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Protective effects of ethanolic beetroot (*Beta vulgaris L.*) extract against heat- and biomass smoke-induced hematological and pulmonary alterations in Wistar rats

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ABSTRACT

Background: Exposure to biomass smoke and heat stress constitutes a significant public health burden, particularly in low-resource settings where traditional cooking practices are prevalent. These environmental stressors induce systemic oxidative stress and inflammation, leading to hematological disturbances and pulmonary damage. Although beetroot (*Beta vulgaris* L.) is rich in bioactive antioxidants with established health benefits, its protective efficacy against the combined effects of heat and biomass smoke exposure remains inadequately characterized. **Objective:** This study aimed to investigate the protective effects of ethanolic beetroot extract against hematological alterations and pulmonary histopathology induced by concurrent exposure to heat and biomass smoke in adult male Wistar rats. **Materials and Methods:** Forty-nine adult male Wistar rats (180–220 g) were randomly assigned to seven groups (n=7): control, smoke only, heat only, smoke + heat, smoke + beetroot, heat + beetroot, and smoke + heat + beetroot. Beetroot extract (400 mg/kg) was administered orally for 28 days. At the end of the 28 days experimental period, the rats were sacrificed under chloroform anesthesia for hematological and histopathological sample collection and analysis. **Results:** Exposure to smoke and heat significantly reduced total white blood cell, red blood cell, hemoglobin, and hematocrit levels ($p < 0.05$), while increasing monocyte counts and altering platelet indices. Beetroot treatment markedly restored these parameters to near-control levels. Histopathological examination revealed that beetroot supplementation attenuated alveolar damage, bronchiolar ulceration, and interstitial inflammation induced by smoke and heat exposure. **Conclusion:** Beetroot extract demonstrates significant hematoprotective and anti-inflammatory effects against heat- and biomass smoke-induced toxicity in Wistar rats, likely attributed to its rich bioactive composition. These findings suggest its potential as a dietary intervention in environmental stress settings, although further studies are warranted to confirm clinical relevance.

Keywords: *Beta vulgaris*, Biomass Smoke Exposure, Heat Stress, Hematological Parameters, Pulmonary Toxicity, Antioxidant Activity.

INTRODUCTION

Hematology encompasses the study of blood, blood-forming organs, and blood disorders, playing a critical role in diagnosing conditions such as anemia, infections, and immune deficiencies [1]. Histopathology, the microscopic examination of tissues, is fundamental for understanding disease mechanisms and evaluating therapeutic interventions [2]. Heat, the transfer of thermal energy through conduction, convection, and radiation, poses significant physiological stress [3]. In developing countries, more than three billion people depend on biomass fuels, including wood, charcoal, crop residues, and animal dung, for cooking and heating, leading to prolonged exposure to hazardous pollutants generated during combustion [4]. This widespread exposure significantly compromises respiratory and systemic health, especially in regions where traditional biomass fuel use remains prevalent [5,6].

Biomass smoke is a complex mixture of harmful pollutants, including fine particulate matter ($PM_{2.5}$ and PM_{10}), carbon monoxide (CO), nitrogen oxides (NOx), polycyclic aromatic hydrocarbons (PAHs), and volatile organic compounds (VOCs) [7,8]. Inhalation of these harmful pollutants induces pulmonary inflammation, oxidative stress, and structural tissue remodeling, which are important underlying

mechanisms contributing to chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma, bronchitis, and lung cancer [9,10]. Concurrently, prolonged or recurrent heat exposure can induce hyperthermia, systemic inflammation, and multi-organ dysfunction through mechanisms involving heat shock protein upregulation, mitochondrial dysfunction, and pro-inflammatory cytokine release [11,12].

The lungs, due to their extensive vascularization and high metabolic activity, are particularly susceptible to environmental insults [13]. Histologically, the respiratory system comprises conducting airways (trachea, bronchi, bronchioles) lined with pseudostratified ciliated columnar epithelium, and respiratory units (alveoli) responsible for gas exchange [14]. Exposure to biomass smoke and heat stress induces marked pulmonary histopathology, including alveolar septal thickening, edema, hemorrhage, and fibrosis [15,16]. These pathological changes are driven by excessive reactive oxygen species (ROS) generation, depletion of antioxidant defenses, and heightened expression of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α) [17,18].

Beetroot (*B. vulgaris* L.) belongs to the Chenopodiaceae family and is a traditional vegetable valued for its vibrant color and nutritional benefits [19]. Nutrient-dense beetroot contains powerful bioactive compounds, including betalains (betacyanins and betaxanthins), ascorbic acid, flavonoids, polyphenols, and dietary nitrates [20]. These compounds exhibit strong antioxidant and anti-inflammatory properties by scavenging ROS, inhibiting lipid peroxidation, and suppressing NF-κB-mediated inflammation while upregulating endogenous antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (SOD) [21,22]. Beetroot's nitrates enhance nitric oxide (NO) bioavailability, promoting vasodilation and improved oxygen transport [20]. Experimental studies have demonstrated its protective effects against oxidative and inflammatory damage in various tissues, including the brain, liver, heart, and lungs [23,24].

Although the antioxidant and protective effects of beetroot against oxidative stress have been well documented, its efficacy in mitigating the combined effects of heat and biomass smoke exposure has not been systematically investigated. This dual-stressor exposure is of growing concern due to climate change and the continued reliance on biomass fuels. The concurrent exposure to heat and biomass smoke may produce synergistic inflammatory and oxidative damage that exceeds the effects of either stressor alone. Understanding the protective potential of a widely available, affordable dietary intervention such as beetroot carries substantial scientific and public health significance, particularly for vulnerable populations in resource-limited settings.

