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ORIGINAL ARTICLE

Dose-Dependent Hepatic and Renal Response to Repeated Oral Exposure to Polyethylene Nanoparticles in Male Wistar Rats

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ABSTRACT

Polyethylene (PE) is widely used, but organ-specific toxicity data for small PE particles remain limited and particle-size dependent. This study evaluated hepatic and renal responses to repeated oral exposure to an ultrasonically generated PE nanoplastics-enriched suspension in male Wistar rats. Particle-metric analysis identified 134 particles with Feret diameters of 35.35 nm–7.33 µm, a median of 83.08 nm, and a mean of 280.50 nm; 94.8% were ≤100 nm and 97.0% were ≤1 µm, indicating nanoscale predominance by number within a polydisperse preparation. Twenty-five rats (90–135 g) were randomized into five groups (n = 5): one control and four groups receiving an 80 mg/mL PE suspension by oral gavage once daily for 28 days at fixed volumes corresponding to initial target doses of approximately 17.5, 35.0, 70.0, and 140.0 mg/kg/day. Serum hepatic and renal indices and electrolytes were assessed, liver and kidney tissues underwent haematoxylin-and-eosin examination. At the highest exposure, ALT, AST, ALP, GGT, and total bilirubin were 556%, 276%, 112%, 129%, and 73% above control, respectively. Albumin decreased significantly in Groups 2–4 ($p<0.05$), while total protein was lowest in Group 3. Creatinine increased significantly at 70 and 140 mg/kg/day by approximately 15% and 27%, respectively ($p<0.05$). Urea, sodium, and chloride remained unchanged, whereas potassium increased significantly ($p<0.05$) and bicarbonate rose modestly at the two highest exposures. Histological examination revealed exposure-associated hepatic and renal abnormalities, most apparent at higher exposures. These findings indicate pronounced, dose-related hepatic injury and comparatively more modest renal impairment accompanied by structural tissue alterations.

Keywords: Polyethylene; Nanoplastics; Sub-acute; Hepatotoxicity; Nephrotoxicity; Wistar rat.

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1. INTRODUCTION

Plastic fragmentation generates micro- and nanoscale particles that can enter food, water, and air and thereby create multiple routes of human and animal exposure. A mass-based assessment estimated that humans may ingest approximately 0.1−5 g of microplastics per week, although it was emphasized that substantial uncertainty in the underlying exposure data (Senathirajah et al., 2021). Microplastics are commonly defined as plastic particles smaller than 5 mm, often operationally classified within the range of 1 µm–5 mm. However, their lower size boundary varies between classification systems (Hartmann et al., 2019). Nanoplastics, on the other hand, are plastic particles whose upper size limit varies between definitions: some use 100 nm, whereas others extend it to 1,000 nm (1 µm). Gigault et al. (2018) define nanoplastics as unintentionally produced plastic particles measuring 1–1,000 nm and exhibiting colloidal behavior (Hartmann et al., 2019; Gigault et al., 2018). Smaller particles are of particular toxicological interest because size influences biological transport, tissue access, and cellular interactions (Pan et al., 2026). The liver and kidney are probable systemic target organs after gastrointestinal exposure. The liver receives portal blood draining the gastrointestinal tract and is central to xenobiotic handling, distribution, metabolic homeostasis, and inflammatory signaling. Reviews of studies on micro- and nanoplastics have identified oxidative stress, inflammatory activation, mitochondrial dysfunction, disturbed lipid/energy metabolism, autophagy, and cell death as recurring hepatic responses (Chiang et al., 2024). The kidney is similarly vulnerable because of high perfusion, filtration of circulating solutes and particles, and the energetic demands of tubular transport; current renal literature links micro- and nanoplastics exposure with oxidative stress, inflammation, tubular and glomerular injury, and altered filtration or electrolyte handling (Bueno de Oliveira et al., 2024). Particle translocation across the intestinal barrier provides a rationale for investigating organs beyond the gastrointestinal tract. Using radiolabeled polystyrene nanoplastics, Zhang et al. (2026) demonstrated hepatic and renal accumulation in rats, with biodistribution influenced by particle size and exposure route. Polymer type, size and particle preparation are critical sources of heterogeneity. Direct experimental evidence for PE is expanding. In rats, PE microplastics have been associated with increased serum ALT, AST, and ALP together with oxidative and inflammatory hepatic responses (Ghafoor et al., 2024). A 28-day rat study of PE microplastics reported dose-dependent increases in creatinine and other renal injury indices, electrolyte disturbances, oxidative stress, mitochondrial impairment, and progressive renal histopathology (Kehinde et al., 2025). Conversely, a recent 28-day study using high-density polyethylene nanoparticles isolated from food packaging did not observe major liver histopathology, underscoring the importance of particle size, dose, surface properties, and preparation method (Dziendzikowska et al., 2026).

The present study therefore evaluated dose-dependent hepatic and renal responses to an ultrasonically polyethylene nanoplastics-enriched suspension in male Wistar rats over 28 days. The study integrated serum hepatic and renal biochemistry, electrolytes, and qualitative histopathology. This study hypothesized that repeated exposure would produce dose-related increases in markers of hepatocellular/cholestatic

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injury and renal functional stress, with concordant structural abnormalities in liver and kidney tissue.

