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*Research Article*

# Biochemical Studies on Toxicity of Yoyo Bitters (A Polyherbal Preparation) on Liver and Kidney Functions of Wistar Rats

**Omotosho I.O.[1], Olusanya T[1] and Onyeaghala, A.O.[2]**

[1]*Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria*
[2]*Medical Laboratory Science Education Unit, University College Hospital, Ibadan, Nigeria.*

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**Abstract**

Management of diseases with herbal preparations is becoming popular, However. there is dirge of scientific evidence on the toxicity and most of the pharmacological claims by manufacturers of these preparations. The toxicity of a popular Nigerian herbal preparation (Yoyo bitters) on the alterations of anatomic and some biochemical functions of the liver of Wister rats was investigated. One hundred Wistar rats in groups of 10 in separate cages over a period of 4, 7, 10, 14, 21, 28, 35 and 42 days respectively were fed with normal rat chow and given the herbal preparation by gavage. Additional two groups, fed only rat chow, served as controls. Blood was collected and analyzed for total and conjugated bilirubin, total protein and albumin levels and activities of alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) and liver samples. Liver and kidney tissues were processed histologically using haematoxylin and eosin (H and E) staining methods. Mean values ranged from 0.60±0.10 -- 1.40±0.06 mg/100ml; 0.17±0.05 – 0.64±0.0.10 mg/100ml and 3.60±0.90 – 7.90±1.90g/100ml; 1.40±0.50—4.2±1.40g/100ml for total and conjugated bilirubin and total protein and albumin respectively. Mean values of 0.92±0.20 and 0.27±0.10mg/100ml, 7.8±0.90 and 4.5±0.50g/100ml were also obtained for total and conjugated bilirubin and total protein and albumin levels respectively in control animals; the variations were significant. Mean values of enzyme activities recorded ranged from 88±35 --201±28, 52±23 --172±100 and 146±90--426±71IU/L for ALP, ALT and AST respectively in comparison to mean of 340±151, 158±61 and 347±136 IU/L for ALP, ALT and AST in controls respectively; only the variations in AST was not significant. Mean values ranging from 0.77±0.12 -1.2±0.3mg/100ml; 5.5±0.7 - 7.7±1.8mg/100ml; 8.6±0.5 - 19.1±3.4mg/100ml and 1.4±0.8mg/100ml - 6.5±2.2mg/ml as against 0.8±0.14mg/100ml, 8.2±1.0mg/100ml, 14.3±2.6 and 1.9±0.2mg/ml were obtained for creatinine, Ca2+, Phosphorus and uric acid in experimental and control animals respectively. No gross adverse changes were observed in the microscopic structure of the liver and kidney. Histological examination of the tissues revealed mild alterations in the kidney and liver of the animals, however, there was upregulation in the level of uric acid and bilirubin and reduction in albumin concentrations. Although, administration of this herbal preparation did not show a significant alteration in liver and kidney histology, prolonged administration of the preparation may trigger inflammatory reactions as indicated by alterations in uric acid, bilirubin and albumin levels.

**Key Words:** Yoyo bitters, toxicity, liver, kidney

## INTRODUCTION

In the past few decades there has been an increase in the use of herbal/natural remedies in both developing and developed countries availability of modern medicines notwithstanding. In Nigeria, consumption of bitter drinks is gradually gaining grounds as a choice of natural remedy for the treatment of various diseases like indigestion, control of obesity and even treatment of chronic ailments like hypertension and cancer.

