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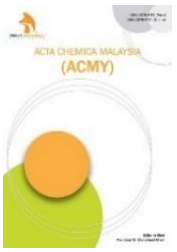

RESEARCH ARTICLE

DOSE-DEPENDENT EFFECTS OF AQUEOUS *Hibiscus sabdariffa* (ZOBO) EXTRACT ON HEPATORENAL FUNCTION IN ADULT WISTAR RATS

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ABSTRACT

Hibiscus sabdariffa (Zobo), widely consumed in Nigeria for its nutritional and medicinal value, is known for its antioxidant and therapeutic properties. This study investigated the effects of aqueous *Hibiscus sabdariffa* (Zobo) extract on kidney and liver function, as well as body weight, in adult Wistar rats. Twenty-four adult Wistar rats were divided into four groups (n=6). Group A served as control, while Groups B, C, and D received 1 mg/kg, 2 mg/kg, and 3 mg/kg of Zobo extract, respectively, for 14 days. Body weights were recorded before and after treatment. Blood samples were collected post-sacrifice for kidney and liver function analysis. Significant weight gain was observed in the control (50.00 ± 9.50 g), 1 mg/kg (46.33 ± 3.23 g), and 3 mg/kg (40.67 ± 7.69 g) groups, while the 2 mg/kg group showed the least gain (26.00 ± 5.77 g). Elevated AST and reduced ALP levels were noted in treated groups, indicating possible hepatocellular stress. Urea levels increased in 1 mg/kg and 2 mg/kg groups, while creatinine decreased in the 2 mg/kg group. Zobo extract influenced body weight and induced dose-dependent alterations in hepatic and renal biomarkers, suggesting both therapeutic and potential toxicological effects.

KEYWORDS

Hibiscus sabdariffa, Zobo, liver enzymes, kidney function, Wistar rats, body weight

1. INTRODUCTION

Hibiscus sabdariffa L., a member of the super order *Malvaceae*, is believed to have originated from East Africa (Ilondu and Iloh, 2007). Commonly known as Roselle or Sorrel in English, it is referred to locally as *zobo* or *Isapa* in Nigeria (Adebayo-Taye and Samuel, 2000). While some *Hibiscus* species are cultivated for their fibrous yield, *H. sabdariffa* is predominantly grown for its culinary and medicinal applications. Various parts of the plant, including the seeds, leaves, fruits, and roots, are utilized in food preparation; however, the fleshy red calyces are the most widely consumed and commercially important component (Yadeng et al., 2005).

In Nigeria, the dried calyces are commonly processed into a popular non-alcoholic beverage known as Zobo. This traditional drink is typically prepared via hot water extraction of the reddish-purple, acid-succulent calyces (Yadeng et al., 2005). Nutritional analyses of the Zobo drink have revealed a proximate composition of approximately 90% water, 0.7% protein, 8% carbohydrate, 1.4% fiber, and 1.1% fat. The beverage also contains micronutrients such as iron, niacin, riboflavin, thiamine, beta-carotene, phosphorus, and calcium in varying concentrations (Fasoyiro et al., 2005).

Beyond its nutritional value, Zobo has garnered considerable attention for its purported medicinal properties. Traditionally, the drink has been used for its antihypertensive, antiseptic, astringent, diuretic, and purgative activities, as well as in the treatment of conditions such as cancer, abscesses, cough, debility, scurvy, and fever (Anjorin et al., 2010). Scientific investigations have also demonstrated its potential to boost the immune system and ameliorate high blood pressure, elevated cholesterol levels, gastrointestinal disorders, and inflammatory conditions (Foline et al., 2011).

As a result of these health-promoting effects, Zobo is often preferred over carbonated soft drinks by many Nigerians, who view it as a more natural, affordable, and mineral-rich alternative (Ogiehor et al., 2008). Its widespread consumption spans diverse socio-economic groups, particularly among youths and in social gatherings where it serves as cost-effective, culturally rooted refreshment (Ogiehor et al., 2008).

The phytochemical and antioxidant capacities of *H. sabdariffa* extracts, especially those derived via aqueous methods have been extensively documented in literature (Yadeng et al., 2005; Zhen et al., 2016). However, most commercially prepared Zobo drinks are conventionally flavored with artificial essences—commonly strawberry—due to their color compatibility. Despite the drink's rising popularity, there remains a significant gap in literature regarding the comparative analysis of traditional Zobo formulations and the potential synergistic effects of natural spices, which may further enhance its health benefits.Given these considerations, the present study was undertaken to investigate the effects of Zobo juice extract on kidney and liver enzyme activities in adult wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The leaves of *H. sabdariffa* used in this research work was gotten from the botanical garden of the department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. It was identified and authenticated

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by a plant taxonomist at the department of Plant Biology and Biotechnology, Faculty of Life sciences, University of Benin, Benin city, Nigeria.

2.2 Extract preparation

Aqueous extraction of the plant was done by freeze-dry method. *H. sabdariffa* leaves was washed with tap water, shade dried and then pounded. The powder obtained was soaked in distilled water for 24 hours in a separating funnel with occasional shaking. The solution was then filtered. The filtrate was allowed to settle and then decanted. The filtrate was then freeze dried with freeze drying machine and refrigerated at -6 celsius.

