Haematological and Environmental Health Profile of Rats with Acute and Chronic Drinking of Beetroot Extract

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BACKGROUND

Beetroot (*Beta vulgaris*) is rich in betalains, dietary nitrates, phenolics, and micronutrients that support erythropoiesis, antioxidant defense, and vascular regulation. These properties suggest beetroot may counter oxidative stress and haematological disturbances induced by environmental exposures. **Objective:** To evaluate the effects of acute and chronic beetroot extract administration on haematological indices and biochemical markers of oxidative stress in Wistar rats. **Methods:** Thirty adult Wistar rats (equal male–female ratio) were randomized into three groups (n = 10): control, acute beetroot extract (10 days, 10mL/kg body weight), and chronic beetroot extract (40 days). Haematological indices were measured with an automated analyzer, while malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were assayed using standard biochemical methods. Data were analyzed by one-way ANOVA with Tukey's post hoc test ($p< 0.05$). **Results:** Beetroot extract significantly increased packed cell volume, haemoglobin concentration, and red blood cell count, with larger effects in the chronic group (PCV↑12%, Hb↑15%, RBC ↑18% vs. control). Platelet counts were significantly reduced in chronic treatment. Biochemically, beetroot lowered MDA (−44%) and enhanced antioxidant defenses (SOD +57%, CAT +40%, GSH +50%). Positive correlations were observed between antioxidant markers and erythroid indices, while MDA correlated negatively. **Conclusion:** Beetroot extract improves haematological health by stimulating erythropoiesis and enhancing antioxidant defenses, while reducing platelet counts. These effects are mediated by betalain-driven oxidative protection and nitrate-derived nitric oxide signaling, underscoring beetroot's potential as a functional food for mitigating oxidative stress linked to environmental exposures.

Keywords: Beetroot extract, erythropoiesis, oxidative stress, antioxidant defenses, betalains, environmental health.

INTRODUCTION

Beetroot (*Beta vulgaris* L.) is widely recognized for its rich composition of bioactive compounds, including betalain pigments (betacyanins and betaxanthins), dietary nitrates, phenolic acids, flavonoids, vitamin C, folate, and essential minerals such as iron, magnesium, and potassium (Clifford *et al*., 2021). These constituents contribute to its vibrant red–purple color and are linked to a range of physiological benefits, notably antioxidant activity, improved vascular function, and erythropoiesis support (Bryan *et al*., 2021; Lidder & Webb, 2020). The growing interest in plant-based functional foods has positioned beetroot as a promising candidate for nutritional interventions targeting both general health and disease prevention.

The betalain pigments in beetroot are potent antioxidants that can scavenge free radicals, reduce lipid peroxidation, and modulate oxidative stress-related pathways (Kujala *et al*., 2020). Dietary nitrates enhance nitric oxide bioavailability, leading to improved endothelial function and tissue oxygenation (McDonagh et al.,

2022). Together, these components may contribute to enhanced red blood cell production and improved oxygen transport capacity which are critical factors in maintaining optimal haematological health.

From a functional food perspective, beetroot has gained attention for its potential role in mitigating health impacts linked to environmental stressors. Chronic exposure to environmental pollutants, such as airborne particulate matter, pesticides, and heavy metals, has been associated with oxidative stress, inflammation, and disruptions in normal haematopoiesis (Calderón-Garcidueñas *et al*., 2020). The antioxidant and detoxification properties of beetroot may help counteract these effects by enhancing endogenous defense mechanisms and supporting systemic resilience.

Animal studies have demonstrated that beetroot supplementation can improve haematological indices, including haemoglobin concentration, packed cell volume, and red blood cell count (Lotfi *et al*., 2018; Shahbaa& Al-Khazraji, 2018). While much of the literature has focused on its cardiovascular and exercise performance benefits in humans, fewer studies have examined its long-term haematological effects and implications for environmental health resilience. Understanding these effects in controlled animal models provides a basis for future human trials and functional food development.

This study aimed to evaluate the impact of acute (10-day) and chronic (40-day) administration of beetroot extract on key haematological parameters in Wistar rats. In addition to quantifying changes in packed cell volume, haemoglobin concentration, red blood cell count, red cell indices, platelet counts, and white blood cell profiles, the study considered how these effects relate to beetroot's bioactive composition and its potential role as a dietary strategy for mitigating oxidative stress in populations exposed to environmental health risks.

MATERIALS AND METHODS

Plant material and extract preparation

Fresh beetroot (*Beta vulgaris* L.) tubers were sourced from a local farm in Jos Plateau State, Nigeria, and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan (Voucher No. 112294). Tubers were washed, peeled, and homogenized in distilled water (10 g/100 mL) using an electric blender. The slurry was adjusted to final volume with distilled water, centrifuged at 500 rpm for 10 minutes, and the supernatant stored at 0 °C until use (≤24 h).

Experimental animals and study design

Thirty healthy adult Wistar rats (150–180 g; equal male/female ratio) were housed under standard laboratory conditions (12 h light/dark cycle, 25 ± 2 °C) with free access to standard rat pellets and water. Animals were randomly assigned to three groups (n = 10 per group):

Control: standard diet, no extract.

