

Original Research Article

Histopathological and haematological assessment of co-administered Odogwu Bitters and Goko Cleanser herbal mixtures on the spleen of adult male Wistar rats

Darlington N Onyejike^{1*}, Nsikak M Umoh², Toheeb L Sulaimon¹, Ambrose E Agulanna¹

¹Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University,

²Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Calabar, Nigeria

*For correspondence: **Email:** dn.onyejike@unizik.edu.ng; **Tel:** +234-8061131867

Sent for review: 2 August 2026

Revised accepted: 16 September 2026

Abstract

Purpose: To investigate the histopathological and haematological impact of individual and co-administered Odogwu Bitters (OB) and Goko Cleanser (GC) on the spleen of adult male Wistar rats.

Methods: Fifty rats were randomized into ten groups (n = 5). Groups B – D received OB only (0.2, 0.4 and 0.8 mL/day, respectively), groups E – G received GC only (0.2, 0.5 and 0.9 mL/day, respectively), and groups H – J received combined doses, while Group A served as the untreated control. After six weeks of oral administration, splenic tissue was processed for histology (H & E staining), and packed cell volume (PCV) and haemoglobin (Hb) concentration were analysed.

Results: Histopathological assessment revealed nodular hyperplasia of the splenic white pulp in groups receiving moderate- and high-dose OB (C, D), low-dose GC (E), and the high-dose combination group (J). Haematologically, a significant decrease (p = 0.046) in PCV occurred exclusively in the group receiving the low dose of GC alone (E). Haemoglobin levels showed non-significant (p > 0.05) variable trends across all experimental groups. All groups exhibited significant (p < 0.05) body weight gain, with no significant (p > 0.05) changes in relative spleen weight.

Conclusion: Co-administration of Odogwu Bitters and Goko Cleanser induces specific splenic lymphoid hyperplasia and haematological alterations in the absence of systemic toxicity. These findings identify the spleen as a target organ and highlight a potential immunohaematological risk associated with the combined use of these herbal mixtures, underscoring the need for stricter safety evaluations.

Keywords: Goko Cleanser, Haematological alterations, Herbal mixtures, Immunotoxicity, Odogwu Bitters, Toxicity screening

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INTRODUCTION

The consumption of herbal mixtures for medicinal purposes remains a cornerstone of traditional healthcare systems globally, particularly in regions such as West Africa where access to and trust in conventional medicine may be limited [1].

In Nigeria, a vast market exists for commercially packaged herbal drinks, including bitters and cleansers, which are promoted for a wide range of benefits from immune support and digestion to libido enhancement and chronic disease management [2]. Despite their popularity, these products often operate with minimal regulatory

scrutiny regarding their safety profiles, especially under conditions of combined or prolonged use, raising significant public health concerns about herb-induced organ injury [3].

Odogwu Bitters and *Goko Cleanser* are two prominently consumed herbal products in this category. *Odogwu Bitters* is an alcoholic formulation containing a blend of botanicals such as ginger and honey, marketed as a general health tonic [4]. Phytochemical analyses reveal it contains saponins (0.13 %), alkaloids (0.09 %), terpenoids (0.30 %), flavonoids (0.12 %), carbohydrates, cardiac glycosides and reducing sugars [5]. In contrast, *Goko Cleanser* is a non-alcoholic mixture composed of *Vernonia amygdalina*, *Saccharum officinarum*, *Allium sativum*, *Cajanus cajan*, *Caramel* and *Zingiber officinale*, promoted as a detoxifier and treatment for infections and urinary disorders [6]. Studies on *Goko Cleanser* have reported significant increases in haematological parameters such as total white blood cell count, packed cell volume, platelet count, red blood cells, and haemoglobin in female Wistar rats, suggesting potential haematopoietic benefits, though it may also suppress appetite [7]. It is notably rich in tannins (0.2 %), saponins (7.35 %), and flavonoids (4 %) [5]. The co-administration of these drinks, a common practice among consumers seeking compounded effects, introduces a complex phytochemical milieu. This combination may precipitate unpredictable interactions where certain compounds like alkaloids and tannins could potentiate hepatotoxic risks [8], while saponins and flavonoids might offer hepatoprotective benefits via antioxidant pathways [9]. The net physiological outcome of this interplay, particularly on lymphoid organs, remains largely uncharacterized.

