

## Original Research Article

# Histopathological evaluation of D'General Bitters on renal health in adult male Wistar rats

Darlington N Onyejike<sup>1,2\*</sup>, Darlington C Akukwu<sup>3</sup>, Fortune O Abrukwe<sup>1</sup>, Emmanuel N Ezeokafor<sup>4</sup>, Ifeoma F Okwuonu<sup>4</sup>, Chukwudi F Afuberoh<sup>4</sup>, Ambrose E Agulanna<sup>1</sup>, Dominic E Ejiofor<sup>5</sup>

<sup>1</sup>Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nigeria, <sup>2</sup>Department of Forensic Medicine, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa, <sup>3</sup>International Institute for Biomedical Tissue Engineering and Regenerative Medicine, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, <sup>4</sup>Department of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, <sup>5</sup>Department of Human Physiology, Faculty of Basic Medical Sciences, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria

\*For correspondence: **Email:** [dn.onyejike@unizik.edu.ng](mailto:dn.onyejike@unizik.edu.ng); **Tel:** +2348061131867

Sent for review: 15 March 2025

Revised accepted: 18 June 2025

## Abstract

**Purpose:** To evaluate the potential nephrotoxic effects of D'General Bitters through histopathological analysis.

**Methods:** A total of 32 male Wistar rats weighing between 195 and 230 g were used for the study. Twelve rats were used for acute toxicity test while another 20 was randomly divided into 2 groups; study group (n = 15) and control group (n = 5). Study group was further divided into 3 groups based on doses administered. Control group received standard feed and water while study group received varying doses of D'General Bitters (0.2, 0.4, and 0.8 mL for 6 weeks). Acute toxicity was assessed using the OECD Guideline 425. Histological analysis was conducted to evaluate renal structural integrity after administration of the herbal preparation.

**Results:** Histopathological examinations showed no significant renal structural damage across all dosage groups.

**Conclusion:** While D'General Bitters did not cause significant histological and gross morphological damage, further studies are recommended to assess long-term effects and ensure safe use.

**Keywords:** Acute toxicity, Herbal medicine, Nephrotoxicity, Renal

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

## INTRODUCTION

Herbal medicine is an integral part of traditional healthcare systems worldwide, with roots dating back to ancient civilizations such as those of Egypt, India, and China [1]. Widespread reliance on herbal formulations persists today, driven by their perceived safety, accessibility, and efficacy in treating various ailments [2]. In Nigeria, herbal

mixtures, commonly referred to as 'bitters', are particularly popular, marketed for their purported benefits ranging from detoxification and immune enhancement to improving sexual performance [3,4].

Despite their popularity, safety profiles of these formulations often remain poorly understood, raising concerns about potential toxicological

effects, particularly on vital organs like the kidneys. D'General Bitters, a recently introduced herbal mixture, has rapidly gained popularity in Nigeria. Promoted for its diverse health benefits, its composition includes a blend of water, honey, citrus, alcohol, and other natural herbal extracts [5]. Its phytochemical constituents include proteins, alkaloids, flavonoids, reducing sugars, glycosides, and amino acids [5].

Lack of rigorous preclinical evaluation raises concerns about its safety, particularly considering evidence linking other herbal formulations to adverse renal effects [6,7]. Given that kidneys are central to detoxifying and eliminating herbal compounds, prolonged or high-dose exposure to certain herbal mixtures may result in cumulative nephrotoxicity [2,4]. Nephrotoxic effects, manifesting as impaired renal function or structural damage, have been documented for several herbs due to components like alkaloids, glycosides, and tannins [8,9]. As the primary site for the elimination of exogenous substances, including herbal compounds, the kidneys are particularly vulnerable to toxic effects [10]. Specific histopathological conditions linked to nephrotoxicity include tubular necrosis, glomerular damage, mesangial cell hyperplasia, and interstitial fibrosis [6,8]. These changes may compromise the kidney's filtration capacity and exacerbate chronic kidney diseases.

Previous studies have highlighted the potential nephrotoxic effects of herbal bitters. For instance, Onyejike *et al* [4] revealed the presence of hemorrhagic lesions and reduced glomerular function in Wistar rats administered Goko Cleanser, another popular herbal bitters. Similarly, Bolawa *et al* [11] reported significant alterations in renal biochemical markers following exposure to an alcoholic herbal mixture. These findings underscore the need for further investigation into the safety of herbal mixtures, particularly those with widespread usage.