Yoyo bitters is one of the most popular of these bitters. Yoyo bitters is an aggregation of the following plants: Aloe – *Vera, Acinos arvensis, Citrus aurantifolia, Chenopodium murale, Cinomoniun aromticum*. It also contains water soluble vitamins and minerals e.g. Vitamins (B1, B2, B3, B6, &B12), Mineral (copper, zinc, iron) added to fortify the herbal preparation. Yoyo bitters has been widely reported to have different effects and functions including the ability to help regulate blood pressure and dilate arteries, aid the process of digestion, prevent disorders like ulcers, gastritis, insomnia, stress and depression, and to also prevent kidney and bladder infections, overweight and excess body fats (Bussman *et al*., 2010). Its usage has been popularly attached to its claimed effect as blood cleanser as well as having other uses such as enhancement of effective function of the secretory glands, stimulation of the liver for effective functioning, elimination of cholesterol, sugar, triglycerides, creatinine, and uric acid (Abllat Nigeria Ltd., 2011). Quite a number of works have also reported on other uses of yoyo bitters including its beneficial effects in management of diabetes, immunomodulatory function like precipitation of inflammatory responses and a role in combating oxidative stress (Oyewo *et al*., 2013).

Despite these widely documented medicinal uses of this bitter, there exists a paucity of experimental data on the likely toxic effect of the herbal drink especially as it relates to vital organs of the body such as the liver and kidney. The objective of this research is therefore to evaluate the anatomical alterations and changes in biochemical liver and kidney function indices occasioned by the consumption of this herbal preparation using Wister rats as the animal model

**Author for Correspondence:* Tel: +234-8023342999 E-mail: iomotosho2014@gmail.com

## MATERIALS AND METHODS

**Food supplements**:
This herbal drink (Yoyo bitters) was collected directly from the manufacturers (Abllat Nigeria Ltd) for the purpose of this project.

**Animals:** A total of one hundred Wistar rats purchased from the University of Ibadan animal house weighing averagely 120g were used for this study. The rats were randomly selected in groups of 10 animals into experimental and control groups. Each group was kept in separate wooden cages with good ventilation for the duration of the evaluation. The animals were allowed to acclimatize to their new environment for two weeks and fed with normal rat chow and water.

**Administration of the Herbal Preparation:** After two weeks of acclimatization, the experimental group was administered yoyo bitters by gavage while the control group had normal saline through oral route once daily in addition to the normal rat chow and water for 42 days. The animals were monitored and changes observed documented towards determining acute, subchronic and chronic toxicity effects

**Sample collection**: Animals that displayed signs of toxicity were removed from their cages and sacrificed in order to obtain samples for laboratory investigation. Blood samples were collected for laboratory analysis from the animal on day 4, 7, 10, 14, 21, 28, 35 and 42. Samples were transferred into heparinized tubes, centrifuged at 5000 rpm for 10 min, plasma separated into a plain container and stored at -4°c until time of analysis. Additionally, liver tissues of the animals were harvested on the specific days listed above and preserved in 10% formol saline for histopathological preparations. The tissues were later processed for evaluation of histolgical changes.

**Biochemical analysis:** Liver function test parameters assayed include total protein, albumin, total bilirubin, conjugated bilirubin, alanine transaminase, aspartate transaminase, and alkaline phosphatase. Plasma total protein level was determined using Biuret method (Frankel and Bess, 1942), plasma albumin level was also determined using the modified BCG dye binding method (Tietz , 1987) while plasma total and conjugated bilirubin levels were estimated using the method of (Buckner, 1961). Plasma Alkaline phosphatase activity was determined using the method of (Jendrassik and Grof, 1938;), Alanine amino transferase and Aspartate amino transferase activities were also estimated in plasma samples of the experimental animals using the modified method of Reitman and Frankel, 1957; Doumas, et al., 1971, DGKC, 1972 as described in the Randox kit (Randox Laboratory UK). Kidney function was evaluated by determining plasma levels of creatinine, calcium and uric acid. Creatinine was determined using the spectrometric method of Bartels and Bohmer, 1972, calcium was determined spectrophotometrically using the modified Ortho-cresolphtalein complex method (Biggs and Moorehead, 1974) while plasma uric acid level was determined by uricase-POD enzymatic colorimetric reaction, according to the method described by Yunsheng et al, (2009).

**Histology:** Sections of liver tissues collected and preserved in 10% formalin at various days of the experiment as highlighted above were carefully cut and hydrated through ascending grades of alcohol (50%-100%). The hydrated sections were treated with xylene and embedded in paraffin wax in preparation for sectioning. Tin sections (5-7µ) were cut on Rotary microtome and then stained with standard Hematoxylin and Eosin stain before examination for possible anatomic changes.