The spleen is a critical secondary lymphoid organ integral to immune modulation, haematopoiesis, and blood filtration [10]. Histopathological alterations in splenic architecture serve as sensitive indicators of systemic toxicity, inflammatory status, or immunomodulatory effects of xenobiotics [11]. Emerging toxicological assessments of these individual and combined herbal drinks have focused primarily on renal, hepatic, cardiac, reproductive and haematological endpoints. Independent studies report that both *Odogwu Bitters* and *Goko Cleanser* can induce biochemical and structural kidney damage [5,12], alter liver enzyme profiles [13] and cause dose-dependent cardiac histological changes [14]. Interestingly, co-administration has been associated with significant biochemical alterations in renal and hepatic markers without

concomitant structural damage in kidneys or testes [12,15], suggesting organ-specific vulnerability. However, the histopathological and haematological impact of this combined phytochemical exposure on the spleen (a primary immune organ) has not been investigated, representing a critical gap in the safety assessment of these widely consumed products.

Therefore, this study aimed to evaluate the histopathological and haematological effects of the individual and co-administered *Odogwu Bitters* and *Goko Cleanser* herbal mixtures on the spleen of adult male Wistar rats. By examining changes in splenic cytoarchitecture, organ weight, and key haematological parameters, this research provides essential data on the potential immunotoxic and systemic risks associated with the concurrent use of these popular herbal remedies.

EXPERIMENTAL

Animals

Fifty (50) adult male Wistar rats, weighing 195 – 230 g, were procured from a local farm at Nsukka, Enugu State, Nigeria. Before the rats were humanely transported to the study location, their health status was confirmed by a veterinarian. Upon arrival at the study location (research facility), the rats were allowed to acclimatize for two weeks before the experimentation. All the rats were given *ad libitum* access to feed and water. They were housed in spacious stainless-steel cages within a climate-controlled, well-ventilated room maintaining a 12-hour light/dark cycle. Standard care included a balanced diet, fresh water, and regular enclosure cleaning with sawdust bedding to minimize infection risks. Routine health monitoring was conducted for the duration of the study.

Acute toxicity assessment

Dosages were determined using established median lethal dose (LD₅₀) values for male Wistar rats: 2154.17 mg/kg for *Odogwu Bitters* and 3184.34 mg/kg for *Goko Cleanser* [12].

Preparation of stock solutions

Each herbal drink was concentrated by oven-drying 200 mL at 50 °C, yielding 5 g of solid residue. This 5 g portion was then dissolved in 100 mL of distilled water to prepare the stock solution. Given that 5 g equals 5000 mg, the resulting concentration was calculated as 5000 mg/100 mL, equating to a stock solution of 50

mg/mL. This standardized concentration was subsequently used for all dosage calculations based on individual animal weight.

Protocol

This study received ethical approval from the Research Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University (Reference: NAU/CHS/NC/FMBS/574, 8 August 2023). All procedures followed the National Research Council's Guide for the Care and Use of Laboratory Animals (8th edition, 2011) [16] and were reported according to ARRIVE guidelines. After acclimatization, the rats were weighed and randomly divided into 10 groups (A – J; n = 5). Group A served as the untreated control. The experimental design comprised three dosing regimens. First, for individual substance evaluation, *Odogwu Bitters* was administered daily to three groups at 0.2 mL (B), 0.4 mL (C), and 0.8 mL (D), while *Goko Cleanser* was given to three groups at 0.2 mL (E), 0.5 mL (F), and 0.9 mL (G). Second, to evaluate co-administration, three combination groups received both drinks daily at matched volumes: Group H (0.2 mL of each), Group I (0.4 mL *Odogwu Bitters* + 0.5 mL *Goko Cleanser*), and Group J (0.8 mL *Odogwu Bitters* + 0.9 mL *Goko Cleanser*). This sample size aligns with common practices in Wistar rat toxicology, ensuring statistical reliability while adhering to the 3Rs principles (refinement, reduction, replacement) [17]. To reflect common usage patterns, the combined doses were administered orally twice daily for six weeks via gavage, with *Odogwu Bitters* given in the morning (6:00 – 8:00 am) and *Goko Cleanser* administered in the evening (5:00 – 7:00 pm).

Euthanasia

Following the 6-week treatment, rats were humanely euthanized via inhalant carbon dioxide (CO₂) overdose in a specialized chamber. The gas flow rate was maintained at 10 – 30 % chamber volume displacement per minute to minimize distress. Animals were monitored until unconsciousness, and death was confirmed by the absence of respiration, cardiac activity, and corneal reflexes. Carcass disposal complied with institutional biosafety policy, and all steps adhered to strict ethical and welfare regulations [16].

