

Ethanol Extract of *Sphenostylis stenocarpa* Mitigates Potassium Bromate-Induced Hepatorenal and Hematological Toxicity in Wistar Rats

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Abstract

Introduction Potassium bromate (KBrO₃) is a commonly used dough improver associated with oxidative stress-mediated liver and kidney toxicity. This study evaluated the protective effects of the ethanol extract of *Sphenostylis stenocarpa* against KBrO₃-induced hepatorenal toxicity in adult male Wistar rats.

Methods Twenty-five male Wistar rats (100–160 g) were randomly assigned into five groups (n = 5). Group A received 25 mg/kg KBrO₃ only. Groups B–D received KBrO₃ plus 50, 100, and 200 mg/kg of the extract, respectively, for 28 days. Group E served as the control. Hepatic enzymes, serum urea, and creatinine were assessed spectrophotometrically. Phytochemical screening of the extract was also performed.

Results Potassium bromate induced significant hepatorenal and hematological toxicity, evidenced by marked elevations in liver enzymes (ALP, ALT, AST: ~160%, 123%, and 253%) and renal markers (urea ~768%, creatinine ~133%), alongside reduced RBC (~28%), Hb (~21%), HCT (~15%), and elevated WBC (~125%) and platelets (~12%). Treatment with *Sphenostylis stenocarpa* ethanol extract produced dose-dependent amelioration of these alterations, reducing liver enzymes (up to ~57%), urea (~85%), creatinine (~50%), and WBC (~30%), while improving RBC (~21%) and Hb (~23%), indicating significant hepatoprotective, nephroprotective, and hematoprotective effects, with optimal efficacy at 100–200 mg/kg.

Conclusion The protective effects of *Sphenostylis stenocarpa* ethanol extract are likely mediated through its antioxidant and free radical scavenging activities, which reduce oxidative stress, stabilize cellular membranes, and prevent lipid peroxidation. This mechanism helps preserve liver and kidney function and protects hematopoietic cells, thereby restoring biochemical and hematological homeostasis.

Keywords Hepatorenal · Hematological · Potassium Bromate · Toxicity · *Sphenostylis stenocarpa* · Oxidative Stress · Hepatoprotective · Nephroprotective

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Introduction

Potassium bromate (KBrO_3) is a widely used oxidizing agent in industrial processes, including food production and flour treatment. However, extensive evidence indicates that KBrO_3 exposure induces oxidative stress, leading to hepatorenal injury, hematological disturbances, and potential carcinogenicity (Altoom et al., 2018; De Vico et al., 2018; Safavi et al., 2017). In experimental models, sub-chronic KBrO_3 administration in Wistar rats elevates serum urea, creatinine, and liver enzymes, while depleting endogenous antioxidants and damaging renal and hepatic tissues (Nazir et al., 2025; Abd El-Wahab et al., 2018; Manzoor et al., 2021).

Several plant extracts have demonstrated protective effects against KBrO_3 -induced toxicity through antioxidant and anti-inflammatory mechanisms, yet few studies have critically examined legumes with underexplored pharmacological potential. *Sphenostylis stenocarpa* Hochst (African yam bean) is a nutrient-dense legume containing bioactive compounds such as flavonoids, phenolics, saponins, and alkaloids, which are known for their antioxidant, anti-inflammatory, and hepatoprotective properties (Chiaka-Onyemeze et al., 2025; Gbenga-Fabusiwa, 2021; Adewale and Nnamani, 2022). These phytochemicals can scavenge free radicals, enhance endogenous antioxidant defenses, and stabilize cellular membranes, suggesting a mechanistic basis for protection against oxidative organ injury. Despite this potential, the efficacy of *S. stenocarpa* in mitigating KBrO_3 -induced hepatorenal toxicity and hematological disruptions has not been systematically evaluated.

Given the prevalence of environmental toxicants and the need for safe, accessible plant-based interventions, this study investigated whether ethanol extract of *Sphenostylis stenocarpa* can ameliorate potassium bromate-induced liver and kidney damage and restore hematological parameters in Wistar rats, with the hypothesis that the extract exerts dose-dependent hepatoprotective, nephroprotective, and hematoprotective effects.

