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Original Research Article

Effect of Odogwu Bitters on Biochemical Indices of Albino Rats

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Abstract

The study assessed the impact of Odogwu bitters on biochemical indices of rats. In this experimental study, 16 adult male rats were used. The rats were randomly divided into four (4) groups. Groups 1-3 were given 100, 200, and 400mg/kg of Odogwu bitters. Group 4 served as the normal control. Serum liver enzyme activity, kidney function enzyme activity, Lipid profile test and haematology tests were carried out. The collected data were analyzed using SPSS, ANOVA, and LSD tests. Elevated levels of ALT and AST in rat plasma indicated potential liver tissue stress due to herbal exposure, aligning with findings from similar herbal studies. However, at higher doses, a reduction in these enzyme levels was observed. Conversely, ALP levels increased significantly with the highest dosage, suggesting potential liver damage. Elevated levels of urea, creatinine, and uric acid in the treated groups indicate potential adverse effects on renal function. Urea, vital for waste nitrogen transport and maintaining osmolarity, showed significant alterations, suggesting potential kidney stress. Similarly, increased creatinine levels, a marker of muscle metabolism and renal health, imply compromised kidney filtration. The disturbance in uric acid metabolism, crucial for various physiological functions, points towards potential renal impairment. Haematological results showed a concentration-dependent increase in hemoglobin levels, potentially supporting hemoglobin synthesis. Platelet counts exhibited a dose-related decrease, suggesting an influence on platelet production or function. Red blood cell levels were maintained, possibly due to antioxidant compounds protecting against oxidative damage and stimulating blood cell production. White blood cell levels remained steady, indicating potential immune-boosting effects. Different doses of Odogwu herbal bitters influenced lipid metabolism in Wistar albino rats, with Group 2 showing a reduction in TG levels and Group 3 having the lowest total cholesterol (TCHO). Groups 2 and 3 also had increased HDL levels, while the control group exhibited the lowest LDL levels, suggesting varied effects of the bitters on lipid profiles. The findings collectively suggest a detrimental impact of Odogwu bitters on kidney indices in the studied animal model and could be toxic at a higher dose upon consumption on the liver. But for the lipid status, Odogwu herbal bitters may influence lipid profiles, their effects are not uniformly beneficial across all parameters.

Keywords: Odogwu bitters, Liver, Kidney, Lipid, Hematology.

1. INTRODUCTION

Herbal medicine has played significant role in treating of diseases since time immemorial and has recorded some feats in curing these diseases which makes developing countries adopt it as alternative to the conventional pharmacotherapy (Patrick-Iwuanyanwu *et al*., 2012). This is possible due to the strong belief system that cut across the ethnic groups existing in Nigeria and as such, Nigeria is blessed with so much natural resources including herbs (Ojekale *et al*., 2019). According to the World Health Organization (WHO), herbal medicines are medications prepared from one or more herbs or plant parts (roots, stem bark, seeds and/or fruits) (Oshikoya *et al*., 2013). Some published reports of bitters include blood cleansers, detoxifiers, hypoglycemic agents, immunomodulatory actions, etc and some of these commercial herbals, including bitters, have specialized therapeutic potentials, while others are sold as a one-mixture-fits-all remedy (Ojekale *et al*., 2019).

As previously mentioned, these "bitters" are now employed as a beverage—typically alcoholic—that is flavored with recognized herbal essences to treat illnesses that seem to have a bitter or bittersweet flavor. All herbal bitters and bitter liquors fall under this general category. Herb and root extracts, derived mostly from tropical and subtropical plants and spices, are used to make bitters. They are often dark in color and are used as

a patent medicine, aid in digestion, and flavoring for cocktails because of their recognized capacity to stimulate hunger and digestion (Anionye & Onyeneke, 2016). Alcoholic beverages (bitters) primarily consist of water, ethanol, and sugar, and are often considered nutritionally deficient as they lack the essential nutrients required for cellular metabolism. Alcohol consumption can have detrimental effects on blood-forming organs, precursor blood cells, and mature red blood cells, white blood cells, and platelets, leading to reduced numbers or impaired function of these blood components. This can result in serious medical complications among individuals who abuse alcohol, such as anemia, fatigue, shortness of breath, dizziness, decreased cognitive abilities, irregular heartbeats, increased susceptibility to infections, and disruptions in blood clotting mechanisms (Odey *et al*., 2022). Many herbal bitters have not been well-researched, and their formulations and sales are insufficiently regulated. Consequently, they may be adulterated and carry risks of adverse effects and toxicity (Oshikoya *et al*., 2013) as these herbal bitters comprises of many herbs in it.

Odogwu bitters being a product of study consists of Ginger, Honey, *Garcinia kola* and bitter leaf. Individually, these medicinal plants it contains are known for some pharmacologically activities. Odogwu Bitters follows this tradition, incorporating local herbs and ingredients reputed for their health benefits. These ingredients are macerated in alcohol, allowing the active compounds to infuse into the liquid, which is then bottled and sold. Like many herbal products, Odogwu Bitters has faced scrutiny over its health claims. Regulatory authorities often warn against attributing medicinal properties to alcoholic beverages, emphasizing the need for scientific validation of health benefits. There is currently no government-generated data in the public domain on the aetiology of the rising cases of nephrotoxicity in Nigeria, which may be influenced by the rise in the use of herbal formulations, given the view held by those who use them that "if it's natural, it's safe." Also, the fact that drug-drug interactions between the various plant constituents in the bitters could be potentially benefiting or harmful necessitates this study. This present study aims at investigating the effect of this popular Odogwu bitters among other bitters on some biochemical indices of wistar rats to ascertain its safety level.

2. MATERIALS AND METHOD

2.1. *Materials:*

Odogwu herbal bitters (OHB) was purchased from the market and used directly.

Equipment: Spectrophotometer, centrifuge, refrigerator (Haier thermocool), weighing balance, measuring cylinder, glass wares (pyrex), sample containers.

2.1.1. Chemicals/reagents:

All the chemicals and reagents used in this research were of the purest analytical grade commercially available.

2.2. Methods:

2.2.1. Sample Collection

Odogwu Bitters product was purchased from reputable pharmaceutical stores along Poly-ihiagwa Road Nekede, Owerri, Imo State, Nigeria. The Odogwu bitters were bought as liquid formulations and stored at room temperature (30-36 ^{o}C) throughout the period of the experiment. Reagent kits and other reagents used were of standard quality and were purchased from qualified/accredited dealers/ suppliers or their manufacturers' representatives in Nigeria.

2.2.2. Animal Management

Adult male rats were used for this study. These animals were purchased from a local breeder in Ihiagwa Owerri-West L.G.A of Imo State. The animals were kept in well-aerated stainless steel wire cages in the animal house of the Department of Biochemistry, Federal Polytechnic Nekede. The rats were given standard feed for at least one week after purchase to acclimatize them to the laboratory environment before use. They were kept at room temperature and maintained ad libitum on water and feed; weighed prior to commencement of experiment and weekly till the end of the experiment.

2.2.3. Experimental Design

Dosages of 0.11ml, 0.22ml and 0.43 ml/kg body weight of OHB, equivalent to 100, 200 and 400 mg of OHB/120 kg body weight of rats daily, were chosen for the study. Sixteen (16) albino rats were divided into four groups of four rats each as follows also as shown in table 2.2.3:

(a) **Group 1:** Healthy rats treated with 0.11 ml/kg body weight of OHB.
(b) **Group 2:** Healthy rats treated with 0.22 ml/kg body weight of OHB.
(c) **Group 3:** Healthy rats treated with 0.43 ml/kg body weight of OHB.
(d) **Group 4:** Normal control rats.