Organ harvesting

Each rat was placed on a dissection board, and a midline abdominal incision was made. The

spleen was carefully excised, gently rinsed in phosphate-buffered saline (PBS) to remove residual blood, and subsequently fixed in 10 % neutral buffered formalin for histological preparation and examination. This organ harvesting and blood collection procedure adhered to established protocol for experimental rat models [16].

Blood collection and haematological analysis

Blood was collected via ocular puncture into sterile tubes containing an anticoagulant. Following collection, samples were processed for specific haematological parameters. Packed Cell Volume (PCV) was determined using the microhematocrit method and Haemoglobin (Hb) concentration was measured spectrophotometrically using the cyanmethemoglobin method. This procedure adhered to an established protocol for experimental rat models [7].

Histological analysis

Tissue specimens were processed for histological evaluation through standard stages of fixation, dehydration, clearing, and paraffin embedding. Sections were then cut and stained with haematoxylin and eosin (H&E). Imaging was performed using a Novex compound microscope equipped with an Amscope 14MP digital camera. The resulting microphotographs were annotated using Photoscape software (version 3.7). All histological procedures adhered to established protocols for rat tissue analysis [6].

Statistical analysis

Data were analysed using IBM SPSS Statistics software (version 25). Descriptive results were expressed as mean $\pm$ standard error of the mean (SEM). Group mean differences were assessed using one-way ANOVA, applied after verifying homogeneity of variance with Levene's test. For outcomes where the ANOVA indicated statistical significance ($p < 0.05$), *post hoc* tests were performed to identify specific differences between the control and treatment groups.

RESULTS

Histopathological examination of splenic tissue

Histological analysis of splenic sections from the control group (Group A) revealed normal cytoarchitecture, characterized by clearly defined white pulp (WP) and red pulp (RP) compartments. The white pulp consisted of

periarteriolar lymphoid sheaths (PALS) with dense aggregates of lymphocytes surrounding central arterioles. The red pulp displayed typical splenic sinuses and cords (of Billroth; Figure 1 A). Splenic morphology in treatment groups B, F, G, H, and I was comparable to the control, with preserved histological architecture and no evidence of pathological alteration (Figure 1 and Figure 2). In contrast, distinct histopathological changes were observed in groups C, D, E, and J. These sections exhibited features of nodular hyperplasia within the white pulp, characterized by focal, expansive aggregates of lymphocytes (Figure 1 C and Figure 2 b-d). Despite this hyperplastic change in the lymphoid compartment, the overall demarcation between white and red pulp was maintained, and the red pulp appeared structurally normal with intact sinuses and cords.

Haematological parameters

Packed cell volume (PCV) was reduced across all experimental groups (B - D, F - J). However, this decrease reached statistical significance only in group E ($p = 0.046$) compared to control group (A; Table 1). Haemoglobin concentration showed a non-significant decrease in groups B – F and J, while a non-significant increase was observed in groups G – I relative to controls.

Morphometric results

All groups, including the control, showed significant body weight gain from baseline to the end of the study ($p < 0.05$). This weight gain, observed in the *Odogwu Bitters*, *Goko Cleanser*, and combination groups, suggests normal growth and good tolerance of the formulations (Table 2).

