**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 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. 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; Ajiboye et al., 2024; Chen et al., 2023). 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).

Moreover, flavonoid-rich calyx extracts ameliorated cisplatin-induced renal oxidative stress by enhancing antioxidant defenses(Wokocha el al 2024.,: Ezekwe et al., 2021:). However, excessively high dosing of *H. sabdariffa* extracts has occasionally resulted in elevated serum urea and creatinine in rodent models, indicating potential nephrotoxicity (Wokocha et al.,2025: Haris el 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 (Nguyen et al., 2015; Al-Masoudi et al., 2020). 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; Varghese 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; Pereira et al., 2018; 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. Animals and Ethical Clearance**

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).

**2.2. Zobo Preparation and Sweetening**

Dried *Hibiscus sabdariffa* calyces (100 g) were boiled in 1 L distilled water for 15 minutes. After cooling, the filtrate was divided into three portions:

* Zobo-only group: Unsweetened
* Zobo + Honey group: Honey added at 10 mL/kg body weight
* Zobo + Aspartame group: Aspartame added at 40 mg/kg body weight

**2.3. 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.

**2.4. 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.

**2.5. Hematological and Biochemical Analysis**

* Hematology: RBC count, hemoglobin (Hb), hematocrit (HCT), WBC, and platelet (PLT) counts were analyzed using Sysmex KX-21N automated analyzer.
* Biochemistry: Serum urea, creatinine, Na+, and K+ were measured using Randox diagnostic kits.

**2.6. Histopathological Analysis**

Kidneys were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Slides were examined under a light microscope by a blinded pathologist for evidence of glomerular and tubular changes.

**2.7. 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 (×10^6/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 (×10^3/µL) | 7.4 ± 0.6 | 7.6 ± 0.5 | 7.3 ± 0.4 | 6.1 ± 0.3\* |
| PLT (×10^3/µL) | 850 ± 45 | 840 ± 38 | 830 ± 42 | 720 ± 36\* |

* p < 0.05, \*\* p < 0.01 vs. 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 | 5.1 ± 0.3\* |

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

**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**

Our findings reveal that chronic ingestion of *Hibiscus sabdariffa* (zobo) sweetened with honey preserves hematological and renal function in Wistar rats, while aspartame negates these benefits and induces significant hematotoxicity and nephrotoxicity. These results align with and expand upon existing literature.

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

Flavonoid- and anthocyanin-rich extracts of *H. sabdariffa* have been shown to protect against nephrotoxicity in various rat models. Ajiboye et al. (2024) demonstrated that leaf flavonoids ameliorate diabetic nephropathy via downregulation of KIM-1 and TGF‑β signaling. Similarly, Chen et al. (2023) observed protective effects in hyperuricemic nephropathy through attenuation of oxidative stress and inflammation mediated via TGF‑β/Smad pathways. Previous models using adenine-induced renal injury also revealed significant reductions in serum creatinine, urea, and histopathological damage after prolonged zobo administration (Garcia-Pinilla et al., 2017). These nephroprotective effects are primarily attributed to antioxidative and anti-fibrotic mechanisms (Ajiboye et al., 2024; Amarachukwu et al., 2024; Amakiri et al., 2024; Chen et al., 2023; Wokocha et al., 2024; Ezekwe et al., 2021.)

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

Natural honey contains phenolic antioxidants, enzymes, and organic acids that confer protective effects. In cisplatin-induced nephrotoxicity, both manuka and talh honeys ameliorate renal biochemical and histopathological alterations via suppression of NF‑κB and COX‑2 expression, enhanced catalase activity, and reduced apoptosis (Al-Waili et al., 2018; Ibrahim et al., 2015). In human cancer patients receiving cisplatin, honey has been shown to lower elevations in creatinine and urea compared to controls (Ghoneim et al., 2017). Additionally, honey supports hematopoiesis, likely contributing to the stable RBC, Hb, and HCT levels observed in our Zobo+Honey group.

**4.3 Aspartame-Induced Hematotoxicity and Nephrotoxicity**

In contrast, aspartame has been associated with oxidative stress–mediated organ damage. Animal studies report decreased antioxidant enzyme activities, increased lipid peroxidation, elevated serum creatinine, and histopathological alterations in renal tissue following prolonged aspartame exposure (Nembhard et al., 2015; Pereira et al., 2018; Al-Eisa et al., 2018). Mechanistically, aspartame metabolism yields phenylalanine, aspartic acid, and methanol, culminating in reactive oxygen species and formaldehyde formation. These toxic metabolites can induce apoptosis, erythrocyte fragility, and tubular necrosis, consistent with our study’s findings—most notably, a 27% decline in RBC counts and over 78% rise in serum creatinine in the Zobo+Aspartame group.

**4.4 Histological Correlations**

Our histopathological observations—glomerular atrophy and pronounced tubular necrosis—corroborate previous reports of aspartame's nephrotoxic effects (Pereira et al., 2018; Al-Eisa et al., 2018). In contrast, zobo with honey maintained normal renal architecture, aligning with the structural improvements observed in prior models using zobo and honey against nephrotoxic insults.

**4.5 Implications for Herbal Beverage Formulation**

These findings underscore the importance of sweetener selection. Honey not only preserves zobo’s inherent bioactivity but may also synergize via antioxidant pathways. Conversely, aspartame appears to override zobo’s positive effects. For consumers and producers of herbal drinks, these results caution against artificial sweeteners in favor of natural alternatives when designing functional beverages for long-term consumption.

**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.

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