Table 2.2.3: Grouping for Experimental animals

Groups	Induction	Drug	Doses (mg/kg Bw)
Test group	-	OHB	100
Test group	-	OHB	200
Test group	-	OHB	400
Normal Control	-	Water	-
Period	14 days Treatment	14 days Treatment	

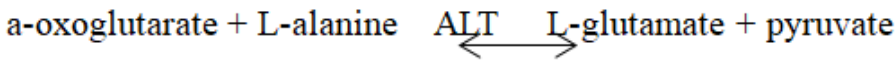
The treatment lasted for 14 days. At the end of the experiment, blood samples were collected from the rats via ocular and cardiac puncture on the first day of post-treatment. The blood samples were collected in anticoagulant free bottles, allowed to clot, and centrifuged at 3000 rpm for 15 min. The serum collected was used for the biochemical assays.

2.2.4. Liver function:

2.2.4.1. Assay of alanine amino transferase (ALT) activity

Serum ALT activity was estimated by the method of Reitman and Frankel (1957).

Principle: This method is based on the production of pyruvate by the transamination activity of alanine amino transferase. Pyruvate reacts with 2, 4 dinitrophenylhydrazone (DNPH) to give a brown coloured hydrazone that is measured colorimetrically at 546 nm.



Reagent composition

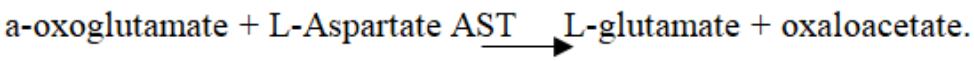
- R_1 is a reagent containing Phosphate buffer (100 mmol/L, pH7.4), L- Alanine (200 mmol/L) a-oxoglutarate (2 mmol/L).
- R_2 is a reagent containing 2, 4dinitrophenyl hydrazine (2 mmol/L).

Procedure: In two separate test tubes, a volume, 0.1 ml of serum and water were mixed with 0.5 ml of R_1 as test and blank, respectively. The solutions were mixed and incubated, respectively for 30 minutes at 37˚C. Subsequently, 0.5ml of R2 was added to both test-tubes, incubated for another 20 minutes at 25˚C, and followed by addition of 5ml of sodium hydroxide (NaOH) solution. The resultant solutions were mixed and the absorbance of test sample against reagent blank was read after 5 minutes at 546 nm. Alanine amino transferase activity was extrapolated from a standard curve.

2.2.4.2. Assay of aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) activity was determined according to the method of Reitman and Frankel (1957).

Principle: Oxaloacetate reacts with AST and is decarboxylated spontaneously to pyruvate. The pyruvate is measured by hydrazone formation after pyruvate reacts with 2,4 dinitrophenylhydrazine (DNPH) to give a brown coloured hydrazone which can be measured at 546nm.



Reagent composition

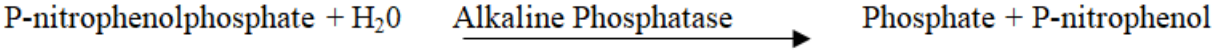
- R_1 is a reagent containing Phosphate buffer (100 mmol/L, pH7.4), L-Aspartate (100 mmol/L), a-oxoglutarate (2 mmol/L)
- R_2 is a reagent containing 2, 4-dinitrophenyl hydrazine (2 mmol/L).

Procedure: A volume, 0.1 ml of serum and blank in different test-tubes were mixed with 0.5 ml of R_1. The solution was incubated for 30 minutes at 37˚C. 0.5 ml of R_2 was added to both test-tubes and allowed to stand for 20 minutes at 25˚C. 5 ml of sodium hydroxide (NaOH) was added, the solution was mixed. The absorbance of sample was read against reagent blank after 5 minutes at 546 nm. Aspartate aminotransferase activity was extrapolated from a standard curve.

2.2.4.3. Assay of serum alkaline phosphatase (ALP) activity

The activity of alkaline phosphatase (ALP) was assayed using the method of Kochmar and Moss (1976).

Principle: This is based on the principle that in the presence of magnesium and zinc ions, p-nitrophenol phosphate is hydrolyzed by phosphatase to form phosphate and p-nitrophenol. The p-nitrophenol released is proportional to the alkaline phosphatase (ALP) activity and can be measured photometrically at 405 nm.



Procedure: To 0.1ml of serum in a test tube labeled 'test', 0.5ml of reagent (p-nitrophenol phosphate) was added, mixed and the initial absorbance read immediately while timer was started simultaneously. It was read again after I, 2 and 3mins. p-nitrophenol was estimated spectrophotometrically at 405 nm.

Calculation:

The mean absorbance per minute was used in the calculation:

$$Activity\ of\ ALP\ (U\ /\ l) = \frac{absorbance\ of\ sample}{asorbance\ of\ standard} \times 2760$$

2.2.5. Kidney Function Test:

2.2.5.1. Urea

Urea concentration was determined using the method of Bartels and Bohmer (1972) as described in Randox Kit.

Principle: Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured spectrophotometrically.

Urease

$$Urea + H_2O \longrightarrow 2NH_3 + CO_2$$

$$NH_3 + Hypochlorite + Phenol \longrightarrow Indophenol\ (blue\ compound)$$

Reagents (R)
R1: EDTA (116mmol/L), Sodium Nitroprusside (6 mmol/L), Urease (1g/L)
R2: Phenol (120mmol/L)
R3: Sodium hypochlorite (27mmol/L), Sodium (0.14N)

Procedure: Ten micro liter (10µl) of distilled water (blank), standard calibrator (urea) and sample were added to three test tubes. This was followed by the addition of 100 µl of reagent1 to each of the test tubes. They were subsequently mixed and incubated at 37^0C for 10 minutes.

The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) against the blank was read at 546nm

Calculation:
Urea Conc. = $\frac{\text{AbsofSample}}{\text{Abs of Standard}}$x Standard conc (mmol/L or mg/dl)

1 mg of urea corresponds to 0.467mg of urea nitrogen.

2.2.5.2. Creatinine

The serum creatinine was determined using the method of Bartels and Bohmer (1972) as outlined in Randox kit.

Principle: Creatinine in an alkaline solution reacts with picric acid to form a coloured complex. The amount of the coloured complex formed is directly proportional to the creatinine concentration.

Reagent
R1a: Picric acid 35 (mmol/L)
R1b: Sodium Hydroxide (0.32 mol/L)

Procedure: Two milliliter (2ml) of the working reagent was mixed with 1ml of standard (creatinine) and incubated for 30 seconds. The same was done for the blood sample. The absorbance A1 of the sample and standard were taken at 492 nm. Exactly 2 minutes later, the absorbances A2 of the sample and standard were taken again. The serum creatinine was calculated thus:
Serum Creatinine Conc.=$\frac{\Delta\text{AbsofSample}}{\Delta\text{Abs Standard}}$x standard conc. (mg/dl)

A1= absorbance 1
A2= absorbance 2
ΔA =A2-A1= change in absorbance (ΔA sample or ΔA standard)
This could either be in mg/dl or µmol/L

2.2.5.3. Determination of Serum uric acid

Serum uric acid level was determined using uricase method as described by Trivedi *et al*., (1978). Thoroughly 1.00 ml of serum (3) and 3.00 ml of the dilute acetic acid were mixed in stoppered centrifuge tubes and Placed in a boiling water bath for 5 mm, later cooled under tap water, and centrifuge for 5 mm. A water blank and the uric acid working standard were treated similarly. Then 1.00 ml of the supernatant fluid was transferred into 15 mm X 125 mm test tubes. Two (2.00) ml of uricase reagent was added, then the test tubes was stoppered and the time recored, then mixed gently by inversion. The reaction proceeded for 30 mm at room temperature. 2.0 ml of 0.10 molar hydrochloric acid, was added followed by 3.00 ml of the chromogen reagent. Then the reaction proceeded for 5 mm. The solution was gently extracted once with 5.00 ml of n-butyl acetate and the absorbance of the butyl acetate against the blank was measured at 492 nm.

