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CHRONIC TOXICITY OF POLYSTYRENE MICROPLASTICS IN BLOOD AND ORGANS OF ALBINO MICE: A HISTOPATHOLOGICAL AND BIOCHEMICAL ASSESSMENT

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ABSTRACT

Microplastics (MPs) are emerging as a profound environmental and health hazard and their omnipresence in ecosystems endangers all life forms. This study delves into PS-MPs' repercussions on albino mice (*Mus musculus*), analyzing body weight and mortality, hematological parameters, and histopathological alterations in crucial organs. A total of seventy-five albino mice (n=75) were segregated into five principal groups, each comprising three subgroups, to receive oral administration of PS-MPs (0.7mm) over five weeks. The regimen included a control group (0.0mg/g) and four escalating doses for the experimental groups: 0.1mg/g (Treatment 1), 0.2mg/g (Treatment 2), 0.4mg/g (Treatment 3), and 0.8mg/g (Treatment 4). No mortality was observed in the study following exposure to PS-MPs in a 5 weeks trial. The investigation revealed a significant weight decrease in the highest dose group (Treatment 4) ($P<0.05$), in contrast to minor weight increases in the control, and other treatments, which were not statistically significant ($P>0.05$) thereby indicating association of higher doses with a decrease in overall weight of mice. Hematological assessments indicated a dose-dependent impact on liver and kidney functions, blood formation, and immune responses even at lower doses ($P<0.05$ for Treatment 3 and 4). Histological evaluations exhibited no changes in the control group, whereas a dosage increase led to notable tissue damage, including hemosiderosis, necrosis, congestion, vacuolization, degeneration, karyolysis, cellular hypertrophy, interstitial fibrosis, infiltration, atrophy, hyperplasia and granulation in contrast to the control animals. These pathologies correlated with significant health conditions ($P<0.05$ for higher dose groups), suggesting a possible link between PS-MP exposure and the development of diseases such as hepatitis, chronic granulomatous disease, and asthma. This study underscores the chronic toxicity of PS-MPs in albino mice, evidenced by hematological and histopathological disturbances that become more pronounced with increased exposure.

Keywords: Microplastics, Polystyrene, Growth alteration, Hematological system, Tissue damage, emerging contaminants

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INTRODUCTION

Plastic pollution is an extensive and increasing issue in today's world (Lau *et al.,* 2020) with microplastics (MPs) becoming a pervasive contaminant in various ecosystems (Brahney *et al.,* 2020). Anthropogenic activities produce plastics and are widely known as the most prevalent category of artificial products (Zalasiewicz *et al.,* 2016). Approximately 8.3 billion metric tons of plastics were made in 2017 (Geyer *et al.,* 2017). Literature indicates an annual release of 8 million metric tons of plastics into oceans (Jambeck *et al.,* 2015), in which approximately 236,000 tons is MPs (Van Sebille *et al.,* 2015).

MPs are generally less than 5mm in diameter (Akdogan and Guven, 2019), and formed by natural activities like ultraviolet radiation, biodegradation, and the natural weathering of plastic fragments (Yin *et al.,* 2021). MPs are responsible for unpredictable environmental damage and specific internal injuries like gastric ruptures, necrosis, oxidative stress and hepatic stress (Browne *et al.,* 2013). MPs also affect mammalian cells and tissues (Wright and Kelly, 2017; Rubio *et al.,* 2020). A study conducted by Dissanayake *et al.,* (2022) showed that MPs effects on the soil are detrimental and beneficial at the same time, which raises the concerns, "Are MPs harmful to everyone?" Due to their small size, MPs can readily permeate tissues through multiple food sources (Kim *et al.,* 2018; Smith *et al.,* 2018), drinking water (Pivokonsky *et al.,* 2018; Koelmans *et al.,* 2019), and plastic water containers (Obmann *et al.,* 2018; Zuccarello *et al.,* 2019). Approximately 74,000-121,000 per capita of MPs are estimated to be ingested or inhaled annually (Cox *et al.,* 2019). A study concluded that per capita MPs intake by animals is 39,000-52,000 (Borriello *et al.,* 2023). Of these, 37-1000 MPs are derived from sea salt, 4000 from tap water, and nearly 11000 from shellfish (Prata, 2018). Moreover, children may ingest about 184 ng/capita/d of MPs, while adults may ingest 583 ng/capita/d of MPs through various sources (Mohamed *et al.,* 2021). Additionally, MPs are also present in both tap water (81% of MPs particles size 0.1 to 5mm) (Kosuth *et al.,* 2018) and bottled water (325 MPs size 6.5µm to 5mm) (Mason *et al.,* 2018, Gambino *et al.,* 2022).

Polystyrene (PS), the prevalent plastic widely present in the environment, is formed through the polymerization of styrene monomers (Kik *et al.,* 2020; Meng *et al*., 2022a). However, styrene is usually derived from benzene and ethylene (Vaughan *et al.,* 2015). The catalytic dehydrogenation of ethylbenzene is the procedure that produces styrene monomers (Wunsch, 2000).

Polystyrene microplastics (PS-MPs) induce histopathological changes in tissues that are observable through microscopic examination. These alterations provide information about the severity and intensity of a disease, aiding in the diagnostic process and guiding treatment strategies. Typical histopathological alterations include cellular and inflammatory changes, fibrosis and scarring, infiltration and accumulation and neoplastic, vascular, and metabolic changes (Ersan *et al.,* 2010). PS-MPs also induced a notable reduction in the white blood cells count in peripheral blood and hindered the colony forming capacity of bone marrow cells in mice (Sun *et al.,* 2021).

Detrimental effects of MPs on mammals are still unknown and only limited data is available on mammalian research. The rising prevalence of MPs is increasing their impact on the terrestrial environment and causing direct contamination (Yu *et al.,* 2020) PS-MPs are predominantly associated with metabolic and lipid peroxidation abnormalities (Liu *et al.,* 2022). Recent studies focus on using rodent models to understand the effects of exposure to specific plastic polymers (da-Silva Brito *et al.,* 2022). Albino mice (*Mus musculus*) are commonly used as a standard model for evaluating health risks associated with ecological impurities due to their well-preserved metabolic and physical features, rapid responsiveness, and sensitivity to toxic effects of environmental chemicals, accessibility of well-designed genome, physio-anatomical similarities with humans, and a comparable metabolic profile (Kararli, 1995). Due to their potential health risks, MPs are administered in mice orally or through food to investigate their harmful impacts (Banerjee and Shelver, 2021). The present work aimed to evaluate how variable but consistent dietary exposure to PS-MPs affect the blood parameters and main organs of Albino mice. This involved examining the changes and abnormalities in blood and in tissues within organs such as liver, kidney, stomach, and intestines, caused by exposure to different levels of PS-MPs. Through hematological and histopathological analysis, the study aimed to provide insights into how these MPs may affect the health and integrity of blood and vital organs in Albino mice.