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 to powder 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 subsequent 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

Prior to ultrasonication, particle morphology and dimensions were examined by scanning electron microscopy (SEM) at 15 kV and a 537-µm field of view. Particle-metric analysis was conducted on a representative SEM image; 142 particles were identified and analyzed using circle-equivalent diameter, major/minor axes, circumference, projected area, aspect ratio, circularity, convexity, and elongation. Elemental composition was assessed by SEM-coupled energy-dispersive X-ray spectroscopy (EDX), using one region analysis and four spot analyses at 15 kV. Polymer chemistry was evaluated by attenuated-total-reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) on a Bruker ALPHA II instrument with a Platinum Diamond single-reflection ATR accessory. Pre-sonication spectra were collected using 24 scans at 4 cm−1 resolution. Following ultrasonication, particle-metric analysis identified 134 discrete particles in the analyzed digital image, and projected area, perimeter, maximum/minimum Feret diameters, and associated parameters were calculated. 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 Animal procurement

Twenty-five apparently healthy male Wistar albino rats weighing 90–135 g were obtained from Animal House, Rhema University, Aba, Abia State. The animals were housed under standard laboratory conditions, maintained on a 12-h light/dark cycle, and provided commercial rat feed and water ad libitum. They were acclimatized for 14 days, from 19 March to 1 April 2026, before commencement of exposure.

Animal care and experimental procedures were approved by the appropriate institutional ethics committee (approval number VPP/EC/2025/005). The study was reported in accordance with the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

2.5 Experimental design and exposure

Following acclimatization, the rats were randomly assigned to five groups of five animals each. Group 1 served as the control and received feed and deionized water. Groups 2–5 received an 80 g/L (80 mg/mL) polyethylene nanoplastics-enriched suspension by oral gavage once daily for 28 consecutive days, from 2 to 29 April 2026.

Fixed daily volumes of 21.96, 45.05, 103.75, and 233.89 µL/rat were administered to Groups 2–5, respectively. Based on initial group mean body weights of 100.40, 102.96, 118.57, and 133.65 g, these volumes corresponded to nominal initial doses of approximately 17.5, 35.0, 70.0, and 140.0 mg/kg/day, respectively. The administered volumes remained fixed throughout the exposure period; therefore, the actual doses expressed per kilogram of body weight varied as the animals gained weight.

Immediately before each dose was withdrawn, the suspension was mixed using a Vortex-Genie 2 mixer (Scientific Industries, Bohemia, NY, USA) at 2,500 rpm for 60 s to resuspend settled particles.

2.6 Sample collection

Following the final exposure on 29 April 2026, the animals were fasted for 12 h and euthanized by cervical dislocation on 30 April 2026. Blood was collected by cardiac puncture into plain sample tubes and processed for serum biochemical analysis. The liver and kidneys were excised and fixed in 10% neutral-buffered formalin for histological examination.

2.7 Serum biochemistry and electrolytes

Liver biomarkers (Serum total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin), and renal biomarkers (urea, creatinine, sodium, potassium, chloride, and bicarbonate were all determined using commercially available diagnostic kits according to the manufacturers' instructions and the procedures described by Ugbogu et al. (2018) and Obasi et al. (2023). Absorbance was measured using a 722N spectrophotometer (China).

2.7.1 Determination of hepatic biochemical parameters

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using Randox kits. ALT and AST catalyzed the formation of pyruvate and oxaloacetate, respectively, from their corresponding amino-acid substrates and α-oxoglutarate. The resulting keto acids reacted with 2,4-dinitrophenylhydrazine to form coloured hydrazones. Following incubation with the respective substrate reagents at 37°C for 30 min, colour was developed with 2,4-dinitrophenylhydrazine and sodium hydroxide. Absorbance was measured at 546 nm against the reagent blank, and enzyme activities were obtained from the manufacturer's calibration tables and expressed as U/L. Serum alkaline phosphatase (ALP) activity was determined using a Randox kit based on the hydrolysis of p-nitrophenylphosphate by ALP in an alkaline medium containing magnesium ion. The formation of p-nitrophenol was monitored spectrophotometrically at 405 nm, and ALP activity was expressed as U/L according to the manufacturer's instructions. Serum total bilirubin was determined using a Randox kit by the diazo method. Bilirubin reacted with diazotized sulphanilic acid to form a coloured azobilirubin complex, while caffeine facilitated the release of albumin-bound bilirubin for measurement of total bilirubin. After colour development, absorbance was measured at 560 nm against the sample blank. Total bilirubin concentration was calculated using the manufacturer's conversion factor and expressed as mg/dL. Serum total protein was determined using a Randox kit by the biuret method. In an alkaline medium, cupric ion reacted with peptide bonds to produce a purple-coloured complex whose intensity was proportional to the protein concentration. Absorbance was measured at 560 nm against the reagent blank, and total protein concentration was calculated relative to the supplied 5.95 g/dL protein standard.

