



Research Article

Hepatotoxicity of Styrene-acrylonitrile Microplastics in Mice: Size-Dependent Effects on Liver Function, Oxidative Stress and Histopathology

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ABSTRACT

Microplastic pollution poses environmental and public health risks, yet the hepatotoxicity of styrene-acrylonitrile (SAN) copolymers remains understudied relative to polystyrene and polyethylene. This study evaluated the hepatotoxicity of SAN microplastics in male mice. SAN-MPs isolated from commercial takeaway food containers were characterized by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX), and fractionated into ≤ 150 , ≤ 300 and ≤ 400 μm particles. Ninety-five male mice (6 weeks old; 36.2 g) were randomized into 19 groups ($n = 5$). Treatment mice received once-daily oral gavage of SAN-MP suspensions (1 mg/5 mL Milli-Q water, ultrasonicated before administration) at progressively escalating doses (0.2–1.0 mL/kg) over 42 days; controls received an equal volume of vehicle. Hepatotoxicity was assessed by serum liver function markers, hepatic oxidative stress indices (MDA, GSH, catalase) and H&E histopathology. Exposure induced significant time-dependent elevations in ALT, AST and total bilirubin, with the ≤ 300 μm fraction producing the most pronounced biochemical perturbations; albumin increased at later time points while total protein remained unchanged. Oxidative stress responses were size-specific: hepatic GSH was significantly elevated in the ≤ 300 μm group at days 28 and 42, whereas catalase activity was suppressed in the ≤ 400 μm and ≤ 150 μm groups at terminal time points. Histopathological injury progressed from periportal inflammation and sinusoidal congestion (day 7) to widespread necrosis and architectural disorganization (day 42), with the ≤ 300 μm and ≤ 400 μm groups exhibiting more severe lesions. These findings demonstrate size-dependent SAN-MP hepatotoxicity and highlight the need for polymer-specific risk assessment of food-contact plastics.

Keywords: Food packaging; Hepatotoxicity; Liver injury; Mice; Oxidative stress; Size-dependent toxicity; Styrene-acrylonitrile microplastics

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INTRODUCTION

Global production of plastics has led to a considerably persistent environmental crisis (Shukla et al., 2024). Discarded plastics fragment into microplastics and nanoplastics under the influence of UV radiation and biological degradation. These plastics have infiltrated terrestrial and atmospheric areas worldwide (Prata, 2018). Consequently, human exposure to these particles is unavoidable. Quantitative estimates suggest that humans may ingest tens of thousands of

microplastic particles annually, and microplastics have since been detected in human blood and placenta, confirming systemic exposure (Cox et al., 2019; Leslie et al., 2022; Ragusa et al., 2021). Exposure occurs primarily through the ingestion of contaminated food and beverages and the inhalation of airborne particles (Kadac-Czapska et al., 2024; Roslan et al., 2024). Of particular concern are food-contact plastics such as polystyrene (PS) and styrene-acrylonitrile (SAN). These copolymers are extensively

employed in disposable takeaway containers and food packaging, and their fragmentation generates microplastic particles that can migrate into food matrices (Du et al., 2020; Smith et al., 2018).

Microplastics can traverse the gastrointestinal barrier once ingested, entering the systemic circulation and distributing to distant organs (Wright et al., 2013). The liver is a primary target organ for microplastic accumulation and toxicity (Beyzaei et al., 2025) and this tropism is underpinned by several anatomical and physiological features. First, particles absorbed across the intestinal epithelium drain into the portal venous system, which delivers blood directly to the liver before systemic distribution. The liver therefore encounters the highest absorbed particle burden first (first-pass effect) (Beyzaei et al., 2025). Second, the hepatic sinusoids are lined by resident macrophages (Kupffer cells), which form the largest macrophage population of the mononuclear phagocyte system and actively phagocytose and sequester circulating particulates, thereby promoting retention of microplastics in hepatic tissue with related studies reporting gut microbiota dysbiosis and hepatic lipid metabolism disorder in PS-exposed mice (Deng et al., 2017; Jin et al., 2019; Lu et al., 2016). Finally, as the principal site of xenobiotic biotransformation, the liver is intrinsically susceptible to oxidative injury because particle-induced overproduction of reactive oxygen species can overwhelm endogenous antioxidant defenses (Chen et al., 2024; Kadaczapska et al., 2024). Consistent with this tropism, ingested microplastics accumulate in murine liver tissue within days of oral exposure (Lu et al., 2016), microplastics have been histologically demonstrated in human liver tissue (Horvatits et al., 2022), and a recent scoping review confirmed the presence of microplastics in multiple human organs, including the liver (Roslan et al., 2024).

A growing body of in vivo evidence demonstrates that microplastic exposure induces hepatic injury across polymer types and animal models. In mice, oral administration of 5 μm and 20 μm polystyrene microplastics resulted in hepatic accumulation accompanied by altered inflammatory marker expression and disturbances in lipid metabolism (Deng et al., 2017). Subsequent mechanistic studies identified pyroptosis and ferroptosis as downstream pathways of PS-induced hepatotoxicity (Mu et al., 2022) and demonstrated that PS microplastics promote liver inflammation through the formation of macrophage extracellular traps (Yin et al., 2023). Oral PS exposure in mice further triggers significant hepatic oxidative injury, manifested by increased lipid

peroxidation and perturbations in antioxidant enzyme kinetics (Alexandrova et al., 2026). Comparable responses have been reported in non-rodent models: exposure to PET microplastics modified hepatic transcriptomic profiles and oxidative stress markers in immature piglets, confirming the cross-species conservation of this toxicological response (Golubska et al., 2025). These experimental findings are corroborated by a recent meta-analysis showing that micro- and nanoplastic exposure consistently elevates hepatic malondialdehyde (MDA) levels, depletes reduced glutathione (GSH) and suppresses catalase (CAT) activity across rodent models, confirming oxidative stress as a reproducible endpoint of microplastic hepatotoxicity (Chen et al., 2024). Reviews of mammalian exposure studies consistently identify intestinal, immune and hepatic endpoints as principal targets of micro- and nanoplastic toxicity (Hirt & Body-Malapel, 2020; Prata et al., 2020; Yong et al., 2020).

The toxicological effect of microplastics is also modulated by particle size, which governs bioavailability and the magnitude of oxidative stress (Beyzaei et al., 2025). Smaller particles possess a higher surface-area-to-volume ratio, facilitating enhanced interaction with cellular membranes and more severe antioxidant depletion (Schirizzi et al., 2017). Comparative toxicokinetic studies show that 100 nm nanoplastics distribute rapidly across multiple organs whereas larger 3–10 μm microplastics show limited biodistribution (Beyzaei et al., 2025). However, larger micro-sized particles can induce significant physical injury to the intestinal epithelium, indirectly compromising liver function through the gut–liver axis (Liu et al., 2025). In hepatocytes, size-dependent differences in oxidative stress induction have been documented, as smaller PS microplastics elicit more pronounced lipid peroxidation and GSH depletion than larger counterparts (Zou et al., 2023). Several critical gaps thus persist. First, the majority of in vivo hepatotoxicity studies have employed polystyrene or polyethylene microplastics, whereas SAN remains understudied. Second, while acute and subchronic exposures have been characterized extensively, the dynamics of SAN microplastic toxicity over a multi-week exposure window with progressive dose escalation remain unexplored. This study therefore examined the hepatotoxic impacts of styrene-acrylonitrile microplastics (SAN-MPs) in a murine model with progressive oral exposure over 42 days.

MATERIALS AND METHODS

Study Area

This study was undertaken at the laboratory of National veterinary research institute (NVRI), located in Vom, Plateau State, Nigeria. The animal handling and laboratory procedures were performed in compliance with standards established by the Institutional Animal Care and Use Committee guidelines and the guidelines for Animal Research reporting of In Vivo Experiments (ARRIVE). Experimental protocols were granted by the University Ethical Review Board.

Experimental Animals

Adult healthy male mice (*Mus musculus*), aged 6 weeks that weigh averagely 36.2kg were acquired from the animal house. A total of 95 mice were divided randomly into 19 groups and caged 5 per group (n=5). The mice were allowed to acclimatize for 7 days prior to the experiment under standard laboratory conditions, including a light/dark cycle of 12 h, a temperature of 22 ± 2 °C and humidity of 40–60%. They were provided with standard rodent pellets and water *ad libitum*. One of the cages containing 5 mice served as the control where mice were treated with water free of microplastics. Six cages were treated with ≤ 150 μ m SAN-MPs; six cages received treatment with ≤ 300 μ m SAN-MPs and six others were given ≤ 400 μ m SAN-MPs treatment.