Methods

Animals

Adult male Wistar rats (100–160 g) and adult Swiss mice (20–30 g) of both sexes were obtained from the Animal Holding Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Animals were housed in suitable cages under standard laboratory conditions (25 ± 2 °C, 12-h light/dark cycle) and handled

according to conventional guidelines for the care and use of laboratory animals.

Plant Material

Seeds of *Sphenostylis stenocarpa* were procured from Orba Market, Nsukka, Enugu State, Nigeria. The seeds were identified and authenticated by Mr. Felix Nwafor of the Herbarium Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Extraction of Plant Material

The seeds were washed, oven-dried at 40 °C to a constant weight, and ground into a fine powder using an electric milling machine (Strathclyde, Scotland). A total of 480 g of the powdered material was extracted with 95% ethanol using a Soxhlet apparatus for 8 hours. The extract was filtered through a 0.45 μm membrane filter under vacuum and concentrated using a rotary evaporator (Model 349/2, Corning Ltd., England) at 40 °C. The dried extract was weighed to determine the extraction yield, which was calculated as the weight of extracted material divided by the weight of dried extract, multiplied by 100. The extract was reconstituted in distilled water to a known concentration of 100 mg/mL for administration and stored at 4 °C until use.

Oral Acute Toxicity Test (LD_{50})

The oral acute toxicity of *Sphenostylis stenocarpa* extract was evaluated using Lorke's method (Lorke, 1983). A total of 12 albino mice were used, divided into two phases. In Phase 1 (pre-test), nine mice were assigned to three groups ($n = 3$ per group) and fasted for 4 hours prior to dosing, with free access to water, receiving 10, 100, and 1000 mg/kg body weight respectively. The animals were monitored for 4 hours for signs of toxicity and mortality; no adverse reactions or mortality were observed, and no mortality was recorded at 24 hours. In Phase 2 (main test), the remaining three mice were assigned to three groups ($n = 1$ per group) and received 1600, 2900, and 5000 mg/kg body weight respectively. No signs of toxicity or mortality were observed within 24 hours or during a 2-week follow-up period. These results indicate that the oral LD_{50} of *Sphenostylis stenocarpa* extract is greater than 5000 mg/kg, suggesting a high margin of safety.

Experimental Design

Animal Grouping

Twenty-five male albino Wistar rats were randomly assigned to five experimental groups ($n = 5$ per group) to minimize allocation bias. Group A (negative control) was administered 25 mg/kg body weight (b.w.) of potassium bromate (KBrO_3) without treatment. Group B was administered 25 mg/kg b.w. KBrO_3 and treated with 50 mg/kg b.w. *Sphenostylis stenocarpa* extract. Group C was

administered 25 mg/kg b.w. $KBrO_3$ and treated with 100 mg/kg b.w. *S. stenocarpa* extract. Group D was administered 25 mg/kg b.w. $KBrO_3$ and treated with 200 mg/kg b.w. *S. stenocarpa* extract. Group E (positive control) received 0.5 mL of distilled water.

Induction of Hepatorenal Toxicity

Hepatorenal damage was induced in Groups A–D by oral administration of $KBrO_3$ (25 mg/kg b.w.) daily for 28 days.

Sample Collection

At the end of the treatment period, rats were anesthetized with chloroform, and 5 mL of whole blood was collected via the medial canthus of the eye. Blood samples were transferred into EDTA and lithium heparinized tubes and stored at 4 °C for subsequent biochemical, renal, and hematological analyses.

Liver Function Tests

Aspartate aminotransferase (AST) activity was measured according to the method of Mañourová et al., 2019. Briefly, 0.1 mL of serum was added to the sample tubes, while 0.1 mL of distilled water was added to the blank. Both tubes received 0.5 mL of Reagent 1, containing phosphate buffer, L-aspartate, and β -oxoglutarate, before incubation at 37 °C for 30 minutes. After incubation, 0.5 mL of Reagent 2 (2,4-dinitrophenylhydrazine) was added, and the mixtures were allowed to stand at 25 °C for 20 minutes. Finally, 5.0 mL of 0.4 N sodium hydroxide was added and absorbance was measured at 546 nm after 5 minutes.