2.7.2 Determination of hepatic biochemical parameters

Serum urea and creatinine concentrations were determined using Randox kits. Urea was measured by the urease–Berthelot method, in which urea

was hydrolyzed by urease to produce ammonia. The ammonia subsequently reacted with phenol and hypochlorite to form a coloured compound whose intensity was proportional to the urea concentration. Creatinine was determined by the kinetic Jaffé method, based on the formation of a coloured complex between creatinine and picrate in an alkaline medium. Absorbance was recorded at 30 s and again after 2 min, and creatinine concentration was calculated from the change in absorbance relative to the supplied standard. Urea and creatinine concentrations were expressed as mg/dL. Serum sodium, potassium, chloride and bicarbonate concentrations were determined using TECO diagnostic kits. Sodium was measured by precipitation as sodium magnesium uranyl acetate, followed by colorimetric determination of the residual uranyl ions using ferrocyanide. Potassium was determined turbidimetrically following its reaction with sodium tetraphenylboron. Chloride was measured by the mercuric thiocyanate method, based on the formation of a coloured ferric thiocyanate complex. The concentrations of sodium, potassium and chloride were calculated relative to their corresponding standards.

Bicarbonate was determined enzymatically using phosphoenolpyruvate carboxylase and malate dehydrogenase. Bicarbonate reacted with phosphoenolpyruvate to form oxaloacetate, which was subsequently reduced to malate with the simultaneous oxidation of NADH. The resulting decrease in absorbance was measured at 340 nm, and bicarbonate concentration was calculated relative to the supplied 30 mmol/L standard. Serum electrolyte concentrations were expressed as mmol/L.

2.8 Histopathology

Liver and kidney tissues were fixed in 10% formalin, routinely processed, paraffin-embedded, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E) dyes. The slides were then mounted and viewed under a light microscope by an experienced histopathologist. Representative photomicrographs were captured at ×400.

2.9 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. RESULTS

3.1 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.

3.2 Hepatic biochemical responses

PE exposure produced pronounced dose-related increases in serum enzyme activities (Table 1). ALT increased from 14.75 ± 4.11 U/L in controls to 96.80 ± 3.83 U/L in group 5 (140 mg/kg), corresponding to an approximately 556% increase. AST rose from 22.25 ± 3.86 to 83.60 ± 4.16 U/L (approximately 276%), while GGT increased from 35.00 ± 4.08 to 80.00 ± 7.68 U/L (approximately 129%). ALP also increased progressively, reaching 108.40 ± 9.34 U/L at the highest dose (approximately 112% above control). Total bilirubin increased with dose from 0.59 ± 0.07 mg/dL in controls to 1.02 ± 0.09 mg/dL in Group 5 (140 mg/kg) (approximately 73% above control). Albumin was reduced in Groups 2 (17.5mg/kg), 3 (35.0 mg/kg), and 4 (70.0 mg/kg), with the largest decrease at Group 3 (1.98 ± 0.42 vs 3.05 ± 0.07 g/dL; approximately 35% lower). Total protein also reached its minimum at Group 3 (4.33 ± 0.71 vs 5.61 ± 0.27 g/dL). In Group 5 (140 mg/kg), total protein returned near the control mean, while albumin remained lower. Globulin varied less consistently across groups.

3.2 Renal biochemical and electrolyte responses

Renal responses were more selective than hepatic responses (Table 2). Serum urea remained statistically unchanged across all groups. Creatinine increased from 0.74 ± 0.03 mg/dL in controls to 0.85 ± 0.04 mg/dL in group 4 (70 mg/kg) and 0.94 ± 0.09 mg/dL in Group 5 (140 mg/kg); these represent approximately 15% and 27% increases, respectively, and both upper-dose groups were statistically distinct from control. Sodium and chloride did not differ significantly among groups. Potassium increased significantly ($p < 0.05$) in Groups 4 and 5, reaching 8.94 ± 0.39 and 8.76 ± 0.38 mEq/L, respectively, compared with 8.08 ± 0.43 mEq/L in controls. Bicarbonate rose modestly but significantly at the two upper doses (20.38 ± 0.26 and 20.44 ± 0.11 mmol/L vs 20.03 ± 0.13 mmol/L in controls). This pattern suggests an early or moderate disturbance in filtration and electrolyte/acid-base handling rather than widespread renal failure.

Table **1**. Serum hepatic biochemical indices after 28-day PE-NP exposure.