Microplastics Collection and Preparation

Styrene-acrylonitrile microplastics (SAN-MPs) samples were obtained from plastic food packages popularly known as takeaway package that were sourced from the Sabon Gari market in Kano, Nigeria (12° 00' 52.12" N, 8° 32' 23.21" E). The plastics were crushed into smaller pieces using a Shuliy crushing machine (SLSP-600, Made in China) at Sharada.

The sample plastics were further grinded using blender to obtain desired particle-size fractions. The crushed materials were sieved sequentially using stainless-steel laboratory sieves of appropriate mesh sizes.

Characterization of SAN-NMPs

Scanning Electron Microscopy (SEM) was carried out to assess morphology, size of particle and surface features of the prepared styrene-acrylonitrile microplastics (SAN-MPs). The SAN-MPs were first dried and put on an aluminum called SEM stub by using carbon adhesive tape. In order to prevent charging during imaging and then the samples subjected to a thin carbon-coating treatment using a quorum SC7620 sputter coater. Imaging was then taken using Scanning Electron Microscope (JEOL Ltd.,

Tokyo, Japan) operating at 15kV. Multiple magnifications were used to assess particle shape, homogeneity and sizes. Similar observations were carried out by Deng et al. (2017).

Particle sized selection and administration

Three SAN-MP particle-size fractions were selected for the exposure experiment: ≤ 150 μ m, ≤ 300 μ m and ≤ 400 μ m. The particle fractions were suspended in sterile, distilled water and sonicated before administration. The SAN-MP stock suspension was prepared at an initial concentration of 0.2 mg/mL in sterile Milli-Q water. 0.5 mL of freshly prepared SAN-MP suspension was administered to each mouse once daily by oral gavage. The suspension concentration was progressively increased by 0.2 mg/mL each week over the 6-week exposure period, giving concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL during Weeks 1–6, respectively. At the fixed administration volume of 0.5 mL/mouse/day, these concentrations corresponded to 0.10, 0.20, 0.30, 0.40, 0.50, and 0.60 mg/mouse/day, respectively.

Collection of Sample

Samples of blood were obtained via retro-orbital venous sampling during the exposure period. Prior to the collection, the animals were subjected to overnight fasting and anesthetized with chloroform. The blood was collected into ethylenediaminetetraacetic (EDTA) tubes and gently inverted to prevent clotting.

The mice were humanely euthanized by exposure to isoflurane vapor in a beaker containing cotton soaked with the anesthetic following blood collection. The liver was carefully excised and immediately stored in phosphate-buffered saline (PBS) to preserve physiological pH and osmolarity then fixed in 10% formalin to carry out histopathological examination and stored at -80 °C for oxidative stress assays.

Biochemical Analysis

Serum biochemical parameters were analyzed using an automated chemistry analyzer at 37° C. Liver function markers include the total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin.

Oxidative Stress Analysis

Homogenates of the liver tissues were homogenized in ice-cold phosphate buffer (pH 7.4) to maintain stability. The homogenates were then centrifuged using a refrigerated Eppendorf 5810R centrifuge at -4 °C and 10000 rpm for 15 minutes. The supernatant was obtained and used for oxidative stress analysis. The analyzed oxidative stress markers include: Malondialdehyde (MDA) for lipid peroxidation,

Catalase (CAT) for antioxidant enzyme activities and reduced glutathione (GSH) levels.

Histopathological Examination

Formalin-fixed tissues were processed by standard paraffin, sectioned at 5 μ m thickness and with Hematoxylin and Eosin staining. The histology of liver sections were examined under a light microscope for lesions such as necrosis, inflammation, congestion, fibrosis and structural damage. Photomicrographs were taken for record purposes.

Data Analysis

All experimental data were analyzed statistically on the GraphPad Prism (v9). Data were subjected to normality test using Shapiro-Wilk's normality test and presented as mean $\pm$ SD. One-way ANOVA was used to compare between test groups across the different time points (days). Differences were considered statistically significant at $p < 0.01$.

RESULTS

Elemental Composition of styrene-acrylonitrile microplastics (SAN-MPs)

Spectroscopic energy-dispersive x-ray showed polymeric identity of the isolated microplastics. The

elemental profile was dominated by carbon (89.26 at%, 84.27 wt%) and nitrogen (7.37 at%, 8.11 wt%) which is consistent with the styrene-acrylonitrile copolymer structure. Aluminum was detected as a minor contaminant (2.66 at%, 5.64 wt%) that likely reflects inorganic filler or processing residue. Trace quantities (<0.5 at%) of calcium, silicon, iron, sulfur, magnesium, sodium and chlorine were also present. Elements such as Phosphorus, potassium, titanium and manganese were below the detection limit (Table 1).

Characterization of SAN-MPs

Scanning electron microscopy revealed that SAN-MPs were irregular, flaky and fractured fragments with rough, torn surface textures (Figure 1). Particles were polydisperse with dimensions ranging from tens to hundreds of micrometres. Bright, high-contrast inclusions observed within the polymer matrix (Figure 1A–C) corresponded to the aluminum signal detected by EDS. No crystalline or spherulitic morphologies were observed in agreement with the amorphous nature of styrene-acrylonitrile copolymers.

Table 1: Elemental Profiles of Microplastics

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	89.26	84.27
7	N	Nitrogen	7.37	8.11
13	Al	Aluminum	2.66	5.64
20	Ca	Calcium	0.29	0.9
14	Si	Silicon	0.26	0.58
26	Fe	Iron	0.05	0.24
16	S	Sulfur	0.06	0.15
12	Mg	Magnesium	0.04	0.07
11	Na	Sodium	0.01	0.03
17	Cl	Chlorine	0.01	0.02
15	P	Phosphorus	0	0
19	K	Potassium	0	0
22	Ti	Titanium	0	0
25	Mn	Manganese	0	0

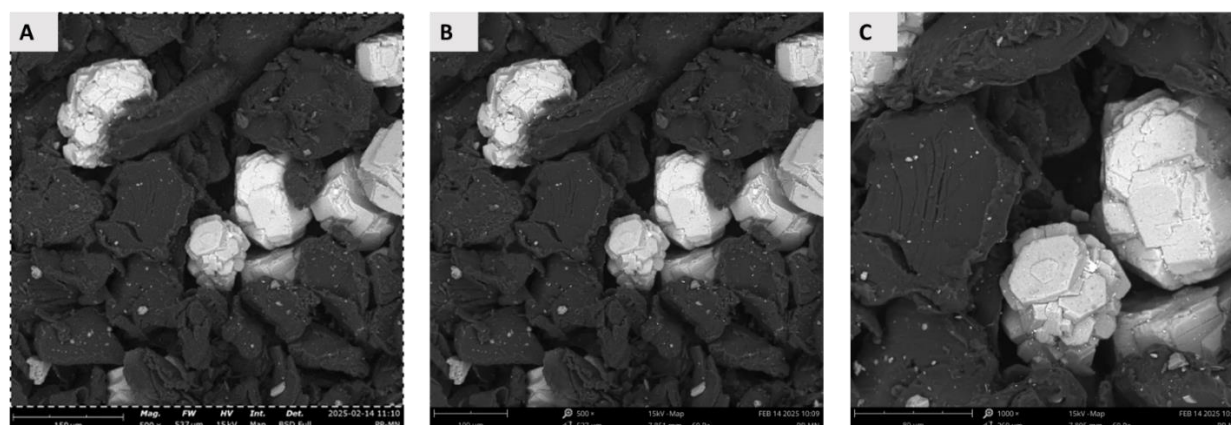


Figure 1: Scanning Electron Microscopy images showing the morphology of styrene-acrylonitrile particles
(A) 2000× magnification, scale bar = 150 µm. **(B)** 1000× magnification, scale bar = 100 µm. **(C)** 500× magnification, scale bar = 80 µm

Effect of microplastics on Liver Parameters in mice

One-way ANOVA revealed particle-size- and time-dependent alterations in liver function markers (Figure 2).

Total protein.

No statistically significant differences were detected among the treatment groups at any time points ($p > 0.01$). Mean values across all groups ranged from 67.10 ± 4.35 to 77.10 ± 0.46 [g/L] (Figure 2A).

Albumin.

Significant inter-group differences emerged on Days 7, 28 and 42. On Day 7, the ≤ 300 µm group (40.00 ± 0.72 [g/L]) was significantly higher than the ≤ 150 µm group (35.80 ± 0.95 [g/L]; $p = 0.0039$). By Day 28, the ≤ 150 µm group (42.90 ± 0.62 [g/L]) exceeded the control (37.80 ± 0.61 [g/L]; $p = 0.0092$). At Day 42, both the ≤ 300 µm (46.10 ± 1.01 [g/L]) and ≤ 400 µm (45.80 ± 0.10 [g/L]) groups were elevated significantly compared with the control (38.70 ± 0.36 [g/L]; $p = 0.0077$ and $p = 0.0028$, respectively) (Figure 2B).

Total bilirubin.