**Statistical analysis**:
All biochemical results were expressed as mean ± standard deviation (SD) for animals in each group. All the grouped data were statistically analyzed using SPSS version 21.0. comparison was done using T-test and analysis of variance (ANOVA) where appropriate. Statistical significance was set at $P < 0.05$.

## RESULTS

**Biochemical Indices of Liver and kidney functions:** Table 1 shows the acute effect of the herbal drug on liver biochemical function indices of bilirubin, ALP, AST, ALT, total protein and albumin levels in the experimental animals compared with the controls. The mean values of some of the biochemical parameters analyzed were significantly lower (ALP= 340 ± 151/103 ± 51) ($P < 0.05$) while AST and ALT were higher in the experimental animals as compared to the controls.

Alkaline phosphatase and albumin maintained consistent significantly lower values in the experimental animals as compared with the controls. There was however a reversal in the outcome of the amino transferases; as mean AST in the experimental animals was 146 ± 90 while that of control was 347 ± 136 and mean ALT became 52 ±23 in the experimental animals and 158 ± 61 in the controls. These differences were significant ($P = 0.034, 0.014$) respectively. At day 10 as shown in the table, there were not much significant changes between the cases and control.

By Day 14 it was observed that the mean alkaline phosphatase activity, total protein and albumin levels were significantly affected by administration of the herbal preparation ($P= 0.001, 0.025$ and $0.009$) respectively. The table also reveals that by day 21, significant differences between cases and controls in most of the biochemical parameters investigated. While some were lower than what was obtained in controls, such as albumin (0.000) and alkaline phosphatase ($P = 0.011$), others had the mean values higher in cases than in control especially Total bilirubin and Direct bilirubin ($P = 0.000, 0.001$) respectively. Also. for the animals sacrificed on 28, there was a significantly higher level of total bilirubin ($P = 0.015$) and Direct Bilirubin ($P = 0.004$). Other values remained lower than the controls.

However, most parameters measured were significantly altered on day 35 and day 42 of treatment with the herbal preparation.

The pattern was not totally different in the kidney function biomarkers analyzed, however, the uric acid level was upregulated throughout the period of the experiment. Although levels of main kidney function markers (creatinine, ca and Pi) significantly varied over the days of the experiment, the variations were not significant compared to the control.

**Histology:** Representative photomicrographs of sections of the liver and kidney from the control rats administered Yoyo bitters for 4, 7, 10, 14, 21, 28 35 and 42 days are presented in Plates 1 and 2. In all the treated groups there was no visible signs of abnormalities seen.

**Table 1**

Effect of Yoyo bitters on liver biochemical parameters animals and controls on herbal preparation. Results are expressed as mean ± S.E.M. (n = 5/group).

