyse microbiologique, évaluation histopathologique de rat, effet immune-potentialisant,*

Introduction

Herbal medicinal products have been used over the years for the management of various disease conditions and infections with significant

contributions to healthcare despite the great advances in modern medicine [1]. The use of herbal medicines has been earlier adduced to their low price, easy availability and affordability; the use was more prominent in developing countries. However, with the increase in the prevalence of some chronic diseases to which modern medicine has not been able to proffer solution, attention has been shifted to herbal medicinal products. There is a significant increase in the demand and use of natural phytopharmaceuticals [2], with the advocates emphasizing their safety and efficacy due to long empirical use and natural origin [3]. Several studies have highlighted the therapeutic benefits of several medicinal plants. The WHO reported that 70 - 80% of Africans and Indians depend on traditional medicine for their primary healthcare [4, 5].

In Nigeria, the use of herbal medicinal products cuts across the different strata of economic classes from the low income to the elites. This has resulted in increased use of such products. This has also triggered an increase in the commercialization of the herbal preparations which are presented in various dosage forms ranging from powders; solutions, tablets, capsules, creams to ointments.

Most herbal preparations contain complex mix of phytochemicals whose component raw materials are usually from variety of plant species with different parts of the plants being used. Plants comprise of complex secondary metabolites as mixtures of phytochemicals; including alkaloids, saponins, terpenes, tannins and other acidic constituents. The mixture of different plants could result in chemical interactions of the constituent compounds from the different plants used. The interactions of the constituent secondary metabolites are the basis for the observed biological effects of such herbal preparations which could be either beneficial or adverse [6]. In some countries, including the USA, botanicals are marketed as dietary supplements and not intended for curing, mitigation, treatment or diagnosis of any ailment. Dietary supplements are used to maintain health, which is a level of functional and metabolic efficiency of a living being. There are various types of herbal products of which herbal bitters constitutes a key member of this group of products [7, 8].

Medicinal, herbal bitters contain blended ingredients in water or as alcoholic tincture. They were originally sold as digestive aids because of their ability to increase the production of saliva and digestive juices. Bitters became popular in Europe in the 1600s.

There are various types of herbal bitters available in Nigeria of which *Yoyo* '*Cleanser*' bitters is about the first herbal bitters made in the country and sold as dietary supplement and marketed as a digestive stimulant and detoxifier. *Yoyo* bitters is reportedly formulated to support the immune system and body's ability to resist disease by reducing free radical damage and removal of harmful toxins in the body. Thus, it is proposed to effectively maintain the overall health and well-being in the management of diverse human ailments. It is composed of five medicinal plants; *Aloe vera, Acinos arvensis, Citrus aurantifolia, Chenopodium murale, Cinnamomum aromaticum* [9]. Previous studies on Yoyo bitters with focus on the toxicity and immunomodulatory effects reported induced hypokaeleamia and raised plasma marker enzymes for liver function at sub-chronic administration [10].

The general perception is that herbal drugs are very safe, and free from side effects. This has been found not to be entirely true. It is also known that herbs can produce undesirable side effects and can be toxic [11]. The need for the assurance of safety, quality and efficacy of herbal products is imperative in view of the high level of commercialisation of herbal medicines in Nigeria [12]. Some of the factors required for the promotion and maintenance of good health in humans and animals include microbial infection control, adequate levels of haematological parameters; packed cell volume, red blood cell count, haemoglobin level, white blood cell count, platelet levels etc, and oxidative stress control.

The current widespread distribution and use of *Yoyo* bitters calls for an assessment of the actual extent to which this product achieves the labelled claim. Although efforts on the elucidation of the toxic effects of some herbal bitters has been attempted [10, 13], this present study focuses on the quality assessment of Yoyo bitters with the aim of evaluating the possible antibacterial and antioxidant properties of the phytochemicals. The study also evaluated the biological effect of sub-chronic administration of the herbal product (at higher doses) on weight, haematological parameters, liver enzyme levels and tissue pathology of healthy rats.

Materials and methods

Herbal sample

Yoyo *'Cleanser'* bitters (NAFDAC No. 04-5347L, manufactured by Abllat Company Nigeria Limited, Batch no. 777AD) was purchased from a Pharmacy Store in Ibadan, Oyo State, Nigeria.

