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Original Research Article

Toxicological Evaluation of Co-Administration of Odogwu Bitters and Goko Cleanser Herbal Drinks on the Kidney of Adult Male Wistar Rats

Ifechukwu J. Obiesie;[1] Darlington N. Onyejike;[1,2] Ifeoma M. Onyejike;[1] Sonia K. Okechukwu;[1] Emeka A. Okubike.[1]

1. *Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nigeria.*
2. *Department of Forensic Medicine, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa.*

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ABSTRACT

The consumption of herbal drinks is on the rise among Nigerians, especially within younger and artisan communities. This study investigates the potential histopathological impacts on kidney function when Odogwu Bitters and Goko Cleanser herbal drinks are co-administered to adult male Wistar rats. Forty male adult Wistar rats were used in the study, organized into ten groups with four rats each. Group A served as the control, while groups B, C, and D received 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, I, and J received varying doses of both Odogwu Bitters and Goko Cleanser. The experiment was conducted over six weeks. At the end of the six-week period, blood samples were drawn for kidney function analysis, and the kidneys were collected for histological examination. The histological analysis indicated that co-administration of Odogwu Bitters and Goko Cleanser did not cause noticeable structural damage to the kidneys of adult male Wistar rats within the study period. However, biochemical analysis revealed that increasing doses of the herbal drinks led to significant changes in serum creatinine, urea, and uric acid levels ($p = .001$ for each parameter). This suggests that while no structural damage was observed in kidney tissue, the biochemical markers indicate potential renal effects at higher doses. The findings highlight the importance of cautious consumption of these herbal drinks to avoid potential kidney-related issues.

Keywords: Creatinine, Interstitial haemorrhage, Mesangial Hypercellularity, Urea, Uric acid.

Introduction

Recently, there has been a significant rise in the consumption of herbal beverages across developing nations, such as Nigeria. This trend is largely driven by the belief in their potential to effectively treat a wide range of ailments.[1,2] These herbal drinks are common across different societies and cultures, varying in their contents, recipes and proposed actions.[3-5] These herbal mixtures offer variety of benefits, ranging from an increase in sexual performance, treatment of several diseases and even aiding in weight loss.[6,7] So many herbal drinks are alcohol-based and are often used as digestive aid and appetite stimulant.[5,8] In Nigeria, different herbal products have been widely distributed into the market, with more brands emerging every day.[1,9] Odogwu Bitters and Goko Cleanser are among the popular herbal drinks that have dominated the Nigerian market due its high demand.[10-13]
In Nigeria, it is common among artisans to consume multiple popular herbal drinks simultaneously, often before, during, or after their daily work.[11] This widespread practice may stem from the common belief that herbal drinks, being natural, are free from adverse effects, potentially contributing to their high demand and misuse.[14]

*Corresponding author. Email: dn.onyejike@unizik.edu.ng
Tel: +2348061131867

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While herbal mixtures can offer various health benefits, they may also pose risks to consumers. Some herbs contain powerful toxins, and many compounds derived from plants can be harmful, potentially causing adverse effects.[15] Studies have shown that several active compounds in herbal medicines can induce nephrotoxicity, leading to structural and functional damage in kidney tissues.[1,11,13,16] Therefore, the co-administration of herbal drinks, such as Odogwu Bitters and Goko Cleanser, raises concerns regarding potential cumulative or synergistic toxic effects, especially on renal function. The kidneys are essential organs that perform several crucial functions, including filtering waste products from the blood, producing urine, and stimulating the production of red blood cells.[17] The kidneys are sites for pathology of any harmful fluid taken into the body.[18-20] A vast majority of these herbal drinks have not been scientifically investigated properly.[1,5,12,21] This study examined the histopathological impact of combined administration of the herbal mixtures Odogwu Bitters and Goko Cleanser on the kidney tissue of adult male Wistar rats.

Materials and Method

Ethics approval
Ethical approval for this study was granted by the Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus. The certification number, NAU/CHS/NC/FMBS/578, was issued on July 26, 2023.

