**Comparative Study of Hematological and Renal Profiles in Rats Fed *Hibiscus sabdariffa* (Zobo) Drink Sweetened with Natural versus Artificial Sugars**

**Abstract:** Hibiscus sabdariffa (commonly known as "zobo") is widely consumed for its antioxidant, antihypertensive, and nephroprotective effects. However, the impact of sweeteners—natural (honey) vs. artificial (aspartame)—on these benefits remains underexplored. This study assessed hematological and renal changes in rats administered zobo drink sweetened with honey or aspartame for eight weeks. Twenty-four adult male Wistar rats were randomized into four groups (n=6): Control (distilled water), Zobo-only, Zobo + Honey, and Zobo + Aspartame. Hematological indices, serum urea and creatinine levels, and renal histopathology were analyzed using standard methods. Rats in the Zobo + Aspartame group showed significant reductions in RBC (27%), hemoglobin (25%), hematocrit (23%) and increases in serum urea (58%) and creatinine (78%) compared to controls (p < 0.01). Histological examination revealed glomerular atrophy and tubular necrosis. Zobo + Honey preserved hematologic and renal profiles comparable to controls. These findings suggest artificial sweeteners may attenuate zobo's benefits, whereas honey preserves them.

**Keywords:** Hibiscus sabdariffa, zobo, honey, aspartame, hematology, nephrotoxicity, Wistar rats

**1. Introduction**

*Hibiscus sabdariffa* L., commonly known as “zobo,” is valued for its high anthocyanin, flavonoid, and polyphenol content, which impart antioxidant, antihypertensive, and nephroprotective effects (Nwachukwu et al., 2016; Chen et al., 2023; Ajiboye et al., 2024) In hypertensive human participants, consumption of aqueous *H. sabdariffa* improved renal function markers such as urine volume and creatinine clearance (Nwachukwu et al., 2016). Further, its extracts have been shown to mitigate hyperuricemic nephropathy by modulating TGF‑β/Smad signaling in vitro and in vivo (Chen et al., 2023) and reduce diabetic nephropathic changes via KIM‑1 and TGF‑β downregulation in streptozotocin-induced rats (Ajiboye et al., 2024).

Flavonoid-rich calyx extracts of Hibiscus sabdariffa have demonstrated significant nephroprotective effects, particularly in models of drug-induced renal injury. For example, they have been shown to ameliorate cisplatin-induced oxidative stress by enhancing endogenous antioxidant defenses (Ezekwe et al., 2021; Wokocha et al., 2024). However, while moderate doses confer protection, excessively high dosing of H. sabdariffa extracts has occasionally led to elevated serum urea and creatinine levels in rodent models, suggesting a dose-dependent risk of nephrotoxicity (Harris et al., 2025; Wokocha et al., 2025).Natural sweeteners like honey are known to possess nephroprotective and hematopoietic properties. Honey has been shown to protect against cisplatin-induced kidney injury and improve hematological parameters in rats In contrast, artificial sweeteners such as aspartame—metabolized to phenylalanine, aspartic acid, and methanol—can induce oxidative stress and impair renal function and hematology in animal studies (Abd El Wahab et al., 2017; Finamor et al., 2017; Yadav & Gupta, 2016). Long-term aspartame intake has also been shown to deplete glutathione, elevate serum creatinine, and cause histopathological damage in rat kidneys (Nembhard et al., 2015; **Adaramoye, O. A., & Akanni, O. O., 2015.** ; Abd ElFatah et al., 2012). Aspartame-induced oxidative damage—evident in increased biomarkers of lipid peroxidation and reduced antioxidant enzyme activity—has been demonstrated in erythrocytes and renal tissues (Nembhard et al., 2015; Al-Eisa et al., 2018).

Although *H. sabdariffa*’s nephroprotective potential is well-supported, the choice of sweetener (honey vs. aspartame) remains understudied. Honey may synergize with zobo’s bioactives to maintain renal and hematologic integrity, while aspartame could negate these benefits. To address this, the current study evaluates hematology, renal biomarkers, and histopathology in rats fed zobo with either honey or aspartame over eight weeks—providing critical insights into safe formulation of herbal beverages for regular intake.

**2. Materials and Methods**

**2.1. Zobo Preparation and Sweetening**

Dried Hibiscus sabdariffa calyces (100 g) were boiled in 1 L of distilled water for 15 minutes, following the extraction method described by Da-Costa-Rocha et al. (2014). After cooling, the filtrate was divided into three portions. Zobo-only group: Unsweetened

* The dose of honey (10 mL/kg) was selected based on previous studies demonstrating protective effects in rodent models of renal or hematological injury ( Hamad et al., 2015; Al-Waili et al., 2018). The aspartame dose (40 mg/kg) approximates the upper limit of the human acceptable daily intake (ADI) extrapolated to rats, and has been used in prior toxicity studies evaluating long-term consumption (Adaramoye & Akanni, 2015; Finamor et al., 2017).

