

Original Research Article

Reproductive effects of Odogwu Bitters and Goko Cleanser on testicular histology, sperm parameters, and hormonal profiles in adult male Wistar rats

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Abstract

Purpose: To study the impact of Odogwu Bitters and Goko Cleanser on testicular morphology, sperm characteristics, and hormonal balance in adult male Wistar rats.

Methods: Acute toxicity assessment (LD_{50}) was carried out for both formulations. Fifty male Wistar rats were then randomly divided into ten groups of five each. Groups B – D were administered Odogwu Bitters at 0.2, 0.4, and 0.8 mL, respectively. Groups E – G received Goko Cleanser at 0.2, 0.5, and 0.9 mL, respectively. Groups H – J were treated with combinations: H (0.2 mL Odogwu Bitters + 0.2 mL Goko Cleanser), I (0.4 mL Odogwu Bitters + 0.5 mL Goko Cleanser), and J (0.8 mL Odogwu Bitters + 0.9 mL Goko Cleanser). All treatments were administered orally, twice daily for six consecutive weeks. At the end, animals were sacrificed via overdose of CO₂ followed by testicular histology, semen analysis, and hormonal assays for testosterone and follicle-stimulating hormone (FSH).

Results: Histological analysis revealed no significant structural damage. However, sperm morphology was significantly altered in low-dose Goko Cleanser group ($p = 0.012$), and sperm count significantly reduced in high-dose Odogwu Bitters group ($p = 0.037$). Hormonal assays showed significant increases in testosterone and FSH levels across groups ($p < 0.05$) in comparison with control. These findings demonstrate that Odogwu Bitters and Goko Cleanser impair male reproductive function following sub-chronic exposure, as evidenced by reduced sperm count, altered morphology, and elevated reproductive hormones.

Conclusion: While no histological damage was observed in Wistar rats, the functional disruptions suggest possible endocrine-related effects. These outcomes raise concern over the reproductive safety of these formulations and highlight the need for further toxicological studies to determine their long-term systemic impact.

Keywords: Herbal medicine, Hormonal effects, Phytochemicals, Sperm quality, Toxicity

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INTRODUCTION

Herbal medications continue to play a vital role in healthcare, often serving as the primary treatment method for various diseases [1]. The increasing global interest in herbal medicines as alternatives to conventional therapies has contributed to a surge in demand. However, lack of standardized preparation and dosage, as well as potential toxicity or herb-drug interactions, underscores the need for integrating traditional medicine with modern healthcare systems [2].

Products like Goko Cleanser and Odogwu Bitters are marketed widely in Nigeria without sufficient evaluation of their potential toxicological risks, raising concerns about their safety and the need for regulation to integrate herbal remedies safely into modern medicine [3]. These products are marketed with claims of curing various ailments and enhancing libido, especially in males, making them particularly popular among male youths and older male adults looking to improve sexual performance [4]. However, this trend raises concerns regarding the potential health risks associated with their abuse, especially regarding male reproductive health. The increasing use of aphrodisiac herbal mixtures, such as Odogwu Bitters and Goko Cleanser, in Nigeria has coincided with a rise in testicular health issues, including infertility and malignancies [5]. Male infertility accounts for 40–50% of global infertility cases, often associated with low sperm concentration, poor motility, or abnormal morphology [3]. These issues underline the necessity of investigating unregulated herbal remedies like Odogwu Bitters and Goko Cleanser, particularly their impact on testicular function.

Goko Cleanser herbal mixture contains active ingredients like *Vernonia amygdalina*, *Saccharum officinalis*, *Allium sativum* and *Cajanus cajan*, and has been reported to contain phytochemical constituents like saponin, flavonoids, and tannin [6]. Excessive consumption of herbal drinks which contain some of these phytochemical constituents has been linked to adverse health outcomes such as epigastric discomfort, nausea, diaphoresis, liver fibrosis and sensations of lightheadedness [1,7]. Previous investigations indicate that Goko Cleanser may exert some positive influence on haematological indices, including total white blood cell count (TWBC), haemoglobin concentration (Hb), red blood cell count (RBC), packed cell volume (PCV), and platelet levels [8], overuse could lead to complications, including appetite suppression [6], potential liver issues such as hepatic lobular inflammation, mild

necrosis and haemorrhage [9], and haemorrhagic effect on the renal tissue [10]. Similarly, Odogwu Bitters contain various ingredients which include *Zingiber officinale* and honey, and contains phytochemicals like alkaloids, saponins, flavonoids, and terpenoids in varying proportions [11], have been implicated in reduced oxidative tissue stress and enhanced antioxidant protection [12]. This raises concern about the impact of the combined mixture of both herbal drinks on testicular health.

