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**BIOCHEMICAL ASSESSMENT OF THE THERAPEUTIC PROPERTIES OF
YOYO^(R) HERBAL BITTERS**

IMAGA NOA* AND OGUNNUSI OT

Department of Biochemistry, College of Medicine of the University of Lagos, P.M.B. 12003,
Idi-araba, Lagos State, Nigeria

*Corresponding Author: E Mail: noaimaga@gmail.com; nimaga@unilag.edu.ng

ABSTRACT

Yoyo^(R) bitters is a herbal bitter tonic widely used in Nigeria for detoxification and its efficiency in aiding digestion. This study was designed to analyze the phytochemical, chemical and proximate nutrient composition of Yoyo bitters and its effect on glucose and lipid profiles in-vivo using rat models. Blood and liver samples of the rats were analyzed for possible glycemic and/or lipidemic effects of the tonic. Phytochemicals present in the Yoyo bitters sample were majorly tannins, flavonoids, phenols and cardiac glycosides, with tannins as the most preponderant phytochemical. Results of the total reducing power and DPPH radical scavenging activity of the Yoyo bitters administered rats showed that the herbal tonic had appreciably high antioxidant property. Results from the biomarkers for liver function and antioxidant status showed that Yoyo bitters caused a slight but negligible reduction in glucose and lipid profiles.

Keywords: Yoyo Bitters^(R), Glycemic Index, Lipid Profile, Antioxidant, Phytochemical
INTRODUCTION

Herbal bitters are much sought after for their health benefits and they have become regular medicines in many Nigerian homes. The use of herbal bitters is an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy [1]. Herbal medication has been reported

ethnomedicinally to prevent, treat, manage and cure several diseases from cough to cancer. This proven efficacy has resulted in great patronage for any product that comes with the name 'herbal'. The manufacturers of these herbal bitters claim they are recipes for indigestion, weight loss, youthfulness,

strength among others. However, the indiscriminate use of herbal bitters may have possible toxic effects on several organs of the body such as the spleen, pancreas, kidney and heart.

In developing countries, herbal prescriptions and natural remedies are mostly used for the treatment of various disorders, such as diabetes and obesity [2], but there is insufficient scientific evidence as regard safety and efficacy of these herbal bitters to back up the continued therapeutic application of their remedies. With the increase in the use of herbal medicines in Nigeria and worldwide, a thorough scientific investigation of these bitters will go a long way in validating their folkloric usage.

Yoyo^(R) bitters is a plant-based medicine in the class of herbal bitters that was launched into the market in 2003 by Abllat Company Nigeria Limited. Abllat Nigeria limited is an indigenous manufacturer of nature green medicines. After it was introduced into the Nigerian drug market, Yoyo cleanser bitters tonic has received wide acceptance and usage by the general populace. The ingredients used for the production of Yoyo bitters as published by the manufacturers are: *Acinos avensis*, *Chenopodium murale*, *Citrus aurantifolia* and *Cinamomum aromaticum*. Each of these components has

been reported to posses several medicinal properties:

Acinos avensis: It serves as antiseptic, stimulant tonic. It is also used for shortness of breath, improving digestion, treating bruises, toothache, Sciatica and neuralgia.

Chenopodium murale: It is commonly known as nettle leaf goosefoot and is an important annual weed. It is distributed throughout the temperate and tropical regions and found in Pakistan in almost every field in winter season..

Citrus aurantifolia: Citrus species are among the native plants of Iran and the history of their cultivation dates back to 4000 years ago from which time, they have been widely used in the ethnomedicine. These species with the wide range of bioactive ingredients have been found to exert anti-infection and anti-inflammatory properties [3, 4, 5]. In addition, Citrus fruits have been found to be beneficial for cancer prevention in an epidemiological survey [6]. These fruits contain several classes of phytochemicals and micronutrients such as limonoids and flavonoids, which have been reported to have antitumor effects in vitro and in vivo [7, 8, 9].

Cinamomum aromaticum: Aqueous Cinnamon Extract from the bark of *Cinamomum cassia* (*Cinamomum aromaticum*) causes apoptosis in human

cervical cancer cell line through loss of mitochondrial membrane potential [8].