Calculations:
Uric acid, mg/liter = $\frac{\text{AbsofSample}}{\text{Abs of Standard}}$ x Standard conc (mg/L)

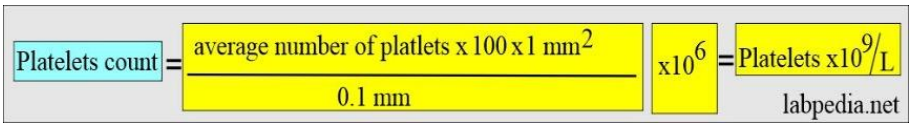
2.2.6. Haematological Tests:

2.2.6.1. Platelets count

Principle: Platelets are the smallest form of formed elements of blood. Platelets are formed by the megakaryocytes in the bone marrow. Basically, these are the detached portion of the megakaryocytes.

Method: Using Neubeur chamber method, 20 µL of blood was taken. 1.8 mL of 1% ammonium oxalate was added. Ammonium oxalate will lyse the RBCs and WBCs while Platelets will remain intact. It was left for 15minutes for complete lysis of RBC's. Neubauer chamber was mounted. The chamber was left for 15 minutes in high humidity and the large central square was counted and labelled as P.

Calculation:



2.2.6.2. Determination of Haemoglobin (Hb) Concentration

Principle: When whole blood is added to Drabkin's reagent (a solution containing KCN and $K_3Fe(CN)_6$), KCN converts Hb-Fe^{2+} (ferrous) to Hb-Fe^{3+} (ferric) state to form methaemoglobin which then combines with KCN to form a stable pigment, cyanmethaemoglobin complex. The colour intensity of this mixture is measured in a spectrophotometer at a wavelength of 540 nm. The optical density (OD) of the solution is proportional to the haemoglobin concentration. All forms of Hb (Hb-C, Hb-O, etc) except Hb-S are measured with this cyanmet-method.

Method: Exactly 5.0 ml of Drabkin's reagent was pipetted into two test tubes 1 and 2 and a well-mixed sample of blood (0.02 ml) in EDTA bottle was pipetted into the tubes, the pipette was rinsed severally with the reagent until all the blood was washed from the pipette. The solutions were well mixed and allowed to stand at 25.0 ^{0}C for 10 min in order to allow the formation of cyan-met-haemoglobin. The mixtures were transferred into cuvettes and read in a spectrophotometer at a wavelength of 540 nm. The Drabkin's reagent in tube 1 was used to blank the machine (setting the percentage transmittance at 100 %). The readings were recorded and transferred into a pre-calibrated chart and the actual Hb values in g/dl were determined.

Calculation:

$$\text{Hb (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}}\ x\ \text{Concentration of standard (mg/dl)}$$

2.2.6.3. Determination of Red Blood Cells (RBCs)

Principle: When whole blood is diluted with an isotonic fluid, it prevents lysis and facilitates counting of the red cells. Some isotonic solutions in use include Hayem's solution, Gower's solution or physiologic saline.

Method: Using the Thoma (manual counting) method, anti-coagulant treated blood was drawn up to the 0.5 ml mark in the RBC count pipette and diluted to a 101 mark with RBC diluting fluid (1: 200 dilution). Dilution was repeated with the replicate tube. Counting chamber was cleaned; both pipettes were shaken three times; counting chamber filled (first expelling the first 4 drops of the mixture), allowing approximately three minutes for the RBCS to settle. Red cells were counted using the counting steps as follows:

1. The filled counting chamber was carefully placed on the microscope stage.
2. Using low power (x10 objective) the large center square was placed in the middle of the field of vision and the entire large square was carefully examined for even distribution of RBCs.
3. The high-dry objective was carefully changed, moving the counting chamber so that the small upper left corner square (this square is further sub-divided into 16 even smaller squares) is completely in the field of vision.
4. All the RBCs were counted in the squares, also counting the cells on the two of the margins but excluding those lying on the other two sides.

Calculation:

The RBCs (in mm^3) = cells counted × correction for volume × correction for dilution:

= RBCs counted in 5 small squares × 200 × 1.0/0.2 (or 50)

= number of RBCS counted in five squares $\times 10^4$

2.2.6.4. Determination of White Blood Cells (WBCs)

Principle: When whole blood is mixed with weak acid solution, it dilutes the blood and haemolyses the RBCs, enabling the WBCs to be counted.

Method: Manual WBC counting method was used as follows:

Dilution of Blood:

(a) The blood specimen was mixed approximately for one minute. Then using the aspirator and WBC pipette, blood was drawn to the 0.5 mark in the pipette.
(b) Blood was removed from the outside of the pipette with clean gauze.
(c) Holding the pipette almost vertically, the tip was placed into the counting diluting fluid to draw it slowly. While gently rotating the pipette, to ensure proper mixing, the diluting fluid was aspirated until it reached the 11 mark.
(d) The pipette was placed in a horizontal position and firmly holding the index finger of either hand over the opening in the tip of the pipette, the aspirator was detached from the other end of the pipette. This is 1:20 dilution.
(e) Having now completed the dilution of blood, the counting chamber and cover glass were cleaned with a lint-free cloth.

Filling the counting chamber: Approximately 0.02 ml of well mixed EDTA- anticoagulated venous blood sample was added to 0.38 ml of diluted fluid dispensed into a small container. One of the grids of the counting chamber was filled with re-mix of the diluted blood sample using a Pasteur pipette, taking care not to overfill the area. The filled area was left undisturbed for two minutes to allow time for the white blood cells to settle, after which the underside of the chamber was dried and placed on the microscope stage.

Counting the white blood cells: Using the10x objective with the condenser iris closed sufficiently to give good contrast, the ruling of the chamber and white cells were focused until the cells appeared as small black dots. The cells in the four large squares of the chamber were then squarely counted.

Calculation:

- The number of white cells per liter of blood was calculated as follows:
- The total number of cells counted was divided by 2
- The number obtained was then divided by 10
- The result was then multiplied by 10^9 to give the white cell count.

2.2.7. Lipid profile tests:

2.2.7.1. Determination of serum total cholesterol concentration

The *in-vitro* determination of serum cholesterol concentration was done by the method of Abell *et al.*, (1952) using RANDOX Laboratories (Crumlin, United Kingdom) test kit.

Principle: Cholesterol concentration was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Method: Three (3) test tubes were set up in a test tube rack and labeled blank, standard and sample respectively. To the blank, was added 10 µl of distilled H_2O, 10µl of standard specimen to the standard test tube and 10µl of sample (serum) to the sample test tube. To each of these test tubes was added 1000µl of the cholesterol reagent. It was thoroughly mixed and incubated for 10min at room temperature (20-25^{o}C). The absorbance of the sample (A_{sample}) against the blank was taken within 60 min at 500nm.

Calculation:

$$\text{Cholesterol concentration}\left(\frac{\text{mmol}}{\text{L}}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 5.2$$

2.2.7.2. Determination of serum high density lipoprotein (HDL) concentration

Determination of the concentration of the serum total HDL concentration was done, using the method described by Kameswara *et al.*, (1999).

Principle: Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the high density lipoproteins (HDL) fraction, which remains in the supernatant is determined.

Method: The precipitant solution 0.1ml was added to 0.3ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuged at 2,000 x g for 15 min. The cholesterol concentration in the supematant was determined.

