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Toxicity Study and Hypoglycaemic Effect of Yoyo Bitters in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IFU and TDG designed and supervised this study, author MPN wrote the protocol, and wrote the first draft of the manuscript and managed the analyses of the study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To determine the toxicity and hypoglycemic effect of Yoyo Bitters in animal model.
Study design: Mention the design of the study here.
Place and Duration of Study: Sample: Department of Medicine (Medical Unit IV) and Department of Radiology, Services Institute of Medical Sciences (SIMS), Services Hospital Lahore, between June 2009 and July 2010.
Methodology: The Lethal Dose 50 (LD50) of the bitters in mice was determined and was found to be 80ml/kg which gave a clue on the safety dose of this herbal remedy. This study further compared the effect of a standard anti-diabetic drug (glibenclamide with the effect of Yoyo Bitters in alloxan induced diabetic rat. In this study twenty-four (24) wistar albino rats were used and was grouped into four (4). Alloxan 150mg/kg was induced intra-peritoneally and subsequently given Yoyo Bitters and antidiabetic drug (glibenclamide).
Results: The result of the hypoglycemic effect of Yoyo Bitters low dose(8.84±0.4) showed no significant difference as compared to anti-diabetic drug (8.3±1.0) treated group of rats on day 7 and 21.But the low dose of Yoyo Bitters fed rats showed a significant reduction on day 21 (5.66±0.6) as compared to the high dose of Yoyo Bitters fed rats on day 21(8.82±1.0) and control group that were given distilled water(8.48±0.8).Statistically, the low dose of Yoyo Bitters (mean value 5.66±0.6) and antidiabetic drug ((mean value 5.7 ±0.5) group of rats produced a significant result as compared to the control that were given distilled water. While the high dose of Yoyo Bitters group of rat produced

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no significant result as compared to the control group(F-value=1.48,P>0.05).The third phase of this study comprised of three groups with six(6) rats each; the control group which were given distilled water, the high dose of Yoyo Bitters fed group of rats(7.75ml/kg) and the low dose of Yoyo Bitters fed group of rats and the findings were as follows; Low dose of Yoyo Bitters group produced no significant difference for all the parameters analyzed (liver and kidney function parameters) as compared with the control, but the group of high dose of Yoyo Bitters fed rat gave a significant result in three of the parameters analyzed (Urea, Direct bilirubin, and Bicarbonate) while others were not significant as compared with the control group. The histological examination of the liver and kidney of Yoyo Bitters administered rats showed a mild necrosis and fragmented proximal and distal tubules and preserved glomerulus in the kidneys of the low dose administered rat as compared to the high dose administered rat. The liver of a low dose yoyo biters administered rats showed a normal hepatocyte with minor vacoulation and fragmentation; and also, a mild necrotic change observed which is contrary to the high dose administered rat which presented a high tissue injury. platelets are significantly but negatively associated with esophageal varices grades.

Conclusion: In conclusion, this study has shown that Yoyo Bitters have hypoglycemic potential and has influence on some of the liver and kidney function parameters and based on the histology results Yoyo Bitters can be toxic to the organs of the body (liver and kidney) if precautions and right dosage is not administered. This study would recommend that more research be carried out especially on the hypoglycemic potential and toxicity levels of Yoyo Bitters.

Keywords: Toxicity; hypoglycaemic; yoyo bitters; wistar albino rats.

1. INTRODUCTION

Diabetes is a chronic disorder (disease) that affects man either when insulin is not produced in a considerable amount by the pancreas or the body cannot effectively use the insulin it produced. Hyperglycemia or raised blood sugar (glucose) can be as a result of uncontrolled diabetes, merely exposing the body to a serious damage especially the nerves and blood vessels.

Diabetes is one of the leading causes of death as estimated by WHO in 2015, of which 1.6million deaths were directly caused by diabetes. In 2012, deaths of 2.2 million people were attributed to high blood glucose occurrence before the age of 70 years, [1]. Yoyo Bitters is one example of indigenous herbal mixture made from different plants and it is consumed in many Nigeria homes because of its health benefits. Although it's widely consumed, there is a few data that has been proven scientifically on the safety and toxicity profile of Yoyo Bitters. Recently, concerns have risen about indiscriminate use of packaged herbal bitters which may have a toxic effect on the spleen, pancreas, kidney and heart. Prior to the advent of modern drugs, medicinal plants had been known and used by all culture throughout history. These medicinal plants are the oldest form of healthcare known to mankind.