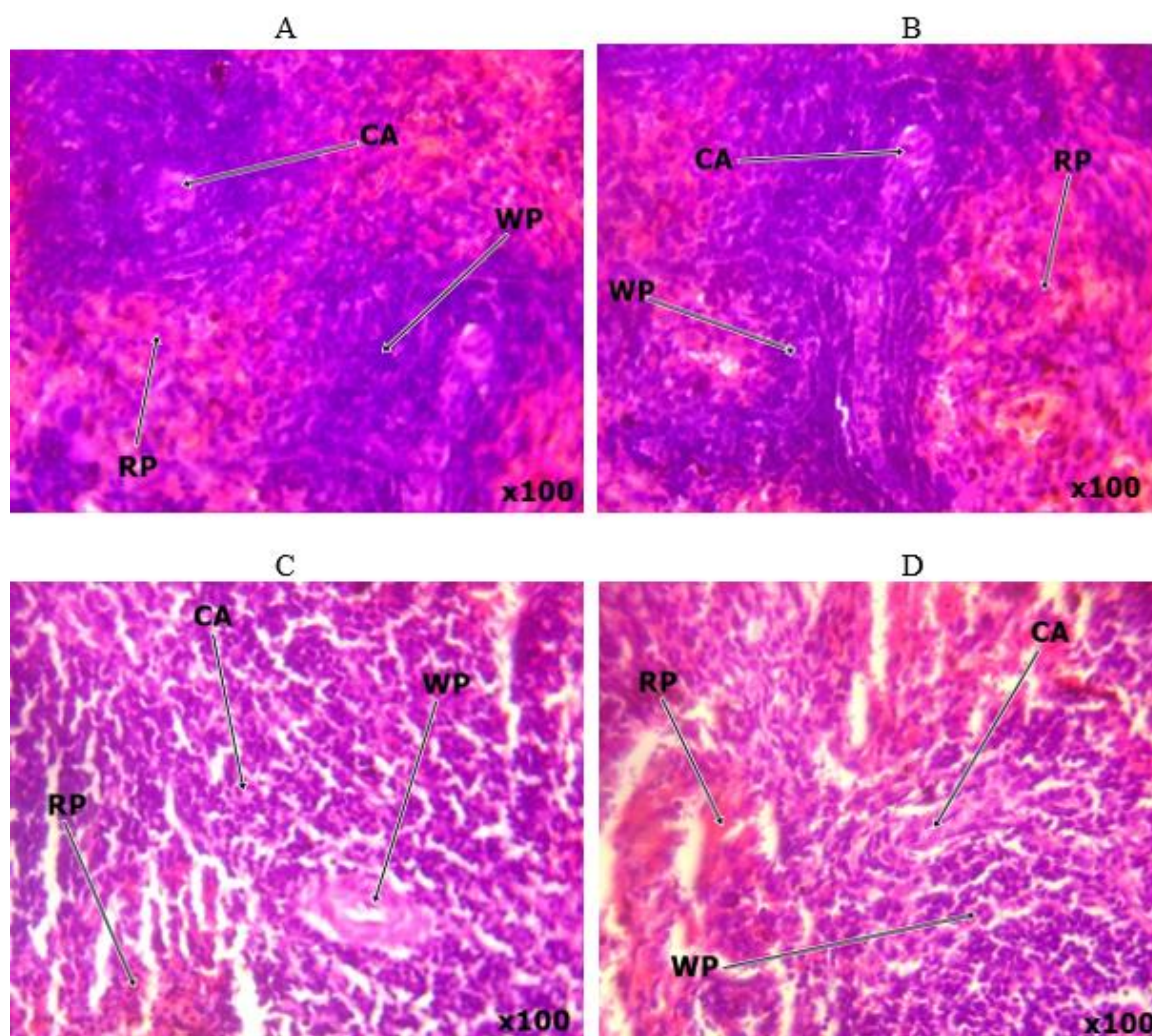


Figure 1: Photomicrograph of the spleen of a rat in (A) control group (Group A); (B) Group B (low dose OB); (C) Group C (moderate dose OB); (D) Group D (high dose OB): CA = Central arteriole, WP = White pulp, RP = Red pulp; 100x magnification; H&E stain.