Materials
Feed, herbal drinks, reagents and chemicals
The following materials were used for to carry out this study: 200mili litres bottles of the herbal drinks (Odogwu Bitters and Goko Cleanser herbal mixture) were procured from the distributors at Nkwo Nnewi market, Nnewi, Anambra state; Top feeds Grower's mash Super-Deluxe Animal Feed produced be Eastern Premier Feed mills Limited (Ltd), a subsidiary of Premier Feeds Mills company Ltd, Plateau state,

Nigeria; 10% Formal saline, normal saline, chloroform, distilled water, alcohol (100%), xylene, sodium citrate, ethanol (100%), and paraffin wax supplied by the Department of Anatomy, Nnamdi Azikiwe University; Haematoxilin (produced by Number Laboratory Chemicals, India); Eosin (produced by Kem Light laboratory, India); Analytical grade reagents (produced by Syntron Bioresearch Incorporated, United States of America).

Animals

The study utilized 40 male adult Wistar rats, each weighing between 195g and 230g. The animals were sourced from a local farm in Nsukka, Enugu State, Nigeria. Before the study, the rats were given a two-week acclimatization period during which they had unrestricted access to food and water. Their health was evaluated by a certified veterinarian prior to their humane transport to the research facility.

The rats were housed in spacious, ventilated stainless-steel cages maintained under controlled temperatures and exposed to a 12-hour light/dark cycle. Their health was regularly monitored, and they were provided with a standard diet along with fresh water. To ensure cleanliness and minimize infection risk, sawdust was used on the cage floors, and the cages were thoroughly cleaned each day.

All experimental procedures followed the ethical guidelines set by the Faculty of Basic Medical Sciences' ethics committee at Nnamdi Azikiwe University, Nnewi Campus, Nigeria.

Methods

Duration of the study

This study was conducted over a three-month period. The first two weeks were dedicated to determining the LD-50, performing phytochemical analysis of the herbal drinks, and acclimatizing the animals. The subsequent six weeks involved the administration of herbal drinks, followed by two weeks for histological and biochemical analyses. The final two weeks were devoted to statistical analysis and the documentation of results.

Experimental Design

After a two-week acclimatization period, the rats were weighed and then randomly assigned to 10 groups, labelled A through J, with four rats per group. Herbal drinks were administered orally twice daily, in the morning (6:00–8:00 am) and evening (5:00–7:00 pm), reflecting typical times of use by artisans before and after work. Odogwu Bitters was given in the morning, and Goko Cleanser in the evening, over a 42-day period. All administrations were performed orally, using a syringe equipped with a cannula to ensure precise delivery into the oral cavity. Group A served as the control group and was fed with water and feed only. Group B received 0.2 ml of Odogwu Bitters herbal mixture daily while group C received 0.4 ml of Odogwu Bitters herbal mixture daily and Group D received 0.8 ml of the Odogwu Bitters herbal mixture daily. Group E received 0.2 ml of Goko Cleanser herbal mixture daily while Group F received 0.5 ml of Goko Cleanser herbal mixture daily and Group G received 0.9 ml of Goko Cleanser herbal mixture daily. Group H received 0.2 ml of both Odogwu Bitters and Goko Cleanser herbal mixtures daily while Group I received 0.4 ml and 0.5 ml of both Odogwu Bitters and Goko Cleanser herbal mixtures respectively daily and Group J received 0.8 ml and 0.9 ml of both Odogwu Bitters and Goko Cleanser herbal mixtures respectively daily.

Acute Toxicity Evaluation of Odogwu Bitters and Goko Cleanser Herbal Mixture in Rats

This study utilized OECD Guideline 425 – the Up-and-Down Procedure – which administers doses sequentially, adjusting each dose based on prior outcomes. This method enables a more precise estimation of LD_{50} while reducing the number of animals required.[22] The Odogwu Bitters herbal beverage utilized in this study was manufactured by Cubana Trading and Investment Company Limited, Lagos, Nigeria, in September 2023, and is registered under NAFDAC number 08-3849L. Similarly, the Goko Cleanser herbal beverage was produced by Kayfahd Herbaceuticals, FCT Abuja, Nigeria, in August 2023, with NAFDAC registration number A7-0804L. This study aimed to assess the acute toxicity of Odogwu Bitters and Goko Cleanser Herbal Mixture through oral administration in rats, conducted in two phases.