Because of the health benefit of Yoyo Bitters, it is now used as herbal medicine by many people, which correspond to the WHO's definition of herbal medicine as any plant which one or more of its parts can be used for therapeutic purposes or as precursor for the synthesis of useful drugs [2].

It's important to treat hyperglycemia because if left untreated can become severe leading to serious complications that will require emergency care and one of such cases is diabetic coma. Although, it might not be severe in some cases but can lead to complications affecting the eyes, kidney, nerves and heart. Therefore, recognizing early signs and symptoms of hyperglycemia will be of benefit in the treatment of the condition. Routine diagnosis of kidney function includes determination of electrolyte (Sodium, Potassium, Chloride and Bicarbonate) plasma/serum urea and creatinine.

This study (research) entails induction of rats with Alloxan (a chemical substance) to establish a transient diabetes, while yoyo herbal bitters and a hypoglycemic drug known as glibenclamide were used to establish a comparison between both substances. Therefore, the aim of this study was to determine the toxicity and hypoglycemic effect of Yoyo Bitters in animal model.

2. MATERIALS AND METHODS

2.1 Study Area/Setting

The research study was conducted at Rivers State University (RSU). The laboratory animals

were housed in the animal house of the biology department where the practical was carried out, and laboratory analysis was done in the Medical Laboratory Department of Rivers State University Medical Center with due consent of the heads of both departments.

2.2 Experimental Animals and Procedure

2.2.1 First phase of the study

Determination of LD 50 (lethal dose 50) of Yoyo Bitters: In order to ascertain the safety dose of this herbal remedy, the LD50 was determined. Twelve mice were used for the study and were grouped into two (2) each group containing six (6). The rats were weighed and Yoyo Bitters administered intra-peritoneally with various doses, 60ml/kg and 80ml/kg for groups 1, and 2 respectively. The mice were observed for the interval of 24hours for various signs that may be caused by herbal remedy. Hence, concluded the lethal dose to be. This observed effect was a guide to the dosage of the herbal bitters used in the second and third phase of this research work.

2.2.2 Second phase of the study

The twelve to fourteen weeks old albino rats were grouped into four comprising of six rats per group. The rats were housed in cages made of plastic frames and metal netting. The rats were allowed for 14 days to acclimatize and were kept fasting overnight.

A fasting blood sample was collected from the tail tip for glucose analysis using glucometer (Accu check) to ascertain the baseline. After which the rats were given alloxan 150mg/kg intra-peritoneally and allowed for 3 days. A fasting blood sample were collected and analysed for glucose using glucometer and this was taken to be the first day after the induction of diabetes.

The group one (1): The Glibenclamide treated group (5mg.)

Group two (2): High dose Yoyo Bitters (23.4ml/kg)
Group three (3): Low dose Yoyo Bitters (7.75ml/kg)
Group four (4): control group (Distilled water)

The group one (1) received glibenclamide which is the antidiabetic drug (standard drug) for the treatment /control of diabetes and was used as control against the Yoyo Bitters treated group.

Group 2: This group was given High dose of Yoyo Bitters
Group 3: This group was given low dose of Yoyo Bitters.
Group 4: This group was given distilled water. This group was used as the general control for the glibenclamide treated group and the Yoyo Bitters treated group.

A fasting blood sample was collected on day 7 and 21 to monitor the blood glucose after which the rats were sacrificed.

2.2.3 Third phase of the study

This study comprised of three (3) groups and each contained 6 rats which weighed between the ranges of 189.2g to 208.4g. Group A received distilled water, group B received 23.4ml/kg of Yoyo Bitters (taken as high dose) and group C received 7.75ml/kg of yoyo bitter (low dose).

