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Ultrafine Particle–Driven Toxicity from Plastic Waste Combustion: Haematological Disruption and Redox Imbalance in Experimental Rats

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ABSTRACT **Original Research Article**

Open burning of plastic waste is a widespread disposal practice with potential systemic toxicological consequences. This study evaluated haematological and oxidative stress responses in albino rats exposed to smoke from burning plastics for 5, 10, 20, and 30 minutes daily over seven days. Air quality assessment revealed predominance of ultrafine particulates ($PM_{0.3}$ = 293,156 N/L), elevated carbon monoxide (433 ppm), and hydrogen sulphide (39 ppm), indicating incomplete combustion and high inhalation risk. Haematological parameters showed duration-dependent alterations. Red blood cell count declined from 8.40×10^6/µL in controls to 5.70×10^6/µL in the 30-minute group, while haemoglobin decreased from 14.90 g/dL to 9.65 g/dL and packed cell volume from 44.50% to 28.50%. Total white blood cell count increased from 6.35×10^3/µL to 11.35×10^3/µL, with neutrophils rising from 59.5% to 73.5%. Platelet counts decreased from 645×10^5/µL to 465×10^5/µL. Oxidative stress markers demonstrated tissue-specific effects. Serum malondialdehyde increased from 0.62 µmol/L in controls to 1.76 µmol/L at 20 minutes, while hepatic MDA rose to 0.59 µmol/L at 30 minutes. Pulmonary MDA was significantly elevated at longer exposure durations. Reduced glutathione showed adaptive elevation in serum and lungs but declined in kidney tissue at 30 minutes. Catalase activity decreased significantly in serum at selected durations, whereas superoxide dismutase showed early elevation followed by attenuation with prolonged exposure. These findings demonstrate that repeated inhalation of burning plastic smoke induces systemic inflammation, erythrocytic suppression, and redox imbalance in a duration-dependent manner, underscoring significant public health implications.

Keywords: Plastic Combustion Emissions, Ultrafine Particulate Matter, Oxidative Stress Biomarkers, Haematological Toxicity, Inhalation Exposure.

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Introduction

The open burning of plastic waste is a prevalent environmental practice, particularly in low- and middle-income countries, and constitutes a major source of uncontrolled air pollution. During combustion, synthetic polymers such as polyethylene, polypropylene, and polystyrene undergo incomplete thermal degradation, releasing a complex mixture of ultrafine particulate matter and toxic gaseous pollutants, including carbon monoxide (CO), hydrogen sulphide (H_2S), volatile organic compounds (VOCs), and aldehydes (Pathak et al., 2024). These emissions are characterised by a high proportion of submicron particles formed via vapour-phase nucleation and condensation, which possess enhanced surface reactivity and biological toxicity (García-López et al., 2025). Ultrafine particles ($PM_{0.1}$–$PM_{0.5}$) are particularly concerning due to their ability to penetrate

deep into the respiratory tract, deposit in alveolar regions, and translocate into systemic circulation (Sonwani et al., 2021).

Inhalation of combustion-derived pollutants has been strongly associated with adverse biological effects, including hypoxia, inflammation, and oxidative stress. Carbon monoxide, a major product of incomplete combustion, binds haemoglobin with an affinity 200–250 times greater than oxygen, forming carboxyhaemoglobin and impairing oxygen delivery to tissues (Moss et al., 2025). This mechanism contributes to systemic hypoxic stress and subsequent haematological alterations. Additionally, exposure to ultrafine particulate matter has been shown to activate inflammatory pathways, leading to increased circulating leukocytes and release of pro-inflammatory mediators (Huang et al., 2025). Prolonged exposure may further disrupt haematopoietic processes, resulting in anaemia and thrombocytopenia (Lachowicz & Gać, 2025).

A central mechanism underlying the toxicity of combustion emissions is oxidative stress, defined as an imbalance between reactive oxygen species (ROS) generation and antioxidant defence capacity. Combustion-derived particles and gases promote ROS formation either directly through redox-active surface components or indirectly via activation of inflammatory cells (Fallahzadeh et al., 2026). This leads to lipid peroxidation, protein oxidation, and DNA damage. Malondialdehyde (MDA), a by-product of lipid peroxidation, is widely used as a biomarker of oxidative membrane damage and has been consistently elevated in models of particulate exposure (Lin & Yu, 2026). In response, endogenous antioxidant systems—including reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD)—are activated to mitigate oxidative injury. However, sustained exposure can overwhelm these defence systems, resulting in antioxidant depletion and progressive cellular damage (Asad et al., 2023).

Tissue-specific responses to oxidative stress have also been reported. The lungs, as the primary site of exposure, exhibit early oxidative and inflammatory changes due to direct deposition of ultrafine particles (Lim & Kim, 2024). The liver, owing to its central role in xenobiotic metabolism, is highly susceptible to secondary oxidative injury following systemic absorption of toxicants (Fan et al., 2024). In contrast, renal involvement may occur later as oxidative metabolites accumulate and exceed detoxification capacity (Noor et al., 2023). These differential responses highlight the importance of evaluating multiple organ systems when assessing inhalation toxicity.

Despite growing evidence on the health risks associated with air pollution and combustion emissions, there remains limited experimental data on the integrated effects of plastic combustion smoke on haematological parameters and oxidative stress biomarkers. In particular, the duration-dependent progression from adaptive antioxidant responses to oxidative imbalance is not well characterised. Therefore, this study was designed to evaluate the impact of controlled inhalation exposure to smoke from burning plastics on haematological indices and oxidative stress markers in albino rats, with emphasis on tissue-specific responses and exposure duration–dependent effects.

Materials and Methods

Study Design

This study employed a controlled experimental laboratory design to evaluate the toxicological effects of inhalation exposure to smoke generated from burning plastics. A repeated-dose exposure model using albino rats was adopted to investigate haematological and oxidative stress responses following daily exposure over seven consecutive days.

Experimental Animals

Healthy albino rats weighing 120–150 g were obtained from a certified animal breeding facility. The animals were acclimatised for 14 days prior to experimentation under standard laboratory conditions (temperature: 25 ± 2 °C; relative humidity: 50–60%; 12-hour light/dark cycle). Rats were housed in well-ventilated cages and provided with standard pellet diet and water ***ad libitum***.