Microplastic-exposed groups exhibited progressive bilirubin elevation. Significant differences were detected on Days 21, 28 and 42. On Day 21, the ≤ 150 µm group (7.90 ± 0.26 [µmol/L]) exceeded the control (6.70 ± 0.20 [µmol/L]; $p = 0.0059$). On Day 28, the

≤ 300 µm group (8.80 ± 0.00 [µmol/L]) was higher than the control (6.80 ± 0.10 [µmol/L]; $p = 0.0008$). By Day 42, the ≤ 300 µm group (9.30 ± 0.10 [µmol/L]) remained significantly elevated versus control (6.80 ± 0.10 [µmol/L]; $p = 0.0001$) (Figure 2C).

Alanine aminotransferase (ALT).

Hepatocellular injury markers increased over time in exposed groups. Significant elevations were observed on Days 14, 21 and 42. On Day 14, the ≤ 150 µm group (31.60 ± 0.35 [U/L]) exceeded the control (28.80 ± 0.17 [U/L]; $p = 0.0023$). On Day 21, both the ≤ 300 µm (36.90 ± 0.44 [U/L]) and ≤ 400 µm (35.00 ± 0.60 [U/L]) groups were significantly higher than the control (29.00 ± 0.10 [U/L]; $p = 0.0003$ and $p = 0.0074$, respectively). At Day 42, the ≤ 400 µm group (42.10 ± 0.53 [U/L]) remained significantly elevated versus control (32.40 ± 1.40 [U/L]; $p = 0.0077$) (Figure 2D).

Aspartate aminotransferase (AST).

Significant increases were noted on Days 7, 21 and 42. On Day 7, the ≤ 400 µm group (56.9 ± 1.54 [U/L]) exceeded the control (49.0 ± 1.42 [U/L]; $p < 0.0001$). On Day 21, the ≤ 400 µm group (58.0 ± 0.82 [U/L]) was again higher than the control (51.2 ± 1.47 [U/L]; $p = 0.0096$). By Day 42, the ≤ 300 µm group (62.8 ± 1.35 [U/L]) was significantly elevated compared with the control (53.3 ± 1.97 [U/L]; $p = 0.0043$) (Figure 2E).

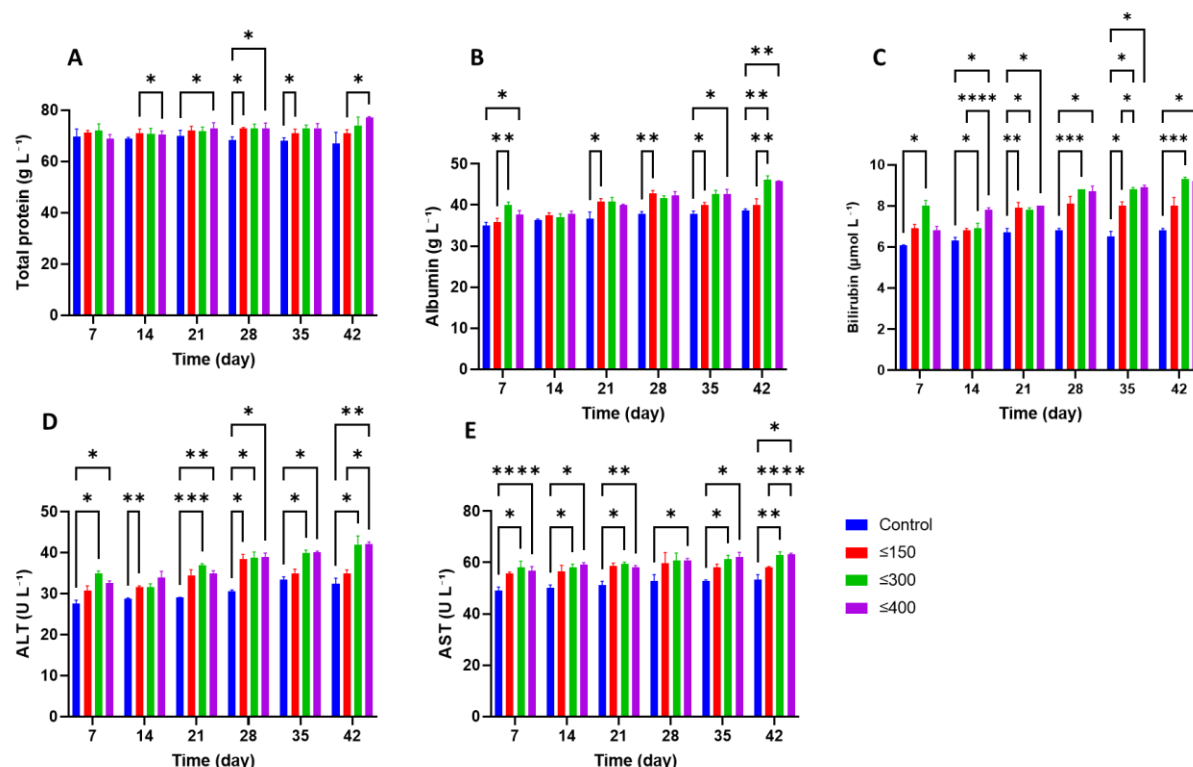


Figure 2: Assessment of (A) Total Protein (B) Albumin (C) Bilirubin (D) ALT (E) AST in liver of microplastic-exposed mice

Compared to control and treatment groups; Statistics data are calculated as mean $\pm$ SD; Mixed repeated measures ANOVA was used to assess differences groups; ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

Hepatic Oxidative Stress

Table 2 shows that MDA levels varied across the experimental period. At Day 7, MDA ranged from 0.85 ± 0.06 in the $\leq 300 \mu\text{m}$ group to 1.56 ± 0.06 in the $\leq 150 \mu\text{m}$ group, with no significant differences at $p < 0.010$. Similarly, no significant differences were observed among groups at Days 14, 21 and 28 ($p > 0.010$). At Day 35, the $\leq 300 \mu\text{m}$ group recorded a significantly lower MDA level than the $\leq 150 \mu\text{m}$ group (0.90 ± 0.02 vs. 1.41 ± 0.06 ; $p = 0.0092$). At Day 42, the $\leq 300 \mu\text{m}$ group also had a significantly lower MDA level than the $\leq 400 \mu\text{m}$ group (0.84 ± 0.12 vs. 1.45 ± 0.14 ; $p = 0.0090$). No other pairwise differences reached the predefined significance threshold of $p < 0.010$.

Table 3 showed the effect of microplastic exposure on hepatic GSH concentration in mice. On Day 7, GSH concentrations ranged from 3.75 ± 0.279 to 5.00 ± 0.070 , with no significant difference among groups ($P > 0.01$). No differences were observed significantly on Days 14 and 21, with concentrations ranging from 4.23 ± 0.780 to 5.35 ± 0.360 and 4.45 ± 0.433 to 5.25

± 0.282 , respectively. On Day 28, GSH concentration ranged from 4.05 ± 0.351 to 6.00 ± 0.387 , with a significant difference observed between the 300 and 400 μm groups ($P < 0.0001$). No other pairwise comparison was significant at $P < 0.01$. On Day 35, concentrations also ranged from 3.25 ± 0.082 to 5.25 ± 0.269 , with no significant difference among groups ($P > 0.01$). On Day 42, GSH concentration ranged from 3.85 ± 0.160 to 5.25 ± 0.282 , with a significant difference between the 300 and 400 μm groups ($P = 0.0068$), while all other comparisons were non-significant ($P > 0.01$).

Table 4 revealed catalase activity in the liver showed no significant differences among the treatment groups from Days 7 to 28 ($p > 0.01$). At Day 35, CAT activity was significantly elevated in the 400 group than in the Control and 150 groups ($p < 0.01$). At Day 42, the $\leq 150 \mu\text{m}$ group showed significantly lower CAT activity than the Control group ($p < 0.01$). Other pairwise comparisons were not significant at the predefined threshold of $p < 0.01$.

Table 2. Assessment of Malondialdehyde (MDA) in liver of microplastic-exposed mice

Time (days)	Control	≤150 µm	≤300 µm	≤400 µm
7	1.11 ± 0.02 ^a	1.56 ± 0.06 ^a	0.85 ± 0.06 ^a	1.31 ± 0.10 ^a
14	0.92 ± 0.07 ^a	1.62 ± 0.24 ^a	1.33 ± 0.03 ^a	1.41 ± 0.12 ^a
21	1.18 ± 0.06 ^a	1.28 ± 0.13 ^a	1.43 ± 0.24 ^a	1.19 ± 0.08 ^a
28	0.87 ± 0.07 ^a	1.16 ± 0.01 ^a	0.76 ± 0.07 ^a	1.23 ± 0.06 ^a
35	0.98 ± 0.09 ^{ab}	1.41 ± 0.06 ^a	0.90 ± 0.02 ^b	1.10 ± 0.08 ^{ab}
42	0.93 ± 0.05 ^{ab}	1.05 ± 0.05 ^{ab}	0.84 ± 0.12 ^b	1.45 ± 0.14 ^a

Values are presented as mean ± SD. Differences were considered statistically significant at $p < 0.010$ and means with different superscript letters within the same row differ significantly.

