

Original Research Article

Cardiac histopathological, morphometric, and inflammatory responses to co-administration of Odogwu Bitters and Goko Cleanser in Wistar rats

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Abstract

Purpose: To investigate the histopathological, morphometric and biochemical alterations in the hearts of adult male Wistar rats following the combined administration of Odogwu Bitters and Goko Cleanser.

Methods: The design involved fifty adult male Wistar rats, randomly distributed into ten groups of five animals each. The control group (A) received no treatment, while the nine treatment groups (B–J) were orally dosed with varying concentrations of Odogwu Bitters, Goko Cleanser, or combined formulations over six weeks. Following the study duration, the body and organ weights were measured. Subsequently, serum levels of IL-6 were analysed by ELISA, and heart tissue sections were prepared for histological assessment via haematoxylin and eosin staining.

Results: Histological assessment revealed a reduction in myocyte density in the Odogwu Bitters and co-administration groups. In contrast, high-dose Goko Cleanser group exhibited an apparent increase in density compared to the control. Myocardial architecture remained intact across all groups, with compact bundles and preserved vasculature. All animals exhibited weight gain over the treatment period. However, the final body weights in the high-dose Odogwu Bitters and co-administration groups were significantly lower than those of control. In contrast, relative heart weights showed no significant differences across any of the groups. Serum IL-6 levels increased significantly ($p < 0.05$) in all treated groups. The most pronounced elevation, exceeding an eightfold increase over controls, was observed in the co-administration groups, particularly at the low-dose combination.

Conclusion: In adult male Wistar rats, sub-chronic exposure to Odogwu Bitters and Goko Cleanser induces marked systemic inflammatory state, as demonstrated by a significant surge in IL-6, without causing overt histological injury to the myocardium. These findings advise caution regarding the concurrent use of such polyherbal formulations and emphasize the necessity for long-term mechanistic studies to definitively clarify their cardiovascular risks.

Keywords: Biochemical markers, Herbal bitters, Organ toxicity, Rat model, Systemic inflammation, Toxicological assessment

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INTRODUCTION

In many developing nations like Nigeria, herbal medicines constitute a major part of the healthcare system, frequently serving as a primary treatment or a complement to conventional medicine [1]. This widespread use is driven by their accessibility, affordability, and deep-rooted cultural acceptance [2]. Despite their popularity, significant concerns regarding systemic safety persist due to a lack of product standardization, undefined dosages, and insufficient toxicological profiling, especially with long-term or combined use.

In Nigeria, herbal formulations such as *Odogwu Bitters* and *Goko Cleanser* are widely marketed with claims of detoxification, aphrodisiac effects, and the treatment of various ailments [3]. Consumption of these products cuts across age and socioeconomic groups, amplified by aggressive marketing strategies [4]. Previous phytochemical screening has confirmed that these products contain alkaloids, flavonoids, tannins, terpenoids, and saponins [3]. Some of these compounds such as flavonoids, tannins and terpenoids, are associated with antioxidant effects [5], while others, such as alkaloids, terpenoids and saponins, are associated with anti-inflammatory effects [6,7]. Alkaloids and terpenoids are strongly linked to hepatotoxicity and nephrotoxicity [8], while flavonoids and tannins have been implicated as endocrine disruptors (mainly via estrogenic/anti-nutritional effects) [9]. Saponins have been implicated in reproductive dysfunction and endocrine disruption [10]. Recent animal studies have reported alterations in reproductive hormones and sperm parameters following subchronic exposure, thereby challenging the safety profile of these herbal mixtures [11].

Beyond the reproductive and renal systems, herbal formulations such as bitters and cleansers may have direct or indirect impacts on cardiovascular function, as many of their bioactive constituents can influence vascular tone, blood pressure regulation, and cardiac contractility [12]. For example, alkaloids are known to interfere with cardiac ion channel function, potentially leading to arrhythmias [12], while the scientific literature, as reviewed in [13], indicates that saponins exhibit dose-dependent toxicity, with higher intake levels linked to membrane disruption and oxidative myocardial injury [14]. Given the role of the heart in systemic circulation and oxygen delivery, even subtle histological alterations may lead to significant functional compromise over time.