MATERIALS AND METHODS

Study animals and ethical approval: Seventy-five healthy adult Albino mice (n=75) aged (4-5) weeks and weighing between 25 to 30g were selected after thorough screening and physical examination from the animal breeding house of the University of Veterinary and Animal Sciences, Lahore. Ethical approval was obtained from the Ethical Review Committee of the University vide letter no. DR/176/12-04-2023. All the experiments followed the ethical guidelines for veterinary practice (Carbone, 2022).

Housing and management: Selected mice were acclimatized to laboratory conditions for one week in a controlled temperature room (29±3°C) with 50-70% relative humidity, regulated light and dark cycles (Jin *et al.,* 2019), and a thermostat-controlled heater to maintain optimal conditions and prevent stress. Bottom of each cage was covered with sterile sawdust, and the mice were provided with commercial feed pellets. The bedding was routinely changed every two days, and fresh sawdust was added to minimize stress and prevent infections.

Experimental setup: The mice were randomly assigned to five experimental groups with five mice per group. Triplicates were maintained for each group, hence fifteen mice per experimental group. Group 1 was the control group which was fed standard diet with no MPs added. Group 2 (Treatment 1) was given 0.1 mg MPs/gm of BW, group 3 or treatment 2 was given 0.2 mg MPs/gm of BW. Group 4 labelled as Treatment 3 and group 5 labelled as treatment 4 were administered 0.4 mg MPs/gm of BW and 0.8 mg MPs/gm of BW, respectively (Li *et al.,* 2020). The dose selection of MPs used in this study was based on previously published literature and intended to reflect a gradient of environmentally relevant levels to high exposure levels. PS-MPs in the form of micro fragments was utilized which were obtained by shredding disposable cutlery in a shredder. The composition of the product was confirmed through FTIR analysis as detailed below. The fragments were then passed through sieves of different mesh sizes and a size of 0.7mm was selected being the dominant size. The PS-MPs fragments were then incorporated into the standard feed containing crude protein content of 30%. Defined doses of PS-MPS were added in the feed pellets. Pellets were prepared with an average weight of 15 grams per five mice per day as mice feed on an average 3 grams per day (Kader *et al*., 2014).

FTIR analysis of PS-MPs: Fig. 1 represents the molecular fingerprint of the material, with peaks corresponding to the vibrational modes of the molecules. Here is a breakdown of the key features and typical peaks we might expect in the FTIR spectrum of polystyrene. Polystyrene has aromatic rings, and the C-H stretching vibrations of these rings typically appear in this region. We might see peaks around 3080-3020 cm^{-1}. Peaks in the aliphatic region correspond to the C-H stretching vibrations of the aliphatic hydrogen atoms in the polymer backbone. Commonly, these peaks appear around 2920 cm^{-1} and 2850 cm^{-1}. The vibrations of the aromatic ring appear as multiple peaks in this region, typically around 1600 cm^{-1} and 1450 cm^{-1}. Polystyrene shows characteristic out-of-plane bending vibrations of the aromatic C-H bonds in this range. Peaks around 750 cm^{-1} and 700 cm^{-1} are commonly observed.

The feeding trial was continued for a duration of thirty-five days in which the mice were daily provided with feed containing defined doses of PS-MPs. Body

weight was regularly noted for each mouse which had been tagged through dye for easy identification. No mortality was reported during the feeding trial. At the end of trial, the impact of different doses of PS-MPs (0.0mg/g, 0.1mg/g, 0.2mg/g, 0.4mg/g, 0.8mg/g) on the growth, hematology and histopathology of the experimental animals was observed. Hematological parameters, serum biochemistry parameters (LFTs, RFTs and Serum electrolytes) were determined for each experimental group. Histopathological investigation of vital organs of mice (liver, stomach, intestine, and kidney) was also carried out to determine how MPs ingestion affected organ integrity.