Administration of the Yoyo Bitters was performed orally once daily between 10:30am ± 30minutes, using sterile syringe. The administration lasted for 21 days. On day 21, blood sample were collected for electrolyte, urea and creatinine, and liver function parameters. After which the animals were dissected and the internal organs (liver and kidney) were harvested each per group for Histology Analysis.

2.2.4 Laboratory estimations

Assessment of Hepatic Function: Liver function was assessed by measuring the activities of AST and ALT in serum. Total protein and Albumin concentrations was also assessed using the principles of Biuret reaction [3] and Bromo cresol green reaction [4] respectively.

Alkaline phosphatase was analysed using the principles of kinetic determination of ALP according to the following reaction.

Bilirubin was assessed using the principle of Diazotized reaction. Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. Total bilirubin reacts with diazotized sulfanilic acid in the presence of TAB to form azobilirubin.

Assessment of Renal Function: The following test were carried out for renal function;

electrolyte (sodium, potassium, chloride, bicarbonate). Sodium was analyzed using spectrum-diagnostics sodium reaction. This method is based on reaction of sodium with a selective chromogen producing a chromophore whose absorbance varies directly as the concentration of sodium in the test specimen. Potassium was analyzed using the principle of Turbidimetric method. Chloride was assessed using the principle: in an acid medium chloride ions and mercury – II – thiocynate form thiocynate ions. These ions react with HNO_3 and iron III-ions and effect a red colour. The intensity of the colour is directly proportional to the concentration of chloride ions.

Bicarbonate was assessed using the spectrum Diagnostic kits with the following assay principle: colorimetric test for the quantitative determination of carbon dioxide (Co_2) in serum.

Urea was analyzed using urease method and lastly, creatinine using the principle of Jaffe's reaction.

Determination of Chloride: In an acid medium chloride ions and mercury 11-thiocynate form thiocynate ions. These ions react with HNO3 and iron-111-ions and effect a red color. The intensity of the color is directly proportional to the concentration of chloride ions.

Determination of Alanine Aminotransferase: According to the following reaction

$$\text{L-Alanine + alpha-ketoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate + L-Glutamate}$$

$$\text{Pyruvate + NADH + } H^+ \xrightarrow{\text{LDH}} \text{L-lactate + } NAD^+$$

Determination of Albumin (using Agape kit): Sulphonphthalein dyes as bromocresol purple or bromocresol green yield with albumin in the presence of detergents in a blue-green complex suitable for the photometric determination.

$$\text{Albumin concentration (g/l)} = \frac{\text{A sample}}{\text{A standard}} \times \text{concentration of standard.}$$

Determination of serum electrolytes: The instrument used for the analysis of (Na^+, K^+, Cl^- and HCO_3^-), was the Ion Selective electrode (ISE) electrode autoanalyzer.

The ion selective electrode membrane for sodium and potassium respectively undergoes a specific reaction with the ion (sodium or potassium) contained in the sample to be analyzed. The membrane reacts to the electric charge in the ion (sodium or potassium) causing a change in the membrane potential which is built up in the film between the sample and membrane. A difference in the sodium or potassium ion concentration between the sodium or potassium solution inside the electrode and the sample causes an electrochemical potential to form across the membrane of the active electrode. The potential is conducted by the electrode to an amplifier. This is compared with the potential of a reference electrode. Rolytes were determined using an ion selective electrode analyzer.

Estimation of serum urea concentration: This method is based on urease, urea will undergo hydrolysis to form NH_3 and Co_2. The ammonia will then react with phenol and hypochlorite to form indophenols (blue) which is measured spectrophotometrically at 546nm.

Urea $+H_20$ urease $2NH_3 + Co_2$
NH_3 + Hypochlorite + Phenol -------- indopenol (blue)

Estimation of serum creatinine: This method is based on the jaffe reaction; in this reaction creatinine reacts with picrate ion formed in alkaline medium to develop a red-orange color. The color produced from the sample is then compared in a colorimeter at wave length of 505nm with that produced by a known amount of creatinine under the same condition.

