Original Research Article

Dual Herbal Impact: Biochemical and Nephrotoxic Effects of Odogwu Bitters and Goko Cleanser on Wistar Rat Kidneys

ABSTRACT

The rising popularity of herbal remedies, perceived as safer alternatives to synthetic drugs, has led to increased consumption of various herbal mixtures, including Odogwu Bitters and Goko Cleanser in Nigeria. This study investigates the biochemical and nephrotoxic effects of these herbal products when co-administered to adult male Wistar rats. A total of 40 rats were acclimatized and divided into ten groups, receiving varying doses of Odogwu Bitters and Goko Cleanser for 42 days. Biochemical analyses were performed to measure kidney function markers—blood urea nitrogen (BUN), creatinine, and uric acid. Results indicated significant alterations in these parameters; elevated creatinine and urea levels in certain experimental groups suggested potential nephrotoxic effects. Specifically, groups receiving higher doses of Goko Cleanser showed a marked increase in creatinine and urea levels compared to the control group ($P = .001$). Phytochemical analyses revealed the presence of saponins, flavonoids, and alkaloids, which may influence renal function and oxidative stress. Despite saponins being known for their nephroprotective properties, the overall findings underscore the dual nature of these herbal mixtures, where beneficial effects may coexist with risks of renal impairment. This research highlights the necessity for cautious consumption of herbal products and suggests further investigations into the long-term renal safety of these widely used herbal remedies.

KEYWORDS: Bitters, Creatinine, herbal drinks, kidney function test, Urea, Uric acid.

INTRODUCTION

In recent years, the use of herbal remedies has gained popularity globally, largely due to a perception that natural products may have fewer side effects than synthetic drugs [1]. Herbal products can be a source of very potent toxins, especially when contaminated [2]. Herbal drinks are common across different societies and cultures, varying in their contents, recipes and proposed actions [3]. Herbal mixtures offer a variety of benefits, ranging from an increase in sexual performance, treatment of several diseases and even aid in weight loss [4-6]. In Nigeria, these mixtures have been widely distributed, with more brands emerging every day. Herbal bitters are alcohol-based herbal drinks which are often used as a digestive aid and an appetite stimulant [7,8]. Among these are herbal mixtures such as Odogwu Bitters and Goko Cleanser, widely marketed for their purported health benefits, including detoxification and enhancement of bodily functions. Both products are commercially available and have become particularly popular in West African countries, where they are frequently used either alone or in combination. However, despite their widespread use, scientific research on the safety and biochemical effects of these formulations is limited.

Odogwu bitters, which is a brand of bitters, contains various ingredients which include bitter leaf, African basil, ginger, bitter kola, lemon grass, honey and alcohol [9, 10]. These ingredients are commonly believed to have antioxidants and anti-inflammatory properties, which theoretically might provide some protection to organs such as the liver and kidneys. Goko cleanser, is one of the popular herbal mixtures used by the Nigerian populace [11]. This mixture offers to aid in weight loss, prevention of the development of diabetes and hypoglycemia, detoxification and even in the treatment of several infections, including urinary tract infections. It contains active

ingredients like *Vernonia Amygdalina, Saccharum Officinalis, Allium Sativum and Cajanus Cajan* [12]. The use of these products in tandem may be expected to produce combined or even synergistic effects, which, while potentially beneficial, could also pose a risk for toxicity, particularly to the kidneys, the primary organs responsible for filtering and excreting waste products from the body [13].

Reports from similar studies indicate that certain herbal combinations can induce oxidative stress in the kidneys, leading to cellular damage if antioxidant defenses are insufficient [14]. Additionally, some herbal products may have toxic components like alkaloids, tannins, or heavy metals that could contribute to renal toxicity when used over extended periods or in combination with other substances [15]. Thus, the biochemical impact of co-administering Odogwu Bitters and Goko Cleanser on kidney health is an area warranting close examination to understand potential interactions and toxicity levels, especially given their increasing popularity and the risks associated with self-medication and high-dose consumption. Examining the biochemical parameters related to kidney functions such as blood urea nitrogen (BUN), creatinine, and electrolyte levels provides insight into potential nephrotoxic effects [16]. Alterations in these parameters following exposure to herbal preparations may indicate oxidative stress or inflammation within renal tissue. Some studies have suggested that specific herbal components in products like Odogwu Bitters and Goko Cleanser might influence these parameters, either by conferring antioxidant protection or by causing damage due to the accumulation of toxic metabolites [17].