Physicochemical evaluation

Organoleptic properties was determined by transferring 5 mL of the sample into a clean test-tube and viewed to determine the clarity and colour.

A little quantity was also poured on the tongue to determine the taste. The pH was determined using a pHmeter. Thin Layer Chromatographic analysis (TLC) was carried out using Silica gel GF_{254} as stationary phase and five mobile phases [100% ethyl acetate; ethyl acetate: glacial acetic acid: water (6.6 :0.8:1.9); ethyl acetate : n-hexane (6 : 4); n-hexane : ethyl acetate : ammonia (4 :5:1); dichloromethane: methanol: cyclohexane: ammonia (7.8:3.9:2.3:0.9) and dichloromethane: methanol: cyclohexane: ammonia (12:6:6:3)]. Visualisation was done using daylight, ultraviolet light (254 nm and 365 nm), and Dragendorff and vanillin as spray reagents. Trace metal content for copper, chromium, zinc and lead were determined using atomic absorption spectroscopic analysis using an earlier described procedure [14].

Phytochemical screening

Tests for alkaloids, tannins, saponins, cardenolides and anthraquinones were done according to standard procedures [15].

Determination of total phenolic acid content

Folin-Ciocalteau method was used to estimate total phenolic acid content of the sample product [16]. To 100 µL of the sample was added 2 mL of 0.2% w/v Na_2CO_3 solution, the solution was mixed properly and incubated for 2 minutes. Folin-Ciocalteau reagent (100 µL of 500 mg/L solution) was added and allowed to stand for 30 minutes at 25°C. The absorbance was measured at 750nm against a reagent blank. The total phenolic content was determined using the standard gallic acid calibration curve and the result expressed as (mg/GAE) mL.

Determination of antioxidant activity

Antioxidant capacity and the free radical scavenging activity of the sample were estimated using the DPPH radical scavenging method [17]. To 1 mL of 0.3 mM DPPH solution in methanol was added 2.5 mL of different concentrations (0, 5, 10, 15, 20, 25, 30, 35 µg/mL) of the sample solution and properly mixed, the sample was incubated at room temperature for 30 minutes, after which the UV absorbance was taken at 520 nm. The negative control was 2.5 mL of methanol and 1 mL of DPPH. The procedure was also carried out with gallic acid and ascorbic acid at similar concentrations respectively, which served as standard. The DPPH radical-scavenging activity was calculated using the following equation;

% DPPH scavenging = [Abs (control) – Abs (sample) /Abs (control)] x 100

where Abs (control) is the absorbance of the control reaction; containing all the reagents except the test compound, while Abs (sample) is the absorbance of the sample.

Microbiological evaluation

Determination of microbial load: Sample concentrations (1 mL) at serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} were plated on nutrient agar (NA), potato dextrose agar (PDA) and McConkey agar (MCA) plates. All the agar plates were incubated at 37°C for 48 h except PDA which was incubated for 72 h. Antibacterial susceptibility testing: Clinical isolates of *Escherichia coli* (3 strains), *Pseudomonas aeruginosa* (2 strains), *Staphylococcus aureus* (2 strains), and one strains each for *Bacillus subtilis, Proteus mirabilis, Klebsiella species* obtained from the Department of Microbiology, University of Ibadan were used for the study. Susceptibility tests were carried out using agar-well diffusion and turbidimetry methods.

The agar-well diffusion method involved the use of half, normal and double strength concentrations (25 µL) of the sample; it was introduced into agar wells (8 mm) bored into agar plates seeded with the different microorganisms. The agar plates were incubated at 37°C for 24 h. Zones of inhibition (mm) for each well were identified and measured at the end of incubation period. Streptomycin and colistin were used as positive controls, while sterile distilled water was used as negative control.

Turbidimetry method was done by adding nutrient broth (9 mL) to 1 mL of microorganism (10^6 cfu/mL) in labelled test tubes, these were carefully mixed. Initial absorbance was adjusted to optical density of 0.4. The sample (100 µL) was added to the labelled tubes, incubated at 37°C for 24 h after which the absorbance was taken at 560 nm using UV spectrophotometer (Perkin Elmer, Lambda 25, Singapore). Similar test tubes without the samples were prepared and incubated under the same conditions. All the determinations were done in triplicate.