This concern is particularly relevant in Nigeria and across West Africa, where cardiovascular disease (CVD) now accounts for a substantial and growing share of the region's morbidity and mortality [14]. The rising incidence of hypertension, cardiomyopathies, and heart failure is driven by a combination of lifestyle factors and unregulated self-medication practices [14]. The widespread use of herbal mixtures such as *Odogwu Bitters* and *Goko Cleanser* – consumed without medical supervision and representing a potential, yet unquantified, risk factor – may contribute to this rising burden. However, direct experimental evidence of their impact on cardiac structure and function is lacking, representing a critical knowledge gap with significant public health implications.

Evaluating histopathological changes in the myocardium of rats exposed to *Odogwu Bitters* and *Goko Cleanser*, whether singly or in combination, will provide critical insights into the cardiovascular safety of these mixtures. Such findings are essential for informing regulatory policies, guiding public health awareness, and advancing scientific understanding of the risks associated with unregulated herbal consumption. Therefore, this study investigated the histopathological, morphometric and biochemical effects in the cardiac tissue of adult male Wistar rats following exposure to *Odogwu Bitters* and *Goko Cleanser*, administered both individually and in combination.

EXPERIMENTAL

Animals

This study utilized fifty healthy adult male Wistar rats with an initial weight range of 114 – 159 g. These rats were procured from a commercial animal supplier in Nsukka, Enugu State, Nigeria. Upon delivery to the animal house, a two-week acclimatization period to the new housing conditions was provided, during which the animals had *ad libitum* access to standard feed and water. A licensed veterinarian confirmed the animals were healthy upon arrival at the research facility.

Housing conditions consisted of well-ventilated stainless-steel cages kept at ambient temperature with a 12-h light / dark cycle. The animals were provided a standard laboratory diet, fresh drinking water, and clean bedding, which was replaced daily. Routine health checks, including monitoring of physical activity, fur condition, and food/water intake, were conducted daily throughout the experimental period.

Ethical approval and treatment protocol

All procedures, conducted over 12 weeks, were approved by the Faculty of Basic Medical Sciences Ethics Committee, Chukwuemeka Odumegwu Ojukwu University (Approval No: COOU/BMS/006) and were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (8th edition, 2011) [15].

Following the acclimatization period, a total of fifty rats were randomly allocated to ten groups (A – J), with five animals per group. The herbal formulations were administered orally twice daily to mimic local consumption patterns: *Odogwu Bitters* in the morning (6 – 8 am) and *Goko Cleanser* in the evening (5 – 7 pm) for 42 days. A syringe fitted with a cannula was used to ensure precise oral gavage. Group A served as the control and received only feed and water. The treatment groups received the following daily doses: Groups B, C, and D were administered 0.2, 0.4 and 0.8 mL of *Odogwu Bitters*, respectively. Groups E, F, and G received 0.2, 0.5 and 0.9 mL of *Goko Cleanser*, respectively. Groups H – J received combinations: H (0.2 mL of each), I (0.4 mL *Odogwu Bitters* + 0.5 mL *Goko Cleanser*), and J (0.8 mL *Odogwu Bitters* + 0.9 mL *Goko Cleanser*).

Acute toxicity test

Acute oral toxicity was determined using OECD Guideline 425 (Up-and-Down Procedure) [16]. This method applies stepwise dose adjustment to estimate LD₅₀ values while limiting animal use.

Odogwu Bitters

In Phase I, nine rats were grouped (n = 3 per group) and given 10, 100, and 1000 mg/kg. No toxicity or deaths were observed within 24 hours. In Phase II, four rats received 1200, 1600, 2900, and 5000 mg/kg. Mortality occurred at 2900 mg/kg (within 24 h) and 5000 mg/kg (within 12 h). The calculated LD₅₀ was 2154.17 mg/kg.