Since the testes play a central role in hormone production and spermatogenesis [13], examining the toxicological effects of these herbal remedies is crucial. Male reproductive functions are intricately regulated by hormones such as testosterone, oestradiol and follicle-stimulating hormone (FSH). Testosterone influences libido, fertility, muscle mass, and secondary sexual characteristics. Oestradiol supports erectile function and spermatogenesis, while inhibin B controls follicle-stimulating hormone (FSH) through negative feedback, and anti-Müllerian hormone is critical for sexual differentiation [13]. Disruption in the levels of these hormones can lead to a cascade of pathological outcomes, including impaired spermatogenesis, testicular atrophy, erectile dysfunction, infertility, and even hypogonadism [14].

These products' popularity and unregulated distribution coincide with rising concerns over male fertility issues, such as oligospermia and azoospermia, suggesting a need for more research into their long-term effects on reproductive health [4]. The aggressive marketing of these products as aphrodisiacs further underscores the need for strict regulation and greater public awareness of the risks involved with their consumption [8]. While consumers often rely on anecdotal evidence for their effectiveness, the lack of comprehensive studies linking these products to fertility problems calls for urgent attention from health authorities. Research using Wistar rats to assess the co-administration of these products could provide insights into their effects on testicular structure and function. Accordingly, the present investigation was designed to assess the toxicological impact of Odogwu Bitters and Goko Cleanser – both individually and in combination – on testicular health in adult male Wistar rats. The study specifically sought to determine their effects on testicular structure, sperm parameters (motility, count, and morphology), and reproductive hormones such as testosterone and follicle-stimulating hormone (FSH). Such studies are pivotal for informing public health policies, understanding the risks of unregulated herbal

consumption, and devising strategies to combat male infertility [3]. Moreover, they could enhance the broader understanding of testicular pathophysiology, supporting the development of therapeutic interventions for conditions like oligospermia and testicular cancer [13].

EXPERIMENTAL

Animals

Fifty adult male Wistar rats, weighing 195 – 230 g, were utilized. The animals were obtained from a farm in Nsukka, Enugu State, Nigeria. A licensed veterinarian certified their health status prior to transportation, which was carried out under humane conditions. After transportation to the animal farm of the College, they were kept in a spacious, clean environment with good ventilation and allowed to acclimatize for two weeks. The rats were kept in large stainless-steel cages, with room temperature maintained and a 12-hour light–dark cycle observed. Standard care was employed, which included free access to a balanced diet and fresh drinking water, as well as daily cage cleaning with sawdust bedding to prevent infections. Regular health checks were conducted throughout the study.

Ethical approval

Ethical clearance was obtained from the Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University (approval no. NAU/CHS/NC/FMBS/570, issued August 4, 2023).

Protocol

The design followed guidelines used for studies carried out in animals [15]. The experiment spanned 12 weeks, which included two weeks for LD₅₀ determination and acclimatization of the animals; oral administration of herbal formulations to the animals was done over six weeks; two weeks were for both biochemical and histological analyses; and two weeks were for statistical analysis and result compilation.

