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ORIGINAL ARTICLE

Effects of Oral Exposure to Polyethylene Nanoplastics Enriched Fraction on Body Weight and Haematological Indices in Male Wistar Rats

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ABSTRACT

Polyethylene particle exposure may alter somatic growth and circulating blood-cell profiles. This study evaluated body-weight and haematological responses following 28-day oral exposure to a polyethylene nanoplastics-enriched fraction in male Wistar rats. Particle-metric analysis identified 134 particles (35.35 nm–7.33 µm), with a median Feret diameter of 83.08 nm; 94.8% were ≤100 nm. Twenty-five rats were assigned to five groups (n = 5): control and groups exposed once daily to approximately 17.5, 35.0, 70.0, or 140.0 mg/kg/day. Initial body weights differed significantly among groups ($p < 0.001$). All groups gained weight, but absolute and percentage weight gains were significantly lower at 70 and 140 mg/kg/day than in the control and two lower-dose groups (both $p < 0.001$). Haemoglobin concentration differed significantly among groups ($p = 0.003$) and was lower at 35 and 140 mg/kg/day than in controls. White blood cell count increased significantly ($p = 0.014$), with higher values at 70 and 140 mg/kg/day than in controls. Platelet count increased significantly ($p < 0.001$), including elevations at 17.5, 70, and 140 mg/kg/day. Mean corpuscular haemoglobin concentration decreased at 70 and 140 mg/kg/day ($p = 0.024$), whereas RBC count, PCV, MCV, and MCH did not differ significantly. Neutrophil percentage decreased and lymphocyte percentage increased at all exposure levels (both $p < 0.001$); monocytes increased from 35 mg/kg/day ($p < 0.001$), while eosinophils were unchanged ($p = 0.633$). These findings indicate reduced unadjusted growth at higher exposures and selective erythrocytic, leukocytic, and platelet alterations.

Keywords: Polyethylene; Nanoplastics; Haematotoxicity; Body-weight gain; Subacute toxicity; Wistar rats;

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1.0 INTRODUCTION

Plastic pollution has become a persistent environmental problem because the extensive production and use of synthetic polymers are accompanied by substantial waste accumulation. A global material-flow analysis estimated that approximately 8.3 billion metric tons of virgin plastics had been produced by 2015, with most accumulated plastic waste either discarded in the environment or retained in landfills. Polyethylene is particularly relevant because of its large production volume and widespread use in packaging. Its contribution to plastic waste provides a strong rationale for investigating the biological effects of polyethylene particles rather than assuming that findings obtained with other polymers apply directly to this material (Geyer et al., 2017).

The environmental significance of plastic debris extends beyond visible litter to particles generated through fragmentation. Evidence that polyethylene microbeads can be fragmented into sub-micrometer particles during ingestion by Antarctic krill illustrates one biological pathway by which smaller plastic particles may form, although this process should not be assumed to occur identically in mammals (Dawson et al., 2018). Definitions of nanoplastics vary, with upper size limits of either 100 nm or 1,000 nm, the latter emphasizing colloidal behaviour (Gigault et al., 2018). Oral exposure is relevant to experimental assessment because ingested particles interact directly with the gastrointestinal barrier, although uptake and biological effects depend on the exposure system. Stock et al. (2019) observed uptake of only a small fraction of polystyrene microparticles and no histologically detectable inflammatory response under their experimental conditions. Evidence of internal human exposure has nevertheless strengthened interest in systemic effects. Leslie et al. (2022) detected polymeric material, including polyethylene, in human whole blood using a method targeting plastic particles of at least 700 nm. Smaller plastic particles are of particular toxicological concern because their reduced dimensions can facilitate transport across biological barriers, enhance cellular internalization and increase their potential for systemic distribution and interactions within internal tissues (Pan et al., 2026). Therefore, these support investigation of blood as a potentially relevant compartment. Experimental studies are therefore needed to distinguish evidence of exposure from evidence of adverse biological responses (Stock et al., 2019; Leslie et al., 2022). Body-weight progression provides an integrated measure of animal growth that can complement more specific toxicological endpoints. Its interpretation in plastic-particle studies is supported by evidence of gastrointestinal and metabolic disturbances that might affect physiological condition. Jin et al. (2019) reported intestinal-barrier dysfunction, altered gut microbiota and metabolic changes in mice exposed to polystyrene microplastics. In a polyethylene-specific study, Li et al. (2020) observed changes in gut microbial composition, increased serum interleukin-1α and intestinal inflammation in mice. Kehinde et al. (2024) further reported altered thyroid and reproductive hormones together with disturbed serum antioxidant indices following polyethylene-microplastic exposure in male Wistar rats. These studies identify plausible pathways through which exposure could influence growth, while leaving the direction and magnitude of any body-weight response to be determined experimentally. Growth responses to polyethylene particles are not uniform. Lee et al. (2022) found no significant changes in body weight or

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food consumption after 28-day oral exposure of ICR mice to 10–50 µm polyethylene microplastics, whereas Farag et al. (2023) reported increased body weight at the fifth week in rats receiving intermediate and high doses of polyethylene microplastics. These contrasting observations emphasize the need to assess growth directly in each exposure model. They also show why final body weight, absolute weight gain and percentage weight gain should be interpreted together (Lee et al., 2022; Farag et al., 2023).