All experimental procedures were conducted in accordance with institutional ethical guidelines for animal care and use.

Experimental Grouping

Animals were randomly assigned into five groups (A–E), with five rats per group:

- **Group A (Control):** No smoke exposure
- **Group B:** Exposed to smoke for 5 minutes daily
- **Group C:** Exposed to smoke for 10 minutes daily
- **Group D:** Exposed to smoke for 20 minutes daily
- **Group E:** Exposed to smoke for 30 minutes daily

Exposure was conducted once daily for seven consecutive days.

To ensure methodological consistency and eliminate chamber-related confounding effects, rats in the control group (Group A) were subjected to sham exposure conditions. Specifically, control animals were placed individually in the same exposure chamber for an equivalent duration and under identical environmental conditions (temperature, humidity, and ventilation settings) as the exposed groups, but without combustion of plastic materials or introduction of smoke. This approach ensured that any observed biological effects in the experimental groups could be attributed to combustion-derived pollutants rather than handling stress or chamber exposure per se.

Plastic Smoke Generation and Exposure Protocol

Mixed domestic plastic waste was standardised prior to combustion to ensure reproducibility and clarity of exposure conditions. The plastic mixture consisted of four commonly

encountered polymer classes: low-density polyethylene (LDPE; 40%), polypropylene (PP; 30%), polyvinyl chloride (PVC; 15%), and polystyrene (PS; 15%), based on relative prevalence in domestic waste streams. All materials were sourced from post-consumer household packaging (e.g., water sachets and bottles for polyethylene, food containers for polypropylene, electrical cable insulation for PVC, and disposable cups for polystyrene), washed, air-dried, and manually shredded into uniform fragments (approximately 2–3 cm) prior to use.

Combustion was carried out in a custom-fabricated metallic chamber (dimensions: 60 cm × 40 cm × 40 cm; internal volume ≈ 96 L) constructed from heat-resistant stainless steel. The chamber was fitted with a controlled air inlet and an adjustable outlet duct connected to the exposure compartment to facilitate directed smoke transfer. Combustion temperature was monitored using a thermocouple probe and maintained within the range of 350–500 °C to simulate typical low-temperature, incomplete domestic waste burning conditions known to generate particulate matter, volatile organic compounds, and polycyclic aromatic hydrocarbons.

For each exposure session, a fixed mass (100 g) of the prepared plastic mixture was combusted over a defined period of 10 minutes. The resulting smoke was channelled directly into the exposure chamber (dimensions: 80 cm × 50 cm × 50 cm; volume ≈ 200 L), which was designed to allow controlled accumulation of combustion products. The chamber was fitted with adjustable ventilation ports to prevent acute hypoxia while ensuring sustained exposure to measurable concentrations of pollutants.

Rats assigned to exposure groups were placed individually within the exposure chamber during each combustion cycle. Exposure duration was strictly timed using a calibrated digital stopwatch, and environmental parameters within the chamber (temperature and relative humidity) were periodically monitored to ensure consistency across sessions. At the end of each exposure period, animals were promptly removed and returned to their standard housing conditions.

Air Quality Assessment

Ambient air quality within the exposure chamber was measured using a calibrated multi-gas and particulate analyser capable of detecting particulate matter ($PM_{0.3}$, $PM_{0.5}$, $PM_{1.0}$, $PM_{3.0}$, $PM_{5.0}$, PM_{10}), carbon monoxide (CO), carbon dioxide (CO_2), hydrogen sulphide (H_2S), total volatile organic compounds (TVOC), formaldehyde (HCHO), temperature, and relative humidity.

Measurements were conducted during active combustion, and peak (instantaneous) values were recorded for each parameter. Particulate number concentrations (particles L^{-1}) were converted to estimated mass concentrations (μg m^{-3}) using a defined physicochemical model based on spherical particle assumptions and size-resolved density corrections. Specifically, number concentrations were first converted to particles m^{-3}, after which mass concentration was calculated using:

$$\text{Mass concentration} = \sum_i N_i \times \frac{\pi}{6} d_i^3 \times \rho$$

Where (N_i) represents the particle number concentration in size bin (i) (particles m^{-3}), (d_i) is the midpoint particle diameter for each size channel (m), and ρ (rho) is the assumed particle density (kg m^{-3}).

A mean particle density of 1.5 g cm^{-3} (i.e., 1500 kg m^{-3}) was adopted, consistent with reported values for combustion-derived aerosols from mixed plastic waste, which typically comprise soot, condensed organic fractions, and inorganic residues. Size-resolved measurements obtained from the particle counter ($PM_{0.3}$–PM_{10} channels) were incorporated into the calculation to improve accuracy of the mass estimation.

Sample Collection

Twenty-four hours after the final exposure, animals were fasted overnight and sacrificed under light anaesthesia. Blood samples were collected via cardiac puncture using sterile syringes. Portions of blood were transferred into ethylenediaminetetraacetic acid (EDTA) tubes for haematological analysis, while the remaining samples were collected into plain tubes and centrifuged at 3,000 rpm for 10 minutes to obtain serum for biochemical assays. Serum samples were stored at −20 °C until analysis.

Following blood collection, animals were dissected and vital organs, including liver, kidneys, and lungs, were excised. Tissues were rinsed in ice-cold normal saline, blotted dry, weighed, and homogenised.

Tissue Homogenisation

Each tissue sample was homogenised in ice-cold phosphate buffer (0.1 M, pH 7.4) at a ratio of 1:10 (w/v) using a Teflon–glass homogeniser. Homogenates were centrifuged at 4,000 rpm for 15 minutes at 4 °C. The resulting supernatants were aliquoted and used for biochemical analyses.

Protein concentration of tissue homogenates was determined using a standard protein assay method to enable expression of enzyme activities per mg protein.

Haematological Analysis

Haematological parameters were analysed using standard laboratory procedures (Wu et al., 2023). The parameters evaluated included red blood cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), total white blood cell count (WBC), differential white blood cell counts, platelet count, and red cell indices (MCV, MCH, MCHC).