Calculation:

$$\text{HDL concentration}\left(\frac{\text{mmol}}{\text{L}}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 5.2$$

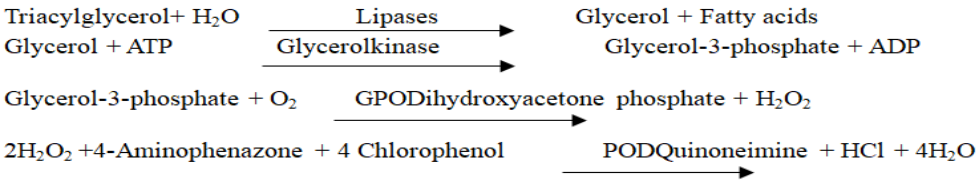
2.2.7.3. Determination of serum triacylglycerol concentration

Triacylglycerol (TAG) concentration was determined using the method of Tietz (1990).

Clinical significance: Triacylglycerol measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g. diabetes mellitus, nephrosis and liver obstruction.

Principle

The Triacylglycerolare determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.



Method: A quantity of the sample (0.1 ml) was pipetted into a clean labeled tube and 1.0 ml of trichloroacetic acid (TCA) was added to it, mixed and then centrifuged at 250 rpm for 10 min. The supernatant was decanted and reserved for use. The assay procedure was carried out as shown in Table 1.

Table 1: Serum triacylglycerol concentration reaction medium

S/N	Blank	Standard	Sample
1. Distilled water	0.5	-	-
2. Standard solution (ml)	-	0.5	-
3. TCA (ml)	0.5	0.5	-
4. Supernatant (ml)	-	-	1.0
5. Reagent mixture (ml)	1.0	1.0	1.0

The mixtures were allowed to stand for 20 min at 25 ^{0}C and the absorbance of the sample and standards read against the blank was taken at 540 nm.

Calculation: The concentration of triacylglycerol in serum was calculated as follows:

$$\frac{\text{Absorbanceofsample}}{\text{Absorbanceofstandard}} \times \text{Standardconcentration(mmol/l)} = \text{mmol/l}$$

2.2.7.4. Determination of serum low density lipoprotein (LDL) concentration

Determination of serum LDL concentration was done with the method of Assmann *et al*., (1984) using RANDOX Laboratories (Crumlin, United Kingdom) test kit.

Principle: LDL-C was determined using the following relationship

$$\text{LDL cholesterol}\left(\frac{\text{mmol}}{\text{L}}\right) = \left(\text{Total cholesterol} - \frac{\text{Triacylglycerol}}{2.2}\right) - \text{HDL}$$

2.3. Statistical analysis

Statistical analysis was performed for the data obtained using the IBM Statistical software for Social Science version 23 to determine the mean, and standard deviation and One-way ANOVA was done to determine their significant differences.

3. RESULTS

3.1. Liver function Tests

The effect of OHB on the liver enzymes (u/l) of the rats is presented in Table 2 outlining the impact of different doses (100mg/kg, 200mg/kg, and 400mg/kg body weight) of a substance, possibly Odogwu bitters, on liver enzymes AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), and ALP (Alkaline Phosphatase) in rats, comparing them to a control group. The results indicate varied effects on liver enzymes: the 100mg/kg group displayed slightly elevated AST and ALT levels compared to the control, suggesting potential hepatocellular stress. Conversely, the 200mg/kg group showed a notable increase in AST and ALT levels, indicating more pronounced liver injury. Additionally, the 400mg/kg group demonstrated lower AST but slightly elevated ALT levels, possibly signifying a different pattern of liver response. ALP levels in the treated groups displayed fluctuations, with the 200mg/kg group exhibiting a marked decrease.

Table 2: Effect of Odogwu bitters on the Liver functions indices of wistar rats

Groups	No of Rats	AST (u/L)	ALT (u/L)	ALP (u/L)
1	4	18.00 ± 0.00^c	20.00 ± 0.01^c	141.01 ± 0.01^c
2	4	35.00 ± 0.00^d	30.00 ± 0.01^d	69.00 ± 0.01^a
3	4	11.00 ± 0.00^a	19.00 ± 0.01^b	156.00 ± 0.01^d
4	4	14.00 ± 0.01^b	10.00 ± 0.01^a	101.0 ± 0.00^b

Group 1: Healthy rats treated with 0.11 ml/kg (100mg) body weight of OHB.
Group 2: Healthy rats treated with 0.22 ml/kg (200mg) body weight of OHB.
Group 3: Healthy rats treated with 0.43 ml/kg (400mg) body weight of OHB.
Group 4: Normal control rats.

3.2. Kidney function Tests

The effect of OHB on the biochemical analysis of urea, creatinine, and uric acid levels in rats administered different doses (100mg/kg, 200mg/kg, and 400mg/kg body weight) compared to a control group was presented in Table 3. In the 100mg/kg group, urea levels showed a notable elevation (17.50 ± 0.70 mg/dl) compared to the normal control (10.00 ± 0.00 mg/dl), suggesting potential kidney stress or dysfunction. However, the 200mg/kg group displayed decreased urea levels (10.50 ± 0.70 mg/dl), closer to the control range, implying a possible dose-dependent response. Creatinine levels in all groups showed slight variations; the 100mg/kg and 400mg/kg groups displayed higher values (0.90 ± 0.00 mg/dl and 0.72 ± 0.03 mg/dl, respectively) compared to the control (0.60 ± 0.00 mg/dl), indicating a potential effect on kidney function. Uric acid levels in the treated groups remained within a comparable range to the control, except for the 200mg/kg group (4.10 ± 0.00 mg/dl), displaying a slight elevation. These findings suggest a dose-dependent impact of the substance on urea and creatinine levels, potentially affecting renal function, while uric acid levels appear less affected overall, warranting further investigation into the substance's effects on renal parameters.

Table 3: Effect of Odogwu bitters on the Kidney functions of wistar rats

Groups	No of Rats	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
1	4	17.50 ± 0.70^{b}	0.90 ± 0.00^{c}	3.80 ± 0.00^{c}
2	4	10.50 ± 0.70^{a}	0.62 ± 0.04^{a}	4.10 ± 0.00^{d}
3	4	21.00 ± 0.00^{c}	0.72 ± 0.03^{b}	3.60 ± 0.00^{b}
4	4	10.00 ± 0.00^{a}	0.60 ± 0.00^{a}	2.10 ± 0.00^{a}

Group 1: Healthy rats treated with 0.11 ml/kg (100mg) body weight of OHB.
Group 2: Healthy rats treated with 0.22 ml/kg (200mg) body weight of OHB.
Group 3: Healthy rats treated with 0.43 ml/kg (400mg) body weight of OHB.
Group 4: Normal control rats.

3.3. Haematological Indices

3.3.1. Platelet count

The mean Platelet count values of rats fed graded doses of Odogwu bitters are shown in figure 1. In the result of study, group 1, 2 and 3 fed with 100mg/kg, 200mg/kg and 400mg/kg respectively of the herbal bitters had relatively a non-significant (P>0.05) number of platelet count but group 1 had significantly (P<0.05) low platelet count number when compared to the control group. Groups 2 and 3 fed with 200mg/kg and 400mg/kg of the bitters showed a non-significant (P>0.05) change in the platelet count of the rats when compared to the control group fed with distilled water only. From the overall result as seen in figure 1, the smaller the error bar, the better the estimate of the mean. However, large error bars indicate unreliable measurement.

3.3.2. Haemoglobin level

Data from mean Hb values of rats fed graded doses of Odogwu bitters are shown in figure 2. In this result of study, Hb level of group 4 was not significantly different (P>0.05) from those of other groups. Group 1 and 2 Hb levels were not significant (P>0.05) when compared together. Odogwu bitters significantly (P<0.05) raised the Hb levels of rats in group 3 when compared to group 1 and 2, although the Hb level of rats in group 3 was not statistically different (P>0.05) from that of the rats in group 4. The study result maintained that the Hemglobin level of rats across all groups were not altered upon consumption of Odogwu bitters. From the overall result as seen in figure 2, the smaller the error bar, the better the estimate of the mean. However, large error bars indicate unreliable measurement.