Biological evaluation

Twenty four male albino Wistar rats (180 – 230 g) obtained from the Central Animal House University of Ibadan, were used for this study. The rats kept at ambient temperature and humidity with 12 h light and dark cycle, were allowed to acclimatize for one week. The animal study was done in accordance with the National Institute of Health Guidelines for Care of Laboratory animals of 1985. They were fed with rat pellets (Ladokun feeds) and water *ad libitum*. The rats were randomly distributed into four groups comprising three experimental test groups [half dose

(YH), normal dose (YN), and double dose (YD)] and control group i.e. negative control (CN). Each group comprised 6 rats.

Yoyo bitters at different doses; 15 mL/kg, 30 mL/kg and 60 mL/kg representing half dose (YH), normal dose (YN) and double dose (YD) respectively were administered to the appropriate groups using oral cannula for 28 days.

The weights and blood glucose levels were determined using glucometer (AccuCheck®, Roche, India) were determined on the first day before treatment commenced (day 0), and at 7, 14, 21 and 28 days. Haematological parameters including; haemoglobin concentration (Hb), packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC) and platelets were determined at the end of the treatment period (24 h after the last dose) using blood (5 mL) collected through the ocular vein into labelled heparinised bottles [18].

The serum obtained from blood (3 mL) collected into labelled non-heparinised bottles from ocular vein were analysed for two liver enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using RANDOX enzyme kits Histopathological examination of the organs; heart, spleen, kidney, liver, intestine and testes were analyzed using conventional methods.

Statistical analysis

The results obtained were presented as mean ± SEM and subjected to statistical analysis using SPSS 17.0. Student t-test and ANOVA were applied with the Duncan's Multiple Range test used for the post test where appropriate. Differences were considered significant at $p < 0.05$.

Results

Physicochemical evaluation

Yoyo bitters is a dark brown liquid with very bitter taste and pH 5.46. The TLC gave a maximum of four spots with two of the mobile phases. Trace elements content revealed the absence of copper, chromium and lead, while the zinc was present at low concentration of 0.110 µg/L.

Phytochemical screening

Phytochemical screening showed the presence of saponins, tannins, alkaloids, anthraquinones and cardenolides at very low quantities.

Total phenolic acid content and antioxidant activity

Total phenolic acid content was 537.7 ± 22.38 mgGAE /mL, while the DPPH radical scavenging activities increased with increase in concentration, the IC_{50} for DPPH inhibition was 855.27± 85.8 mg/mL compared with 1.27± 0.03 and 1.24 ± 0.02 mg/mL for gallic acid and ascorbic acid respectively.

Microbiological evaluation

Absence of microorganism was observed in all the media used at the various concentrations i.e. diluted and undiluted. Furthermore, the *'bitter'* exhibited weak antibacterial activity on only one strain of *Escherichia coli*; zone of inhibition of 5.0 ± 3.54 mm against that of reference streptomycin (28.5 ± 5.5 mm), while it was not active on all the other microorganisms used in the study using both agar well diffusion and turbidimetry assay.

Effects of Yoyo bitters on haematological and biochemical parameters

A non-significant increase in weight at normal and double dose with a non-significant decrease with half dose administration at 28 days was observed with administration of *Yoyo* bitters when compared with the weight before the administration (Table 1).

On the other hand, no significant effect was observed on the blood glucose level of the healthy rats. Similarly, the slight increase in packed cell volume (PCV), haemoglobin level (Hb) and red blood cell (rbc) was found not to be significant (Table 1).

Table 1: Body weight, glucose level and haematological parameters obtained after administration of yoyo bitters to wistar rats for 28 days.

Group	Body Weight (g)		Blood Glucose (mg/dL)		Haematological parameters at 28days				
	Day 0	Day 28	Day 0	Day 28	PCV (%)	Hb. (g/dL)	RBC ($x10^{12}$/L)	WBC (/Ul)	Platelet (/Ul)
YN	185.0± 6.2	220.0±12.9	122.5±5.7	119.7±3.2	44.2 ±1.3	14.3± 0.6	7.3 ± 0.3	9,491.7 ±760.9[a]	164,500 ±32,406[b]
YD	196.7 ±13.8	226.7±16.7	113.7±8.37	113.0 ±4.5	43.8±0.9	14.3± 0.4	7.3 ± 0.2	11,191.7 ± 160.9[a]	130,500 ±18,518[b]
YH	195.0±17.3	173.3±36.4	110.2±10.6	96.7 ±19.7	44.2 ±1.9	14.5± 0.8	7.4 ± 0.3	9,590.0 ±885.1[a]	118,800 ± 11,599
CN	195.0±7.6	195.0 ±8.7	110.5±1.7	117.3±2.1	42.8 ±1.4	13.6± 0.5	7.0 ± 0.3	6,650.0 ± 156.5	125,200 ± 10,370

