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Research Article

SAFETY CONCERNS OF THE REPEATED ADMINISTRATION OF YOYO BITTERS IN MALE WISTAR RATS

OYEWO, EMMANUEL BUKOYE AND FAKUNLE, PONLE BAMIDELE

Oyewo, Emmanuel BukoyeDepartment of Biochemistry,Faculty of Basic Medical Sciences,Ladoke Akintola University of Technology, P. M. B. 4000, Ogbomoso, Oyo State. Nigeria. Email: askafterbukoye@yahoo.com/eboyewo@lautech.edu.ng

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ABSTRACT

Objective: The safety of sub-chronic administration of Yoyo bitters at the manufacturer's recommended doses for adult human was assessed in male Wistar rats.

Methods: Eighteen rats were assigned into three groups (n=6) with mean body weights of 86.2±4.43g. Group A received distilled water, while 0.308 and 0.462 ml/kg body weight of Yoyo bitters were administered daily for 56 days to groups B and C respectively. The toxic index of Yoyo bitters was determined by liver function indices, the activities of respective 'enzyme markers', such as, lactate dehydrogenate (LDH), alkaline phosphatase (ALP) and acid phosphatase (ACP) in the liver, kidney, small intestine, heart, brain, lungs, spleen, serum and the histological studies on the organs.

Results: Dose dependent decreases ($p<0.05$) were obtained in the LDH activities in the liver, small intestine and lungs. ALP activities were decreased ($p<0.05$) in the liver, kidney, small intestine and heart, while the activities of ACP were reduced ($p<0.05$) in the liver and kidney. Significant enzyme inductions were recorded in the spleen and brain of rats administered Yoyo bitters ($p<0.05$). Liver function indices indicated the interference of Yoyo bitters with hepatic functions. Structural alterations were revealed in the histoarchitecture of the small intestine and spleen.

Conclusion: Therefore, this study discouraged the regular usage of Yoyo bitters as a dose below the recommended dose was toxic to the spleen and small intestine.

Keywords: Safety, Sub-chronic, Yoyo bitters, Recommended dose, Discouraged, Regular usage

INTRODUCTION

Herbal medicine is renowned as the most common type of alternative medicine. It was reportedly said to be used by about 80% of the world population both in the developing countries and in the developed countries where modern medicines are predominant [1, 2]. The rising popularity of phytomedicines could be attributed to the alleged advantages of being efficacious and also a cheap source of medical care. In the same vein, there is growing disillusion with modern medicines coupled with the misconception that herbal supplements might be devoid of adverse and toxic effects, which are associated with convectional and allopathic medicines.

Herbal bitters are most often 'polyherbal formulations prepared from mixtures of many plant parts, obtained from various plant species and families. Thus, they contain multiple bioactive constituents that could have interacted with one another in solution, thereby posing some difficulties in their characterization [3]. Herbal supplements are administered in most clinical conditions over a long period of time, without the consideration of toxic effects that might result from such prolonged usage and also, the proper dosage monitoring [4]. Although, folkloric herbal supplements are alleged to be safe. However, some herbal products have been reported to be toxic at high doses, while some others were associated with potential adverse effects after prolonged usage. In most cases, these herbal products are not often prescribed by the physician and neither were they dispensed by the pharmacist. The individual reports of their adverse effect are largely inaccurate and oftentimes excommunicated. Therefore, the danger associated with the potential toxicity of many of these herbal products and other herbal therapies, which are being used over long period of times, demands that the practitioners be kept abreast of the reported incidence of any tissue toxicities.

Yoyo bitters is a poly-herbal formula, produced by Abllat Company Nigeria Limited, Ikeja, Lagos State. Ethnomedicinally, it is allegedly taken once daily and preferably at night (30 ml for adult) for the treatment of indigestion, muscle pains (colic), joint pains, backache, menstrual pain, regulating blood sugar, blood cleanser, keeping healthy (immune booster) among others. The present study was, therefore, carried out to evaluate the sub-chronic toxicity profile of Yoyo bitters in male Wistar rats.

MATERIALS AND MATERIALS

Herbal bitters

Yoyo bitters was a product of Abllat Company Nigeria Limited, 12 Ajayi Close, Ikotun-Ijegun Road, Ikeja, Lagos State.

Quantitative assay kits and other reagents

Acid phosphatase (ACP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, albumin and bilirubin concentrations were products of LABKIT, CHEMELEX, S.A. Pol. Canovelles-Barcelona, Spain. All the chemicals and reagents used in the study were of analytical grade and were purchased from the Bristish Drug House (BDH) Poole England and Sigma Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.

Laboratory animals

Eight to ten weeks old male Wistar rats were obtained from the Animal Care Facility II, Ladoke Akintola University of Technology (LAUTECH), Osogbo, Osun State. The rats were fed with rat pellet (product of Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria).