Estimation of serum bilirubin: Serum Bilirubin by Malloy and Evelyn, Bilirubin reacts with diazotized sulphalinic acid to form a purple colour compound, azobilirubin. Conjugated bilirubin reacts in an aqueous solution (direct reaction) whereas unconjugated bilirubin requires an accelerator or solubilizer such as alcohol (indirect reaction). After incubating for 5 minutes, it will be read at 540nm.

Determination of carbon dioxide (CO_2): Colorimetric test for the quantitative determination of carbon dioxide (Co_2) in serum and plasma

$$\text{Phosphoenolpyruvate + Bicarbonate + NADH} \xrightarrow{\text{PEPC \& MDH}} \text{phosphate + malate + NADH}$$

Determination of Alkaline phosphatase: Colorimetric method was used.

Alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which, at alkaline pH values, turn into a pink colour that can be spectrophotometrically determined.

3. RESULTS AND DISCUSSION

Table 2 Compared hypoglycemic effect in different doses of Yoyo Bitters and anti-diabetic drug, the result shows a statistical significance on low dose of Yoyo Bitters treated diabetic rats as compared to the control group (given distilled water). The high dose of Yoyo Bitters treated group shows no significant difference as compared to the control group (given distilled water). The group treated with anti-diabetic drug (glibenclamide) gave a significant result with p-value 0.04 ($p<0.05$) when compared with the control. The blood glucose level significantly reduced in the low dose of Yoyo Bitters treated rats and the anti-diabetic drug treated diabetic rats.

Table 3 Compared the hyperglycemic effect of Yoyo Bitters (low dose) and anti-diabetic drug From the result there was no significant difference between the Yoyo Bitters treated group of rats and anti-diabetic drug (glibenclamide) treated group of rats.

Table 4 The toxicity study of Yoyo Bitters when compared to the control group with the low dose of Yoyo Bitters treated group, showed no significant difference for all the parameters analysed ranging from creatinine, urea, albumin, Total protein,chloride, conjugated and direct bilirubin, sodium (Na^+), Alkaline phosphatase, Bicarbonate, Alanine amino transferase (ALT) to Aspartate amino transferase (AST).

The result shows a toxicity study of different parameters; electrolytes, urea, creatinine and liver functions of control group compared with the high dose of Yoyo Bitters treated group. There was a statistical significance in the following parameters; urea, direct bilirubin and bicarbonate, but there was no significance result in the following parameter; creatinine, albumin, Total protein, chloride, Total bilirubin, potassium (K^+), sodium (Na^+), Alkaline phosphatase, bicarbonate, AST and ALT.

Table 1. Result of the Lethal Dose 50 (LD50) of Yoyo Bitters at different interval

Yoyo Bitters (ml/kg)	Observation (5minutes)	Observation (8 minutes)	Observation (22 hours)	Observation (24 hours)
Group A(60 ml)	Circular movement	Normal movement	Dizzinesss	Dizzinesss
Group B(80ml)	Circular movement	Dizziness/ Sunkened eyes	Red eyes/ sluggishness	Death of 3 mice

Table 2. Comparing hypoglycemic effect in different doses of yoyo bitters and anti-diabetic drug

Parameter	Control (Mean±SEM)	7days (Mean±SEM)	21days (Mean±SEM)	F-value	P-value	Remark
Low dose	8.48±0.8	8.84±0.4	5.66±0.6	8.11	0.006	SS
High dose	8.48±0.8	10.68±1.3	8.82±1.0	1.48	0.27	NS
Anti-diabetic drug	8.48±0.8	8.36±1.0	5.7±0.5	4.43	0.04	SS

Key:
SS: Statistical Significance
NS: Non-Significance

Table 3. Comparing the hypoglycemic effect of yoyo bitters (low dose) and anti-diabetic drug

Periods	Low Dose (Mean ± SEM)	Anti-diabetic drug (Mean ± SEM)	t-value	P-value	Remark
7days	8.84±0.4	8.36±1.0	0.37	0.36	NS
21days	5.66±0.6	5.7±0.5	0.06	0.48	NS

Key:
NS: Non-Significant
T-Value: Calculated difference

Table 4. Toxicity study of yoyo bitters (low dose)