Alanine aminotransferase (ALT) activity was measured according to the method of Reitman and Frankel, 1957. Serum (0.1 mL) was added to the sample tube and distilled water (0.1 mL) to the blank, and both received 0.5 mL of ALT substrate buffer containing phosphate buffer, L-alanine, and α -oxoglutarate. The reaction mixtures were incubated at 37 °C (pH 7.4) for 30 minutes, after which 0.5 mL of 2,4-dinitrophenylhydrazine was added and allowed to stand at 25 °C for 20 minutes. Finally, sodium hydroxide was added and absorbance was measured at 546 nm after 5 minutes.

Alkaline phosphatase (ALP) activity was measured using the method of Babson et al., 1966 as described in the Randox diagnostic kit. Serum (50 μ L), standard solution (50 μ L), and distilled water (50 μ L) were added to test, standard, and blank tubes respectively, followed by 50 μ L of ALP

substrate, and incubated at 37 °C for 10 minutes. After incubation, 2.5 mL of ALP color developer was added at timed intervals and absorbance was measured at 630 nm.

Kidney Function Tests

Urea concentration was determined using the urease–sodium nitroprusside method. Serum (10 μ L), standard, and distilled water were added to serum, standard, and blank tubes, each receiving 100 μ L of urease–sodium nitroprusside reagent (R1) and incubated at 37 °C for 10 minutes. Reagents R2 and R3 (2.5 mL) were then added sequentially and incubated again at 37 °C for 15 minutes, and absorbance was measured at 546 nm.

Serum creatinine was measured using the method of Burtis and Ashwood, 1994, following the Randox (UK) kit protocol. Serum (50 μ L), standard (50 μ L), and distilled water (50 μ L) were added to test, standard, and blank tubes respectively, followed by 500 μ L of working reagent, and absorbance was measured at 510 nm.

Haematological Methods

Blood samples were collected via puncture of the medial canthus of the eye using heparinized capillary tubes and transferred into clean tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Red blood cells (RBCs) were counted using an improved Neubauer hemocytometer with Hayem's solution as a diluent and expressed in millions per mm^3 . White blood cells (WBCs) were counted using the same hemocytometer with Turk's solution as the diluent and expressed in thousands per mm^3 . Packed cell volume (PCV) was measured using the microhematocrit method. Hemoglobin (Hb) concentration was determined using the cyanmethemoglobin method with Drabkin's solution, measured spectrophotometrically (JENWAY 6305 UV/Vis) at 540 nm against a standard hemoglobin solution.

Statistical Analysis

All data were expressed as mean \pm standard deviation (SD). Statistical analyses were performed using SPSS software version 26 (IBM Corp., Armonk, NY, USA). Differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value \leq 0.05 was considered statistically significant.

Results

Table 1. Effect of ethanol extract of *S. stenocarpa* on liver enzymes against potassium bromate–induced hepatorenal toxicity.

Group	ALP (U/L)	ALT (U/L)	AST (U/L)
A ($KBrO_3$ 25 mg/kg)	520.4 \pm 234.4	231.8 \pm 181.9	413.4 \pm 142.2
B ($KBrO_3$ 25 mg/kg + 50 mg/kg extract)	426.2 \pm 50.4	136.2 \pm 31.6	371.2 \pm 81.1
C ($KBrO_3$ 25 mg/kg + 100 mg/kg extract)	369.0 \pm 39.0	98.7 \pm 18.4	216.2 \pm 108.9
D ($KBrO_3$ 25 mg/kg + 200 mg/kg extract)	313.0 \pm 47.0	133.5 \pm 40.1	181.2 \pm 40.0

Group	ALP (U/L)	ALT (U/L)	AST (U/L)
E (Positive Control)	200.2 ± 34.4	104.2 ± 8.5	117.2 ± 49.0

Values are presented as mean ± SD, n = 5. Values are statistically significant at $p \leq 0.05$.