Hence, the study was designed to: (1) evaluate the protective effects of ethanolic beetroot extract against hematological alterations induced by concurrent heat and biomass smoke exposure in adult male Wistar rats; (2) assess the histopathological changes in lung tissues following exposure and beetroot treatment; and (3) determine the potential mechanisms underlying the protective effects of beetroot extract.

MATERIALS AND METHODS

Materials

During this research, the following materials were used: Animal feed, sterilized water, beetroot (roots), dissecting set, cotton swab, rat cages, syringes, sample bottles, alcohol, wire gauze, charcoal, binding wire, plastic containers and pipes.

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and obtained from commercial suppliers. The following reagents were used: absolute ethanol (≥99.8% purity; CAS: 64-17-5) obtained from Sigma-Aldrich (St. Louis, MO, USA); chloroform ($CHCl_3$; CAS: 67-66-3) obtained from Merck KGaA (Darmstadt, Germany); 10% neutral buffered formalin (CAS: 50-00-0) obtained from Leica Biosystems (Wetzlar, Germany); hematoxylin (CAS: 517-28-2) and eosin Y (CAS: 17372-87-1) obtained from Thermo Fisher Scientific (Waltham, MA, USA); and DPX mounting medium obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade and purchased from standard suppliers. Distilled water was used throughout the experimental procedures.

Beetroot Extract Preparation

Fresh beetroots were purchased from a local market in Benin City, Edo State, Nigeria. The beetroots were washed, peeled, cut into small pieces, and shade-dried at room temperature. The dried materials were pulverized into a coarse powder using a mechanical grinder. Extraction was performed using the cold maceration method [25]. Briefly, 500 g of powdered material was soaked in 1500 mL of 70% ethanol for 72 h with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper, and the marc was re-macerated twice with fresh solvent [26,27]. The combined filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator (Heidolph, Germany). The resulting semi-solid extract was stored at -20 °C until use [28]. The yield was calculated as 12.5% w/w.

Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic beetroot extract was conducted using standard protocols to confirm the presence of major bioactive constituents including betalains, flavonoids, phenolics, saponins, and alkaloids [29,30].

Experimental Animals and Ethical Clearance

Forty-nine (49) adult male Wistar rats (180-220 g) were obtained from the Animal House of the Department of Anatomy, University of Benin. The rats were housed in polypropylene cages under standard laboratory conditions: temperature (25 ± 2 °C), relative humidity (55 ± 5%), and a 12:12 h light/dark cycle. Animals had free access to standard rodent pellet diet and water *ad libitum*. All procedures complied with the NIH (1985) Procedural Manual for animal handling and care.

Ethical approval for this study was obtained from the Institutional Animal Ethics Committee of the University of Benin (Approval Number: CMS/REC2025/422).

Experimental Design

After a two-week acclimatization period, rats were randomly divided into seven groups (n=7 per group). The beetroot extract dose of 400 mg/kg was selected based on previous studies demonstrating its protective efficacy without toxicity [31]. Treatments were administered via oral gavage daily for 28 days.

Group A (Control): Received normal saline (1 mL/kg/day) and was exposed to fresh air.

Group B (Smoke only): Exposed to biomass smoke only.

Group C (Heat only): Exposed to elevated ambient temperature only.

Group D (Smoke + Heat): Exposed to both biomass smoke and heat stress.

Group E (Smoke + Beetroot): Exposed to smoke and treated with beetroot extract (400 mg/kg/day).

Group F (Heat + Beetroot): Exposed to heat and treated with beetroot extract (400 mg/kg/day).

Group G (Smoke + Heat + Beetroot): Exposed to both smoke and heat, and treated with beetroot extract (400 mg/kg/day).

All treatments were administered one hour after the daily exposure period.

Biomass Smoke Exposure

Rats were placed in a whole-body inhalation chamber (50 L volume) and exposed to smoke generated from the combustion of 50 g of a mixture of dry wood and charcoal, as previously described [32]. The exposure lasted for one hour daily. The particulate matter ($PM_{2.5}$) concentration inside the chamber was monitored and maintained at approximately 500–600 µg/m³. An improvised rectangular exposure chamber (90 cm × 45 cm × 45 cm) was used, with the rats allowed to move freely. The chamber had an inlet for smoke introduction and an outlet pipe fitted at the top to direct smoke out during exposure [33].

Heat Stress Exposure

Heat stress was simulated using a perforated heated wooden chamber (30 cm × 50 cm × 25 cm) fitted with a digital thermometer. The chamber was heated using a non-light heat emitter ceramic bulb and regulated using a heat switch. The chamber was maintained at 38 ± 1 °C [34], for one hour daily (9:00 am – 10:00 am), immediately following the smoke exposure for the combined exposure groups [35].

Blood Sample Collection and Hematological Assay

At the end of the 28-day experimental period, rats were anaesthetized using chloroform ($CHCl_3$) inhalation. Blood samples (approximately 5 mL) were collected by cardiac puncture into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles for full blood count analysis. Hematological parameters, including total white blood cell (WBC) count, lymphocyte (LYM) count, monocyte (MID) count, granulocyte (GRAN) count, red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW-CV, RDW-SD), platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV), plateletcrit (PCT), and platelet-large cell ratio (PLCR), were analyzed at the Department of Hematology, University of Benin Teaching Hospital, Edo State, Nigeria.