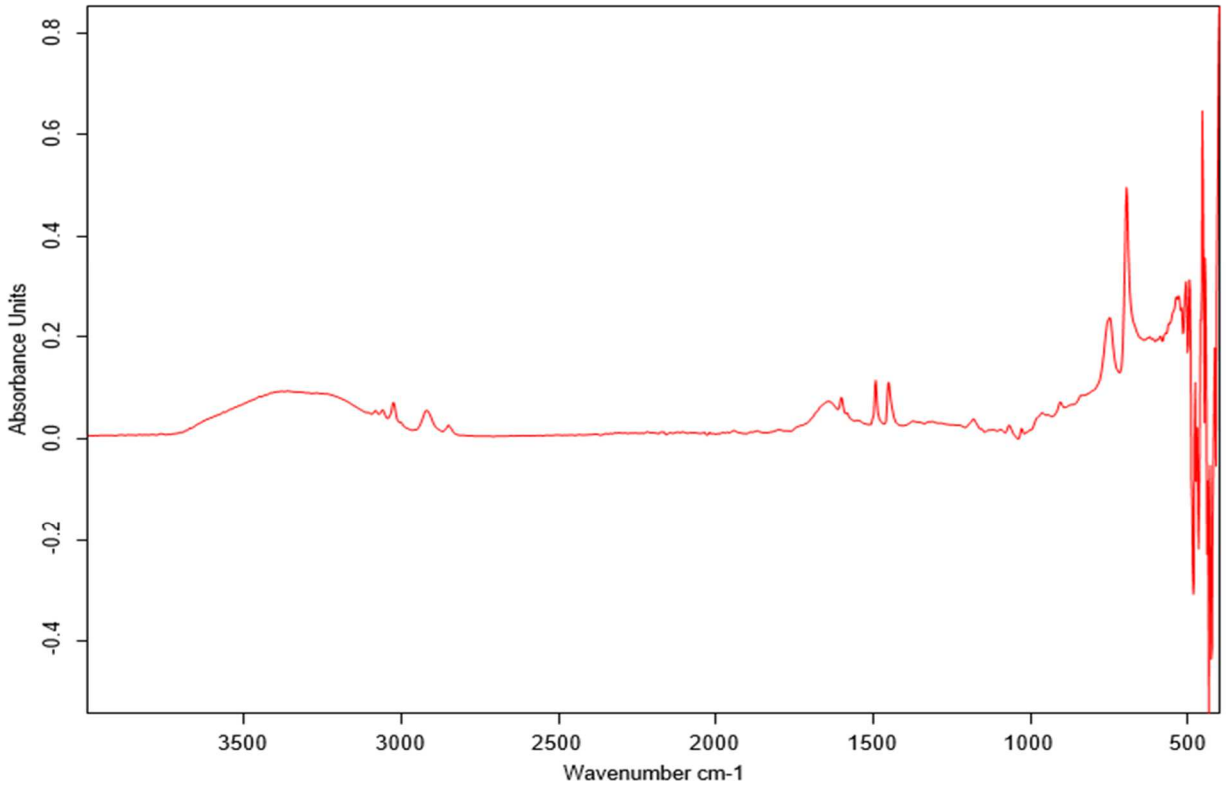


Fig. 1. FTIR analysis of PS-MPs

Hematotoxicity: Following the conclusion of the trial, a volume of 2ml of blood was extracted from each animal within the experimental cohort utilizing a sterile needle. The extracted blood samples were carefully transferred into clean EDTA tubes to inhibit coagulation. Subsequently, these samples were transported to the University Diagnostic Laboratory, UVAS Lahore for comprehensive analysis. Parameters such as RBCs, WBCs, lymphocytes, monocytes, granulocytes, Packed Cell Volume (PCV), Hemoglobin (Hb), Mean Platelet Volume (MPV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were assessed using the URIT-2900 Vet Plus Automated Hematology Analyzer (URIT Medical Electronics, China). This analysis employed specific reagents provided by URIT Medical Electronics, located in Guangxi541001, PR China.

LFT (liver function test, and RFT (renal function test) were analyzed by automatic analyzer. The levels of various biochemical markers including Total Bilirubin, ALT, AST, Alkaline Phosphatase, Total protein, Albumin, A/G Ratio, Serum Creatinine, Urea, Sodium, Potassium, and Chloride in both control and experimental groups were determined using calorimetric tests conducted on an automatic biochemistry analyzer (BK-200, Biobase China).

Histopathological analysis: Following the conclusion of the feeding trial, the animals underwent anesthesia with chloroform, dissected and organs (Kidney, liver, spleen, lungs, stomach, small intestine and large intestine) excised. The organs were fixed using Conroy fixative, a solution comprising 85% absolute alcohol, 10% formaldehyde, and 5% glacial acetic acid (Beely *et al.,* 2016). Subsequently, the organs underwent dehydration, progressing through different concentrations of alcohol, namely 50%, 70%, 90% and 100% for 24 hours each. A xylene solution was applied for 5-6 hours to facilitate clearing the organs, followed by embedding them in molten paraffin wax (50^0C-58^0C) for 3-5 hours. Paraffinized blocks of organs were prepared using glass cavities of various sizes. Serial sections of 2 µm thickness were acquired using a rotatory microtome. These sections were extended onto glass slides coated with albumen, rehydrated with a descending series of alcohol grades, and stained with hematoxylin for the sections and eosin for counter staining (Luna, 1968). Dehydration of slides was carried out using ascending alcoholic grades, and sections were affixed using Canada balsam before

covering with a cover slip. Prepared histopathological slides were examined under a microscope at magnification of 40X, 100X, and 400X.

Statistical analysis: Alterations induced by the independent variable (Inclusion rate of PS-MPs) on dependent variables i.e. growth, blood parameters (haematology, serum biochemistry) of mice were measured by one-way ANOVA technique (Steel and Torrie, 1980) using SPSS 27.0. Following this analysis, LSD multiple comparison test was conducted by using the SPSS statistical package 27.0, with a 95% confidence interval. Changes in different organs (liver, stomach, small intestine, large intestine, kidney, spleen) of mice were measured by descriptive analysis.

RESULTS

Key findings from the net weight gain, hematological parameters, serum biochemistry and histopathological results are highlighted below.

Growth of mice: Regular monitoring of the body revealed no significant weight loss in control group and treatment 1, 2 and 3 all of which demonstrated modest weight gain over a period of five weeks. However, treatment 4 shows a significant decrease in the weight of mice after 5 weeks (Fig 2). This indicates that the higher doses are associated with a decrease in the overall weight of mice.

Blood profile: The results of the hematological analysis indicated that an increase in MP concentration had a significant impact on the evaluated parameters at a significance level of 0.05. The level of RBCs and hemoglobin decreased in treatment 4 as compared to the control group while parameters indicative of inflammation and other underlying health issues such as WBCs, HCT, MCH, PCT, and MPV were found to be elevated in experimental group treated with 0.8 mg/kg PS-MPs. Furthermore, the serum electrolyte levels were found to decrease significantly with an increase in MP level given to each experimental group (Table 1).

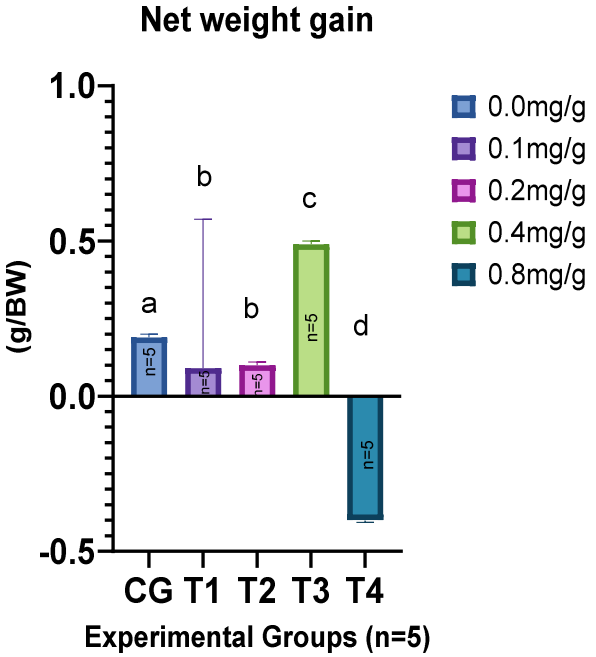


Fig. 2. Effect of different doses of PS-MPs upon body weight of albino mice over a duration of thirty-five days. [a-b]Means without common superscript differed significantly (P<0.05).