**2.2. Experimental Design**

Rats were randomly assigned to four groups (n=6):

* **Group I (Control):** Distilled water
* **Group II:** Zobo only
* **Group III:** Zobo + Honey
* **Group IV:** Zobo + Aspartame

All treatments were administered daily via oral gavage for eight weeks.

**2.3. Blood and Tissue Collection**

At the end of the experiment, rats were anesthetized and sacrificed. Blood samples were obtained via cardiac puncture for hematological and biochemical analyses.

**2.4 Hematological and Biochemical Analysis**

**Hematology**

Red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), white blood cell (WBC), and platelet (PLT) counts were determined using the **Sysmex KX-21N automated hematology analyzer** (Sysmex Corporation, Kobe, Japan), following the **manufacturer’s protocol** and standardized hematology procedures in accordance with the **Clinical and Laboratory Standards Institute (CLSI, 2018)** guidelines.

**Biochemistry**

Serum levels of urea, creatinine, sodium (Na⁺), and potassium (K⁺) were measured using **Randox® diagnostic kits** (Randox Laboratories Ltd., UK). All biochemical assays were performed according to the **manufacturer’s instructions** and validated using procedures aligned with the **Association of Official Analytical Chemists (AOAC, 2019)** standards.

**2.5 Histopathological Analysis**

* Kidney tissues from all experimental groups were harvested and fixed in 10% neutral buffered formalin for 48 hours. Following fixation, tissues were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were obtained using a rotary microtome and mounted on glass slides. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope at ×400 magnification for structural abnormalities.
* Histopathological evaluation focused on glomerular morphology, tubular integrity, interstitial inflammation, and vascular changes. The **Zobo + Aspartame** group displayed **moderate to severe glomerular atrophy, tubular dilation, epithelial desquamation, and necrosis**, with interstitial congestion and leukocytic infiltration. These changes are consistent with **toxicant-induced nephropathy** as previously characterized by **Feldman & Wolfe (2014)** and **Boorman et al. (1990)**.
Conversely, kidneys from the **Zobo-only** and **Zobo + Honey** groups exhibited largely preserved architecture, with minimal or no histological alterations compared to controls.

**2.6. Statistical Analysis**

Data were analyzed using GraphPad Prism 9.0. Results were presented as mean ± standard deviation (SD). One-way ANOVA followed by Tukey’s post hoc test was used. p < 0.05 was considered statistically significant.

**3. Results**

**3.1. Hematological Parameters**

Table 1- Hematological analysis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Control** | **Zobo-only** | **Zobo + Honey** | **Zobo + Aspartame** |
| RBC (×106/µL) | 7.12 ± 0.31 | 7.00 ± 0.29 | 6.85 ± 0.34 | 5.20 ± 0.41\*\* |
| Hb (g/dL) | 13.6 ± 0.6 | 13.2 ± 0.7 | 13.0 ± 0.5 | 10.1 ± 0.5\*\* |
| HCT (%) | 42.1 ± 2.2 | 41.3 ± 2.4 | 40.8 ± 2.1 | 32.5 ± 1.7\*\* |
| WBC (×103/µL) | 7.4 ± 0.6 | 7.6 ± 0.5 | 7.3 ± 0.4 | 6.1 ± 0.3\* |
| PLT (×103/µL) | 850 ± 45 | 840 ± 38 | 830 ± 42 | 1. 36\*
 |

\* p < 0.05, \*\* p < 0.01 vs. Control

(Rats in the Zobo + Aspartame group showed a **27% decrease in RBC count**, **25.7% reduction in hemoglobin**, and **22.8% decrease in hematocrit** compared to controls. WBC and platelet counts were also reduced by **17.6%** and **15.3%**, respectively. Zobo-only and Zobo + Honey groups showed slight, non-significant variations compared to the control)

**3.2. Renal Function Biomarkers**

Table 2- Biochemical Analysis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Control | Zobo-only | Zobo + Honey | Zobo + Aspartame |
| Urea (mmol/L) | 8.7 ± 0.6 | 8.9 ± 0.8 | 9.1 ± 0.7 | 13.8 ± 0.9\*\* |
| Creatinine (µmol/L) | 76 ± 8 | 78 ± 7 | 80 ± 9 | 136 ± 12\*\* |
| Na+ (mmol/L) | 142 ± 3 | 140 ± 4 | 141 ± 5 | 145 ± 4 |
| K+ (mmol/L) | 4.5 ± 0.3 | 4.6 ± 0.2 | 4.7 ± 0.2 | * 1. ± 0.3\*
 |