The kidneys are especially vulnerable to toxic effects from herbal products due to their role in filtering blood and excreting metabolites, which can concentrate potentially harmful compounds. In the context of herbal medicine, nephrotoxicity is a serious concern, especially when multiple herbs with unknown interactions are used simultaneously [18]. Studies on animal models, including Wistar rats, are often conducted to assess the effects of herbal substances on kidney function. Wistar rats are commonly chosen for such studies due to their physiological and biochemical similarities to humans, making them an appropriate model for toxicological research [19].

Research using animal models, like the Wistar rat, offers insights into the biochemical effects of these herbal supplements on kidney health. Wistar rats are commonly used in biomedical research due to their well-understood physiology and their sensitivity to various substances. Studies on the co-administration of Odogwu Bitters and Goko Cleanser may investigate parameters such as blood urea nitrogen (BUN), creatinine levels, antioxidant enzymes (e.g., catalase, superoxide dismutase), and markers of oxidative stress to assess kidney function and health. Such biochemical markers are indicators of kidney integrity and functionality, and alterations in these values could suggest potential nephrotoxicity. Given the high prevalence of herbal product usage, especially in regions where access to conventional medical care is limited, assessing the safety of these products is essential. This study aims to evaluate the biochemical effects of co-administering Odogwu Bitters and Goko Cleanser on the kidneys of adult male Wistar rats, providing insight into the potential renal risks or benefits of such combined herbal use.

MATERIALS AND METHOD

Materials

Animals

A total of 40 adult male Wistar rats were used for this study. The rats weighed between 195g to 230g. The rats were supplied by a local farm (in Nsukka, Enugu state, Nigeria), and were allowed to acclimatize for period of one week with free access to food and water. The health status of the animals was certified by a veterinarian before it was humanely transported to the research facility.

The rats were housed in a spacious well-ventilated stainless-steel cage under normal temperatures (27^{o}-31^{o}C) with 12-hour dark / light cycle throughout the course of the experiment. The health status of the rats was closely monitored during the experiment. The rats were fed with standard diet and water. The floors of the cages were sprinkled with sawdust and the cages cleaned every day to prevent infections.

All the procedures were carried out in strict compliance with the guidelines of the ethics committee of Faculty of Basic Medical Sciences of Nnamdi Azikiwe University, Nnewi campus, Nigeria.

Feed, reagents, chemicals and drugs

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); Dragendorff's reagent; Benedict's reagent; Analytical grade reagents (produced by Syntron Bioresearch Incorporated, United States of America).

Methods

Experimental Protocol

The rats were weighed using a 5kg capacity weighing scale after two weeks of the acclimatization period. The rats were divided into 10 groups with four rats in each group labelled group A-J. The herbal drinks were administered twice daily – morning and evening. Odogwu Bitters was administered in the morning whereas Goko Cleanser was administered in the evening which lasted for 42 days. All administrations were done orally by carefully inserting a syringe with cannula affixed on it into the oral cavity of the rat.

Group A served as the control group and was fed with water and feed only. Group B received 0.2ml of Odogwu Bitters herbal mixture daily while group C received 0.4ml of Odogwu Bitters herbal mixture daily and Group D received 0.8ml of the Odogwu Bitters herbal mixture daily. Group E received 0.2ml of Goko Cleanser herbal mixture daily while Group F received 0.5ml of Goko Cleanser herbal mixture daily and Group G received 0.9ml of Goko Cleanser herbal mixture daily. Group H received 0.2ml of both Odogwu Bitters and Goko Cleanser herbal mixtures daily while Group I received 0.4ml and 0.5ml of both Odogwu Bitters and Goko Cleanser herbal

mixtures respectively daily and Group J received 0.8ml and 0.9ml of both Odogwu Bitters and Goko Cleanser herbal mixtures respectively daily.