Parameter	Control	Group 2	Group 3	Group 4	Group 5	P-values
Total protein (g/dL)	5.61 ± 0.27^{b}	4.94 ± 0.67^{ab}	4.33 ± 0.71^{a}	4.68 ± 0.80^{ab}	5.52 ± 0.69^{b}	0.038
Albumin (g/dL)	3.05 ± 0.07^{b}	2.43 ± 0.45^{a}	1.98 ± 0.42^{a}	2.27 ± 0.57^{a}	2.57 ± 0.33^{ab}	0.015
Globulin (g/dL)	2.55 ± 0.23^{ab}	2.51 ± 0.24^{ab}	2.36 ± 0.33^{a}	2.41 ± 0.56^{ab}	2.95 ± 0.53^{b}	0.186
ALT (U/L)	14.75 ± 4.11^{a}	27.17 ± 10.40^{b}	47.60 ± 4.16^{c}	83.80 ± 4.82^{d}	96.80 ± 3.83^{e}	<0.001
AST (U/L)	22.25 ± 3.86^{a}	29.50 ± 3.99^{b}	40.40 ± 6.11^{c}	69.40 ± 6.03^{d}	83.60 ± 4.16^{e}	<0.001
ALP (U/L)	51.25 ± 5.91^{a}	80.83 ± 16.46^{b}	87.40 ± 5.03^{b}	90.80 ± 4.60^{b}	108.40 ± 9.34^{c}	<0.001
GGT (U/L)	35.00 ± 4.08^{a}	42.33 ± 3.27^{b}	49.00 ± 2.45^{c}	59.80 ± 5.31^{d}	80.00 ± 7.68^{e}	<0.001
Total bilirubin (mg/dL)	0.59 ± 0.07^{a}	0.65 ± 0.08^{ab}	0.73 ± 0.05^{b}	0.93 ± 0.10^{c}	1.02 ± 0.09^{c}	<0.001

Values are presented as mean ± standard deviation (n = 5); and values with different letter superscripts are significantly ($p < 0.05$) different across the row.

Table **2**. Serum renal biochemical indices and electrolytes after 28-day PE-NP exposure.

Parameter	Control	Group 2	Group 3	Group 4	Group 5	P-values
Urea (mg/dL)	22.58 ± 1.28^{a}	23.02 ± 2.75^{a}	21.54 ± 1.94^{a}	21.48 ± 1.96^{a}	22.42 ± 0.90^{a}	0.649
Creatinine (mg/dL)	0.74 ± 0.03^{a}	0.76 ± 0.05^{a}	0.77 ± 0.05^{a}	0.85 ± 0.04^{b}	0.94 ± 0.09^{c}	<0.001
Na^{+} (mEq/L)	139.13 ± 6.52^{a}	142.00 ± 2.89^{a}	144.54 ± 5.58^{a}	143.98 ± 4.94^{a}	137.46 ± 6.11^{a}	0.192
K^{+} (mEq/L)	8.08 ± 0.43^{a}	8.47 ± 0.42^{ab}	8.62 ± 0.33^{ab}	8.94 ± 0.39^{b}	8.76 ± 0.38^{b}	0.038
Cl^{-} (mEq/L)	88.08 ± 1.12^{a}	90.53 ± 2.32^{a}	90.52 ± 1.66^{a}	90.24 ± 1.73^{a}	90.54 ± 1.32^{a}	0.212
HCO^{3-} (mmol/L)	20.03 ± 0.13^{a}	20.12 ± 0.18^{a}	20.10 ± 0.17^{a}	20.38 ± 0.26^{b}	20.44 ± 0.11^{b}	0.006

Values are presented as mean ± standard deviation (n = 5); and values with different letter superscripts are significantly ($p < 0.05$) different across the row.

3.3 Histopathology

Representative H&E micrographs are shown in Figures 1 and 2. Control kidney sections showed preserved glomerular and tubular architecture. Exposure-group micrographs showed increasingly conspicuous departures from the control appearance, especially at the intermediate-to-high doses, with focal tubular/interstitial abnormalities marked by arrows in the supplied images. Similarly, control liver sections showed preserved hepatic architecture around the central vein, whereas higher-dose sections displayed focal architectural and cellular abnormalities indicated in the photomicrographs.