Methods

Experimental animals and procedure

The eighteen male Wistar rats were randomly grouped into three, Comprising of six rats per group with average body weight of 86.2 ± 4.43 g. The rats were housed in cages made of wooden frames and metal netting, and were fed *ad libitum* with rat pellet and tap water with 12-hours light/dark cycle. The cages were cleaned every morning and disinfected at intervals of 3 days. The rats were allowed to acclimatize for 14 days before the administration of Yoyo bitters was commenced. Calculated doses of Yoyo bitters in ml/kg body weight from the recommended doses in the manufacturer's pamphlet (20 and 30 ml respectively for adult human) were administered to male Wistar rats as illustrated:

Group A: control, Received 1.0 ml distilled water

Group B: received 0.308 ml/kg body weight of Yoyo bitters (YB)

Group C: received 0.462 ml/kg body weight of Yoyo bitters (YB)

Administration of the herbal bitters was performed orally, once daily between 7:20 pm ± 30 minutes, using metal cannula attached to a 1.0 ml syringe. The administration of the herbal bitters lasted for 56 days, after which the rats were fasted for 12 hours and the organs of interest (brain, liver, kidney, small intestine, heart, spleen and lung) were exercised, cleansed and blotted with filter paper. This study was conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals [5].

Determination of enzyme activities

The lactate dehydrogenase (LDH) activity was determined by pyruvate kinetic liquid reaction, according to the method described by [6]. The activity of alkaline phosphatase (ALP) was determination by p-nitrophenylphosphate kinetic reaction, according to the method described by [7]. The activity of acid phosphatase (ACP) was determined by α-naphtyphosphate kinetic reaction, according to the method described by [8]. The serum and tissue homogenates were stabilized with 50 µl of acetic acid (R_4) per ml of sample.

Serum proteins determination

The determination of serum total protein concentration was determined by Biuret colourimetric reaction by the method of [9], while serum albumin concentration was determined by bromocresol green colourimetric reaction as described by [10]. The globulin concentration was obtained by the formulae:

Globulin (g/dl)= Total protein (g/dl) – Albumin (g/dl)

Serum bilirubin determination

The serum bilirubin concentrations were determination by dimethylsulphoxide (DMSO) colourimetric reaction as described by [11].

Histology

The histological studies were performed on liver, kidney, brain, heart, spleen, small intestine and lung following the procedure described by [12].

Statistical analysis

This research work employed a completely randomised design (CRD) model and the results were expressed as mean of 5 replicates ± standard error of mean (SEM). Results were analyzed using statistical package for social sciences (SPSS) 16.0 for Window software. Results were subjected to one way analysis of variance (ANOVA) to test the effect of each dose level on the parameter under investigation at 95% level of confidence. The Duncan Multiple Range Test (DMRT) was conducted for the pair-wise mean comparisons, to determine the significant treatment dose at 95% level of confidence. Values were considered statistically significant at ($p<0.05$) and denoted by different alphabets [13].

RESULTS

Enzyme activities

The activities of the 'enzymes markers' determined in some tissues in male rat administered with Yoyo bitters are depicted in Table 1. The administration of Yoyo bitters at 0.462 ml/kg body weight produced significant reductions ($p<0.05$) in the lactate dehydrogenase (LDH) activity of male rats liver. In the small intestine and lungs of rats administered with the herbal bitters, dose dependent marked reductions ($p<0.05$) were obtained in the LDH activity, while significant increases ($p<0.05$) were recorded in the LDH activity in the heart, brain and serum. At 0.462 ml/kg body weight of Yoyo bitters, significant reductions ($p<0.05$) were obtained in the ALP activity in liver, kidney, small intestine and heart of rats. The activity of ALP increased significantly ($p<0.05$) in the brain, lungs, spleen and serum in male rats administered with Yoyo bitters. The activity of acid phosphatase (ACP) was significantly reduced ($p<0.05$) in the liver and kidney of rats administered with the herbal bitters, while the enzyme activity was markedly increased ($p<0.05$) in the spleen and serum of the rats (Table 1).

Liver function indices

Administration of the herbal bitters at 0.308 ml/kg body weight of Yoyo bitters reduced ($p<0.05$) the concentration of the total bilirubin, while the concentration of conjugated bilirubin was not significantly changed ($p>0.05$) at both doses of the herbal bitters (Table 2). The concentrations of unconjugated bilirubin and total protein were significantly increased ($p<0.05$) at 0.462 ml/kg body weight of the Yoyo bitters. Rats administered with Yoyo bitters produced a significant increase ($p<0.05$) in the serum albumin concentration at 0.308 ml/kg body weight, while the globulin and A/G ratio were increased significantly ($p<0.05$) at both doses of the herbal bitters (Table 2).