After the two-week acclimatization, the rats were weighed with a 6000 g capacity balance (Model WT6000GT, Want Balance Instrument Co., China) and then randomly distributed into ten groups (A–J), each consisting of five animals. A syringe fitted with a cannula ensured accurate and consistent oral delivery of the herbal mixtures. The herbal preparations were obtained from the sole distributors from Nkwo Nnewi market, Anambra state and administered orally to

the rats for a duration of 42 days. Odogwu Bitters is manufactured by Cubana Trading and Investment Company Limited, while Goko Cleanser is produced by Dr Iguedo Herbal Mixture Nigeria Limited. Group A functioned as the control and was given only standard feed and water. Groups B – D received Odogwu Bitters once daily at doses of 0.2, 0.4, and 0.8 mL, respectively. Groups E – G were treated with Goko Cleanser once daily at 0.2, 0.5, and 0.9 mL, respectively. Groups H–J were administered combined regimens to simulate common local consumption practices with Odogwu Bitters given in the morning (6:00–8:00 am), while Goko Cleanser was administered in the evening (5:00 – 7:00 pm); Group H received 0.2 mL of each formulation, Group I was given 0.4 mL of Odogwu Bitters plus 0.5 mL of Goko Cleanser, while Group J received 0.8 mL of Odogwu Bitters along with 0.9 mL of Goko Cleanser.

Acute toxicity test

Acute oral toxicity of Odogwu Bitters and Goko Cleanser was determined using the OECD Guideline 425 (Up-and-Down Procedure) [16], as previously described by Obiesie *et al* [11]. Briefly, graded doses were administered sequentially with observation for clinical signs of toxicity and mortality. The LD₅₀ was calculated according to the method outlined in the guideline.

Animal euthanasia

All procedures involving euthanasia adhered to institutional animal welfare and ethical guidelines established by Nnamdi Azikiwe University. The animals were humanely sacrificed through CO₂ inhalation at overdose levels according to the WHO guidelines on animal research [17]. Gas flow was adjusted to replace approximately 10 – 30 % of the chamber volume per minute, thereby minimizing distress. Unconsciousness was confirmed before euthanasia was finalized by monitoring the cessation of respiratory and cardiac activity. Reflex absence, including the corneal reflex, was used to confirm death. The carcasses were disposed of following institutional biosafety protocols.

Blood collection and organ harvesting

About 0.5 mL of blood samples were drawn from each rat through ocular puncture and transferred into sterile tubes. After 30 minutes of clotting, samples were centrifuged at 2500 rpm for 10 minutes using an 800D electric centrifuge with a 6 × 20 mL rotor. The serum obtained was stored under refrigeration for subsequent hormonal evaluation.

For organ retrieval, rats were secured on a dissection board and subjected to a lower abdominal incision to expose the testes. The testes were excised, rinsed in sterile phosphate-buffered saline (PBS) to eliminate residual blood, and fixed in 10% formalin saline for histological processing.

Histological analysis

Testicular tissues were processed for histology through the standard sequence of fixation, dehydration, clearing, embedding, sectioning, and H&E staining. Photomicrographs were taken with a Novex compound microscope fitted with Hi-PLAN objectives and an Amscope 14MP digital camera, with magnifications displayed on the images. Labelling and annotations were carried out using Photoscape v3.7. All steps followed established histological protocols for rat experimental studies [9].

Method of sperm analysis

Sperm parameters were evaluated following standard procedures for rodents, including assessment of motility, concentration, and morphology, with eosin–nigrosin staining used for morphological classification [17,18]. For motility, a drop of sperm suspension was placed on a pre-warmed glass slide, covered with a coverslip, and examined under a light microscope ($\times 400$). The proportion of motile to immotile sperm was expressed as a percentage. Sperm concentration was determined by loading a diluted aliquot into a haemocytometer and counting under the microscope. Morphology was assessed using eosin–nigrosin staining, with sperm classified as normal or abnormal based on head and tail features, and the percentage of abnormal forms calculated.

Hormonal assay

Blood samples were left to clot at room temperature and centrifuged at 2500 rpm for 10 minutes to obtain serum, which was stored at -20°C until analysis. Serum testosterone and follicle-stimulating hormone (FSH) concentrations were determined using ELISA kits, following the manufacturer's instructions [19]. Absorbance was read at 450 nm with a microplate reader, and hormone levels were calculated from standard curves.

Data analysis

Data were analysed using IBM SPSS Statistics (version 25). Descriptive statistics were

generated, and group comparisons were performed using one-way ANOVA followed by post hoc testing where applicable, with significance set at $p < 0.05$ (95% confidence level). Normality of data distribution was assessed using Levene's test.

Hormone concentrations were reported in units provided by the assay kits (e.g., ng/mL, IU/L). Sperm parameters were expressed as mean percentages for motility and morphology, and as mean concentration (million/mL) for count.