3.3.3. Red Blood cell count

Data obtained from RBC count of rats treated with Odogwu bitters showed evidently as seen in Figure 3 that there is no significant change (P>0.05) in the RBC count of both treated and healthy rat group. However, In animal study research, the Red Blood Cell (RBC) count holds substantial clinical significance as it serves as a fundamental indicator of an animal's overall health status and physiological functioning. RBC count provides crucial insights into the animal's oxygen-carrying capacity, reflecting the efficiency of oxygen delivery to tissues and organs. Changes in RBC count can signal various pathological conditions such as anemia, dehydration, blood loss, or certain diseases affecting red blood cell production or destruction. Additionally, alterations in RBC count due to experimental treatments or interventions can help researchers assess the potential toxicity or therapeutic effects of drugs, chemicals, or environmental factors on hematopoiesis and overall blood health in the animal model under investigation.

3.3.4. White Blood cell count

Data obtained from WBC count of rats treated with Odogwu bitters showed evidently as seen in Figure 4 that there is no significant change (P>0.05) in the WBC count of both treated and healthy rat group. The White Blood Cell (WBC) count holds significant clinical importance in animal study research as it serves as a pivotal indicator of an animal's immune system function and overall health. WBC count variations can provide crucial insights into an animal's response to infections, inflammation, stress, or exposure to toxins. Elevated WBC counts often signify an ongoing infection or

immune system activation, while decreased counts might indicate immunosuppression or bone marrow dysfunction.

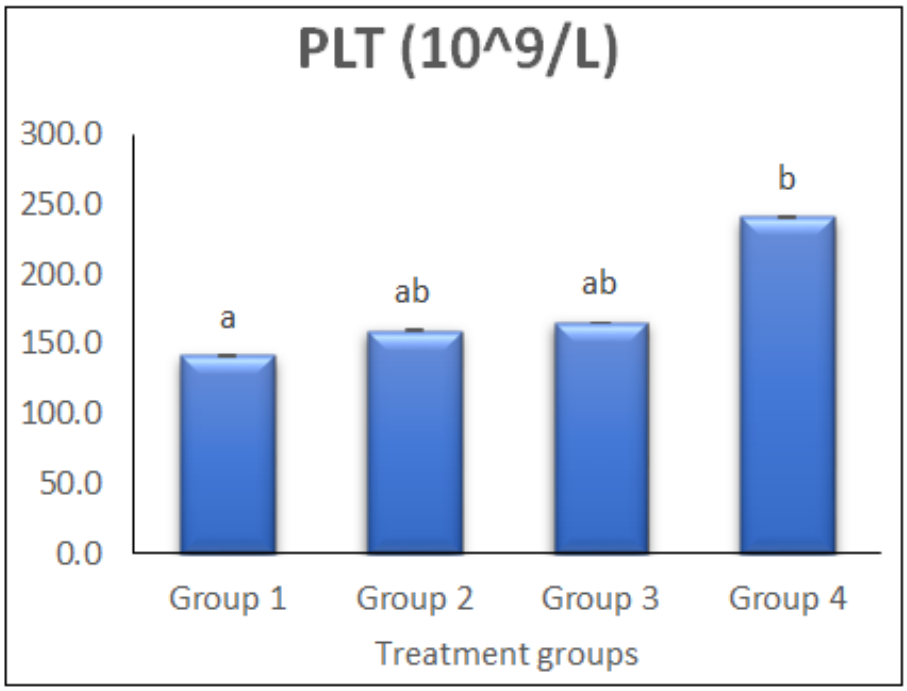


Figure 1: Effect of the Odogwu herbal bitters on the Platelet count of rats.
Group 1: Fed with 100mg/kg of Odogwu bitters
Group 2: Fed with 200mg/kg of Odogwu bitters
Group 3: Fed with 400mg/kg of Odogwu bitters
Group 4: Fed with Distilled water only

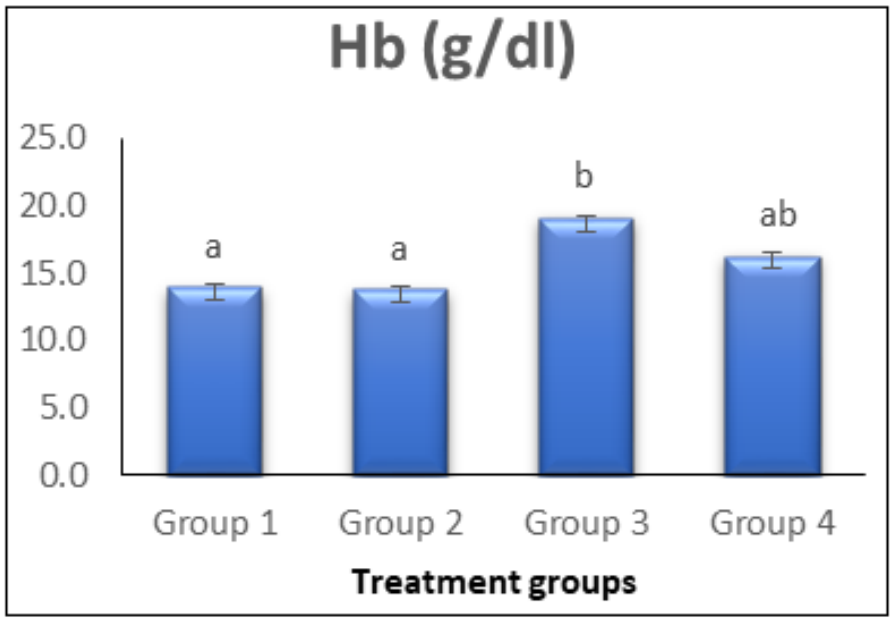


Figure 2: Effect of the Odogwu herbal bitters on the Haemoglobin level of rats
Group 1: Fed with 100mg/kg of Odogwu bitters
Group 2: Fed with 200mg/kg of Odogwu bitters
Group 3: Fed with 400mg/kg of Odogwu bitters
Group 4: Fed with Distilled water only

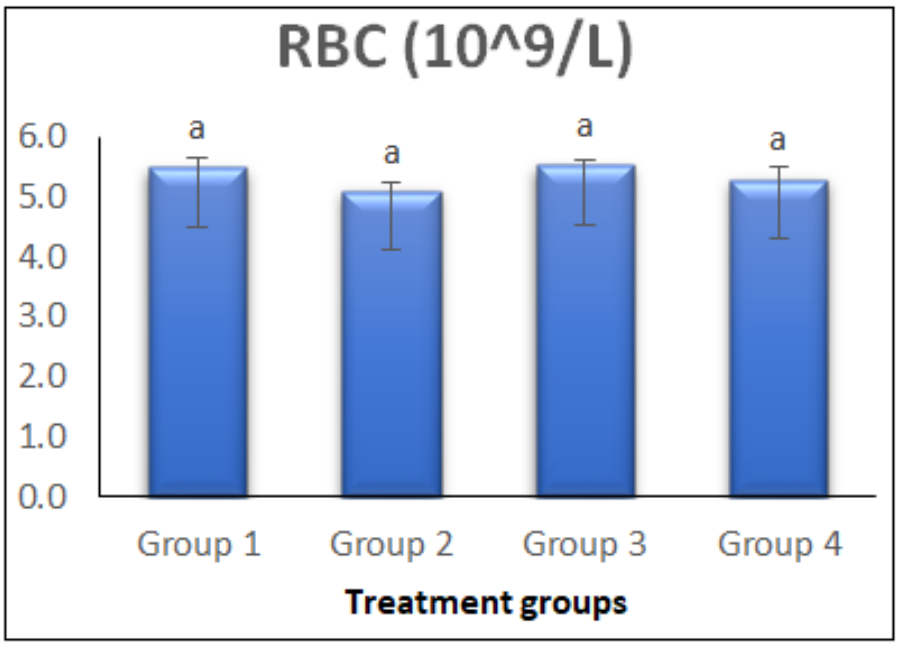


Figure 3: Effect of the Odogwu herbal bitters on the Red Blood cell count of rats.
Group 1: Fed with 100mg/kg of Odogwu bitters
Group 2: Fed with 200mg/kg of Odogwu bitters
Group 3: Fed with 400mg/kg of Odogwu bitters
Group 4: Fed with Distilled water only