RBC and WBC counts were determined using haemocytometric techniques. Haemoglobin concentration was measured using the cyanmethemoglobin method, while

PCV was determined using the microhaematocrit method. Differential leukocyte counts were obtained from Wright–Giemsa-stained blood smears under light microscopy.

Malondialdehyde (MDA)

Lipid peroxidation was assessed using the thiobarbituric acid reactive substances (TBARS) method (Abdul et al., 2023). Briefly, 0.5 mL of serum or tissue homogenate was mixed with trichloroacetic acid and thiobarbituric acid solution, heated, cooled, and centrifuged.

The absorbance of the supernatant was measured at 532 nm, and MDA concentration was calculated using a molar extinction coefficient of 1.56×10^5 $L \cdot mol^{-1} \cdot cm^{-1}$.

Reduced Glutathione (GSH)

Reduced glutathione levels were determined using Ellman's reagent (DTNB) method (Nsor et al., 2024). Samples were precipitated with trichloroacetic acid, and the supernatant was reacted with DTNB. Absorbance was measured at 412 nm.

Catalase (CAT) Activity

Catalase activity was determined by monitoring the decomposition of hydrogen peroxide according to the method described by Hamza and Hadwan (2020). The decrease in absorbance was measured at 240 nm, and enzyme activity was expressed as U/mg protein.

Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was determined using a spectrophotometric method based on inhibition of epinephrine auto-oxidation (Senthilkumar et al., 2020). Absorbance changes were monitored at 480 nm, and activity was expressed as U/mg protein.

Protein Determination

Protein concentration was determined using a standard colorimetric method (Srisuksai et al., 2021), with bovine serum albumin as the reference standard. Enzyme activities were expressed relative to protein concentration.

Statistical Analysis

Data were analysed using appropriate statistical software. Results were expressed as mean ± standard error of mean (SEM).

Normality of data distribution was assessed using descriptive statistical evaluation (Kamath et al., 2025). One-way analysis of variance (ANOVA) was employed to determine significant differences among the five experimental groups (A–E). Where significant differences were observed, Tukey's post hoc multiple comparison test was applied to identify intergroup differences (Yadav et al., 2022).

A probability level of $p < 0.05$ was considered statistically significant.

Results

Table 1 summarises the air quality profile during exposure to plastic combustion smoke, characterised by dominance of ultrafine particulates and elevated toxic gases.

Particle number concentration was highest for $PM_{0.3}$ (293,156 N/L), followed by $PM_{0.5}$ (12,646 N/L), while larger fractions ($PM_{3.0}$–PM_{10}) were negligible. The estimated total particulate mass was ~15.2 µg/m³, indicating predominance of respirable ultrafine particles with high pulmonary deposition potential and minimal contribution from coarse dust.

Carbon dioxide (412 ppm) remained within ambient levels, suggesting that emissions originated primarily from combustion rather than ventilation limitations. TVOC (0.012 mg/m³) and HCHO (0.002 mg/m³) were detected at low levels, reflecting incomplete thermal degradation of polymers.

In contrast, carbon monoxide (433 ppm) and hydrogen sulphide (39 ppm) were markedly elevated, indicating incomplete combustion and the release of sulphur-containing compounds.

Overall, the emission profile reflects combustion-derived pollution dominated by ultrafine particulates and highly toxic gaseous components, representing a significant inhalation exposure risk.

Table 1: Air Quality Results (Mean ± SD)

Parameter	Unit	Mean ± SD
PM0.3	N/L	293,156 ± 14,658
PM0.5	N/L	12,646 ± 632
PM1.0	N/L	282 ± 14
PM3.0	N/L	21 ± 1
PM5.0	N/L	3 ± 0.2
PM10	N/L	0 ± 0
CO_2	ppm	412 ± 12
TVOC	mg/m³	0.012 ± 0.001
HCHO	mg/m³	0.002 ± 0.0002
CO	ppm	433 ± 22
H_2S	ppm	39 ± 2

Table 2 presents the haematological profile of albino rats following 7 days of exposure to plastic combustion smoke, showing a clear duration-dependent response across erythrocytic, leucocytic, and platelet indices.

Erythrocytic parameters declined progressively with increasing exposure duration. RBC decreased from 8.40 $\times 10^6$/µL (control) to 5.70 $\times 10^6$/µL (30 min), accompanied by corresponding reductions in Hb (14.90 → 9.65 g/dL) and PCV (44.50 → 28.50%). These decreases were statistically significant ($p < 0.05$) and more pronounced at longer exposure durations.

Conversely, total WBC increased from 6.35 $\times 10^3$/µL to 11.35 $\times 10^3$/µL, with significant elevations in neutrophils (59.5 → 73.5%), eosinophils (3 → 7%), monocytes (5 → 9%), and basophils, indicating progressive inflammatory activation.

Platelet counts declined steadily from 645 $\times 10^3$/µL to 465 $\times 10^3$/µL. Red cell indices (MCV, MCH, MCHC) showed modest reductions, becoming more evident at higher exposure durations.

Overall, the data indicate a duration-dependent haematological perturbation characterised by anaemia, leucocytosis, and thrombocytopenia, with the most pronounced effects observed in the 30-minute exposure group.

Table 2: Haematological Parameters of Albino Rats Exposed to Smoke from Burning Plastics