Table 1: Showing the activities of some 'enzyme markers' in Wistar in rat administered with Yoyo bitters

	Control	0.308 ($kb^{-1}$of YB)	0.462 ($kb^{-1}$of YB)
Activity (U/L)			
LDH	189.13±7.51[a] 460.34 ±13.65[a]	183.62 ± 5.55[a]	139.33 ± 5.27[b]
Liver	189.55±6.65[a] 220.15±9.65[a] 425.45±12.67[a] 62.65 ± 5.34[a]	215.65 ± 6.94[b]	189.50 ± 11.05[c]
Small intestine	141.04 ± 4.11[a]	291.57 ± 6.74[b]	302.34 ± 11.25[b]
Heart	169.31 ± 5.51[a]	315.05 ± 13.24[b]	330.55 ± 8.85[b]
Brain	180.34 ± 9.93[a]	285.66 ± 9.87[b]	210.65 ± 8.65[c]
Lung	202.15±7.28[a] 95.45±3.85[a] 187.20±6.17[a] 165.95±5.64[a] 115.56 ± 3.35[a]	172.45 ± 8.80[b]	227.50 ± 9.95[c]
Serum	235.37 ±11.26[a]	140.61 ± 5.25[a]	97.22 ± 4.71[b]
ALP	352.44±7.54[a] 178.34 ± 9.35[a]	153.22 ± 5.31[a]	121.31 ± 4.81[b]
Liver	87.35 ± 4.25[a]	172.45 ± 6.65[a]	145.64 ± 4.34[b]
Kidney		254.65 ± 5.94[b]	262.35 ± 6.25[b]
Small intestine		135.85 ± 5.55[b]	172.72 ± 6.15[c]
Brain		178.17 ± 5.05[a]	155.15 ± 5.25[b]
Lung		191.55 ± 4.95[b]	190.65 ± 3.75[b]
Heart		195.45 ± 4.45[b]	191.25 ± 4.65[b]
Spleen		202.31 ± 9.24[b]	155.67 ± 10.43[c]
Serum		321.84 ± 9.65[b]	309.67 ± 11.05[b]
ACP		245.55 ± 7.86[b]	261.50 ± 8.25[b]
Liver		172.20 ± 7.30[b]	206.75 ± 9.65[c]
Kidney			
Spleen			
Serum			

Values are means ± SEM; n=5. Values bearing different alphabets are significantly different ($p<0.05$).

kb^{-1} of YB (kg body $weight^{-1}$ of Yoyo bitters), LDH (lactate dehydrogenase), ALP (alkaline phosphatase), ACP (acid phosphatase).

Table 2: Showing the effect of Yoyo bitters on the liver function indices in male rat

	Control	0.308 (kb^{-1} of YB)	0.462 (kb^{-1} of YB)
Concentration (mg/dl)			
Total bilirubin	37.44 ± 2.11^a	32.52 ± 1.02^b	41.72 ± 3.01^a
Conjugated bilirubin	28.88 ± 1.67^a	26.01 ± 2.81^a	29.87 ± 1.24^a
Unconjugated bilirubin	10.47 ± 0.89^a	9.92 ± 1.22^a	16.99 ± 2.42^b
Total protein	5.72 ± 0.82^a	6.01 ± 0.54^a	6.38 ± 0.21^b
Albumin	3.22 ± 0.90^a	3.61 ± 0.68^b	3.05 ± 0.38^a
Globulin	2.67 ± 0.16^a	3.01 ± 0.29^b	4.01 ± 0.87^c
A/G	1.08 ± 0.28^a	1.12 ± 0.20^a	0.80 ± 0.06^b

Values are means ± SEM; n=5. Values bearing different alphabets are significantly different ($p<0.05$).

kb^{-1} of YB (kg per body weight of Yoyo bitters)

Histology

The photomicrographs of the organs were classified as percentages of inflammation or compromise to the integrity of cells in the captured area of the organs, in which < 25% is non-significant and > 25% is significant. The administration of Yoyo bitters at the different doses did not reveal any obvious tissue damage in the liver (Figures 1-3), kidney (Figures 4-6), brain (Figures 13-15), heart (Figures 16-18) and lungs (Figures 19-21). However, dose dependent significant alterations were revealed in the photomicrographs of the spleen of rats administered the herbal bitters, compared to the control (Figures 7-9). The sinosoids and lobes (lymphoid tissues) were enlarged and the red pulps were degenerated with the infiltration of the white pulp (Figures 7-9). The small intestines showed a compromised in the structure and histoarchitecture of the small intestines that was seen as enlargement and aggregation of the lymphoid tissues that lead to the eroding of the villi and lyses of the epithelia cells (Figures 10-12).