Table 1: Hematology and serum biochemistry levels for each experimental group after feeding on PS-MPs for thirty five days

	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
	Mean+SD	Mean+SD	Mean+SD	Mean+SD	Mean+SD
Hematological parameters					
RBC	11.14+1.21[a]	8.67+0.66[b]	8.88+0.20[b]	4.89+0.24[c]	9.07+5.10[a,b,c,]
WBC	5.33+4.03[a]	10.46+1.31[a]	21.62+15.97[a]	9.06+3.26[a]	14.39+6.27[a]
Hb	21.13+10.22[a]	15.40+5.49[a]	11.51+2.38[a]	7.03+2.88[a]	13.72+6.08[a]
HCT	58.92+46.35[a]	55.45+23.34[a]	75.47+30.21[a]	27.81+16.13[a]	72.66+26.06[a]
MCV	46.03+27.84[a]	31.68+18.19[a]	42.96+13.49[a]	71.90+35.43[a]	83.89+66.29[a]
MCH	11.47+9.05[a]	18.27+9.93[a]	16.39+7.17[a]	11.53+7.51[a]	23.10+10.00[a]
MCHC	24.84+12.76[a]	50.53+16.40[a]	19.64+13.36[a]	28.27+11.17[a]	37.41+28.78[a]
Platelet	561.81+307.03[a]	584.24+513.01[a]	1480.82+740.61[a]	329.48+139.49[a]	1239.15+824.65[a]
RDW(CV)	11.48+12.23[a]	25.97+17.71[a]	17.55+8.73[a]	22.68+10.32[a]	16.61+6.15[a]
RDW(SD)	53.86+10.05[a]	44.17+29.10[a]	28.38+17.57[a]	42.87+26.47[a]	31.47+13.63[a]
PCT	0.24+0.01[a]	0.31+0.04[a]	0.48+0.07[a]	0.25+0.03[a]	0.54+0.04[b]
MPV	6.22+3.31[a]	6.92+3.73[a]	7.37+3.71[a]	6.67+0.95[a]	9.96+3.13[a]
PDW	19.25+15.25[a]	22.31+8.52[a]	16.41+3.78[a]	19.72+3.04[a]	12.46+10.53[a]
Serum biochemistry parameters					
Serum Sodium	129.64+67.49[a]	106.90+74.25[a]	136.65+84.48[a]	137.88+66.81[a]	88.86+53.26[a]
Serum Potassium	11.11+2.68[a]	7.1758+4.41[a,b]	9.27+4.04[a,b]	8.36+3.10[a,b]	3.90+1.75 [b]
Serum Chloride	122.56+95.24[a]	105.70+86.98[a]	161.20+118.54[a]	189.80+65.66[a]	99.44+57.92[a]

Note: The comparison is within rows. [ab] Means that values not sharing common superscript differed significantly (P<0.05).

Table 2. The performance of liver and kidneys after being fed upon varying doses of PS-MPs in Albino mice.

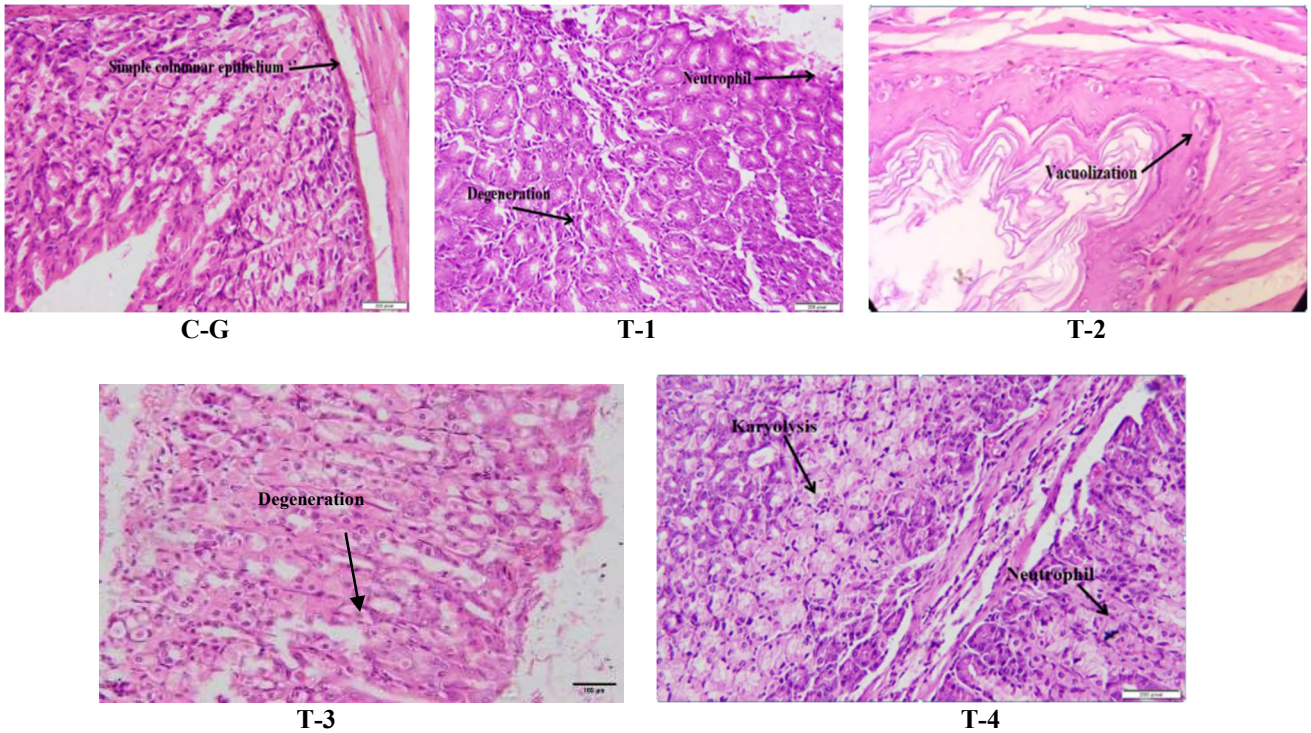
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Parameters	Mean+SD	Mean+SD	Mean+SD	Mean+SD	Mean+SD
LFTs					
Bilirubin total	0.49+0.01^{a}	0.7614+0.05^{b}	0.50+0.00^{a}	0.49+0.00^{a}	0.50+0.00^{a}
ALT	8.72+3.78^{a,b}	37.82+16.76^{a}	15.89+6.43^{a,b}	6.84+6.16^{b}	12.57+4.30^{a,b}
AST	9.69+3.70^{a}	113.61+60.25^{a}	96.71+78.78^{a,b}	34.31+10.96^{b}	124.70.+92.11^{a,b}
Alkaline Phosphatase	7.38+5.06^{a}	7.14+4.56^{a}	5.18+3.19^{a}	7.78+1.91^{a}	3.60+1.87^{a}
Protein Total	5.39+2.83^{a,c,d}	3.82+1.58^{a,b}	2.64+0.43^{a,c}	0.77+0.03^{d}	2.83+0.66^{b,c,e}
Albumin	3.78+1.15^{a,c}	4.37+2.64^{a,b,c}	1.84+0.07^{a}	0.43+0.05^{b}	2.29+0.06^{c}
A/G Ratio	3.63+0.80^{a}	5.96+1.86^{a}	2.90+0.84^{a}	2.34+1.93^{a}	3.15+1.52^{a}
RFTs					
Serum Creatinine	0.40+0.02^{a}	0.43+0.03^{a}	0.39+0.01^{a}	0.39+0.01^{a}	0.40+0.01^{a}
Serum Urea	2.85+0.04^{a}	1.17+0.05^{b}	1.19+0.05^{b}	1.19+0.05^{b}	1.22+0.04^{b}

Note: The comparison is within rows. ^{ab} Means that values not sharing common superscript differed significantly (P<0.05).