Table 3. Assessment of reduced glutathione (GSH) in liver of microplastic-exposed mice

Time (Day)	Control	≤150 µm	≤300 µm	≤400 µm
7	4.65 ± 0.079 ^a	4.35 ± 0.217 ^a	5.00 ± 0.070 ^a	3.75 ± 0.279 ^a
14	4.23 ± 0.780 ^a	5.35 ± 0.360 ^a	4.50 ± 0.219 ^a	4.50 ± 0.211 ^a
21	4.45 ± 0.433 ^a	5.15 ± 0.046 ^a	5.25 ± 0.282 ^a	4.75 ± 0.185 ^a
28	4.85 ± 0.508 ^{ab}	5.15 ± 0.409 ^{ab}	6.00 ± 0.387 ^b	4.05 ± 0.351 ^a
35	5.25 ± 0.269 ^a	4.45 ± 0.755 ^a	3.75 ± 0.226 ^a	3.25 ± 0.082 ^a
42	4.65 ± 0.243 ^{ab}	4.65 ± 0.214 ^{ab}	5.25 ± 0.282 ^a	3.85 ± 0.160 ^a

Values are presented as mean ± SD. Differences were considered statistically significant at $p < 0.010$ and means with different superscript letters within the same row differ significantly.

Table 4. Assessment of Catalase (CAT) in the liver of microplastic-exposed mice

Time (Day)	Control	≤150 µm	≤300 µm	≤400 µm
7	6.07 ± 0.21 ^a	7.02 ± 0.43 ^a	5.04 ± 0.04 ^a	6.67 ± 0.98 ^a
14	6.17 ± 0.15 ^a	6.07 ± 0.33 ^a	4.64 ± 0.36 ^a	5.43 ± 0.34 ^a
21	6.26 ± 0.26 ^a	6.61 ± 0.66 ^a	6.32 ± 0.76 ^a	5.46 ± 0.38 ^a
28	6.23 ± 0.58 ^a	5.54 ± 0.25 ^a	6.10 ± 0.18 ^a	6.01 ± 0.50 ^a
35	5.97 ± 0.25 ^a	6.28 ± 0.42 ^a	8.52 ± 0.14 ^a	3.32 ± 0.25 ^b
42	6.81 ± 0.58 ^a	5.49 ± 0.50 ^b	4.86 ± 0.08 ^{ab}	5.76 ± 0.19 ^{ab}

Values are presented as mean ± SD. Differences were considered statistically significant at $p < 0.010$ and means with different superscript letters within the same row differ significantly.

Histopathological Examination

Light microscopy of H&E-stained liver sections revealed that the control animals have normal hepatic architecture throughout the 42-day study period which is characterized by hepatocytes with distinct nuclei, hepatic sinusoids and uncongested central veins (Plate 1A–6A).

In SAN-MP-exposed mice, liver pathology showed progression in severity with exposure duration. By Day 7, the ≤400 µm group exhibited inflammatory infiltrates, sinusoidal congestion and early parenchymal hemorrhage (Plate 1D), whereas the ≤300 µm group showed mild inflammation with sinusoidal narrowing (Figure 1C). The ≤150 µm group displayed preserved architecture with only slight central vein congestion (Figure 1B).

By Day 14, exposed groups demonstrated patchy necrosis which is characterized by karyolytic nuclei and cytoplasmic eosinophilia. This is accompanied by sinusoidal compression and central vein congestion

(Plate 2B–D). Inflammatory cell infiltration was evident in the ≤300 µm group (Plate 2C).

At Day 21, more pronounced degenerative changes were visible. The ≤150 µm and ≤300 µm groups showed extensive inflammatory infiltrates, widespread necrosis with pyknotic nuclei and sinusoidal compression which indicates hepatocellular enlargement (Plate 3B, 3C). The ≤400 µm group exhibited similar inflammatory changes with hypertrophied hepatocytes and narrowed sinusoids (Plate 3D).

By Day 28, all exposed groups displayed progressive injury. The ≤150 µm group showed parenchymal hemorrhage and nuclear pyknosis (Plate 4B). The ≤300 µm group presented with inflammation and loss of normal lobular architecture (Plate 4C). The ≤400 µm group demonstrated pyknotic nuclei and pronounced sinusoidal compression (Figure 4D).

At Day 35, necrotic changes became prominent. The ≤150 µm group exhibited focal necrosis with karyolytic nuclei and central vein congestion (Plate

5B). The $\leq 300 \mu\text{m}$ group showed intense inflammatory infiltration and extensive necrosis (Plate 5C). The $\leq 400 \mu\text{m}$ group displayed massive inflammatory cell infiltrates and hepatocellular hypertrophy (Plate 5D).

By Day 42, the $\leq 150 \mu\text{m}$ group showed persistent central vein congestion and nuclear pyknosis (Plate 6B). The $\leq 300 \mu\text{m}$ and $\leq 400 \mu\text{m}$ groups both exhibited severe inflammation and diffuse sinusoidal compression (Plate 6C, 6D). Pyknotic nuclei were also

consistently observed across all particle-size groups at this terminal time point. Collectively, SAN-MP exposure induced a time-dependent spectrum of hepatic injury ranging from early inflammation and congestion to advanced necrosis and architectural disorganization. Larger particle sizes ($\leq 300 \mu\text{m}$ and $\leq 400 \mu\text{m}$) generally elicit more severe histopathological changes at intermediate and late time points.

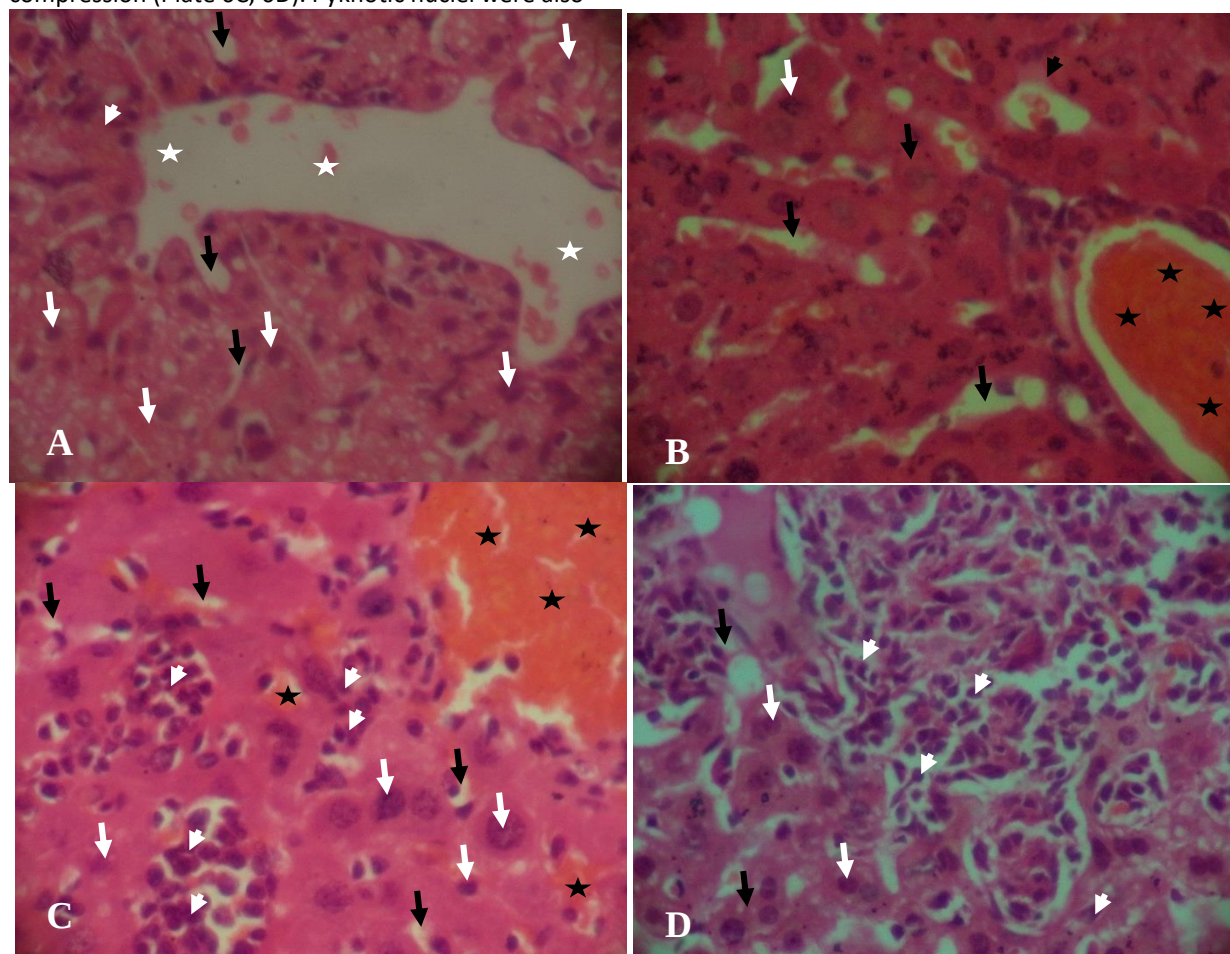


Plate 1: Liver of Albino mice on Day 7.