Acute Toxicity Test

The median lethal dose (LD-50) of Odogwu Bitters was carried determined by using Dietrich Lorke's method [20].

Animal Euthanasia

The rats were humanely euthanized via cervical dislocation.

Blood collection

Blood samples were collected via ocular puncture into plastic plain tubes. The blood samples were allowed to stand for 20 minutes to ensure complete clotting. The clotted blood sample was centrifuged (using 800D Electric Centrifuge Machine with 4000RPM W/ 6X20Ml Rotor capacity) at 2500rpm (rotary per minutes) for 10 min and clear serum samples were aspirated off and stored frozen at -2^0C until required for biochemical analysis.

Phytochemical analysis

The following constituents were qualitatively and quantitatively evaluated – saponins, tannins, flavonoids, steroids, alkaloids, cardiac glycosides, reducing sugars, proteins, carbohydrates, and terpenoids. The study employed standard phytochemical analyses procedures [21].

Kidney Function Test

The biochemical parameters determined on the sera specimen include urea, creatinine and uric acid. The study adopted the procedure documented by for kidney function test [22].

Data Analysis

Data were descriptively and inferentially analyzed. The difference between the control group and the experiment groups were analyzed using a two-way analysis of variance (ANOVA). Independent samples t-test was employed to compare between groups. The confidence level for the hypothesis test was set at 95%.

Duration of the study

This study was carried out within three months. The first two weeks was used for LD-50 determination, phytochemical analysis of the herbal drinks, and acclimatization of the animals; the following six weeks was used for administration of the herbal drinks which was followed by biochemical that lasted for two weeks; and then, the two weeks was used for statistical analysis and documentation of findings.

RESULTS

Results of the kidney function test

The descriptive statistics of the creatinine test showed a high negative mean difference between the control group and group D (Table 1; Table 2). The descriptive statistics of the urea test showed a high negative mean difference between the control group and groups E and F; and a high positive mean difference between the control and groups D, G, and H (Table 1; Table 3). The descriptive statistics of the uric acid test showed a high negative mean difference between the control group

and groups D and F; and a high positive mean difference between the control group and group H (Table 1; Table 4).

The ANOVA result of the kidney function test showed a statistically significant difference (P = .001) between the control and experimental groups for creatinine, urea and uric acid (Table 5). The kidney function test was further analysed independently using t-test. The independent comparison result showed that there was a significant difference between control and experimental groups for creatinine and urea (Tables 2 and 3). The independent comparison result for uric acid showed that there was a significant difference between the control group and groups B (p = .006), D (p = .001), F (P = .001), H (P = .001) and I (P = .018); but showed a non-significant difference between the control group and groups C (P = .828), E (P = .828), G (P = .828) and J (P = .471) (Table 4).

Results of Phytochemical analysis of Goko Cleanser and Odogwu Bitters

Results from both the qualitative and quantitative analyses of Goko Cleanser herbal mixture showed that it contained saponin, flavonoids and tannin (Table 6; Table 7). However, saponin had the highest concentration (7%), followed by flavonoids (4%), and tannin (0.2) with the least concentration in the herbal drink (Table 7).

The results of the qualitative and quantitative phytochemical analyses of Odogwu Bitters showed that it contained traces of saponin (0.13%), alkaloid (0.09%), terpenoid (0.30), flavonoid (0.12), carbohydrate, cardiac glycosides, and reducing sugar (0.11) (Table 8; Table 9). However, the quantitative analyses could not compute the amount of cardiac glycoside and carbohydrate in the herbal drink (as seen in Table 9).