Histopathological analysis: Histopathological analysis of different organs indicated that the control group exhibited no tissue damage. However, an increase in the administrated PS-MPs dose led to noteworthy damage to the structural integrity of the tissues. Histopathological findings for each selected organ are discussed below:

Digestive tract: Sections of stomach, small and large intestine were prepared for histological examination as described in figures 3a, 3b and 3c.

Stomach: Significant histological alterations observed in stomach of mice from various experimental groups (Fig 3a). The control group exhibited intact simple columnar epithelium indicating no pathological changes. In contrast, treatment group 1 (T1) which was exposed to the lowest dose (0.1mg/g) of PS-MPs showed degeneration. Treatment Group 2 (T2) that was exposed to medium dose (0.2mg/g) indicated vacuolization. Meanwhile, treatment group 3 (T3) given a higher dose (0.4mg/g) showed the degeneration at some points. On the other hand, dense neutrophil and karyolysis was observed in the group treated with the highest dose (0.8mg/g) of PS-MPs.



C-G **T-1** **T-2**

T-3 **T-4**

Fig. 3a. Histological investigation of sections of stomach of mice in different experimental groups fed with varying doses of PS-MPs (Hematoxylin and Eosin stain). The slides were observed at 40X.

Small and Large intestine: Histological changes in both the small and large intestine were observed in mice treated with different doses of PS-MPs. The control group and treatment group 1 (T1) showed no significant morphological changes, indicating preserved intestinal tissue histology. Treatment group 2 (T2) treated with

0.2mg/g dose of PS-MPs showed mild hemosiderin in the small intestine while karyolysis in the large intestine. Likewise, the degree of damage increased in treatment group 3 (T3) given medium dose (0.4mg/g) demonstrating infiltration in the small intestine visible at some points and necrosis in the tissues of the large intestine. In contrast, hyperplasia was observed in sections of the small and large intestine at the highest dose of PS-MPs (0.8 mg/g) in treatment group 4 (T4) (Fig 3b, fig 3c).

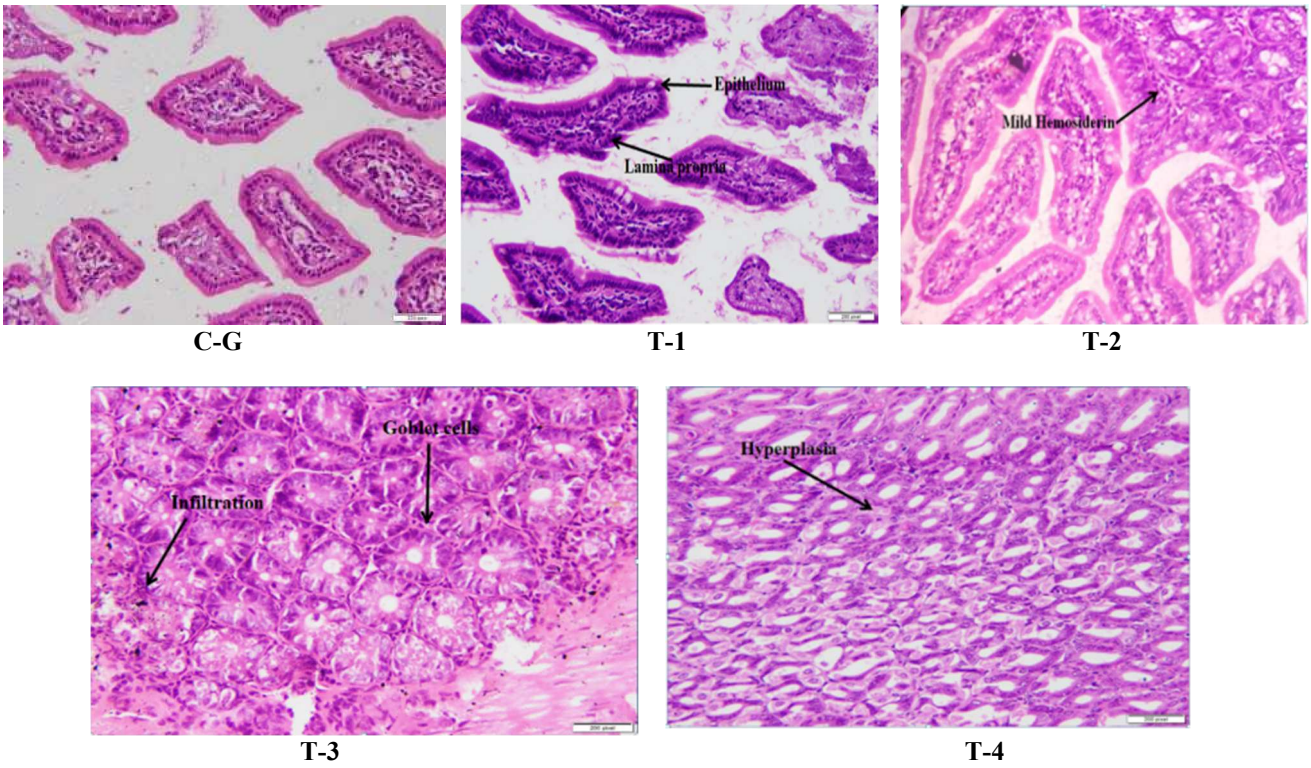


Fig 3b: Histological investigation of sections of small intestine of mice in different experimental groups fed with varying doses of PS-MPs (Hematoxylin and Eosin stain). The slides were observed at 40X.