Haematological evaluation provides a complementary means of examining systemic responses. Red blood cell count, haemoglobin concentration and packed cell volume describe the circulating erythrocyte profile, while mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration help characterize changes in cell size and haemoglobin content. The relevance of these endpoints is supported by the erythrocyte morphological abnormalities and serum redox disturbances reported in polyethylene-microplastics-exposed rats by Farag et al. (2023). Such findings justify testing whether exposure also changes conventional erythrocyte indices. Total and differential leukocyte counts, together with platelet counts, broaden this assessment by characterizing additional circulating cell populations. Polyethylene-related immune effects have been demonstrated using endpoints beyond routine blood counts. Li et al. (2020) reported changes in CD4-positive T-cell subsets alongside increased serum interleukin-1α, while Jo et al. (2023) found alterations in selected cytokine and immunoglobulin ratios after four weeks of oral microplastics exposure, with responses differing by polymer, particle size, dose and sex. Major thymic and splenic immune-cell populations were not significantly altered in the latter study. These findings support investigation of immune-related responses while showing that different immune endpoints need not change in parallel. Differential percentages therefore require interpretation alongside total leukocyte counts, and shifts in leukocyte or platelet numbers should not be treated as direct evidence of a particular inflammatory pathway (Li et al., 2020; Jo et al., 2023).

Accordingly, this study evaluated body-weight changes and haematological responses in male Wistar albino rats following once-daily oral exposure to a polyethylene nanoplastics-enriched preparation for 28 days. The assessment included absolute and percentage weight gain, red blood cell count, haemoglobin, packed cell volume, erythrocyte indices, total white blood cell count, platelet count and differential leukocyte percentages. The study tested the hypothesis that repeated exposure would alter growth and circulating blood-cell profiles, with more pronounced responses at higher exposure levels. This combined approach was intended to characterize the pattern of subacute growth and haematological effects attributable to the tested polyethylene-nanoplastics enriched preparation because body-weight gain and haematological indices have been assessed jointly in experimental rats to characterize physiological responses to dietary interventions, including supplementation under restricted feeding conditions (Nwaigwe et al., 2022).

2. MATERIALS AND METHODS

2.1 Polyethylene starting material and particle preparation

High-density polyethylene (HDPE) resin pellets (grade HFW00346, Indorama Eleme Petrochemicals Limited, Eleme, Rivers State, Nigeria) were purchased from the Ariaria International market, Aba, Abia State, and mechanically pulverized using an electric blender (Boscon® BOS-WBM4630S). The blending process used shear and impact forces to produce irregular fragments. Pulverized material was stored in airtight containers before characterization and further processing. For further size reduction, pulverized HDPE was dispersed in filtered deionized water at 80 g/L and sonicated using an Ultramet II Sonic Cleaner (Buehler-Met AG, CH-4018, Basel, Switzerland). The polyethylene suspension was prepared gravimetrically by weighing 80 g of pulverized polyethylene microplastics using an electronic balance (Model YX30002, 3000 g/0.01 g) and adding deionized water to a final suspension volume of 1,000 mL. The nominal mass concentration was calculated as the mass of polyethylene divided by the final suspension volume, yielding 80 g/L (80 mg/mL). Mass conc. (c) = mass (g)/Vol (L). The suspension was sonicated continuously for 60 min at 40kHz, with the sample vessel immersed in a water bath and periodically removed for cooling to maintain the suspension below 30 °C. Following sonication, the suspension was decanted to remove residual micro fragments and obtain a particle fraction targeted at <100 nm, with a stated subpopulation in the tens-to-hundreds-of-nanometers range.

2.2 Particle characterization

Before ultrasonication, pulverized PE morphology was examined by scanning electron microscopy (SEM) at 15 kV with a 537-µm field of view, and particle-metric analysis was performed on a representative micrograph (142 particles). Elemental composition was assessed by SEM-coupled energy-dispersive X-ray spectroscopy using one region and four spot analyses. Polymer chemistry was evaluated by attenuated-total-reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy on a Bruker ALPHA II instrument with a Platinum Diamond single-reflection ATR accessory; pre-sonication spectra used 24 scans at 4 cm^{-1} resolution, and post-sonication spectra used 32 sample and 32 background scans over 4000–400 cm^{-1} at 4 cm^{-1} resolution.

