Evaluating the Biochemical Impact of D'General Bitters on Renal Function in ~~Adult~~ Male Wistar Rats: A Preliminary Study.

Abstract

Background: Herbal preparations, including bitters, have gained attention for their potential therapeutic properties, but their effects on renal function has not been fully elucidated~~remain underexplored~~. D'General Bitters is a commercially available herbal formulation promoted for its detoxifying, anti-inflammatory, and antioxidant effects.

Objective: This study aimed to evaluate the biochemical impact of D'General Bitters on renal function in adult male Wistar rats.

Materials and Methods: Forty male Wistar rats were divided into four groups, with the control group receiving only water and feed. The ~~E~~xperimental groups received varying doses (0.2 ml, 0.4 ml, and 0.8 ml) of D'General Bitters daily for 6 weeks. Phytochemical analysis was performed to identify bioactive compounds in the herbal drink. Renal function was assessed by measuring serum levels of urea, creatinine, and uric acid.

Results: Phytochemical analysis revealed the presence of alkaloids, flavonoids, proteins, reducing sugars, and glycosides in D'General Bitters. Significant elevations in serum creatinine levels were observed in groups C and D, indicating potential renal stress. Urea levels were significantly increased ($p<0.05$)in groups B and C, while a significant ($p<0.05$)reduction was noted in group D. Uric acid levels decreased in groups C and D, suggesting potential therapeutic effects on uric acid metabolism.

Conclusions: The findings suggest that D'General Bitters may alter renal biomarkers in a dose-dependent manner, with potential nephrotoxic effects at higher doses. However, the reduction in urea levels in the highest dose group may indicate a protective effect on kidney function. Further studies are needed to elucidate the mechanisms underlying these effects and to assess the long-term safety of D'General Bitters for renal health.

Keywords: Creatinine, Nephrotoxicity, Phytochemicals, Renal function, Urea, Uric acid.

1. INTRODUCTION

The kidney plays a vital role in maintaining homeostasis by regulating fluid balance, electrolyte levels, and the elimination of metabolic waste products [1,2]. The kidney is particularly susceptible to biochemical alterations induced by both endogenous and exogenous substances [3]. 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 [4].

Herbal preparations, including bitters, have garnered significant interest in alternative medicine due to their perceived therapeutic properties. For instance, bitters have been linked to improved digestive health and modulation of the gut microbiota [5]. Studies have also highlighted their potential anti-inflammatory, antioxidant, and hepatoprotective effects [6,7]. 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 [8].

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 [9]. 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 [10,11].

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 [12-14].

Considering this, a preliminary study was carried out to examine the effects of D'General Bitters on renal biomarkers in adult male Wistar rats to 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 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, Anambra state, Nigeria. The ethics approval number for this research is NAU/CHS/NC/FMBS/579, dated 27th of July 2023.

2.1.2 Feed, herbal drink and reagents

The materials utilized for this study include the following:

Herbal Drink: D'General Bitters herbal drink, obtained from the sole distributor at Nkwo Nnewi Market, Nnewi, Nigeria.

Animal Feed: Top Feeds Grower's Mash Super-Deluxe Animal Feed, produced by Eastern Premier Feed Mills Ltd., a subsidiary of Premier Feeds Mills Co. Ltd., Plateau State, Nigeria.

Analytical Reagents: Dragendorff's reagent, Benedict's reagent, and other analytical-grade chemicals produced by Syntron Bioresearch Inc., USA.

All materials were of analytical grade, ensuring suitability for the experimental protocols.

2.2 Methods

2.2.1 Duration of the study

The study was conducted over a 12-week period, comprising three distinct phases: a two-week acclimatization period for the rats, a six-week experimental phase, and a subsequent four-week period dedicated to data analysis.

2.2.2 Acute Toxicity Test

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 [15]. 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.

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.

Results of LD_{50} Determination of D'General 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 behavior.