Data presented as mean ± SEM, n = 6, superscripts represents statistical difference at $p < 0.05$

However, the increase in white blood cell at the different concentrations was found to be statistically significant ($p = 0.003$) (Table 1). The normal dose (YN) showed a statistically significant increase in platelets when compared with the control group, while the double dose (YD) and half dose (YH) did not show any significant difference. Non-significant decrease in ALT and AST was observed at different concentrations (Table 2).

Table 2: Effect of Yoyo bitters administration on the liver enzymes of the rats after administration for 28 days.

Group	ALT Activities (U/L ± SEM)	AST Activities (U/L ± SEM)
YN	3.17 ± 2.03	8.95 ± 2.72
YD	3.16 ± 1.24	8.07 ± 2.86
YH	2.50 ± 0.40	3.68 ± 3.72
CN	5.11 ± 2.34	7.16 ± 4.75

Data presented as mean ± SEM

Effects of Yoyo bitters on the organs

Histopathological evaluation of the selected organs showed the development of gut associated lymphoid tissues (GALT) in the intestine after administration for 28days in both normal and double doses (Figure 1). Liver lesions (fatty degenerations) were observed with the normal and half dose administrations. No visible lesions were observed in the other organs.

Discussion

Much has been said about the health benefits of bitters which include; an increase in the production of digestive juices (hydrochloric acid and bile), activation of immune function through sensors in the small intestine and playing important roles in weight loss [19, 20]. However, little attention has been paid to the empirical evidences of such activities, functions and benefits. This present study elucidates the antioxidant, antibacterial and mucosal immune modulation properties of *Yoyo* bitters which have not been previously reported in Nigeria.

The physicochemical analysis of the herbal bitters showed a weakly acidic product, dark brown liquid with a sharp bitter taste which is a result of the constituent five bitter herbs. The thin layer chromatography showed a maximum of four spots as against five constituent plants. This may indicate the constituents with the highest proportion during the mixture or it may be as a result of possible dilute extract of the different active plants being used. Plants with such aromatic properties included in the formulation are *Cinnamomum aromaticum* and *Citrus aurantifolia* [21].

The presence of zinc at 0.110 µg/L in the preparation which is equivalent to a daily intake of 0.0033 µg is far below the recommended daily limit of 3 – 8 mg [22]. Hence, the zinc content at this low level in the bitter could not have any negative effect on the body homeostasis. The absence of copper, lead and chromium and low level of zinc indicated a certain level of safety with respect to trace metal content.