After ultrasonication, 134 discrete particles were identified by image analysis. Projected area, perimeter, maximum/minimum Feret diameters, and associated dimensional parameters were measured and converted using a calibration factor of 25 nm $pixel^{-1}$. For descriptive reporting, particles were classified as ≤100 nm, >100 nm to ≤1 µm, or >1 µm. This operational ≤100-nm class was retained for consistency with the experimental analysis, while acknowledging that nanoplastics definitions in the literature are not uniform (Gigault et al., 2018). Post-sonication FTIR spectra were collected from 4000 to 400 cm^{-1} using 32 sample and 32 background scans at 4 cm^{-1} resolution and major bands were compared with the starting material.

2.3 Dose preparation and administration of the polyethylene suspension

The polyethylene nanoplastics-enriched suspension was prepared at a nominal concentration of 80 g/L (80 mg/mL). Rats in Groups 2–5 were assigned target doses of 17.5, 35.0, 70.0 and 140.0 mg/kg/day, respectively. Group-specific gavage volumes were calculated from the initial mean body weights using the following equation:

$$Gavage\ volume = \frac{Target\ dose\left(\frac{mg}{kg}\right) \times Initial\ body\ weight\ (g)}{Suspension\ concentration\ (mg/mL)} \quad (1)$$

The initial mean body weights of Groups 2–5 were 100.40, 102.96, 118.57 and 133.65 g, respectively, yielding calculated gavage volumes of 21.96, 45.05, 103.75 and 233.89 µL/rat/day.

2.4 Animals and exposure design

Twenty-five male Wistar albino rats were housed under standard conditions with a 12-h light/dark cycle and free access to commercial feed and deionized water. Following two weeks of acclimatization, the rats were randomly allocated to five groups (n = 5 per group). Group 1 served as the control and received feed and deionized water. Groups 2–5 received a polyethylene nanoplastics-enriched suspension at 80 g/L (80 mg/mL) by oral gavage once daily for 28 consecutive days. Fixed group-specific volumes of 21.96, 45.05, 103.75, and 233.89 µL/rat/day were administered to Groups 2–5, respectively. These volumes corresponded to nominal initial doses of approximately 17.5, 35.0, 70.0, and 140.0 mg/kg/day, calculated using the respective initial group mean body weights of 100.40, 102.96, 118.57, and 133.65 g. Gavage volumes remained unchanged throughout the exposure period; consequently, individual doses expressed per kilogram of body weight varied with each rat's body weight and subsequent growth. The polyethylene nanoplastics suspension was vortex-mixed using a Vortex-Genie 2 mixer (Scientific Industries, USA) at 2,500 rpm for 60 seconds immediately before each dose withdrawal to resuspend settled particles. Animal care and experimental reporting were undertaken in accordance with institutional ethical requirements with ethical approval number VPP/EC/2025/005 and followed established laboratory-animal guidance as stated by ARRIVE guidelines. (Percie du Sert et al., 2020). At the end of 28 days, animals were fasted for 12 hours and sacrificed the following day via cervical dislocation. Blood was collected by cardiac puncture into EDTA tubes for haematological analysis. Analysis was done within 2 hours after sample collection. Blood smears for manual differential leukocyte counts were prepared immediately after collection.

2.5 Haematological analysis and body weight measurements

The haematological parameters determined include red blood cell count (RBCC), Packed Cell Volume (PCV), Haemoglobin concentration (HbC), White Blood Cell counts (WBCC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin

concentration (MCHC) and Platelets (PLT) counts. These parameters were obtained at once for each blood sample using an Automated Haematology Analyzer model 2800 BC produced by Mindray company, India, following standard procedures outlined by the producer. Before sample analysis, the Mindray BC-2800 haematology analyzer underwent a background check. Daily internal quality control was performed using low-, normal- and high-level control materials, and results were assessed against the lot-specific acceptance ranges. Calibration and subsequent verification were conducted using manufacturer-recommended materials according to the instrument's operating instructions. Samples were analyzed only after acceptable quality-control results were obtained.

Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using the following formulae:

$$MCV\ (fL) = \frac{PCV\% \times 10}{RBC\ count\ (10^6/\mu L)} \quad (2)$$

$$MCH\ (pg) = \frac{Haemoglobin\ (g/dL) \times 10}{RBC\ count\ (10^6/\mu L)} \quad (3)$$

$$MCHC\ (g/dL) = \frac{Haemoglobin\ (g/dL) \times 100}{PCV\ (\%)} \quad (4)$$

Body weight was measured at baseline and at completion of the exposure period between 8 AM and 10 AM. Body weight was measured using Electronic Balance, Model YX30002, 3000 g/0.01 g. Absolute gain was calculated for each animal as final minus initial weight. Percentage gain was calculated as 100 × (final − initial)/initial weight.