RESULTS

Histological analysis of testicular tissue

Histological examination across all groups showed preserved seminiferous tubules and interstitial tissue, with germ cell maturation occurring in an orderly fashion (Figures 1 A – J).

In the control group (A), the mean seminiferous tubule density was 77 per LPF, with spermatid counts averaging 370 per tubule. Groups B – D showed comparable tubule integrity, with mean spermatid densities of 280, 305, and 345 per tubule, respectively. Groups E and F maintained similar architecture, with higher spermatid counts averaging 365 and 385 per tubule. Group G recorded an average of 325 per tubule. In the combination treatment groups, spermatid counts were 420 (H), 350 (I), and 360 (J) per tubule.

Sperm analysis results

Sperm motility did not differ significantly between control and treated groups, with mean values remaining comparable (Table 1; Figure 2). Sperm morphology was also generally unaffected, except in Group E (0.2 mL Goko Cleanser), which showed a significant increase in abnormal forms ($p = 0.012$; Table 1; Figure 2). Similarly, sperm counts were consistent across most groups, except for Group D (0.8 mL Odogwu Bitters), where a significant reduction was observed ($p = 0.037$; Table 1; Figure 3).

Hormonal assay results for FSH and testosterone

Testosterone and FSH levels differed significantly between control and treated groups ($p = 0.001$; Table 2). Post hoc comparisons indicated that testosterone was significantly altered in most treatment groups, except B and E (both $p = 1.000$) and H ($p = 0.383$), which received lower doses (Table 3). For FSH, significant differences were observed across groups, except for group H ($p = 1.000$; Table 4).

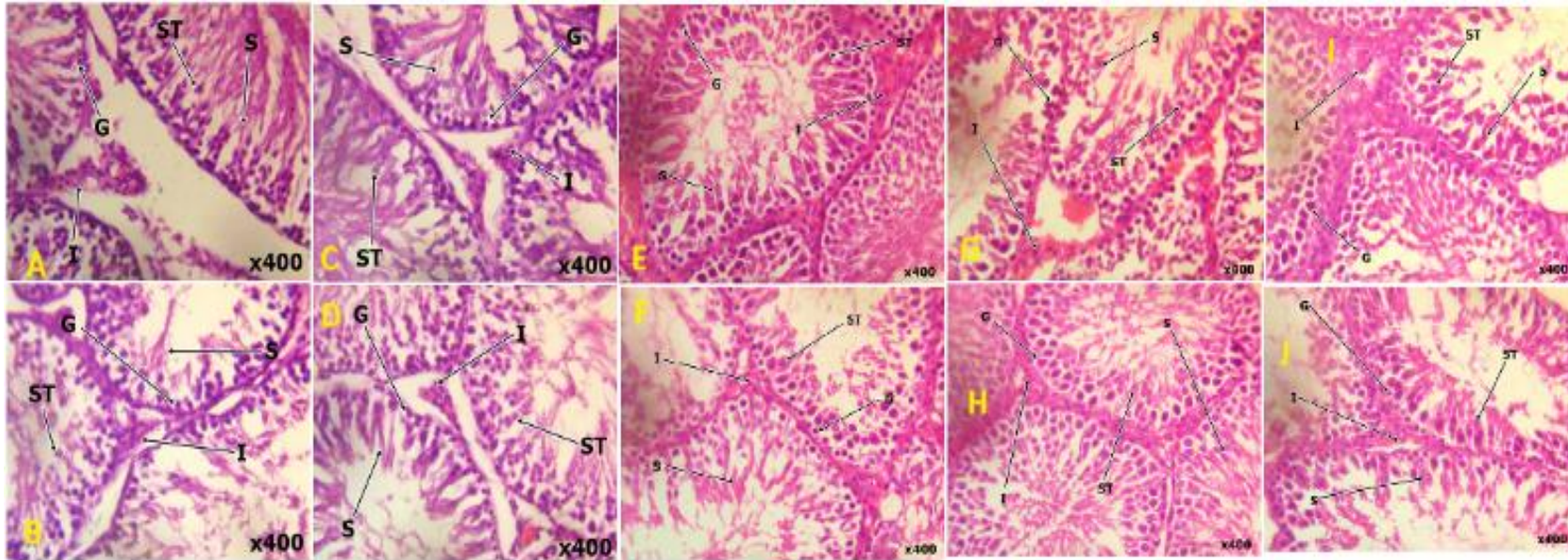


Figure 1: (A – J) Representative photomicrograph of the testis of rats in Groups A – J, respectively. G = Germinative layer, I = Interstitial cells of Leydig, ST = Sertoli cell, S = Mature Spermatid