Yoyo cleanser bitters is reported to be formulated in such a way that the ingredients have a synergistic effect on the management of the digestive, circulatory, nervous, urinary and systems by decreasing the stomach acidity in cases of ulcer, diminishing the irregular production of gastric juice, stimulating the liver to ensure proper and complete digestion of heavy and fatty food, enhancing blood circulation, assists in the elimination of cholesterol, sugar triglycerides, creatine and uric acid, enhances effective function of the secretive glands and is beneficial in the treatment of disorders such as insomnia, stress, depression, kidney stones and bladder infections.

It is also acclaimed to dissolve any encased toxic materials in the body, enhance cell formation and growth, reduces excess body fat and boost healthy weight loss [Abllat Company Nigeria Limited (2009) "Yoyo bitters" leaflet].

The aim of this research was to verify these claims biochemically, by analyzing the phytochemical, proximate nutrient composition and antioxidant potential of Yoyo bitters and its effect on haematological, glucose and lipid profiles via in vivo studies with rat models.

MATERIALS AND METHODS

The materials used for this study were Yoyo bitters, which was purchased at YEM-YEM stores in University of Lagos, Lagos, Nigeria. All equipment and chemicals used were of analytical grade and obtained from reputable companies, Sigma and Merck, without further purification.

Animal Study

In this study, fourteen female albino rats were used. The rats were allowed to acclimatize for a period of fourteen days during which they were fed ad-libitum with standard rodents feed (rat chow) and tap water. Then, the rats were equally divided (7 rats/group) into two groups; the test and the control group. The weights of the rats in the test group were measured and used to calculate the dosage of Yoyo bitters to be administered to each rat throughout the three weeks of treatment. Administration was done via the oral route with the aid of oral cannula and syringe.

During the administration phase, freshly prepared Yoyo bitters extract was administered daily to the rats in the test group in addition to their chow and tap water, while the rats in the control group were fed with chow and tap water only.

After three weeks of administration, blood was collected from the rats into labeled lithium-heparin, fluoride and EDTA bottles

via *retro-orbital sinus* technique. The rats were then euthanized via cervical dislocation and their livers were collected into plain bottles and placed in ice. The blood samples and livers were then analyzed for various parameters.

The dosage of Yoyo bitters for an adult human being (70 kg) is 15ml of Yoyo bitters in 1 cup (100ml) of water. From this stock solution, the volume that would be given to a rat weighing Xkg is; $(Xkg \times 100ml) \div 15ml$. (In this study, the rats in the test group weighed 175g; hence 1.3ml of the diluted Yoyo bitters was administered to each rat and for rats with weight of 150g 1.1ml of the diluted Yoyo bitters was administered).

Phytochemical Screening

Quantitative and qualitative phytochemical tests for tannins, phlobatannins, flavonoids, cardiac glycosides, steroids and saponins were carried out on Yoyo bitters to identify the constituents using standard procedures described previously [10, 11, 12]. A diluted solution was made by mixing 2ml of sample with 20ml of distilled water.

Proximate Analysis

Tests to determine the ash content, moisture content, crude fibre, lipid content, protein content and carbohydrate content of the Yoyo herbal bitters were carried out using previously described methods [12].

In vitro Antioxidant Assay of Yoyo Bitters

0.2g of the yoyo bitters was weighed in a beaker and diluted with 100ml of water. From this solution, 20ml solution with concentrations of; 100, 75, 50 and 25 were obtained and labelled appropriately.

Reducing Power Capacity

1ml of the solution prepared above was mixed with 2.5ml of phosphate buffer and 2.5ml of potassium ferrocyanide and incubated at 50°C for 30 minutes. Since no precipitate was formed, 2.5ml of the mixture was added 0.5ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700nm

DPPH (Diphenylpicrylhydrazyl) Radical Scavenging Assay

Hydrogen donating activity was examined in the presence of DPPH stable radical [13]. 1ml of each fraction of the solution made above was added 3ml of methanol, 2ml of DPPH and placed in the dark for 30minutes after which the absorbance was read at 514nm Mixture of 4ml of methanol and 2ml of DPPH was used as negative control and Ascorbic acid as the positive control. 2ml of methanol was used as blank.