2.6 Statistical analysis

Results were analyzed statistically using SPSS 22 statistical software (SPSS, Chicago, IL, USA). Statistical data were expressed as mean ± SD. Comparison of groups was performed using a one–way ANOVA followed by a Duncan's multiple comparison post–tests. A probability of less than 5% ($p < 0.05$) was considered to be statistically significant.

3.0 RESULTS

3.1 Morphology of polyethylene particle

SEM examination of the pulverized polyethylene prior to ultrasonication revealed a heterogeneous population of irregularly shaped particles with marked variation in particle size and morphology (Figure 1). The particles were predominantly non-spherical and occurred as angular, elongated, plate-like and fragmented structures. The heterogeneous morphology was consistent with the mechanically pulverized nature of the starting polyethylene material.

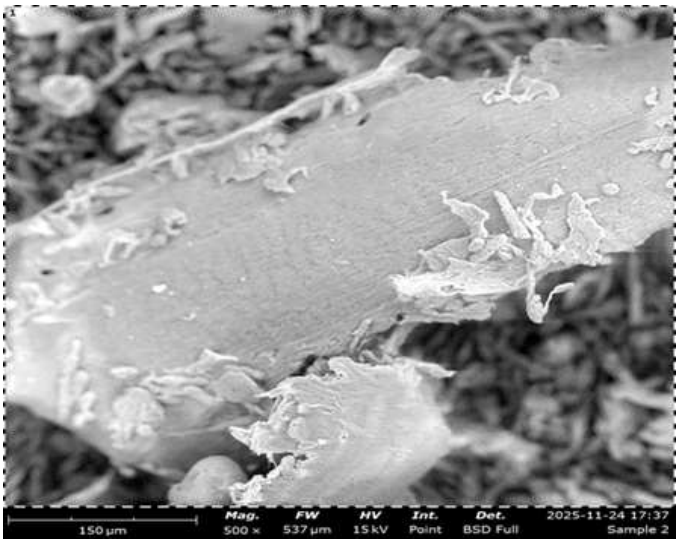


Figure **1**. SEM image of the pulverized polyethylene starting material

3.2 Particle-size characteristics of the ultrasonicated PE suspension

Particle-metric analysis identified 134 discrete post-sonication particles. Feret diameters ranged from approximately 35.35 nm to 7.33 µm. The median Feret diameter was 83.08 nm, whereas the mean was 280.50 nm, indicating a strongly right-skewed distribution driven by a small number of larger residual particles or aggregates. Of the 134 particles, 127 (94.8%) had Feret diameters ≤100 nm, 3 (2.2%) were >100 nm to ≤1 µm, and 4 (3.0%) exceeded 1 µm. Thus, 130/134 particles (97.0%) were ≤1 µm. On a number basis, the analyzed preparation was therefore strongly dominated by nanoscale particles, while remaining polydisperse and containing a small larger-particle fraction.

3.3 Body weight

All groups showed positive mean weight gain (Table 1). Mean gain was 33.05 ± 4.33 g in controls, 33.76 ± 5.05 g at 17.5 mg/kg, 28.67 ± 4.93 g at 35 mg/kg, 13.33 ± 9.48 g in Group 4 (70 mg/kg) and 15.70 ± 6.83 g in Group 5 (140 mg/kg). The two upper-dose groups had significantly lower absolute and percentage gain than controls and both lower-dose groups ($p < 0.05$); the two upper-dose groups did not differ significantly from one another. Their mean gains were approximately 59.7% and 52.5% lower than the control mean, respectively. Initial mean body weight was 91.66 ± 2.15 g in controls and 133.65 ± 9.75 g in Group 5. Initial weights in the two upper-dose groups were significantly ($p < 0.05$) higher than in controls, and the highest-dose group (Group 5) was also significantly ($p < 0.05$) heavier than the Group 4. Final weight of group 5 was significantly ($p < 0.05$) higher than in all other groups, while the remaining final-weight means shared the same significance grouping. The results therefore indicate reduced weight gain.