A

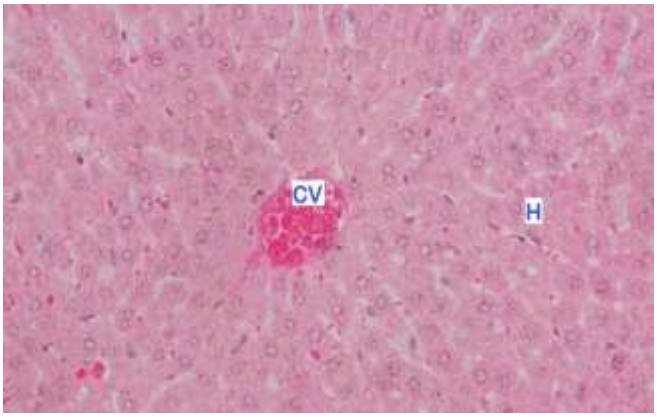


Plate 1

B

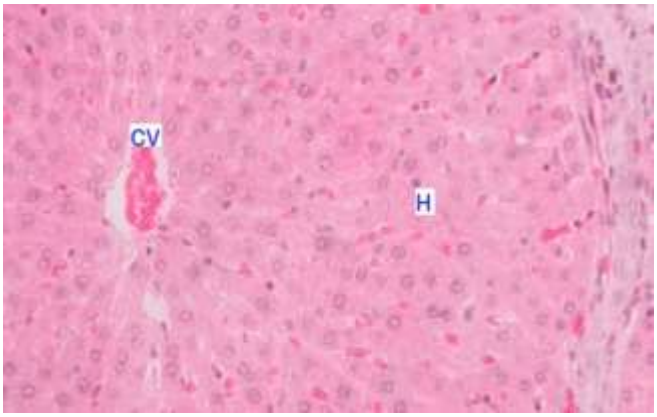


Plate 2

C

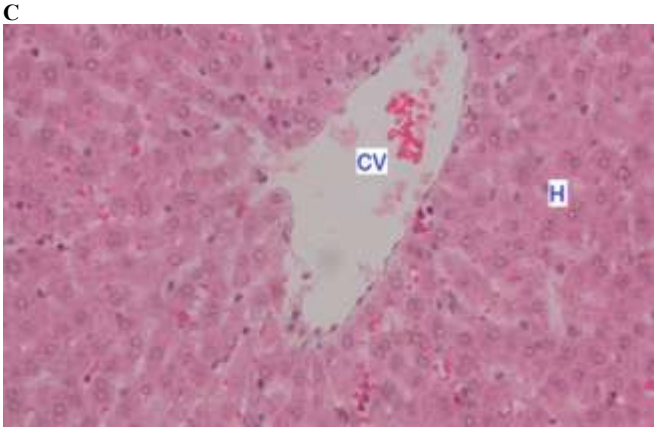


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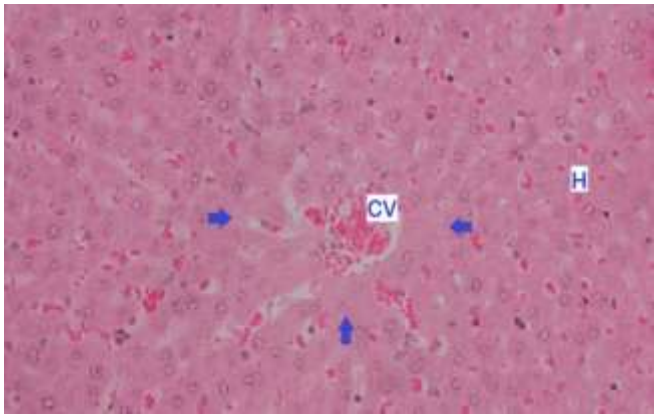


Plate 4

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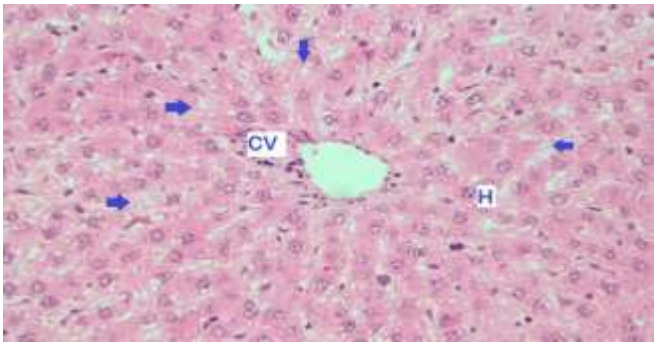


Plate 5

*Figure **1**. Representative liver sections (H&E, ×400) after 28-day PE-NP exposure: A, control; B, Group 2 (17.50 mg/kg); C, Group 3 (35.00 mg/kg); D, Group 4 (70.00 mg/kg); E, Group 5 (140.00 mg/kg). CV, central vein; H, hepatocytes. Degeneration and necrosis of the hepatocytes (arrow)*

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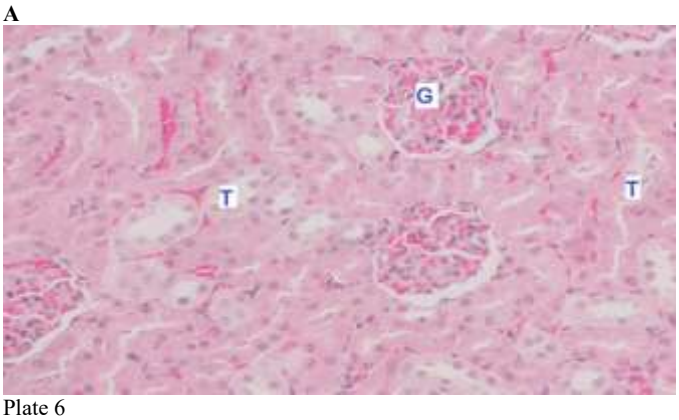


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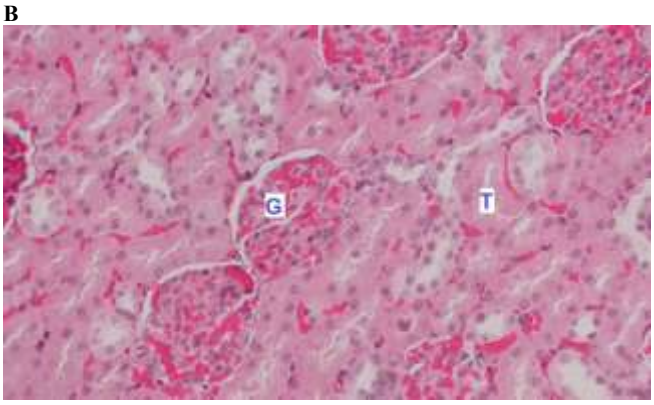


