Evaluating the Biochemical Impact of D'General Bitters on Renal Function in Adult Male Wistar Rats

Abstract

The present study evaluating the Biochemical Impact of D'General Bitters on Renal Function in Adult Male Wistar Rats.Herbal preparations, including bitters, have garnered significant interest in alternative medicine due to their perceived therapeutic properties. D'General Bitters, a commercially available herbal formulation, is promoted for its purported detoxifying, anti-inflammatory, and antioxidant properties.Animal models, particularly Wistar rats, are frequently used to study the renal effects of herbal remedies. Wistar rats are preferred due to their physiological and genetic similarities to humans, as well as their ability to metabolize xenobiotics in a manner comparable to humans.The rats were weighed before the commencement of experiment using a 6000g capacity weighing scale (with model number WT6000GT produced by Want balance instrument company limited, China).The impact of D'General Bitters on renal function markers such as creatinine, urea, and uric acid is particularly revealing, as each marker provides insight into different aspects of kidney function and metabolic processes.The observed results suggest that while certain constituents may have potential health benefits, others could pose risks, especially concerning kidney function at higher doses or prolonged exposure. These effects are consistent with documented outcomes from similar herbal compounds, reinforcing the need for cautious use.

Keywords: genetic similarities, xenobiotics, blood urea nitrogen, kidney health

1. INTRODUCTION

The kidneys play a vital role in maintaining homeostasis by regulating fluid balance, electrolyte levels, and the elimination of metabolic waste products. They are particularly susceptible to biochemical alterations induced by both endogenous and exogenous substances. Renal function assessments often involve evaluating levels of key biomarkers, including serum creatinine, serum uric acid and blood urea nitrogen (BUN), which are critical indicators of glomerular filtration and overall kidney health [1].

Herbal preparations, including bitters, have garnered significant interest in alternative medicine due to their perceived therapeutic properties. D'General Bitters, a commercially available herbal formulation, is promoted for its purported detoxifying, anti-inflammatory, and antioxidant properties. However, the precise biochemical impact of D'General Bitters on renal physiology remains largely unexplored. This is particularly important as the kidneys are frequently involved in the detoxification of ingested compounds, making them susceptible to potential nephrotoxic effects [2].

Previous studies have indicated that certain plant-based formulations may induce either protective or adverse effects on renal function, often depending on the bioactive constituents and their metabolic pathways [3]. Common constituents in bitters include phytochemicals such as saponins, flavonoids, and alkaloids, which are known for their antioxidants and anti-inflammatory effects but may also pose nephrotoxic risks under prolonged or high-dose exposure [4,5].

Animal models, particularly Wistar rats, are frequently used to study the renal effects of herbal remedies. Wistar rats are preferred due to their physiological and genetic similarities to humans, as well as their ability to metabolize xenobiotics in a manner comparable to humans. Studies on rats have shown that exposure to various herbal extracts can lead to significant alterations in renal biomarkers, oxidative stress levels, and histopathological changes, providing insight into potential mechanisms of toxicity or protection [6-8].

Considering this, examining the effects of D'General Bitters on renal biomarkers and kidney tissue morphology in adult male Wistar rats could provide valuable insights into its safety and efficacy. Understanding these impacts is crucial, given the growing usage of herbal supplements globally, and it will inform safer consumption practices and guide further clinical research on herbal formulations' renal effects.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Animals

The animal models used for this study were 20 male adult Wistar rats. The rats weighed between 90g to 160g. 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, and when the animals were housed at the research facility. The rats were housed in comfortable spacious cages with a 12-hour dark / light cycle. The health status of the rats was closely monitored during the experiment. 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. The ethics approval number for this research is NAU/CHS/NC/FMBS/573, dated 4th August 2023.

2.1.2 Feed, reagents, chemicals and drugs

The following materials were used for to carry out this study: D'General Bitters herbal drink supplied by the sole distributor in Nkwo Nnewi market, Nnewi; Top feeds Grower's mash Super-Deluxe Animal Feed produced be Eastern Premier Feed mills Ltd, a subsidiary of Premier Feeds Mills Co. 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 Lab. Chemicals, India); Eosin (produced by Kem Light Lab. India); Dragendorff's reagent; Benedict's reagent; Analytical grade reagents (produced by Syntron Bioresearch Inc., USA).