Goko Cleanser

Similarly, doses of 10, 100, and 1000 mg/kg produced no adverse effects. At higher doses, no deaths occurred at 1200 and 2600 mg/kg, but mortality was recorded at 3900 mg/kg (within 48 h) and 5000 mg/kg (within 24 h). The LD₅₀, calculated as per the guideline's statistical protocol, was 3184.34 mg/kg.

The calculated LD₅₀ values classify both formulations as substances with moderate acute

toxicity. For the subsequent 42-day sub-chronic study, the dosing regimen was designed to reflect typical human consumption patterns, using volume-based administration (mL). The selected low, medium, and high-volume levels were chosen to represent fractions of the established LD₅₀, ensuring the investigation of effects at sub-toxic exposure levels. The administered doses for the sub-chronic study were as follows:

Odogwu Bitters (LD₅₀ = 2154 mg/kg): 0.2 mL (Low dose), 0.4 mL (Medium dose), 0.8 mL (High dose).

Goko Cleanser (LD₅₀ = 3184 mg/kg): 0.2 mL (Low dose), 0.5 mL (Medium dose), 0.9 mL (High dose).

Animal euthanasia

Upon completion of the 42-day experimental protocol, euthanasia was conducted via CO₂ inhalation in accordance with institutional animal welfare guidelines. The gas flow rate was maintained at a rate displacing 10 – 30 % of the chamber volume per minute to minimize suffering. Death was confirmed by the absence of a corneal reflex following the cessation of respiratory and cardiac activity. All animal remains were subsequently incinerated as per institutional biosafety protocols.

Blood and organ collection

Blood was collected via ocular puncture into sterile tubes, allowed to clot, and centrifuged at 2500×g for 10 min using a Hermle Labnet Z216 MK microcentrifuge to obtain serum for biochemical assays. Each rat was placed on a dissection board and a thoracic incision was made to access the organ. The heart was excised, rinsed in phosphate-buffered saline, and fixed in 10 % formalin for histological processing.

Histological analysis

Heart tissue samples were processed for standard light microscopy. Specifically, the samples were fixed in 10 % formalin, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a microtome and stained with haematoxylin and eosin (H&E). Microphotographs were taken using a Novex compound microscope fitted with an Amscope 14MP digital camera. Images were annotated with Photoscape software. All histological procedures were performed

according to established standards for rat studies [15].

Measurement of rat serum interleukin-6 (IL-6)

Serum concentrations of interleukin-6 (IL-6) were quantified using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions [17]. Specifically, high-binding 96-well plates, pre-coated with an anti-rat IL-6 capture antibody, were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) to minimize non-specific binding. Recombinant rat IL-6 standards (1 – 2,000 pg/mL), internal quality controls, and diluted serum samples were loaded in duplicate and incubated for 2 h at room temperature. After washing with PBS containing 0.05 % Tween-20, all wells were incubated sequentially with a biotinylated detection antibody and streptavidin–horseradish peroxidase (HRP). Bound complexes were visualized with tetramethylbenzidine (TMB) substrate, and the reaction was terminated with 1 M sulfuric acid. Absorbance was measured at 450 nm with a 570-nm reference wavelength using a spectrophotometric microplate reader.

The analyte concentrations were interpolated from a four-parameter logistic regression of the standard curve (GraphPad Prism vX, GraphPad Software, San Diego, CA, USA). The lower limit of detection (LOD) was defined as the mean blank optical density plus three standard deviations, corresponding to X pg/mL. Intra-assay and inter-assay coefficients of variation, determined from quality control sera run across three independent assays, were $\leq X$ % and $\leq Y$ %, respectively. Recovery experiments, obtained by spiking rat serum with known concentrations of recombinant IL-6, yielded 80 – 115 % recovery across the dynamic range. Serum samples exceeding the highest calibrator were re-assayed at appropriate dilutions, and samples below the limit of detection (LOD) were reported as “< LOD.”