2.3 Experimental Design

Twenty-four (24) adult Wistar rats weighing between 160g to 185g were randomly assigned into four (4) groups; Groups A – E comprising of six rats per group. The rats were obtained from the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Edo State, and acclimatized for two weeks prior to the start of the study. During this time, the animals were given unlimited access to conventional animal feed (Top feed growers mash.) and clean water. Each animal procedure was carried out in accordance with established procedures and recommendations for the proper management and exploitation of laboratory animals employed for research (Buzek and Chastel, 2010).

Group A served as control

Group B was administered 1mg/kg of Zobo extract

Group C was administered 2mg/kg of Zobo extract

Group E was administered 3mg/kg of Zobo extract

The experiment lasted for 14 days and the animals were sacrificed on day 15 following chloroform anesthesia.

After fourteen (14) days, the rats were anesthetized with chloroform and sacrificed by making a midline incision in the ventral abdominal wall. Blood was collected directly from the abdominal aorta to assess serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), conjugated bilirubin, total bilirubin, sodium ion (Na^+), potassium ion (K^+), chloride ion (Cl^-), bicarbonate ion (HCO_3^-), urea and creatinine levels.

2.4 Statistical analysis

Results obtained were expressed as Mean ± SEM (standard error of means). Differences between the means were determined by one-way analysis of variance (ANOVA). Values were considered statistically significant if P value is less than 0.05 ($p < 0.05$). LSD Post Hoc test was used to determine where the significance lay. Statistical package Graph Pad Prism Version 9.0 for Windows (GraphPad Software Inc.) was used to analyze the data obtained in this study.

3. Results

The effect of Zobo extract on body weight was evaluated by comparing initial and final weights across all groups. The initial body weights of the experimental animals were not significantly different, with values ranging from 158.3 ± 3.48 g in the 1 mg/kg Zobo group to 172.3 ± 6.57 g in the 3 mg/kg group ($p = 0.4148$), indicating that all groups started at a relatively similar baseline. Following administration, the final weights showed an increase across all groups. Statistically significant weight gain was observed in the control (217.3 ± 15.07 g), 3 mg/kg Zobo (213.0 ± 14.19 g), and 1mg/kg (214.0 ± 7.81 g) groups when compared with their respective initial weights ($p < 0.05$). However, the 2 mg/kg Zobo group did not exhibit significant weight increases, with the 2 mg/kg group recording the lowest final weight (184.3 ± 4.70 g).

In terms of weight change, the control group had the highest increase (50.00 ± 9.50 g), followed by the test and 3 mg/kg groups. The 1 mg/kg and 2 mg/kg groups showed notably lower weight gains, especially the 2 mg/kg group with a mean change of 26.00 ± 5.77 g. Despite these differences, the variations in weight change across the groups were not statistically significant ($p = 0.1559$), suggesting a dose-dependent trend that warrants further investigation.

Regarding kidney function, urea levels were significantly elevated in the 1 mg/kg and 2 mg/kg Zobo groups when compared with the control group, although the overall variation across groups was not statistically significant ($p = 0.2763$). Creatinine levels were significantly reduced in the 2 mg/kg group (0.60 ± 0.10 mg/dL) compared to control (0.90 ± 0.06 mg/dL), yet this difference also did not reflect a statistically significant trend across all groups ($p = 0.2096$). Electrolyte levels (sodium, potassium, bicarbonate, and chloride) remained relatively stable across all treatment groups, with no statistically significant alterations observed.

Liver function tests revealed that treatment with Zobo extract significantly influenced several parameters. Alkaline phosphatase levels were significantly reduced in all Zobo-treated groups compared to control, with the greatest decrease observed in the 1 mg/kg group ($p = 0.0489$). Aspartate transaminase (AST) levels were markedly elevated in all treatment groups, particularly in the 3 mg/kg group, indicating a possible hepatocellular effect ($p = 0.0098$). Alanine transaminase (ALT), bilirubin (total and conjugated), total protein, albumin, and globulin levels did not show significant differences across groups.

Table 1: Result of weight changes across all groups, before and after the experiment

Groups Test	Control	1mg/kg of Zobo extract	2mg/kg of Zobo extract	3mg/kg of zobo extract	P-value
Initial weight	167.7±4.33	167.3±7.36	158.3±3.48	172.3±6.57	0.4148
Final weight	214.0±7.81*	217.3±15.07*	184.3±4.70	213.0±14.19*	0.2183
Weight change	46.33±3.23	50.00±9.50	26.00±5.77	40.67±7.69	0.1559

Values are expressed as mean ± Standard Error of Mean (SEM): **The mean difference is significant at the P<0.05 level of the final weight compared with the initial weight.*

Table 2: Kidney function test of experimental animals across all groups after administration.