2.2 Methods

2.2.1 Experimental Protocol

The rats were divided into four groups of five rats each; and then housed in four big, meshed cages. Group A served as the control group while groups B, C and D served as the experiment groups. Rats in group A were fed with water and feed only. Group B received 0.2ml of D'General Bitters solution daily, group C received 0.4mls of D'General Bitters solution daily, and Group D received 0.8mls of D'General Bitters daily.

The rats were weighed before the commencement of experiment using a 6000g capacity weighing scale (with model number WT6000GT produced by Want balance instrument company limited, China). The rats were fed *ad-libitum* on standard pelleted mash and clean tap-water during the entire course of the acclimatization and experiment periods. The cages and environment were kept clean and disinfected daily.

2.2.2 Acute Toxicity Test

The median lethal dose of D'General Bitters was carried determined by using Dietrich Lorke's method [9].

2.2.3 Animal Euthanasia

The rats were humanely euthanized via humane cervical dislocation.

2.2.4 Blood collection

Blood samples were collected via ocular puncture into plastic plain tubes. The blood samples were allowed to stand for 30 mins 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.

2.2.5 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 [10].

2.2.6 Kidney Function Test

The biochemical parameters analyzed in the serum samples included urea, creatinine, and uric acid. The study followed the established procedures outlined for kidney function testing [11].

2.3 Statistical Analysis

The data collected in this study were analyzed with IBM's Statistical Package for Social Sciences (SPSS) version 25. A 95% confidence level was used for the hypothesis testing. Both descriptive and inferential analyses were conducted. A two-way ANOVA was applied to examine the differences between the control and experimental groups.

2.4 Duration of the study

The study spanned a total of 12 weeks, with a two-week acclimatization period for the rats, followed by a six-week experimental phase. Data analysis was conducted over the final four weeks.

3. RESULTS

3.1 Impact of D'General Bitters on Levels of Urea, Creatinine, and Uric Acid

Creatinine levels were markedly elevated in groups C and D relative to the control, with a modest elevation observed in group B (Figure 1). Statistical analysis confirmed that the increase was significant exclusively for group C, while the elevations in groups B and D did not reach statistical significance (Table 1).

Urea levels in Groups B and C were markedly elevated relative to the control, whereas Group D demonstrated a significant reduction (Figure 2). Statistical analysis confirmed these variations to be significant across all experimental groups (Table 1).

Uric acid levels were markedly decreased in Groups C and D, while a slight elevation was observed in Group B relative to the control (Figure 3). Hypothesis testing confirmed these changes as statistically significant across all experimental groups (Table 1).

3.2 Phytochemical analysis results of D'General Bitters

The phytochemical analysis of D'General Bitters identified multiple compounds, such as proteins (0.07 mg/ml), alkaloids (0.23% w/v), flavonoids (0.4% w/v), reducing sugars (0.16% w/v), glycosides, and amino acids (refer to Tables 2 and 3). However, the study did not quantify the exact levels of amino acids and glycosides present in the herbal drink (Table 3).

4. DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 Discussion

The impact of D'General Bitters on renal function markers such as creatinine, urea, and uric acid is particularly revealing, as each marker provides insight into different aspects of kidney function and metabolic processes.

Creatinine, a waste product formed from muscle metabolism, is a primary marker for assessing renal function. Elevated levels of creatinine are indicative of compromised kidney function, as the kidneys are primarily responsible for filtering creatinine out of the blood. The significant elevation in creatinine levels observed in group C, with a modest, non-significant increase in groups B and D, suggests a potential nephrotoxic effect associated with certain dosages or durations of D'General Bitters administration. This pattern aligns with findings in related studies on herbal supplements, where certain phytochemicals in high concentrations can impair kidney function by causing oxidative stress or by interfering with renal filtration processes [12, 13].

Urea levels, another critical indicator of renal function, were markedly elevated in groups B and C, whereas a significant reduction was observed in group D. The elevated urea in groups B and C may indicate a disruption in protein metabolism or reduced renal clearance, aligning with findings in studies that connect some herbal compounds to increased nitrogenous waste products[14]. Interestingly, the reduction in urea levels in group D might be attributed to a potential adaptive response of the kidneys to prolonged or different exposure levels to D'General Bitters, although this warrants further investigation.