DISCUSSION AND CONCLUSIONS

Discussion

The results of the kidney function test indicate notable differences between the control and experimental groups in levels of creatinine, urea, and uric acid. Elevated levels of these biomarkers can signal impaired kidney function, as the kidneys are responsible for their clearance from the bloodstream. Creatinine, a waste product from muscle metabolism, is widely used to evaluate renal function. In this study, the control group displayed a significant negative mean difference in creatinine compared to group D, implying reduced clearance and suggesting potential nephrotoxicity in this group. This aligns with prior research demonstrating the role of elevated serum creatinine in indicating kidney stress or injury, especially when associated with chronic herbal or medicinal intake [23, 24].

In terms of urea, a byproduct of protein metabolism excreted by the kidneys, similar patterns emerged. Groups E and F showed a high negative mean difference from the control, indicating elevated urea levels and pointing to potential renal impairment. Conversely, groups D, G, and H exhibited a positive mean difference, suggesting improved urea clearance or metabolic response. Studies have shown that various factors, such as the specific type of herbal supplements or their components, can influence urea metabolism, either enhancing or diminishing renal function [25, 26].

Uric acid results further underscore the variability in response among the experimental groups. High levels of uric acid, typically associated with conditions like gout, may also indicate kidney stress due to reduced filtration capacity. The observed positive mean difference in uric acid in groups D and H relative to the control group suggests a possible strain on renal excretory function

in these groups, while the negative mean differences seen in groups D and F could indicate either a protective effect or differences in metabolic response. These findings align with studies linking certain phytochemicals with both uric acid reduction and excretory modulation in the kidneys [27].

The ANOVA analysis showed statistically significant differences ($P = 0.001$) between the control and experimental groups across all tested markers (creatinine, urea, and uric acid). This outcome underscores a distinct overall response to the interventions across the groups. Furthermore, independent t-tests provided detailed insight, showing specific significant differences between the control group and experimental groups for creatinine and urea (Tables 2 and 3). Interestingly, the results for uric acid demonstrated group-specific variations, with significant differences found between the control and groups B, D, F, H, and I, while no significant differences were noted with groups C, E, G, and J. This variability may suggest differential metabolic or renal responses to the herbal compounds tested, potentially linked to individual phytochemical profiles and their interactions with kidney function [28, 29].

The phytochemical analysis results for the Goko Cleanser and Odogwu Bitters provide additional context. Goko Cleanser contained saponins, flavonoids, and tannins, with saponins in the highest concentration (7%). Saponins are known for their nephroprotective properties, as well as for anti-inflammatory and diuretic effects, which may influence renal function markers like creatinine and urea [30]. Flavonoids, present at 4% in Goko Cleanser, are also associated with antioxidant and nephroprotective effects, which could mitigate oxidative stress on the kidneys and influence biomarker levels [31]. The low tannin content (0.2%) suggests minimal impact on kidney function,

as tannins have been linked to both protective and adverse renal effects depending on dosage and duration of intake [32].

The phytochemical profile of Odogwu Bitters, though different, included traces of saponins (0.13%), alkaloids (0.09%), terpenoids (0.30%), flavonoids (0.12%), carbohydrates, cardiac glycosides, and reducing sugars. Saponins, even in low concentrations, may have contributed to renal modulation in certain experimental groups. Alkaloids, although known for diverse biological activities, have been associated with nephrotoxicity in some cases, particularly when present in herbal supplements consumed regularly [33]. Terpenoids, known for their anti-inflammatory properties, could play a role in reducing renal inflammation, thus influencing markers like urea and uric acid [34]. However, the precise effect of Odogwu Bitters on kidney function remains complex, as the phytochemical diversity likely leads to multiple overlapping effects on renal physiology, both protective and adverse.

Conclusions

The study results highlight significant differences in kidney function markers - creatinine, urea, and uric acid between the control and experimental groups, indicating varied renal responses to herbal supplements tested. Elevated creatinine and urea levels in some experimental groups suggest potential nephrotoxic effects, aligning with established knowledge of these markers as indicators of kidney stress. The variations in uric acid levels further point to differential responses in renal excretory function, potentially due to the distinct phytochemical compositions in the tested herbal mixtures.