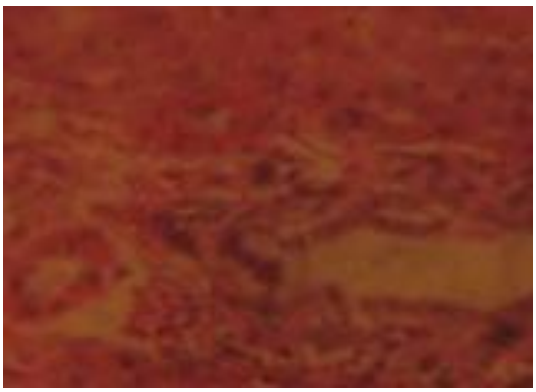

Fig. 1: Photomicrograph showing the liver of male rat administered distilled water (Mag x 100; H & E). A normal liver.

Fig. 2: Photomicrograph showing the liver of rat administered with 0.308 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A normal liver.

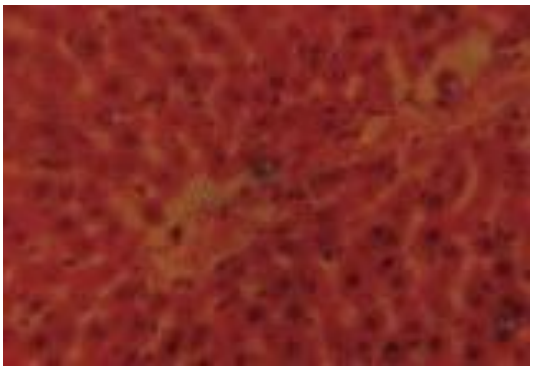

Fig. 3: Photomicrograph showing the liver of rat administered 0.462 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A liver with non-significantly enlarged hepatocytes which are acidophilic in nature.

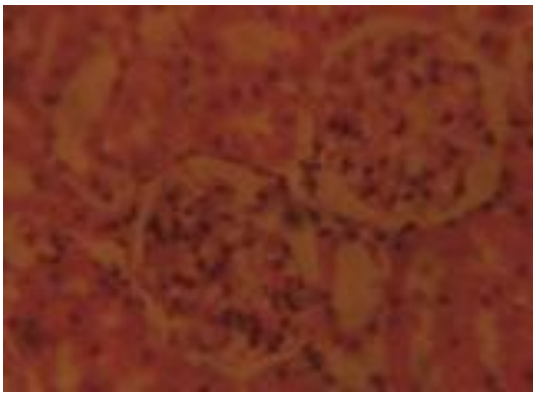

Fig. 4: Photomicrograph showing the kidney of male rat administered distilled water (Mag x 100; H & E). A normal kidney with non-significantly enlarged cells at the connective tissues.

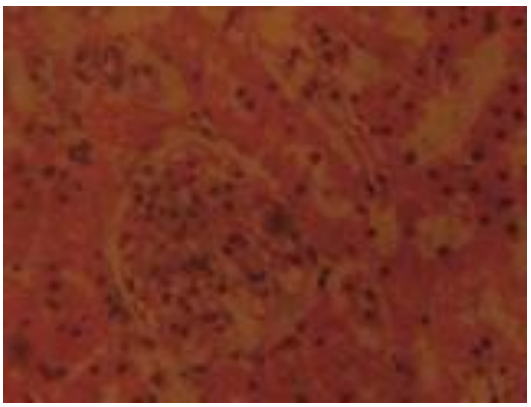

Fig. 5: Photomicrograph showing the kidney of rat administered with 0. 308 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A normal kidney.

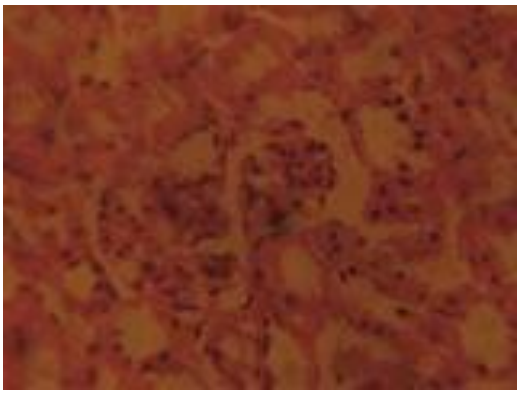

Fig. 6: Photomicrograph showing the kidney of rat administered 0.462 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A normal kidney.

Fig. 7: Photomicrograph showing the spleen of male rat administered distilled water (Mag x 100; H & E). (RP (red pulp). A spleen with non-significant increase in the red pulp.