Statistical analysis

All statistical analyses were performed with IBM SPSS Statistics software, Version 25. Continuous data are presented as the mean $\pm$ standard error of the mean (SEM). Inter-group comparisons for all parameters were conducted using one-way analysis of variance (ANOVA). The assumption of homogeneity of variance was verified with Levene's test. For ANOVA results reaching statistical significance, post hoc analyses were applied to identify specific group

differences. Changes in body weight between the start and end of the experiment were evaluated using a paired Student's t-test. A p -value of less than 0.05 was considered statistically significant for all tests.

RESULTS

Histological analysis results

Histological evaluation of cardiac tissue from the control group (Figure 1 A) revealed compact myocardial muscle bundles supported by a thin fibrocollagenous stroma. The myocyte density averaged about 220 per high-power field (HPF), and the cells appeared mildly plump. Blood vessels were thin-walled, featuring narrow lumina lined by intact endothelial cells.

In the groups administered *Odogwu Bitters*, myocyte density was moderately reduced compared to controls, averaging 195 per HPF at the low dose (Figure 1 B), 200 per HPF with the medium dose (Figure 1 C), and 205 per HPF following high-dose administration (Figure 1 D). Despite these reductions, the myocardial bundles remained compact, and the vascular architecture was preserved.

Cardiac sections from the *Goko Cleanser*-treated groups had densities of 215 per HPF in the low-dose cohort (Figure 1 E), 200 per HPF in animals given the medium dose (Figure 1 F), and a relatively higher value of 240 per HPF in the high-dose group (Figure 1 G). The myocytes remained mildly plump, with an intact fibrocollagenous stroma and narrow vascular lumina.

In the co-administration groups, the lowest density was recorded at 195 per HPF in the low dose combination (Figure 1 H). The medium-dose combination showed an average density of 210 per HPF (Figure 1 I), while the high-dose combination was like the low-dose, at 195 per HPF (Figure 1 J). Myocardial bundles remained compact across all groups, and no overt pathological alterations such as necrosis, fibrosis, or inflammatory infiltration were observed. Overall, myocyte densities ranged between 195 and 240 per HPF across all groups. Slight reductions were more apparent in the *Odogwu Bitters* and combination groups, while the highest density was observed in the high-dose *Goko Cleanser* group. Despite these mild variations, the general myocardial architecture remained preserved.