| Biochemical Parameter | Control | Day 4 | Day 7 | Day 10 | Day 14 | Day 21 | Day 28 | Day 35 | Day 42 | F | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Calcium** | 8.2 ±1.0 | 5.5 ± 0.7 | 5.2 ± 0.38 | 8.3 ± 0.46 | 7.4 ± 3.8 | 7.6 ± 1.2 | 7.9 ±0.95 | 5.9 ± 1.9 | 7.7 ± 1.8 | 1.58 | 0.165 |
| **Inorganic phosphate** | 14.3 ±2.6 | 8.6 ± 0.5 | 17.5 ± 3.0 | 24.9 ± 10.0 | 8.6 ± 4.5 | 8.7 ±1.7 | 14.5 ± 3.3 | 7.5 ± 1.8 | 19.1 ± 3.4 | 9.82 | 0.000* |
| **Uric acid** | 1.9 ± 0.2 | 1.4 ± 0.8 | 2.6 ± 1.60 | 3.6 ± 0.84 | 2.2 ± 0.9 | 2.7 ±0.42 | 4.4 ± 2.3 | 2.8 ± 0.7 | 6.5 ± 2.2 | 8.13 | 0.000* |
| **Total bilirubin** | 0.92 ± 0.2 | 0.6 ± 0.1 | 0.92 ± 0.17 | 0.93 ± 0.83 | 1.1 ± 0.3 | 1.4 ±0.06 | 1.3 ± 0.1 | 1.3 ±0.07 | 1.1 ± 0.21 | 2.95 | 0.012* |
| **Direct bilirubin** | 0.27 ± 0.1 | 0.17 ± 0.58 | 0.35 ± 0.06 | 0.33 ± 0.32 | 0.4 ± 0.2 | 0.64 ± 0.1 | 0.6 ± 0.1 | 0.6 ±0.13 | 0.5 ± 0.2 | 4.02 | 0.002* |
| **ALP** | 340 ± 151 | 103 ± 51 | 89 ± 23 | 201 ± 28 | 88 ± 35 | 115 ± 46 | 111 ±45 | 92 ± 36 | 115 ± 78 | 4.07 | 0.002* |
| **AST** | 347 ± 136 | 426 ± 71 | 146 ± 90 | 258 ±17.7 | 312 ± 207 | 326 ±124 | 343 ±131 | 332 ± 42 | 254 ± 179 | 1.09 | 0.390 |
| **ALT** | 158 ± 61 | 172 ± 100 | 52 ±23 | 83 ± 66 | 120 ± 80 | 64 ± 26 | 54 ± 6.0 | 93 ± 52 | 75 ± 4.8 | 2.48 | 0.031* |
| **Total protein** | 7.8 ± 0.9 | 4.8 ± 1.0 | 4.6 ± 3.9 | 7.9 ± 1.9 | 5.2 ± 2.3 | 5.7 ± 1.1 | 5.5 ± 0.7 | 3.6 ± 0.9 | 5.4 ±2.0 | 2.74 | 0.018* |
| **ALB** | 4.5 ± 0.5 | 1.7 ± 0.3 | 1.9 ± 0.72 | 4.2 ± 1.4 | 2.5 ± 1.4 | 2.5 ± 0.5 | 2.2 ± 0.4 | 1.4 ±0.5 | 2.4 ± 1.3 | 6.02 | 0.000* |

*Significant at p<0.05 ALP: AST: ALT: ALB:

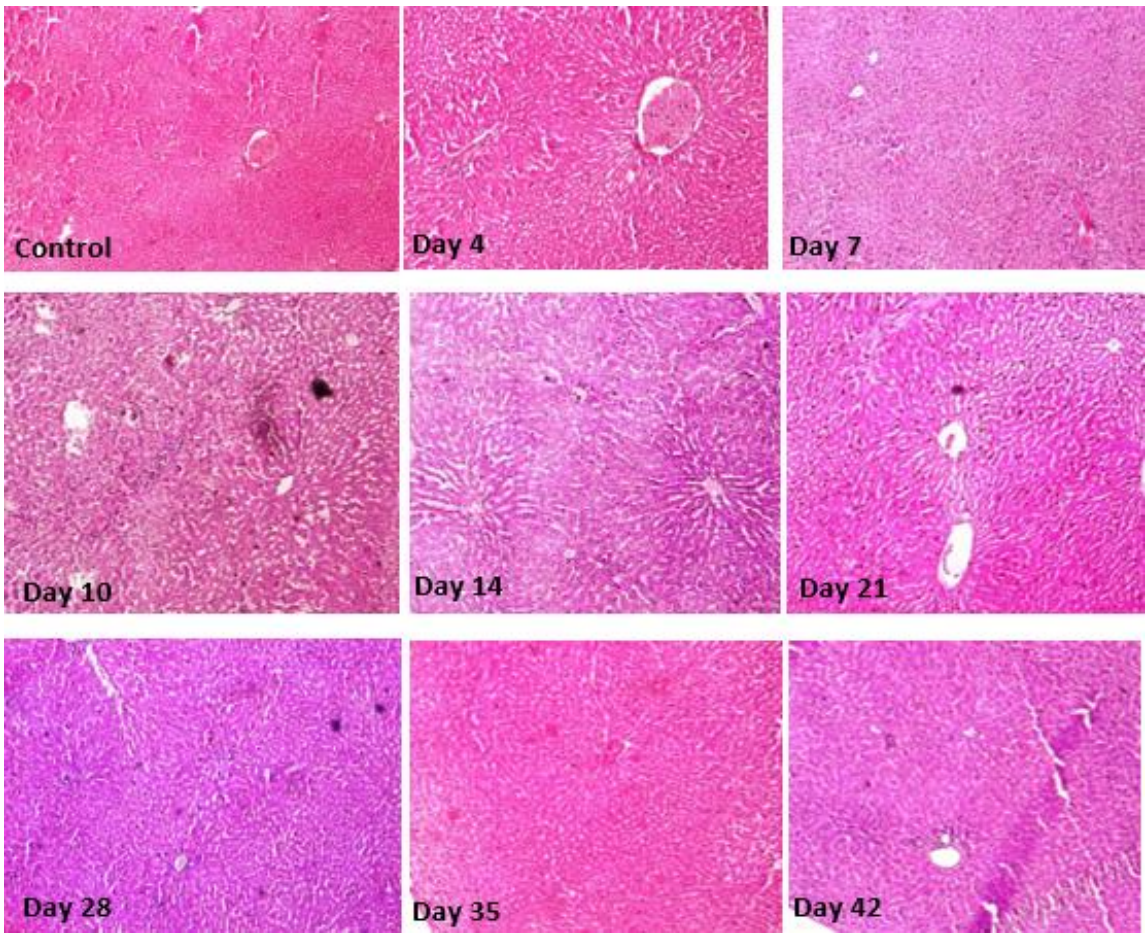


**Plate 1**

Representative photomicrograph of liver tissue from control group and those treated with Yoyo bitters for 4, 7, 10, 14,21, 28, 35 and 42 day respectively. No visible differences in the treated groups when compared with the control (H and E staining, x100 magnification)