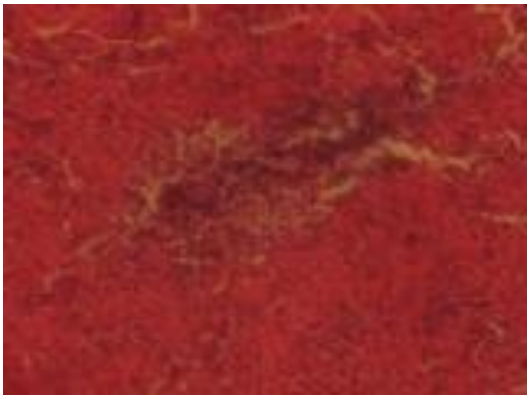

Fig. 8: Photomicrograph showing the spleen of rat administered with 0.308 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A spleen with significantly enlarged sinosoids.

Fig. 9: Photomicrograph showing the spleen of rat administered with 0.462 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A spleen with significantly enlarged lymphoid tissues.

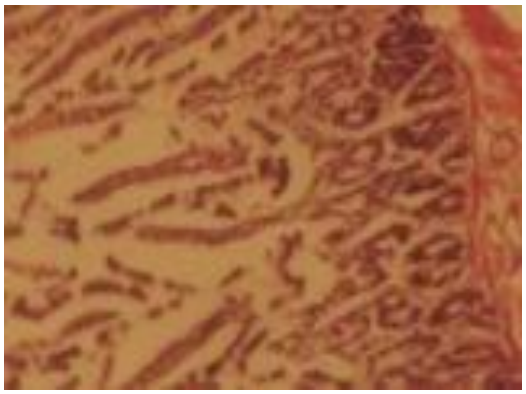

Fig. 10: Photomicrograph showing the small intestine of male rat administered distilled water (Mag x 100; H & E). A normal intestine with non significantly enlarged lymphoid tissues and separated gastric pith.

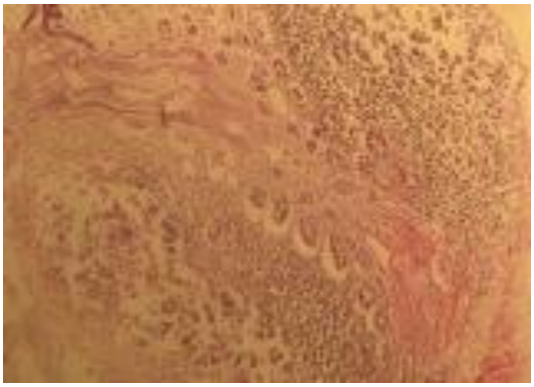

Fig. 11: Photomicrograph showing the small intestine of rat administered with 0.308 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). Intestine with significantly increased lymphoid tissues and cell lysis.

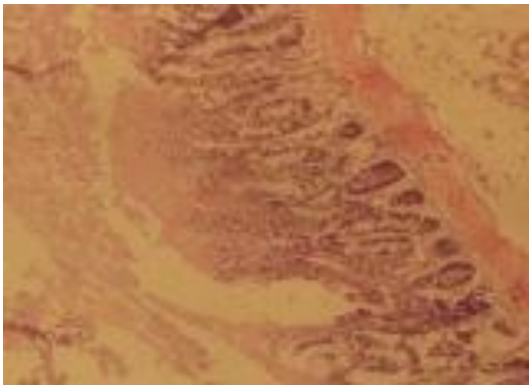

Fig. 12: Photomicrograph showing the small intestine of rat administered with 0.462 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). Intestine with significantly increased and aggregated lymphoid tissues and epithelial cell lysis.

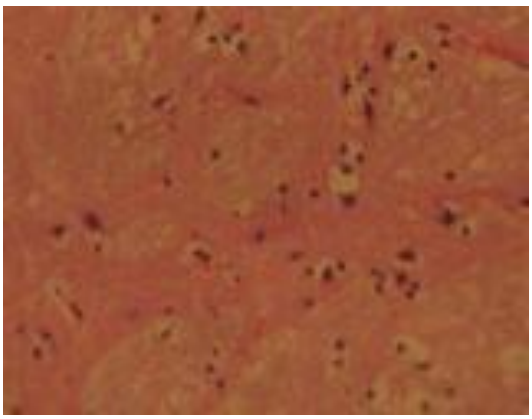

Fig. 13: Photomicrograph of brain showing the male rat administered distilled water (Mag x 100; H & E). A normal brain.

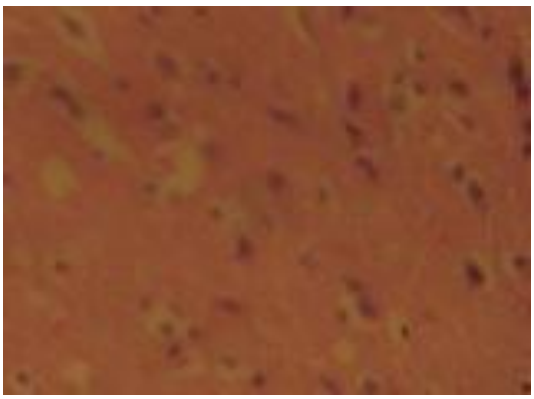

Fig. 14: Photomicrograph showing the brain of rat administered with 0.308 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A normal brain.