Odogwu Bitters

Phase I: Thirteen rats were divided into three groups, each consisting of three animals, and monitored over a 24-hour 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: Four additional rats were introduced, each receiving a single dose. These rats were similarly monitored for morbidity and mortality over a subsequent 24-hour period.

LD_{50} Determination for Odogwu Bitters

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

Phase II: At higher doses of 1200 mg/kg and 1600 mg/kg, no fatalities occurred, and animals remained calm. However, at 2900 mg/kg and 5000 mg/kg, mortality occurred within 24 and 12 hours, respectively.
The calculated LD50 was:
$LD50=\sqrt{AB}$
A=Maximum dose with 0% mortality
B= Minimum dose with 100% mortality
$LD50= \sqrt{1600\times2900}=2154.17$mg/kg

Goko Cleanser Herbal Mixture

Phase I: Nine rats were divided into three groups of three male Wistar rats each. They were observed for 24 hours, with no mortality recorded, leading to the progression of Phase II.
Phase II: The rats were again monitored for 24 hours at higher doses.

LD50 Determination for Goko Cleanser Herbal Mixture

Phase I: No mortality was observed at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg.
Phase II: At doses of 1200 mg/kg and 2600 mg/kg, no fatalities occurred, with the rats remaining calm. However, at 3900 mg/kg and 5000 mg/kg, mortality was observed within 48 and 24 hours, respectively. The LD50 was calculated as:
$LD50 = \sqrt{A \times B}$
A = Maximum dose with 0% mortality (2600mg/kg)
B = Minimum dosed with 100% mortality (3900mg/kg)
LD50 of Goko Cleanser = $\sqrt{2600 \times 3900} = 3184.34$mg/kg
The LD50 values determined for both Odogwu Bitters (2154.17 mg/kg) and Goko Cleanser Herbal Mixture (3184.34 mg/kg) indicate a moderate level of acute toxicity, with higher doses resulting in mortality. Further studies are recommended to explore the safety profile of these herbal products for potential therapeutic use.

Animal Euthanasia

The euthanasia of the Wistar rats adhered strictly to Nnamdi Azikiwe University animal welfare regulations and ethical considerations.
Animals were euthanized using inhalant anaesthesia overdose (CO_2 gas).[23] The gas flow rate was adjusted to displace 10–30% of the chamber's volume each minute to maintain comfort and prevent distress. The animal was monitored for unconsciousness, which was followed by euthanasia confirmation after complete cessation of respiratory and cardiac function. Euthanasia was confirmed by assessing the absence of heartbeat, respiration, and reflexes (e.g., corneal reflexes).

Blood collection and organ harvesting

Blood samples were collected from the eye using ocular puncture and placed into sterile plastic tubes. The samples were then allowed to sit for 30 minutes to ensure full clotting. Once clotted, they were centrifuged at 2500 rpm for 10 minutes using an 800D Electric Centrifuge Machine (operating at 4000 RPM and equipped with a 6 x 20 mL rotor). The resulting clear serum was carefully separated and stored in a refrigerator until it was needed for biochemical testing.

The rats were positioned on a flat board, with their limbs gently and securely fastened using optical pins. A careful abdominal dissection was then performed with a dissecting kit to expose the kidney. The kidney was excised, rinsed in sterile phosphate-buffered saline (PBS) to eliminate blood and surface contaminants, and subsequently preserved in 10% formalin saline for histological examination.