A: Liver of mouse exposed to normal atmospheric and nutritional conditions (Control), showing normal morphology as seen by the hepatocytes presenting with their polygonal shape and hepatic sinusoids (black arrows) linked to the central vein (white stars). White arrows= nuclei

B: Liver of mouse exposed to $\leq 150 \mu\text{m}$ of plastic, showing normal morphology as seen by the hepatocytes presenting with their polygonal shape and hepatic sinusoids (black arrows) linked to the central vein which appear congested (black stars). White arrows= nuclei. **C:** Liver of mouse exposed to $\leq 300 \mu\text{m}$ of plastic showing inflammation as seen by the presence of inflammatory cells (white arrowheads) within the tissue and parenchymal hemorrhage/ congestion as seen by the presence of red blood cells (black stars) within the tissue. Interspersed within the inflamed tissue are normal cells with normal nuclei (white arrows). The liver is slightly hypertrophied as seen by the reduction in sinusoidal spaces (black arrows). **D:** Liver of mouse exposed to $\leq 400 \mu\text{m}$ of plastic showing massive inflammation (white arrowheads) and generalized tissue degeneration with few cells presenting with normal nuclei (white arrows). **H&E X400.**

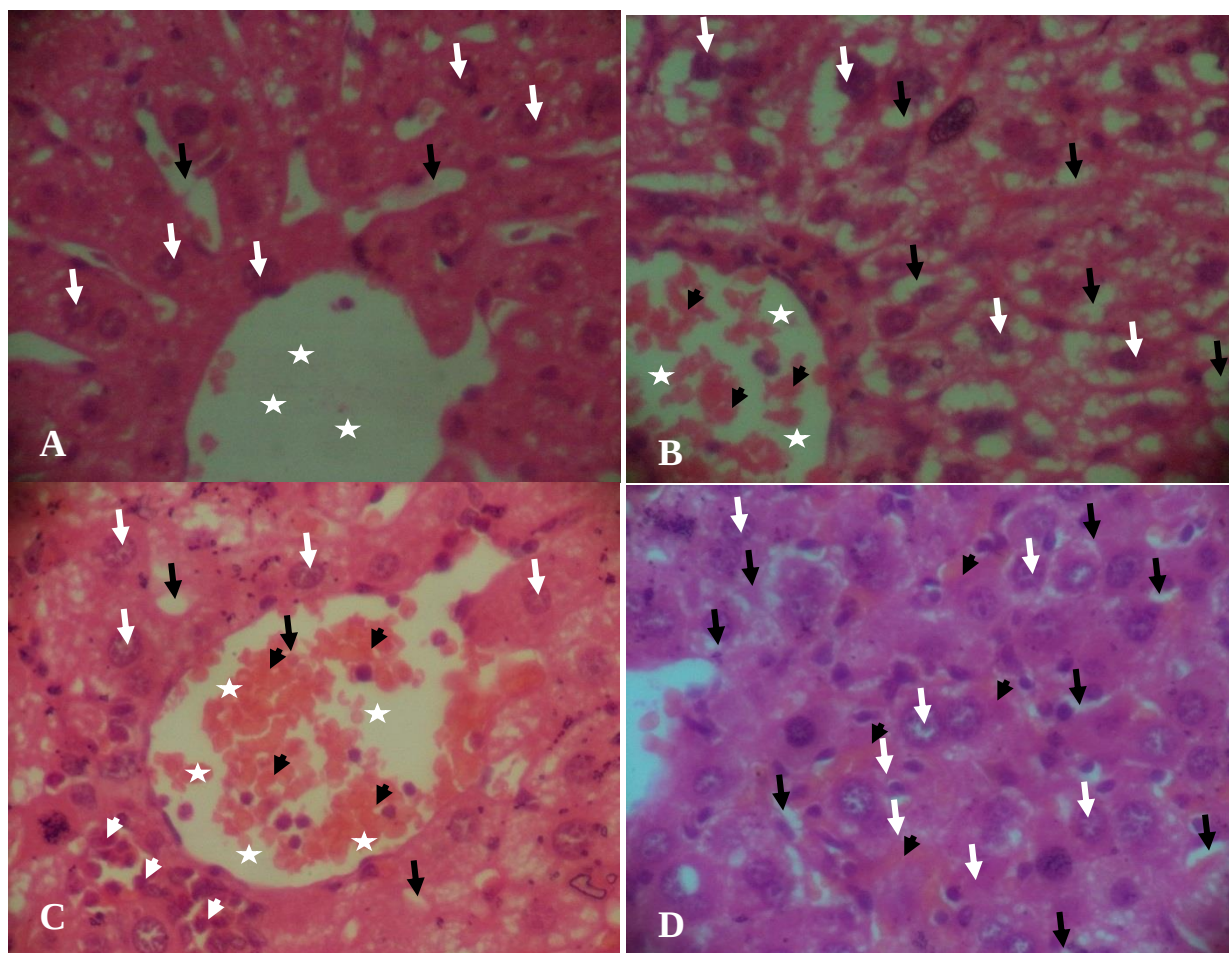


Plate 2: Liver of Albino mouse on Day 14

A: Liver of mouse exposed to normal atmospheric and nutritional conditions, showing normal morphology as seen by the hepatocytes with polygonal shape and interspersed by hepatic sinusoids (black arrows) linked to the central vein (white stars).

White arrows= nuclei **B:** Liver of mouse exposed to $\leq 150 \mu\text{m}$ of plastic, showing mild morphological distortion and necrosis as seen by the presence of karyolytic nuclei (white arrows) and reduced sinusoidal spaces (black arrows).

The central vein (white stars) is slightly congested with red blood cells (black arrowheads). Black arrowheads= necrotic areas with complete loss of nuclei.

C: Liver of mouse exposed to $\leq 300 \mu\text{m}$ of plastic showing mild inflammation (white arrowheads). There is hepatic hypertrophy as seen by the reduction of the sinusoidal spaces (black arrows).

The central vein (white stars) is congested with red blood cells (black arrowheads). **D:** Liver of mouse exposed to $\leq 400 \mu\text{m}$ of plastic showing mild congestion (black arrowheads) and hypertrophy as seen by the reduction in the sinusoidal spaces (black arrows).