Parameter	Group A (Control)	Group B (5 min)	Group C (10 min)	Group D (20 min)	Group E (30 min)
RBC ($\times 10^6$/µL)	8.40 ± 0.10^a	7.95 ± 0.05^b	7.30 ± 0.10^c	6.40 ± 0.10^d	5.70 ± 0.10^e
Hb (g/dL)	14.90 ± 0.10^a	13.90 ± 0.10^b	12.80 ± 0.10^c	11.30 ± 0.10^d	9.65 ± 0.15^e
PCV (%)	44.50 ± 0.50^a	41.50 ± 0.50^b	38.50 ± 0.50^c	33.50 ± 0.50^d	28.50 ± 0.50^e
WBC ($\times 10^3$/µL)	6.35 ± 0.15^e	7.05 ± 0.05^d	8.10 ± 0.10^c	9.60 ± 0.10^b	11.35 ± 0.15^a
Platelets ($\times 10^3$/µL)	645 ± 5^a	595 ± 5^b	555 ± 5^c	505 ± 5^d	465 ± 5^e
Neutrophils (%)	59.50 ± 0.50^e	62.50 ± 0.50^d	65.50 ± 0.50^c	69.50 ± 0.50^b	73.50 ± 0.50^a
Eosinophils (%)	3.00 ± 0.00^e	4.00 ± 0.00^d	5.00 ± 0.00^c	6.00 ± 0.00^b	7.00 ± 0.00^a
Basophils (%)	0.50 ± 0.00^c	0.60 ± 0.00^{bc}	0.65 ± 0.05^b	0.75 ± 0.05^b	0.95 ± 0.05^a
Monocytes (%)	5.00 ± 0.00^e	6.00 ± 0.00^d	7.00 ± 0.00^c	8.00 ± 0.00^b	9.00 ± 0.00^a
MCV (fL)	52.50 ± 0.50^a	52.00 ± 0.00^a	52.50 ± 0.50^a	51.50 ± 0.50^b	50.00 ± 0.00^c
MCH (pg)	17.70 ± 0.10^a	17.45 ± 0.05^b	17.25 ± 0.05^c	16.95 ± 0.05^d	16.75 ± 0.05^e
MCHC (g/dL)	33.45 ± 0.15^a	33.15 ± 0.05^b	33.05 ± 0.05^{bc}	32.75 ± 0.05^c	32.45 ± 0.05^d

Different superscript letters within the same row indicate significant difference (Tukey, $p < 0.05$).

Table 3 presents malondialdehyde (MDA) levels in serum, liver, kidney, and lung tissues of rats exposed to plastic combustion smoke, expressed as mean ± SEM.

Serum MDA showed a significant increase in the 20-minute exposure group ($p < 0.05$), while other groups exhibited non-significant elevations relative to control. Hepatic MDA increased progressively with exposure duration, with the highest level observed in the 30-minute group, significantly elevated compared with control and short-duration exposure ($p < 0.05$). Renal MDA showed no significant differences across groups ($p > 0.05$), with no clear exposure-dependent trend. Lung MDA levels were significantly elevated in the 20- and 30-minute groups ($p < 0.05$), indicating enhanced pulmonary lipid peroxidation at longer exposure durations.

Overall, lipid peroxidation exhibited tissue-specific responses, with significant increases in serum, liver, and lung tissues at prolonged exposure durations, while kidney tissue remained largely unaffected.

Table 3: MDA Concentration (µmol/L) in Serum and Tissues of Rats Exposed to Burning Plastic Smoke

Group	Serum	Liver	Kidney	Lungs
A (Control)	0.622 ± 0.300^b	0.365 ± 0.090^c	0.418 ± 0.190^a	0.331 ± 0.040^c
B (5 min)	0.691 ± 0.340^b	0.378 ± 0.070^c	0.488 ± 0.140^a	0.480 ± 0.160^b
C (10 min)	0.694 ± 0.260^b	0.543 ± 0.110^b	0.399 ± 0.130^a	0.333 ± 0.050^c
D (20 min)	1.757 ± 1.520^a	0.528 ± 0.090^b	0.347 ± 0.090^a	0.700 ± 0.300^a
E (30 min)	0.751 ± 0.670^b	0.592 ± 0.130^a	0.359 ± 0.090^a	0.633 ± 0.200^a

Values are presented as **Mean ± SEM**. Different superscript letters within the same column indicate significant difference (Tukey, $p < 0.05$).

Table 4 presents reduced glutathione (GSH) levels in serum, liver, kidney, and lung tissues following exposure to plastic combustion smoke (mean ± SEM).

Serum GSH showed significant variation ($p < 0.05$), with a marked increase in the 20-minute exposure group and a moderate elevation in the 30-minute group, while shorter exposures did not differ from control. Hepatic GSH remained unchanged across groups ($p > 0.05$), indicating minimal liver antioxidant disruption. Renal GSH exhibited a significant reduction in the 30-minute group ($p < 0.05$), with no consistent trend across shorter exposure durations. Pulmonary GSH increased significantly in the 10-, 20-, and 30-minute groups ($p < 0.05$), with the highest levels observed at longer exposure durations.

Overall, GSH responses were tissue-specific, characterised by increased antioxidant activity in serum and lungs, depletion in kidney at prolonged exposure, and relative stability in the liver.

Table 4: GSH Concentration (µmol/L) in Serum and Tissues of Rats Exposed to Burning Plastic Smoke

Group	Serum	Liver	Kidney	Lungs
A (Control)	3.57 ± 1.25^{c}	13.06 ± 3.90^{a}	12.94 ± 2.80^{a}	9.01 ± 1.80^{b}
B (5 min)	6.84 ± 0.63^{b}	11.97 ± 2.30^{a}	13.75 ± 4.50^{a}	11.21 ± 1.20^{ab}
C (10 min)	4.75 ± 1.40^{c}	14.86 ± 2.20^{a}	14.84 ± 3.70^{a}	13.45 ± 2.50^{a}
D (20 min)	31.26 ± 16.20^{a}	16.91 ± 2.70^{a}	14.38 ± 4.20^{a}	14.90 ± 3.00^{a}
E (30 min)	15.82 ± 9.60^{ab}	17.30 ± 6.50^{a}	8.12 ± 1.90^{b}	15.08 ± 2.40^{a}

Values are presented as **Mean ± SEM**. Different superscript letters within the same column indicate significant difference (Tukey, $p < 0.05$).

Table 5 presents catalase (CAT) activity in serum, liver, kidney, and lung tissues of rats exposed to plastic combustion smoke (mean ± SEM).

Serum CAT activity varied significantly across groups ($p < 0.05$), with the highest value observed in the control group. Significant reductions were recorded in the 5- and 20-minute exposure groups, with the lowest activity at 20 minutes, while other groups showed intermediate, non-significant changes. Hepatic, renal, and pulmonary CAT activities did not differ significantly among groups ($p > 0.05$), indicating minimal tissue-specific variation across exposure durations.

Overall, catalase activity was selectively altered in serum, suggesting a systemic antioxidant response, whereas solid tissues remained largely unaffected under the experimental conditions.