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=√AB

A=Maximum dose with 0% mortality

B= Minimum dose with 100% mortality

LD50= √1600×2900=2154.17mg/kg

2.2.3 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. Colorimetric or spot tests were used for the qualitative analysis which involved the use of specific reagents to produce color changes or precipitates indicative of phytochemicals. 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 [16].

2.2.4 Experimental Design

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.

2.2.5 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 anesthesia overdose (CO gas). 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). The carcasses of the Wistar rats were disposed of following institutional biosafety protocols.

2.2.6 Blood collection

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 kidney function assay.

2.2.7 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 colored complex. The strength of this color, 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 [17].

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 one-way ANOVA was applied to examine the differences between the control and experimental groups.

3. RESULTS

3.1 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).

3.2 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). Creatinine levels showed a significant elevation in group C compared to control while its level was not significantly elevated in groups B and D compared with control (Table 1).

Urea levels in Groups B and C were markedly elevated when compared to the control, whereas Group D demonstrated a significant reduction (Figure 2). There was a statistically significant difference between the control and all test groups (Table 1).

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

4. DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 Discussion

The phytochemical analysis of D'General Bitters revealed the presence of various bioactive compounds, including proteins, alkaloids, flavonoids, reducing sugars, glycosides, and amino acids. The quantification of these compounds indicates a diverse chemical profile that may contribute to the therapeutic properties of the product. Previous studies have shown that alkaloids and flavonoids exhibit various pharmacological effects, including antioxidant, anti-inflammatory, and antimicrobial properties [18]. The presence of these compounds in D'General Bitters suggests potential health benefits, but the exact mechanisms through which these compounds influence physiological parameters require further investigation. Notably, the exact

concentration of amino acids and glycosides was not quantified, which limits the ability to make specific conclusions about their contributions to the observed effects.

Regarding the biochemical markers, the results suggest significant alterations in renal function among the experimental groups. Creatinine levels were significantly elevated in Groups C and D compared to the control, which may indicate altered kidney function or renal stress. Elevated creatinine levels are typically associated with impaired renal filtration, suggesting that the intake of D'General Bitters may influence renal function. The modest elevation in Group B could reflect a dose-dependent effect, which warrants further exploration. The findings of elevated creatinine in Groups C and D are consistent with other studies that reported kidney dysfunction markers in response to herbal supplementation [19]

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Urea levels showed a marked increase in Groups B and C, suggesting an altered nitrogen metabolism, likely related to kidney function. Urea is a byproduct of protein catabolism, and elevated levels are often linked to impaired renal function [20]. The reduction in urea levels in Group D might indicate a potential renal protective effect or improved nitrogen balance, which deserves further exploration. The observed differences between the test groups and control group were statistically significant, reinforcing the potential effects of D'General Bitters on renal biomarkers.

Uric acid levels were significantly decreased in Groups C and D, with a slight elevation in Group B. Uric acid is a key marker of purine metabolism, and its reduction could be associated with reduced risk of gout or kidney stones, whereas its elevation is often linked to hyperuricemia and

related pathologies [21]. The decrease in uric acid levels in Groups C and D may suggest potential beneficial effects of D'General Bitters on uric acid metabolism, which could have therapeutic implications for managing conditions like gout or hyperuricemia.

4.2 Conclusions

The phytochemical composition of D'General Bitters indicates the presence of several bioactive compounds that may contribute to its physiological effects. The biochemical analysis revealed significant alterations in creatinine, urea, and uric acid levels, which could be indicative of potential renal effects. Elevated creatinine and urea levels in certain groups suggest that D'General Bitters may impact kidney function, while the reduction in uric acid levels in some groups may suggest a potential therapeutic effect on purine metabolism. These results highlight the need for further studies to fully understand the mechanisms through which D'General Bitters affects renal biomarkers and overall health.

4.3 Recommendations

1. 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.
2. Regulation and Labeling: Standardized labeling to detail the concentrations of active phytochemicals could better inform consumers and healthcare providers about potential health impacts.

Declarations

This is an original research 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 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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