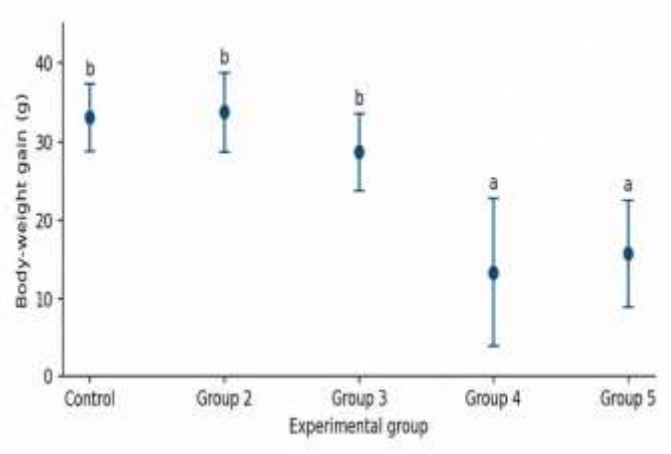


Figure **2**. Observed 28-day body-weight gain, mean ± SD (n = 5). Different letters indicate $p < 0.05$.

3.4 Erythrocyte and platelet responses

RBC count and PCV were significantly lower than control in only Group 5 (Table 2). RBC declined from 6.24 ± 0.43 to 5.58 ± 0.23 ×10⁶/mm³ and PCV from 45.37 ± 1.32% to 41.80 ± 1.95%, corresponding to reductions of 10.6% and 7.9%. Haemoglobin was significantly ($p < 0.05$) lower in Group 3 and Group 5, falling from 14.83 ± 0.54 g/dL to 13.52 ± 0.88 and 13.00 ± 0.76 g/dL, respectively. Group 4 did not differ significantly ($p > 0.05$) from control and lower-dose groups. RBC, PCV and haemoglobin did not decrease monotonically across the full dose series. MCV did not differ significantly among groups. MCH in Group 5 was lower than in Group 1 but did not differ significantly from control. MCHC was significantly lower in Groups 4 and 5 than in controls, reaching 31.06 ± 0.65 and 31.10 ± 1.16 g/dL compared with 32.67 ± 0.64 g/dL. Platelet count increased significantly at Groups 2, 4, and 5, but the Group 3 did not differ significantly from control. The two upper-dose groups had similar counts of 668.20 ± 76.68 and 670.20 ± 92.95 ×10³/mm³, approximately 73.0% and 73.5% above control. Total WBC count increased numerically across the dose series, from 8.03 ± 1.44 ×10³/mm³ in controls to 11.84 ± 1.86 ×10³/mm³ in Group 5 (Table 2). Groups 4 and 5 differed significantly from control, whereas Groups 2 and 3 did not.

Table **1**. Initial and final body weight and growth over 28 days of PE-NP oral exposure.

Group	Initial (g)	Final (g)	Gain (g)	Gain (%)
Control	91.66 ± 2.15^{a}	124.70 ± 5.18^{a}	33.05 ± 4.33^{b}	36.00 ± 4.04^{b}
Group 2	100.40 ± 10.87^{a}	134.16 ± 10.42^{a}	33.76 ± 5.05^{b}	34.14 ± 7.31^{b}
Group 3	102.96 ± 4.25^{a}	131.63 ± 7.75^{a}	28.67 ± 4.93^{b}	27.82 ± 4.44^{b}
Group 4	118.57 ± 12.03^{b}	131.90 ± 15.47^{a}	13.33 ± 9.48^{a}	11.29 ± 7.82^{a}
Group 5	133.65 ± 9.75^{c}	149.35 ± 10.43^{b}	15.70 ± 6.83^{a}	11.87 ± 5.42^{a}
p-values	<0.001	0.028	<0.001	<0.001

Values are mean ± SD, n = 5. Within each column, means with no shared superscript letter differ at p < 0.05.

Table **2**. Erythrocyte indices, white blood cell and platelet counts after 28 days PE-NP exposure.

Treatment groups	Control	Group 2	Group 3	Group 4	Group 5	p-values
RBC (x10^{6}/mm^{3})	6.24 ± 0.43^{b}	6.07 ± 0.52^{a,b}	5.68 ± 0.53^{a,b}	5.88 ± 0.12^{a,b}	5.58 ± 0.23^{a}	0.119
PCV (%)	45.37 ± 1.32^{b}	45.10 ± 2.14^{b}	42.72 ± 2.79^{a,b}	44.36 ± 1.78^{a,b}	41.80 ± 1.95^{a}	0.059
Hb (g/dL)	14.83 ± 0.54^{b}	14.87 ± 0.88^{b}	13.52 ± 0.88^{a}	13.78 ± 0.71^{a,b}	13.00 ± 0.76^{a}	0.003
WBC (x10^{3}/mm^{3})	8.03 ± 1.44^{a}	9.17 ± 1.02^{a,b}	10.14 ± 1.22^{a,b,c}	10.96 ± 2.22^{b,c}	11.84 ± 1.86^{c}	0.014
PLT (x10^{3}/mm^{3})	386.25 ± 44.42^{a}	538.17 ± 82.61^{b}	490.60 ± 107.66^{a,b}	668.20 ± 76.68^{c}	670.20 ± 92.95^{c}	<0.001
MCV (fL)	72.88 ± 3.21^{a}	74.51 ± 3.05^{a}	75.42 ± 4.85^{a}	75.37 ± 1.69^{a}	74.95 ± 0.88^{a}	0.735
MCH (pg)	23.80 ± 0.81^{a,b}	24.55 ± 0.93^{b}	23.84 ± 0.74^{a,b}	23.41 ± 0.84^{a,b}	23.31 ± 0.82^{a}	0.151
MCHC (g/dL)	32.67 ± 0.64^{b}	32.95 ± 0.62^{b}	31.68 ± 1.75^{a,b}	31.06 ± 0.65^{a}	31.10 ± 1.16^{a}	0.024