Table 2. Effect of ethanol extract of *Sphenostylis stenocarpa* on kidney markers against potassium bromate–induced hepatorenal toxicity.

Group	Urea (U/L)	Creatinine (μmol/L)
A (KBrO ₃ 25 mg/kg)	62.5 ± 13.2	131.39 ± 52.7
B (KBrO ₃ 25 mg/kg + 50 mg/kg extract)	38.80 ± 13.9	75.95 ± 21.7
C (KBrO ₃ 25 mg/kg + 100 mg/kg extract)	22.81 ± 7.3	67.83 ± 5.8
D (KBrO ₃ 25 mg/kg + 200 mg/kg extract)	9.70 ± 2.5	65.63 ± 14.2
E (Positive Control)	7.20 ± 1.4	56.50 ± 8.9

Values are presented as mean ± SD, n = 5. Values are statistically significant at $p \leq 0.05$.

Table 3. Effect of ethanol extract of *Sphenostylis stenocarpa* on hematological parameters against potassium bromate–induced hepatorenal toxicity.

Group	RBC (×10 ⁹ /L)	WBC (×10 ⁹ /L)	Hb (g/dL)	HCT (%)	PLT (×10 ⁹ /L)
A (KBrO ₃ 25 mg/kg)	6.48 ± 0.99	24.33 ± 7.84	11.74 ± 0.97	38.5 ± 1.45	461 ± 75
B (KBrO ₃ 25 mg/kg + 50 mg/kg extract)	7.12 ± 0.77	30.10 ± 5.36	13.20 ± 1.95	39.4 ± 3.72	596 ± 215
C (KBrO ₃ 25 mg/kg + 100 mg/kg extract)	7.87 ± 0.63	16.94 ± 5.86	14.44 ± 1.53	43.8 ± 2.40	567 ± 147
D (KBrO ₃ 25 mg/kg + 200 mg/kg extract)	7.57 ± 0.68	17.49 ± 4.07	13.51 ± 0.89	41.5 ± 1.85	351 ± 62
E (Positive Control)	8.94 ± 1.70	10.82 ± 1.49	14.83 ± 1.45	45.1 ± 1.49	413 ± 86

Values are presented as mean ± SD, n = 5. Values are statistically significant at $p \leq 0.05$.

In Table 1, potassium bromate (KBrO₃) administration significantly elevated liver enzymes compared to the positive control, with ALP, ALT, and AST increasing by ~160%, 123%, and 253%, respectively ($p < 0.05$). Treatment with *S. stenocarpa* ethanol extract resulted in a dose-dependent reduction of these enzymes. Notably, the 100 mg/kg and 200 mg/kg doses restored enzyme levels closer to normal, with ALP decreasing by ~40%, ALT by ~57%, and AST by ~56%, indicating significant hepatoprotective activity.

In Table 2, KBrO₃ caused marked renal impairment, evidenced by a ~768% increase in urea and ~133% increase in creatinine ($p < 0.05$). Administration of the extract significantly improved renal function in a dose-dependent manner, with the highest dose (200 mg/kg) reducing urea by ~85% and creatinine by ~50% relative to the negative control. These findings demonstrate substantial nephroprotective effects of *S. stenocarpa*.

In Table 3, exposure to KBrO₃ induced hematotoxicity, characterized by reductions in RBC (~28%), hemoglobin (~21%), and HCT (~15%), alongside a ~125% increase in WBC and ~12% increase in platelet count ($p < 0.05$). Treatment with the ethanol extract improved hematological indices in a dose-dependent manner. The 100 mg/kg dose produced the most pronounced effect, increasing RBC by ~21%, hemoglobin by ~23%, and normalizing WBC counts by up to ~30%, highlighting the extract's hematoprotective potential.