Parameter	Control	Yoyo bitter	t-value	P-value	Remark
Creatinine	67.8	65.8	0.24	0.41	NS
Urea	4.6	4.7	0.21	0.42	NS
Albumin	30	29.4	0.67	0.26	NS
Total Protein	57.8	53.2	0.90	0.20	NS
Chloride	106.5	100.2	0.86	0.21	NS
T.bilirubin	37.3	43.2	0.41	0.35	NS
D. bilirubin	10.3	9.5	0.25	0.41	NS
Potassium	5.2	4.9	0.36	0.37	NS
Sodium	139.3	140.8	0.38	0.36	NS
ALP	256.7	371	0.92	0.20	NS
Bicarbonate	15.7	18.4	1.38	0.11	NS
AST	24.2	21.4	0.80	0.22	NS
ALT	19.3	21	0.51	0.31	NS

Table 5. Toxicity study of Yoyo bitter (High dose)

Parameter	Control	Yoyo bitter	t-value	P-value	Remark
Cr	67.8	74.8	0.73	0.24	NS
Urea	4.6	5.8	1.83	0.048	SS
Albumin	30	24.7	1.17	0.13	NS
Total Protein	57.8	55.5	0.67	0.26	NS
Chloride	106.5	106.2	0.08	0.47	NS
TB	37.3	23.7	1.29	0.11	NS
D. bil	10.3	5.5	1.83	0.048	SS
K	5.2	6.5	1.19	0.13	NS
Na	139.3	132.2	1.07	0.16	NS
ALP	256.7	209.7	0.83	0.21	NS
HCO3	15.7	19	1.95	0.04	SS
AST	24.2	19.7	1.40	0.10	NS
ALT	19.3	17	0.86	0.21	NS

3.1 Histological Examination Results

The LD50 simply means The Lethal Dose at 50 which is the dose of a test substance that is lethal for 50% of the experimental animals in a dose group. The result of the Lethal Dose at 50 (LD50) of Yoyo Bitters was observed to be 80ml/kg and this actually proved a safe dose of the herbal remedy. It also gave an idea on the administered dose in the hypoglycemic and toxicity studies carried out in this research work.

The result presented in table 2 compared the hypoglycemic effect in different doses of Yoyo Bitters and anti-diabetic drug (glibenclamide). The low dose of Yoyo Bitters on day 7 and day 21 showed a significant reduction (effect) on the blood glucose levels with a mean value 8.84 ± 0.4 and 5.66 ± 0.6 respectively. When compared with the control group (8.48 ± 0.8) shows a significant result $p<0.05$. The high dose of Yoyo Bitters gave a mean value of 10.68 ± 1.3 on day 7 and 8.82 ± 1.0 on day 21 and when compared with the control group (8.48 ± 0.8) shows no significance result ($p>0.05$). The Anti-diabetic drug on day 7 gave a mean value of 8.36 ± 1.0 and on day 21(5.7 ± 0.5). The results gave a

clear indication that the low dose of Yoyo Bitters gave a hypoglycemic effect likewise the anti-diabetic drug. While the high dose of Yoyo Bitters produced no significant effect [5].

From the Table 3 which compared the hypoglycemic effect of low dose Yoyo Bitters and anti-diabetic drug, a similar effect was presented as both enhanced a reduction in blood glucose level. This research is in concordance with the research of Jimmy and Udofia [6] with a little difference in the high dose of Yoyo Bitters results. This research has shown that Yoyo Bitters to an extent can regulate blood glucose level, for example in hyperglycemic condition. Obviously, Yoyo Bitters may have influenced the pancreas and promoted the uptake of glucose by the cells of the tissues.

