



Research Article

Styrene-acrylonitrile Microplastics Alters Renal Redox Homeostasis and Induce Inflammatory Histopathology in Mice in a Particle Size-Dependent Manner

Suleiman Kabiru¹ and *Sada M. Salamatu²

¹Department of Biological Sciences, Faculty of Sciences, Bayero University, Kano, Nigeria

²Department of Biological Sciences, Faculty of Sciences, Federal University of Kashere Gombe, Nigeria

*Corresponding Author's email: salliesada89@gmail.com; Phone: +2347031001863

ABSTRACT

Styrene-acrylonitrile (SAN) copolymers are employed in disposable food-packaging materials, yet the nephrotoxic potential of the microplastics remains poorly characterised. This study investigated the particle size-dependent renal toxicity of SAN microplastics in mice following oral exposure. SAN-MPs were mechanically fragmented from commercial takeaway containers into ≤ 150 , ≤ 300 and ≤ 400 μm fractions. Ninety-five adult male mice were allocated to control and three treatment groups, exposed via oral gavage to doses over 42 days. Renal function was examined by serum creatinine, urea and sodium. Oxidative stress was determined by malondialdehyde, reduced glutathione and catalase. Histopathological alterations were also examined by H&E staining. SEM revealed irregular, flaky particles, while EDS confirmed carbon and nitrogen profiles with aluminium and trace contaminants. Serum creatinine and urea trended upward as the ≤ 300 μm group showed significantly elevated urea at Day 21. MDA was significantly increased in the ≤ 150 μm group at Days 14 and 28 and in the ≤ 300 μm group at Day 42. GSH and CAT showed early responses followed by exhaustion. Histopathology revealed progressive inflammatory infiltration and collagen deposition, with the ≤ 300 μm fraction causing the earliest and most severe damage. These findings show that SAN-MPs induce particle size- and time-dependent nephrotoxicity with the ≤ 300 μm fraction having the greatest pathological risk. Oxidative stress and inflammatory injury represent central mechanisms that underscore the need for toxicological scrutiny of SAN-containing food-contact plastics.

Keywords: Food-packaging plastics; Kidney histopathology; Mice; Nephrotoxicity; Oxidative stress; Particle size-dependent toxicity; Styrene-acrylonitrile microplastics

Citation: Kabiru, S., & Sada, S.M. (2026). Styrene-acrylonitrile Microplastics Alters Renal Redox Homeostasis and Induce Inflammatory Histopathology in Mice in a Particle Size-Dependent Manner. *Sahel Journal of Life Sciences FUDMA*, 4(3): 446-461. DOI: <https://doi.org/10.33003/sajols-2026-0403-47>

INTRODUCTION

The increasing plastic production has led to the widespread accumulation of plastic waste across terrestrial and aquatic bodies that create environmental and public-health challenge. Through photodegradation, and mechanical abrasion, larger plastic materials can progressively fragment into smaller particles. Microplastics (MPs) have consequently become known contaminants of surface water, drinking water, food, soil and air. Their small size, persistence, mobility and resistance to conventional environmental degradation facilitate

their transport across ecosystems (de Oliveira *et al.*, 2024; Haleem *et al.*, 2024; WHO, 2022).

Exposure by human to MPs occurs principally via inhalation and ingestion. Dermal exposure represents an additional potential route. Ingestion is relevant because MPs have been observed in water and foods, while plastic particles may also be released from materials in contact with food during manufacturing, handling, storage and use. Importantly, exposure is not limited to the polymer particles themselves. MPs can contain residual monomers and may adsorb environmental contaminants onto their surfaces,

thereby providing potential routes for combined exposure to particulate and chemical hazards. The WHO has identified uncertainties concerning the human health effects of MPs and emphasized the need for improved characterization of exposure and biological effects (WHO, 2022). Recent reviews similarly indicate that polymer type, particle size, morphology, surface characteristics and associated chemicals can influence the biological responses elicited by MPs (de Oliveira *et al.*, 2024; Haleem *et al.*, 2024).

The kidney is of particular interest in the toxicological assessment of MPs because it is an organ responsible for filtration of circulating substances. It is also responsible for regulation of fluid, acid–base homeostasis and urinary excretion of metabolic waste. The renal filtration and tubular handling of circulating particles and particle-associated substances may therefore expose the kidney to internally absorbed MPs. Evidence of renal exposure has become increasingly compelling. Massardo *et al.* (2024) identified plastic particles in human urine and kidney tissue using micro-Raman spectroscopy, with characterized particles in kidney samples ranging from approximately 1 to 29 μm . Experimental evidence further suggests that MPs can adversely affect renal structure and function. Also, a growing body of *in vitro* and *in vivo* finding has associated MP exposure with inflammation, oxidative stress and disturbances of homeostasis in renal tissues.

Oxidative stress is a consistently reported mechanism that underlies MP-induced toxicity. Plastics exposure may increase the generation of reactive oxygen species while impairing endogenous antioxidant defence. Lipid peroxidation which is often assessed through malondialdehyde (MDA), provides an indicator of oxidative damage to cellular membranes. Reduced glutathione (GSH) and Catalase (CAT) are important components of enzymatic and non-enzymatic antioxidant systems, respectively. Disruption of these systems may initiate or amplify inflammatory signalling, mitochondrial dysfunction and also, membrane injury.

The toxicological effects of MPs are not necessarily uniform across particle sizes. Particle size can influence gastrointestinal uptake and tissue distribution. Smaller particles may have greater potential for cellular uptake and systemic translocation, whereas larger particles may produce different biological responses through gastrointestinal retention or physical interaction with tissues. Importantly, recent evidence suggests that the relationship between particle size and renal

toxicity is not necessarily linear. Tan *et al.* (2025) reported that MP-induced nephrotoxicity is associated with particle size but that particular size ranges may produce greater toxicity than either smaller or larger particles.

Despite the environmental relevance of SAN-containing plastics, their toxicological characterization as microplastic particles remains limited. The kidney provides useful organ for evaluating these potential effects because renal injury can be assessed at biochemical, oxidative and histological levels. Serum creatinine and urea are commonly used indicators of renal functional impairment while serum sodium provides information concerning electrolyte homeostasis.

The present study, therefore investigated the toxicological impacts of orally administered Styrene-acrylonitrile microplastics on the kidneys of mice after exposure to different particle-size fractions and over different exposure durations. Renal toxicity was evaluated using serum biochemical indices of kidney function, tissue oxidative stress biomarkers that include MDA, CAT and GSH and histopathological examination of renal tissue.

MATERIALS AND METHODS

Study Area

The study was undertaken at the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The handling of animal and laboratory analyses were done in accordance with institutional guidelines for the care and in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. The experimental protocol was reviewed and granted by the NVRI institution ethical committee (AEC/02/220/25).

Study Design

Adult male mice (*Mus musculus*), aged approximately 6 weeks and weighing an average of 36.2 g were gotten from the animal facility of the institution. Total of 95 animals were divided randomly into 19 groups and caged 5 per group (n=5). The animals were acclimatized for 7 days prior to the commencement of the experiment under controlled laboratory conditions with a dark cycle of 12-h light/12-h, an ambient temperature of 22 ± 2 °C and relative humidity maintained at 40–60%. Commercial rodent pellets and potable water *ad libitum* throughout the acclimatization and experimental periods.

The mice were allocated to a control and three experimental groups according to the particle-size fraction of the Styrene-acrylonitrile microplastics (SAN-MPs). The experimental groups received SAN-

MPs with particle-size fractions of ≤ 150 , ≤ 300 and ≤ 400 μm , respectively. The control group similarly provided an equivalent volume of distilled water without microplastics. Animals from each treatment group were taken for sacrifice at 7, 14, 21, 28, 35 and 42 days of exposure for assessment of toxicity.

Collection and Preparation of Styrene-acrylonitrile Microplastics

Styrene-acrylonitrile (SAN) plastic materials were obtained from commercially available food-packaging containers ("takeaway" packages) purchased from Sabon Gari Market, Kano, Nigeria (12°00'52.12" N, 8°32'23.21" E). The collected plastic materials were washed and prepared for particle-size reduction.

The plastic materials were initially reduced to smaller pieces using a Shuliy crushing machine (SLSP-600, China) at Sharada, Kano State. The crushed materials were further ground using a laboratory blender to obtain smaller particles. The material was fractionated by sequential sieving using stainless-steel laboratory sieves of appropriate mesh sizes to obtain three non-overlapping, sieve-defined particle-size fractions: ≤ 150 μm , >150 – 300 μm , and >300 – 400 μm .

Morphological Characterization of SAN-MPs

The scanning electron microscopy (SEM) was employed to examine the characteristics of the prepared SAN-MPs. Prior to imaging, samples were dried and put on aluminium SEM stubs with the use of conductive carbon adhesive tape. The specimens were sputter-coated with a thin carbon layer using a Quorum SC7620 sputter coater. SEM imaging was performed using scanning electron microscope (JEOL Ltd., Tokyo, Japan) which was operated at 15 kV. Images were acquired at multiple magnifications to determine morphology of particle, surface characteristics and particle-size distribution. However, quantitative particle-size measurements were not performed from the SEM images.

Particle-Size Selection and Preparation of Exposure Suspensions

Three SAN-MP particle-size fractions were selected for the exposure experiment: ≤ 150 μm , ≤ 300 μm and ≤ 400 μm . 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.