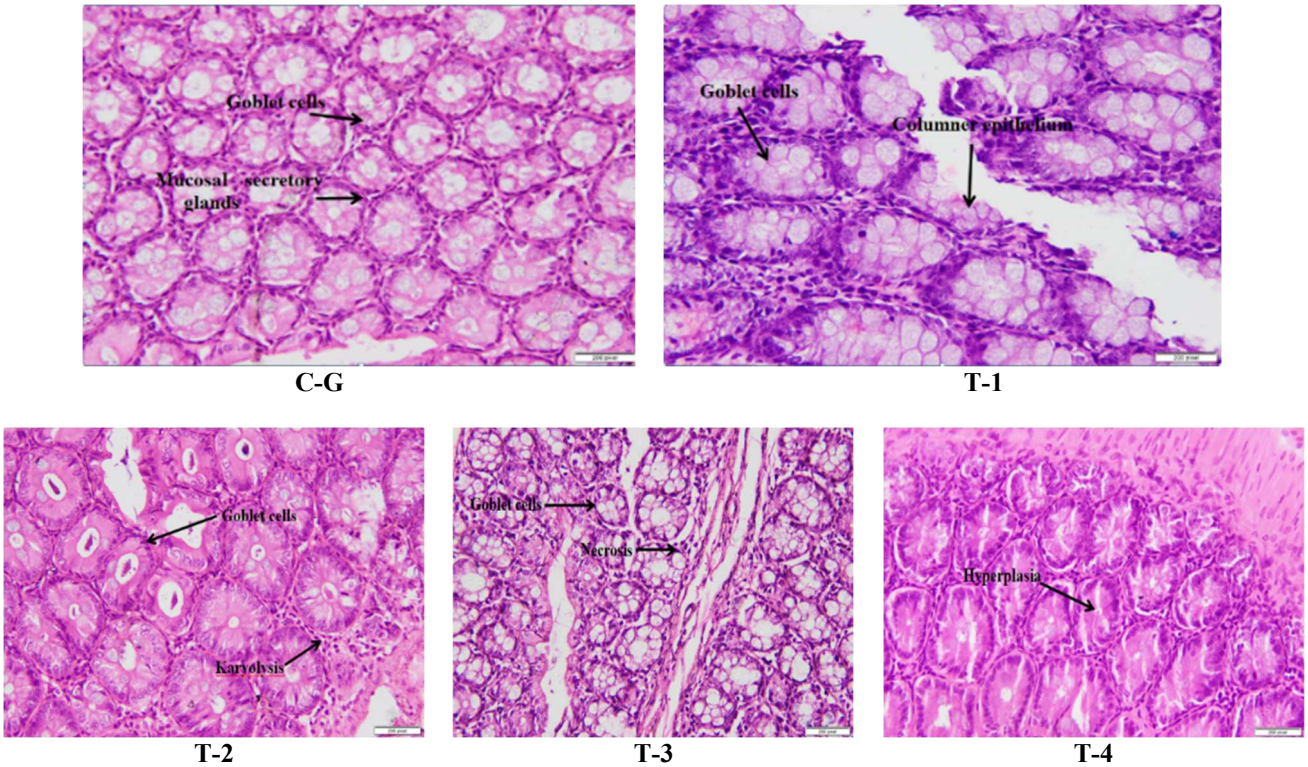


Fig 3c: Histological investigation of sections of the large intestine of mice in different experimental groups fed with varying doses of PS-MPs (Hematoxylin and Eosin stain). The slides were observed at 40X.

Kidney: Several histopathological alterations were observed in kidney tissues after administration of different concentrations of PS-MPs. (Fig 4). Control group exposed no histopathological changes while treatment group 1 (T1) treated with 0.1mg/g of PS-MPs showed vacuolization. In contrast, treatment group 2 (T2) medium dose (0.2mg/g) of PS-MPs exhibited cellular hypertrophy. Treatment group 3 (T3) given high dose (0.4mg/g) of PS-MPs demonstrated interstitial fibrosis. Whereas, infiltration was observed in the treatment group 4 (T4) treated with highest dose (0.8mg/g) of PS-MPs

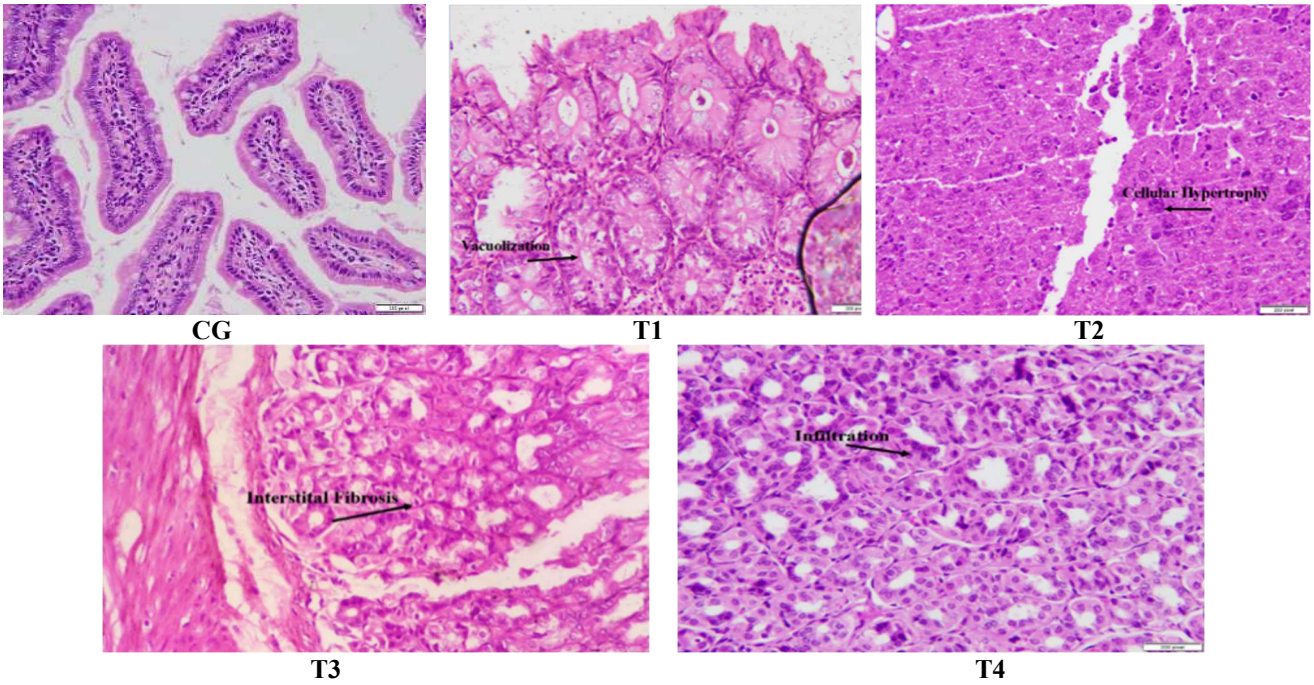


Fig 4: Histological investigation of sections of kidneys of mice in different experimental groups fed with varying doses of PS-MPs (Hematoxylin and Eosin stain). The slides were observed at 40X.