Antioxidant Enzymes Assay

The following antioxidant enzymes activities were determined spectrometrically as follows:

Determination of Superoxide Dismutase (SOD) Activity

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described previously [14]. The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. $\Sigma = 4020M^{-1} cm^{-1}$

Catalase Activity Determination

Catalase activity was determined [15]. It was assayed colorimetrically at 620nm and expressed as μ moles of H_2O_2 consumed/min/mg protein at 25⁰C. The reaction mixture (1.5ml) contained 1.0ml of 0.01M phosphate buffer (pH 7.0), 0.1ml of tissue homogenate and 0.4ml of 2M H_2O_2 . The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). $\Sigma = 40M^{-1} cm^{-1}$

Reduced Glutathione Determination

The reduced glutathione (GSH) content of liver tissue as non-protein sulphhydryls was

estimated according to previously described methods [16]. To the homogenate 10% TCA was added, then centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm. $\Sigma = 1.34 \times 10^4 M^{-1} cm^{-1}$

Lipid Peroxidation

Malondialdehyde (MDA) an index of lipid peroxidation was determined [17]. 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100⁰C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA-complex of $1.56 \times 10^5 M^{-1} CM^{-1}$.

Determination of Total Protein

This was determined using the Biuret method [18]. 5.0 ml of blank Biuret reagent prepared by dissolving $CuSO_4 \cdot 5H_2O$ crystal in 500 ml of distilled water was added to sample blank. These were mixed well and allowed to stand for 20 min at room

temperature 25 - 27°C. Absorbance was read for one test and standard against a blank at 540 nm. The concentration of protein was calculated using: optical density of standard \times concentration of standard.

Liver Function Test

Liver function test was carried out on the livers of the rats to determine the levels of; AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), Creatinine, Albumin, Alkaline Phosphatase, Total protein, Serum Glucose and Urea. Also, the level of total bilirubin was analyzed. These were carried out using specialized Randox assay kits.

Haematological Assay

A complete blood count was carried out on the blood of the sacrificed rats using an Automated Analyzer. The levels of; WBC (white blood cell), RBC (red blood cell), HB (haemoglobin), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), Neutrophils and Lymphocytes.

Lipid Profile

This was done to determine the levels of HDL (High density lipoproteins), LDL (Low density lipoproteins), Total Cholesterol and Triglycerides using standard protocols and an automated analyzer.

Statistical Analysis

Data are expressed as mean \pm standard error of the mean (SEM). The students' T-test was used for comparison of the experimental groups. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening of Yoyo Bitters

Results of the Phytochemical screening of Yoyo bitters sample showed majorly tannins, flavonoids, phenols and cardiac glycosides, with tannins as the most preponderant phytochemical (**Table 1; Figure 1**).

Proximate Analysis

The percentage nutrient composition in yoyo bitters was determined and the result presented in **Figure 2**.

Antioxidant Assay

The total reducing power (compared to the standard garlic acid) and DPPH radical scavenging activity of the yoyo bitters administered rats was determined and the results show that the herbal tonic had significantly higher antioxidant property than the standard (**Figures 3 and 4**).

Antioxidant Enzyme Assay and Lipid Peroxidation

The concentration of antioxidant enzymes and lipid peroxidation potential in the yoyo bitters administered rats was determined and the result presented in **Figure 5**.

Liver Function Test

Effects of yoyo bitters on biological parameters were determined and the result presented in **Figure 6**.

Lipid Profile

Effects of yoyo bitters on lipid parameters in the test group compared to the control are presented in **Figure 7**.

Haematological Analysis

The effect of yoyo bitters on haematological parameters was analysed and the results obtained showed no significant difference between test and control groups (**Figure 8**).

The result of the phytochemical tests shows that Tannins, Flavonoids, Cardiac Glycosides and phenols are present in Yoyo bitters while Phlobatannins, Saponins and Alkaloids are absent.

The result showed that Tannins are present in higher concentration than any other phytochemical. Tannins are the preponderant phytochemical in the herbal tonic.