Experimental Exposure and Sample Collection

Animals in the SAN-MP treatment groups were exposed continuously for 42 days. At each designated time points (days 7, 14, 21, 28, 35 and 42), animals scheduled for that time point were anaesthetized and sampled. Blood was obtained through retro-orbital venous sampling after overnight fast. The animals were anaesthetized with chloroform via inhalation. Blood samples were collected into appropriate blood collection tubes. Blood for biochemical analysis was allowed to clot and subsequently centrifuged at -4 °C and 10000 rpm for 15 minutes to obtain serum, which was later stored under appropriate conditions until analysis.

The kidneys were rapidly excised and rinsed with cold phosphate-buffered saline to remove adhering blood and divided as appropriate for biochemical and analyses of histopathology. Tissues for histopathological examination were fixed immediately in 10% neutral buffered formalin whereas portions intended for oxidative stress analyses were stored at -80 °C.

Renal Function Biochemical Analysis

Serum biochemicals were performed using an automated chemistry analyser at 37 °C according to the manufacturer's instructions for the respective assay procedures (Cobas c311, Roche Diagnostics, Mannheim, Germany).

Renal function was assessed by determining serum creatinine, urea and sodium concentrations (mg/dL, mmol/L and mmol/L respectively).

Preparation of Kidney Homogenates

Frozen kidney tissue samples were thawed on ice and put in ice-cold phosphate buffer (pH 7.4) for oxidative stress analyses. The homogenates were centrifuged using a refrigerated Eppendorf 5810R centrifuge at 10,000 rpm for 15 min (temp. 4 °C). The supernatants were carefully obtained and used for oxidative stress biomarkers determination.

Oxidative Stress Markers

Oxidative stress in renal tissue was examined by measuring reduced glutathione (GSH), malondialdehyde (MDA) and catalase (CAT) activity. MDA was determined as an index of lipid peroxidation, whereas GSH concentration and CAT activity were used as indicators of the antioxidant status of the kidney tissue.

The assays were performed using established biochemical methods and commercial assay kits

according to the instructions of the manufacturer. For MDA, colorimetric measurement of MDA-TBA adduct was done at 532 nm (Elabscience, Cat# E-BC-K025-M) (Janero, 1990). GSH was performed using DTNB colorimetric assay measuring yellow derivative at 412 nm (Abcam, Cat# ab239727) (Guo *et al.*, 2026) while for CAT was carried out using spectrophotometric measurement of hydrogen peroxide decomposition/reduction (Sigma-Aldrich / Merck, Cat# CAT100-1KT) (Aebi, 1984).

Kidney Histopathological Examination

Kidney tissues were put in 10% neutral buffered formalin. They were routinely processed, dehydrated with concentrations of ethanol, cleared in an appropriate clearing agent and embedded in paraffin wax. Embedded tissues were sectioned at approximately 5 µm thickness with the use of a rotary microtome.

The sections of tissue were put on glass slides and also stained with hematoxylin and eosin using standard histological procedures. Sections were examined under a light microscope for evidence of pathological alterations.

Data Analysis

Statistical analyses were done with GraphPad Prism v 9.0 (GraphPad Software, USA). Data were first examined for normality using the Shapiro-Wilk test. Normally distributed data are presented as mean ± standard deviation.

The effects of SAN-MP particle size and exposure duration on the measured biochemical and oxidative

stress parameters were analyzed using two-way ANOVA. Where significant effects were observed, Tukey's multiple-comparisons test was performed for post hoc pairwise comparisons. Statistical significance was set at $p < 0.01$ for all analyses.

RESULT

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

The elemental profile was predominantly composed of carbon (89.26 at%; 84.27 wt%) and nitrogen (7.37 at%; 8.11 wt%). Aluminum was detected at 2.66 at% (5.64 wt%), while calcium (0.29 at%; 0.90 wt%), silicon (0.26 at%; 0.58 wt%), iron (0.05 at%; 0.24 wt%), sulfur (0.06 at%; 0.15 wt%), magnesium (0.04 at%; 0.07 wt%), sodium (0.01 at%; 0.03 wt%) and chlorine (0.01 at%; 0.02 wt%) were present at relatively low concentrations. Phosphorus, potassium, titanium and manganese were not detected under the analytical conditions employed (Table 1).

Morphological Characteristics of SAN-MPs

Scanning electron microscopy (SEM) revealed that the prepared SAN-MPs consisted predominantly of irregular, flaky and fractured particles with rough and torn surface features (Figure 1). The particles exhibited morphological heterogeneity and a broad size distribution which extends from tens to hundreds of micrometres. Bright and high-contrast inclusions were also visible within some particles.

No clearly defined crystalline or spherulitic structures were observed in the examined particles.

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

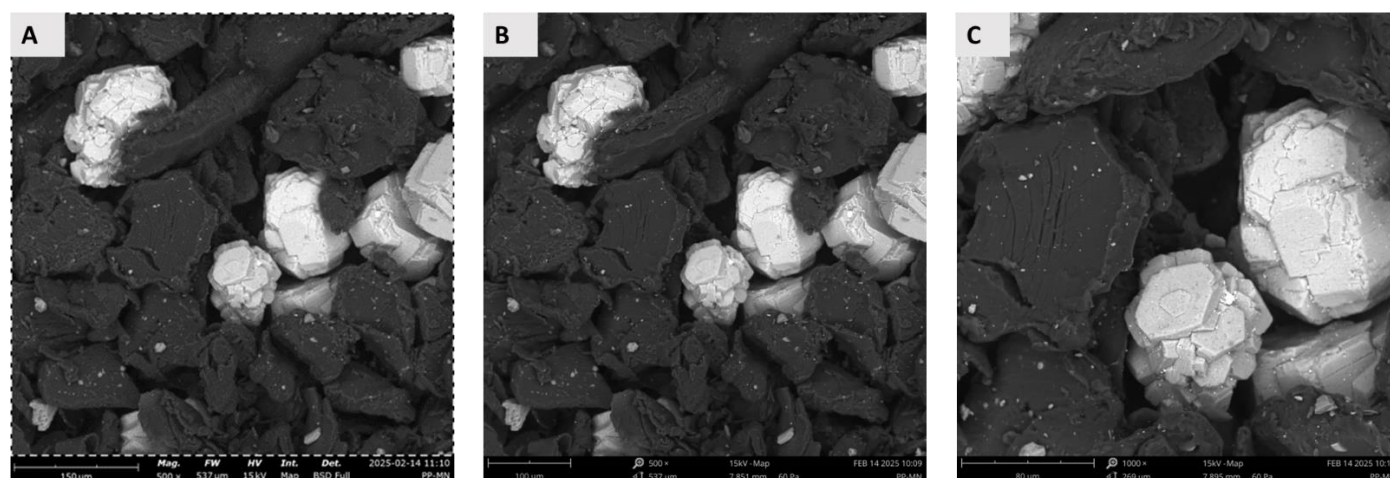


Figure 1: Scanning Electron Microscopy images indicating the morphology of Styrene-acrylonitrile particles

(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 Kidney Parameters in mice

Two-way ANOVA revealed particle-size- and time-dependent alterations in kidney function markers (Figure 2). Creatinine levels generally showed increased across the study period in all groups. At Day 7, the ≤ 300 µm group recorded a significantly higher creatinine level than the ≤ 150 µm group (40.00 ± 0.95 vs. 33.50 ± 0.75 ; $p = 0.0001$), while the other pairwise differences were not significant at the threshold of $p < 0.010$. No significance among the groups were observed at Days 14, 21, 28, 35, or 42 ($p > 0.010$). Although the 300 and ≤ 400 µm groups recorded higher creatinine levels than the Control group at Day 42, the p -values (0.0318 and 0.0363, respectively) did not meet the predefined significance threshold (Figure 2A).

Figure 2B shows that urea levels generally increased across the experimental period, especially in the 300 and ≤ 400 µm groups. No differences were observed significantly among the groups at Days 7 and 14 ($p > 0.010$). At Day 21, the ≤ 300 µm group had a significantly higher urea level than the Control group (7.60 ± 0.20 vs. 6.50 ± 0.20 ; $p < 0.0001$) while the other pairwise differences were not significant. No differences were also observed significantly at Days 28 and 35 ($p > 0.010$). At Day 42, although the 300 and ≤ 400 µm groups recorded higher urea levels than the Control group (8.70 ± 0.36 and 9.00 ± 0.30 vs. 6.90 ± 0.10), the differences were not significant at the threshold of $p < 0.010$ ($p = 0.0399$ and 0.0242 , respectively).

Sodium levels generally showed increased across the experimental period in all groups. At Day 7, the ≤ 300 µm group recorded the highest sodium level (152.00 ± 7.25) while the ≤ 150 µm group had the lowest

(141.90 ± 4.39), although none of the pairwise differences were significant at the predefined threshold of $p < 0.010$. Similarly, no significant differences were observed among groups at Days 14, 28, 35, or 42 ($p > 0.010$). At Day 21, sodium ranged from 146.20 ± 4.40 in the Control group to 153.00 ± 1.37 in the ≤ 400 µm group with no significant differences between groups (Figure 2C).

Oxidative Stress Markers of the Kidney

Malondialdehyde concentrations

Table 2 shows that kidney MDA levels (nmol/mg) in the Control group remained relatively stable which range from 0.90 ± 0.03 at Day 14 to 1.58 ± 0.13 at Day 35. The ≤ 150 µm group peaked at Day 14 (1.94 ± 0.09), while the ≤ 300 µm group reached its highest level at Day 42 (2.02 ± 0.05) and its lowest at Day 7 (0.89 ± 0.08). The ≤ 400 µm group showed minimal variation across the study period.