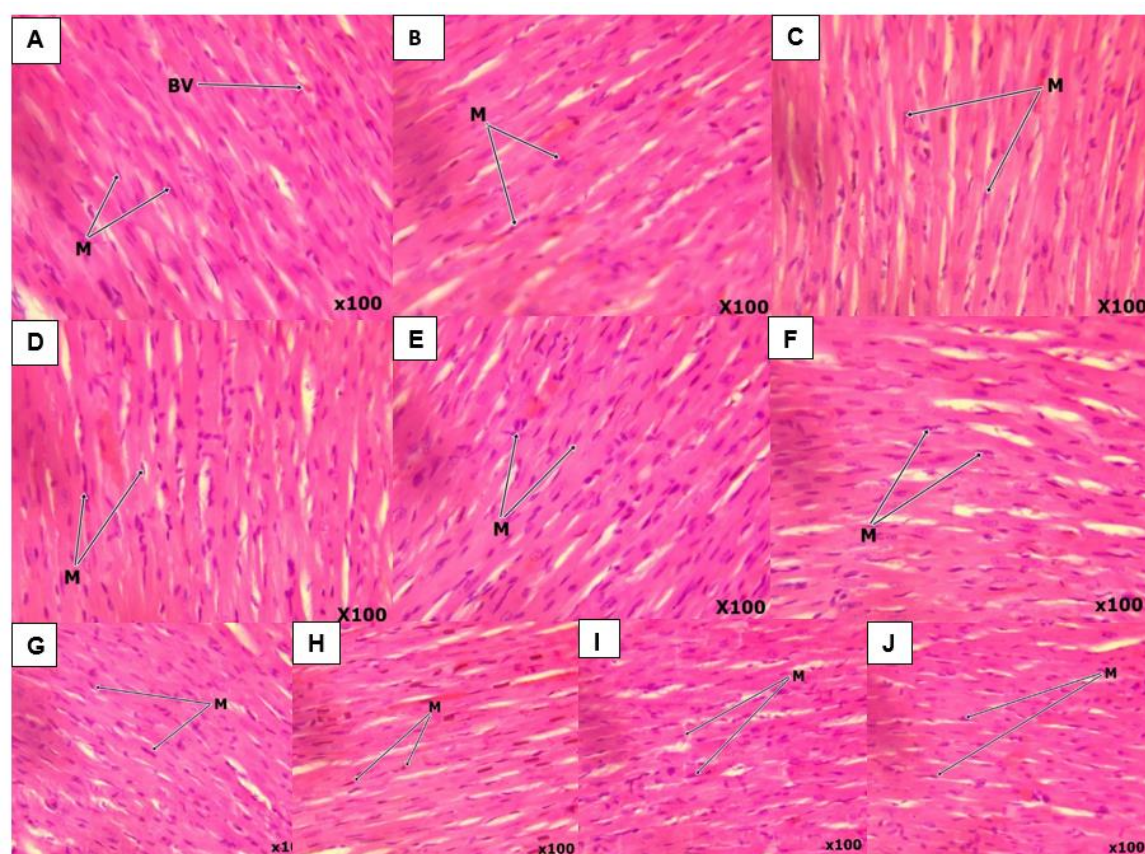


Figure 1: (A – J) Representative photomicrographs of the heart of rats in Groups A – J; M = Cardiac muscle cells (myocytes), BV = Blood vessel; 400x magnification; H&E stain

Table 1: Initial and final body weights of the rats across all groups

Group	Initial body weight (g)	Final body weight (g)	P-value	t-value
A	133.50±3.41	244.25±11.50	0.001 [#]	-14.903
B	139.50±5.19	230.50±19.02	0.005 [#]	-7.601
C	133.75±5.62	250.25±8.61	0.000 [#]	-40.157
D	148.75±6.99	222.50±38.18	0.045 [#]	-3.311
E	152.25±4.34	241.25±20.90	0.004 [#]	-8.091
F	121.50±2.38	215.00±16.99	0.002 [#]	-9.815
G	126.50±4.23	212.00±12.03	0.002 [#]	-10.722
H	113.50±5.92	212.75±11.32	0.001 [#]	-11.760
I	152.50±7.32	242.75±12.04	0.002 [#]	-11.247
J	158.75±11.32	231.00±11.46	0.002 [#]	-10.665

[#] $p < 0.05$ vs initial weight, values are mean $\pm$ standard deviation

Morphometric results

All groups had significant increases in body weight between baseline and the end of the study ($p < 0.05$). Weight gain was evident in the control as well as in the *Odogwu Bitters*, *Goko Cleanser*, and combination groups, indicating normal growth and tolerance of the formulations (Table 1).

Among the *Odogwu Bitters*-treated animals, the medium dose group showed the greatest increase, while the high dose group had the smallest weight gain compared to the control

(Table 1). In the *Goko Cleanser* groups, both the low and high doses produced relatively higher gains, whereas the medium dose group showed more modest changes. For the combination groups, weight increases were consistent across all doses, with the medium dose combination producing the highest final body weight. Overall, while the pattern of weight gain varied slightly among treatment regimens, all groups followed the same trend of significant increase in initial body weight (Table 1).

At the end of the experiment, a decrease in body weight was observed in rats across most groups,

except in Group C (Table 2). However, these changes were only significant in Groups F, G, and H, when compared with controls ($p < 0.05$); changes in other groups were not statistically significant (Table 2).