Liver: In the liver, histopathological analysis revealed significant histological alteration in different treatment groups in a dose-dependent manner. The control group showed normal nucleoli and hepatocytes. In treatment group 1 (T1) intact lymphocytes and neutrophils were observed. Treatment group 2 (T2) given medium dose (0.2mg/g) of PS-MPs showed karyolysis indicating nuclear degeneration. Meanwhile, treatment group 3 (T3) exposed to high dose (0.4mg/g) of PS-MPs demonstrated vacuolization and hyperplasia at some points. In contrast, treatment group 4 (T4) which received the highest dose (0.8mg/g) of PS-MPs indicated dense lymphocytes and congestion.

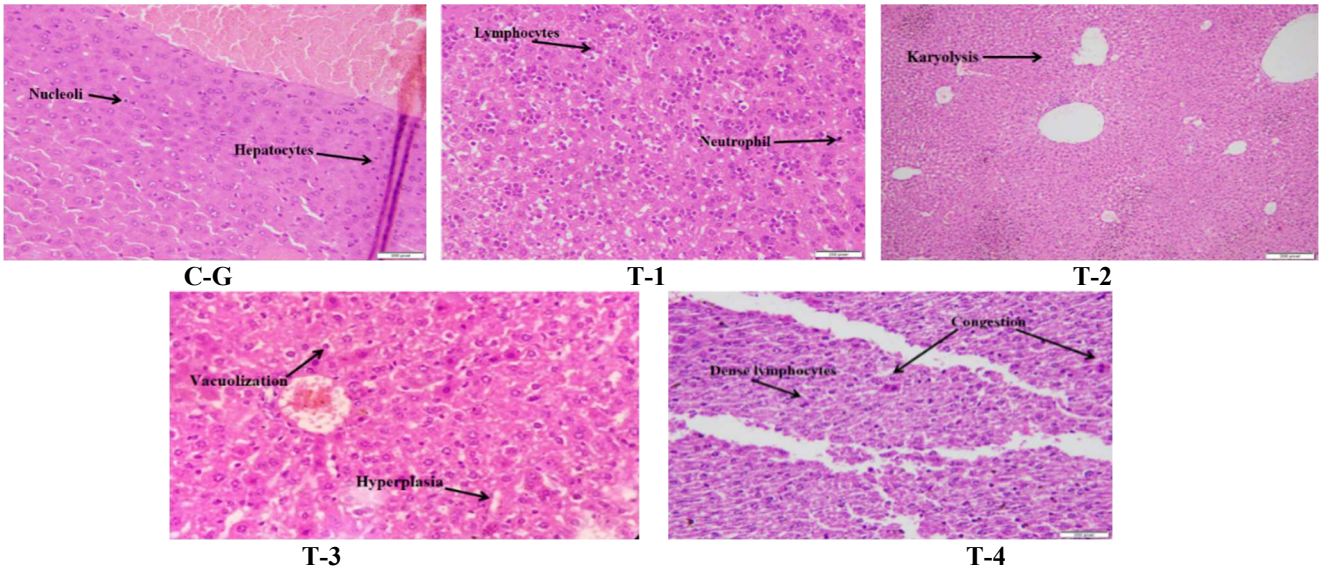


Fig. 5. Histological investigation of sections of liver of mice in different experimental groups fed with varying doses of PS-MPs (Hematoxylin and Eosin stain). The slides were observed at 40X.

Spleen: Histological analysis of spleen tissue revealed distinct morphological changes in treatment groups as compared to control group. The control group showed a typical structure of lymphocytes indicating normal histological integrity. Similarly, treatment group 1 (T1) administered 0.1mg/g of PS-MPs showed standard lymphocytes structure. In treatment group 2 (T2) given the medium dose (0.2mg/g) of PS-MPs, atrophy was observed. Treatment group 3 (T3) receiving 0.4mg/g of PS-MPs exhibited fibrosis. Hyperplasia and necrosis was observed in treatment group 4 (T4) which was exposed to the highest dose (0.8mg/g) of PS-MPs. (Fig 6).

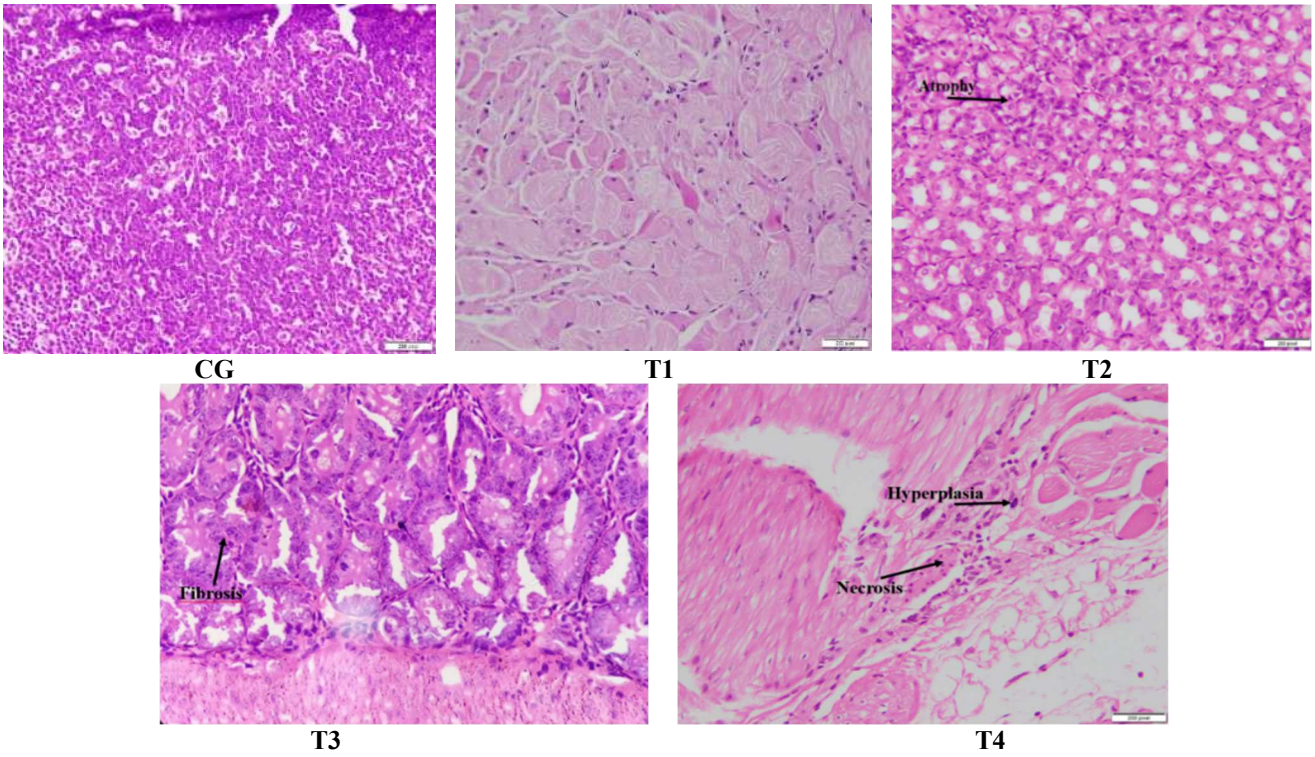


Fig. 6: Histological investigation of sections of spleen of mice in different experimental groups fed with varying doses of PS-MPs (Hematoxylin and Eosin stain). The slides were observed at 40X.