Histopathological Examination

Following euthanasia, lung tissues were carefully dissected and fixed in 10% neutral buffered formalin. Tissue processing was performed using an automatic tissue processor (Leica TP2010) through fixation, dehydration using ascending grades of isopropyl alcohol, clearing in xylene, and infiltration with molten paraffin wax. Tissues were embedded in paraffin wax, sectioned at 5 µm thickness using a semi-automated rotary microtome (Thermo Scientific), and stained with hematoxylin and eosin (H&E) to demonstrate general tissue architecture [36-38]. Stained slides were mounted in DPX mounting medium and examined under a light microscope (Olympus BX43) at x10 and x40 magnifications. Photomicrographs were captured using a digital camera. Histological changes were evaluated qualitatively for the presence of necrosis, inflammatory infiltrates, and architectural alterations. All histopathological examinations were performed at the Department of Histopathology, University of Benin Teaching Hospital, Edo State, Nigeria.

Statistical Analysis

Data were expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed using GraphPad Prism software (version 9.0). Differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Phytochemical Profile of Beetroot Extract

Qualitative phytochemical screening of the ethanolic beetroot extract revealed the presence of betalains, flavonoids, phenolics, saponins, and terpenoids, consistent with previous reports [29,30]. The presence of these bioactive compounds provides a chemical basis for the observed antioxidant and anti-inflammatory activities.

Hematological Parameters

Leukocyte Profile

Exposure to smoke and heat significantly altered leukocyte populations (Figures 1A–D). Total WBC count was significantly decreased in the smoke only, heat only, and smoke + heat groups compared to the control group ($p < 0.05$). Beetroot treatment effectively restored WBC counts to near-normal levels in the smoke + beetroot, heat + beetroot, and smoke + heat + beetroot groups ($p < 0.05$). Similarly, granulocyte (GRAN) counts showed significant decreases in the exposure groups, with restoration observed following beetroot treatment. Monocyte (MID) counts were significantly elevated in the smoke only and heat only groups ($p < 0.05$), which was attenuated by beetroot treatment. Lymphocyte (LYM) counts remained unchanged across all groups ($p > 0.05$).

Erythrocyte Profile

Exposure to smoke and heat significantly compromised erythrocyte parameters (Figures 2A–D). RBC count was significantly decreased in the smoke only and smoke + heat groups compared to control ($p < 0.05$), with beetroot treatment restoring RBC levels in the smoke + heat + beetroot group ($p < 0.05$). Hemoglobin (HGB) concentration was significantly reduced in the smoke only and heat only groups ($p < 0.05$), while HGB levels were maintained in the beetroot-treated groups. Hematocrit (HCT) levels showed significant decreases in the smoke only, heat only, and smoke + heat groups ($p < 0.05$), with restoration observed in all beetroot-treated groups ($p < 0.05$). Red cell indices (MCV, MCH, MCHC, RDW-CV, RDW-SD) remained unchanged across all groups ($p > 0.05$), indicating a normocytic, normochromic anemia pattern.

Platelet Profile

Significant alterations in platelet indices were observed in the exposure groups (Figures 3A–D). Platelet count (PLT) was significantly elevated in the smoke only and heat only groups ($p < 0.05$), with beetroot treatment normalizing PLT levels in the heat + beetroot group ($p < 0.05$). Mean platelet volume (MPV) and platelet-large cell ratio (PLCR) were significantly increased in the heat only group ($p < 0.05$), which was attenuated by beetroot treatment. Plateletcrit (PCT) showed a similar pattern, with significant elevation in the heat only group ($p < 0.05$) and normalization following beetroot supplementation. Platelet distribution width (PDW) remained unchanged across all groups ($p > 0.05$).

Histopathological Findings

Histological examination of lung tissues revealed significant architectural alterations following exposure to smoke and heat, which were attenuated by beetroot treatment (Figures 4A–D and 5A–C).

DISCUSSION

This study investigated the protective effects of ethanolic beetroot extract against heat- and biomass smoke-induced hematological and pulmonary alterations in adult Wistar rats. The findings demonstrate that exposure to these environmental stressors induces significant hematological disturbances and pulmonary histopathology, while beetroot supplementation effectively attenuates these alterations. The

protective effects are likely mediated through the antioxidant and anti-inflammatory properties of beetroot's bioactive constituents, including betalains, polyphenols, and dietary nitrates [20,21].

The significant decrease in total WBC and granulocyte counts observed in the exposure groups suggests a state of stress-induced leukopenia, contrary to the anticipated inflammatory leukocytosis [39]. This paradoxical response may indicate early immunosuppression or, more likely, a large-scale migration of leukocytes from the circulation into damaged tissues, such as the lungs, resulting in reduced blood counts [40]. This interpretation is supported by the histopathological findings of inflammatory cell infiltration in lung tissues. The restoration of WBC and granulocyte counts in beetroot-treated groups underscores the extract's immunomodulatory potential, likely mediated through inhibition of pro-inflammatory pathways such as NF-κB, thereby reducing chemotactic signals that promote leukocyte egress from the vasculature [41].

The elevation in monocyte counts observed in the smoke-only and heat-only groups is consistent with the known ability of these stressors to induce systemic inflammation [7,42]. Monocytes are attracted to sites of tissue damage and serve as important mediators of inflammation. The significant reduction in MID counts following beetroot treatment further supports its anti-inflammatory properties, likely through inhibition of pro-inflammatory cytokine release [23].