Phytochemical analysis
The bioactive compounds examined encompassed saponins, tannins, flavonoids, steroids, alkaloids, cardiac glycosides, reducing sugars, proteins, carbohydrates, and terpenoids. These compounds were evaluated using both qualitative and quantitative techniques. For quantification, High-Performance Liquid Chromatography (HPLC) was utilized due to its ability to separate, identify, and measure phytochemicals based on their retention times compared to established standards. This analysis adhered to standardized protocols, as established in rat-based experimental studies.[24]

Histology analysis
The kidney samples were processed through several stages, which involved fixation, dehydration, clearing, embedding, sectioning, and staining using haematoxylin and eosin (H&E). Photomicrographs were obtained using an Amscope 14MP digital camera mounted on a Novex compound microscope, outfitted with Hi-PLAN objectives. Image annotation was carried out using Photoscape v3.7. All procedures followed standardized protocols for rat experimental studies.[25]

Kidney Function Test
The study conducted a biochemical assay to quantify the levels of urea, creatinine, and uric acid in sera collected from rats.
Urea Determination: Urea concentration was measured using the enzymatic (urease) method. In this method, urease breaks down urea into ammonia and carbon dioxide, and the resulting ammonia was quantified through a colorimetric assay.
Creatinine Measurement: The Jaffe reaction was employed to measure creatinine levels. In this process, creatinine interacts with picric acid in an alkaline solution, resulting in the formation of a coloured complex. The strength of this colour, which can be quantified using spectrophotometry, is directly proportional to the concentration of creatinine.
Uric Acid Analysis: Uric acid is typically measured using a uricase-based method. Uricase converts uric acid to allantoin, producing hydrogen peroxide as a byproduct, which is then measured colorimetrically or enzymatically to determine uric acid levels.
Each assay required the use of standards and controls to ensure accuracy. Results were read with a spectrophotometer, with the absorbance values compared to those of known standards to calculate the concentrations in the serum samples. These procedures followed a standard protocol used in rat experimental models.[26]

Statistical Analysis
The data from this study were analysed using IBM SPSS Statistics software (version 25). A 95% confidence level was set for hypothesis testing. Both descriptive and inferential statistical methods were applied in the analysis. To examine differences between the control and experimental groups, a one-way analysis of variance (ANOVA) was conducted. Additionally, independent samples t-test were used to compare group differences.

Results and Discussion

Histopathological effects of Odogwu Bitters and Goko Cleanser on the Kidney
The histological examination of the control group (Group A) revealed normal renal architecture, characterized by uniformly distributed glomeruli of similar size, normal mesangial cellularity, open glomerular capillaries, and viable tubular epithelium (Figure 1). These findings establish a baseline for comparison. In Groups B and C, which received lower doses of Odogwu Bitters, glomeruli remained evenly spaced with normal mesangial cellularity, while mild mesangial hypercellularity was observed in Group D, which received the highest dose (Figures 2-4). This hypercellularity may signify early renal stress, potentially due to bioactive compounds in the herbal mixture. Previous studies have indicated that excessive saponin exposure can disrupt glomerular integrity.[1]

In Groups E, F, and G, treated with Goko Cleanser, histological analysis showed predominantly normal kidney architecture, although mild interstitial haemorrhage was evident in Groups F and G at higher doses (Figures 5-7). Such findings suggest a dose-dependent response, where higher concentrations of flavonoids and saponins might exert stress on renal tissues, as documented in studies on nephrotoxic plant derivatives.[27]

Table 1: Result of Phytochemical Analysis of Goko Cleanser herbal mixture

Constituents	Qualitative test	Quantity (% w/v)
Saponin	+	7.35
Flavonoid	+	4.0
Tannin	+	0.2
Alkaloid	-	-
Steroid	-	-
Reducing sugar	-	-
Cardiac glycoside	-	-
Protein	-	-
Carbohydrate	-	-

The combined administration in Groups H, I, and J produced histological patterns comparable to the control, with no significant abnormalities (Figures 8-10). This outcome highlights potential interactions between the compounds in Odogwu Bitters and Goko Cleanser that may neutralize adverse effects, supporting earlier reports on the synergistic nephroprotective effects of flavonoids and terpenoids.[28]

Table 2: Result of Phytochemical analysis of Odogwu Bitters

Phyto-constituents	Qualitative test	Quantitative test (% w/v)
Saponin	+	0.13
Alkaloid	+	0.09
Terpenoid	+	0.30
Cardiac glycoside	+	N/D
Flavonoid	+	0.12
Carbohydrate	+	N/D
Reducing sugar	+	0.11
Protein	-	-
Steroid	-	-