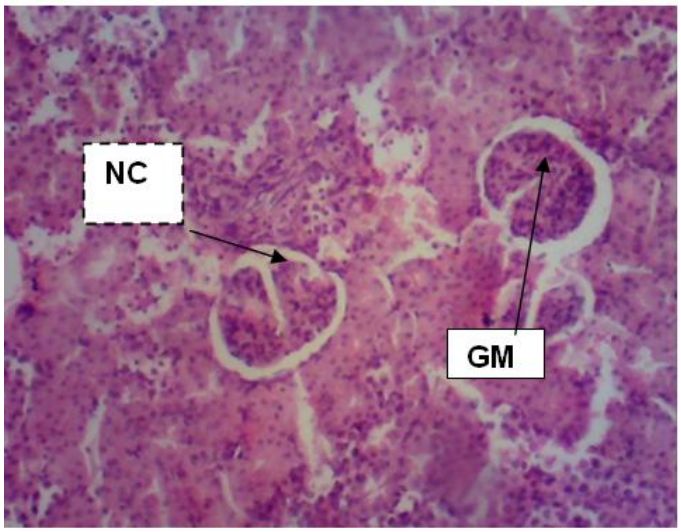

Fig. 1. Micrograph of the Kidney of Low Dose of Yoyo Bitters fed rats: Shows kidney tissue with necrotic and fragmented proximal and distal tubules and preserved glomerulus. There is nuclear pyknosis and tissue inflammation

Key:
NC: Necrotic changes
GM: Glomerulus

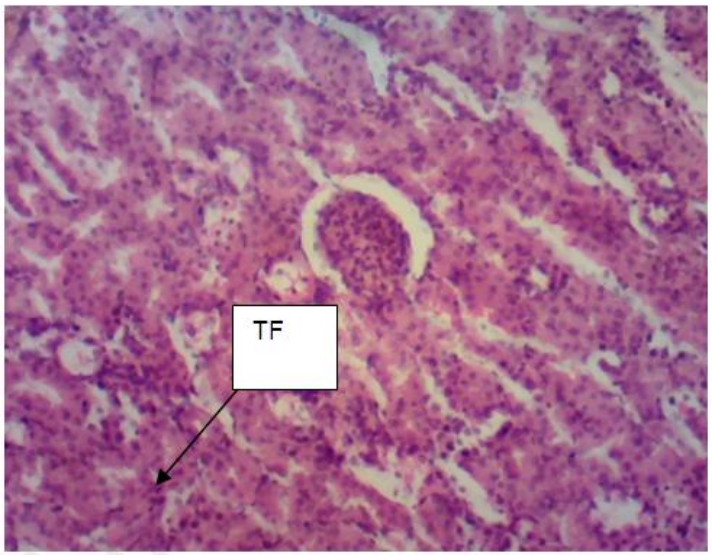

Fig. 1.1. Photomigrograh shows pyknotic nucleus in the tubules and necrotic changes

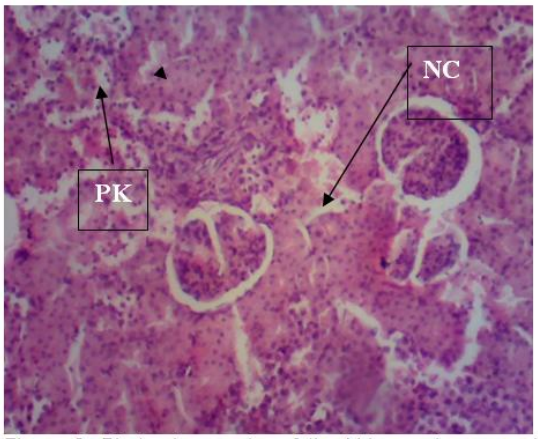

Fig. 2. Photomicrograph of the kidney shows proximal and distal tubules necrosis and nuclear pyknosis. There is also glomerular tissue degeneration and necrosis

Key:
PK: Pyknosis
Nc: Necrosis

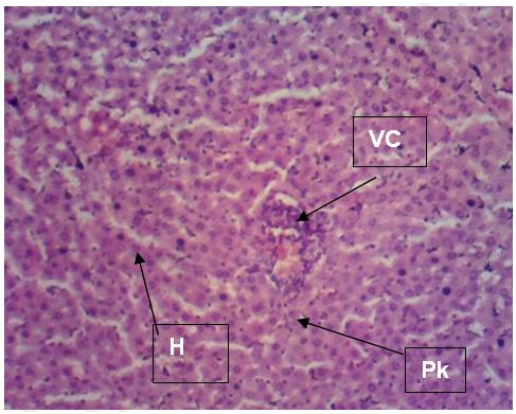

Fig. 3. Micrograph of the liver of low Dose of Yoyo Bitters fed rats: Liver section shows normal hepatocytes with minor vacoulation and fragmentation. There is mild necrotic change observed