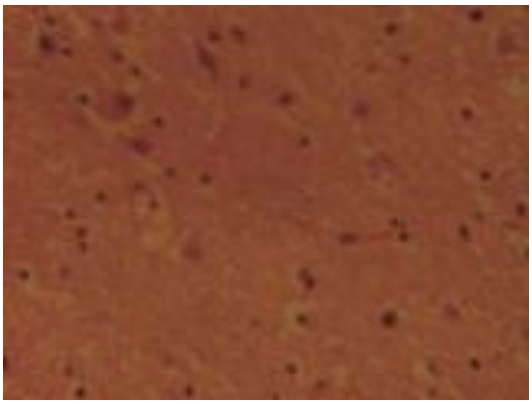

Fig. 15: Photomicrograph showing the brain of rat administered with 0.462 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A normal brain.

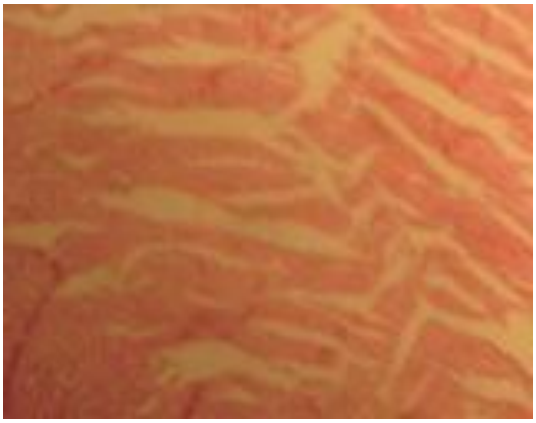

Fig. 16: Photomicrograph of heart showing the male rat administered distilled water (Mag x 100; H & E). A normal heart.

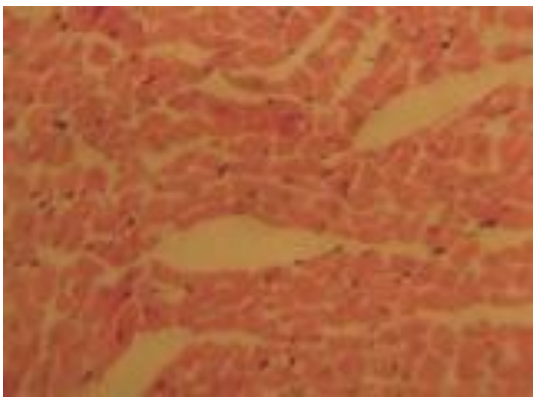

Fig. 17: Photomicrograph showing the heart of rat administered with 0.308 ml/kg ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A normal heart.

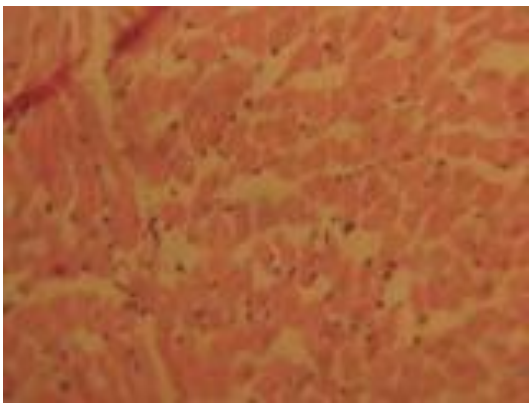

Fig. 18: Photomicrograph showing the heart of rat administered with 0.462 ml/kg ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A normal heart.

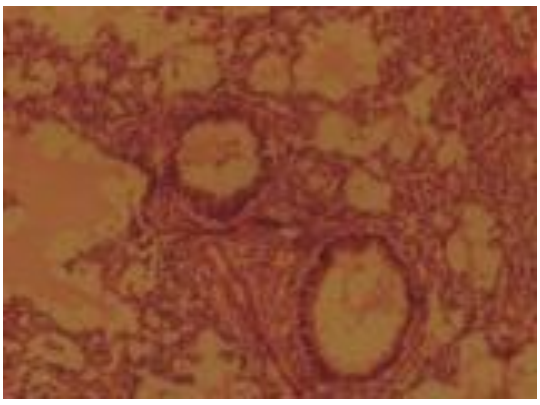

Fig. 19: Photomicrograph showing the lungs of male rat administered distilled water (Mag x 100; H & E). A normal lungs.