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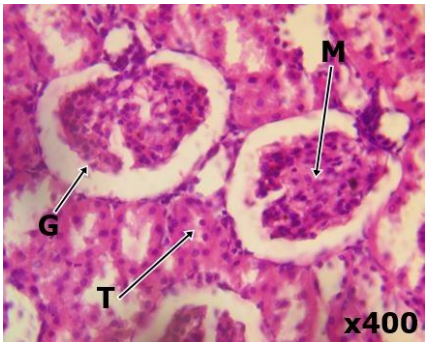

Figure 1: Photomicrograph of kidney of Group A rats (Control group)

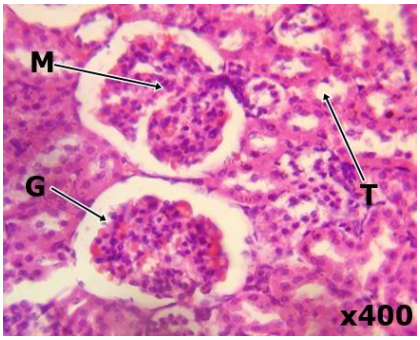

Figure 2: Photomicrograph of kidney of Group B rats

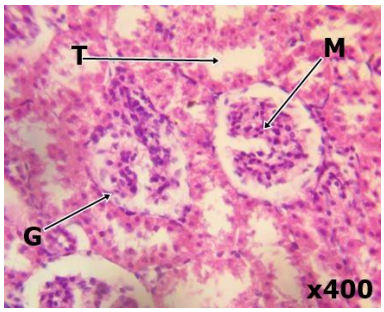

Figure 3: Photomicrograph of kidney of Group C rats

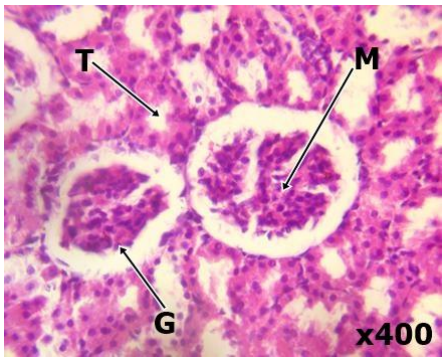

Figure 4: Photomicrograph of kidney of Group D rats

Key:
M = Mesangium
G = Glomerulus
T = Tubule

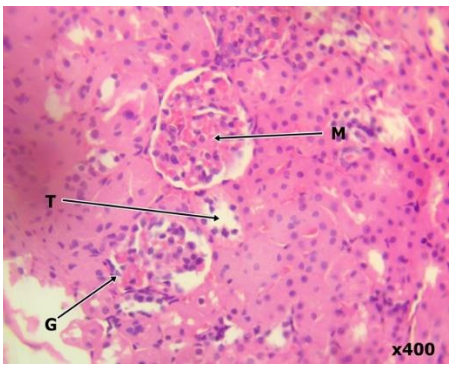

Figure 5: Photomicrograph of kidney of Group E rats

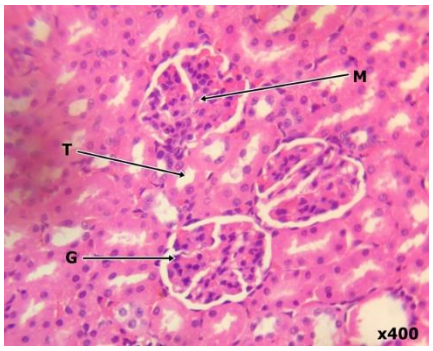

Figure 6: Photomicrograph of kidney of Group F rats

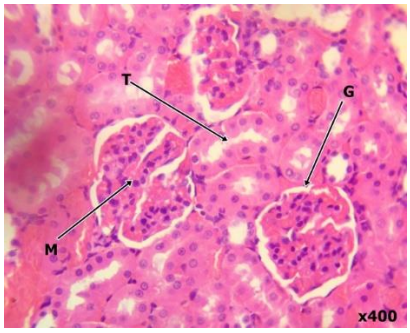

Figure 7: Photomicrograph of kidney of Group G rats

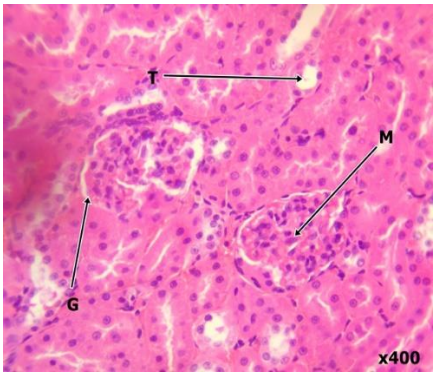

Figure 8: Photomicrograph of kidney of Group H rats

Key:
M = Mesangium
G = Glomerulus
T = Tubule

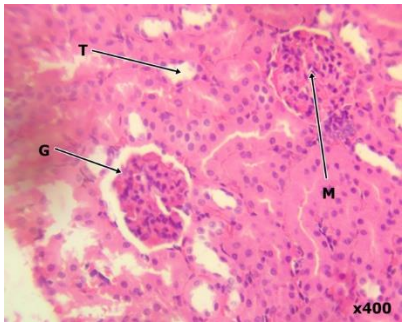

Figure 9: Photomicrograph of kidney of Group I rats

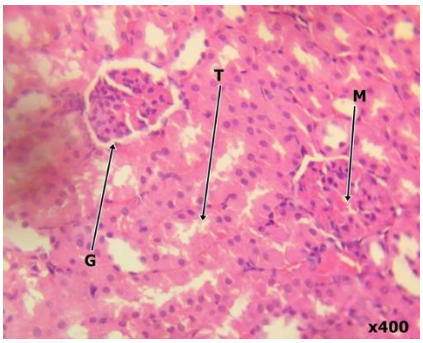

Figure 10: Photomicrograph of kidney of Group J rats

Key:
M = Mesangium
G = Glomerulus
T = Tubule

Phytochemical Composition of Goko Cleanser and Odogwu Bitters
Phytochemical analysis revealed substantial concentrations of saponins (7%) and flavonoids (4%) in Goko Cleanser (Table 1), while Odogwu Bitters contained trace amounts of saponins (0.13%) and flavonoids (0.12%), alongside terpenoids and alkaloids (Table 2). The high levels of these bioactive compounds in Goko Cleanser are consistent with its observed mild renal stress at higher doses. Saponins are known to modulate renal inflammation, whereas flavonoids act as antioxidants, mitigating oxidative damage.[29]
The negligible levels of saponins in Odogwu Bitters might explain the limited histological changes observed in Groups B and C. However, the presence of terpenoids, even in trace amounts, has been linked to potential modulation of renal oxidative stress pathways, as shown in earlier nephroprotective studies.[27,30]

Results of the kidney function test
Kidney function tests revealed significant changes in serum creatinine, urea, and uric acid levels across experimental groups (Table 3). Groups D, F, and H showed elevated creatinine levels, indicative of renal stress at higher doses. Uric acid levels increased significantly in Groups B, D, F, H, and I, reflecting dose-dependent biochemical stress, while Groups E, G, and J exhibited no significant differences from the control. These variations align with the histological findings, where structural integrity was preserved despite biochemical fluctuations.
The biochemical alterations suggest that higher doses of these herbal mixtures may influence renal clearance mechanisms. Saponins and flavonoids, as noted in phytochemical analyses, could enhance renal oxidative resilience at moderate doses but impose stress when consumed excessively.[27,28]

Body and Kidney weight Analysis
The relative weight of the kidney did not differ significantly across groups (p = 0.697, Table 4). This finding supports the histological