Table 1: Results of sperm analysis

Group	Sperm motility (%)	P-value	Semen morphology (%)	P-value	Total sperm count	P-value
A	92.50±2.50	0	94.00±1.00	0	765.00±35.00	0
B	87.50±2.50	0.293	82.50±7.50	0.268	734.00±16.00	0.505
C	87.5±2.50	0.293	93.50±1.50	0.808	818.50±6.50	0.271
D	83.33±4.41	0.292	88.00±4.00	0.319	634.33±76.30*	0.037
E	85.00±5.00	0.312	85.00±0.00*	0.012	679.00±21.50	0.173
F	80.00±5.00	0.155	80.00±10.00	0.298	590.00±94.00	0.223
G	91.00±1.00	0.633	93.00±0.00	0.423	676.00±74.00	0.391
H	85.00±5.00	0.312	87.50±7.50	0.481	785.50±10.50	0.631
I	87.5±2.50	0.293	91.00±1.00	0.168	809.00±1.00	0.336
J	89.00±4.00	0.535	93.50±1.50	0.808	719.50±30.50	0.430

Values are Mean ± SEM, * $P < 0.05$ vs control (A)

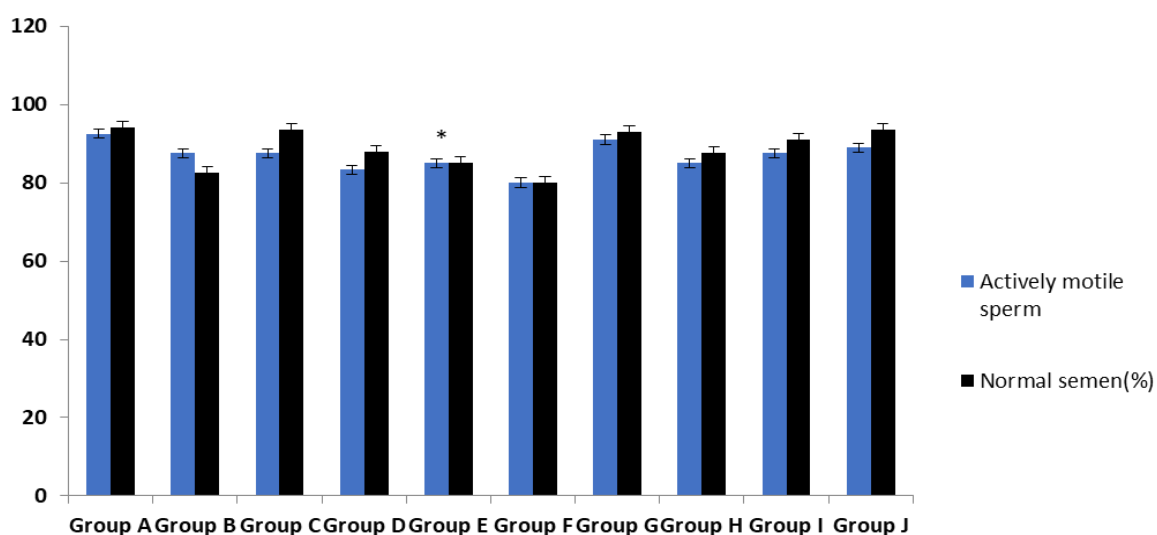


Figure 2: Bar chart showing the results of sperm motility and sperm morphology of the rats across all groups. * $P < 0.05$