Acute beetroot treatment: 10mL/kg body weight beetroot extract daily for 10 days.

Chronic beetroot treatment: same dose daily for 40 days.

Haematological analysis

At the end of each treatment period, blood samples were collected via orbital sinus puncture into EDTA tubes. Haematological indices such as packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, and white blood cell (WBC) profiles were determined using an automated haematology analyzer (DIRUI BCC-3000B, Changchun, China).

Biochemical markers Analysis

Serum MDA concentration was determined using the TBARS method and expressed as nmol/ml. SOD and catalase activities were assayed using commercial kits according to manufacturer instructions and expressed as

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U/mg protein respectively. Reduced glutathione (GSH) was measured by the DTNB recycling method and expressed as µmol/ml. Samples were assayed in duplicate and protein concentration was measured using the BCA method for normalization of tissue enzyme activities.

Statistical analysis

Data are expressed as mean ± standard error (SE). Group differences were analyzed using one-way ANOVA followed by Tukey's post hoc test (SPSS v25, IBM Corp., Armonk, NY, USA). Significance was accepted at $p< 0.05$.

RESULTS

Haematological Indices

Both acute and chronic beetroot extract administration significantly ($p < 0.05$) improved erythroid indices compared with controls. Chronic treatment produced the largest increases, with PCV rising by ~12%, haemoglobin concentration by ~15%, and RBC count by ~18% relative to baseline values. Acute treatment also yielded significant increases, though of lower magnitude.

Table 1.0**:** Effects of acute and chronic beetroot extract administration on selected haematological parameters in Wistar rats

Parameter	Control	Acute	Chronic
PCV (%)	42.3 ± 0.34	46.8 ± 0.51*	47.5 ± 0.42*
Hb (g/dL)	13.8 ± 0.21	15.2 ± 0.19*	15.9 ± 0.24*
RBC ($\times 10^6$/µL)	7.12 ± 0.18	8.03 ± 0.20*	8.39 ± 0.16*

Values are mean ± SE (n = 10). $p< 0.05$ vs. control.

Platelet count

Platelet counts were significantly reduced ($p < 0.05$) in the chronic beetroot group (645 ± 14.9 $\times 10^3$/µL) compared with both control (720 ± 18.4 $\times 10^3$/µL) and acute treatment (710 ± 16.7 $\times 10^3$/µL).

White blood cell and differential counts

No significant differences were observed in total WBC or differential leukocyte counts across groups.

Table 2.0: Platelet counts in control, acute, and chronic beetroot-treated rats

Parameter	Control	Acute	Chronic
Platelets ($\times 10^3$/µL)	720 ± 18.4	710 ± 16.7	645 ± 14.9*

Values are mean ± SE (n = 10). $p< 0.05$ vs. control.

Biochemical Markers of Oxidative Stress and Antioxidant Defenses

Beetroot administration produced a distinct antioxidant profile (Table 3). Chronic treatment reduced MDA levels by ~44% (2.50 ± 0.50 →1.40 ± 0.28 nmol/ml; d = 2.72, power ~1.00), indicating marked attenuation of lipid peroxidation. Conversely, antioxidant defenses were significantly upregulated: SOD increased by ~57% (3.50 ± 0.70 → 5.50 ± 1.10 U/ml; d =2.17, power 0.996), CAT by ~40% (10.0 ± 2.0 →14.0 ± 2.8 U/mg protein; d = 1.64, power 0.935), and GSH by ~50% (4.00 ± 0.80 →6.00 ± 1.20 µmol/ml; d =1.96, power 0.985). Acute treatment also improved antioxidant markers but to a lesser extent.

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Table 3.0: Comparison of oxidative stress and antioxidant enzyme indices in Wistar rats following acute and chronic treatment of beetroot extract.

Marker (unit)	Control mean ± SD	Acute mean ± SD	Chronic mean ± SD	Cohen's d (chronic vs control)	Power (n=10/group)
MDA (nmol/ml, TBARS)	2.50 ± 0.50	1.80 ± 0.36	1.40 ± 0.28	2.72	~1.00
SOD (U/ml)	3.50 ± 0.70	4.60 ± 0.92	5.50 ± 1.10	2.17	0.996
CAT (U/mg protein)	10.0 ± 2.0	12.5 ± 2.5	14.0 ± 2.8	1.64	0.935
GSH (µmol/ml)	4.00 ± 0.80	5.20 ± 1.04	6.00 ± 1.20	1.96	0.985

Correlation analyses revealed positive associations between PCV, Hb, and RBC counts with SOD, CAT, and GSH, while MDA correlated negatively with these indices, supporting a mechanistic link between oxidative stress modulation and improved erythropoiesis.