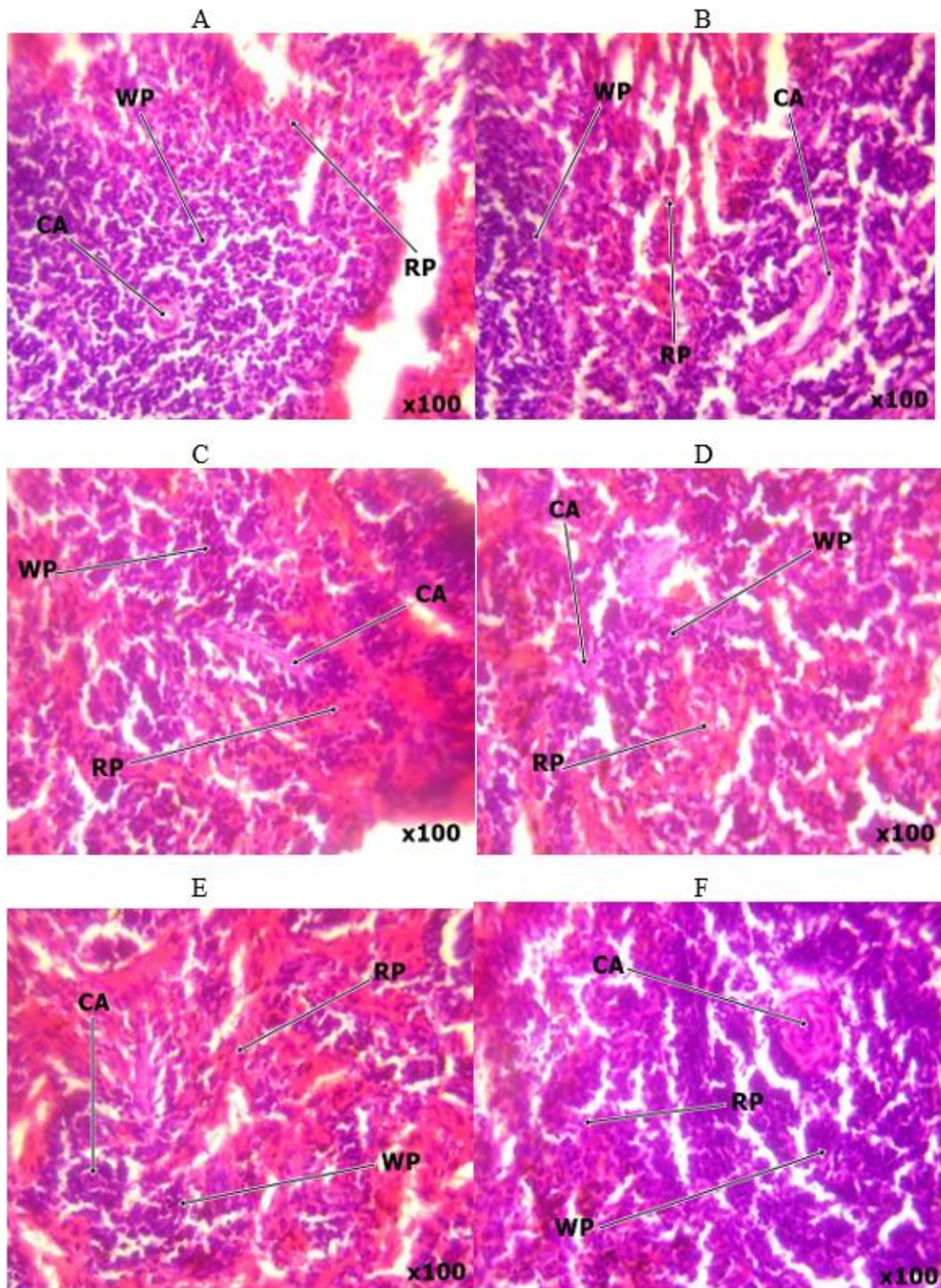


Figure 2: Photomicrograph of the spleen of a rat in (A) Group E (low dose GC); (B) Group F (moderate dose GC); (C) Group G (high dose GC); (D) Group H (low dose combination); (E) Group I (moderate dose combination); (F) Group J (high dose combination): CA = Central arteriole, WP = White pulp, RP = Red pulp; 100x magnification; H&E stain

Table 1: Effect of co-administration of *Odogwu Bitters* and *Goko cleanser* on Packed cell volume and Haemoglobin concentration

Group	Packed cell volume (%)	P-value	Haemoglobin (g/dL)	P-value
A (Control)	44.00±1.00		13.30±0.30	
B	43.50±0.50 [#]	0.698	11.75±0.25 [#]	0.058
C	39.50±2.50 [#]	0.236	11.70±0.30 [#]	0.064
D	41.00±0.00 [#]	0.095	12.00±0.00 [#]	0.059
E	34.00±2.00 [*]	0.046	11.35±0.65 [#]	0.112
F	38.50±2.50 [#]	0.178	12.85±0.85 [#]	0.667
G	42.00±2.00 [#]	0.465	13.75±0.45 [#]	0.493
H	40.50±1.50 [#]	0.192	13.55±0.35 [#]	0.642
I	43.50±0.50 [#]	0.698	13.45±0.15 [#]	0.076
J	40.00±1.00 [#]	0.106	12.40±0.60 [#]	0.312