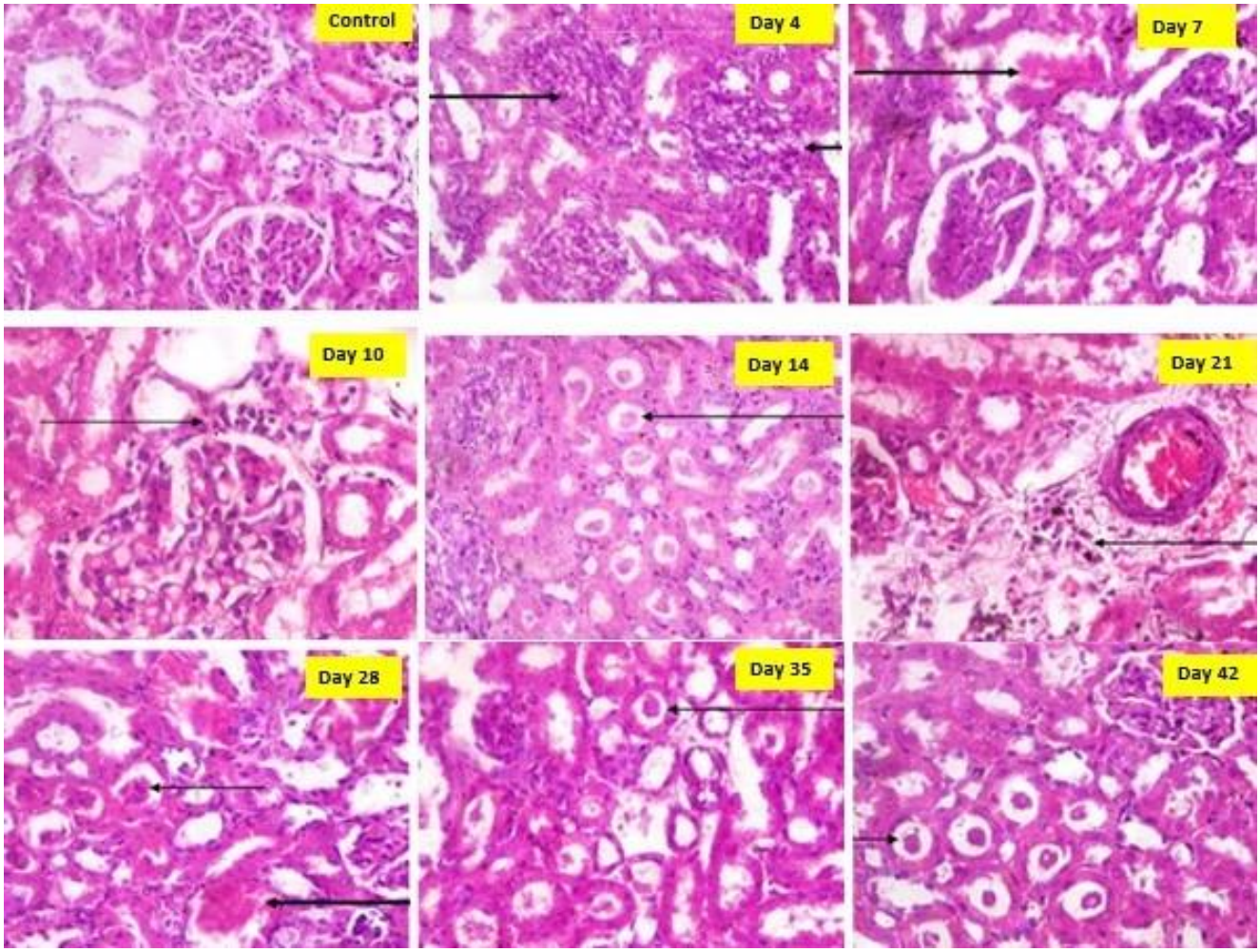


**Plate 2**

Representative photomicrograph of kidney tissue from control group and those treated with Yoyo bitters for 4, 7, 10, 14,21, 28, 35 and 42 day respectively. (H and E staining, x400 magnification). **Control** (normal glomeruli, bowman capsule and tubules. No significant lesion seen). **Day 4** - hypercellularity of glomerulus and marked absence of Bowman's space (black arrow**). Day 7**- mild hemorrhagic lesion (black arrow) and presence of eosinophilic substance (thin arrow) in the tubules. **Day 10**: moderate infiltration of inflammatory cells to the periglomerular region (thin arrow), mild peritubular inflammation (blue arrow) and presence of eosinophilic substance (Casts) within the tubules (black arrow). **Day 14**: mild vascular congestion (black arrow) and mild presence of eosinophilic substance in the tubules (thin arrow). **Day 21:** mild perivascular inflammation (thin arrow) and mild hemorrhagic lesion (black arrow). **Day 28**: mild haemorrhagic lesion (black arrow) and presence of eosinophilic material within the tubules (thin arrow). **Day 35**: slight eosinophilic substance within the tubules (thin arrow). **Day 42**: moderate presence of eosinophilic substance within the tubules (thin arrow).

## DISCUSSION

The growing acceptance and use of herbal mixtures such as yoyo bitters has necessitated the need to evaluate the effect of these natural remedies on the body organs vis-à-vis their functions. This study not only evaluated the effect of use by comparing control and experimental animals, but also attempted to highlight patterns of liver and kidney function indices that may be generated over duration of time when using this bitter.

The basic kidney function indices investigated in this work are plasma creatinine, uric acid, calcium and inorganic phosphorus. Plasma creatinine is considered to be a good traditional marker of kidney function especially because of the fact that it is not influenced by diet and many other extra renal factors. The observed averagely lower level of creatinine in the experimental rats on most days compared to the controls in this study may support the claim of yoyo bitters that the herbal preparation works as a blood cleanser by aiding the kidney in the excretion of waste products from the body. Other kidney function markers were also not affected abnormally.