Conditions such as acute tubular necrosis and interstitial inflammation have been observed in cases of excessive or prolonged herbal mixture consumption, highlighting the potential for long-term renal damage [7,8]. This study evaluated the histopathological effects of D'General Bitters on the kidneys of adult male Wistar rats. By examining morphological changes, and histological alterations, this research elucidated the potential risks associated with consumption of D'General Bitters. Such findings will provide critical insights into its safety profile, contributing to informed public health recommendations and regulatory policies.

## EXPERIMENTAL

### Sample

The herbal drink, D'General Bitters, was obtained from the authorized distributor at Nkwo Nnewi Market, Nnewi, Nigeria.

### Animals

The study involved 40 male adult Wistar rats, each weighing between 195 and 230 g, obtained from a local farm in Nsukka, Enugu State, Nigeria. The animals underwent a two-week acclimation period with unrestricted access to feed (Premier Feeds Mills Co. Ltd., Plateau State, Nigeria) and water. A certified veterinarian assessed their health before they were ethically transported to the research facility. The rats were housed in spacious, well-ventilated stainless-steel cages, under standard conditions (12-hour light/dark cycle, temperature of  $22 \pm 2$  °C, and humidity level of 50 – 60 %). To maintain hygiene and reduce the risk of infection, sawdust was used as bedding, and the cages were cleaned daily. The research spanned 12 weeks and was divided into three phases: a two-week acclimatization period, a six-week experimental stage, and a final four weeks allocated for data analysis.

The study received ethical approval from the Ethics Committee, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria (approval no. NAU/CHS/NC/FMBS/579), and the animal study was conducted in compliance with the internationally accepted guide for the Care and Use of Laboratory Animals [12].

### Acute toxicity test

This study followed the Organization for Economic Co-operation and Development (OECD) Guideline 425, known as the Up-and-Down Procedure, which involves sequential dose administration, and adjusting each dose based on the previous outcome. This approach allows for a more accurate determination of the LD<sub>50</sub> while minimizing the number of animals used [13]. Acute toxicity test was carried out in two phases.

#### Phase I

A total of 9 rats were randomly divided into three groups, each consisting of 3 rats, and monitored over a 24 h period for signs of morbidity and mortality. No adverse effects were observed, and all rats maintained normal health throughout the

observation period, prompting progression to Phase II.

### Phase II

In phase II, 3 additional rats were introduced, each receiving a single dose. These rats were similarly monitored for morbidity and mortality over a subsequent 24 h period.

### Design

Rats were assigned to 2 groups, viz, study (n = 15) and control groups (n = 5). Control group received standard feed and water while study groups received varying doses of D'General Bitters (0.2, 0.4, and 0.8 mL for 6 weeks) designated as A, B, and C, respectively. The cages and surrounding environment were cleaned and disinfected daily to maintain hygiene.

### Animal euthanasia

The animals were euthanized using an overdose of inhalant anesthesia (CO<sub>2</sub> gas) in accordance with the guidelines set by the American Veterinary Medical Association (AVMA) [14]. The gas flow rate was regulated to replace 10 – 30 % of the chamber's volume per minute, ensuring minimal discomfort and preventing distress. Each rat was closely monitored for loss of consciousness, followed by confirmation of euthanasia after complete cessation of respiratory and cardiac activity. This was verified by checking for the absence of heartbeat, respiration, and reflex responses, such as corneal reflexes. The carcasses were disposed of in compliance with institutional biosafety regulations.

### Organ harvesting

The rats were positioned on a flat board, with their limbs securely held in place using optical pins. A dissecting kit was used to carefully open the abdominal cavity, and the kidneys were harvested. The organs were rinsed in a plain tube containing normal saline before being preserved in a 10 % formal saline (Syntron Bioresearch Inc., USA) solution for histological examination.

### Histological examination

The kidney samples underwent fixation, dehydration, clearing, embedding, sectioning, and staining following standard tissue processing

protocols [15]. Photomicrographs were captured using an Amscope 14MP digital microscope camera mounted on a Novex compound microscope equipped with Hi-Plan objectives. Image labeling was performed using Photoscape v3.7.