Mean ± SD, n = 5. Within each row, groups with no shared letter differ at p < 0.05. Hb, haemoglobin; PCV, packed cell volume; WBC, white blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Table **3**. Differential leukocyte percentages after 28 days PE-NP exposure.

Treatment groups	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophil (%)	Basophils (%)
Control	51.75 ± 2.75^{c}	43.50 ± 3.42^{a}	3.50 ± 0.58^{a}	1.25 ± 0.50^{a}	0.00 ± 0.00
Group 2	43.33 ± 5.75^{b}	51.17 ± 5.19^{b}	4.00 ± 0.63^{a,b}	1.50 ± 0.55^{a}	0.00 ± 0.00
Group 3	34.60 ± 3.21^{a}	59.20 ± 3.19^{c}	4.60 ± 0.55^{b,c}	1.60 ± 0.55^{a}	0.00 ± 0.00
Group 4	32.00 ± 2.12^{a}	61.20 ± 1.92^{c}	5.20 ± 0.45^{c,d}	1.60 ± 0.55^{a}	0.00 ± 0.00
Group 5	31.00 ± 2.00^{a}	61.60 ± 2.30^{c}	5.60 ± 0.55^{d}	1.80 ± 0.45^{a}	0.00 ± 0.00
p-values	<0.001	<0.001	<0.001	0.633	-----

Mean ± SD, n = 5. Within each row, groups with no shared letter differ at p < 0.05.

3.5 Differential leukocytes

Neutrophil percentage fell from 51.75 ± 2.75% in controls to 43.33 ± 5.75%, 34.60 ± 3.21%, 32.00 ± 2.12% and 31.00 ± 2.00% across increasing doses. Every exposed group differed from control, and the three upper exposure groups shared the same grouping. Lymphocyte percentage rose from 43.50 ± 3.42% to 51.17 ± 5.19%, 59.20 ± 3.19%, 61.20 ± 1.92% and 61.60 ± 2.30%; all exposed groups differed from control. Monocyte percentage increased from 3.50 ± 0.58% in controls to 5.60 ± 0.55% in Group 5. Values of Group 3, Group 4 and Group 5 differed significantly from control, while Group 1 did not. Eosinophil percentage did not differ significantly among groups. Basophils were reported as 0.00 ± 0.00% in every group and carried no inferential comparison symbols.