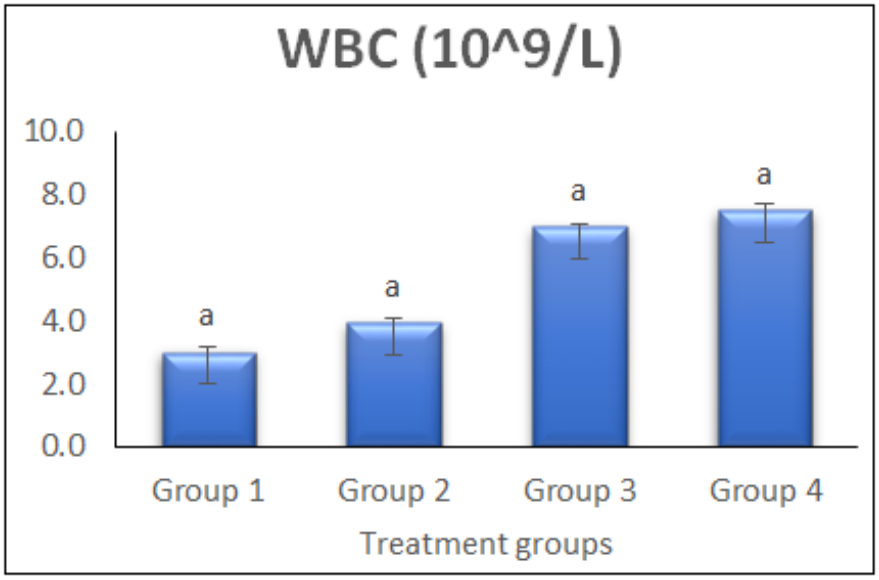


Figure 4: Effect of the Odogwu herbal bitters on the White Blood cell count of rats
Group 1: Fed with 100mg/kg of Odogwu bitters
Group 2: Fed with 200mg/kg of Odogwu bitters
Group 3: Fed with 400mg/kg of Odogwu bitters
Group 4: Fed with Distilled water only

3.4. Lipid Profile

In the result of the study, the TG levels differ significantly ($P<0.05$). Group 2 TG levels were seen to be significantly lower ($P<0.05$) when compared to the control group (see Table 4). However, a significant increase ($P<0.05$) in TG level was observed in groups 1 and 3 when compared to the control group. Nevertheless, in a lipid profile, triglyceride is the main non-cholesterol fat measured, detected by enzymatic reactions that release glycerol from its breakdown. Glycerol levels are then compared to determine the total triglyceride concentration, providing crucial information about fat storage, cardiovascular health, and potential risks for metabolic diseases.

In the result of the study, the TC levels differ significantly (P<0.05). Group 2 TC levels were seen to be significantly higher (P<0.05) when compared to the control group (see Table 4). However, a significant decrease (P<0.05) in TC levels was observed in groups 1 and 4 but group 3 had significantly lower (P<0.05) TC levels when compared to the control. In a lipid profile, total cholesterol represents the aggregate amount of different cholesterol types circulating in blood. It's calculated by summing your HDL ("good" cholesterol), LDL ("bad" cholesterol), and a fraction of your triglyceride levels. Analyzing total cholesterol provides a general picture of risk for cardiovascular disease, highlighting whether total cholesterol falls within healthy ranges or indicates a need for dietary or lifestyle adjustments.

The HDL levels differ significantly (P<0.05). The HDL levels decreased significantly (P<0.05) with an increase in the dose of Odogwu bitters administered orally to the rats (see Table 4). HDL level of healthy (control) was significantly higher (P<0.05) when compared to the test groups. In a lipid profile, high-density lipoprotein (HDL), often called "good" cholesterol, plays the superhero role: it scoops up excess cholesterol from your bloodstream and ferries it back to the liver for disposal. Measuring HDL provides vital information about your cardiovascular health. Higher HDL levels generally offer protection against heart disease and stroke, while lower levels may raise your risk.

In the result of the study, the LDL levels differ significantly (P<0.05). The LDL levels increased significantly (P<0.05) in group 1 as Odogwu bitters were administered orally to the rats (see Table 4). LDL level of group 2 rats decreased significantly (P<0.05) and was non-significant when compared to the control group. In addition, group 3 experienced a significant rise (P<0.05) in LDL level but was significantly lower (P<0.05) when compared to group 1 rats. LDL, the notorious "bad" cholesterol, plays a starring villain role. It transports cholesterol around the bloodstream, potentially depositing it in artery walls and forming dangerous plaques. Measuring LDL levels is crucial, as high levels signal a significant cardiovascular risk.

Table 4: Effect of Odogwu bitters on the Lipid profile status of wistar rats

Groups	No of Rats	TG (mg/dl)	TCHO (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group 1	4	124.05 ± 0.05^{d}	76.05 ± 0.07^{c}	11.05 ± 0.07^{a}	98.00 ± 0.00^{c}
Group 2	4	101.00 ± 0.00^{a}	98.05 ± 0.07^{d}	13.00 ± 0.00^{b}	68.05 ± 0.07^{a}
Group 3	4	106.00 ± 0.00^{c}	43.00 ± 0.00^{a}	14.00 ± 0.00^{c}	83.05 ± 0.07^{b}
Group 4	4	102.00 ± 0.00^{b}	55.00 ± 0.00^{b}	23.05 ± 0.07^{d}	68.00 ± 0.00^{a}

Group 1: Healthy rats treated with 0.11 ml/kg (100mg) body weight of OHB.
Group 2: Healthy rats treated with 0.22 ml/kg (200mg) body weight of OHB.
Group 3: Healthy rats treated with 0.43 ml/kg (400mg) body weight of OHB.
Group 4: Normal control rats

4. DISCUSSION

There is an increasing demand for herbal products as alternative medicines. Unfortunately, the use of herbal products is not strictly regulated in Nigeria thus making them freely available, a scenario which predisposes to possible abuse by consumers (Osemene *et al*., 2012). Although herbal mixtures enjoy wide patronage in Nigeria, little is known about the likely toxicity that may be associated with repeated consumption. Herbal remedies may have recognizable therapeutic effects; they also may have toxic side effects. More so, many herbal preparations lack scientific facts to back up acclaimed medicinal benefits. Currently, there is no available empirical data on Odogwu bitters herbal mixture. The present study sought to determine the influence of oral and repeated administration of the Odogwu herbal mixture on rat biochemical and morphological parameters. This is coming against the background that previous studies have demonstrated toxic potentials of packaged herbals (Adeyemi and Orekoya, 2014).