\* p < 0.05, \*\* p < 0.01 vs. Control

(Serum urea and creatinine levels were markedly elevated in the Zobo + Aspartame group, showing a **58.6% increase in urea** and a **78.9% increase in creatinine** relative to controls. The potassium level increased by **13.3%**, while sodium showed a non-significant rise).

**3.3. Histopathology**

**Fig 1 control**



Fig 2 zobo alone



Fig 3 zobo + honey



Fig 4 zobo + Aspartame



The control and Zobo + Honey groups exhibited normal renal architecture. In contrast, the Zobo + Aspartame group showed marked glomerular atrophy, tubular dilation, and necrosis. Mild tubular degeneration was observed in the Zobo-only group.

**4. Discussion**

**4.1 Nephroprotection by *H. sabdariffa***

Our findings affirm the nephroprotective role of *Hibiscus sabdariffa*. While the Zobo-only and Zobo + Honey groups exhibited slight increases in urea and creatinine (Zobo + Honey: urea +4.6%, creatinine +5.3%), these changes were not statistically significant, indicating the preservation of renal function. This aligns with previous studies reporting *H. sabdariffa*–induced reductions in serum creatinine and histopathological damage through antioxidative and anti-fibrotic mechanisms (Ajiboye et al., 2024; Garcia-Pinilla et al., 2017).

**4.2 Honey’s Renal and Hematologic Benefits**

The Zobo + Honey group retained hematological values close to control. The minor reductions in RBC (3.8%), hemoglobin (4.4%), and hematocrit (3.1%) suggest honey's potential hematopoietic and cytoprotective properties. These findings are consistent with prior reports where honey mitigated hematologic and renal toxicity in models of drug-induced nephrotoxicity (Ghoneim et al., 2017; Al-Waili et al., 2018., Patel, S., et al. 2020.).

**4.3 Aspartame-Induced Hematotoxicity and Nephrotoxicity**

In contrast, aspartame consumption led to significant hematological and renal impairment. The **Zobo + Aspartame** group showed a **27.0% reduction in RBC**, **25.7% in Hb**, and **22.8% in HCT**, indicating hematotoxicity. Concurrently, serum **urea increased by 58.6%** and **creatinine by 78.9%**, suggesting compromised renal function. These findings correlate with previous studies linking aspartame metabolism to reactive oxygen species generation, formaldehyde toxicity, and subsequent organ damage (**Adaramoye, O. A., & Akanni, O. O. 2015.;**  Al-Eisa et al., 2018).

**4.4 Histological Correlations**

Histopathological analysis reinforced the biochemical data. The Zobo + Aspartame group showed **pronounced glomerular atrophy and tubular necrosis**, consistent with oxidative stress–induced nephrotoxicity. In contrast, the Zobo + Honey group maintained normal renal architecture, further supporting its protective role.

**4.5 Implications for Herbal Beverage Formulation**

These results highlight the critical impact of sweetener selection on the safety and efficacy of functional herbal beverages. While natural sweeteners like honey preserve the bioactivity of *H. sabdariffa*, artificial agents like aspartame may compromise its benefits. This underscores the need for evidence-based formulation strategies in herbal drink production.

**4.6 Limitations and Future Directions**

While our rat model provides compelling preclinical data, translation to humans will require clinical trials. Future studies should also explore mechanistic markers (e.g., oxidative stress enzymes, pro-inflammatory cytokines) and behaviorally relevant dosing regimens. Additionally, evaluating other artificial sweeteners (e.g., sucralose) and lower-dose honey formulations would inform broader dietary recommendations.

**Conclusion**

In summary, honey is a protective adjuvant to zobo, sustaining hematologic homeostasis and renal integrity, whereas aspartame is detrimental when combined with the same beverage. These findings advocate for natural sweetening agents in functional herbal products and warrant further research to substantiate translational benefits.

Ethical Approval

Twenty-four adult male Wistar rats (180–220 g) were procured from the University of Port Harcourt animal house. They were housed in standard cages, with 12-hour light/dark cycles, and allowed free access to rat chow and water. Ethical approval was obtained from the University of Port Harcourt Animal Care and Use Committee (Ref: UPH/ACUC/2024/03).

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