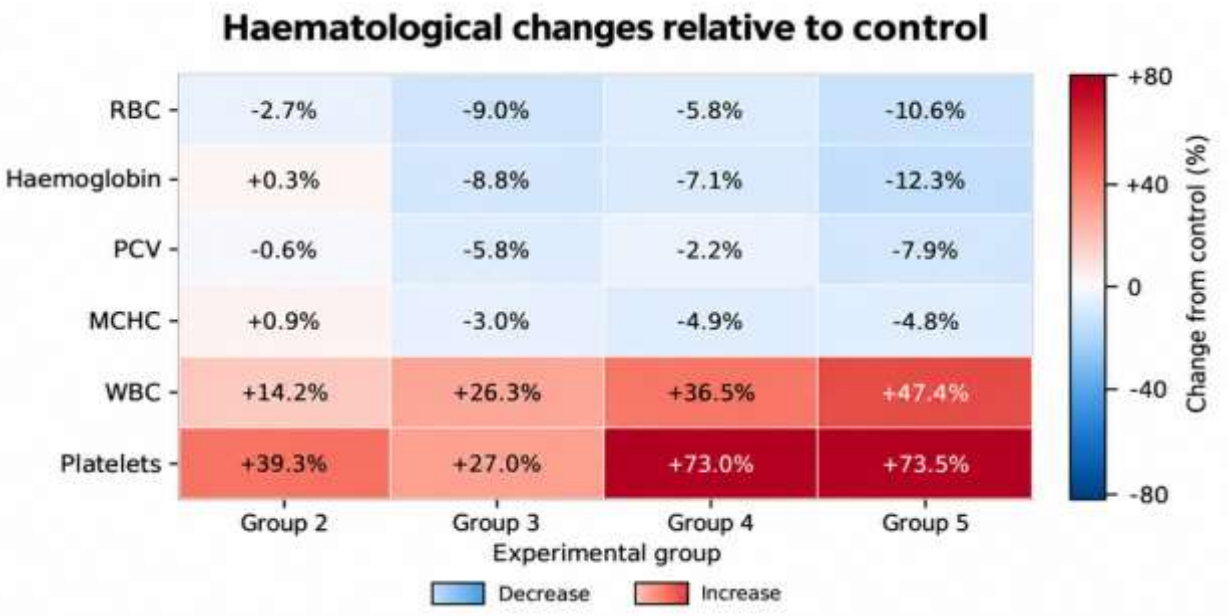


Figure **3**. Descriptive percentage differences from the control mean for selected haematological parameters.

4.0 Discussion

The median Feret diameter of 83.08 nm and the 94.8% of analyzed particles being ≤100 nm demonstrate strong nanoscale predominance on a number basis; however, the larger mean (280.50 nm), the 7.33-µm upper extreme, and the residual >1-µm fraction show that the preparation was slightly polydisperse but nanoplastics rich. This distinction matters because particle size influences transport, surface area, aggregation, and biological interaction. Hwangbo et al. (2024) similarly demonstrated that ultrasound-based fragmentation can generate irregular PE nanoplastics in the tens-to-hundreds-of-nanometers range, supporting the plausibility of the present preparation approach though did not imply identical particle characteristics. Repeated oral exposure to the polyethylene nanoplastics-enriched preparation for 28 days was associated with suppressed body-weight gain at the two highest nominal doses and alterations in erythrocyte, leukocyte and platelet indices. These findings suggest effects on growth and blood physiology under the experimental conditions. However, the responses were not uniformly monotonic. All groups gained weight, but the gains at Group 4 and Group 5 were significantly lower than those reported for the control and lower-dose groups. Compared with the control gain of 33.05 ± 4.33 g, gains of 13.33 ± 9.48 and 15.70 ± 6.83 g represent reductions of approximately 59.7% and 52.5%, respectively. This shows that there is suppression of weight gain rather than loss of existing body mass. The absence of a statistically significant reduction at Groups 2 and 3, followed by reduced gains at the two higher doses, suggests that growth effects were more evident at higher exposure levels. The slightly greater gain at Group 5 than at Group 4 also means that growth suppression did not increase monotonically with dose. Reduced food intake, impaired nutrient utilization or altered energy allocation are possible explanations. Polyethylene-microplastics exposure has produced serum oxidative and endocrine alterations in male Wistar rats after 28 days, providing indirect support for physiological pathways that might accompany altered growth (Kehinde et al., 2024). That study used 34–50 µm particles, however, and does not establish the mechanism underlying the present response. Contrastingly, Lee et al. (2022) reported no significant body-weight or food-consumption changes in ICR mice given 10–50 µm polyethylene microplastics at 500–2,000 mg/kg/day for 28 days. Farag et al. (2023), in contrast, reported increased body weight at the fifth week in rats receiving intermediate and high polyethylene-microplastics doses. The reductions in red blood cell count, haemoglobin and packed cell volume, particularly at higher exposure levels, indicate an adverse shift in the erythrocyte profile. At the highest dose, haemoglobin and packed cell volume were 13.00 ± 0.76 g/dL and 41.80 ± 1.95%, compared with 14.83 ± 0.54 g/dL and 45.37 ± 1.32% in controls. These findings are compatible with an anaemia-like response (Yahaya et al., 2024). Reductions in haemoglobin concentration and packed cell volume have also been reported in male Wistar rats following 28-day administration of calcium carbide-ripened banana juice (Onwuka et al., 2022), providing a comparison with chemically induced disturbances in erythrocyte indices. A similar haematological pattern was reported by Obasi et al. (2023), in which inhalation of particulate matter from burning tyres reduced RBC count, haemoglobin, and PCV while increasing WBC and platelet counts in male Wistar rats. Although the exposure material and route differed from those used in the present study, the comparable changes suggest that particle-associated oxidative and inflammatory stress may adversely affect erythrocyte integrity and haematopoietic regulation. The unchanged mean corpuscular volume, together with lower mean corpuscular haemoglobin concentration, suggests reduced haemoglobin concentration within erythrocytes without a detectable change in average cell size. This pattern is compatible with a normocytic, relatively hypochromic erythrocyte profile. Mean corpuscular haemoglobin showed only modest numerical variation. Oxidative injury is a plausible contributor, Farag et al. (2023) observed erythrocyte morphological abnormalities alongside reduced serum antioxidant indices and increased lipid peroxidation after 35 days of polyethylene-microplastics exposure. This supports a possible connection between redox disturbance and erythrocyte injury. White blood cell means increased progressively from 8.03 ± 1.44 to 11.84 ± 1.86 × 10^3/µL. This exposure-associated increase is consistent with a leukocytosis-like response and possible inflammatory or immune stimulation. This corresponds with the studies by Petrov et al., (2026) and Alexandrova et al., (2026). This leukocytosis indicates activation of the immune system and possible systemic inflammatory responses via recognition of PE-NPs as foreign particles, activation of macrophages and neutrophils, increased production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and oxidative stress-induced inflammation. The elevated WBC count supports the premise that PE-NPs trigger chronic inflammatory responses following repeated exposure. The dose-dependent increase in WBC count indicates activation of inflammatory and immune responses following PE-NP exposure, likely mediated through cytokine production and oxidative stress pathways (Skaba et al., 2025; Panizzolo et al., 2023).