The phytochemical analysis of Goko Cleanser and Odogwu Bitters revealed active compounds known to influence renal function, such as saponins, flavonoids, and tannins, which are generally associated with nephroprotective or diuretic effects. Odogwu Bitters contained additional components like alkaloids and terpenoids, which may contribute to complex renal responses. These findings underscore the need for cautious use of these herbal mixtures, as some constituents may exert both protective and adverse effects on kidney function, dependent on concentration and metabolic interaction.

Overall, the study emphasizes the potential renal impacts of chronic intake of herbal supplements and supports further investigation into the safe dosage and long-term effects of specific phytochemicals to better understand their implications for kidney health.

ABBREVIATIONS

ANOVA – Analysis of variance

Co – Company

LD – Lethal dose

Ltd – Limited

Mls – Mili litres

RPM – Rotary per minutes

SD – Standard deviation

Sig - Significance

Std – Standard

Declarations

This research article is an original article. It has not been submitted for review to another journal and has not been published in any journal or conference proceedings.

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

Ethics approval

The Ethical Approval was obtained from the Ethics Committee of the Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra state. The approval number is COOU/BMS/008.

Availability of data and materials

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

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Table 1: Descriptive statistics of creatinine, urea and uric acid analysis.

GROUPS		Creatinine	Urea	Uric acid
CONTROL GROUP A	Mean	.2325	51.0250	12.4825
	N	2	2	2
	Std. Deviation	.00354	.03536	.00354
EXPERIMENTAL GROUP B	Mean	.5250	47.4250	12.3425
	N	2	2	2
	Std. Deviation	.03536	.03536	.00354
EXPERIMENTAL GROUP C	Mean	3.0250	52.0250	12.5250
	N	2	2	2
	Std. Deviation	.03536	.03536	.03536
EXPERIMENTAL GROUP D	Mean	6.0250	43.0250	14.5825
	N	2	2	2
	Std. Deviation	.03536	.03536	.00354
EXPERIMENTAL GROUP E	Mean	1.3325	59.8250	12.5250
	N	2	2	2
	Std. Deviation	.00354	.03536	.03536
EXPERIMENTAL GROUP F	Mean	2.0250	68.5250	15.8925
	N	2	2	2
	Std. Deviation	.03536	.03536	.00354
EXPERIMENTAL GROUP G	Mean	2.0250	45.3250	12.5250
	N	2	2	2

	Std. Deviation	.03536	.03536	.03536
EXPERIMENTAL GROUP H	Mean	1.5250	42.0250	10.8825
	N	2	2	2
	Std. Deviation	.03536	.03536	.00354
EXPERIMENTAL GROUP I	Mean	2.6725	48.4250	12.3625
	N	2	2	2
	Std. Deviation	.00354	.03536	.00354
EXPERIMENTAL GROUP J	Mean	1.5250	54.4250	12.4225
	N	2	2	2
	Std. Deviation	.03536	.03536	.00354
Total	Mean	2.0912	51.2050	12.8542
	N	20	20	20
	Std. Deviation	1.58355	7.90165	1.34794

Table 2: Individual comparison of the groups for creatinine test

Dependent Variables	GROUPS	GROUPS	Mean Difference	Standard (Std.) Error	Significance (Sig.)	95% Confidence Interval	
						Lower Bound	Upper Bound
Creatinine	CONTROL GROUP A	GROUP B	-.29250*	.02964	.000	-.4471	-.1379
		GROUP C	-2.79250*	.02964	.000	-2.9471	-2.6379
		GROUP D	-5.79250*	.02964	.000	-5.9471	-5.6379
		GROUP E	-1.10000*	.02964	.000	-1.2546	-.9454
		GROUP F	-1.79250*	.02964	.000	-1.9471	-1.6379

		GROUP G	-1.79250*	.02964	.000	-1.9471	-1.6379
		GROUP H	-1.29250*	.02964	.000	-1.4471	-1.1379
		GROUP I	-2.44000*	.02964	.000	-2.5946	-2.2854
		GROUP J	-1.29250*	.02964	.000	-1.4471	-1.1379