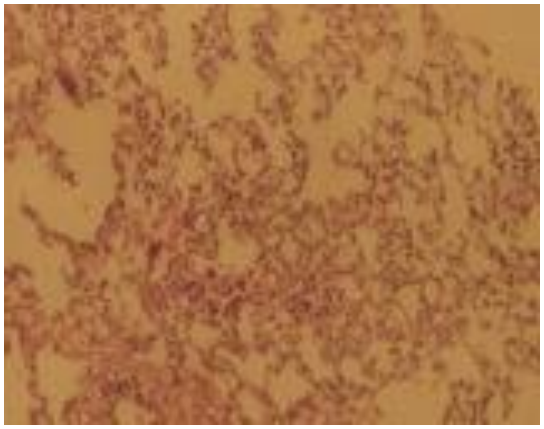

Fig. 20: Photomicrograph showing the lungs of rat administered with 0.308 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A normal lungs

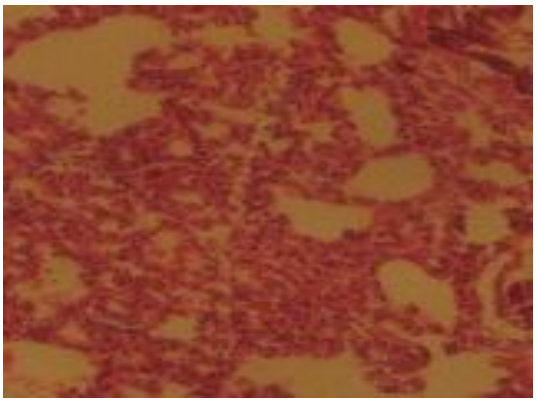

Fig. 21: Photomicrograph showing the lungs of rat administered with 10.462 ml/kg body weight of Yoyo bitters (Mag x 100; H & E). A normal lungs

DISCUSSION

Herbal medicines have received greater attention as alternative to clinical therapy in recent times leading to subsequent increase in their demand [14]. They are commonly employed in developing countries for the treatment of various diseases, which is an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy [15]. However, [16], reported that the damaging effects of herbal preparations to the human body is generally considered to be minimal compared to their synthetic counterpart (drugs), and as such, herbal medicines are generally regarded as safe (GRAS).

'Marker enzymes' are biochemical parameters associated with health indices, which are always of diagnostic significance in the routine clinical evaluation of the state of health. The result obtained in the alkaline phosphatase (ALP) activity in the kidney, small intestine and heart in rats administered 0.308 ml/kg body weight of Yoyo bitters (Table 1) indicated that at that dose, Yoyo bitters did not interfere with the activity of ALP and its cellular functions in the membrane, which includes the preservation of the structural integrity of cell. This could have been due to the stabilization of plasma membrane thereby protecting the cells from assaults and tissue damage [17]. However, at 0.462 ml/kg body weight of Yoyo bitters, the reduction recorded in ALP activity in the kidney, small intestine and heart might be as a result of the compromise in the structural integrity of phospholipids in the plasma membranes or the inactivation of the enzyme *in situ*.

In the liver of rats administered with herbal bitters, the dose dependent reduction in ALP activity may be due to the disorientation of the lipid bilayer of the plasma membrane and/ the inactivation of the enzyme *in situ*. Yoyo bitters might have challenged or exerted pressure on the liver, which could have adversely affected the normal functioning of hepatocytes, as ALP activity was reported to be related closely to the health status of the liver [18]. Reductions in the ALP activity in the liver have been reported to be as a result of a compromised in the functionalities of the hepatocytes, bile ducts or gall bladder system [19]. The reductions in the ALP activity in the tissues could have led to the inadequate transportation of needed ions or molecules across their cell membranes and the inhibition of metabolic processes, such as the synthesis of nuclear proteins, and nucleic acids.

The increase obtained in the ALP activity in the brain and spleen might indicate that the administration of Yoyo bitters increased the functional activity of ALP that probably led to *de novo* synthesis of the enzyme molecules *in situ*. It is imperative to note that this hyper-activity of ALP could lead to a threat to the survival status of the cells dependent on a variety of phosphate esters, as indiscriminate hydrolysis of phosphate esters in the tissues could result in cytolysis. [20] reported that cytolysis would adversely affect the facilitation of the transfer of metabolites across the cell membrane. The result of the serum ALP activity in rats administered with Yoyo bitters supported the results obtained in the enzyme activities in the tissues (liver, kidney, heart, small intestine and lungs), which indicated the loss of ALP into the extra-cellular spaces. The increase in serum ALP activity, however, might lead to autolysis and consequently hemolysis.