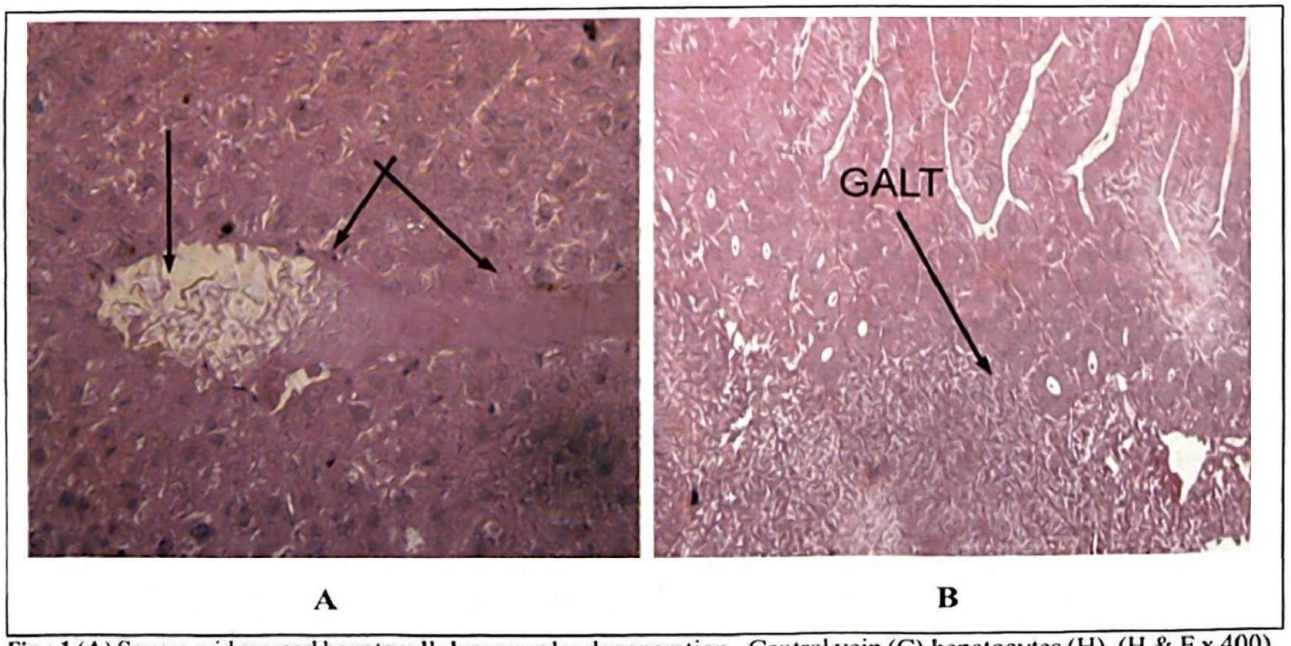

Fig.: 1 (A) Severe widespread hepatocellular vacuolar degeneration. Central vein (C).hepatocytes (H). (H & E x 400). B) Gut associated lymphoid tissue (GALT) in the intestine. (H & E x 100).

Phytochemical investigation showed the presence of low quantities of tannins, saponins, cardenolides, anthraquinones and alkaloid. These plant secondary metabolites are known to possess a wide range of pharmacological activities [23]. In view of the fact that almost all the plants listed in the formulary of the product have been reported to contain these secondary metabolites, their presence in little quantities may be as a result of dilution, possible reaction between the metabolites or process of preparation and parts of the plants used.

Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals [24]. Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers [25, 26]. The total phenolic acid content obtained for *Yoyo* bitters in this study was 537.7 ± 22.38 mgGAE /mL. The inhibition of DPPH is widely used to measure antioxidant and free radical scavenging power of chemical compounds [27, 28]. The higher the percentage (%) inhibition of DPPH and lower IC_{50} value the higher the free radical scavenging activity and antioxidant power [26]. The antioxidant activity of plants has been related to the active compounds present in them. The result of this study showed that the DPPH radical scavenging activities of Yoyo bitters increased from 0.81 to 2.72% at sample concentration 5 to 35 µg/mL compared to ascorbic and gallic acid whose activities increased from 76.06 to 97.39% and 76.31 to 98.17% respectively. Similarly, the radical scavenging activity of Yoyo bitters (IC_{50} = 855.27 mg/mL) was too small compared to 1.24 mg/mL and 1.27 mg/mL) for ascorbic acid and gallic acid respectively. This DPPH radical scavenging activity showed that *Yoyo* bitters is a weak free radical scavenger. The DPPH radical scavenging corroborates the low total phenolic acid content obtained in this study.

Microbial assay of the *Yoyo* bitters sample showed an absence of all types of microorganism, also no evidence of significant antimicrobial properties was observed. The observed weak antimicrobial effect shows that the microorganism species were not susceptible/sensitive to the herbal bitter except for one strain of *E. coli* used which showed little inhibition zone which may be a bacteriostatic effect or indicate a weak strain of the organism. The absence of antimicrobial activity coupled with the absence of microorganism in the *Yoyo* bitters sample may be indicative of the presence of some substances (preservatives) that mask the two properties (susceptibility and microbial load). This could be explained by the fact that some of the component plants in the formulation such as *Acinos arvensis, Citrus aurantifolia, Cinnamomum aromaticum* [29, 30] have been reported to possess antibacterial and antifungal activities, with *Cinnamomum aromaticum* specifically indicated for prevention of food spoilage due to bacterial contamination [31]. These findings could indicate the level of care used in the preparation and or composition of the formulation of the Yoyo bitters to prevent microbial spoilage of the product.