Table 3: Result of creatinine, urea and uric acid analysis

GROUPS		Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
CONTROL GROUP A	Mean	.2325	51.0250	12.4825
	Std. Deviation	.00354	.03536	.00354
	N	2	2	2
EXPERIMENTAL GROUP B	Mean	.5250	47.4250	12.3425
	Std. Deviation	.03536	.03536	.00354
	Sig.	.001	.001	.006
EXPERIMENTAL GROUP C	Mean	3.0250	52.0250	12.5250
	Std. Deviation	.03536	.03536	.03536
	Sig.	.001	.001	.828
EXPERIMENTAL GROUP D	Mean	6.0250	43.0250	14.5825
	Std. Deviation	.03536	.03536	.00354
	Sig.	.001	.001	.001
EXPERIMENTAL GROUP E	Mean	1.3325	59.8250	12.5250
	Std. Deviation	.00354	.03536	.03536
	Sig.	.001	.001	.828
EXPERIMENTAL GROUP F	Mean	2.0250	68.5250	15.8925
	Std. Deviation	.03536	.03536	.00354
	Sig.	.001	.001	.001
EXPERIMENTAL GROUP G	Mean	2.0250	45.3250	12.5250
	Std. Deviation	.03536	.03536	.03536
	Sig.	.001	.001	.828
EXPERIMENTAL GROUP H	Mean	1.5250	42.0250	10.8825
	Std. Deviation	.03536	.03536	.00354
	Sig.	.001	.001	.001
EXPERIMENTAL GROUP I	Mean	2.6725	48.4250	12.3625
	Std. Deviation	.00354	.03536	.00354

	Sig.	.001	.001	.018
EXPERIMENTAL GROUP J	Mean	1.5250	54.4250	12.4225
	Std. Deviation	.03536	.03536	.00354
	Sig.	.001	.001	.471
Total	Mean	2.0912	51.2050	12.8542
	Std. Deviation	1.58355	7.90165	1.34794
	Sig.	.001	.001	.001

Data was analysed using paired t-test and values were considered significant at $p \leq .05$.

Table 4: Descriptive statistics of the relative weight of kidney

GROUPS	Mean	N	Std. Deviation
GROUPS A	.3760315	2	.03845855
GROUPS B	.3923530	2	.03673235
GROUPS C	.3703704	2	.02909904
GROUPS D	.2166556	2	.25339552
GROUPS E	.3627768	2	.01728628
GROUPS F	.3592902	2	.00780171
GROUPS G	.3723751	2	.00764050
GROUPS H	.3829528	2	.00731861
GROUPS I	.3380057	2	.01388811
GROUPS J	.3706386	2	.06237808
Total	.3541450	20	.07888775

results, indicating minimal structural impact. Moreover, all groups demonstrated consistent increases in body weight over the study period (Figure 11), suggesting adequate nutritional intake and general health maintenance. The weight gain observed might reflect a metabolic response to the phytochemicals' nutritional or therapeutic properties, as documented in prior studies on herbal formulations.[31]

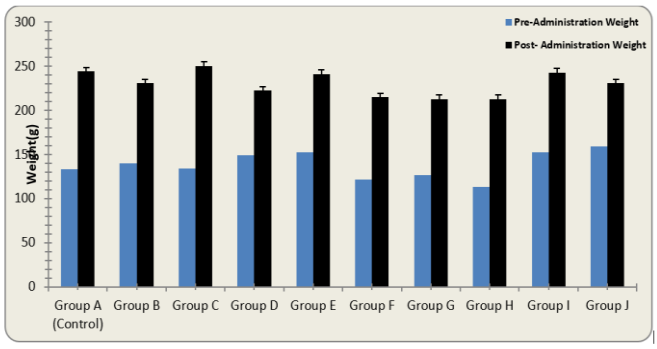

Figure 11: Effect of co-administration of Odogwu Bitters and Goko Cleanser on body weight of the animals

Conclusion

The histological and biochemical findings indicate that Odogwu Bitters and Goko Cleanser do not induce significant structural damage to kidney tissues, even at higher doses. However, the observed biochemical alterations at elevated concentrations underscore the importance of regulated consumption. The nephroprotective effects attributed to flavonoids and saponins highlight their therapeutic potential, but excessive intake may negate these benefits by imposing renal stress. Further studies are needed to elucidate the long-term implications and optimize dosage for safe use. These findings contribute to the growing body of evidence advocating for cautious and informed use of herbal products to maintain renal health.

Conflict of interest disclosure
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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