The result of the lactate dehydrogenase (LDH) activity in the liver of rats administered Yoyo bitters at 0.308 and 0.462 ml/kg body weight supported the results obtained in the liver ALP activity at the same doses (Table 1). This further strengthened the fact that Yoyo bitters at 0.308 ml/kg body weight did preserve the structural integrity of cell and functional state, but did otherwise at 0.462 ml/kg body weight. In addition, the activity of LDH in the small intestine and lungs in rats administered with Yoyo bitters, supported the result obtained in the ALP activity in the small intestine and lungs. This is logical as LDH is in close proximity to the

plasma membrane [21]. However, in the brain and heart, the induction of LDH activity was indicated by the result obtained in rats administered with Yoyo bitters (Table 1). This might be due to an increase in functional activity or size of the organs that could be reflected in an increased endogenous glucose production and cholesterol synthesis. The increase in the serum LDH activity in the rats administered with the herbal bitters, supported the reported loss of cellular contents into extra-cellular spaces, as indicated in the tissues with reduced LDH activity (Table 1). The increase in LDH activity may further indicated hemolysis, as serum LDH activity is known to be increased in hemoloytic conditions [22].

Acid phosphatase (ACP) is a membrane bound enzyme that is involved in active transport in the membranes of the lysosomes [23]. The decrease in the ACP activity in the liver and kidney indicated an increased in the activities of the lysosomes, which could be due to inflammatory processes arising from tissue injury and damage. The increases obtained in the ACP activity in some tissues in the rats administered with Yoyo bitters could indicate increased rate in membranes active transport that could lead to loss of other proteins by the indiscriminate hydrolysis of phosphate esters contents [23, 24]. The result of the serum ACP activity in rats administered the herbal bitters complemented the decreases recorded in the liver and kidney of the rats. This supported fact that administration of Yoyo bitters induced injury in such organs in male rats.

The administration of Yoyo bitters at 0.308 ml/kg body weight enhanced the metabolism and/ excretion of haem in the liver of male Wistar rats (Table 2). This is indicated by the result obtained in the serum total bilirubin concentration and was supported the results obtained in the liver ALP and LDH actitivies (Table 1). However, hemolysis was indicated following the administration of Yoyo bitters at 0.462 ml/kg body weight. Serum protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney and liver diseases and many other conditions [25]. The increase in serum total protein concentration indicated that there could be impaired renal function or tissue inflammation. The increase in serum albumin concentration in rats administered 0.308 ml/kg body weight of Yoyo bitters supported the hepatoprotective role of the herbal bitters at the dose. Yoyo bitters stimulated the differentiation and proliferation of B lymphocytes in rats, as indicated by the result of the serum globulin concentration and albumin/globulin index (Table 2).

The results of 'enzyme markers' that were determined in the study were further corroborated with histopathological studies of the tissues. The photomicrograph of the liver revealed that the hepatocytes were enlarged in an acidophilic manner at 0.462 ml/kg body weight (Figure 3), although, it was < 25% of the captured area, but supported the trends obtained in the 'enzyme markers' activities in the liver. This is so, because, [24] reported that pathogenesis of tissue damages are initially revealed in deficiencies in the activity of cellular markers, before histogical examinations are substantial enough. Thus, the photomicrograph of the liver at the dose might suggest potential damage to the hepatocytes. This supported the previous report that the administration Yoyo bitters enhanced free radical generation and ensuing lipid peroxidation in rat liver [26]. The enlargement and aggregation of the lymphoid tissues that eroded the villi and lysed of the epithelia cell wall in the small intestine in the rats administered with Yoyo bitters (Figures 11 and 12), indicated that the herbal bitters might have affected the digestion and absorption of food nutrients. Herbal bitters are known to have the purgative capabilities that involved the expulsion of accumulated mucus from the gastrointestinal tract, thereby enhancing digestion, absorption and excretion of food substances [27]. The sub-chronic use of Yoyo bitters might have led to the continuous removal of the mucus, which latter compromised the integrity of the wall of the gastrointestinal tract. This is supported by the results presented in the ALP and LDH activities in the small intestine (Table 1). The enlarged sinosoids and lobes (lymphoid tissues), the degeneration of the red pulps and the infiltration of the white pulp (Figures 8 and 9) as revealed in the spleen in the rats administered with Yoyo bitters, supported the increased activities indicated by the result of the ALP and ACP activities in the spleen (Table 1). Although, the spleen/body weight index was not determined, but localized inflammation was indicated by the enlarged lymphoid tissues and might suggest an increased expression of the enzymes markers (ALP and ACP) *in situ*.

CONCLUSION

The sub-chronic administration of Yoyo bitters at the manufacturer's recommended dose precipitated toxicities in some tissues in male Wistar rats. Therefore, the use of the herbal bitters should be under medical supervision and the habitual use is calls for caution.

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