At Day 7, the ≤ 300 µm group had significantly lower MDA than the Control group (0.89 ± 0.08 vs. 1.37 ± 0.04 ; $p = 0.0078$). At Day 14, the ≤ 150 µm group was significantly higher than both the Control group (1.94 ± 0.09 vs. 0.90 ± 0.03 ; $p = 0.0045$) and the ≤ 400 µm group (1.94 ± 0.09 vs. 0.91 ± 0.05 ; $p = 0.0094$).

At Day 28, the ≤ 150 µm group exceeded both the Control group (1.25 ± 0.03 vs. 1.15 ± 0.04 ; $p = 0.0086$) and the ≤ 400 µm group (1.25 ± 0.03 vs. 0.80 ± 0.03 ; $p = 0.0049$). At Day 42, the ≤ 300 µm group was significantly higher than both the Control group (2.02 ± 0.05 vs. 1.07 ± 0.04 ; $p < 0.0001$) and the ≤ 400 µm group (2.02 ± 0.05 vs. 1.44 ± 0.06 ; $p < 0.0001$), while the Control group was also significantly lower than the ≤ 400 µm group (1.07 ± 0.04 vs. 1.44 ± 0.06 ; $p = 0.0081$). No other pairwise comparisons reached the $p < 0.010$ threshold.

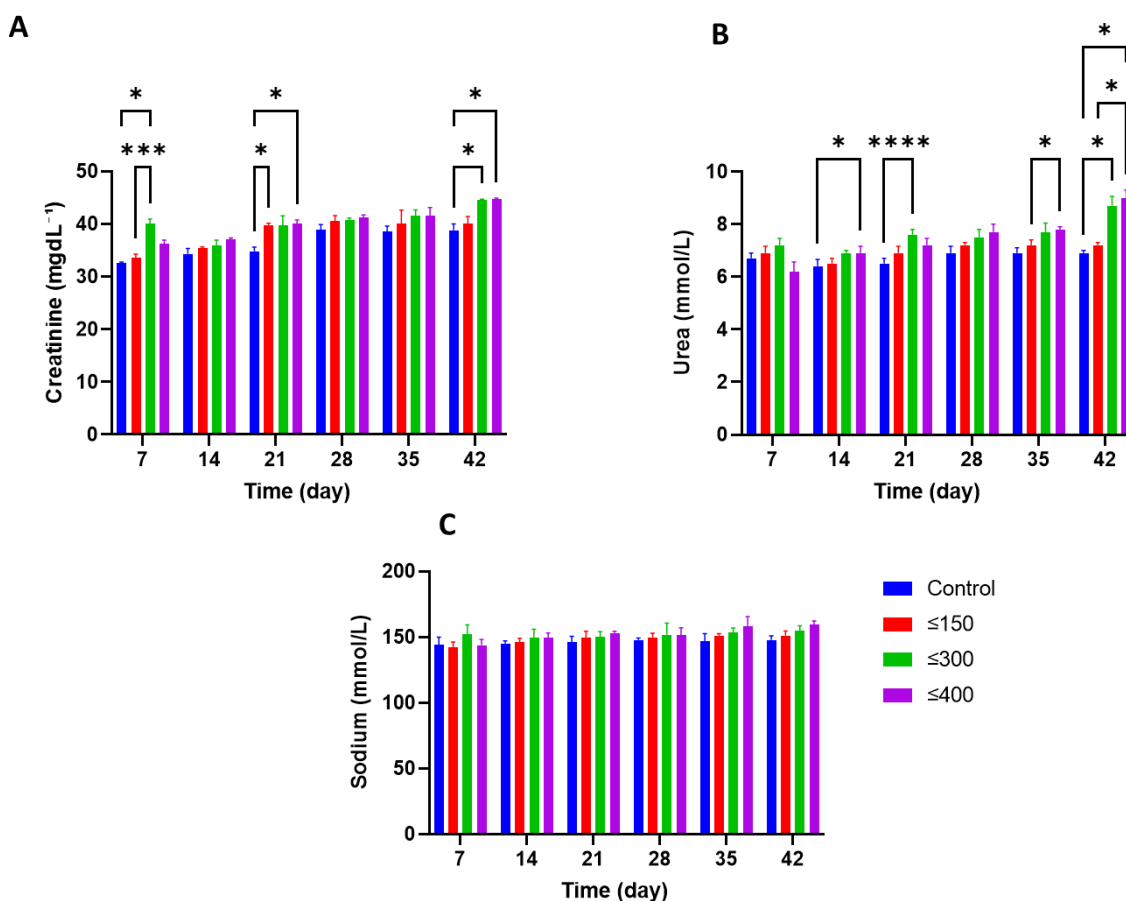


Figure 2: Assessment of (A) Creatinine (B) Urea (C) Sodium in the kidney of microplastic-exposed mice

Compared to control and treatment groups; Statistics data are calculated as mean $\pm$ SD; Two-way ANOVA was used to assess differences between groups, ** $P < 0.01$

Table 2. Assessment of Malondialdehyde (MDA; nmol/mg) in kidney of microplastic-exposed mice

Time (days)	Control	$\leq 150 \mu\text{m}$	$\leq 300 \mu\text{m}$	$\leq 400 \mu\text{m}$
7	1.37 ± 0.04^a	1.18 ± 0.10^{ab}	0.89 ± 0.08^b	1.39 ± 0.20^{ab}
14	0.90 ± 0.03^a	1.94 ± 0.09^b	1.21 ± 0.10^{ab}	0.91 ± 0.05^a
21	1.24 ± 0.01^a	1.10 ± 0.11^a	1.42 ± 0.14^a	1.43 ± 0.14^a
28	1.15 ± 0.04^a	1.25 ± 0.03^b	0.89 ± 0.09^{ab}	0.80 ± 0.03^a
35	1.58 ± 0.13^a	1.19 ± 0.07^a	1.28 ± 0.06^a	1.21 ± 0.07^a
42	1.07 ± 0.04^a	1.38 ± 0.14^{abc}	2.02 ± 0.05^b	1.44 ± 0.06^c

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

Reduced glutathione

Table 3 shows that GSH concentrations ($\mu\text{mol/g}$) varied across the experimental period among the Control and microplastic-exposed groups. On Day 7, the 150 and $\leq 300 \mu\text{m}$ groups recorded higher mean GSH concentrations than the Control group (5.55 ± 0.12 and 5.25 ± 0.28 vs. 4.15 ± 0.28), with the difference between the Control and $\leq 300 \mu\text{m}$ groups being significant ($p = 0.0069$) whereas the difference

involving the $\leq 150 \mu\text{m}$ group was not significant ($p = 0.0148$).

At Day 14, the $\leq 400 \mu\text{m}$ group recorded the highest GSH value (6.50 ± 0.51) while the Control group recorded the lowest (4.45 ± 0.11). Although the difference was not significant at the predefined threshold ($p = 0.0468$).

At Day 21, GSH was highest in the $\leq 300 \mu\text{m}$ group (4.75 ± 0.07) and lowest in the $\leq 400 \mu\text{m}$ group ($3.75 \pm$

0.09) with a significant difference between the two groups ($p = 0.0064$) while the other comparisons were not significant ($p > 0.010$).

At Day 28, the $\leq 150 \mu\text{m}$ group recorded the highest GSH value (6.55 ± 0.28) followed by the $\leq 300 \mu\text{m}$ group (5.75 ± 0.25). Although, none of the pairwise differences met the predefined significance threshold ($p > 0.010$).

At Day 35, GSH concentrations ranged from 3.45 ± 0.27 in the $\leq 400 \mu\text{m}$ group to 5.35 ± 0.56 in the $\leq 150 \mu\text{m}$ group, with the 300 and $\leq 400 \mu\text{m}$ groups showing a significant difference (5.25 ± 0.28 vs. 3.45 ± 0.27 ; $p < 0.0001$). At Day 42, GSH concentrations ranged from 4.00 ± 0.52 in the Control group to 5.05 ± 0.26 in the $\leq 150 \mu\text{m}$ group, with no statistical significance among the groups ($p > 0.010$).

Catalase Activity

Table 4 shows that CAT activity (U/mg) varied across the experimental period. At Day 7, the $\leq 150 \mu\text{m}$ group recorded a significantly higher CAT level than the $\leq 300 \mu\text{m}$ group (8.02 ± 0.12 vs. 6.09 ± 0.14 ; $p < 0.0001$) while the other pairwise differences were not statistically significant ($p > 0.010$).

At Day 28, CAT was significantly higher in the $\leq 150 \mu\text{m}$ than the Control (7.11 ± 0.32 vs. 6.19 ± 0.25 ; $p = 0.0051$) while the $\leq 150 \mu\text{m}$ group also differed significantly from the $\leq 400 \mu\text{m}$ group (7.11 ± 0.32 vs. 3.78 ± 0.11 ; $p = 0.0087$). At Day 35, the $\leq 300 \mu\text{m}$ group recorded significantly higher CAT than the $\leq 400 \mu\text{m}$ group (7.11 ± 0.80 vs. 4.94 ± 0.60 ; $p = 0.0078$). No other comparisons met the predefined significance threshold of $p < 0.010$.