The reducing power of yoyo bitters was significantly high compared to the standard. At 100% concentration, Yoyo bitters has more reducing properties as indicated by the highest absorbance of 0.385, at 75% the absorbance was 0.181 while the lowest absorbance of 0.310 was recorded at 50%.

Free radical scavenging activity of tonic was highest at its 100% concentration. At 100% the lowest scavenging activity of 0.4 was

recorded, while the highest was at 50%, a declination of this activity was recorded at 25%. At 75% it was found to be 8.2.

Again, in attestation to its potent antioxidant activity, Yoyo bitters was found to slightly lower the activity of antioxidant enzymes (SOD, Catalase) while sharply increasing Malonaldehyde (MDA) formation, which is a marker of lipid peroxidation. However, the MDA findings were not significant at $p < 0.05$.

Aspartate amino transferase was high in the test sample compared to the Control. Alanine amino transferase was low in the test Sample compared to the Control. Creatinine was low in the test sample compared to the Control. Urea had low concentration compared to the Control. Total protein had the same concentration in Test sample and Control. Albumin had a lower concentration in test sample compared to the Control. Alkaline phosphatase had a higher concentration in test Samples compared to the Control. Glutamine had a higher concentration in test Samples compared to the Control. However the levels of significance were insignificant at $p < 0.05$.

High density lipoprotein was observed to be lowered in the test sample compared to the Control. Total bilirubin was observed to be higher in the test sample compared to the Control

Triacylglycerol, Low density lipoprotein and total cholesterol were observed to be lowered in the test sample compared to the

Control. There was however no significant difference at $p < 0.05$.

Table 1: Phytochemicals in Yoyo Bitters

PHYTOCHEMICAL TEST	
TANNIN	+
PHLOBATANIN	-
SAPONINS	-
FLAVONOIDS	+
CARDIAC GLYCOSIDES	+
ALKALOIDS	-
PHENOL	+

NOTE: +Positive Indicates Presence While (-) Negative Indicates Absence

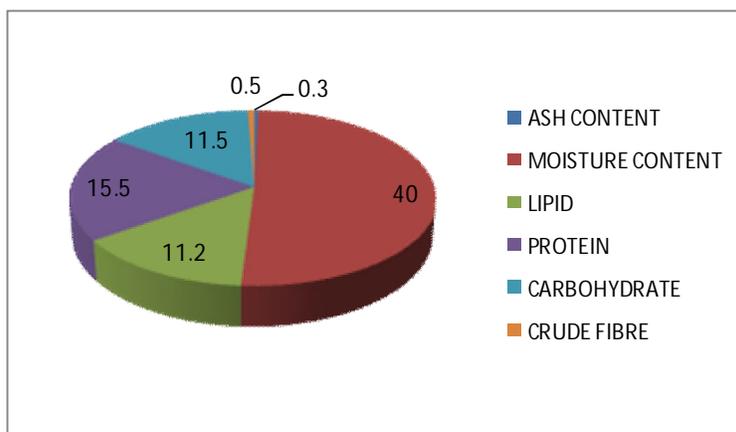
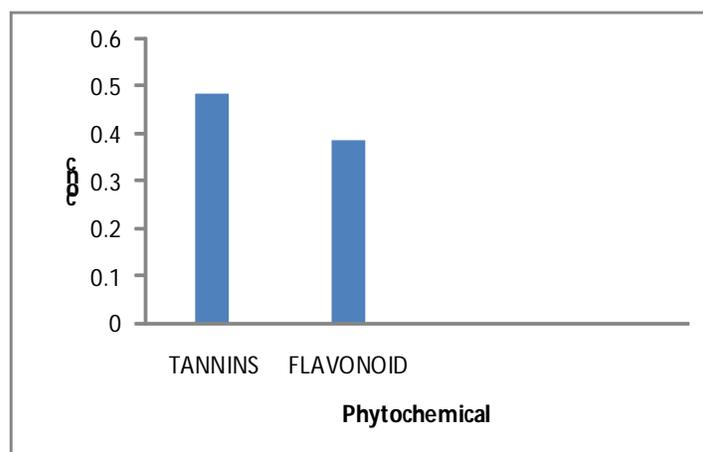