Platelet counts also increased, reaching approximately 668–670 × 10^3/µL at the two highest doses compared with 386.25 ± 44.42 × 10^3/µL in controls. This corresponds with the study by Yahaya *et al*., (2024) on microplastics exposure and, with the studies by Petrov et al., (2026) and Alexandrova et al., (2026). The increase suggests reactive thrombocytosis, commonly associated with inflammation and tissue injury. The differential leukocyte results show a shift towards higher lymphocyte and monocyte proportions and a lower neutrophil proportion. Neutrophils declined from 51.75% to 31.00%, whereas lymphocytes increased from 43.50% to 61.60% and monocytes from 3.50% to 5.60%. These are compositional measurements: an increase in one leukocyte population can reduce the percentage represented by another. The higher lymphocyte and monocyte proportions are consistent with altered immune-cell distribution. Eosinophil percentages did not differ significantly, and basophils were recorded as zero across groups. These findings indicate no detected treatment-related change in these measured populations. The findings support suppressed growth at higher nominal exposure levels and an altered haematological profile.

Limitations

The relatively small sample size and exclusive use of male Wistar rats may limit statistical power and the generalizability of the findings, particularly regarding potential sex-dependent differences in haematological responses. The 28-day exposure period allowed the assessment of subacute toxicity but did not establish the long-term persistence or reversibility of the observed alterations. Body-weight measurements were obtained at the beginning and end of the experimental period without intermediate assessments, limiting the characterization of weight-gain trajectories and the identification of when growth disturbances emerged. Furthermore, the absence of quantitative food-intake data limited the ability to distinguish reduced weight gain associated with altered feeding behaviour from other metabolic or toxicological effects. Although the haematological analyses identified alterations in erythrocyte indices, leukocyte profiles and platelet counts, the absence of complementary investigations, including reticulocyte counts, peripheral blood smear examination and bone marrow assessments, limited the identification of the underlying mechanisms of these changes. Finally, the experimental exposure conditions may not directly reflect environmentally relevant human exposures, necessitating caution in extrapolating the findings. Future investigations incorporating larger cohorts, both sexes, serial body-weight measurements, food-consumption monitoring, extended exposure and recovery periods, and targeted haematological mechanistic assessments are warranted. Despite these limitations, the study provides useful evidence of altered body-weight gain and haematological responses following repeated oral exposure to a polyethylene nanoplastics-enriched preparation.

Conclusion

Twenty-eight-day oral exposure to the polyethylene nanoplastics-enriched preparation was associated with selective erythrocyte, leukocyte and platelet changes in male Wistar rats. The strongest combined erythrocyte response occurred at group 5, with lower RBC, haemoglobin and PCV; WBC and platelets were increased at upper exposures. Reduced neutrophil percentage and increased lymphocyte percentage were present throughout the exposed groups. Weight gain was lower at group 4 and group 5. These findings support a haematological toxicity signal and suppressed growth.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Data availability
The datasets generated and analyzed during the present study are available from the corresponding author upon reasonable request.

Author contributions
Ifeanyi C. Obasi: Conceptualization, methodology, experimental design, investigation, data curation, and writing—original draft. **Yusuf N. Omeh**: Conceptualization, methodology, supervision, experimental design, and writing—review and editing. **Simeon I. Egbachukwu**: Conceptualization, methodology, supervision, experimental design, and writing—review and editing. **Bayim, P-R. Bayim**: Investigation, animal care, data collection, formal analysis, and writing—review and editing. **Nwuke, Chinedu**: Investigation, animal care, data collection, formal analysis, and writing—review and editing. **Ngozi K. Achi** and **Victor N. Obasi**: Laboratory investigation, validation, interpretation of biochemical and writing—review and editing. All authors read and approved the final manuscript and agreed to be accountable for the integrity of the work.

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