* Represents values significant at $p \leq .05$

Table 3: Individual comparison of the groups for urea test

Dependent Variables	GROUPS	GROUPS	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Urea	CONTROL GROUP A	GROUP B	3.60000*	.03536	.000	3.4157	3.7843
		GROUP C	-1.00000*	.03536	.000	-1.1843	-.8157
		GROUP D	8.00000*	.03536	.000	7.8157	8.1843
		GROUP E	-8.80000*	.03536	.000	-8.9843	-8.6157
		GROUP F	-17.50000*	.03536	.000	-17.6843	-17.3157
		GROUP G	5.70000*	.03536	.000	5.5157	5.8843

		GROUP H	9.00000*	.03536	.000	8.8157	9.1843
		GROUP I	2.60000*	.03536	.000	2.4157	2.7843
		GROUP J	-3.40000*	.03536	.000	-3.5843	-3.2157

* Represents values significant at $p \leq .05$

Table 4: Individual comparison of the groups for uric acid test

Dependent Variables	GROUPS	GROUPS	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Uric Acid	CONTROL GROUP A	GROUP B	.14000*	.01959	.006	.0379	.2421
		GROUP C	-.04250	.01959	.828	-.1446	.0596
		GROUP D	-2.10000*	.01959	.000	-2.2021	-1.9979
		GROUP E	-.04250	.01959	.828	-.1446	.0596
		GROUP F	-3.41000*	.01959	.000	-3.5121	-3.3079

GROUP G	-.04250	.01959	.828	-.1446	.0596
GROUP H	1.60000*	.01959	.000	1.4979	1.7021
GROUP I	.12000*	.01959	.018	.0179	.2221
GROUP J	.06000	.01959	.471	-.0421	.1621

* Represents values significant at $p \leq .05$

Table 5: ANOVA Result of kidney function test

TESTS			Sum of Squares	df	Mean Square	F	Sig.
Creatinine	Between Groups	(Combined)	47.636	9	5.293	6023.197	.000*
	Within Groups		.009	10	.001		
	Total		47.645	19			
Urea	Between Groups	(Combined)	1186.272	9	131.808	105446.400	.000*
	Within Groups		.013	10	.001		

	Total	1186.285	19			
Uric acid	Between Groups (Combined)	34.518	9	3.835	9994.434	.000*
	Within Groups	.004	10	.000		
	Total	34.522	19			

* Represents values significant at $p \leq .05$

df = Degrees of freedom

Table 6: Result of Qualitative Analysis of Goko Cleanser herbal mixture

Constituents	**Goko Cleanser Herbal mixture**	**Interpretation**
Saponin	++	Moderately present
Flavonoid	+	Present in trace
Tannin	+	Present in trace
Alkaloid	-	Absent
Steroid	-	Absent
Reducing sugar	-	Absent

Cardiac glycoside	-	Absent
Protein	-	Absent
Carbohydrate	-	Absent
Terpenoid	-	Absent

Table 7: Result of Quantitative Analysis of Goko Cleanser herbal mixture

Phyto-constituents	**Quantity (% w/v)**
Saponin	7.35
Flavonoid	4
Tannin	0.2

Table 8: Result of Qualitative analysis of Odogwu Bitters

Phyto-constituents	Odogwu Bitters	Interpretation
Saponin	+	Present in trace
Alkaloid	+	Present in trace
Terpenoid	+	Present in trace
Cardiac glycoside	+	Present in trace
Flavonoid	+	Present in trace
Carbohydrate	+	Present in trace

Reducing sugar	+	Present in trace
Protein	-	Absent
Steroid	-	Absent
Tannin	-	Absent

Table 9: Result of quantitative analysis of Odogwu Bitters

Phytochemical	**Quantity (% w/v)**
Saponin	0.13
Alkaloid	0.09
Terpenoid	0.30
Flavonoid	0.12

Reducing sugar	0.11