The cell nuclei (white arrows) are intact. **H&E X400.**

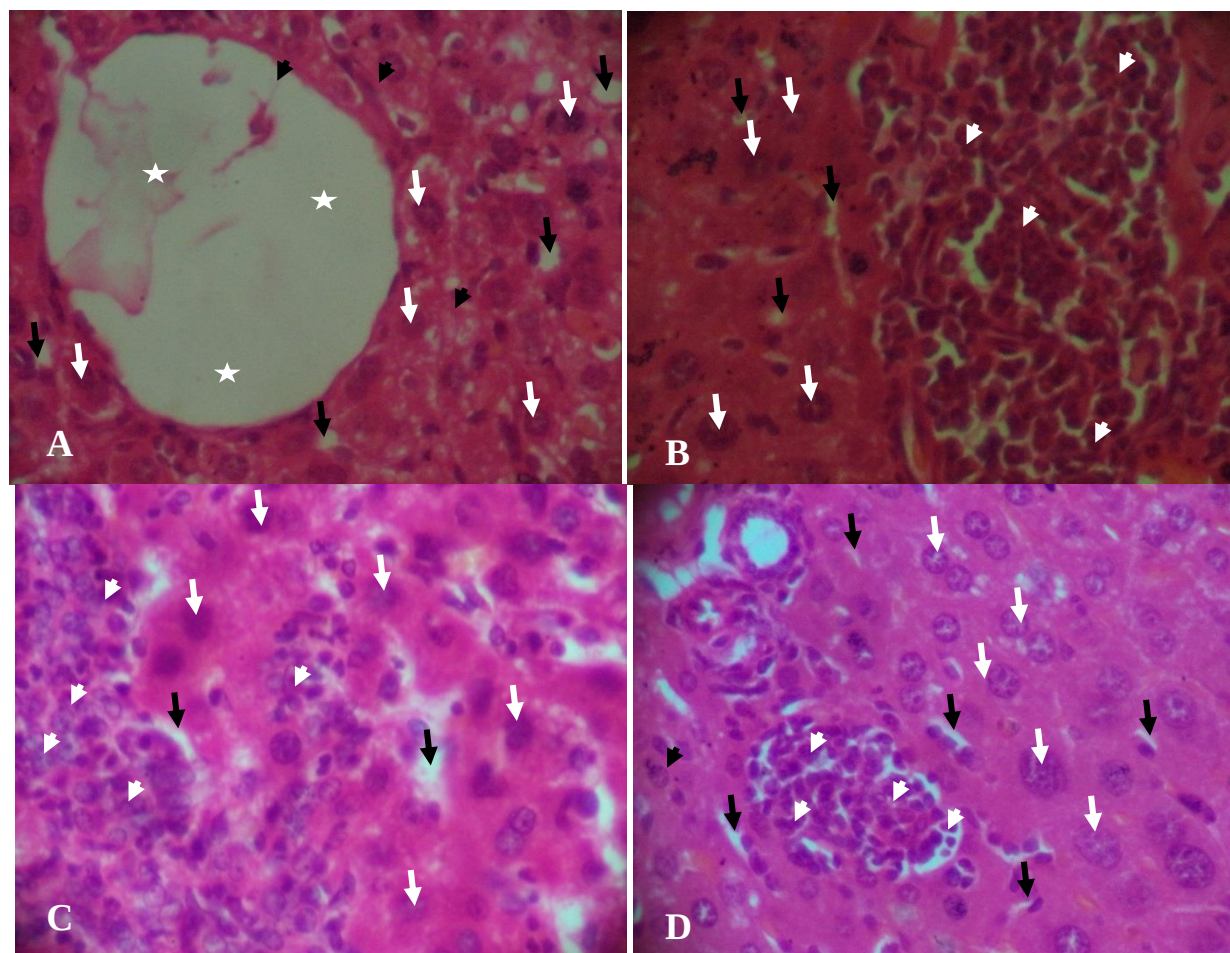


Plate 3: Liver of Albino mouse on Day 21

A: Liver of mouse exposed to normal atmospheric and nutritional conditions, showing normal hepatic architecture, characterized by polygonal-shaped hepatocytes interspersed with hepatic sinusoids (black arrows) that converge toward the central vein (white stars).

White arrows= nuclei. **B:** Liver of mouse exposed to $\leq 150 \mu\text{m}$ of plastic showing massive inflammation (white arrowheads) and generalized tissue degeneration with few cells with pyknotic nuclei (white arrows). There is hypertrophy as seen by the reduction in sinusoidal spaces. **C:** Liver of mouse exposed to $\leq 300 \mu\text{m}$ of plastic showing massive inflammation (white arrowheads) and generalized tissue degeneration with few cells presenting with pyknotic nuclei (white arrows). There is hypertrophy as seen by the reduction in sinusoidal spaces (black arrows). **D:** Liver of mouse exposed to $\leq 400 \mu\text{m}$ of plastic showing inflammation (white arrowheads). The hepatocytes are hypertrophied as seen by the reduction in sinusoidal spaces (black arrows) while the nuclei (white arrows) appear normal **H&E X400**.

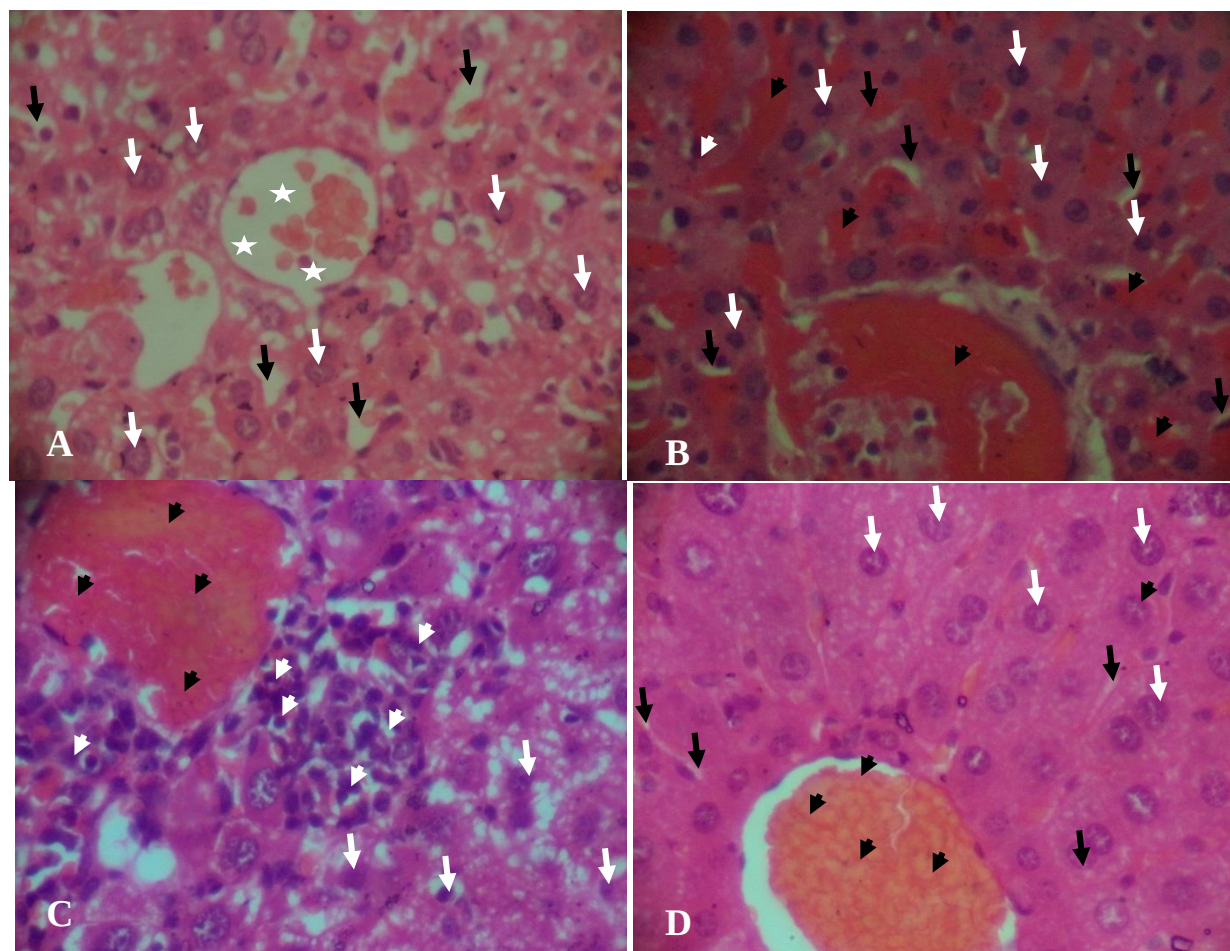


Plate 4: Liver of Albino mouse on Day 28

A: Liver of mouse exposed to normal atmospheric and nutritional conditions, showing normal hepatic architecture, characterized by polygonal-shaped hepatocytes interspersed with hepatic sinusoids (black arrows) that converge toward the central vein (white stars).

White arrows= nuclei **B:** Liver of mouse exposed to $\leq 150 \mu\text{m}$ of plastic for 28 days showing parenchymal hemorrhage (black arrowheads) and congestion as seen by the reduced sinusoidal spaces (black arrows). The cell nuclei (white arrows) are slightly pyknotic. **C:** Liver of mouse exposed to $\leq 300 \mu\text{m}$ of plastic, showing inflammation (white arrowheads) and congestion (black arrowheads). **D:** Liver of mouse exposed to $\leq 400 \mu\text{m}$ of plastic, showing pyknotic nuclei (white arrows) and hypertrophy as seen by the reduction in sinusoidal spaces (black arrows). The central vein is congested (black arrowheads) **H&E X400.**