Table 3. Assessment of reduced glutathione (GSH; $\mu\text{mol/g}$) in kidney of microplastic-exposed mice

Time (days)	Control	$\leq 150 \mu\text{m}$	$\leq 300 \mu\text{m}$	$\leq 400 \mu\text{m}$
7	4.15 ± 0.28^a	5.55 ± 0.12^{ab}	5.25 ± 0.28^b	4.25 ± 0.48^{ab}
14	4.45 ± 0.11^a	5.45 ± 0.30^a	5.25 ± 0.70^a	6.50 ± 0.51^a
21	4.00 ± 0.13^{ab}	4.11 ± 0.20^{ab}	4.75 ± 0.07^a	3.75 ± 0.09^b
28	4.00 ± 0.23^a	6.55 ± 0.28^a	5.75 ± 0.25^a	4.75 ± 0.38^a
35	4.75 ± 0.29^{ab}	5.35 ± 0.56^{ab}	5.25 ± 0.28^a	3.45 ± 0.27^b
42	4.00 ± 0.52^a	5.05 ± 0.26^a	4.75 ± 0.17^a	4.05 ± 0.43^a

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

Table 4. Assessment of Catalase (CAT; U/mg) in kidney of microplastic-exposed mice

Time (days)	Control	$\leq 150 \mu\text{m}$	$\leq 300 \mu\text{m}$	$\leq 400 \mu\text{m}$
7	6.18 ± 0.33^{ab}	8.02 ± 0.12^a	6.09 ± 0.14^b	8.10 ± 0.27^{ab}
14	8.62 ± 0.87^a	5.82 ± 0.42^a	7.29 ± 0.13^a	6.51 ± 0.19^a
21	7.34 ± 0.43^a	7.04 ± 0.23^a	5.50 ± 0.40^a	7.34 ± 0.65^a
28	6.19 ± 0.25^a	7.11 ± 0.32^b	6.85 ± 0.42^{ab}	3.78 ± 0.11^a
35	7.30 ± 0.62^{ab}	6.21 ± 0.19^{ab}	7.11 ± 0.80^a	4.94 ± 0.60^b
42	6.28 ± 0.27^a	6.37 ± 0.25^a	5.60 ± 0.38^a	5.92 ± 0.30^a

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

Histopathological Findings in the Kidney

Histopathological examination revealed structural changes in the kidneys of SAN-MP-exposed mice compared with the control animals across the exposure period. Control kidneys retained normal renal architecture at all examined time points. This is

characterized by well-defined glomerular tufts, intact glomerular capsules and preserved renal tubules.

At day 7, the control and $\leq 150 \mu\text{m}$ groups showed generally preserved renal architecture. In contrast, kidneys from the $\leq 300 \mu\text{m}$ group exhibited inflammatory changes, areas of collagen deposition and loss of glomerular structures. The $\leq 400 \mu\text{m}$ group

showed inflammatory cell infiltration. Although, the renal tubules remained relatively preserved (Plate 1). At day 14, the control group continued to exhibit normal renal architecture. The $\leq 150 \mu\text{m}$ group showed prominent inflammatory cell infiltration accompanied by mild interstitial haemorrhage. The ≤ 300 and $\leq 400 \mu\text{m}$ groups showed inflammatory changes and vascular/interstitial congestion. Renal tubular morphology remained relatively preserved in the $\leq 150 \mu\text{m}$ group (Plate 2).

At day 21, pathological alterations were observed in all SAN-MP-exposed groups. The $\leq 150 \mu\text{m}$ group showed inflammatory infiltration, loss of tissue architecture and congestion of the remaining tubules. Similar inflammatory changes and architectural disruption were observed in the ≤ 300 and $\leq 400 \mu\text{m}$ groups (Plate 3).

At day 28, the control group maintained normal renal architecture. In the SAN-MP-exposed groups, the $\leq 150 \mu\text{m}$ group showed inflammation and loss of

tissue architecture with congestion of the remaining tubules. The $\leq 300 \mu\text{m}$ group showed inflammatory infiltration and congestion, whereas the $\leq 400 \mu\text{m}$ group also exhibited inflammatory changes accompanied by degenerative alterations (Plate 4).

At day 35, the control kidneys remained structurally preserved. The $\leq 150 \mu\text{m}$ group showed inflammatory infiltration while the $\leq 300 \mu\text{m}$ group exhibited inflammatory changes with mild interstitial haemorrhage. The $\leq 400 \mu\text{m}$ group showed prominent inflammatory infiltration accompanied by loss of normal tissue architecture (Plate 5).

At day 42, the control group continued to display normal renal architecture. In the SAN-MP-exposed groups, inflammatory cell infiltration was observed. The $\leq 150 \mu\text{m}$ group showed inflammation while the ≤ 300 and $\leq 400 \mu\text{m}$ groups exhibited substantial inflammatory infiltration with areas of relatively preserved renal tubules (Plate 6).

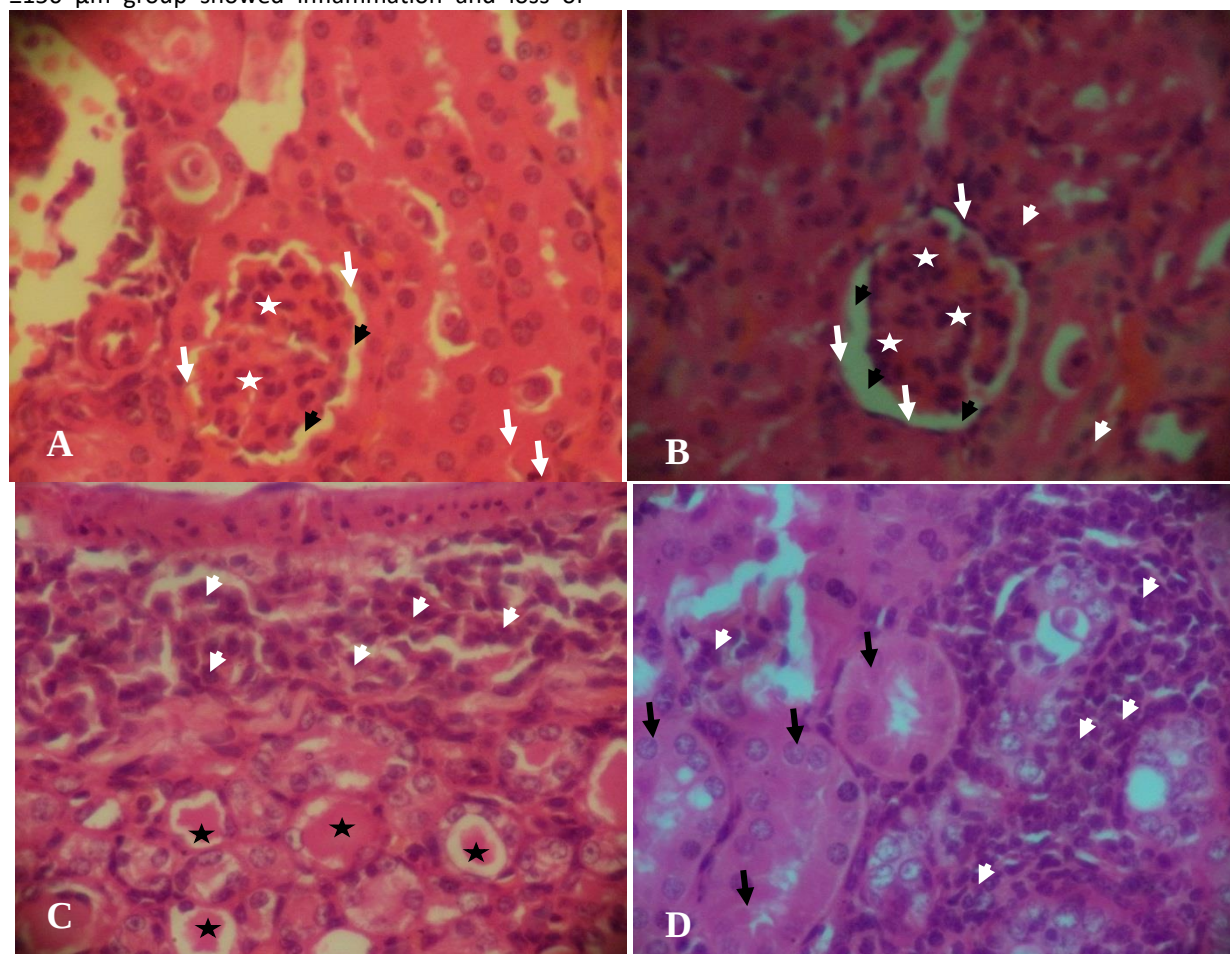


Plate 1: Kidney of Albino mice on Day 7

A: control group, exposed to normal atmospheric and nutritional condition, for 7 days and **B,** group 2 exposed to $\leq 150 \mu\text{m}$, both showing normal architecture of glomerular tufts (white stars) surrounded by clear capsular spaces (black arrowheads) and normal tubules (black arrows). The glomerular capsules (white arrows) present their characteristic simple squamous epithelium.