### Body weight and relative organ weight

Before and after treatment, body weight was measured and relative kidney weight was assessed after harvesting the kidney using a 6000 g capacity balance (Model WT6000GT, Want Balance Instrument Company Limited, China).

### Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS version 22.0, IBM, Armonk, NY, USA). Results were expressed as mean ± standard deviation (SD) and compared using one-way analysis of variance (ANOVA). *P* < 0.05 was considered statistically significant.

## RESULTS

### LD<sub>50</sub> of D'General Bitters

#### Phase I

Doses of 10, 100 and 1000 mg/kg were administered to the respective groups, with no signs of mortality or abnormal behavior.

#### Phase II

At higher doses of 1200 and 1600 mg/kg, no fatalities occurred, and animals remained calm. However, at 2900 and 5000 mg/kg, mortality occurred within 24 and 12 h, respectively. The LD<sub>50</sub> was calculated using Eq 1.

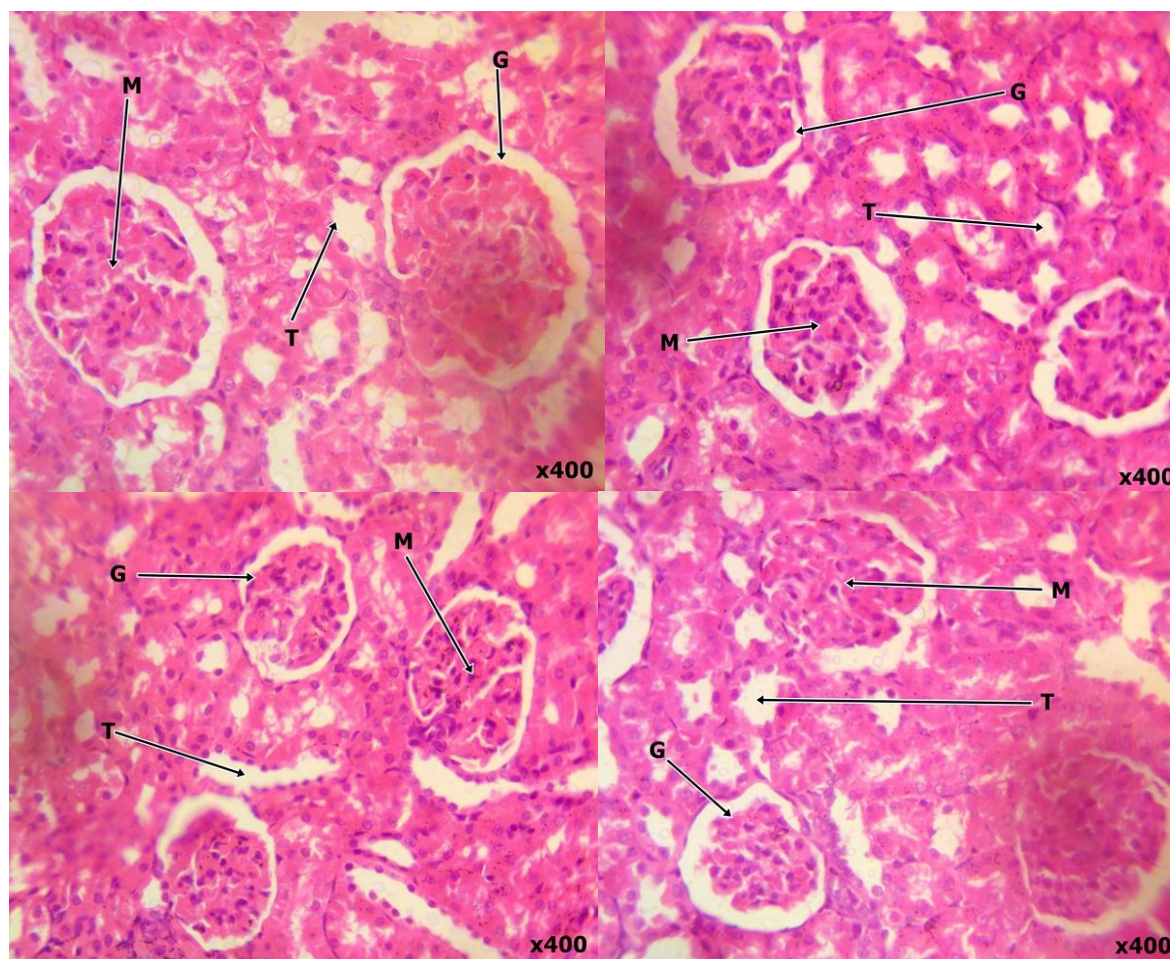
$$LD_{50} = \sqrt{AB} \dots\dots\dots (1)$$