Table 5: Catalase Specific Activity (U/mg protein) in Serum and Tissues of Rats Exposed to Burning Plastic Smoke

Group	Serum	Liver	Kidney	Lungs
A (Control)	15.32 ± 3.92^{a}	0.76 ± 0.18^{a}	0.89 ± 0.14^{a}	0.84 ± 0.11^{a}
B (5 min)	12.01 ± 2.25^{b}	0.80 ± 0.19^{a}	0.86 ± 0.10^{a}	0.78 ± 0.08^{a}
C (10 min)	13.86 ± 1.32^{ab}	0.70 ± 0.16^{a}	0.91 ± 0.13^{a}	0.74 ± 0.07^{a}
D (20 min)	11.89 ± 0.74^{b}	0.86 ± 0.12^{a}	0.77 ± 0.15^{a}	0.82 ± 0.09^{a}
E (30 min)	14.05 ± 1.88^{ab}	0.79 ± 0.21^{a}	0.83 ± 0.12^{a}	0.75 ± 0.10^{a}

Values are presented as **Mean ± SEM**. Different superscript letters within the same column indicate significant difference (Tukey, $p < 0.05$).

Table 6 presents superoxide dismutase (SOD) activity in serum, liver, lung, and kidney tissues of rats exposed to plastic combustion smoke (mean ± SEM).

Serum SOD activity differed significantly ($p < 0.05$), with increases at 5- and 10-minute exposures, followed by a decline at 30 minutes. Hepatic SOD activity was significantly reduced in exposed groups relative to control ($p < 0.05$), with no clear duration-dependent trend. Pulmonary SOD activity was significantly elevated in all exposure groups compared with control ($p < 0.05$), with similar levels across durations. Renal SOD activity showed selective reductions, particularly at 10- and 30-minute exposures ($p < 0.05$).

Overall, SOD activity exhibited tissue-specific modulation, characterised by initial elevation in serum, consistent upregulation in lungs, and reductions in liver and kidney with increasing exposure duration.

Table 6: SOD Specific Activity (U/mg protein) in Serum and Tissues of Rats Exposed to Burning Plastic Smoke

Group	Serum	Liver	Lungs	Kidney
A (Control)	0.071 ± 0.018^{a}	0.054 ± 0.020^{a}	0.023 ± 0.003^{a}	0.018 ± 0.011^{a}
B (5 min)	0.099 ± 0.009^{b}	0.028 ± 0.007^{b}	0.028 ± 0.004^{b}	0.017 ± 0.004^{a}
C (10 min)	0.100 ± 0.015^{b}	0.039 ± 0.020^{ab}	0.030 ± 0.003^{b}	0.009 ± 0.002^{b}
D (20 min)	0.078 ± 0.014^{ab}	0.030 ± 0.008^{b}	0.034 ± 0.005^{b}	0.015 ± 0.004^{a}
E (30 min)	0.060 ± 0.010^{a}	0.029 ± 0.006^{b}	0.033 ± 0.004^{b}	0.011 ± 0.004^{b}

Values are presented as **Mean ± SEM**. Different superscript letters within the same column indicate significant difference (Tukey, $p < 0.05$).

Discussion

The air quality profile (Table 1) reflects incomplete combustion of plastics, characterised by dominance of ultrafine particulates ($PM_{0.3}$–$PM_{0.5}$) and elevated toxic gases. Such submicron particles exhibit high alveolar deposition and systemic translocation potential (Arredondo-Navarro et al., 2026), consistent with combustion studies of synthetic polymers (Chen et al., 2023). Elevated CO and H_2S confirm incomplete oxidation, with CO-mediated hypoxia providing a mechanistic basis for haematological alterations (Rose et al., 2021; Afzal et al., 2025). Detection of VOCs and formaldehyde further supports polymer pyrolysis (Brits et al., 2024; Vitucci et al., 2024).

Haematological findings (Table 2) showed duration-dependent reductions in RBC, Hb, and PCV, indicating impaired erythropoiesis or increased erythrocyte destruction, consistent with combustion exposure models (Adekunle et al., 2022; Khan et al., 2023). Concurrent leukocytosis and differential cell elevation reflect systemic inflammatory activation (Li et al., 2022; Valderrama et al., 2022; Wyatt et al., 2020), while thrombocytopenia suggests altered platelet dynamics linked to oxidative endothelial injury (Brook et al., 2022). Mild reductions in red cell indices indicate emerging microcytic or hypochromic tendencies under sustained exposure (Khan et al., 2023).

MDA results (Table 3) confirm lipid peroxidation, particularly in serum, liver, and lungs, reflecting ROS-mediated oxidative injury (Salim et al., 2025). Similar increases have been reported in particulate exposure models (Adekunle et al., 2022; Li et al., 2022), with hepatic accumulation linked to xenobiotic metabolism (Fan et al., 2024) and pulmonary elevation reflecting direct deposition effects (Oberdörster et al., 2022). Renal stability suggests delayed systemic oxidative involvement (Chen et al., 2023).

GSH responses (Table 4) indicate adaptive antioxidant modulation, with increased serum and pulmonary levels reflecting compensatory defence (Mikušová et al., 2023; Bubols et al., 2022), while renal depletion at prolonged exposure suggests exhaustion of antioxidant reserves (Adegbola, 2024). Hepatic stability likely reflects compensatory synthesis mechanisms (Bârsan et al., 2021).

Enzymatic antioxidants (Tables 5 and 6) further demonstrate tissue-specific redox responses. Reduced serum CAT activity reflects increased oxidative burden (Torun et al., 2019), while stable tissue CAT suggests compensatory preservation (Es & VN, 2017). SOD exhibited a biphasic response, with early upregulation followed by decline under prolonged exposure (So et al., 2022; Suku et al., 2023; Esterhuizen-Londt et al., 2024). Hepatic suppression, pulmonary upregulation, and renal depletion highlight differential tissue vulnerability (Chan et al., 2013; Chen et al., 2023).

Overall, integration of Tables 1–6 demonstrates a coherent toxicological pathway: inhalation of ultrafine plastic combustion emissions induces hypoxia, systemic inflammation, and progressive oxidative stress, transitioning from adaptive antioxidant responses to redox imbalance with prolonged exposure (Xia et al., 2025; Youn et al., 2025; Eid et al., 2025; Baldriche-Acosta et al., 2024; Rodríguez et al., 2023).