Discussion

Exposure to potassium bromate (KBrO₃) resulted in

significant systemic toxicity, as reflected by elevations in liver enzymes, renal markers, and alterations in hematological parameters. The pronounced increases in ALT, AST, and ALP observed in KBrO₃-treated rats are consistent with previous reports of KBrO₃-induced hepatocellular injury in Wistar rats (Nazir et al., 2025; De Vico et al., 2018). These studies similarly attributed hepatotoxicity to oxidative stress and disruption of hepatocyte membrane integrity, although the exact mechanisms were not directly measured in the current experiment.

Renal function was also significantly impaired, as evidenced by elevated blood urea nitrogen (BUN) and creatinine levels. These results align with recent findings by Altoom et al., 2018 and Manzoor et al., 2021, who reported that KBrO₃ exposure causes oxidative injury to renal tubular and glomerular structures, resulting in reduced filtration and nitrogenous waste accumulation. Our observations reinforce the susceptibility of metabolically active organs to oxidative xenobiotics.

Hematological alterations following KBrO₃ exposure, including decreased RBC count, hemoglobin, and hematocrit, as well as changes in WBC levels, are in agreement with Uche et al., 2024, who documented KBrO₃-induced hematotoxicity and potential anemia in experimental models. These changes may reflect systemic stress and impaired erythropoiesis, although mechanistic validation was beyond the scope of this study.

Treatment with ethanol extract of *Sphenostylis stenocarpa* partially restored biochemical and hematological parameters relative to KBrO₃-only controls. Comparable

hepatoprotective and nephroprotective effects have been reported for other legume-derived plant extracts, including *Vigna unguiculata* and *Cajanus cajan*, in chemically induced toxicity models (Chiaka-Onyemeze et al., 2025; Chinma et al., 2021). Although the extract appeared to improve outcomes at higher doses, statistical trend analysis for dose-dependency was not performed, and mechanistic pathways were not directly assessed.

These findings suggest that *S. stenocarpa* has protective potential against $KBrO_3$ -induced organ and hematological damage, likely attributable to its bioactive phytochemicals, which have been associated with antioxidant and anti-inflammatory properties in previous studies (Chiaka-Onyemeze et al., 2025; Ikagu and Ponnann, 2022). However, the lack of histopathological evaluation, oxidative stress marker assessment, and formal dose-response analysis limits mechanistic conclusions. Future studies incorporating tissue-level analyses and quantification of oxidative and inflammatory markers are warranted to validate the observed protective effects and elucidate underlying mechanisms.

Conclusion

Ethanol extract of *Sphenostylis stenocarpa* partially improved hepatic, renal, and hematological parameters in potassium bromate-exposed Wistar rats, suggesting potential protective effects. However, the small sample size and lack of mechanistic and histopathological analyses limit definitive conclusions. Further studies are needed to confirm these preliminary findings and elucidate the underlying mechanisms.

Statements and Declarations

Ethics Approval

Ethical approval was obtained from the Faculty of Pharmaceutical Science Research Ethics Committee, University of Nigeria, Nsukka, Enugu State (approval code: FPSRA/UNN/25/00157; dated 7 November 2025). All animals were handled according to conventional guidelines for the care and use of laboratory animals.

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Limitations of the Study

The study had a small sample size ($n = 5$ per group) and did not include histopathological or mechanistic analyses, limiting confirmation of organ protection and underlying pathways. Dose-response effects were not statistically assessed. Future studies should address these gaps to validate the findings.

CRedit Authorship Contribution Statement

Ndubuisi Nonso Richards: Conceptualization, Methodology, Investigation, Writing – original draft.

Nwobi Princess Somtochukwu: Investigation, Formal analysis, Writing – original draft.

Nyejirime Young Wike: Conceptualization, Supervision, Writing – review and editing.

Uzoefuna Chima Casmir: Methodology, Validation, Writing – review and editing.

Joseph Ikenna Agbor: Investigation, Data curation.

Nwatu C. Francisca: Investigation, Formal analysis.

Akhigbe Omoighele Faith: Methodology, Resources.

Patrick Chinedu Alor: Data curation, Writing – review and editing.

Declaration of Competing Interest

The authors declare that they have no competing interests to disclose.

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