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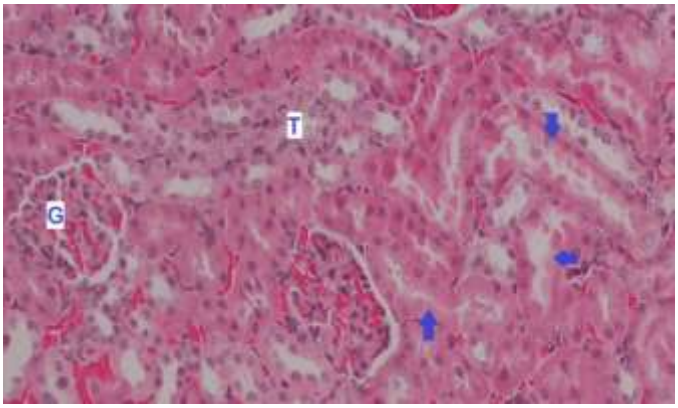


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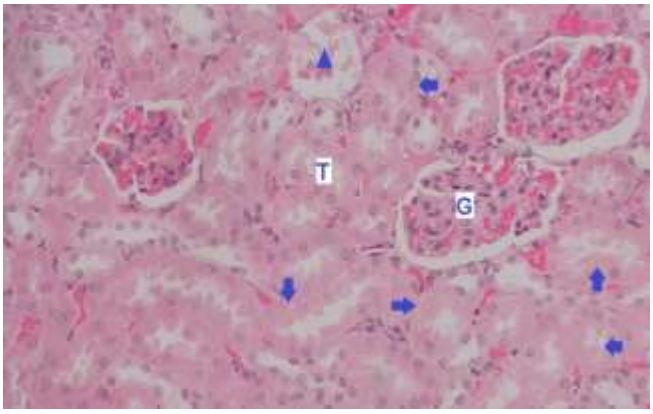


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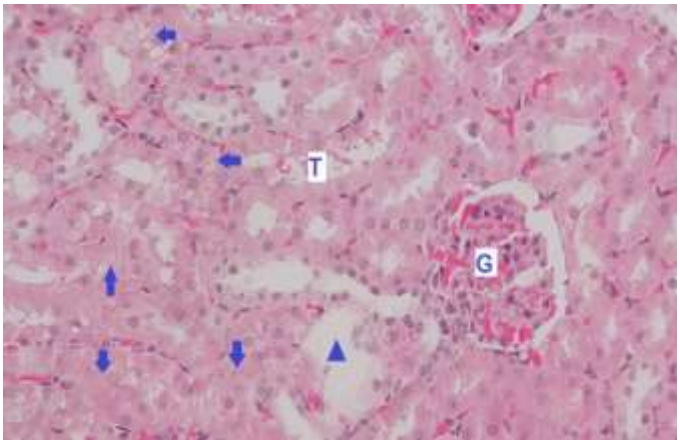


Plate 10

*Figure **2**. Representative kidney sections (H&E, ×400) after 28-day PE-NP exposure: A, control; B, Group 2 (17.50 mg/kg); C, Group 3 (35.00 mg/kg); D, Group 4 (70.00 mg/kg); E, Group 5 (140.00 mg/kg). G, glomerulus; T, renal tubules. Degeneration and necrosis of the tubular epithelium of the renal tubules (arrow)*

4. DISCUSSION

The median Feret diameter of 83.08 nm and the finding that 94.8% of analyzed particles were ≤100 nm indicates strong nanoscale predominance on a number basis. However, the higher mean diameter (280.50 nm), maximum diameter of 7.33 µm, and residual fraction exceeding 1 µm indicate a polydisperse, polyethylene-nanoplastics-rich preparation. This distinction is relevant because particle size influences transport, surface area, aggregation, and biological interactions. Hwangbo et al. (2024) similarly reported that ultrasonic fragmentation generated irregular polyethylene nanoplastics with sizes ranging from tens to hundreds of nanometers, supporting the plausibility of the preparation approach used here without establishing equivalence in particle characteristics. The principal finding is a marked dose-related hepatic response after 28 days of exposure to the prepared PE particle suspension. ALT and AST increased progressively across all dose tiers, while ALP, GGT, and bilirubin also increased. In combination, this pattern is consistent with hepatocellular injury accompanied by a cholestatic or hepatobiliary component rather than an isolated change in a single enzyme. The magnitude of the response was substantial: at the highest dose, ALT was more than sixfold the control mean and AST was nearly fourfold the control mean. Although serum enzyme elevations do not themselves identify the cellular mechanism, their concordance with the qualitative tissue abnormalities strengthens the interpretation that the exposure materially affected hepatic integrity. The hepatic findings are biologically plausible within the broader micro- and nanoplastics literature. Experimental and review evidence repeatedly implicates reactive oxygen species generation, antioxidant depletion, mitochondrial dysfunction, inflammatory signaling, altered lipid metabolism, autophagy, and apoptotic pathways in plastic-particle-associated liver injury (Chiang et al., 2024). In a PE-specific rat model, Ghafoor et al. (2024) reported increased ALT, AST, and ALP together with increased ROS and MDA, suppression of antioxidant systems, and activation of inflammatory and apoptotic