where A is the maximum dose with 0 % mortality, B is the minimum dose with 100 % mortality.

Therefore,  $LD_{50} = \sqrt{1600 \times 2900} = 2154.17$  mg/kg

### Histopathological analysis of renal tissues

There was no significant histological and gross morphological damage following administration of D'General Bitters (Figure 1).



**Figure 1:** Histo-architecture of renal tissues. **A:** The histological examination of renal tissues from the control group revealed evenly distributed glomeruli of uniform size, exhibiting normal mesangial cellularity. The glomerular capillaries appeared widely open with intact endothelial lining. The renal tubules displayed normal density, and the tubular epithelium remained structurally viable. No significant pathological alterations were observed in the interstitial space. **B:** Renal sections of study group (0.2 mL) demonstrated evenly distributed glomeruli of consistent size, with mild mesangial hypercellularity. The glomerular capillaries remained widely open, and the endothelial lining appeared intact. The renal tubules retained normal density, and the tubular epithelium remained viable. No notable pathological changes were identified within the interstitium. **C:** Renal sections of study group (0.4 mL) exhibited histological features comparable to the control group. The glomeruli were uniformly distributed and maintained normal mesangial cellularity. Glomerular capillaries were open, with an intact endothelial lining. The renal tubules showed normal density, and the tubular epithelium remained structurally intact. The interstitial space displayed no significant pathological alterations. **D:** Renal sections of study group (0.8 mL) showed evenly distributed glomeruli of similar size, with mild mesangial hypercellularity, comparable to findings in the low-dose group. The glomerular capillaries remained open, with normal endothelial integrity. Tubular density and epithelial viability were preserved, and no significant interstitial pathology was noted. **Key:** M = Mesangium, G = Glomerulus, T = Tubule

### Body weight and relative organ weight analysis

There was significant difference in body weight between initial and final body weight in all groups ( $p < 0.05$ ). However, there was no significant difference in final body weight between control and study groups (A, B, and C;  $p > 0.05$ ; Table 1).

### Relative organ weight

Relative organ weight analysis showed no significant difference between control and study groups (A, B, and C;  $p > 0.05$ ; Table 2).

**Table 1:** Difference in body weight (mean  $\pm$  SD, n = 5 in each group)

| Group   | Initial weight (g) | Final weight (g)   | P-value            | T-value |
|---------|--------------------|--------------------|--------------------|---------|
| Control | 90.00 $\pm$ 8.71   | 213.00 $\pm$ 17.69 | 0.010 <sup>#</sup> | -9.837  |
| Group A | 146.25 $\pm$ 1.50  | 241.25 $\pm$ 18.41 | 0.002 <sup>#</sup> | -9.816  |
| Group B | 150.75 $\pm$ 3.40  | 247.50 $\pm$ 36.42 | 0.016 <sup>#</sup> | -4.979  |
| Group C | 158.25 $\pm$ 31.69 | 239.50 $\pm$ 15.15 | 0.017 <sup>#</sup> | -4.802  |

Data obtained was analyzed using paired t-test and values were considered significant at <sup>#</sup> $p < 0.05$

**Table 2:** Difference in body and relative organ (mean  $\pm$  SD, n = 5 in each group)

| Group   | Body weight (g)                 | P-value | Relative organ weight (g)        | P-value |
|---------|---------------------------------|---------|----------------------------------|---------|
| Control | 213.00 $\pm$ 17.69              | -       | 0.0035 $\pm$ 0.0006              |         |
| Group A | 241.25 $\pm$ 18.41 <sup>a</sup> | 0.151   | 0.0033 $\pm$ 0.0001 <sup>a</sup> | 0.453   |
| Group B | 247.50 $\pm$ 36.42 <sup>a</sup> | 0.086   | 0.0038 $\pm$ 0.0003 <sup>a</sup> | 0.571   |
| Group C | 239.50 $\pm$ 15.15 <sup>a</sup> | 0.175   | 0.0040 $\pm$ 0.0002 <sup>a</sup> | 0.236   |
| F-ratio | 1.316                           |         | 1.545                            |         |