The observed decrease in uric acid levels in groups C and D, along with a slight increase in group B, underscores an intriguing biochemical modulation by D'General Bitters on purine

metabolism. While elevated uric acid is often associated with gout and other metabolic issues, decreased levels could imply enhanced clearance or altered metabolism. Notably, certain phytochemicals, particularly flavonoids and alkaloids, are known for their uricosuric properties, potentially explaining these findings [15]. However, the pharmacokinetics and dose-dependent effects of D'General Bitters on uric acid metabolism should be investigated further to clarify these results.

The phytochemical profile of D'General Bitters revealed compounds like flavonoids, alkaloids, glycosides, and proteins, each known to influence metabolic pathways and renal function. Flavonoids, for instance, are documented for their antioxidant properties, which might modulate oxidative stress in renal tissues. Alkaloids, while beneficial in some therapeutic contexts, can exert nephrotoxic effects if present in high concentrations or under prolonged exposure [12, 16]. The presence of reducing sugars and glycosides could further interact with kidney functions through their influence on cellular metabolism and membrane permeability.

4.2 Conclusions

This study provides preliminary evidence that D'General Bitters may significantly impact renal function markers, with varied effects on creatinine, urea, and uric acid levels, likely due to its complex phytochemical composition. The observed results suggest that while certain constituents may have potential health benefits, others could pose risks, especially concerning kidney function at higher doses or prolonged exposure. These effects are consistent with documented outcomes from similar herbal compounds, reinforcing the need for cautious use.

4.3 Recommendations

1. Dosage Control and Monitoring: Given the renal implications observed, it is advisable that consumers adhere strictly to recommended dosages, avoiding prolonged or excessive use of D'General Bitters.
2. Further Research: Long-term studies with varied doses are recommended to fully understand the nephrotoxic potential and dose-dependent effects of D'General Bitters, especially on renal filtration markers.
3. Regulation and Labeling: Standardized labeling to detail the concentrations of active phytochemicals could better inform consumers and healthcare providers about potential health impacts.
4. Clinical Assessment: Healthcare professionals should consider inquiring about herbal supplement usage in patients with altered renal function markers and advise accordingly.

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 arecommonly and predominantly used products inour area of research and country. There isabsolutely no conflict of interest between theauthors and producers of the products becausewe do not intend to use these products as anavenue for any litigation but for the advancementof knowledge.

Ethics approval and consent to participate

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/579, on the 27th of July 2023.All the authors gave full consent to participate in the study.

Consent for publication

Authors enlisted in this manuscript have given full consent for this draft article to be submitted to the Asian Journal of Research and Reports in Urology.

Availability of data and materials

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

Competing interests

Authors have declared that no competing interests exist.

Authors' contribution

This work was carried out in collaboration of all authors; and all authors read and approved the final manuscript. Author Darlington Nnamdi Onyejike (DNO) conceptualized the study, designed the study, wrote the experimental protocol, supervised the experiment, carried out the data analysis and wrote the first draft of the manuscript. Author Ugochukwu Samuel Aguwa (USA) reviewed the draft. Author Fortune

Oghenekaro Abrukwe (FOA) carried out the experiment, managed the animals, managed the literature searches and curated the data.

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Table 1: Impact of D'General Bitters on Serum Creatinine, Urea, and Uric Acid Level

Groups	Creatinine (mg/dl)		Urea (mg/dl)		Uricacid (mg/dl)	
	MEAN±STD	P-value	MEAN±STD	P-value	MEAN±STD	P-value
Group A	0.33±0.01	-	41.83±3.60	-	10.80±1.20	-
Group B	0.59±0.02 [a]	0.696	46.78±0.57[#]	0.048	12.67±0.73[#]	0.014
Group C	2.17±1.58[#]	0.024	53.14±1.73[#]	0.001	12.64±0.24[#]	0.015
Group D	1.30±0.27 [a]	0.179	31.59±3.26[#]	0.001	12.83±0.33[#]	0.009
F-ratio	3.114	-	36.864	-	5.159	-

Data was analyzed using ANOVA followed by post Hoc LSD comparison and values were significant at $P \leq 0.05$. [#]: significant, [a]: not significant, STD: standard deviation.