Figure 2: Nutrient Percentage Composition of Yoyo Bitters

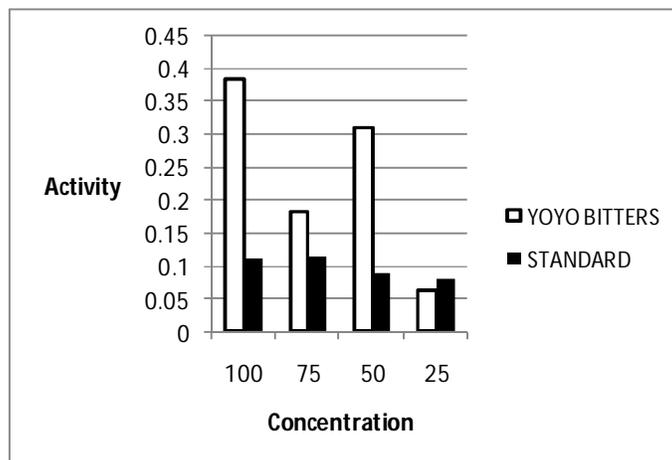


Figure 3: Total Reducing Antioxidant Power of Yoyo Bitters

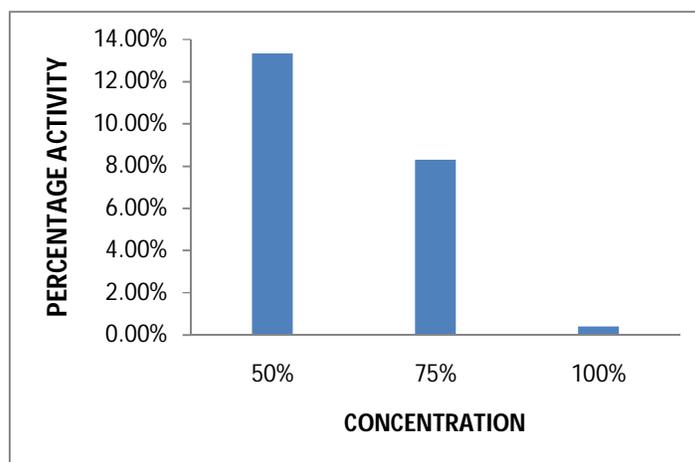


Figure 4: DPPH Radical Scavenging Activity of Yoyo Bitters

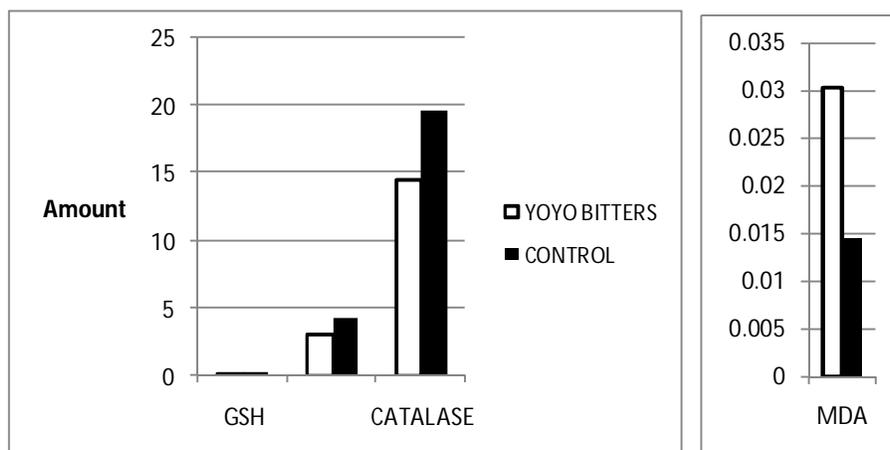


Figure 5: Concentration of Antioxidant Enzymes in the Yoyo Bitters Administered Rats as Compared to the Control