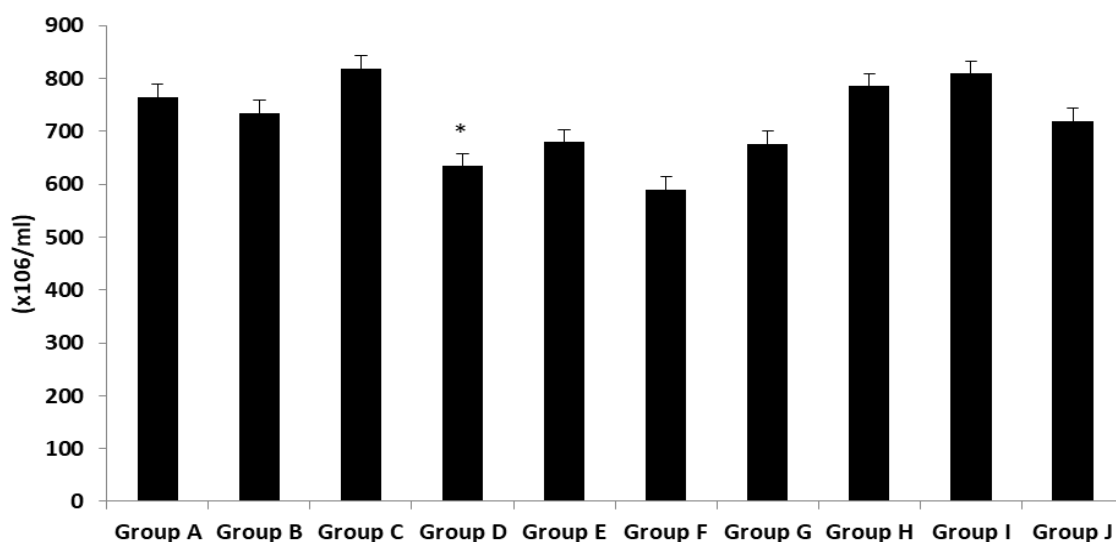


Figure 3: Bar chart showing the result of sperm count of the rats across all groups. * $P < 0.05$

Table 2: ANOVA result of testosterone and FSH analysis

Hormone levels (IU/L)		Sum of Squares	df	Mean square	F	Sig.
Testosterone*	Between Groups	100.130	9	11.126	153.160	0.001
	Within Groups	0.726	10	0.073		
	Total	100.856	19			
FSH*	Between Groups	49.480	9	5.498	2748.889	0.001
	Within Groups	0.020	10	0.002		
	Total	49.500	19			

*P < 0.05 vs control

Table 3: Multiple comparisons of the testosterone analysis results

Hormone levels (IU/L)		Control group	Study group	Mean difference	Std. Error	Sig.	95% Confidence interval	
							Lower bound	Upper bound
Testosterone*	Bonferroni	A	B	0.4650	0.2695	1.000	-0.7528	1.6828
			C	-5.5100*	0.2695	0.001	-6.7278	-4.2922
			D	-2.8100*	0.2695	0.001	-4.0278	-1.5922
			E	0.2750	0.2695	1.000	-0.9428	1.4928
			F	-1.2300*	0.2695	0.047	-2.4478	-0.0122
			G	-5.3250*	0.2695	0.001	-6.5428	-4.1072
			H	-0.8800	0.2695	0.383	-2.0978	0.3378
			I	-3.0750*	0.2695	0.001	-4.2928	-1.8572
			J	-4.9500*	0.2695	0.001	-6.1678	-3.7322