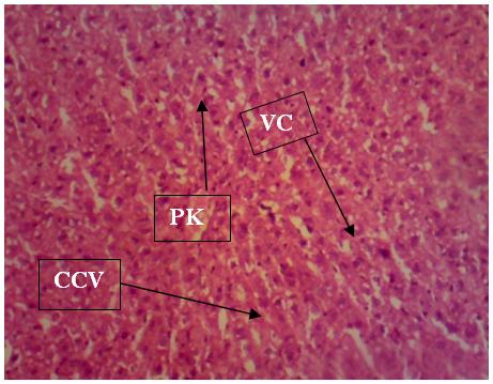

Fig. 4. Micrograph of the liver of High Dose of Yoyo Bitters fed rats: Liver section showing nuclear Pyknosis, vacoulation and congested central vein. The tissues are fragmented with fatty change

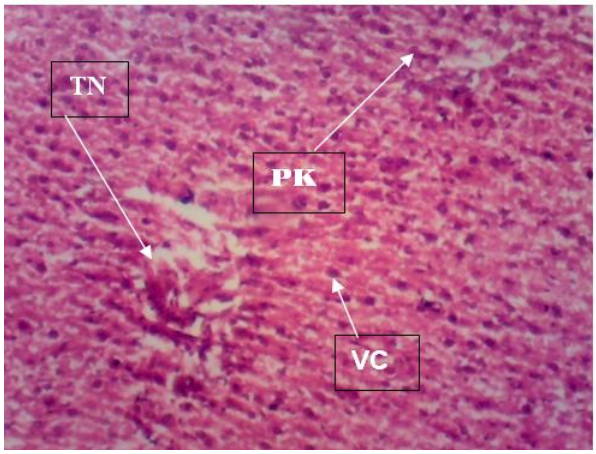

Fig. 5. High Dose of Yoyo Bitters fed rats: Liver section shows tissue necrosis (TN) with vacoulation (VC). The nuclear material shows vacoulation with pyknosis (PK) showing high tissue injury

Key:
TN: Tissue Necrosis
VC: Vacoulation
PK: Pyknosis

From Table 4 which compared the effect of a high dose of Yoyo Bitters on the liver and kidney function parameters with the control group. Urea result increased significantly ($p<0.05$) with the mean value of 5.8 as compared with control group mean value of 4.6. Over the years, measurement of serum/plasma has been used as an indicator for kidney function, although creatinine is said to provide better information Urea which is made when protein is broken down is actually made in the liver and passed out in the kidney. One of the clinical significances of carrying out a urea test is to see if the kidneys are working well. In a situation whereby the kidneys are not able to excrete urea from the blood, eventually, the blood urea nitrogen level rises, and this may result in heart failure, dehydration etc. [5].

An increase in serum/plasma urea concentration characterizes the uremic state. Urea is one of the renal markers and is freely filtered by the glomerulus, therefore a high value gives information on filtering capacity of the kidney. Although the urea value increased significantly, the values of the results that were gotten are majorly within the normal range but may have had a negative influence on the kidney function parameters. (i.e Yoyo Bitters) if high dosage was continued.

Direct bilirubin (conjugated bilirubin) also gave a significance statistical result with the mean control value 10.3 and the Yoyo Bitters high dose mean value 5.5. Direct bilirubin is one of the liver function parameters, when there is a sign of liver damage (e.g hepatitis) there may be a release of both indirect and direct bilirubin into the blood stream. Normally bilirubin is made when old red blood cells are broken down. It is also part of bile which is made in the liver to help digest food. Some of the bilirubin (unconjugated or indirect) is bound to albumin (protein) in the blood while some moves from the liver into the small intestine, allowing a small amount to go into the kidney and excreted in the urine given it a yellow color.