pathways after PE microplastic exposure. Another PE-specific model by Forutan et al., (2026), reported increases in ALT, AST, and ALP. Broadly, cross polymer studies on polystyrene nanoplastics by Dziendzikowska et al., (2026) and Alexandrova et al., (2026) reported increases in ALT, AST, and ALP. AST elevation further supports the occurrence of hepatocellular damage. Although AST is less liver-specific than ALT because it is also found in cardiac and skeletal muscle, concurrent increases in ALT and AST strongly indicate hepatic injury (Giannini et al., 2005). The observed parallel rise in ALT and AST suggests progressive hepatocellular degeneration as PE-NP exposure increased. Elevated ALP suggests impaired bile flow, biliary tract irritation, hepatic inflammation, cholestatic changes induced by nanoplastics accumulation. The increase indicates that PE-NPs may affect not only hepatocytes but also the hepatobiliary system. GGT is a sensitive marker of hepatobiliary injury and oxidative stress. It is concentrated in hepatobiliary membranes and often increases with biliary or canalicular injury, its parallel increase with ALP suggests that the PE-NP effect was not limited to leakage of hepatocellular aminotransferases. Rather, there appears to be involvement of hepatobiliary transport or membrane systems as well. GGT is particularly useful here because ALP can originate from non-hepatic tissues, whereas concordant GGT elevation strengthens evidence for hepatobiliary involvement (Giannini et al., 2005). The broader toxicological evidence supports this result. A systematic review and meta-analysis by Zhang et al. (2024) found that plastic-particle exposure alters liver enzymes and induces oxidative and intracellular toxicity; smaller particles and longer exposure periods were generally associated with stronger hepatic effects. The decrease in albumin across the lower and intermediate exposure groups may indicate impaired hepatic synthetic function, altered protein turnover, inflammatory redistribution, or nutritional effects. However, the pattern was non-monotonic: albumin partially recovered at the highest dose and total protein returned close to control. This divergence from the monotonic enzyme response reflects biological variability, or compensatory responses. Alexandrova et al., (2026) and Yahaya et al., (2024) observed dose-dependent decreases in albumin after exposure to microplastics. Albumin is synthesized exclusively by hepatocytes and is widely regarded as a sensitive marker of hepatic synthetic capacity. The observed hypoalbuminemia suggests impaired protein synthesis due to hepatocellular injury induced by polyethylene nanoplastics (Kwo et al., 2017). The hypoalbuminemia indicates chronic liver dysfunction rather than merely acute hepatocellular leakage. The increase in globulin at the high dose may reflect activation of immune and inflammatory responses following PE-NP exposure. Elevated globulin concentrations are often associated with increased immunoglobulin production during chronic inflammatory conditions (Tizard, 2018). Bilirubin differs conceptually from ALT and AST because it reflects hepatic uptake, conjugation and excretory handling rather than simply hepatocyte enzyme leakage. Therefore, the combination of elevated bilirubin, ALP and GGT suggests that the hepatotoxic response may include impaired hepatobiliary processing or bile excretion, particularly at higher doses (Giannini et al., 2005). Broader experimental and meta-analytic evidence linking micro/nanoplastics exposure to liver injury (Djouina et al., 2023; Zhang et al., 2024; Zhang et al., 2025). The elevations in ALT, AST, ALP and bilirubin observed in the present study suggest disturbances in hepatocellular integrity and hepatobiliary function following PE-NP exposure. A related experimental approach was reported by Obiekwe et al. (2023), who assessed these markers in carbon tetrachloride-challenged rats and reported hepatoprotective activity of *Olax viridis* root extract. Their findings provide a comparison for the responsiveness of circulating hepatic biomarkers to chemical injury and protective interventions. However, differences in the toxicant, exposure schedule and intervention prevent direct equivalence with the present study. Here, the accompanying hepatic histopathological alterations strengthen the interpretation of the biochemical changes as evidence of liver injury, although they do not establish the underlying molecular mechanism. Renal effects were present but less extensive. Creatinine increased significantly only in groups 4 and 5, while urea remained stable. Such a dissociation can occur when renal stress is early, modest, or biomarker-specific. Because creatinine concentration is inversely related to glomerular filtration, an exposure-associated rise can indicate reduced renal clearance. However, serum creatinine is a functional rather than kidney-injury-specific biomarker and is influenced by production, muscle mass and tubular handling (Delanaye et al., 2017; Kashani et al., 2020). The accompanying increases in potassium and bicarbonate at the upper doses suggest altered renal handling of electrolytes and acid-base balance, though the changes were modest and sodium/chloride remained stable. Accordingly, the present findings support renal functional stress at higher doses. The renal pattern is broadly consistent with recent PE-specific evidence. Kehinde et al. (2025) exposed male Wistar rats to PE microplastics for 28 days and reported dose-dependent increases in creatinine and other renal injury markers, hyperkalemia, oxidative stress, mitochondrial dysfunction, and progressive glomerular/tubular histopathology. They are also consistent with broader experimental evidence demonstrating oxidative-stress- and inflammation-associated renal injury following plastic-particle exposure (Wang et al., 2023; Verma et al., 2026). A broader renal review similarly concludes that micro- and nanoplastics can affect kidney tissue through oxidative stress, inflammatory signaling, mitochondrial disturbance, and structural injury, while emphasizing that exposure characterization and human relevance remain unresolved (Bueno de Oliveira et al., 2024). Experimental exposure to other environmental toxicants has also produced hepatic and renal structural injury in rats; Obaji et al. (2024) reported inflammatory changes, necrosis, fatty degeneration and pyknosis following oral exposure to lead and cadmium. Taken together, the biochemical evidence supports a stronger hepatic than renal response under the present 28-day conditions. The liver showed consistent and large changes across multiple enzymes and bilirubin, whereas the kidney showed upper-dose changes in creatinine and selected electrolytes without urea elevation. This organ-level contrast suggests that hepatic biomarkers may be more sensitive early indicators for PE nanoplastics preparation and dosing paradigm, while renal effects become apparent chiefly at higher doses.