C: kidney of mouse administered $\leq 300 \mu\text{m}$ of plastic showing inflammation (white arrowheads) and collagenation (black stars) and loss of glomeruli **D:** kidney of mouse administered $\leq 400 \mu\text{m}$ of plastic showing inflammation (white arrowheads) amidst normal kidney tubules (black arrows). **H&E X400.**

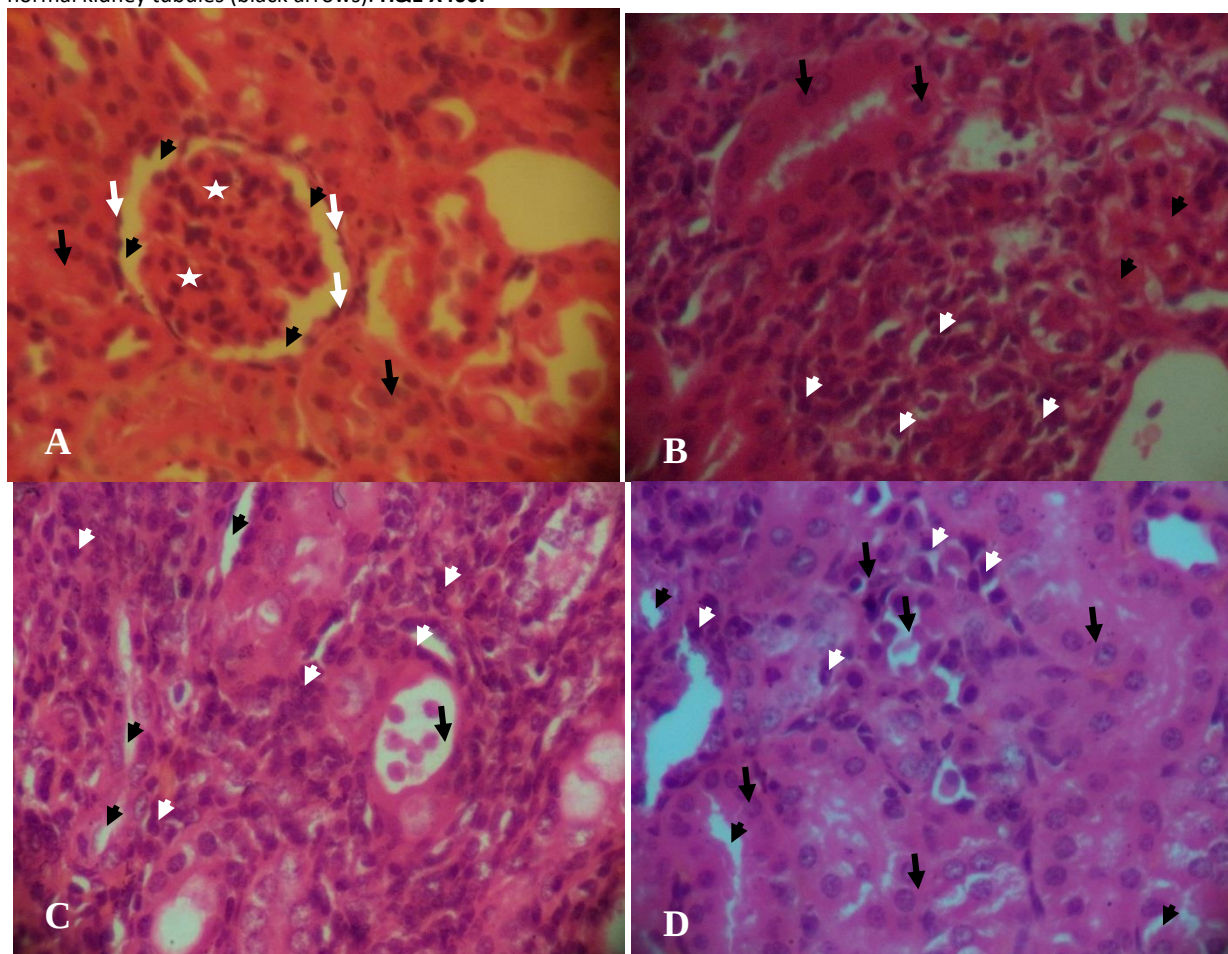


Plate 2: Kidney of Albino mice on Day 14

A: control group, exposed to normal atmospheric and nutritional condition, for 14 days, showing normal architecture of glomerular tufts (white stars) surrounded by clear capsular spaces (black arrowheads) and normal tubules (black arrows). The glomerular capsules (white arrows) present their characteristic simple squamous epithelium **B:** kidney of mouse administered $\leq 150 \mu\text{m}$ of plastic showing inflammation (white arrowheads) amidst normal kidney tubules (black arrows) with mild interstitial haemorrhage. **C and D** Kidney from $\leq 300 \mu\text{m}$ and $\leq 400 \mu\text{m}$ groups respectively, showing inflammation (white arrowheads) and congestion as seen by the loss of interstitial spaces (black arrowheads). The kidney tubules (black arrows) retain their normal morphology in **B**. **H&E X400.**

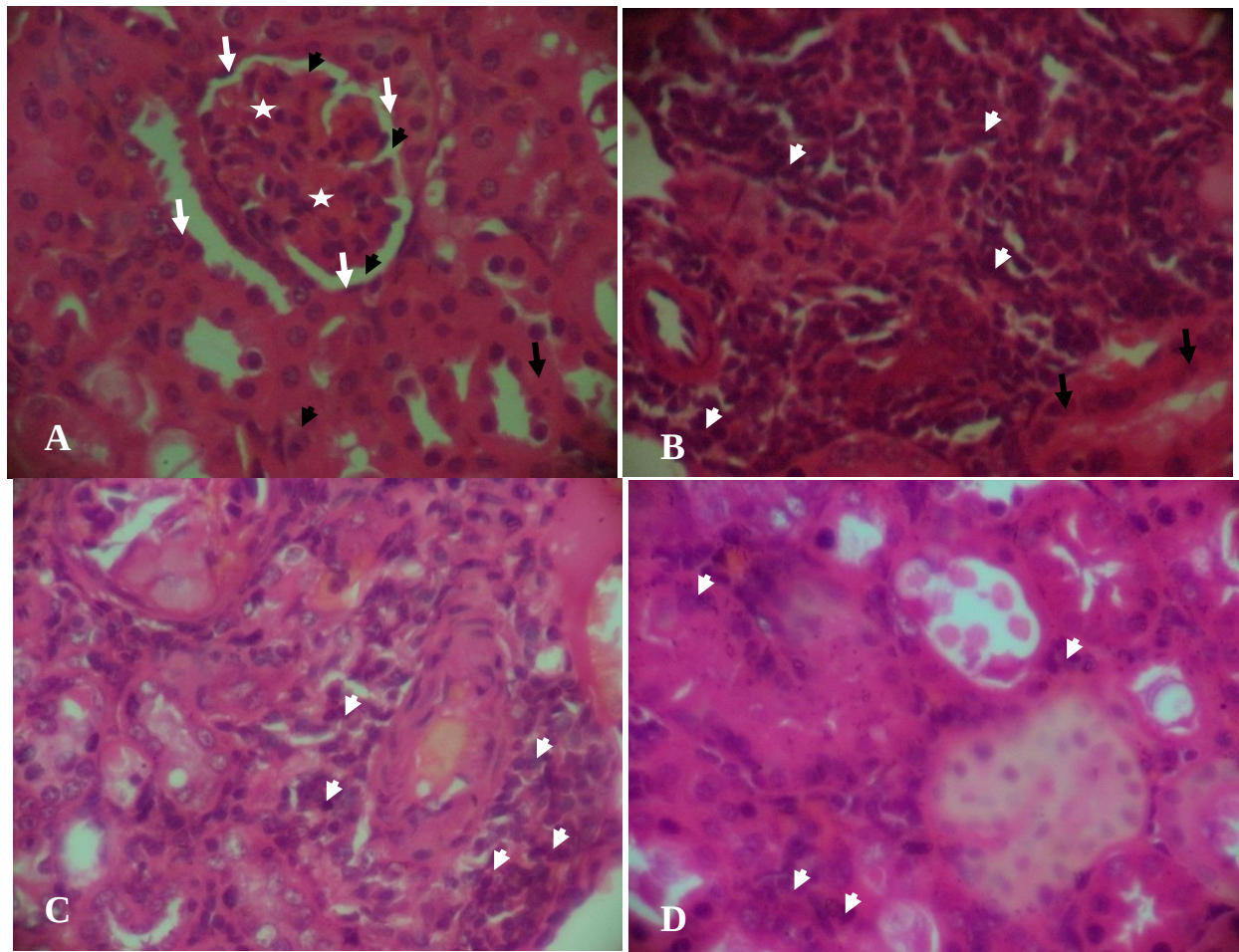


Plate 3: Kidney of Albino mice on Day 21

A: control group, exposed to normal atmospheric and nutritional condition, for 21 days, showing normal architecture of glomerular tufts (white stars) surrounded by clear capsular spaces (black arrowheads) and normal tubules (black arrows). The glomerular capsules (white arrows) present their characteristic simple squamous epithelium **B:** Kidney of mouse exposed to $\leq 150 \mu\text{m}$ of plastic showing inflammation (white arrowheads) and loss of tissue architecture. Few tubules (black arrows) are seen, congested. **C:** Kidney of mouse exposed to $\leq 300 \mu\text{m}$ of plastic showing inflammation (white arrowheads) and loss of tissue architecture **D:** Kidney of mouse exposed to $\leq 400 \mu\text{m}$ of plastic showing inflammation (white arrowheads) and loss of tissue architecture **H&E X400.**