The significant decreases in RBC count, hemoglobin concentration, and hematocrit observed in the exposed groups indicate a state of stress-induced anemia. This hematological disturbance likely results from multiple mechanisms, including oxidative damage leading to hemolysis, inflammatory suppression of erythropoietin production, and bone marrow suppression by systemic toxins [43]. Biomass smoke contains carbon monoxide, which binds irreversibly to hemoglobin to form carboxyhemoglobin, further reducing oxygen-carrying capacity and functional red blood cell lifespan [9]. The stable MCV, MCH, and MCHC values across groups indicate that the anemia was normocytic and normochromic, suggesting that direct oxidative and toxic effects, rather than nutritional deficiencies, were the primary drivers.

Beetroot's effectiveness in restoring erythrocyte parameters is biologically supported by its rich composition. Dietary nitrates increase nitric oxide (NO) bioavailability, improving microvascular blood flow and oxygen delivery [20]. Betalains and vitamin C protect red blood cells from oxidative lysis [22], while iron and folate content supports erythropoiesis [19]. These findings are consistent with previous reports demonstrating the hematoprotective effects of beetroot in anemic models [44].

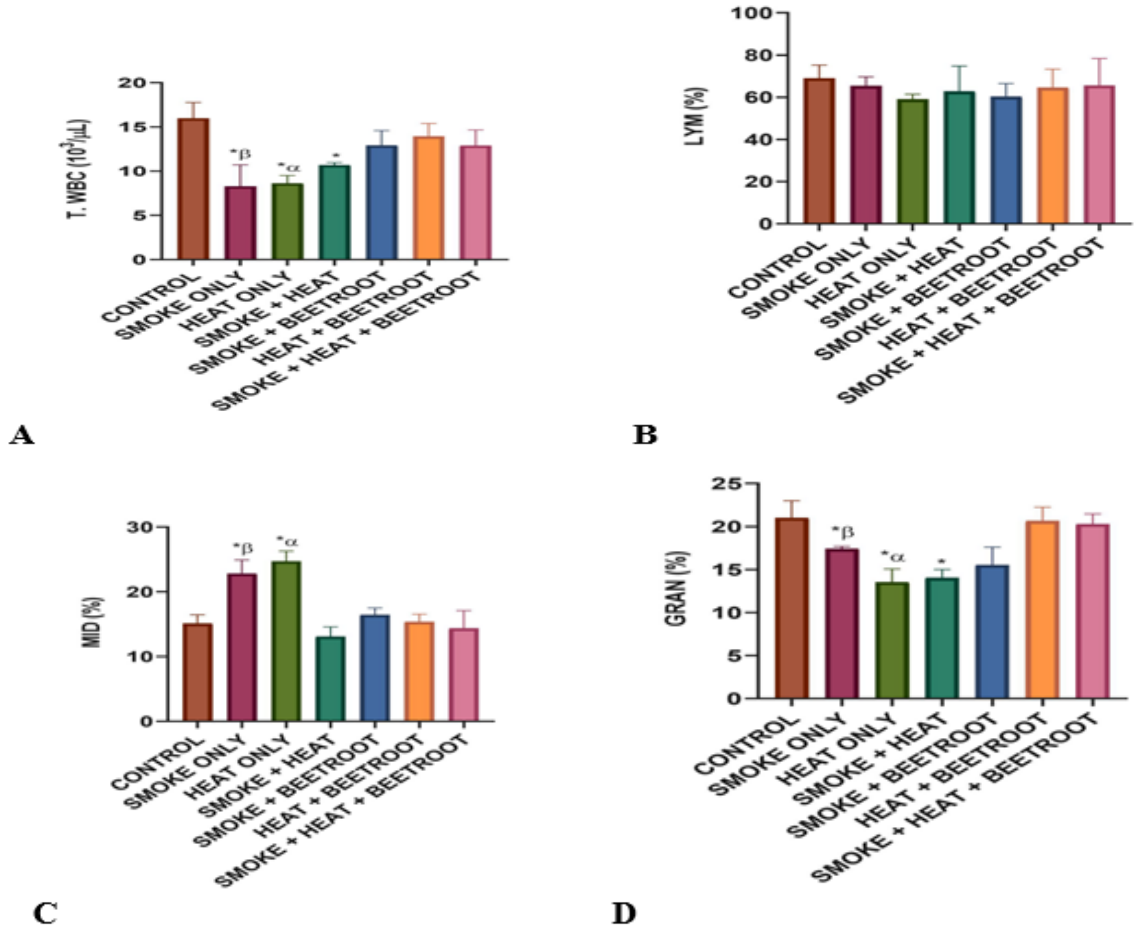

Figure 1: Effect of Smoke, Heat, and Beetroot on Leukocyte Parameters (A–D)
A: Total White Blood Cell Count; **B:** Lymphocyte Count; **C:** Monocyte Count; **D:** Granulocyte Count
Data are presented as mean ± SEM (n=7). $^{*}p < 0.05$ vs. control; $^{\beta}p < 0.05$ vs. smoke only group; $^{\alpha}p < 0.05$ vs. heat only group; $^{\pi}p < 0.05$ vs. smoke + heat group.