Data are mean ± SEM; * $P \leq 0.05$ vs Control. significant, #: not significant

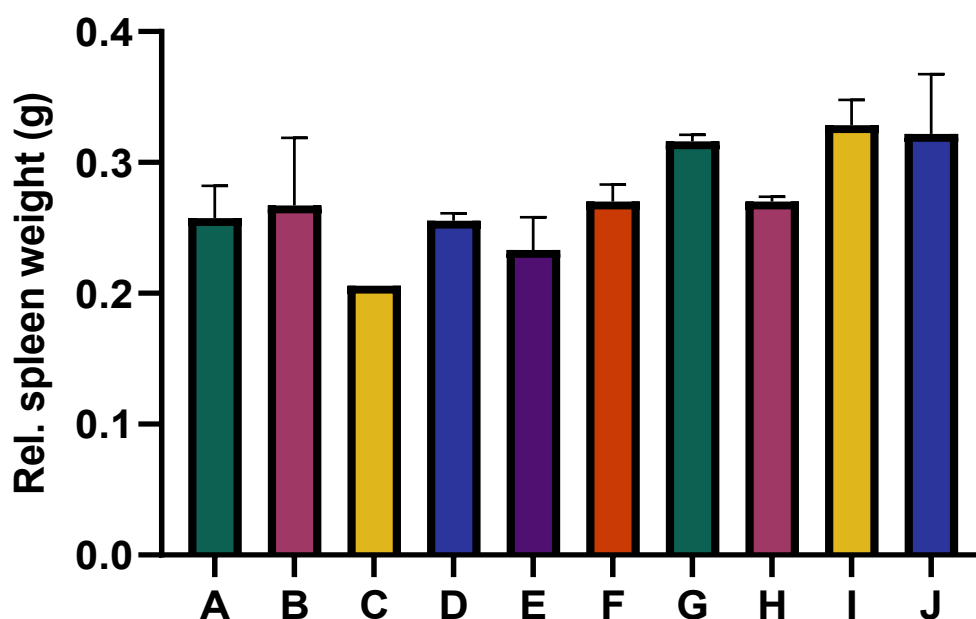
Compared to group A, the relative spleen weight decreased in groups B and E but increased in groups C, D, F, G, H, I, and J (Figure 3). As illustrated in Figure 3, relative spleen weight remained stable across all treatment groups, with

mean values narrowly ranging from 0.21 to 0.33 g. Statistical evaluation confirmed that these minor fluctuations were not significantly different from control ($p > 0.05$).

Table 2: Effect of co-administration of *Odogwu Bitters* and *Goko cleansers* on body weight

Group	Weight (g)		P-value
	Pre-Administration	Post-Administration	
A (Control)	133.5 ± 1.71	244.25 ± 5.75 [*]	0.001
B	139.5 ± 2.59	230.5 ± 9.51 [*]	0.004
C	133.75 ± 2.81	250.25 ± 4.31 [*]	0.000
D	148.75 ± 3.49	222.5 ± 19.08 [*]	0.045
E	152.25 ± 2.17	241.25 ± 10.45 [*]	0.004
F	121.5 ± 1.19	215.00 ± 8.49 [*]	0.002
G	126.5 ± 2.10	212.75 ± 6.18 [*]	0.002
H	113.5 ± 2.96	212.75 ± 5.67 [*]	0.001
I	152.5 ± 3.67	242.75 ± 6.02 [*]	0.002
J	158.75 ± 5.67	231.00 ± 5.73 [*]	0.002

Data are mean ± SEM, * $P \leq 0.05$ vs Pre-Administration weight

**Figure 3:** Chart showing the effect of co-administration of *Odogwu Bitters* and *Goko Cleanser* on relative spleen weight

DISCUSSION

This study provides the first comprehensive assessment of the splenic impact following individual and co-administration of two widely consumed herbal mixtures, *Odogwu Bitters* (OB) and *Goko Cleanser* (GC). The central finding is that a six-week exposure, while not systemically toxic as evidenced by preserved physiological weight gain, induced distinct, treatment-specific effects on splenic histoarchitecture and key haematological indices, revealing a targeted immunohaematological impact.