4.1. Effect of OHB on the Liver

The rat plasma levels of ALT and AST were elevated by exposure to repeated administration of the Odogwu herbal mixture relative to the control. The ALT and AST are normally found in the red blood cells, liver, heart, and kidney tissues. The levels of ALT and AST have long been used to assess the functions of the liver. Increased plasma levels of both ALT and AST have been linked to tissue toxicity (Adeyemi and Akanji, 2011; Liu *et al*., 2018). Normally, a basal level of the enzymes is found in the plasma; however, when there is cellular damage, the enzymes extrude into the extracellular fluid thus raising the concentrations in the plasma. In the present study, the significant alterations to levels of rat plasma ALT and AST may implicate stress imposed on the liver by oral exposure to Odogwu bitters. At a higher dose, the elevated AST and ALT levels were reduced significantly at the dose of 400mg/kg. On the other hand, the rat plasma ALP levels were significantly increased in the group that received the highest dosage of the Odogwu herbal mixture, and a significant reduction as observed in the group treated with 200mg/kg. The decreased level may be a result of inactivation or decreased protein synthesis (Adeyemi and Orekoya, 2014). Previous studies have shown that herbal mixtures can alter the levels of ALT, AST, and ALP in rats. One of the studies that investigated the effects of herbal mixtures on liver enzymes in rats is "Effects of a standardized mixture of

Chinese medicinal herbs on liver injury induced by alcohol and restraint stress in rats" by Shi *et al*., (2014). Another study is "Hepatoprotective effect of a polyherbal formulation against carbon tetrachloride-induced liver injury in rats" by Patel *et al*., (2010). Both studies found that the herbal mixtures used in their experiments were able to decrease the levels of ALT, AST, and ALP in rats with liver injury. Ogechi and Ibioku (2019) concluded that Action Bitters did not induce any obvious biochemical derangement in liver and renal indices at a dose of 0.68ml/kg bodyweight. However, Achionye *et al*., (2019) reported in their findings that Alomo bitters showed no indicative damage on the liver organ.

4.2. Effect of OHB on the Kidney

Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia (Sakami and Harrington, 1963). Urea production occurs in the liver and is regulated by N-acetylglutamate. Urea is found dissolved in the blood and is excreted by the kidneys as a component of urine (Sakami and Harrington, 1963). The handling of urea by the kidneys is a vital part of human metabolism. Besides its role as carrier of waste nitrogen, urea also plays a role in the countercurrent exchange system of the nephrons that allows for re-absorption of water and critical ions from the urine. Urea is reabsorbed in the inner medullary collecting ducts of the nephrons (Walter and Boron, 2004), thus raising the osmolarity in the medullary interstitial surrounding the thin ascending limb of the loop of Henle, which in turn causes water to be reabsorbed. By action of the urea transporter, some of this reabsorbed urea will eventually flow back into the thin ascending limb of the tubule, through the collecting ducts, and into the excreted urine. This mechanism, which is controlled by the antidiuretic hormone, allows the body to create hyperosmotic urine that has a higher concentration of dissolved substances than the blood plasma. This mechanism is important to conserve water, to maintain blood pressure, and to maintain a suitable concentration of sodium ions in the blood plasma (Jacki *et al*., 2007).

Uric acid production and metabolism are complex processes involving various factors that regulate hepatic production, as well as renal and gut excretion of this compound. Uric acid is the end product of an exogenous pool of purines and endogenous purine metabolism. The exogenous pool varies significantly with diet, and animal proteins contribute significantly to this purine pool. The endogenous production of uric acid is mainly from the liver, intestines, and other tissues like muscles, kidneys and the vascular endothelium (Chaudhary *et al*., 2013). Humans cannot oxidize uric acid to the more soluble compound allantoin due to the lack of the enzyme uricase. Normally, most daily uric acid disposal occurs via the kidneys (Jin *et al*., 2016). The kidneys eliminate approximately two-thirds, while the gastrointestinal tract eliminates one-third of the uric acid load. Almost all uric acid is filtered from glomeruli, while post-glomerular reabsorption and secretion regulate the amount of uric acid excretion. The proximal tubule is the site of uric acid reabsorption and secretion, and approximately 90% is reabsorbed into blood. This is primarily accomplished at the proximal tubular level by transporters that exchange intracellular anions for uric acid. Almost all reabsorption of uric acid occurs at the S1 segment of the proximal tubule. In the S2 segment of the proximal tubule, uric acid is secreted to a greater extent than that which undergoes reabsorption. Post-secretory reabsorption occurs at a more distal site of the proximal tubule, and approximately 10% of the filtered uric acid appears in the urine (Chaudhary *et al*., 2013). Hyperuricemia is a key risk factor for the development of gout, renal dysfunction, hypertension, hyperlipidemia, diabetes, and obesity. Hyperuricemia occurs as a result of increased uric acid production, impaired renal uric acid excretion, or a combination of the two (Su *et al*., 2014).

Creatinine is a breakdown product of creatine phosphate in muscle and is usually produced at a fairly constant rate by the body depending on muscle mass. Serum creatinine is an important indicator of renal health because it is an easily-measured by-product of muscle metabolism (Konje *et al*., 1996). Creatinine itself is an important biomolecule because it is a major by-product of energy usage in muscle (Brown, 2006) via a biological system involving creatine, phosphocreatine and adenosine triphosphate. Creatinine is chiefly filtered out of the blood by the kidneys. There is little or no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate creatinine clearance, which reflects the glomerular filtration rate (Allen, 2014).

Elevated levels of creatinine, Urea, and uric acid levels in the rats treated with Odogwu bitters suggest that the herbal mixture has a deleterious effect on the kidneys. Ogechi and Ibioku (2019) report that the use of Action bitters has a non-significant effect on the kidney organ when compared to their control group. Onyeneke *et al*., (2017) reportedly affirmed that Alomo bitters had a non-toxic effect on the kidney organ after assaying for the parameters. However, this present report contradicts their findings as some significant changes were seen in the kidney indices of treated rats when compared to the control. Maybe, this toxic effect could be attributed to the unique formulation for Odogwu bitters as previous studies used Alomo bitters and Action bitters.

4.3. Effect of OHB on Hematology

Hematological parameters are helpful indicators that can be used to evaluate plant extracts' potential for toxicity in living systems (Sunmonu and Oloyede, 2010). They can also be used to illustrate how chemicals and plant extracts relate to blood. These laboratory studies are still the cornerstone of moral and

reasonable research, illness diagnosis, prevention, and therapy since they are extremely sensitive, accurate, and dependable (Okonkwo *et al*., 2011).

Rats given herbal bitters had higher hemoglobin concentrations (Figure 2). Rats given 400 mg/kg of Odogwu bitters experienced a concentration-dependent and statistically significant ($p<0.05$) rise in hemoglobin concentration. This suggests that high dosages of Odogwu herbal bitters may maintain hemoglobin synthesis. Numerous illnesses, such as iron deficiency anemia, thalassemia (a hereditary condition characterized by insufficient globin chain formation), and anemias linked to long-term infections or illnesses, are caused by a failure to make hemoglobin. In addition to being a part of the heme group in hemoglobin, iron is a necessary component of numerous enzymes in cells (which consists of a porphyrin ring containing iron). Red blood cells contain the majority of the body's iron reserves since iron is necessary for the synthesis of hemoglobin. An iron deficit may result from insufficient iron consumption or absorption, excessive iron loss from external bleeding, or disruption of iron metabolism (Ladokun *et al*., 2015).

The platelet count results (Figure 1) in the animal study indicate potential effects of the herbal remedy on platelet levels in rats. A healthy rat group having a platelet count of 241 serves as the baseline for comparison. The groups fed with different doses of the herbal remedy, i.e., 100mg, 200mg, and 400mg, displayed platelet counts of 142, 160, and 166, respectively. Observing these results, it appears that the herbal remedy might have an impact on platelet counts in a dose-dependent manner. The decreasing trend in platelet counts with increasing doses of the herbal remedy suggests a potential dose-related effect on platelet levels. However, it's important to note that the platelet counts in all treated groups are notably lower than those in the healthy rat group, indicating a possible influence of the herbal remedy on platelet production or function. Platelets play an important role in the maintenance of normal homeostasis and MPV is an indicator of platelet function, including platelet aggregation; release of thromboxane A_2, platelet factor 4, and beta- thromboglobulin; and expression of glycogen 1b and glycogen IIb/IIIa receptors (Ofem *et al*., 2012).