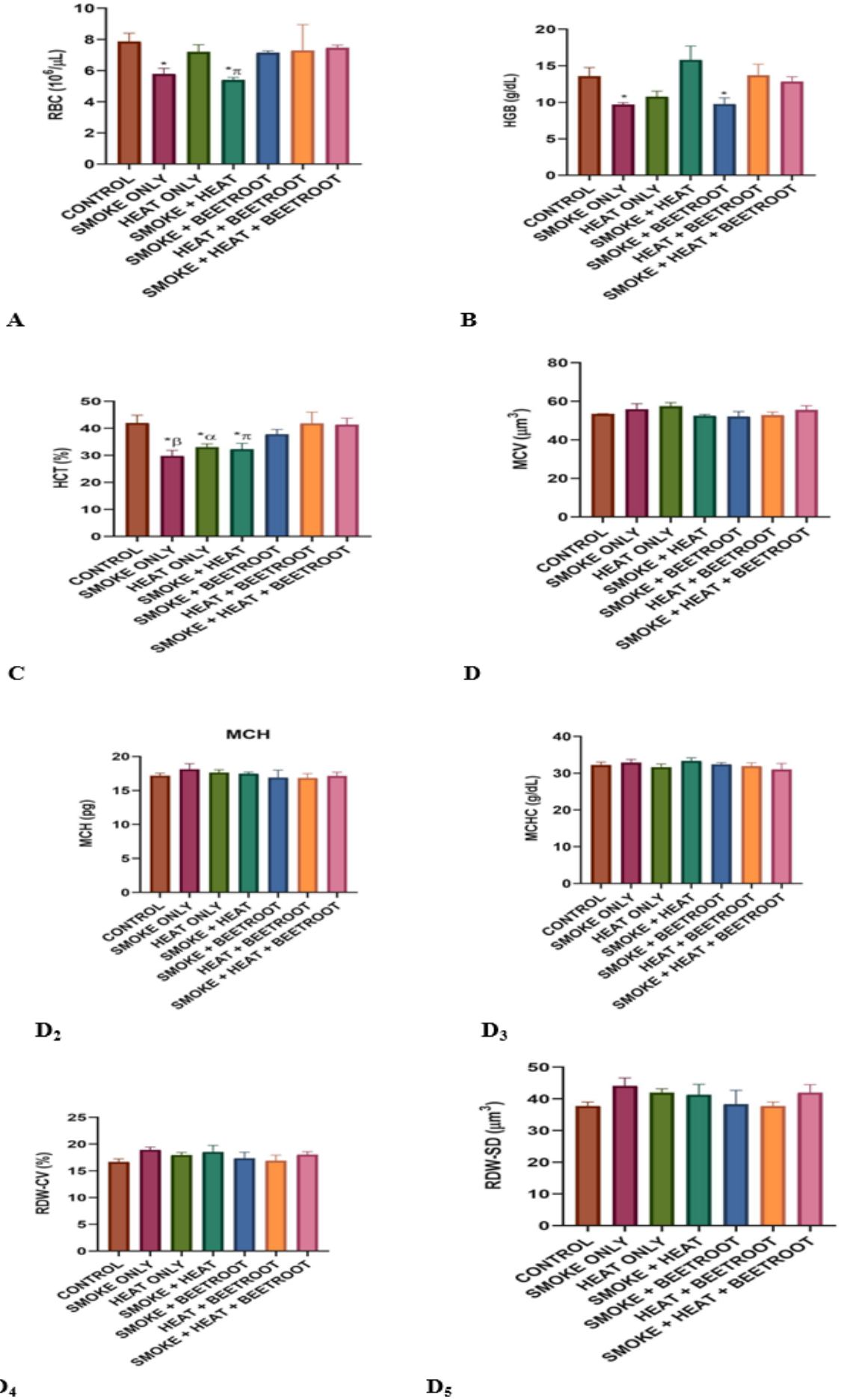

Figure 2: Effect of Smoke, Heat, and Beetroot on Erythrocyte Parameters (A–D)
A: Red Blood Cell Count; **B:** Hemoglobin Concentration; **C:** Hematocrit; **D:** Mean Corpuscular Volume (Red Indices)
Data are presented as mean ± SEM (n=7). *p < 0.05 vs. control; βp < 0.05 vs. smoke only group; αp < 0.05 vs. heat only group; πp < 0.05 vs. smoke + heat group