Table 2: Phytochemical test of D'General Bitters (Qualitative test)

Phytochemical constituent	Test (s)	D' General Bitters
ALKALOIDS	Mayer's	-
	Dragendorf's	+
	Wagner's	
	Hager's	
FLAVONOIDS	Lead acetate test	+
	Alkaline reagent test	-
REDUCING SUGARS	Benedict's test	+
	Fehling's test	+
SAPONINS	Frothing test	-
PROTEINS	Millon's test	+
	Precipitation test	
	Lead acetate	+

	Copper sulphate	+
TANNINS	Ferric Chloride test	-
AMINO ACIDS	Ninhydrin test	+
STEROIDS	Salkowski test	-
TRITERPENOIDS	Salkowski test	-
GLYCOSIDES	General test	+

Table 3: Phytochemical test of D'General Bitters (Quantitative test)

Phytochemical	**Quantity**
Protein	0.07 mg/ml
Alkaloid	0.23 % w/v
Flavonoid	0.4 % w/v
Reducing sugar	0.16 % w/v

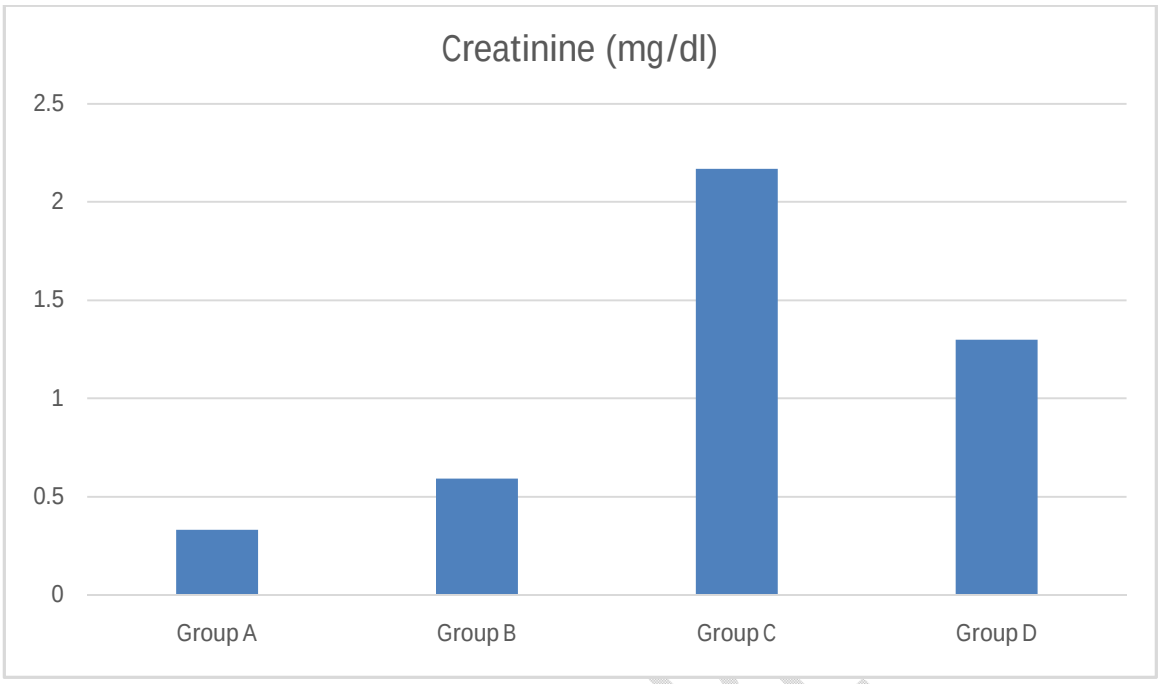

Figure 1: Chart showing the creatinine level of the groups

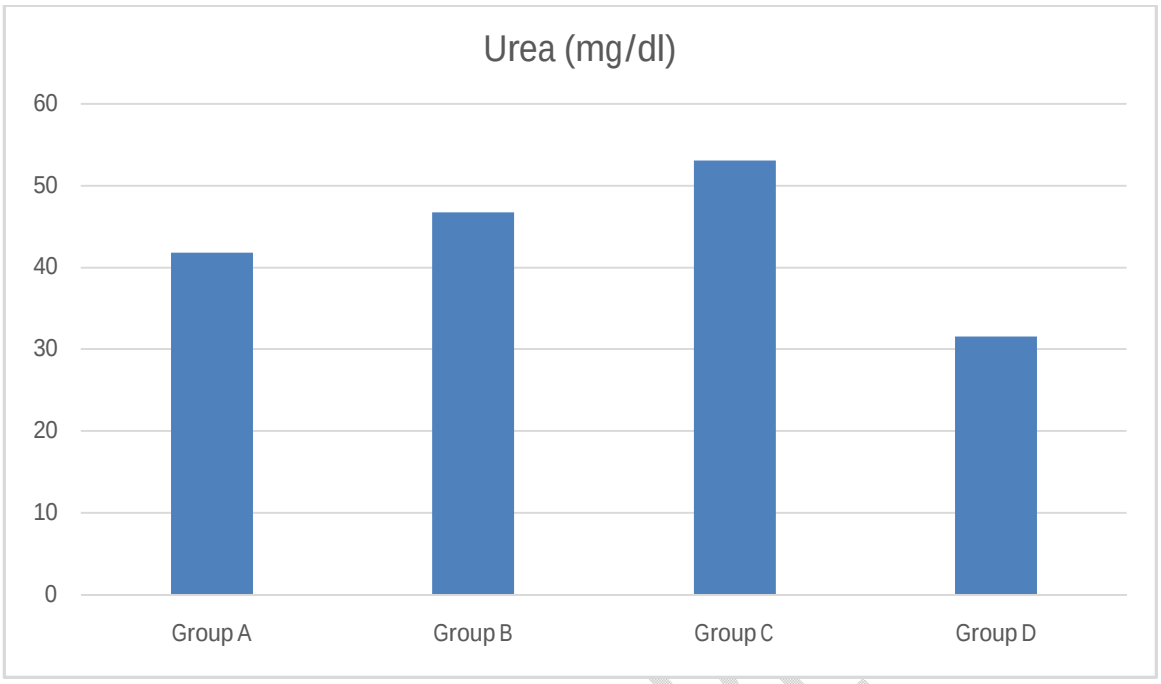

Figure 2:Chart showing urea level of the groups

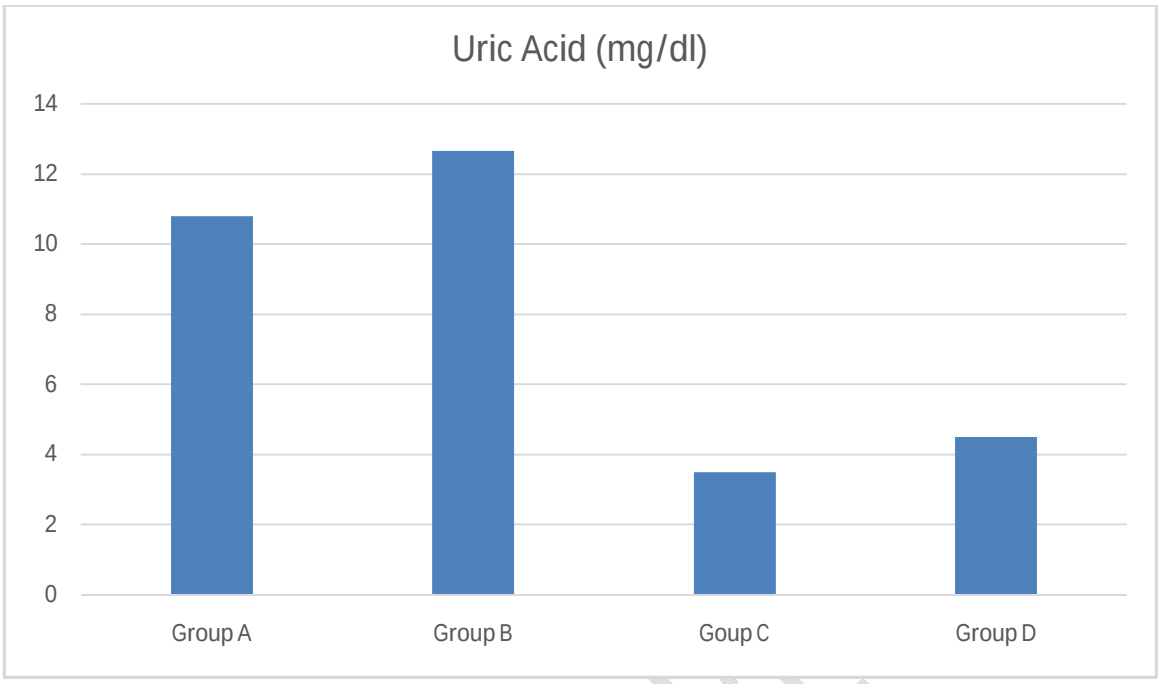

Figure 3:Chart showing uric acid level of the groups