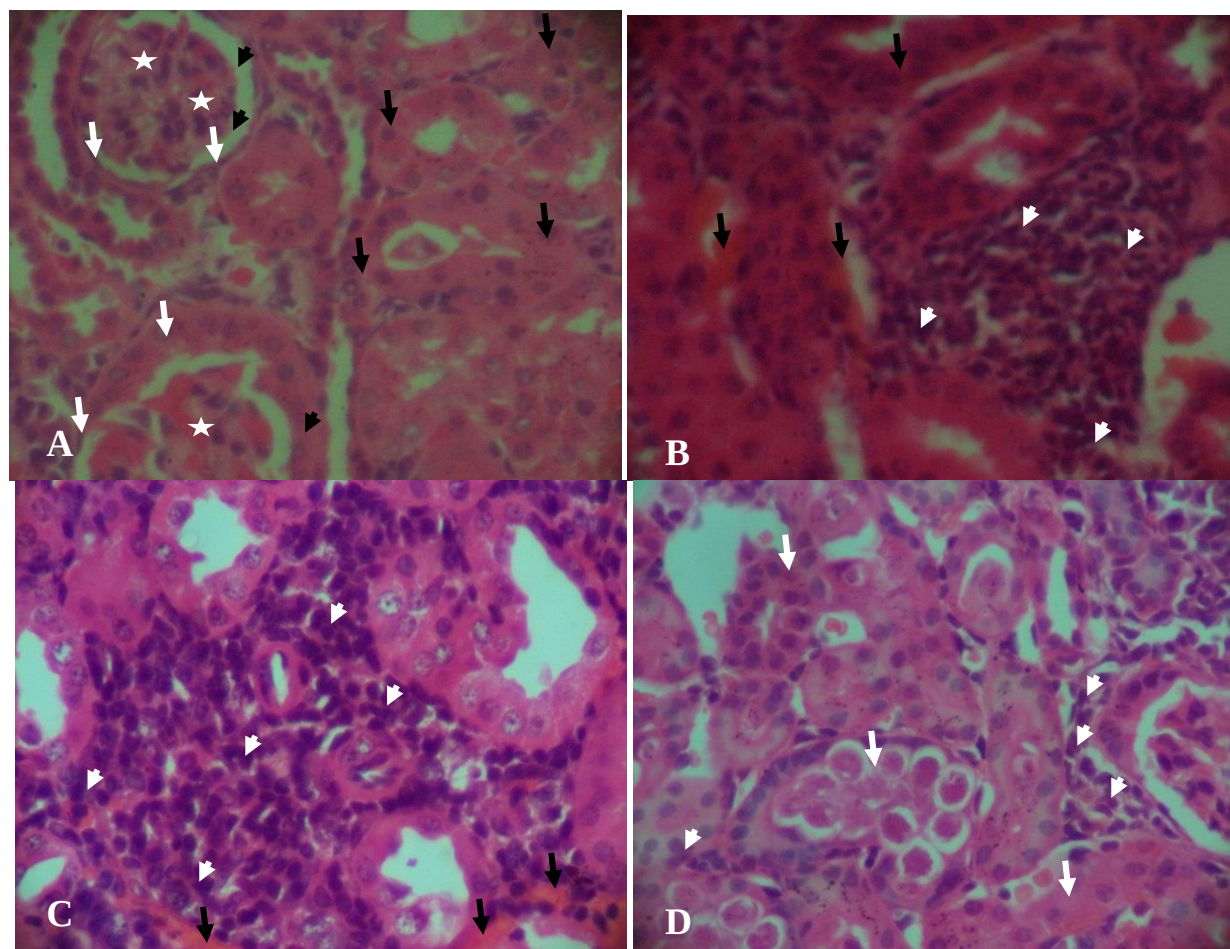


Plate 4: Kidney of Albino mice on Day 28

A: control group, exposed to normal atmospheric and nutritional condition, for 28 days, showing normal architecture of glomerular tufts (white stars) surrounded by clear capsular spaces (black arrowheads) and normal tubules (black arrows). The glomerular capsules (white arrows) present their characteristic simple squamous epithelium **B:** Kidney of mouse exposed to $\leq 150 \mu\text{m}$ of plastic showing inflammation (white arrowheads) and loss of tissue architecture. Few tubules (black arrows) are seen, congested. **C:** Kidney of mouse exposed to $\leq 300 \mu\text{m}$ of plastic showing inflammation (white arrows) and congestion (black arrows) **D:** Kidney of mouse exposed to $\leq 400 \mu\text{m}$ of plastic showing inflammation (white arrows) and degeneration (white arrows) **H&E. X400.**

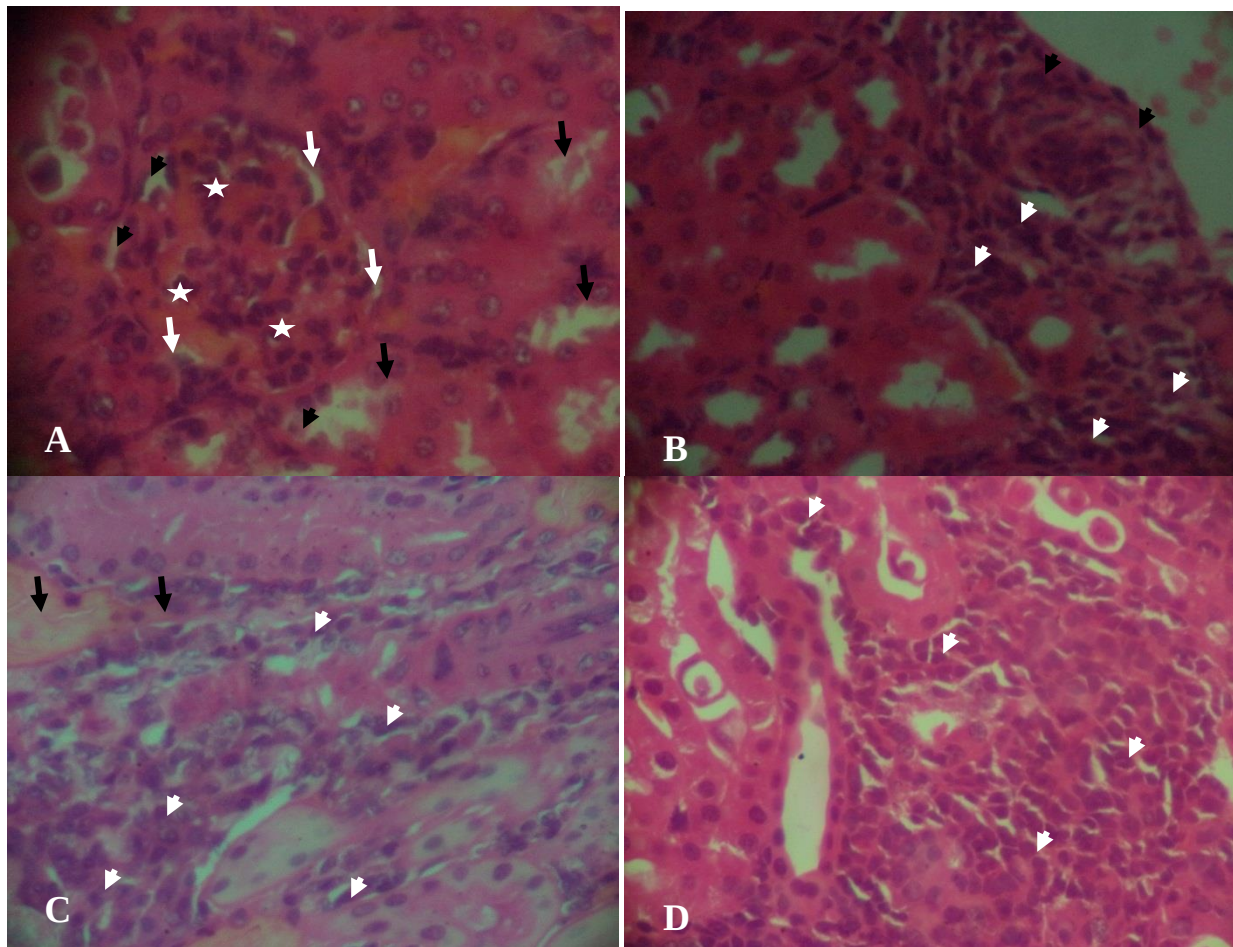


Plate 5: Kidney of Albino mouse on Day 35. **A:** control group, exposed to normal atmospheric and nutritional condition, for 35 days, showing normal architecture of glomerular tufts (white stars) surrounded by clear capsular spaces (black arrowheads) and normal tubules (black arrows). The glomerular capsules (white arrows) present their characteristic simple squamous epithelium **B:** Kidney of mouse exposed to $\leq 150 \mu\text{m}$ of plastic showing inflammation (white arrowheads). **C:** Kidney of mouse exposed to $\leq 150 \mu\text{m}$ of plastic showing inflammation (white arrowheads) and mild interstitial haemorrhage (black arrows). **D:** Kidney of mouse exposed to $\leq 150 \mu\text{m}$ of plastic showing inflammation (white arrowheads) and loss of tissue architecture. **H&E X400.**