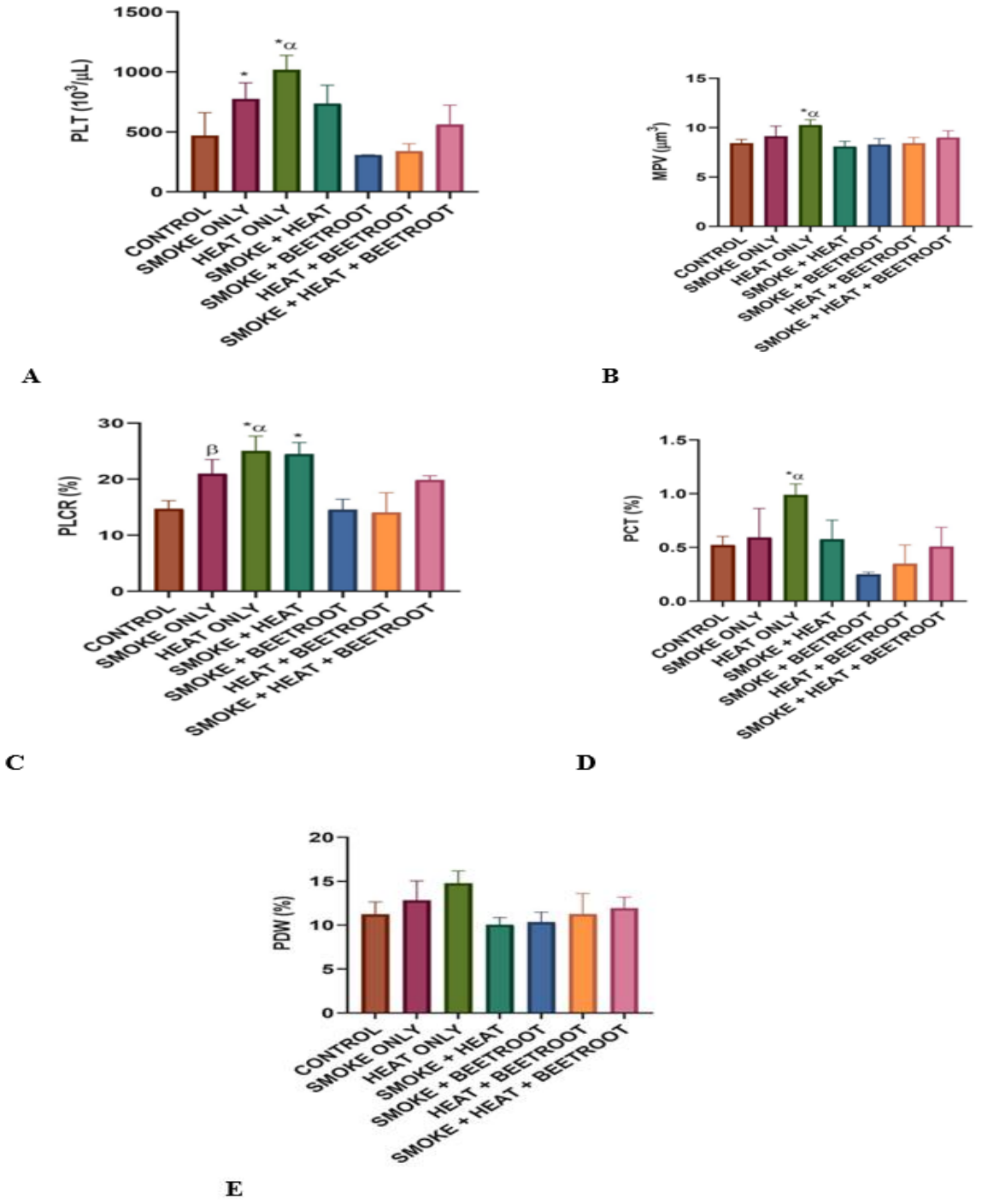

Figure 3: Effect of Smoke, Heat, and Beetroot on Platelet Parameters (A–E)
A: Platelet Count; **B:** Mean Platelet Volume; **C:** Platelet-Large Cell Ratio; **D:** Plateletcrit. Figure 3E: Platelet Distribution Width
Data are presented as mean ± SEM (n=7). $^{*}p < 0.05$ vs. control; $^{\beta}p < 0.05$ vs. smoke only group; $^{\alpha}p < 0.05$ vs. heat only group; $^{\alpha}p < 0.05$ vs. smoke + heat group