Groups/Tests	Control	1mg/kg Zobo	2mg/kg Zobo	3mg/kg Zobo	p-value
Urea	37.00±2.08	55.67±7.62*	49.67±10.71*	41.00±2.52	0.2763
Creatinine	0.90±0.06	0.90±0.15	0.60±0.10*	1.03±0.19	0.2096
Sodium ion	140.70±0.67	139.00±0.58	140.00±1.20	140.70±0.88	0.5093
Potassium ion	5.33±0.28	5.00±0.21	5.20±0.36	5.30±0.12	0.7999
Bicarbonate ion	20.67±1.20	20.33±0.33	19.67±0.33	21.00±1.16	0.7358
Chloride ion	104.30±0.88	102.30±0.88	103.00±2.08	103.00±1.00	0.7512

Values are expressed as mean ± Standard Error of Mean (SEM): **The mean difference is significant at the P<0.05 level compared with control.*

Table 3: Liver function test of experimental animals across all groups after administration.

Groups/Tests	Control	1mg/kg Zobo	2mg/kg Zobo	3mg/kg Zobo	p-value
Alkaline phosphatase	502.00±41.58	284.30±54.48*	365.70±23.38*	402.30±51.90*	0.0489
Aspartate Transaminase	35.67±10.53	86.33±15.45*	121.30±24.74*	149.30±17.13*	0.0098
Alanine Transferase	97.00±2.00	80.00±14.57	103.00±1.20	91.33±9.17	0.3393

Table 3 (cont): Liver function test of experimental animals across all groups after administration.					
Total Bilirubin	0.27±0.03	0.33±0.03	0.27±0.03	0.27±0.03	0.4411
Conjugated Bilirunbin	0.10±0.00	0.10±0.00	0.08±0.02	0.10±0.00	0.4411
Total Protein	7.60±0.15	7.73±0.53	7.30±0.50	7.37±0.07	0.8293
Alanine	3.83±0.33	3.47±0.27	3.43±0.22	3.90±0.12	0.4545
Globulin	3.77±0.35	4.27±0.35	3.53±0.42	3.47±0.18	0.3843

Values are expressed as mean ± Standard Error of Mean (SEM): **The mean difference is significant at the P<0.05 level compared with control.*

4. Discussion

The administration of *Hibiscus sabdariffa* (Zobo) extract produced varying effects on body weight, renal, and hepatic function, with outcomes suggesting a dose-dependent response. A significant increase in body weight was observed in the control, 1 mg/kg, and 3 mg/kg groups, while the 2 mg/kg group failed to show a similar trend. This suggests a biphasic metabolic effect of the extract, possibly linked to the concentration of bioactive compounds such as anthocyanins and organic acids (Nwankwo et al., 2023). At low doses, these compounds may enhance appetite or improve metabolic efficiency due to their antioxidant and digestive-stimulating properties. However, at intermediate levels, they may disrupt energy metabolism or interfere with nutrient absorption, leading to reduced weight gain. This aligns with findings from who reported beneficial metabolic effects at low doses, in contrast to other studies showing diminished or adverse outcomes at higher concentrations (Oladejo et al., 2006; Onyesom et al., 2008).

Renal function markers also revealed significant alterations. Elevated urea levels in the 1 mg/kg and 2 mg/kg groups may indicate compromised renal excretory function or increased protein catabolism, possibly due to mild nephrotoxicity from prolonged extract exposure. This is supported by the known diuretic effects of Zobo, driven by its flavonoid and organic acid content, which can increase renal workload and potentially affect filtration (Nwachukwu et al., 2015). Interestingly, creatinine levels were significantly reduced in the 2 mg/kg group, a result that may reflect increased clearance or altered muscle metabolism. This paradoxical reduction could result from flavonoid-induced modulation of glomerular function or tubular handling of creatinine (Vargas et al., 2018). A group researcher reported similar renal challenges in high-dose Zobo-treated rats, emphasizing the importance of dose consideration in its therapeutic use (Ogundapo et al., 2014). Electrolyte levels remained relatively unaffected, suggesting that despite early signs of renal stress, overall ionic balance was maintained, possibly due to the potassium-sparing and buffering effects of hibiscus phytochemicals.

The liver function profile was notably influenced by Zobo extract, with marked elevation in aspartate transaminase (AST) levels across all treated groups and a significant reduction in alkaline phosphatase (ALP). The rise in AST suggests hepatocellular injury or membrane leakage, especially at higher doses, possibly due to oxidative stress induced by anthocyanins, which may exhibit pro-oxidant behavior under certain conditions (Sen and Chakraborty, 2018). Delphinidin and cyanidin derivatives in Zobo can interfere with mitochondrial enzymes and promote reactive oxygen species (ROS) formation, leading to hepatic stress (Nnachetam et al., 2020). Meanwhile, the consistent reduction in ALP may reflect impaired bile production or a downregulation of enzyme synthesis in response to hepatic burden. Studies also found similar enzyme derangements, supporting the idea that Zobo extract exerts a dose-dependent hepatotoxic effect (Ogundapo et al., 2014). The minimal changes in ALT, bilirubin, total protein, albumin, and globulin suggest that while there is hepatocellular stress, it has not yet progressed to severe hepatic dysfunction or failure.

5. Conclusion

The findings of this study indicate that Hibiscus sabdariffa (Zobo) extract exerts dose-dependent effects on body weight, renal, and hepatic function.

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