Odogwu Herbal bitters is a botanical mixture known for their diverse array of bioactive compounds, which often include phytochemicals such as flavonoids, alkaloids, terpenoids, and polyphenols. These compounds possess antioxidant, anti-inflammatory, and potentially hematopoietic properties, among other biological activities. The maintenance of Red Blood Cell (RBC) (Figure 3) levels in treated rats could be attributed to several factors inherent to herbal bitters. Firstly, certain constituents within herbal bitters, such as flavonoids and polyphenols, are recognized for their antioxidant capabilities. They scavenge free radicals, reducing oxidative stress that might otherwise damage red blood cells and impede their function or production. By protecting RBCs from oxidative damage, the herbal bitters could help sustain their lifespan and functionality, thus contributing to maintaining RBC levels within a normal range in the treated rats. Secondly, Odogwu herbal bitters contain bioactive compounds known to stimulate hematopoiesis, the process of blood cell production in the bone marrow. Certain constituents, like specific alkaloids or terpenoids found in herbs, have been linked to enhancing the production of blood cells, including red blood cells. By potentially stimulating the bone marrow to produce more RBCs, the herbal bitters might have aided in sustaining adequate RBC levels in the treated rats. However, it's essential to consider that the specific composition and concentration of bioactive compounds in herbal bitters, as well as their interactions within the body, could vary, influencing their effects on RBC levels. Further research is warranted to elucidate the precise mechanisms by which herbal bitters may contribute to maintaining RBC levels in treated animal subjects.

Maintained levels of white blood cells (WBC) were also observed in rats after administration of herbal bitters (Figure 4), although the increase was not statistically significant ($P>0.05$). The crucial role of WBC in defending the body against infection and tissue damage is well known. This supports previous reports that ginseng tea and some commonly prescribed medicinal plants contain agents that stimulate the production of leucocytes (Al-Mamary, 2002; Imoru and Buseri, 2005). This suggests that the extract may have immune immune-boosting effect on the animals. Such effects may also be due to an increase in vascular permeability. Administration of Odogwu herbal bitters appears to exhibit a stimulatory effect on the effector cells of the immune system. Immune boosters are usually recommended to strengthen and harmonize degenerative body systems and assist the immune system in fighting invading agents such as bacteria and viruses (Al-mamary, 2002; Ladokun *et al*., 2015).

Previous researches has validated that different bitters exhibit differently on this hematological parameters. Aderonke (2022) found that HB Cleanser Bitters increased white blood cell count and decreased red blood cell count and hemoglobin concentration in rats. Ekor (2010) reported that Yoyo 'Cleanser' Bitters and Fields Swedish Bitters did not significantly affect most haematological parameters but had the potential to induce hypokalaemia and increase liver function markers. However, Elechi-Amadi (2020) discovered that overdose of Action Bitters and Goko Cleanser led to significant alterations in hepatic, renal, and haematological indices. These studies collectively suggest that herbal bitters can have varying effects on haematological parameters in rats, with some potentially causing adverse effects.

4.4. Effect of OHB on Lipid profile Test

Significant evidence suggests that lipid irregularities are a critical factor in the onset and advancement of atherosclerosis and cardiovascular diseases, and environmental influences also contribute to these pathological conditions (Ogunrinola *et al*., 2019). High-density lipoprotein is commonly referred to as "good" cholesterol due to its capacity to extract cholesterol from the arterial walls and transport it to the liver, thus clearing the arteries and reducing the risk of heart attack. Additionally, HDL helps prevent the oxidation of low-density lipoprotein and is believed to possess antioxidant properties (Brunzell *et al*., 2008). More so, Wierzbicki, (2005) submitted the existence of an inverse relationship between high-density lipoprotein level and the risk of cardiovascular diseases. The increase in HDL cholesterol in Groups 2 and 3 compared to Group 1 is promising, as HDL is known for its protective role against heart disease. However, Group 4 shows the highest HDL levels, suggesting that the control diet may inherently support better HDL levels.

The decreased amount of LDL and a concomitant increase in HDL could be attributed to two factors; (1) Direct effect on Lipid metabolism: where the extracts may contain compounds that directly inhibit cholesterol absorption from the intestine, thereby reducing the amount of LDL cholesterol entering the bloodstream and also may increase HDL production, (2) indirect effect on Lipid metabolism: where oxidized LDL is more susceptible to modification and deposition in the arterial wall, contributing to atherosclerosis. Extracts with antioxidant activity may protect LDL from oxidation, reducing the risk of atherosclerosis and indirectly lowering LDL levels (Vu and Chaiwat, 2015). Low LDL-C levels are generally considered beneficial for cardiovascular health hence, the result of the present study demonstrated some potential that the extracts have LDL-lowering ability. The control group has the lowest LDL levels, while Group 1 shows the highest. This pattern suggests that the herbal bitters, at least at certain doses, might not effectively lower LDL cholesterol, which is a critical factor in cardiovascular health.

High triglyceride concentrations are linked to an elevated risk of cardiovascular disease, metabolic syndrome, and pancreatitis. Certain plant-derived extracts, such as those from herbal bitters, have demonstrated the ability to inhibit the activity of enzymes that participate in hepatic fatty acid synthesis (Bays *et al*., 2009). This may result in diminished triglyceride synthesis and decreased serum triglyceride concentrations. Conversely, certain plant extracts, such as those derived from garlic and ginger, have been observed to stimulate enzymes responsible for fatty acid catabolism (Davidson, 2000). This can lead to increased breakdown of triglycerides and lower circulating triglyceride levels. Lipoprotein lipase is an enzyme that breaks down triglycerides in the bloodstream. Some plant extracts, such as those from cinnamon and fenugreek, have been shown to increase lipoprotein lipase activity. This can lead to increased clearance of triglycerides from the circulation (Herber *et al*., 2000). Bile acids help to solubilize and transport triglycerides in the bile. Some plant extracts, such as those from dandelion and milk thistle, have been shown to increase bile acid synthesis. This can lead to increased clearance of triglycerides from the liver and into the bile (Kris-Etherton *et al*., 2002). Group 3 has the lowest total cholesterol, indicating that higher doses may reduce overall cholesterol levels. However, Group 4 (control) shows relatively low TCHO, raising questions about the consistency and reliability of the bitters in managing cholesterol. While Group 1 shows the highest TG levels, Group 2 exhibits a notable reduction. Elevated TG levels are associated with an increased risk of atherosclerosis and pancreatitis, so the reduction in Group 2 suggests a potentially positive effect at this dose.

5. CONCLUSION

A reduction in AST and ALT levels but an increase in ALP level in a rat model can indicate several possible conditions. An obstruction in the bile ducts can lead to increased ALP levels without significantly affecting AST and ALT. Biochemical parameters such as AST, ALT, and ALP were affected by the Odogwu Bitters when administered in Albino rats at the doses of 100mg, 200mg, and 400mg/kg body weight for 14 days. Elevated levels of creatinine, Urea, and uric acid levels in the rats treated with Odogwu bitters suggest that the herbal mixture has a deleterious effect on the kidneys. Also, the maintained levels in the Hb, WBC, Platelet count levels observed in rats administered herbal bitters suggest that Odogwu bitters can be used in anemic conditions, and the maintained level of WBC count indicates that Odogwu herbal bitters contains agents that could stimulate the production of leucocytes, therefore the herbal drink could serve as immune boosters. Lipid profile results suggest that while Odogwu herbal bitters may influence lipid profiles, their effects are not uniformly beneficial across all parameters. The control group's favorable lipid profile raises questions about the overall efficacy of the herbal bitters in improving cardiovascular health. Therefore, Odogwu bitters are considered toxic at a higher dose intake to the liver and kidney.

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