Biological evaluation of Yoyo bitters in an animal model showed that the herbal bitter caused a non-significant increase in weight by the end of the study (28 days) for normal dose and double dose administrations, while the half dose was accompanied by a decrease in weight (Table 1) even below that of the control group. The effect on body weights with normal dose group (YN) is in agreement with earlier reports on *Yoyo* bitters [10, 13], while that of half dose group (YH) was not. There was no significant effect on the blood glucose in healthy rats at the different concentrations used in this study. This is at variance with an earlier report of significant reduction of blood glucose by *Yoyo* bitters at normal dose [13].

The slight increase in PCV, Hb and RBC levels in all the treatment groups of healthy animals used in this study was found not to be statistically significant ($p>0.05$) as shown in table 1. Furthermore, no significant effect was obtained on the lymphocytes, monocytes, eosinophils and neutrophils levels (Table 1). Although, Oyewo *et al*, 2013 [13] reported a significant reduction in PCV, RBC and Hb with a significant increase in lymphocytes, monocytes, eosinophils and neutrophils levels. However, a significant increase in white blood cells and platelets ($p=0.003$) was obtained with all the treatment groups, with the double dose (YD) showing the highest increase when compared with the control group (Table 1). This increase in white blood cells and platelets is an indication of enhanced immune system and clotting activity as earlier opined by Ekor *et al*, [10] and Oyewo *et al* [13].

Most pathological changes induced by xenobiotics are usually assessed from the liver [32]. An initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood. Alanine transaminase (ALT) is an enzyme that helps metabolize protein, while AST is involved in the metabolism of the amino acid: alanine. In the presence of liver damage or compromise of liver function the two enzymes are

released into the bloodstream. Thus, an increase in ALT and AST levels in the blood may indicate liver damage or disease. Alanine transaminase is a cytosolic enzyme, which is more specific for the liver than AST. A significant decrease in ALT was obtained in this study at all the concentration which is accompanied by a non significant increase in AST at the normal dose (YN) and double dose (YD), while a significant decrease was obtained at the half dose (YH). These liver enzymes changes observed, though at variance with the earlier report by Ekwor *et al* [13] corroborates their observation of absence of decline in the metabolic function of the liver. Histopathological evaluation of the liver and intestine showed the presence of some liver lesions with the normal and half dose groups, and gut associated lymphoid tissues (GALT) in the intestine, in the normal and double doses, while no visible lesion was observed in the other organs.

The development of gut associated lymphoid tissues (GALT) in the intestine after the administration of *Yoyo* Bitters for 28 days is indicative of an enhanced mucosal immunity which may in turn protect the animal from intestinal infections. This corroborates the immune modulator effect earlier reported by Oyewo *et al* [10]. On the other hand, the presence of liver lesions may be a sign of possible damage to some of the liver cells. This result thus indicates that though AST and ALT levels were not significantly affected to indicate liver damage within the period of the study, there is the need to exhibit caution as the observed lesions may result in liver necrosis as a result of cumulative effect of prolong use of *Yoyo* bitters. This is very important in view of the lipid peroxidation effect of Yoyo bitters reported by Adeyemi *et al* [33].

The low quantities of phytochemical constituents obtained in this study may explain the weak antioxidant activity obtained with Yoyo bitters which could be easily associated with the low total phenolic acid content and weak radical scavenging activities.

This weak antioxidant activity observed in this study indicated that *Yoyo* bitters may not be of any significant clinical use as an antioxidant. This poses a question as to the effectiveness of this product in the removal of harmful toxins through free radical mechanism as indicated in the product label. On the other hand possible enhancement of immunity as a result of significant increase in white blood cells and platelets, as well as the development of GALT in the intestine corroborates the labelled claim of supporting the immune system.

Conclusion

It may be concluded from this study that the increased white cell production and gut associated lymphoid tissue may be the means by which *Yoyo* 'Cleanser' bitters enhances the general body health. However, there is need for caution with long term use because of the mild liver lesions observed when administered for about one month in this study, which may be aggravated as a result of cumulative effect of the use of *Yoyo* bitters.

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