Although there was an increase in the level of uric acid over the period of the experiment, absence of abnormality in the major kidney function marker (creatinine and inorganic phosphorus) may be a clear indication of an extra renal basis of the hyperuricaemia observed in this study. Oyewo, et al, (2013), also observed a dose dependent increase in the level of uric acid among the experimental animals taking yoyo bitters in their study. The increase in the level of uric acid was explained to be as a result of the inflammatory process that could be going in the animals as aided by the intake of yoyo bitters. According to them, the increase in the serum uric acid concentration supported the trends obtained in the serum IL-6 and TNF-α concentration in rats administered the herbal bitters in their study. Uric acid is a known endogenous adjuvant that drives immune responses in the absence of microbial stimulation (Shi, 2003). An increase in the plasma level of uric

acid concentrations has been implicated in the stimulation of localized inflammatory responses that has been identified as having a key role in the innate immune response through interleukin-mediated inflammation via activation of the NOD - like receptor protein (NLRP)-3 inflammasome, a multimolecular complex whose activation appears to be central to many pathological inflammatory conditions (Choi, 2001; Short, 2005). Cases of hypoalbuminaemia as linked to inflammation in this study gives support to this explanation for increased uric acid. Secondly, there was a significant up-regulation of conjugated bilirubin which may not only be indicative of normal hepatic conjugation but also an indication of possible antioxidative effect of bilirubin in the system. Hence, it can be inferred that the increased uric acid observed in this study may be of extra renal origin especially because of the low levels of creatinine also obtained.

In this study, there was a significantly notable change in the level of plasma albumin during the course of use of the bitter by the experimental animals. Here it was observed that there was a decrease in the level of albumin as the days went on except for the increase in day 10 which however, resumed its downward trend afterward. Hypoalbuminemia is a common outcome in many acute and chronic conditions which can result from decreased albumin production, defective synthesis because of hepatocyte damage, deficient intake of amino acids, increased losses of albumin via the intestinal tract or renal processes, and, most commonly, acute or chronic inflammation. According to the study conducted by Oyewo et al., 2013, it was established that consumption of yoyo bitters may have immunomodulatory effect by precipitating the inflammatory process. In their study, they observed that there was an up regulation of TNF-α as well as IL-6 (dose dependent) in the blood of rats fed with yoyo bitters. These cytokines (TNF, IL-6) released as part of the inflammatory response to physiologic stress (infection, surgery, trauma) can decrease serum albumin by increased vascular permeability (allowing albumin to diffuse into the extravascular space), increased degradation and decreased synthesis (among other mechanisms, by activating TNF-α, which decreases transcription of the albumin gene). Absence of hepatocellular damage as shown by the observed normal liver enzyme activity in the animals over the period in this study may not be supportive of hepatic dysfunction as the basis of hypoalbuminaemia observed. Also, since the kidney function indicators are all grossly normal, the observed hypoalbuminaemia may be said to be unconnected with abnormality in kidney function. Thus, the possibility of increased vascular permeability as a form of inflammatory response may be the most likely cause of the hypoalbuminaemia. The findings of this study may therefore infer induced inflammatory response as the basis of the down regulation of albumin production observed in the animals.

Furthermore, this study showed a decrease in the level of liver enzymes in the experimental animals compared to the controls. There was however an increasing pattern of both total and direct bilirubin which were significantly higher than values obtained from controls. Although bilirubin is included in the markers of liver function, this increase may not necessarily indicate a liver abnormality as there was no corresponding increase in liver enzymes. Bilirubin is known to be one of the non-enzymatic antioxidants in the body. Few studies have indicated that intake of yoyo bitters may aid in combating oxidative stress. Alabi et al., 2013 reported a significant decrease in thiobarbituric acid reactive substances, a marker of lipid peroxidation, in rats fed with yoyo bitters. This slight increase in plasma bilirubin may indicate the capacity of yoyo bitters to improve the total antioxidant status of the body system although further research may be necessary to confirm these findings and possibly extrapolate to humans.

In conclusion, the herbal preparation, Yoyo bitters, seems to be safe on kidney and liver functions over short-term period of administration. However, the effect on long-term consumption of the product needs further investigation.

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