Data was analyzed using ANOVA followed by post hoc LSD comparison. <sup>#</sup> $P < 0.05$ , <sup>a</sup> $p > 0.05$

## DISCUSSION

The findings from this study align with existing literature on the nephrotoxic effects of herbal bitters, providing a better understanding of the impact of D'General Bitters on renal health in Wistar rats. Previous studies have documented varying degrees of nephrotoxicity associated with different herbal bitters. For instance, Onyejike *et al* [4] reported significant renal damage, including hemorrhagic lesions and reduced glomerular function, in Wistar rats administered Goko Cleanser. Similarly, Ekor discussed the growing use of herbal medicines and highlighted issues relating to adverse reactions and challenges in monitoring safety [16]. These studies underscore the potential nephrotoxic risks posed by herbal bitters. Findings from this study revealed no significant structural damage to the kidneys across all dosage groups, which was in contrast with earlier studies; however, with other herbal bitters [4,15-17].

It is also in contrast with the findings of Onyejike *et al* [5] on the effect of D'General Bitters on parameters of kidney function (urea, uric acid and creatinine levels) [5]. This discrepancy may be attributed to many factors which include differences in composition and concentration of bioactive compounds in D'General Bitters, early kidney injury, reversible functional changes, and the limit of detection in the case of light microscopy against electron microscopy. Also, the presence of flavonoids and glycosides in D'General Bitters, which have been shown to possess both therapeutic and toxicological properties, might contribute to its relatively mild impact on renal tissues [18]. Previous studies with other bitters revealed distorted kidney tissue architecture at higher doses [18].

Analysis of body weight and relative organ weight revealed no significant difference between the control and study groups. This suggests that D'General Bitters did not induce systemic toxicity or cause significant changes in overall body mass and organ size. These findings are consistent with other studies that reported no significant alterations in body weight and relative organ weight following the administration of certain herbal mixtures [19,20]. The lack of significant changes in body and organ weights further suggests that D'General Bitters may have a relatively safe profile at the tested doses.

## CONCLUSION

This study demonstrates that D'General Bitters, at varying doses, does not cause significant histological damage or gross morphological changes to the kidneys of adult male Wistar rats. Further long-term experimental and clinical studies to assess the chronic effects of D'General Bitters on renal and other vital organs are required to validate these findings.

## DECLARATIONS

### Acknowledgement/Funding

We wish to extend our sincere gratitude to Dr Albert Nwamaradi of Nnamdi Azikiwe University for his support during this research. This research was supported by Tertiary Education Trust Fund of the Federal Republic of Nigeria (grant no. TETF/DR&D/CE/UNI/AWKA/IBR/2024/VOL.II).

## Conflict of interest

No conflict of interest is associated with this work.

## Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved of the final manuscript.

Darlington Onyejike conceptualized the study, designed the study, wrote the protocol, supervised the experiment and wrote the first draft of the manuscript. Darlington Akukwu reviewed the manuscript. Fortune Abrukwe carried out the experiment, managed the animals, curated the data, and assisted Emmanuel Ezeokafor in euthanizing the animals for sample collection. Emmanuel Ezeokafor carried out the toxicity test and euthanized the animals for sample collection. Ifeoma Okwuonu carried out the phytochemical analysis and biochemical assay. Chukwudi Afuberoh managed the literature searches and assisted Fortune Abrukwe in managing the animals. Ambrose Agulanna carried out the histology analysis. Dominic Ejiofor carried out the data analysis and assisted Darlington Akukwu in reviewing the manuscript.

## Availability of data and materials

The datasets generated during and/or analyzed during the current study are available within the text.