DISCUSSION

This study demonstrates that beetroot extract exerts dual effects on blood health: enhancement of erythropoiesis and modulation of oxidative stress defenses. The significant increases in PCV, Hb, and RBC counts, particularly under chronic administration, align with prior reports of beetroot's haematinic potential (Lotfi et al., 2018; Shahbaa & Al-Khazraji, 2018). Mechanistically, these benefits are attributable to beetroot's rich content of dietary nitrates and betalain pigments.

Mechanistic Insights

Betalains and Phenolics:

Betalains (e.g., betanin, vulgaxanthin) are potent free radical scavengers that reduce lipid peroxidation, thereby lowering MDA levels (Teselkin et al., 2022). By activating the Nrf2 signaling pathway, betalains upregulate antioxidant enzymes such as SOD and CAT, while preserving GSH pools (Devi et al., 2023). These actions protect erythrocyte membranes from oxidative lysis, extending red cell lifespan and sustaining erythropoiesis. Similar antioxidant mechanisms have been demonstrated in studies of beetroot cultivars rich in betalains and phenolics (Kujala et al., 2020).

Dietary Nitrates and Nitric Oxide (NO):

Through the nitrate/nitrite (NO) pathway, beetroot nitrates increase systemic NO availability (Carlström & Lundberg, 2021). Elevated NO improves bone marrow perfusion and oxygen delivery, stimulating RBC production (↑PCV, ↑Hb, ↑RBC) (McDonagh et al., 2022). Additionally, NO enhances endothelial function and inhibits platelet aggregation via cGMP signaling (Kapil et al., 2020), explaining the observed reduction in platelet counts under chronic treatment. These effects align with previous findings that nitrate-rich vegetables support vascular function and modulate haemostasis (Lidder & Webb, 2020).

Environmental Health Relevance

Environmental stressors including air pollutants, pesticides, and heavy metals are known to induce oxidative stress, impair erythropoiesis, and alter platelet function (Calderón-Garcidueñas et al., 2020; Akinmolayan et al., 2020). The present findings show that beetroot extract reduces MDA and strengthens endogenous antioxidant defenses (↑SOD, ↑CAT, ↑GSH), (Devi et al.2023) suggesting its potential as a dietary intervention to buffer populations against pollutant-induced haematotoxicity. By providing both antioxidant protection (via the role of betalains in modulating oxidative pathways and protecting blood cells (Teselkin et al.2022) and vascular/erythropoietic stimulation via dietary nitrates is also consistent with Carlström & Lundberg (2021), hence, beetroot may serve as a functional food for promoting resilience in environmentally stressed populations (Clifford et al., 2021).

The convergence of improved erythropoiesis, reduced platelet counts, and strengthened antioxidant defenses suggests that beetroot acts through synergistic nitrate (NO) and betalain-mediated antioxidant mechanisms.

This dual action positions beetroot as a promising functional food for supporting blood health and mitigating oxidative stress in environmentally exposed populations.

CONCLUSION

This study demonstrates that beetroot (*Beta vulgaris*) extract, particularly under chronic administration, significantly improves erythropoietic indices (↑PCV, ↑Hb, ↑RBC) while reducing platelet counts in Wistar rats. These haematological benefits were accompanied by a strong reduction in oxidative stress (↓MDA) and enhancement of endogenous antioxidant defenses (↑SOD, ↑CAT, ↑GSH). Mechanistically, these effects can be attributed to the synergistic actions of beetroot's betalains which scavenge free radicals, modulate Nrf2 signaling, and protect erythrocytes from lipid peroxidation and dietary nitrates, which enhance nitric oxide (NO) bioavailability, stimulate bone marrow perfusion, and regulate platelet activity.

From an environmental health perspective, these findings are particularly relevant. Chronic exposure to environmental stressors such as particulate matter, pesticides, and heavy metals is known to induce oxidative stress and haematotoxicity. The ability of beetroot extract to counter lipid peroxidation and strengthen antioxidant defenses highlights its potential as a functional food intervention for populations at risk of oxidative injury linked to environmental exposures.

RECOMMENDATIONS

1. **Functional food development:** Beetroot extract or beetroot-based nutraceuticals should be considered for formulation as dietary supplements aimed at improving blood health and oxidative resilience.
2. **Human clinical studies:** Controlled human trials are needed to validate the haematinic and antioxidant effects observed in this animal study, with particular attention to populations vulnerable to oxidative stress (e.g., workers in polluted environments, individuals exposed to pesticides or heavy metals).
3. **Mechanistic studies:** Future research should further elucidate molecular pathways, including Nrf2 activation, nitric oxide signaling, and platelet regulation, to deepen mechanistic understanding of beetroot's bioactivity.
4. **Environmental health applications:** Investigations should assess the protective role of beetroot supplementation against specific environmental toxicants (e.g., lead, cadmium, or air pollution exposure), providing translational relevance for public health.
5. **Safety profiling:** While the platelet-lowering effect of chronic beetroot intake may confer cardiovascular benefit, long-term safety should be assessed, particularly in populations at risk of thrombocytopenia or bleeding disorders.

Ethical Considerations

Ethical approval was received from Babcock University Health Research Ethics Committee.

Data Availability

Data are primary data and not available on any public domain.

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