The most notable histopathological observation was the induction of nodular hyperplasia within the splenic white pulp in groups C, D, E, and J. This lesion, characterized by focal, expansive lymphocyte aggregates, indicates a clear state of lymphoid activation or proliferation [18]. Its absence in groups B, F, G, H, and I demonstrate that this is not a universal effect but rather a threshold- and combination-dependent immunostimulatory response. The phytochemical complexity of these mixtures, particularly their content of saponins and flavonoids, provides a plausible basis for this immunoproliferative effect, as such compounds are known to possess immunomodulatory potential [19]. This proliferative response, occurring without concomitant inflammation or red pulp damage, suggests an adaptive, antigen-driven lymphoid expansion rather than direct cytotoxicity, aligning with patterns where biochemical perturbations precede structural damage in other organs [12,15]. The haematological profile introduces a critical nuance to the safety assessment. A statistically significant reduction in Packed Cell Volume (PCV) was observed exclusively in Group E. This finding indicates that specific treatment conditions can meaningfully compromise erythrocyte mass. The non-significant but divergent trends in haemoglobin concentration across groups further underscore a complex and variable physiological impact on haematopoiesis. The mild increases observed in certain groups may tentatively reflect the purported haematopoietic properties of individual mixture components [7], effects which appear highly context-dependent and can be negated under different dosing or combination regimens [14,15].

A pivotal observation is the dissociation between microscopic pathology and gross morphometry. Despite clear evidence of lymphoid hyperplasia in four groups, there were no statistically significant alterations in relative spleen weight. This underscores a key toxicological principle: sensitive cellular alterations often precede measurable changes in organ mass [20].

Consequently, reliance on organosomatic indices alone would have failed to detect significant immunoproliferative activity, highlighting the indispensable role of histopathology for a complete toxicological evaluation [21]. The consistent body weight gain across all groups further confirms the absence of overt systemic toxicity, localising the observed effects to specific organ systems.

When contextualised within the established toxicological profile of these mixtures, our findings delineate an organ-specific vulnerability of the spleen. Previous research on OB and GC has primarily identified hepatic, renal, and reproductive endpoints [5,12,15]. This study established that the spleen, a primary lymphoid organ, responds with discernible histopathological changes under specific treatment conditions. This identifies a previously unrecognised target organ and suggests the immune compartment may be particularly sensitive to the phytochemical interactions of co-administered herbal products.

The mechanistic implications are rooted in the complex phytochemical synergy. Co-administration creates a milieu where compounds with potential antioxidant benefits [9] interact with others bearing possible toxicological risk [8]. The net effect observed in the spleen – lymphoid proliferation without overt destruction – suggests a dominant low-grade immunostimulatory signal. This state of chronic, low-level immunological activation, if sustained, could have implications for long-term immune homeostasis [22], a prospect requiring further investigation.

Limitations

The sub-acute duration of this study may not capture the consequences of long-term consumption, which is common among users. The assessment was also limited to histology and standard haematology; future work incorporating immunophenotyping of splenic lymphocyte subsets, cytokine profiling, and markers of oxidative stress would provide deeper mechanistic insight. Investigating the reversibility of these changes upon treatment cessation is also essential for a complete risk assessment.

CONCLUSION

Individual and combined administration of *Odogwu Bitters* and *Goko Cleanser* induces significant, condition-specific effects on the spleen and haematological system in Wistar rats. The findings of lymphoid hyperplasia and altered

PCV reveal a discrete immunohaematological impact that occurs in the absence of systemic toxicity or changes in organ weight. These results highlight a previously uncharacterised risk associated with these popular herbal mixtures and underscore the critical need for regulatory safety assessments to include detailed evaluation of lymphoid organs when profiling polyherbal formulations.

DECLARATIONS

Acknowledgement/Funding

None.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) 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 have read and approved the final manuscript. DNO conceptualized and designed the study, developed the experimental protocol, supervised the research, performed data and histopathological analysis, and drafted the initial manuscript. DNO also assisted NMU in literature curation. NMU contributed to data curation, conducted literature searches, and assisted in drafting the manuscript. TLS executed experiments, managed animal models, and supported data curation. AEA performed the haematological assessments and critically reviewed the manuscript.

Ethical approval

Ethical approval was obtained from the Research Ethics Committee of the Faculty of Basic Medical

Sciences, College of Health Sciences, Nnamdi Azikiwe University (Reference: NAU/CHS/NC/FMBS/574, 8 August 2023).