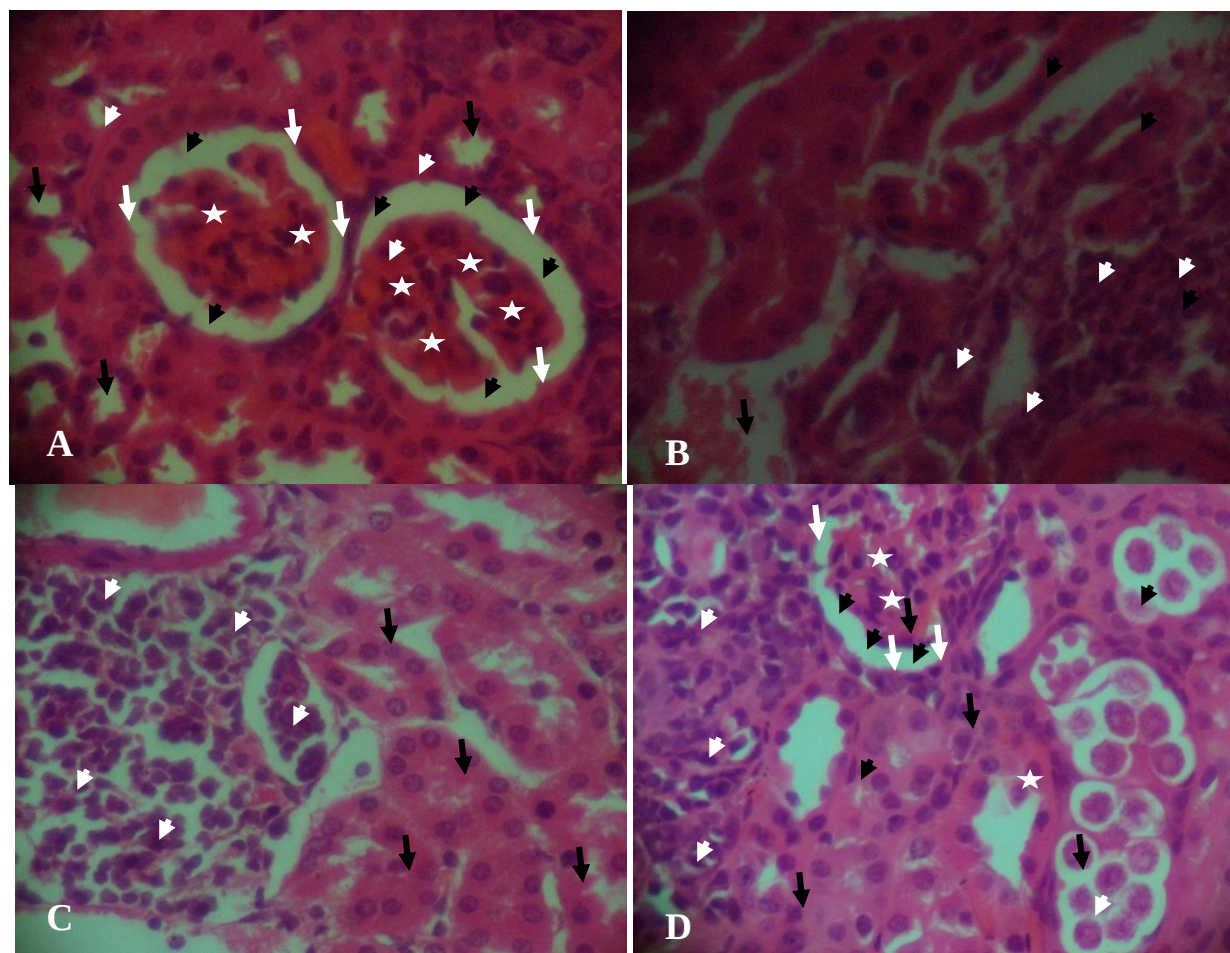


Plate 6: Kidney of Albino mice on Day 42

A: control group, exposed to normal atmospheric and nutritional condition, for 42 days, showing normal architecture of glomerular tufts (white stars) surrounded by clear capsular spaces (black arrowheads) and normal tubules (black arrows). The glomerular capsules (white arrows) present their characteristic simple squamous epithelium **B:** Kidney of mouse exposed to $\leq 150 \mu\text{m}$ of plastic showing inflammation (white arrowheads). **C and D:** Kidney from group 3 ($\leq 300 \mu\text{m}$) and 4 ($\leq 400 \mu\text{m}$), showing inflammation as seen by the observed inflammatory cells (white arrowheads). Interspersing the inflamed areas are normal kidney tubules (black arrows). White arrows= glomerular capsules, black arrowheads= capsular spaces. **H&E X400.**

DISCUSSION

The wide presence of MPs in the environment has raised growing concerns regarding their potential to induce organ-specific toxicity during ingestion. SAN copolymers are used in food-contact applications. These include disposable packaging containers, yet the renal toxicological profile of SAN-derived microplastics remains poorly characterised. This present study shows that oral exposure to SAN-MPs of varying particle-size fractions induces particle-size- and time-dependent nephrotoxicity in mice which is manifested through oxidative stress perturbations, progressive inflammatory histopathological lesions and alterations in renal function biomarkers.

Recent evidence also confirms that MPs accumulate in mammalian kidneys and can traverse the glomerular filtration barrier with urinary excretion identified as a primary elimination route (Jiang *et al.*, 2025). The kidney is vulnerable to MP-induced injury due to its high demand metabolically and role in filtering blood-borne particulates (Augey *et al.*, 2026). In this study, the elemental composition of the prepared SAN-MPs was characterised by SEM-EDS, with carbon and nitrogen constituting the predominant elements. Also notable is the detection of aluminium and trace inorganic contaminants. Their presence is consistent with common inorganic fillers and additives incorporated into food-contact plastics, together with environmental dust, washing residues

and possible minor abrasion from the stainless-steel apparatus used during fragmentation. This suggests that mechanical fragmentation of commercial food-packaging plastics may introduce surface-associated exogenous elements that potentially act as supplementary stressors via the "Trojan horse" mechanism that is previously described for MPs adsorbed to organic pollutants and heavy metals (Jiang *et al.*, 2025).

Serum creatinine and urea are established clinical indicators of glomerular filtration efficiency and nitrogenous waste excretion, respectively. In this study, creatinine and urea levels exhibited upward trends across the exposure period, particularly in the $\leq 300 \mu\text{m}$ and $\leq 400 \mu\text{m}$ groups. Yet most pairwise comparisons did not achieve the predefined significance threshold of $p < 0.01$. This observation aligns with recent reports that indicate that MP-induced renal dysfunction may manifest subclinically. For instance, Shen *et al.* (2023) reported variable effects of PS-MPs on serum creatinine and BUN in mice while Jiang *et al.* (Jiang *et al.*, 2025) documented elevated creatinine in rats exposed to PS-MPs. The absence of statistically significant electrolyte disturbances in the present study suggests that tubular ion-handling capacity was largely preserved during the 42-day exposure window. Although, the observed increases in serum sodium require cautious interpretation given that electrolyte dysregulation has been documented at higher MP exposure burdens (Kehinde *et al.*, 2025).

A central observation of this study was the disruption of renal redox homeostasis after SAN-MP exposure. MDA was significantly elevated in the $\leq 150 \mu\text{m}$ group at Days 14 and 28 and in the $\leq 300 \mu\text{m}$ group at Day 42. This observation indicates oxidative damage with prolonged exposure. These results corroborate evidence that MPs exacerbate nephrotoxicity through the induction of mitochondrial dysfunction and disruption of oxidative homeostasis in renal tissue (Jiang *et al.*, 2025). The divergence in MDA elevation may reflect differences in particle retention or cellular uptake kinetics. Smaller particles possess higher specific surface areas and may more readily initiate early oxidative reactions, whereas larger particles could elicit sustained physical irritation and progressive inflammatory cascades. This a pattern is interpreted as an initial compensatory response to presumed MP-associated ROS generation, followed by attenuation of antioxidant capacity under sustained exposure (Kadac-Czapska *et al.*, 2024).

The antioxidant responses displayed complex, non-linear dynamics. Early exposure (Day 7) was

associated with elevated GSH and CAT in several treatment groups. This is consistent with an adaptive compensatory upregulation of the antioxidant defence system. However, this protective response appeared to wane with continued exposure, as evidenced by reduced GSH in the $\leq 400 \mu\text{m}$ group at Day 35 and variable CAT activity at later time points. Antioxidant enzymes initially rose to counteract ROS generation but subsequently decline as the oxidative burden overwhelms cellular detoxification capacity (Kadac-Czapska *et al.*, 2024). Ahmed *et al.* (Ahmed *et al.*, 2022) similarly reported that polystyrene nanoparticles depleted GSH and glutathione peroxidase in rat kidneys. This underscores the vulnerability of the glutathione system to MP-induced oxidative challenge.

Histopathological examination provided morphological evidence of SAN-MP nephrotoxicity. Control kidneys maintained normal architecture throughout the study whereas exposed groups exhibited progressive inflammatory cell infiltration, interstitial collagen deposition and tubular degeneration. These lesions were pronounced in the $\leq 300 \mu\text{m}$ and $\leq 400 \mu\text{m}$ groups from Day 21 onwards and in the $\leq 150 \mu\text{m}$ group at later time points. The observed inflammatory infiltrates and collagenation are also consistent with MP-activated NF- κ B signalling and downstream cytokine release (Meng *et al.*, 2022). Pan *et al.* (Pan *et al.*, 2024) showed that PS-MPs accelerate tubular epithelial cell senescence and facilitate renal fibrosis while Xiong *et al.* (Xiong *et al.*, 2023) identified renal injury in MP-exposed mice through transcriptomic profiling.