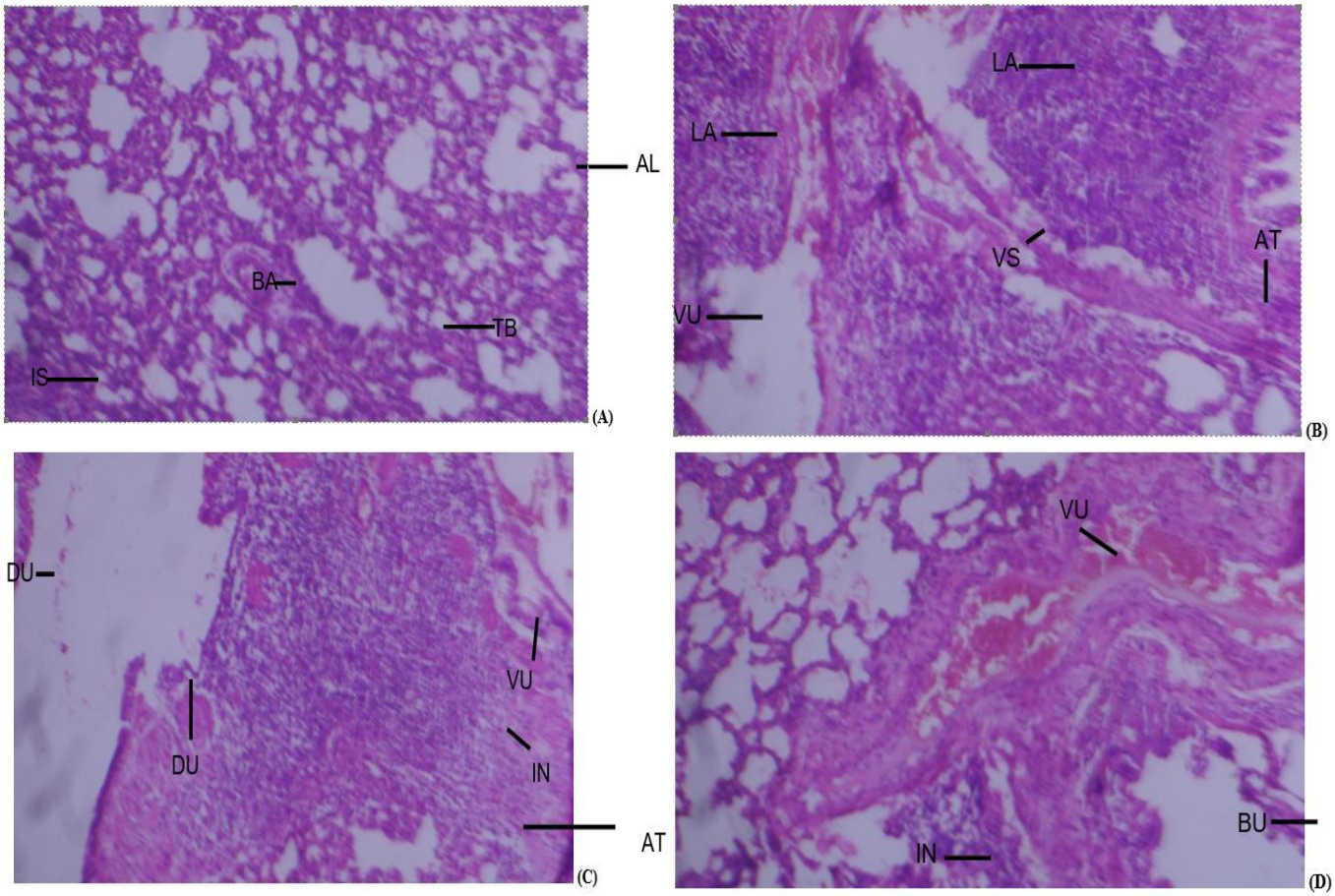

Figure 4: Histopathological Changes in Lung Tissues (A–D)

A: Control Group – Normal lung architecture showing alveoli (AL), interstitial space (IS), bronchial artery (BA), and terminal bronchiole (TB). H&E, ×100. **B:** Smoke Only Group – Lung section showing severe bronchiole-alveolar lymphoid tissue activation (LA), vascular ulceration (VU), stenosis (VS), and atelectasis (AT). H&E, ×100 **C:** Heat Only Group – Lung section showing severe bronchiolar dilation and ulceration (DU), atelectasis (AT), vascular ulceration (VU), and severe inflammation (IN). H&E, ×100. **D:** Smoke + Heat Group – Lung section showing severe vasodilatation and ulceration (VU), bronchiolar ulceration (BU), and interstitial inflammation (IN). H&E, ×100

The significant increases in MPV and PLCR observed in the heat-only group indicate thermally induced changes in platelet production and activation. Heat stress can cause hemoconcentration and endothelial activation, leading to the release of larger, more reactive platelets from the bone marrow, representing a pro-thrombotic risk factor [34,45]. Beetroot consumption normalized these indices, likely through the nitrate-NO pathway, which reduces platelet activation by promoting vasodilation and decreasing endothelial dysfunction [20]. The elevation in platelet count in smoke-only and heat-only groups suggests reactive thrombocytosis in response to inflammatory stress, which was attenuated by beetroot treatment.

Histopathological examination revealed significant pulmonary damage following exposure to biomass smoke and heat stress. The smoke-only group exhibited intense peribronchiolar and interstitial inflammation, vascular ulceration, and alveolar collapse, while the heat-only group showed bronchiolar ulceration, vascular congestion, and edema. These findings align with previous studies demonstrating that particulate matter and heat stress exposure induce oxidative stress, inflammatory cell infiltration, and lung epithelial damage [15,16,46].

The combined exposure group demonstrated more severe pathological changes than either stressor alone, indicating synergistic interaction between oxidative and inflammatory pathways. This finding is particularly significant given the increasing prevalence of concurrent heat waves and air pollution episodes due to climate change [47]. The complex mixture of carbon monoxide, nitrogen oxides, and polycyclic aromatic hydrocarbons in biomass smoke causes tissue damage and vascular congestion by inducing cytokine release from neutrophils and macrophages [48,49].

Beetroot-treated groups demonstrated significant attenuation of histopathological lesions, including reduced inflammatory cell infiltration, vascular ulceration, and preservation of alveolar architecture. This protective effect is consistent with established reports that *B. vulgaris* extracts, rich in phenolic antioxidants and betalains, stabilize cell membranes and inhibit inflammatory responses [20]. Beetroot supplementation has been shown to reduce tissue oxidative stress and improve histological outcomes in models of pulmonary and hepatic injury [24,50]. The present findings therefore support the potential of beetroot extract as a natural antioxidant agent against environmental lung insults.

Study Limitations

Several limitations were acknowledged. First, the absence of biochemical oxidative stress markers (e.g., MDA, SOD, CAT, GPx) and inflammatory cytokines (e.g., TNF-α, IL-6) limits mechanistic interpretation of the observed protective effects. Future studies incorporating these markers would strengthen the mechanistic conclusions. Second, the histopathological evaluation was qualitative rather than quantitative; the use of a semi-quantitative lesion scoring system or histomorphometric analysis would have strengthened the findings. Third, no extract-only control group was included to assess the independent effects of beetroot extract on hematological parameters. Fourth, the absence of quantitative phytochemical standardization (e.g., HPLC quantification of betalains and polyphenols) limits reproducibility and product standardization. Fifth, the study was conducted in a laboratory animal model, and findings may not directly translate to human exposure scenarios. Finally, the relatively small sample size (n=7 per group) may limit statistical power to detect subtle differences.