Availability of data and materials

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

REFERENCES

1. Onukansi FO, Anokwuru CC, Eneh SC, Odey GO, Onah DO, Sokunbi T, et al. Investing in traditional medicine: leveraging evidence and innovative research to strengthen the fight against malaria in Nigeria. *Malar J* 2025; 24(1): 232.
2. Dike DN, Ajala TO, Odeku OA, Afolabi JM, Oyagbemi AA, Oyeyemi MO. The pharmaceutical properties, microbial quality, in vivo aphrodisiac effect and safety of some herbal bitters sold in Southwest Nigeria. *Afr J Biomed Res* 2024; 23(2): 155-164.
3. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. *Front Pharmacol* 2023; 14: 1265178.
4. Ademokoya W. Benefits of Odogwu bitters herbal alcoholic drink. *Just U Magazine*. 2022 (Accessed 12 Dec 2025). Available from: <https://justumagazine.com/news/benefits-of-odogwu-bitters-herbal-alcoholic-drink/>
5. 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.
6. 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.
7. Onyejike DN, McWilliams WC, Mmaju CI, Okeke SM, Obiesie IJ, Eze CE. Hematological study on the effects of Goko Cleanser (Herbal mixture) on adult female Wistar rats. *Int Blood Res Rev* 2021; 12(1): 8–19.
8. Nunes DRDCMA, Monteiro CSJ, dos Santos JL. Herb-induced liver injury: A challenging diagnosis. *Healthcare (Basel)* 2022; 10(2): 278.
9. Kim M, Jee SC, Sung JS. Hepatoprotective effects of flavonoids against benzo(a)pyrene-induced oxidative liver damage along its metabolic pathways. *Antioxidants (Basel)* 2024; 13(2): 180.
10. Lewis SM, Williams A, Eisenbarth SC. Structure and function of the immune system in the spleen. *Sci Immunol* 2019; 4(33): eaau6085.
11. Elmore SA. Enhanced histopathology of the spleen. *Toxicol Pathol* 2006; 34(5): 648-655.
12. Obiesie IJ, Onyejike DN, Onyejike IM, Okechukwu SK, Okubike EA. Toxicological evaluation of co-administration of Odogwu Bitters and Goko Cleanser *Trop J Pharm Res*, September 2026; 25(9): 1244

- herbal drinks on the kidney of adult male Wistar rats. *Trop J Nat Prod Res* 2025; 9(3): 1256-1262.
13. Nduka-Arinze AI, Onyejike DN, Onwumere SH, Nweke EO. Biochemical assessment of hepatic function following co-administration of Odogwu Bitters and Goko Cleanser in male Wistar rats: a preliminary study. *J Med Health Res* 2025; 10(2): 122-131.
14. Okwuonu IF, Onyejike DN, Nduka-Arinze AI, Chukwukaeme CW, Afubero CF. Cardiac histopathological, morphometric, and inflammatory responses to co-administration of Odogwu Bitters and Goko Cleanser in Wistar rats. *Trop J Pharm Res* 2025; 24(12): 1517-1524.
15. Onyejike DN, Onyejike IM, Ejiofor DE, Izuegbunam CJ, Obiesie IJ, Nwamaradi AT, et al. Reproductive effects of Odogwu Bitters and Goko Cleanser on testicular histology, sperm parameters, and hormonal profiles in adult male Wistar rats. *Trop J Pharm Res* 2025; 24(11): 1371-1381.
16. National Research Council. *Guide for the Care and Use of Laboratory Animals*. 8th ed. Washington, DC: The National Academies Press; 2011
17. Hubrecht RC, Carter E. The 3Rs and humane experimental technique: implementing change. *Animals (Basel)* 2019; 9(10): 754.
18. Willard-Mack CL, Elmore SA, Hall WC, Harleman J, Kuper CF, Losco P, et al. Nonproliferative and proliferative lesions of the rat and mouse hematolymphoid system. *Toxicol Pathol* 2019; 47(6): 665-783.
19. Olarewaju OO, Fajinmi OO, Naidoo KK, Arthur GD, Cooposamy RM. A review of the medicinal plants with immune-boosting potential. *J Med Plants Econ Dev* 2022; 6(1): a158.
20. Borgert CJ, Fuentes C, Burgoon LD. Principles of dose-setting in toxicology studies: the importance of kinetics for ensuring human safety. *Arch Toxicol* 2021; 95(12): 3651-3664.
21. Dettmeyer RB. The role of histopathology in forensic practice: an overview. *Forensic Sci Med Pathol* 2014; 10(3): 401-412.
22. Bronte V, Pittet MJ. The spleen in local and systemic regulation of immunity. *Immunity* 2013; 39(5): 806-818.