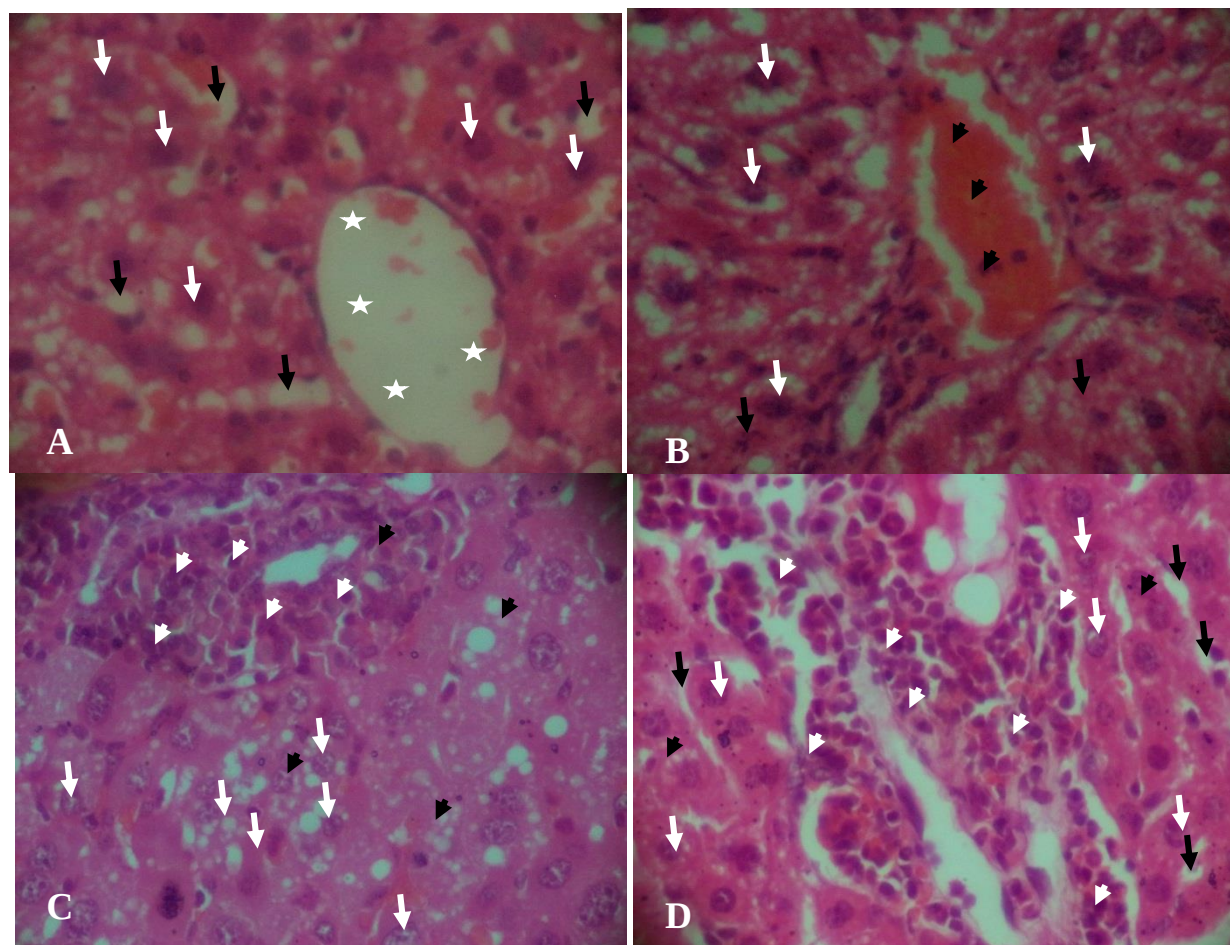


Plate 5: Liver of Albino mice on Day 35

A: Liver of mouse exposed to normal atmospheric and nutritional conditions, showing normal hepatic architecture, characterized by polygonal-shaped hepatocytes interspersed with hepatic sinusoids (black arrows) that converge toward the central vein (white stars). White arrows= nuclei

B: Liver of mouse exposed to $\leq 150 \mu\text{m}$ of plastic showing necrosis as seen by the presentation of karyolytic nuclei (white arrows) and generalized tissue distortion. There is congestion (black arrowheads) of the central vein. **C:** Liver of mouse exposed to $\leq 300 \mu\text{m}$ of plastic showing inflammatory as seen by the presence of inflammatory cells (white arrowheads), with tissue degeneration (necrosis) as seen by the loss of cellular components (black arrowheads) and the appearance of naked and pyknotic nuclei (white arrows). **D:** Liver of mouse exposed to $\leq 400 \mu\text{m}$ of plastic showing massive inflammation as seen by the shown inflammatory cells (white arrowheads) within the tissue. The nuclei (white arrows) are pyknotic H&E X400.

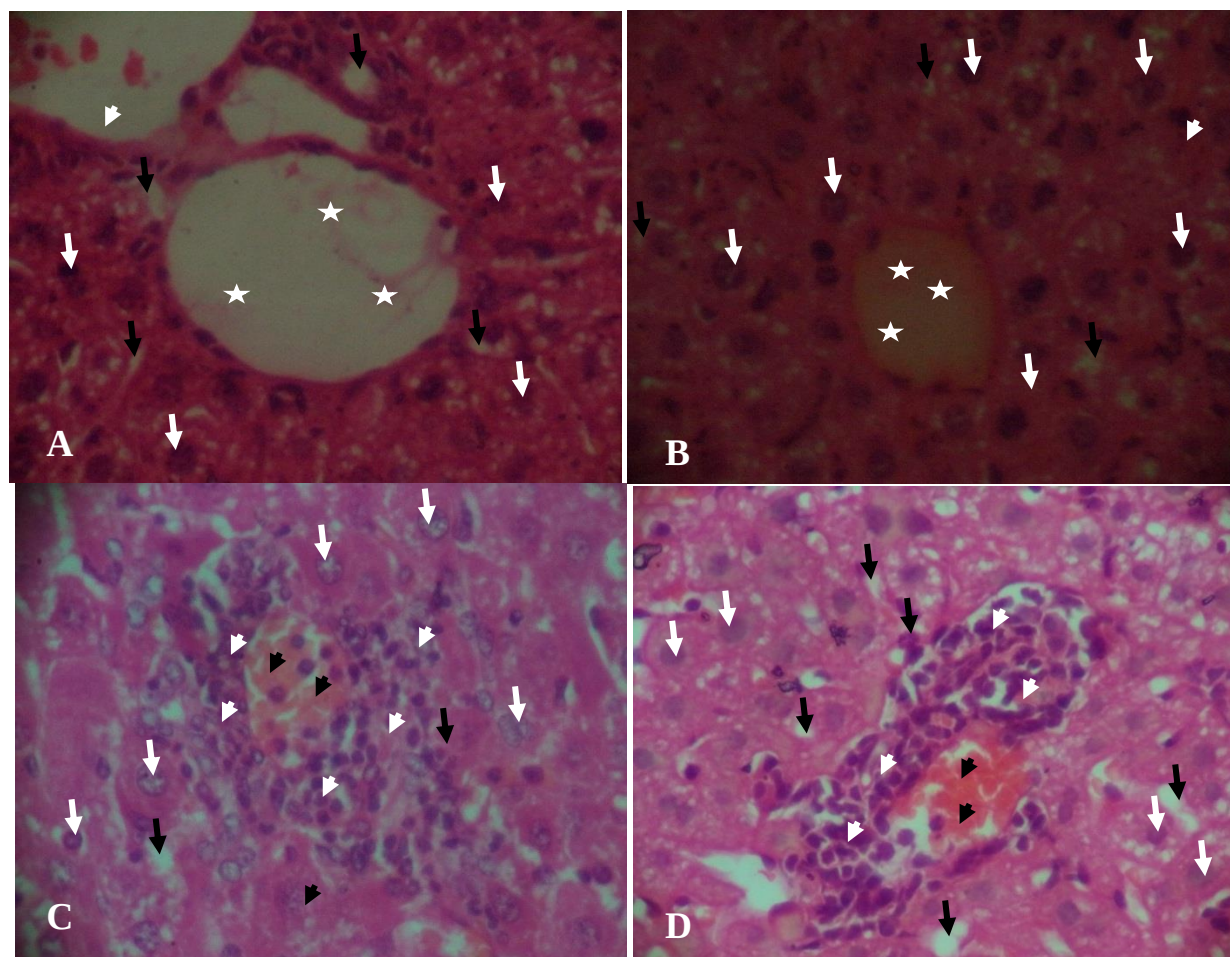


Plate 6: Liver of Albino mice on Day 42

A: Liver of mouse exposed to normal atmospheric and nutritional conditions, showing normal hepatic architecture, characterized by polygonal-shaped hepatocytes interspersed with hepatic sinusoids (black arrows) that converge toward the central vein (white stars).