The particle-size dependency of SAN-MP nephrotoxicity require careful consideration. Contrary to the general paradigm that smaller particles exert greater toxicity due to enhanced cellular internalisation (Gillings *et al.*, 2026), the $\leq 300 \mu\text{m}$ fraction frequently elicited robust biochemical and histopathological responses in this study. This may be attributable to the irregular, flaky morphology of the prepared SAN-MPs which differ from the smooth spherical particles typically used in toxicological studies. Larger angular fragments may cause more severe mechanical irritation to the gastrointestinal and renal tubular epithelia and their reduced clearance efficiency could prolong tissue contact time. Furthermore, the progressive dosing regimen wherein exposure concentrations increased weekly may have favoured accumulation of intermediate-sized particles in renal tissue (Jiang *et al.*, 2025).

Integrating the biochemical and histological findings, the elevations in MDA together with the early fluctuations in GSH and CAT, are consistent with enhanced ROS generation and a perturbed redox balance in the renal parenchyma. Although ROS was not measured directly in this study. According to the current literature, sustained oxidative stress can activate pro-inflammatory signalling, including NF- κ B- and NLRP3-dependent pathways with downstream release of IL-1 β , IL-6 and TNF- α , and such cascades have been implicated in MP-induced tissue injury (Meng *et al.*, 2022; Ramirez-Celis *et al.*, 2026). These mediators are, in turn, considered to drive leukocyte infiltration and tubular epithelial injury, ultimately compromising glomerular and tubular integrity (Aditya *et al.*, 2025).

Limitations

The investigation of this study was restricted to male mice, precluding assessment of sex-specific susceptibility to SAN-MP nephrotoxicity. Also, the use of a progressively escalating dose, while mimicking cumulative environmental exposure, complicates the attribution of effects to specific concentration thresholds. Additionally, the study focused exclusively on SAN and future research should compare nephrotoxic profiles across diverse polymer chemistries. The absence of a recovery group limits conclusions regarding the reversibility of the observed renal lesions.

CONCLUSION

This study demonstrates that oral exposure to SAN-MPs derived from commercial food-packaging plastics induces particle size- and time-dependent nephrotoxicity in mice. The ≤ 300 μ m fraction elicited the most pronounced effects which is characterized by progressive lipid peroxidation, perturbation of antioxidant defences and inflammatory and degenerative histopathological lesions. Although serum creatinine and urea exhibited upward trends across the exposure period, the most sensitive indicators of SAN-MP renal toxicity were oxidative stress biomarkers and histopathological architecture which deteriorated significantly as early as Day 7 and worsened through Day 42. These findings support an oxidative stress–inflammation–fibrosis axis as a central mechanism underlying SAN-MP-induced kidney injury. Given the widespread use of SAN copolymers in disposable food-contact materials and the environmental burden of microplastic pollution, these results reflect the need for chronic low-dose toxicological assessments and informed regulatory

consideration of polymer selection in food-packaging applications to mitigate renal health risks.

REFERENCES

- Aditya, M. R., Muhammad, A. R., Adriansyah, V., Samsu, N., & Sulistomo, H. W. (2025). Microplastic exposure and its consequences for renal and urinary health: systematic review of in vivo studies. *All Life*, 18(1).
<https://doi.org/10.1080/26895293.2025.2457760>
- Aebi, H. (1984). Catalase in Vitro. *Methods in Enzymology*, 105(C), 121–126.
[https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
- Ahmed, Y. H., El-Naggar, M. E., Rashad, M. M., M.Youssef, A., Galal, M. K., & Bashir, D. W. (2022). Screening for polystyrene nanoparticle toxicity on kidneys of adult male albino rats using histopathological, biochemical, and molecular examination results. *Cell and Tissue Research*, 388(1), 149–165. <https://doi.org/10.1007/s00441-022-03581-5>
- Augey, L., Berdougou-Tritz, J., Mbarek, A., Jadoul, M., Massy, Z., Deray, G., & Liabeuf, S. (2026). Effects of microplastics and nanoplastics on the kidneys. *Nephrology Dialysis Transplantation*.
<https://doi.org/10.1093/ndt/gfag034>
- de Oliveira, R. B., Pelepenko, L. E., Masaro, D. A., Lustosa, G. M. M. M., de Oliveira, M. C., Roza, N. A. V., Marciano, M. A., dos Reis, L. M., Kamel, S., Louvet, L., & Mazon, T. (2024). Effects of microplastics on the kidneys: a narrative review. *Kidney International*, 106(3), 400–407.
<https://doi.org/10.1016/j.kint.2024.05.023>
- Gillings, H. L., Rojas-Canales, D. M., Wong, S. W., Bhuskute, K. R., Kaur, A., Delcheva, I., Gleadle, J. M., & MacGregor, M. (2026). Nanoplastic toxicity and uptake in kidney cells: differential effects of concentration, particle size, and polymer type. *Cell Biology and Toxicology*, 42(1).
<https://doi.org/10.1007/s10565-025-10135-2>
- Guo, H., Yang, T., Xia, C., Ni, Z., Lou, Y., Deng, H., Zuo, Z., Zhu, Y., Wang, X., & Fang, J. (2026). Glutathione selenium nanoparticles alleviate oxidative stress-induced reproductive dysfunction by regulating mitochondrial dynamics. *Free Radical Biology and Medicine*, 242, 108–119.
<https://doi.org/10.1016/j.freeradbiomed.2025.10.277>
- Haleem, N., Kumar, P., Zhang, C., Jamal, Y., Hua, G., Yao, B., & Yang, X. (2024). Microplastics and associated chemicals in drinking water: A review of their occurrence and human health implications.

- Science of the Total Environment*, 912. <https://doi.org/10.1016/j.scitotenv.2023.169594>
- Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine*, 9(6), 515–540. [https://doi.org/10.1016/0891-5849\(90\)90131-2](https://doi.org/10.1016/0891-5849(90)90131-2)
- Jiang, N., Zheng, X., Zhang, N., & Cao, Y. (2025). The detrimental effects of microplastic exposure on kidney function. *Frontiers in Medicine*, 12. <https://doi.org/10.3389/fmed.2025.1620733>
- Kadac-Czapska, K., Ośko, J., Knez, E., & Grembecka, M. (2024). Microplastics and Oxidative Stress—Current Problems and Prospects. *Antioxidants*, 13(5). <https://doi.org/10.3390/antiox13050579>
- Kehinde, S. A., Olajide, A. T., Ogunsanya, S. T., Fatokun, T. P., Olulana, D. I., Olabiyi, F. A., Faokunla, O., Tham, C. L., & Chusri, S. (2025). Polyethylene microplastics disrupt renal function, mitochondrial bioenergetics, redox homeostasis, and histoarchitecture in Wistar rats. *Scientific Reports*, 15(1). <https://doi.org/10.1038/s41598-025-24887-8>
- Massardo, S., Verzola, D., Alberti, S., Caboni, C., Santostefano, M., Eugenio Verrina, E., Angeletti, A., Lugani, F., Ghiggeri, G. M., Bruschi, M., Candiano, G., Rumeo, N., Gentile, M., Cravedi, P., La Maestra, S., Zaza, G., Stallone, G., Esposito, P., Viazzi, F., ... Artini, C. (2024). MicroRaman spectroscopy detects the presence of microplastics in human urine and kidney tissue. *Environment International*, 184. <https://doi.org/10.1016/j.envint.2024.108444>
- Meng, X., Yin, K., Zhang, Y., Wang, D., Lu, H., Hou, L., Zhao, H., & Xing, M. (2022). Polystyrene microplastics induced oxidative stress, inflammation and necroptosis via NF-κB and RIP1/RIP3/MLKL pathway in chicken kidney. *Toxicology*, 478. <https://doi.org/10.1016/j.tox.2022.153296>
- Pan, C., Wang, X., Fan, Z., Mao, W., Shi, Y., Wu, Y., Liu, T., Xu, Z., Wang, H., & Chen, H. (2024). Polystyrene microplastics facilitate renal fibrosis through accelerating tubular epithelial cell senescence. *Food and Chemical Toxicology*, 191. <https://doi.org/10.1016/j.fct.2024.114888>
- Ramirez-Celis, C., Martínez-Torres, A. M., & Morán, J. (2026). NLRP3 Inflammasome Activation in Oxidative Stress: A Key Mechanism Driving Neuroinflammation. *Acta Cytologica*, 33(1), 236–263. <https://doi.org/10.1159/000551943>
- Shen, T., Zhang, W., Wang, Y., Li, H., Wu, J., Wang, Q., Qin, L., Zhang, L., Liu, C., & Li, R. (2023). Effects of Microplastic (MP) Exposure at Environmentally Relevant Doses on the Structure, Function, and Transcriptome of the Kidney in Mice. *Molecules*, 28(20). <https://doi.org/10.3390/molecules28207104>
- Tan, R. Y., She, Q. Y., Ma, Y. C., Liu, M. H., Li, L. J., Huang, L. L., Zhong, Y. W., & Bi, H. X. (2025). The threat of microplastics to human kidney health: Mechanisms of nephrotoxicity and future research directions. *Environmental Research*, 283. <https://doi.org/10.1016/j.envres.2025.122124>
- WHO. (2022). Dietary and inhalation exposure to nano- and microplastic particles and potential implications for human health. *Dietary and Inhalation Exposure to Nano-and Microplastic Particles and Potential Implications for Human Health*, 1–154.
- Xiong, X., Gao, L., Chen, C., Zhu, K., Luo, P., & Li, L. (2023). The microplastics exposure induce the kidney injury in mice revealed by RNA-seq. *Ecotoxicology and Environmental Safety*, 256. <https://doi.org/10.1016/j.ecoenv.2023.114821>