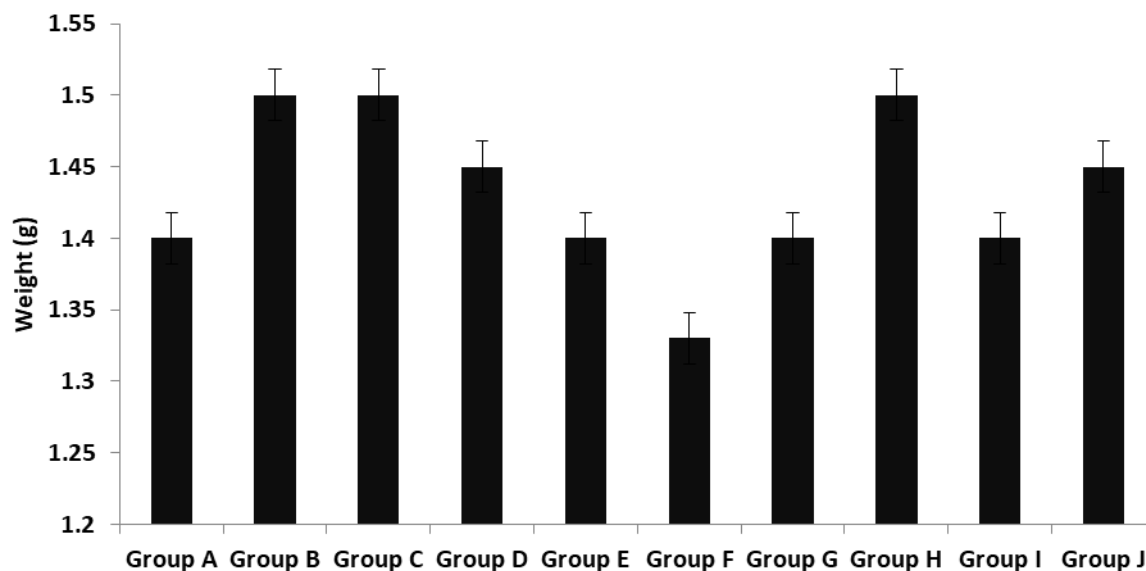


Figure 4: Result of relative weight of testis

Histological evaluation of testicular tissues in all groups revealed preserved architecture, with seminiferous tubules remaining intact and germ cell maturation occurring in an orderly sequence. These findings suggest that neither Odogwu Bitters nor Goko Cleanser, at the administered doses, caused overt testicular damage. Notably, spermatid density remained relatively stable across groups, with only slight reductions in rats treated with 0.2 mL Odogwu Bitters, 0.4 mL Odogwu Bitters, and 0.9 mL Goko Cleanser, compared with controls. Interestingly, animals receiving the lowest combined dose (0.2 mL Odogwu Bitters + 0.2 mL Goko Cleanser) displayed increased spermatid density, suggestive of a hormetic response, whereas those given the highest combination (0.8 mL Odogwu Bitters + 0.9 mL Goko Cleanser) showed reduced spermatid density per tubule. This dose–response trend is consistent with the biphasic effects described by Yakubu *et al*, where compounds beneficial at lower doses may exert harmful effects at higher levels [20]. The observed differences in spermatid density do not appear to translate into clinically significant alterations in spermatogenesis but rather highlight the potential for subtle variations in reproductive parameters such as sperm morphology, sperm count, FSH and testosterone in response to herbal interventions. This functional-histological disconnect mirrors findings from Raji *et al*, demonstrating that certain herbal extracts can impair reproductive function without visible tissue damage [21].

Despite the absence of significant variation in sperm motility among groups, sperm morphology analysis revealed a statistically significant

alteration in Group E, which received the lowest dose of Goko Cleanser (0.2 mL). This alteration in sperm morphology, in the absence of corresponding changes in motility or total count, underscores the need for further studies to assess the potential long-term impact of Goko Cleanser on spermatogenesis and sperm quality. A study on the effects of similar plant-based compounds has reported both beneficial and deleterious effects on sperm morphology, depending on the concentration and duration of exposure [22]. Treatment with 0.8 mL of Odogwu Bitters (Group D) resulted in reduced total sperm count. This observation suggests that higher doses of Odogwu Bitters may exert a negative influence on spermatogenesis, potentially due to its constituent compounds such as saponins and alkaloids. Saponins are known to possess both beneficial and toxic effects on various organs and systems, including the reproductive system [23]. This result underscores the importance of dose optimization when considering herbal treatments, as high concentrations of certain active components could disrupt normal reproductive function.