## REFERENCES

1. World Health Organization. Traditional medicine has a long history of contributing to conventional medicine and continues to hold promise. Available at <https://www.who.int/news-room/feature-stories/detail/traditional-medicine-has-a-long-history-of-contributing-to-conventional-medicine-and-continues-to-hold-promise?> (Accessed: 5th January 2025).
2. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol* 2014; 4: 177.
3. Grover JK, Yadav S. Pharmacological actions and potential uses of *Momordica charantia*: a review. *J Ethnopharmacol* 2004; 93(1): 123-132.
4. Onyejike DN, Aladeyelu SO, Onyejike IM. Histopathological effects of Goko Cleanser (herbal mixture) on the kidney of adult female Wistar rats. *Int J Innov Res Adv Stud* 2018; 5(6): 254–262.
5. Onyejike DN, Aguwa US, Abrukwe FO. Evaluating the biochemical impact of D'General bitters on renal function in male Wistar rats: a preliminary study. *Asian J Res Rep Urol* 2024; 7(1): 101-109.
6. Xu X, Zhu R, Ying J, Zhao M, Wu X, Cao G, Wang K. Nephrotoxicity of herbal medicine and its prevention. *Front Pharmacol* 2020; 11: 569551.
7. Onyejike IM, Onyejike DN, Nweke EO. Biochemical and nephrotoxic effects of Odogwu bitters and Goko Cleanser on Wistar rat kidneys: a preliminary study. *J Med Health Res* 2024; 9(2): 52-61.
8. Velickovic-Radovanovic RM, Mitic B, Kitic D, Kostic S, Cvetkovic T, Djordjevic V. Acute renal failure after licorice ingestion: A case report. *Cent Eur J Med* 2011; 6: 113–116.
9. Yang B, Xie Y, Guo M, Rosner MH, Yang H, Ronco C. Nephrotoxicity and Chinese herbal medicine. *Clin J Am Soc Nephrol* 2018; 13(10): 1605-1611.
10. Bajaj P, Chowdhury SK, Yucha R, Kelly EJ, Xiao G. Emerging kidney models to investigate metabolism, transport, and toxicity of drugs and xenobiotics. *Drug Metab Dispos* 2018; 46(11): 1692-1702.
11. Bolawa EO, Adeogun BM, Alani RA. Genotoxic and histopathological alterations in rats exposed to herbal liquors. *GSC Biol Pharm Sci* 2021; 17(03): 198–216.
12. National Research Council. Guide for the care and use of laboratory animals, National Academies Press. Washington, DC; 2010
13. Organization for Economic Co-operation and Development (OECD). Test No. 425: Acute oral toxicity – Up-and-Down Procedure. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2008. Available from: 10.1787/9789264071049-en. (Accessed: 5th January 2025).
14. American Veterinary Medical Association (AVMA). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. 2020. Available from: <https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>. (Accessed: 5th January 2025).
15. Onyejike DN, Aladeyelu SO, Onyejike IM, Ogbo FO. Effects of Goko Cleanser herbal mixture on the microarchitecture of the liver of adult female Wistar rats. *Int Invent Sci J* 2018; 2(5): 184–200.
16. Obasi DC, Ogugua VN. Effect of Ruzu Herbal Bitters on the kidney function and hematological parameters of alloxan-induced diabetic rats. *Int J Sci Eng Res* 2020; 11(5): 63-86.
17. Ekor M. Toxicity Evaluation of Yoyo 'Cleanser' bitters and fields Swedish bitters herbal preparations following sub-chronic administration in rats. *Am J Pharmacol Toxicol* 2010; 5(4): 159-166.
18. Oforibika G, Oforibika A. Estimation of toxicity level of three different herbal bitters (Confam, G. Winco and 1960 Roots) on some serum lipid profile parameters and the heart of Wister Albino rats. *J Health Sci Res* 2020; 3(6): 15-20.

19. Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN. Evaluation of acute and subchronic toxicity of *Alstonia congensis* Engler (Apocynaceae) bark and *Xylopi aethiopica* (Dunal) A. Rich (Annonaceae) fruits mixtures used in the treatment of diabetes. *Afr J Biotechnol* 2008; 7(6): 701-705.
20. Adebayo AH, Abolaji AO, Opata TK, Adegbenro IK. Effects of ethanolic leaf extract of *Chrysophyllum albidum* G. on biochemical and haematological parameters of albino Wistar rats. *Afr J Biotechnol* 2010; 9(14): 2145-2150.