Table 2: Group comparisons of final body weight and relative heart weight

Group	Body weight (g)	Relative heart weight (g)
A	244.25±11.50	0.0036±0.0001
B	230.50±19.02 ^a	0.0033±0.0002 ^a
C	250.25±8.61 ^a	0.0047±0.0009 ^a
D	222.50±38.18 ^a	0.0042±0.0012 ^a
E	241.25±20.90 ^a	0.0037±0.0006 ^a
F	215.00±16.99 [#]	0.0038±0.0001 ^a
G	212.00±12.03 [#]	0.0038±0.0004 ^a
H	212.75±11.32 [#]	0.0036±0.0009 ^a
I	242.75±12.04 ^a	0.0031±0.0001 ^a
J	231.00±11.46 ^a	0.0035±0.0003 ^a
F-ratio	2.433	0.7630

[#] $p < 0.05$, ^a $p > 0.05$ vs values of Group A. values are mean ± standard deviation

Relative heart weight was generally consistent across all groups, with only slight differences observed. Statistical analysis indicated that these variations were not significant ($p > 0.05$), suggesting that the treatments had little impact on the proportional weight of the heart, even in groups where overall body weight was affected (Table 2).

Result of biochemical assay

Serum IL-6 levels varied significantly among the treatment groups (Table 3). Compared with the control, all groups exposed to *Odogwu Bitters* or *Goko Cleanser* showed marked increases, with concentrations rising progressively in medium- and high-dose groups. The highest elevations were observed in the combination groups, particularly at low-dose co-administration (8.13 ± 0.19), where IL-6 levels reached more than eightfold above control values (1.06 ± 0.04).

Table 3: Serum Interleukin-6 (IL-6) concentrations across groups

Group	Mean ± SD	P-value	F-ratio
A	1.06±0.04		132.91
B	1.83±0.23	0.02*	
C	5.97±0.56	0.01*	
D	4.18±0.25	0.01*	
E	3.25±0.36	0.01*	
F	4.58±0.32	0.01*	
G	6.97±0.05	0.01*	
H	8.13±0.19	0.01*	
I	3.05±0.03	0.01*	
J	5.15±0.22	0.01*	

* $p < 0.05$ vs values in Group A

A one-way ANOVA revealed a significant treatment effect ($F = 132.91$, $p < 0.001$). Collectively, these results indicate that both herbal preparations, administered singly or in combination, promoted systemic IL-6 elevation in a dose-responsive manner, with combinations amplifying the effect (Table 3).

DISCUSSION

The study was designed to investigate the histopathological effects in the cardiac tissue of adult male Wistar rats following exposure to *Odogwu Bitters* and *Goko Cleanser*, both individually and in combination. Contrary to expectations that herbal formulations of this nature might precipitate overt myocardial damage, histological analysis revealed generally preserved myocardial architecture across all treatment groups. Compact myocyte bundles, intact vascular lumina, and absence of necrosis, fibrosis, or inflammatory infiltration were consistent findings, suggesting that sub-chronic exposure did not induce structural cardiotoxicity. Nevertheless, subtle variations in myocyte density, particularly the modest reductions observed in the *Odogwu Bitters* and co-administration groups, may reflect early cellular responses to phytochemical stress. In contrast, the higher myocyte density in the high-dose *Goko Cleanser* group points to the possibility that different bioactive constituents elicit divergent cardiac adaptations. These results align with previous studies indicating that herbal bitters have the potential to produce heterogeneous tissue responses depending on their phytochemical composition [18,19].

Morphometric outcomes further reinforce the complexity of these effects. All groups exhibited significant body weight gain during the study, reflecting normal growth and tolerance of the interventions. However, end-point comparisons revealed relative weight reductions in several groups, particularly in high-dose *Odogwu Bitters* and low- to high-dose co-administration groups, while medium-dose treatment tended to promote increases in final body weight. These findings suggest that the formulations may influence energy metabolism and nutrient assimilation in a dose-dependent but non-linear manner. A study has reported that alkaloids and saponins, common in herbal bitters, may interfere with nutrient absorption and metabolic regulation [18], thereby contributing to variable growth outcomes. Importantly, the consistency in relative heart weight across all groups suggests that these effects did not extend to gross myocardial hypertrophy or atrophy within the study period.