In cases of hepatitis and blockages like gall-stones, direct bilirubin is often very high and thus is a clear indication that the liver may be in danger. This study is in accordance with their findings that Yoyo Bitters can increase liver function marker although in this research is the direct bilirubin.

Bicarbonate is the byproduct of the body's metabolism and it functions in regulating the body's pH or acid balance and also works with other electrolyte such as sodium (Na^+) potassium (K^+) and chloride to achieve this function. One major role of the kidney is to in maintain acid-base balance through excreting acid in the amount that are equal to the extra anal acid production which is made possible by a process of reclamation of filtered bicarbonate on one hand while on the other hand regenerating base through the excretion of ammonium and titratable

acid. When the blood levels of bicarbonate are high, it can suggest a metabolic alkalosis, where the pH level in the tissue is increased. It can also result when there is a loss of acid from the body, for example vomiting and dehydration. Bicarbonate result increased significantly with a mean value of 19 as compared to the control which is 15.7. This result shows the ability of the kidney in reclamation process, maintaining or restoring a normal pH in the general circulation. Therefore, Yoyo Bitters high dose produced a positive effect in the values of bicarbonate such that the acid-base balance was maintained. From the result obtained, it shows the absence of acidosis, but consistently increased dosage of Yoyo Bitters might lead to increased bicarbonate which then might suggest a state of acidosis whereby the body`s reclamation process will be enhanced. The low dose of Yoyo Bitters did not produce a statistically significant result but the mean value also increased (18.4) as compared to the mean control group (15.7).

Histology results: the micrograph of the kidneys of the low dose of Yoyo Bitters fed rat (Figure 1) revealed a necrotic and fragmented proximal and distal tubule, while the glomerulus was preserved. A nuclear pyknosis and tissue inflammation were observed. In the high dose of Yoyo Bitters fed rat (Figure 2) the proximal and distal tubules necrosis and nuclear pyknosis were observed with necrosis and degeneration of glomerulus. This observation correlates with the kidney function parameters of this toxicity study, as abnormal results are often gotten when half of the kidneys are damaged. This result reveals a progressive negative effect on the kidney whereby if intake is not moderated could lead to chronic damage when the kidneys functional integrity is reduced. Photomicrograph of the liver of a low dose of Yoyo Bitters fed rat (Figure 3) showed normal hepatocytes with minor vacuolation and fragmentation. Mild necrotic change was also observed. While the micrograph of the high dose of Yoyo Bitters fed rat (Figure 4 and Figure 5) presented a nuclear pyknosis, vacoulation and congested central vein. The tissues were fragmented with fatty changes; hence the result showed a high tissue injury in the high dose of Yoyo Bitters fed rat group.

Acute tubular necrosis (ATN) is a medical condition which involves death of tubular epithelial cell, and these cells forms the renal tubules of the kidney injury (AKI). Common causes of ATN include low blood pressure and use of nephrotoxic drugs. Acute tubular necrosis (ATN) may be classified into two (i) toxic and (ii) ischemic. When the tubular cells are exposed to a toxic substance, it is categorized under toxic and can be referred to as nephrotoxic ATN. While ischemic ATN, the tubular cells do not get enough oxygen [7]. Most times degeneration is considered as an early indicator of necrosis and is characterized by several morphologic and variable cell features and this degeneration in some cases is preceded by vacuolation [8]. So far, the micrograph has shown that there is tissue injury which suggest that Yoyo Bitters could be a nephrotoxic agent.

Figure 3 Shows the micrograph of the liver of a low dose Yoyo Bitters fed rat which showed a normal hepatocyte with minor vacuolation and fragmentation. It also showed a mild necrotic change. These observations as compared to the high dose of Yoyo Bitters fed rat, are intense with the following observations; nuclear pyknosis, vacuolation, congested central vein and tissue fragmentation with fatty change.

4. CONCLUSION

So far, this study has shown that Yoyo Bitters have hypoglycemic potential and has influence on some of the liver and kidney function parameters. From the histology results, Yoyo Bitters can be toxic to the organs of the body (liver and kidney) if proper precautions are not taken.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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