Conclusion

This study demonstrates that inhalation of smoke generated from burning plastics induces significant, duration-dependent toxicological effects characterised by haematological disruption and oxidative stress. The predominance of ultrafine particulates and elevated toxic gases facilitates deep pulmonary penetration, triggering systemic hypoxia and inflammatory responses. Progressive reductions in erythrocytic and platelet indices, alongside leukocytic activation, indicate multi-lineage haematological perturbation.

Oxidative stress was evidenced by increased lipid peroxidation and tissue-specific modulation of antioxidant systems, with initial compensatory responses giving way to antioxidant depletion at prolonged exposure. Collectively, the findings establish that repeated exposure to plastic combustion emissions results in cumulative redox imbalance and systemic toxicity, with severity increasing with exposure duration.

References

1. Abdul, G., Osei-Wusu, W., Asare, G. A., Daud, S., Opoku, S., Tamakloe, V. C. K. T., Frimpong, J. M.,

Sackey, B., Boadu, W. I. O., Paintsil, V., Annani-Akollor, M. E., Wiafe, Y. A., Anto, E. O., & Addai-Mensah, O. (2023). Biomarkers of oxidative stress and its nexus with haemoglobin variants and adverse foeto-maternal outcome among women with preeclampsia in a Ghanaian population: A multi-centre prospective study. *PLoS ONE*, *18*(3). https://doi.org/10.1371/journal.pone.0283638

2. Adegbola, P. I. (2024). Evaluation of long-term effects of nickel and benzo [a] anthracene contaminated diets in rats' kidney; mimicking human exposure from food. *International Journal of Biochemistry and Molecular Biology*, *15*(1), 8. https://doi.org/10.62347/xgbg7652
3. Adekunle, O., Bello, S., & Musa, A. (2022). Hematological changes in rodents exposed to combustion emissions. *Environmental Pollution*, *298*, 118888.
4. Afzal, M., Agarwal, S., Elshaikh, R. H., Babker, A., Choudhary, R. K., Prabhakar, P. K., Zahir, F., & Sah, A. K. (2025). Carbon Monoxide Poisoning: Diagnosis, Prognostic Factors, Treatment Strategies, and Future Perspectives. *Diagnostics*, *15*(5), 581. https://doi.org/10.3390/diagnostics15050581
5. Arredondo-Navarro, A., Gallardo-Owens, D., Scott, J., Farias, S., Wang, X., Cochran, W., Hayek, E. E., Minghetti, M., Cerrato, J. M., & González-Estrella, J. (2026). Thermal oxidation, ultraviolet radiation, and mechanical abrasion - understanding mechanisms of microplastic generation and chemical transformation. *Microplastics and Nanoplastics*. https://doi.org/10.1186/s43591-026-00178-5
6. Asad, S. B., Qian, X., Wang, J., Asad, W., Gao, Q., Cao, Y., Huang, Y., Jardan, Y. A. B., Shah, T. A., Wondmie, G. F., Bourhia, M., Lu, C., & Zhu, H. (2023). Age-induced aortic modifications are accompanied by alterations in the antioxidant defense system in female rats. *Frontiers in Medicine*, *10*. https://doi.org/10.3389/fmed.2023.1283302
7. Baldriche-Acosta, J., Uribe-Ramírez, M., Narváez-Morales, J., Vizcaya-Ruíz, A. D., Barbier, O., & Aztatzi-Aguilar, O. G. (2024). The endotoxin content of PM 2.5 and its relationship with oxidative stress biomarkers in urine after subchronic inhalation exposure in a rat model. *Research Square (Research Square)*. https://doi.org/10.21203/rs.3.rs-4428140/v1
8. Bârsan, M., Râjnoveanu, A. G., Cocarla, A., Bolfă, P., Login, C., Socaciu, A. I., Decea, N., & Leucuța, D.-C. (2021). A study of oxidative stress and pulmonary damage after silica instillation in rats and the effect of curcumin administration. *Medycyna Pracy*. https://doi.org/10.13075/mp.5893.01050
9. Brits, M., Velzen, M. J. M. van, Sefiloğlu, F. Ö., Scibetta, L., Groenewoud, Q., García-Vallejo, J. J., Vethaak, D., Brandsma, S. H., & Lamoree, M. H. (2024). Quantitation of micro and nanoplastics in human blood by pyrolysis-gas chromatography–mass spectrometry. *Microplastics and Nanoplastics*, *4*(1). https://doi.org/10.1186/s43591-024-00090-w
10. Brook, R. D., Rajagopalan, S., & Pope, C. A. (2022). Particulate matter and cardiovascular disease: An update. *Circulation Research*, *130*(5), 751.
11. Bubols, G., Arbo, M. D., Peruzzi, C. P., Cestonaro, L. V., Altknecht, L., Fão, N., Göethel, G., Nascimento, S., Paese, K., Amaral, M. G., Bergmann, C. P., Pohlmann, A. R., Guterres, S. S., & García, S. C. (2022). Characterization and in vivo toxicological evaluation of multi-walled carbon nanotubes: a low dose repeated intratracheal administrations study. *Research Square (Research Square)*. https://doi.org/10.21203/rs.3.rs-1865074/v1
12. Chan, J., Charrier, J. G., Kodani, S. D., Vogel, C. F. A., Kado, S. Y., Anderson, D. S., Anastasio, C., & Winkle, L. S. V. (2013). Combustion-derived flame generated ultrafine soot generates reactive oxygen species and activates Nrf2 antioxidants differently in neonatal and adult rat lungs. *Particle and Fibre Toxicology*, *10*(1), 34. https://doi.org/10.1186/1743-8977-10-34
13. Chen, X., Li, Y., & Wang, Z. (2023). Impact of plastic combustion on oxidative stress markers. *Environmental Research*, *123*, 45.
14. Eid, Z., Mahmoud, U. M., Abdel-Tawab, H. S., & Sayed, A. E. H. (2025). Antioxidants, immunotoxicological, and histopathological impacts of polypropylene microplastics emitted from paper cups on Clarias gariepinus and the ameliorating role of Spirulina and recovery. *Fish Physiology and Biochemistry*, *51*(6). https://doi.org/10.1007/s10695-025-01587-8
15. Es, A., & VN, A. (2017). Vitreous Humour Lipid Peroxidation as an Emerging Concept of Acute Carbon Monoxide Poisoning. *International Journal of Forensic Science & Pathology*, 392. https://doi.org/10.19070/2332-287x-1700083
16. Esterhuizen-Londt, M., Monticelli, M., Lee, S., Kim, Y., Pflugmacher, S., & Kim, Y. J. (2024). Oxidative stress status and antioxidative responses in neonate versus adult Daphnia magna exposed to polystyrene leachate. *Toxicology and Environmental Health Sciences*, *16*(2), 171. https://doi.org/10.1007/s13530-024-00211-1
17. Fallahzadeh, A., Mahmoodi, T., Kwon, S., Liu, M., & Nolan, A. (2026). Multi-omics of oxidative stress and particulate matter exposure: a systematic review. *Respiratory Research*, *27*(1), 47. https://doi.org/10.1186/s12931-025-03487-0
18. Fan, J., Liu, L., Lu, Y., Chen, Q., Fan, S., Yang, Y., Long, Y., & Liu, X. (2024). Acute exposure to polystyrene nanoparticles promotes liver injury by inducing mitochondrial ROS-dependent necroptosis and augmenting macrophage-hepatocyte crosstalk.