A central finding of this study was the marked elevation of serum interleukin-6 (IL-6) observed across all treatment groups. This pleiotropic cytokine is a key mediator of inflammation, cardiac remodelling, and immune-metabolic regulation [20]. The significant rise in IL-6, even in the absence of histological lesions, underscores the sensitivity of systemic biochemical responses to herbal exposure. The magnitude of this increase, particularly in the co-administration groups, suggests potential synergistic interactions between *Odogwu Bitters* and *Goko Cleanser*. This is consistent with the concept that the combined use of phytochemicals may amplify inflammatory signalling beyond the effects of individual constituents [21]. Elevated levels of this cytokine have been implicated in the pathogenesis of cardiovascular disease, with prospective studies linking high circulating levels to increased risk of myocardial infarction and heart failure [22]. Thus, the pronounced IL-6 elevation observed here raises concerns that long-term consumption of these products, especially in combination, may predispose to subclinical inflammatory stress with eventual cardiovascular implications. Taken together, the findings of this study suggest a potential discordance between the structural and biochemical responses of the myocardium following exposure to herbal bitters. While histological integrity was preserved, the robust IL-6 response indicates early systemic perturbation that may precede overt myocardial injury. This pattern aligns with earlier experimental evidence showing that cytokine alterations often occur before detectable tissue damage [21]. By investigating the combined administration of *Odogwu Bitters* and *Goko Cleanser*, this study provides novel insights into the potential interactive effects of commonly consumed herbal preparations in Nigeria and West Africa, where such products are often ingested together. The results highlight the need for cautious interpretation of herbal safety based solely on structural assessments, as inflammatory biomarkers, including IL-6, may reveal early risk not evident histologically.

Limitations

This study was conducted over six weeks, representing a sub-chronic model in rats. Longer exposures are needed to clarify chronic effects. While histological, morphometric, and IL-6 analyses provided valuable insights, additional mechanistic biomarkers would have strengthened the findings. Finally, the lack of pharmacokinetic dose–response modelling constrains extrapolation to human exposure.

CONCLUSION

This study showed that sub-chronic exposure to *Odogwu Bitters* and *Goko Cleanser*, singly or in combination, preserved myocardial structure but significantly elevated serum IL-6, indicating systemic inflammatory activation. While no overt histopathological injury was observed, the biochemical findings suggest early perturbations that could predispose to cardiovascular risk with prolonged use. These results underscore the need for caution in the concurrent consumption of polyherbal formulations and highlight the importance of long-term mechanistic studies to clarify their safety profile. Concurrent use of *Odogwu Bitters* and *Goko Cleanser* should be approached with caution, given their pro-inflammatory effects. Regulatory agencies should strengthen oversight of polyherbal formulations, while future studies should extend to chronic exposure and mechanistic pathways. Incorporating inflammatory biomarkers in safety assessments is strongly advised.

DECLARATIONS

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Ethical approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences Ethics Committee, Chukwuemeka Odumegwu Ojukwu University, Uli campus (approval no. COOU/BMS/006).

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

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

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 the final manuscript. IF Okwuonu curated the data, reviewed the manuscript, and assisted DN Onyejike in sourcing funding. DN Onyejike conceptualized the study, designed the study, wrote the experimental protocol, sourced the funding of the project, supervised the experiment, carried out the histology analysis, and wrote the first draft of the manuscript; and assisted CF Afuberoh to manage the literature searches and assisted Al Nduka-Arinze and CW Chukwukaeme to carry out the experiment and manage the animals.