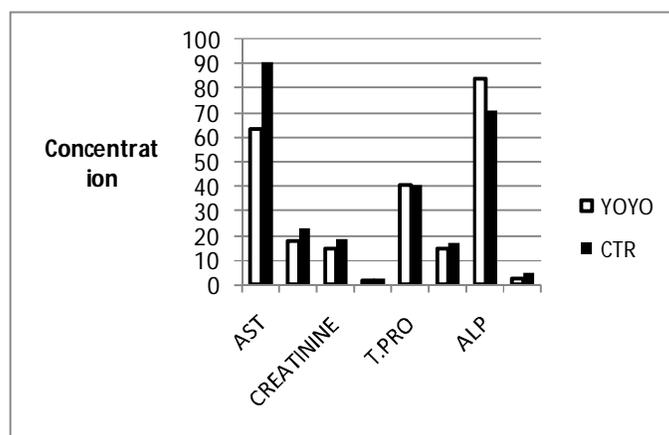


Figure 6: Comparison of Liver Function Assay and blood Glucose Level in Test Sample (Yoyo Bitters Administered Groups) and Control Groups

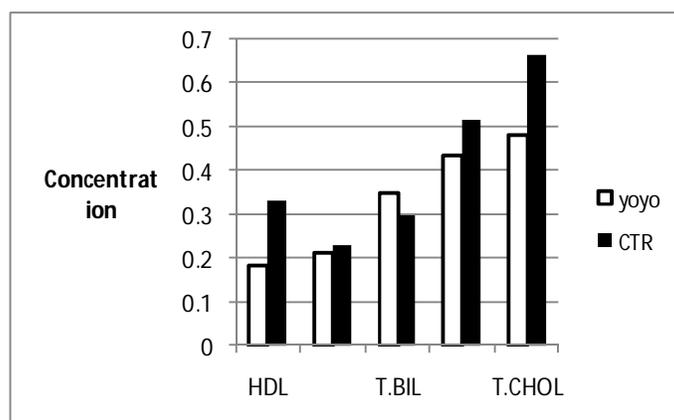


Figure 7: Effects of Yoyo Bitters on Lipid Profile of the Test Rats Compared to Control Group

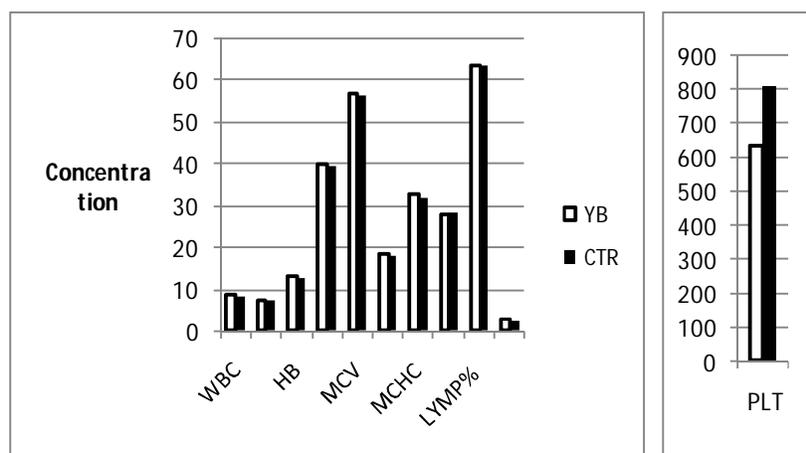


Figure 8: Effect of Yoyo Bitters (YB) on Different Haematological Parameters in the Test (YB) and Control (ctr) Groups

There was no significant difference at $p < 0.05$. Increase in the level of blood sugar and lipid of individuals, due to the unhealthy feeding habits in the tropics and a sedentary lifestyle [19, 20], has led to the occurrence of diseases such as Diabetes, Obesity and Hypertension which have been the major health concerns worldwide [21, 22]. Yoyo bitters, possibly due to its phytochemicals and antioxidant property may possess the ability to reduce blood sugar and lipid in tissues thereby reducing possible occurrence of diseases related to increase in the levels of blood sugar and lipid.

CONCLUSION

From our study, it can be concluded that Yoyo bitters is rich in antioxidant potential and can be used as a health tonic to aid stabilization of the glycemic index and lipid profile of users with little or no marked end organ damage.

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