The hepatic and renal histopathological findings suggest that oral polyethylene nanoplastics (PE-NP) exposure was associated with structural alterations that were more evident in the higher exposure groups. However, the organs differed in the apparent severity of injury: hepatic changes remained mild and focal, whereas renal tubular damage was severe in Group 5. The liver sections from the control group and Groups 2 and 3 retained normal lobular architecture, with organized hepatocyte cords, preserved sinusoidal spaces and identifiable central veins (Plates 1–3). In contrast, Groups 4 and 5 exhibited cytoplasmic pallor and vacuolation, focal sinusoidal widening and irregularity, and limited disruption of hepatic cords, with mild central venous congestion additionally observed in Group 4 (Plates 4 and 5). These findings suggest modest hepatocellular and microvascular disturbances. Nevertheless, the absence of extensive necrosis, prominent inflammatory-cell infiltration or widespread architectural disruption indicates that hepatic injury was relatively limited in the examined sections. The descriptions also do not establish greater lesion severity in Group 5 than in Group 4. The kidneys exhibited a more pronounced pattern of injury across the exposure groups. The control group and Group 2 showed intact glomeruli and renal tubules without observable lesions (Plates 6 and 7). Mild tubular epithelial degeneration was evident in Groups 3 and 4, whereas Group 5 exhibited severe tubular degeneration and reported epithelial necrosis (Plates 8–10). The occurrence of renal lesions in Group 3 despite preserved hepatic architecture suggests that tubular alterations were detectable at an exposure level that produced no overt hepatic lesions. However, this difference alone does not establish greater intrinsic renal susceptibility, because lesion detection depends on tissue sampling and the criteria used to assess each organ.

These findings are broadly consistent with experimental evidence of polyethylene-associated hepatic and renal toxicity. Forutan et al. (2026) reported altered liver biochemical markers, increased lipid peroxidation, reduced antioxidant capacity and increased hepatic caspase-3 expression following four weeks of oral polyethylene microplastic exposure in male Wistar rats. Similarly, Kehinde et al. (2025) reported tubular degeneration, glomerular atrophy and interstitial inflammation alongside disturbances in renal redox balance and mitochondrial enzyme activities after 28 days of oral polyethylene microplastic exposure. Both studies provide relevant supporting evidence, although the studies made use of microplastics during exposure. Preserved architecture in the lower exposure groups indicates an absence of overt lesions in the examined sections, rather than an absence of all biological effects. Generally, PE-NP exposure was associated with mild focal hepatic alterations and more pronounced renal tubular injury at the highest exposure level.

Limitations

Although this study provides evidence of hepatic and renal biochemical alterations following repeated oral exposure to a polyethylene nanoplastics (PE-NP)-enriched fraction, limitations have to be acknowledged. First, the

relatively small sample size and exclusive use of male Wistar rats may limit the statistical power and generalizability of the findings, particularly regarding potential sex-dependent responses. Second, the 28-day exposure period permitted the assessment of subacute toxicity but did not establish the consequences of prolonged exposure or the reversibility of the observed biochemical and histopathological alterations. Although serum biochemical analyses and hepatic and renal histopathology provided complementary evidence of organ-associated toxicity, the study did not directly quantify polyethylene particle accumulation in the liver or kidneys. Similarly, the absence of tissue-specific measurements of oxidative stress, inflammatory mediators and molecular injury pathways limited mechanistic interpretation of the observed alterations. Notwithstanding these limitations, the integration of serum biochemical indices with hepatic and renal histopathological observations provides complementary evidence of adverse organ-associated responses following repeated exposure to the PE-NP-enriched preparation.

5. Conclusion

Repeated oral exposure to the PE nanoplastics-enriched suspension for 28 days produced dose-related hepatic and renal alterations in male Wistar rats. The hepatic response was characterized by increased ALT, AST, ALP, GGT, and total bilirubin, reduced albumin, and exposure-associated histological abnormalities, with the most pronounced changes occurring at the higher exposure levels. These findings indicate hepatocellular and hepatobiliary injury. The renal response was comparatively moderate and comprised increased creatinine, potassium, and bicarbonate concentrations, together with tubular epithelial degeneration, whereas urea, sodium, and chloride remained unchanged. The combined biochemical and histological findings suggest that the liver was more responsive than the kidney to the tested preparation.

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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. **Jane C. Onyeulor:** 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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