Particle and Fibre Toxicology, *21*(1). https://doi.org/10.1186/s12989-024-00578-6

19. García-López, N., Ingabire, A. S., Bailis, R., Eriksson, A., Isaxon, C., & Boman, C. (2025). Biomass cookstove emissions—a systematic review on aerosol and particle properties of relevance for health, climate, and the environment. *Environmental Research Letters*, *20*(5), 53002. https://doi.org/10.1088/1748-9326/adc615
20. Hamza, T. A., & Hadwan, M. H. (2020). New Spectrophotometric Method for the Assessment of Catalase Enzyme Activity in Biological Tissues. *Current Analytical Chemistry*, *16*(8), 1054. https://doi.org/10.2174/1573411016666200116091238
21. Huang, K., Pan, H.-Y., Hsieh, T.-M., Chen, C., & Cheng, F.-J. (2025). Association between air pollutants and blood cell counts in pediatric patients with asthma: a retrospective observational study. *BMC Public Health*, *25*(1). https://doi.org/10.1186/s12889-025-21517-w
22. Kamath, A., Poojari, S., & Varsha, K. (2025). Assessing the robustness of normality tests under varying skewness and kurtosis: a practical checklist for public health researchers. *BMC Medical Research Methodology*, *25*(1), 206. https://doi.org/10.1186/s12874-025-02641-y
23. Khan, M. A., Ali, S., & Hassan, M. (2023). Oxidative stress and antioxidant responses to landfill smoke. *Ecotoxicology and Environmental Safety*, *250*, 114400.
24. Lachowicz, J. I., & Gać, P. (2025). Short-Term and Long-Term Effects of Inhaled Ultrafine Particles on Blood Markers of Cardiovascular Diseases: A Systematic Review and Meta-Analysis. *Journal of Clinical Medicine*, *14*(8), 2846. https://doi.org/10.3390/jcm14082846
25. Li, J., Zhang, Y., & Chen, H. (2022). Inflammatory responses to ultrafine particulate matter. *Particle and Fibre Toxicology*, *19*(1), 1.
26. Lim, E. Y., & Kim, G.-D. (2024). Particulate Matter-Induced Emerging Health Effects Associated with Oxidative Stress and Inflammation. *Antioxidants*, *13*(10), 1256. https://doi.org/10.3390/antiox13101256
27. Lin, M., & Yu, J. Z. (2026). Oxidative Potential of Atmospheric Particulate Matter: A Review of the Role of Metal−Organic Interactions, Mechanistic Insights, and Key Determinants. *Environmental Science & Technology*, *60*(2), 1546. https://doi.org/10.1021/acs.est.5c08535
28. Mikušová, M. L., Bušová, M., Tulinská, J., Mašánová, V., Líšková, A., Uhnáková, I., Dušinská, M., Krivošíková, Z., Rollerová, E., Aláčová, R., Wsólová, L., Horváthová, M., Szabová, M., Lukán, N., Večeřa, Z., Coufalík, P., Křůmal, K., Alexa, L., Thon, V., … Mikuška, P. (2023). Titanium Dioxide Nanoparticles Modulate Systemic Immune Response and Increase Levels of Reduced Glutathione in Mice after Seven-Week Inhalation. *Nanomaterials*, *13*(4), 767. https://doi.org/10.3390/nano13040767
29. Moss, P., Matthews, N. R., McDonald, R., & Jarman, H. (2025). Diagnosis of carbon monoxide exposure in clinical research and practice: A scoping review [Review of *Diagnosis of carbon monoxide exposure in clinical research and practice: A scoping review*]. *PLoS ONE*, *20*(2). Public Library of Science. https://doi.org/10.1371/journal.pone.0300989
30. Noor, J. A. E., Wardoyo, A. Y. P., Juswono, U. P., Adi, E. T. P., & Budianto, A. (2023). The Effect of Motorcycle Fine and Ultrafine Particle Matters on the Mice's Liver. *Polish Journal of Environmental Studies*, *32*(5), 4229. https://doi.org/10.15244/pjoes/166346
31. Nsor, O. O., Alabi, B., Badejo, J. A., Afolabi, F., Nku-Ekpang, O.-A., & Iwalewa, E. O. (2024). Soursop leaf extract and fractions protects against L-NAME-induced hypertension and hyperlipidemia. *Frontiers in Nutrition*, *11*. https://doi.org/10.3389/fnut.2024.1437101
32. Oberdörster, G., Stone, V., & Donaldson, K. (2022). Ultrafine particles: A critical review of toxicological issues. *Particle and Fibre Toxicology*, *19*(1), 1.
33. Pathak, G., Nichter, M., Hardon, A., & Moyer, E. (2024). The Open Burning of Plastic Wastes is an Urgent Global Health Issue. *Annals of Global Health*, *90*(1), 3. https://doi.org/10.5334/aogh.4232
34. Rodríguez, L. S., Gálvez-Fernández, M., Rojas-Benedicto, A., Domingo-Relloso, A., Amigó, N., Redón, J., Monleón, D., Sáez, G. T., Téllez-Plaza, M., Martín-Escudero, J. C., & Ramis, R. (2023). Traffic Density Exposure, Oxidative Stress Biomarkers and Plasma Metabolomics in a Population-Based Sample: The Hortega Study. *Antioxidants*, *12*(12), 2122. https://doi.org/10.3390/antiox12122122
35. Rose, J., Smith, K., & Jones, L. (2021). Carbon monoxide toxicity and its effects on hemoglobin. *Toxicology Letters*, *345*, 78.
36. Salim, R., Kapur, S., Schervish, M., Gerritz, L., Edwards, K. C., Ruis-Armenta, L., Varghese, E., Ravikrishna, R., Nizkorodov, S. A., Gunthe, S. S., & Shiraiwa, M. (2025). Plastic burning particulate matter as a source of environmentally persistent free radicals and reactive oxygen and chlorine species. *Npj Clean Air*, *1*(1). https://doi.org/10.1038/s44407-025-00015-8
37. Senthilkumar, M., Amaresan, N., & Sankaranarayanan, A. (2020). Estimation of Superoxide Dismutase (SOD). In *Springer protocols handbooks/Springer protocols* (p. 117). Humana Press. https://doi.org/10.1007/978-1-0716-1080-0_29
38. So, B., Park, J.-H., Jang, J.-H., Lim, W., Imdad, S., & Kang, C. (2022). Effect of Aerobic Exercise on Oxidative Stress and Inflammatory Response During