In contrast to the effects on sperm morphology and count, hormonal analysis revealed significant disruptions in testosterone and FSH levels in most treatment groups, except in those administered 0.2 mL Odogwu Bitters, 0.2 mL Goko Cleanser, and the combination of 0.2 mL Odogwu Bitters + 0.2 mL Goko Cleanser. The significant increase in these hormonal levels suggests that the herbal treatments may modulate the hypothalamic-pituitary-gonadal axis, influencing male reproductive physiology. These hormonal variations may be associated

with the bioactive phytochemicals present in the mixtures: Goko Cleanser containing saponins, flavonoids, and tannins [6], while Odogwu Bitters contains alkaloids, saponins, flavonoids, and terpenoids [11]. Saponins are well-documented cytotoxic agents. A comprehensive review highlights that steroidal and triterpenoid saponins can induce cell death through apoptosis and membrane disruption, particularly at higher concentrations [24]. Various alkaloids and environmental endocrine disruptors are known to impair Leydig cell function and steroid hormone production, either via endocrine-disrupting activity or through direct cytotoxicity [25]. Flavonoid-rich teas (e.g., rooibos, *Camellia sinensis*) have been shown to act as endocrine disruptors in Leydig cell cultures, reducing testosterone biosynthesis in a dose-dependent manner [26].

While testicular weight remained unchanged, this gross measure is insensitive to subclinical toxicity. Comparable findings were documented by Raji *et al*, who observed reproductive impairment in the absence of changes in organ weight [27]. For unstandardized herbal formulations such as Odogwu Bitters and Goko Cleanser, this underscores the limitation of using morphological assessments alone. Recent Nigerian literatures raise additional caution. Obiesie *et al* and Onyejike *et al* confirmed consistent phytochemical presence in these formulations, but emphasized the need for batch verification due to manufacturing variability [11,28]. Moreover, studies have shown nephrotoxic potential (creatinine and urea elevation) without structural damage after similar dosing regimens, suggesting systemic risks beyond reproduction [11,28]. These findings are particularly relevant to the Nigerian context, where herbal bitters and cleansers are ubiquitously marketed as detoxifiers or sexual enhancers, often sold on street corners and public transport with minimal regulatory oversight [29].

Despite its contributions, this study has several limitations. Although previous work have detailed phytochemical constituents in Odogwu Bitters and Goko Cleanser [6,11,28], batch-specific analyses were not performed here, which are necessary given product variability. Additionally, the sample size ($n = 5$ per group) may limit statistical robustness; larger sample sizes would improve generalizability. The study also did not use ultrastructural microscopy to detect subcellular damage. Finally, the absence of a post-treatment recovery period leaves the reversibility of hormonal and sperm alterations unknown.

In conclusion, despite preserved testicular architecture, Odogwu Bitters and Goko Cleanser especially at moderate-to-high doses or in combination caused significant disruptions in sperm morphology, sperm count, and reproductive hormone levels. These results challenge the assumption of safety for unregulated herbal products in Nigeria, underscoring the urgent need for comprehensive phytochemical profiling, standardization, long-term toxicity studies, and evaluation of recovery potential.

CONCLUSION

This study shows that repeated oral administration of Odogwu Bitters and Goko Cleanser for six weeks resulted in dose-dependent alterations in sperm parameters and reproductive hormone levels, despite preserved testicular histoarchitecture. The study indicates that regular use of these herbal mixtures could pose reproductive risks. While the exact bioactive components responsible remain unclear, the observed endocrine-related effects underscore the need for regulatory scrutiny and further toxicological evaluation. Further research is needed to clarify the mechanisms involved and the long-term effects on male fertility.

DECLARATIONS

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Conflict of interest

No conflict of interest 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. Author Darlington Nnamdi Onyejike (DNO) conceptualized the study, designed the study, supervised the experiment, wrote the experimental protocol and wrote the first draft of

the manuscript. Author Ifeoma Miracel Onyejike (IMO) curated the data, assisted author CJI to carry out the experiment and manage the animals, and assisted author IJO to manage the literature searches. Author Dominic Edward Ejiofor (DEE) carried out the kidney function test. Author Chinaechelum Joan Izuegbunam (CJI) carried out the experiment and managed the animals; and assisted author IMO to curate the data. Author Ifechukwu Justicia Obiesie (IJO) carried out the histology analysis and managed the literature searches. Author Albert Tobechukwu Nwamaradi (ATN) carried out the data analysis. Author Ambrose Echefulachi Agulanna (AEA) reviewed the manuscript.

Ethical approval

The ethical approval was obtained from the ethical committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus. The certification number is NAU/CHS/NC/FMBS/570, on the 4th of August 2023.

Availability of data and materials

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

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