White arrows= nuclei **B:** Liver of mouse exposed to $\leq 150 \mu\text{m}$ of plastic showing mild congestion of the central vein (white arrows), with hypertrophy as seen by the reduced sinusoidal spaces (black arrows). Nuclei (white arrows) are pyknotic. **C:** Liver of mouse exposed to $\leq 300 \mu\text{m}$ of plastic showing massive inflammation as seen by the presence of inflammatory cells (white arrowheads). There is mild reduction of hepatic sinusoids (black arrows). **D:** Liver of mouse exposed to $\leq 400 \mu\text{m}$ of plastic showing massive inflammation as seen by the inflammatory cells (white arrowheads). There is also mild parenchymal hemorrhage as seen by the presence of red blood cells (black arrowheads). There is mild reduction of hepatic sinusoids (black arrows). The nuclei are pyknotic. **H&E X400**

DISCUSSION

The present study evaluated the hepatotoxicity of SAN-MPs in a murine model using particles derived from real-world takeaway food packaging. Morphological and elemental analyses confirmed the test material as an SAN copolymer (C 89.26 at%; N 7.37 at%) occurring as irregular, flaky fragments, consistent with mechanically fractured amorphous SAN. Over 42 days of progressive oral exposure, SAN-MPs produced time-dependent elevations in serum ALT, AST and total bilirubin, accompanied by histopathological lesions that progressed from

inflammation and sinusoidal congestion to widespread necrosis and architectural disorganization. The pattern of injury was particle-size-dependent, with the $\leq 300 \mu\text{m}$ and $\leq 400 \mu\text{m}$ fractions generally eliciting more pronounced biochemical and histological effects than the $\leq 150 \mu\text{m}$ fraction.

Hepatic injury following oral microplastic exposure must be interpreted in the context of the toxicokinetic pathways connecting the intestinal lumen to the liver. Ingested particles are largely eliminated in faeces, but a small fraction translocates

across the intestinal epithelium through enterocyte and M-cell endocytosis, persorption at villus extrusion zones, and paracellular passage where tight-junction integrity is compromised (Campanale et al., 2020; Shukla et al., 2024). Particles and particle-derived solutes absorbed from the gut drain into the portal circulation and therefore encounter the liver before systemic distribution, positioning the liver as the first and most heavily exposed target organ (Beyzaei et al., 2025). Once within the sinusoids, translocated particles are efficiently sequestered by Kupffer cells, which accounts for the rapid hepatic accumulation of orally administered polystyrene particles reported by Deng et al. (2017) and is consistent with the detection of microplastics in human liver tissue (Horvatits et al., 2022; Roslan et al., 2024).

Oxidative stress is widely recognized as the central mechanism of microplastic-induced hepatotoxicity (Chen et al., 2024; Kadac-Czapska et al., 2024). For SAN, chemical and particulate pathways are expected to converge. The acrylonitrile moiety is bioactivated primarily by CYP2E1 to 2-cyanoethylene oxide and detoxified through glutathione-S-transferase-mediated conjugation with reduced glutathione (Agency for Toxic Substances and Disease Registry [ATSDR], 2025). Sustained GSH consumption by this conjugation pathway depletes the principal intracellular antioxidant reserve, disabling glutathione-peroxidase-dependent clearance of lipid hydroperoxides and permitting reactive oxygen species to accumulate. The resulting lipid peroxidation destabilizes mitochondrial and plasma membranes, producing the hepatocellular membrane disruption reflected by leakage of ALT and AST into serum. Concurrently, ROS act as signalling molecules that activate Kupffer cells and promote the release of pro-inflammatory mediators, recruiting the inflammatory infiltrates observed histologically from Day 7 onward; macrophage-driven inflammatory mechanisms of this type have been demonstrated for polystyrene microplastics (de Sá et al., 2018; Wright et al., 2013). Beyond necrosis, GSH depletion is mechanistically relevant to ferroptosis, an iron-dependent, lipid-peroxidation-driven cell death pathway whose execution requires GSH exhaustion; activation of both pyroptosis and ferroptosis has been documented in PS-induced hepatotoxicity (Mu et al., 2022) and the GSH-conjugating behaviour of acrylonitrile provides a plausible chemical route to the same endpoint in SAN-exposed liver.

The oxidative stress readouts in the present study were, however, heterogeneous rather than uniformly

dose- or size-progressive. MDA was not consistently elevated, GSH was paradoxically higher in the ≤ 300 μm group at Days 28 and 42, and CAT activity was suppressed in the ≤ 400 μm group at Day 35 and in the ≤ 150 μm group at Day 42. A biphasic interpretation reconciles these patterns. Early compensatory upregulation of GSH synthesis may buffer ROS during the initial weeks of exposure, followed by eventual exhaustion of antioxidant capacity, manifested as CAT suppression at terminal time points. Non-monotonic GSH kinetics are also compatible with the conjugation dynamics of acrylonitrile, which transiently consumes GSH in proportion to absorbed monomer. This profile deviates partially from the meta-analytic expectation for micro- and nanoplastics of consistently elevated MDA with depleted GSH and CAT (Chen et al., 2024), suggesting that polymer identity and dosing regimen shape the kinetics of oxidative injury. It further supports the conclusion that SAN-MP hepatotoxicity involves pathways operating independently of, or in parallel with, lipid-peroxidation-driven damage. Downstream, the histopathological progression is the expected morphological consequence of peroxidative membrane injury, endothelial damage and inflammatory amplification, and its temporal concordance with the biochemical markers strengthens the causal chain from SAN exposure to oxidative stress to functional liver alteration.

The significant increases in ALT and AST conform to established biomarkers of hepatocellular membrane disruption (Beyzaei et al., 2025). AST rose earliest, in the ≤ 400 μm group by Day 7, whereas ALT rose from Day 14 onward, with the ≤ 300 μm and ≤ 400 μm groups remaining significantly elevated at Day 21 and the ≤ 400 μm group at Day 42. The modest magnitude of these elevations indicates sublethal, cumulative injury rather than acute hepatic failure, which is consistent with the progressive rather than fulminant histopathology. The subsequent rise in total bilirubin in the ≤ 300 μm group at Days 28 and 42 further indicates impaired excretory function and compromised bile processing, as reported for rodents exposed to polystyrene (Beyzaei et al., 2025). Total protein remained unchanged whereas albumin increased significantly at later time points. This is unusual, since severe hepatocellular damage typically reduces albumin synthesis. The elevation may reflect compensatory upregulation of hepatic protein synthesis during sublethal stress or growth-related increases with age under the progressive dosing protocol. Regardless, the absence of hypoalbuminemia indicates that hepatic synthetic reserve was preserved.

The histopathological findings provide direct morphological evidence of SAN-MP-induced liver injury. The progression from periportal inflammation and sinusoidal congestion (Day 7) through patchy necrosis with karyolytic nuclei (Day 14) to widespread necrosis and architectural disorganization (Day 42) reflects the natural history of toxic hepatitis. The presence of parenchymal hemorrhage and karyolytic nuclei at Days 35 and 42 indicates advanced cellular degeneration and corroborates the biochemical evidence of membrane leakage reflected in elevated ALT and AST. These lesions closely mirror those documented in polystyrene-exposed mice, including inflammatory infiltration, congestion, necrosis and hepatocellular degeneration (Alexandrova et al., 2026; Beyzaei et al., 2025; Deng et al., 2017), indicating that SAN shares the histopathological endpoint of better-characterized polymers.

In vitro studies establish that smaller particles are generally more cytotoxic owing to higher surface-area-to-volume ratios, with smaller polystyrene particles eliciting more pronounced lipid peroxidation and GSH depletion in mouse hepatocytes (Zou et al., 2023), and in vivo toxicokinetic studies show greater systemic distribution of smaller particles (Beyzaei et al., 2025). Contrary to this inverse size-toxicity paradigm, our data reveal a non-linear profile: the ≤ 300 μm fraction frequently elicited the most significant biochemical perturbations, while histopathology indicated that both the ≤ 300 μm and ≤ 400 μm fractions caused more severe tissue damage than the ≤ 150 μm fraction at later stages. Several factors may explain this deviation in an intact organism. First, larger irregular fragments inflict more physical abrasion on the intestinal epithelium, provoking barrier disruption and microbiota dysbiosis that indirectly amplify liver injury via the gut-liver axis (Liu et al., 2025). Second, intermediate-sized fractions may represent a balance between epithelial interaction and inflammatory provocation. Cross-polymer comparison further contextualizes these findings: PET microplastics altered hepatic transcriptomic profiles and oxidative stress markers in piglets (Golubska et al., 2025) and meta-analytic evidence across predominantly PS and PE studies confirms oxidative stress as a conserved endpoint (Chen et al., 2024).

Limitation

One limitation of this study is the progressive dose-escalation regimen. Although, the study is designed to mimic chronic environmental exposure, it confounds dose and time effects. Also, the absence of profiling of inflammatory cytokine and transcriptomic

analysis evades definitive mechanistic conclusion. And finally, the study was restricted to male mice. Thus, sex-specific differences in microplastic toxicokinetics remain to be explored.

CONCLUSION

This study shows that oral exposure to SAN microplastics isolated from takeaway food packaging induces significant hepatocellular injury in mice. This is evidenced by elevated serum enzymes, bilirubin and progressive histopathological damage. The toxicity profile is particle-size-dependent and does not conform to a simple inverse size-toxicity relationship with the ≤ 300 μm fraction appearing particularly active. These findings expand the hepatotoxicity literature beyond dominant polystyrene and polyethylene experiments and also reflect the need for polymer-specific risk assessments of food-contact plastics.

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