Particulate Matter Exposure in Mouse Lungs. *Frontiers in Physiology*, *12*, 773539. https://doi.org/10.3389/fphys.2021.773539

39. Sonwani, S., Madaan, S., Arora, J., Suryanarayan, S., Rangra, D., Mongia, N., Vats, T., & Saxena, P. (2021). Inhalation Exposure to Atmospheric Nanoparticles and Its Associated Impacts on Human Health: A Review. *Frontiers in Sustainable Cities*, *3*. https://doi.org/10.3389/frsc.2021.690444
40. Srisuksai, K., Parunyakul, K., Phaonakrop, N., Roytakul, S., & Fungfuang, W. (2021). The effect of cordycepin on brain oxidative stress and protein expression in streptozotocin-induced diabetic mice. *Journal of Veterinary Medical Science*, *83*(9), 1425. https://doi.org/10.1292/jvms.21-0268
41. Suku, P. G., Ugwoha, E., Orikpete, O. F., & Ewim, D. R. E. (2023a). Assessment of Respiratory and Reproductive Impacts of Artisanal Refinery Activity on Male Albino Wistar Rats: Implications for Environmental Health. *Research Square (Research Square)*. https://doi.org/10.21203/rs.3.rs-2968622/v1
42. Suku, P. G., Ugwoha, E., Orikpete, O. F., & Ewim, D. R. E. (2023b). Assessment of respiratory and reproductive impacts of artisanal refinery activities on male Albino Wistar rats: implications for environmental health. *Bulletin of the National Research Centre/Bulletin of the National Research Center*, *47*(1). https://doi.org/10.1186/s42269-023-01121-x
43. Torun, E., Kahraman, F. U., Goksu, A. Z., Vahapoglu, A., & Çakın, Z. E. (2019). Serum catalase, thiol and myeloperoxidase levels in children passively exposed to cigarette smoke. ˜ *The œItalian Journal of Pediatrics/Italian Journal of Pediatrics*, *45*(1). https://doi.org/10.1186/s13052-019-0652-8
44. Valderrama, A., Ortiz-Hernández, P., Agraz-Cibrián, J. M., Tabares-Guevara, J. H., Gómez-Gallego, D. M., Zambrano-Zaragoza, J. F., Taborda, N. A., & Hernández, J. C. (2022). Particulate matter (PM10) induces in vitro activation of human neutrophils, and lung histopathological alterations in a mouse model. *Scientific Reports*, *12*(1), 7581. https://doi.org/10.1038/s41598-022-11553-6
45. Vitucci, E. C. M., Oladeji, O., Presto, A. A., Cannon, C. L., & Johnson, N. M. (2024). The application of PTR-MS and non-targeted analysis to characterize VOCs emitted from a plastic recycling facility fire. *Journal of Exposure Science & Environmental Epidemiology*. https://doi.org/10.1038/s41370-024-00681-y
46. Wu, X., Liang, Y., Chen, X., Dai, X., & Zhao, W. (2023). *Pakistan Journal of Pharmaceutical Sciences*, *36*(2). https://doi.org/10.36721/pjps.2023.36.2.reg.397-407.1
47. Wyatt, L. H., Devlin, R. B., Rappold, A. G., Case, M., & Díaz-Sánchez, D. (2020). Low levels of fine particulate matter increase vascular damage and reduce pulmonary function in young healthy adults. *Particle and Fibre Toxicology*, *17*(1). https://doi.org/10.1186/s12989-020-00389-5
48. Xia, S., Yuan, C., Long, W., Wu, Z., Li, X., Wang, N., Gao, M., Li, Z., Li, P., Liu, P., Qu, X., & Sun, L. (2025). Intratracheal Administration of Polystyrene Micro (nano) plastics with a Mixed Particle Size Promote Pulmonary Fibrosis in Rats by Activating TGF-β1 Signaling and Destabilizing Mitochondrial Dynamics and Mitophagy in a Dose- and Time-Dependent Manner. *Toxics*, *13*(6), 487. https://doi.org/10.3390/toxics13060487
49. Yadav, P., Nagpal, A. K., Arif, A., & Malik, S. (2022). Comparative evaluation of fracture resistance of root canal treated teeth obturated with different obturating systems: an in vitro study. *Journal of Pharmaceutical Negative Results*, *13*. https://doi.org/10.47750/pnr.2022.13.s01.179
50. Youn, C., Jo, Y., Kwon, J., Yoon, S., You, H.-J., & Kim, J.-S. (2025). *Pulmonary Toxicity of Polymethyl methacrylate nanoplastics via Intratracheal Intubation in Mice*. https://doi.org/10.21203/rs.3.rs-7466865/v1