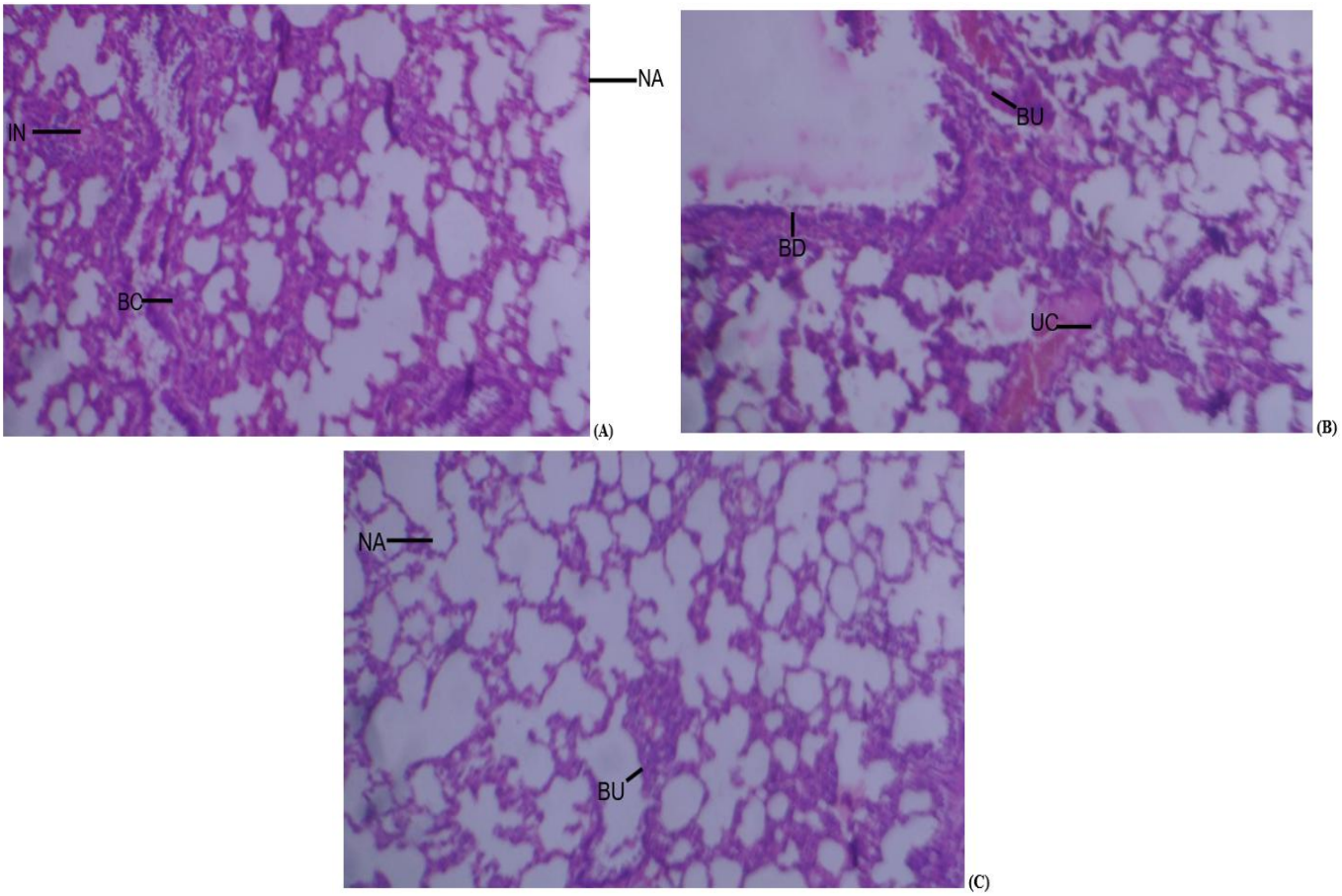

Figure 5: Protective Effects of Beetroot on Lung Histopathology (A–C)
A: Smoke + Beetroot Group – Lung section showing normal alveoli (NA), bronchiolar constriction (BC), and interstitial inflammation (IN). H&E, ×100.
B: Heat + Beetroot Group – Lung section showing bronchiolar dilation (BD), bronchiolar ulceration (BU), and vascular ulceration with congestion (UC). H&E, ×100
C: Smoke + Heat + Beetroot Group – Lung section showing normal alveoli (NA) and focal bronchiolar ulceration (BU). H&E, ×100

Implications and Future Directions

Despite these limitations, the findings contribute to the growing body of evidence supporting beetroot as a dietary intervention against environmental stressors. The study demonstrates that beetroot extract offers significant protection against hematological and pulmonary damage induced by concurrent heat and biomass smoke exposure. Given its affordability, accessibility, and favorable safety profile, beetroot warrants further investigation as a potential public health intervention in resource-limited settings where biomass fuel use is prevalent.

Future research should incorporate oxidative stress and inflammatory biomarkers, quantitative histomorphometric analysis, and phytochemical standardization. Clinical studies are needed to translate these preclinical findings to human populations, particularly vulnerable groups such as women and children in rural areas who face the highest exposure to biomass smoke and heat stress.

CONCLUSION

Exposure to heat and biomass smoke induced significant hematological disturbances and pulmonary histopathology in adult Wistar rats. Beetroot extract supplementation effectively restored leukocyte and erythrocyte parameters, normalized platelet indices, and attenuated pulmonary architectural damage. The protective effects are likely mediated through the antioxidant and anti-inflammatory properties of beetroot's bioactive constituents, including betalains, polyphenols, and dietary nitrates. While these findings are promising, the limitations of this preclinical study—particularly the absence of biochemical oxidative stress and inflammatory markers—suggest that conclusions should be interpreted with appropriate caution. Further well-controlled studies incorporating mechanistic biomarkers and quantitative histopathological assessment are warranted to confirm the therapeutic potential of beetroot as a dietary intervention against environmental stressors.

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Author Contributions

MIO and DOE were involved in concept, design, literature review and execution of research work; MIO conceptualized the manuscript; DOE was involved in statistical analysis and in the data acquisition; DOF was involved in histopathology and examination of histological findings; DOE was involved in hematological examination. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Use of AI in Drafting of Manuscript

The authors declare that they have not used any generative AI/AI-assisted technologies in the writing of this manuscript.

Conflict of interest

The authors declare no conflict of interest.

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