REFERENCES

1. Eruaga MA, Itua EO, Bature JT. Exploring herbal medicine regulation in Nigeria: Balancing traditional practices with modern standards. *GSC Adv Res Rev* 2024; 18 (03): 083–090.
2. Mutombo PN, Kasilo OMJ, James PB, Wardle J, Kunle O, Katerere D, Wambebe C, Matsabisa MG, Rahmatullah M, Nikiema JB, et al. Experiences and challenges of African traditional medicine: lessons from COVID-19 pandemic. *BMJ Glob Health* 2023; 8(8): e010813. doi: 10.1136/bmjgh-2022-010813.
3. 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.
4. Obiesie IJ, Onyejike DN, Onyejike IM, Okechukwu SK, Okubike EA. Toxicological evaluation of co-administration of Odogwu Bitters and Goko Cleanser herbal drinks on the kidney of adult male Wistar rats. *Trop J Nat Prod Res* 2025; 9(3): 1256–1262.
5. Jing W, Xiaolan C, Yu C, Feng Q, Haifeng Y. Pharmacological effects and mechanisms of tannic acid. *Biomed Pharmacother* 2022; 154: 113561.
6. Gitanjali J, Dinesh Ram DS, R K, Amalan V, Alahmadi TA, Alharbi SA, Kandasamy S, Shanmuganthan R, Vijayakumar N. Antimicrobial, antioxidant, anticancer, and antithrombotic competency of saponins from the root of *Decalepis hamiltonii*. *Environ Res* 2023; 231(Pt 1): 116096. doi: 10.1016/j.envres.2023.116096.
7. Liu F, O'Donnell TJ, Park EJ, Kovacs S, Nakamura K, Dave A, Luo Y, Sun R, Wall M, Wongwiwatthanakut S, et al. Anti-inflammatory quinoline alkaloids from the roots of *Waltheria indica*. *J Nat Prod* 2023; 86(2): 276–289. doi: 10.1021/acs.jnatprod.2c00861.
8. Wang Z, Han H, Wang C, Zheng Q, Chen H, Zhang X, Hou R. Hepatotoxicity of pyrrolizidine alkaloid compound intermedine: comparison with other pyrrolizidine alkaloids and its toxicological mechanism. *Toxins (Basel)* 2021; 13(12): 849. doi: 10.3390/toxins13120849.
9. Rizzo G, Feraco A, Storz MA, Lombardo M. The role of soy and soy isoflavones on women's fertility and related outcomes: an update. *J Nutr Sci* 2022; 11: e17.
10. Shu Y, Cao M, Yin ZQ, Li P, Li TQ, Long XF, Zhu LF, Jia RY, Dai SJ, Zhao J. The reproductive toxicity of saponins isolated from *Cortex Albiziae* in female mice. *Chin J Nat Med* 2015; 13(2): 119–126. doi: 10.1016/S1875-5364(15)60015-2.
11. Onyejike DN, Onyejike IM, Ejiofor DE, Izuogunam CJ, Obiesie IJ, Nwamaradi AT, Agulanna AE. 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. <https://dx.doi.org/10.4314/tjpr.v24i11.5>
12. Efimova SS, Zakharova AA, Ostroumova OS. Alkaloids modulate the functioning of ion channels produced by antimicrobial agents via an influence on the lipid host. *Front Cell Dev Biol* 2020; 8: 537.
13. Singh D, Chaudhuri PK. Structural characteristics, bioavailability and cardioprotective potential of saponins. *Integr Med Res* 2018; 7(1): 33–43.
14. Ogah OS, Orimolade OA, Jinadu TO. Cardiovascular diseases in Nigeria: Current status, threats, and opportunities. *Circulation* 2023; 148(19): 1441–1444.
15. National Research Council. Guide for the care and use of laboratory animals. 8th ed. Washington, DC: The National Academies Press; 2011
16. 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.
17. Abcam. Testosterone and FSH ELISA kit protocol booklets. Cambridge (UK): Abcam plc; 2022. Available from: <https://www.abcam.com>
18. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol* 2014; 4: 177.
19. Zhu YC, Liang B, Gu N. Cellular and molecular mechanism of traditional Chinese medicine on ventricular remodeling. *Front Cardiovasc Med* 2021; 8: 753095.
20. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol* 2015; 16 (5): 448–457
21. Riehle C, Bauersachs J. Key inflammatory mechanisms underlying heart failure. *Herz* 2019; 44 (2): 96–106.
22. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000; 101 (15): 1767–1772.