DISCUSSION

The issue of plastic pollution has garnered global attention due to the potential entry of MPs into the human body through inhalation, ingestion, or dermal contact within the food chain. In our current investigation, we have illustrated that orally administrated PS-MPs can traverse the circulatory system, digestive, excretory, immune, and respiratory systems of mice, accumulating in blood and in various vital tissues of the gastrointestinal tract (stomach, small intestine, large intestine) and in liver, kidney, and spleen (da-Silva Brito *et al.,* 2022). Our study highlighted a dose-dependent impact of PS-MPs, with the extent of hematological and histopathological changes varying based on exposure dosage. The results indicated that histopathological alterations were evident in all groups exposed to PS-MPs except the liver, spleen, and both intestines of treatment group 1 fed on the lowest dose of PS-MPs. Nevertheless, hematological and histopathological alterations became more noticeable with an increase in dosage. The vital organs of mice revealed necrosis, karyolysis, vacuolization, congestion, hemosiderin, infiltration, hyperplasia, inflammation, hypertrophy, atrophy, fibrosis, and degeneration of hepatocytes, and the intensity increases with increasing doses of PS-MPs as evident in the figures 2-5. There was an alteration in blood parameters and in serum biochemistry of mice when exposed to PS-MPs and previous reports have also indicated the accumulation and resulting damage caused by PS-MPs in the intestine, liver, and kidneys of mice (Wang *et al.,* 2021; Cheng *et al.,* 2023).

The administration of PS-MPs and PS-NPs in a previous study reported weight loss in mice, elevated mortality rate, markedly altered various biomarkers, and induced histological damage to the kidneys (Meng *et al.,* 2022b). However, in our findings, weight loss was evident only in the group of mice having the greater concentration of PS-MPs. Literature review indicated that upon prolonged exposure, MPs induce changes in absorption, metabolism, excretion, and distribution, potentially causing unforeseen environmental harm. MPs can lead to issues such as clogging of the digestive system, internal injuries including ulcerative lesions and perforated gut, disruptions to the immune system, altered enzyme activity, oxidative stress, hepatic stress, necrosis, and in severe cases, possibly resulting in fatality (Rochman *et al.,* 2013; Pittura *et al.,* 2018; Limonta *et al.,* 2019; Zhang *et al.,* 2019; Wang *et al.,* 2021). Furthermore, the presence of PS-MPs caused histopathological harm to the colonic mucosa in mice

experiencing an imbalance in intestinal immunity. This phenomenon might be attributed to the notable rise in the accumulation of PS-MPs caused by intestinal barrier damage in these mice with disrupted intestinal immune function (Liu *et al.,* 2022). Our study has similar findings of disturbed intestinal barrier that leads to hemosiderin, infiltration, and hyperplasia in the small intestine among subjects in treatment groups 2, 3, and 4. In contrast, the large intestine exhibits karyolysis, necrosis, and hyperplasia in groups subjected to higher doses. Exposure to elevated concentrations of PS-NPs can induce apoptosis in the liver of *M. albus* and disrupt the immune response and structure of the intestinal bacterial community. Consequently, this disruption leads to dysbiosis in the intestinal flora (Zhou *et al.,* 2024). Our results yield similar outcomes, inducing apoptosis in the liver and disrupting intestinal functions. The structural integrity of the intestinal tissue in mice exposed to PS-MPs at concentrations of 1 and 10 mg/l exhibited notable abnormalities, including villus erosion, reduced crypt numbers, and extensive infiltration of inflammatory cells (Li *et al.,* 2024). However, no such abnormalities were seen in our results. In individuals having intestinal disorders, the inflammation in the intestines results in heightened tissue permeability. This, in turn, substantially enhances the efficiency of microplastic transport (Schmidt *et al.,* 2013).

It has been observed that PS-MPs shift the mode of cell death from apoptosis to necrosis by elevating Caspase 8 levels. This disruption then extends to the intestinal vascular barrier, causing intestinal flora disturbances and facilitating the accumulation of lipopolysaccharide. This can hence lead to the translocation of detrimental flora and metabolites to the liver through the liver-gut axis, triggering hepatic immune responses and fostering disorders in hepatic lipid metabolism and apoptosis (Yin *et al.,* 2023). The intestinal tissues of largemouth bass (LMB) accumulated PS-NPs, leading to a substantial reduction in both feeding and growth rates. Histopathological analysis revealed varying degrees of structural damage to the intestine and liver of LMB caused by PS-NPs (Liao *et al.,* 2022). In the current study, mice administered with 0.4 and 0.8mg/g of PS-MPs showed similar damage in both the liver and intestine. Furthermore, administrating PS-NPs orally for two weeks leads to significant accumulation in the liver, kidneys, and intestine of ICR mice. This administration also triggers pronounced inflammatory and oxidative stress responses (Choi *et al.,* 2021). The present study assessed hepatotoxicity and gastrointestinal toxicity by examining the histopathological structures in albino mice treated with PS-MPs. After being given MPs orally for 28 days, mice experience inflammation in the lungs (Lee *et al.,* 2022). Our study describes the similar results in the mice with 0.4mg/g dosage, causing inflammation in the lungs. PS-MPs exposure for 5 weeks lead to inflammation and cell apoptosis in spleen of the Japanese quail (Zhang *et al.,* 2024). Our research has discovered similar results across treatments 2, 3, and 4. Mice that received an oral administration of 50nm PS-NPs exhibited accumulation in various organs, with concentration of 37.4 mg/g tissue in the kidney, 52.8 mg/g tissue in the heart, 98.3 mg/g tissue in the stomach wall, and 94.4 mg/g tissue in the small intestinal wall (Walczak *et al.,* 2015). These results align with our study, where we observed that a higher dosage of PS-MPs with a size of 0.7mm accumulated in the liver, stomach, small intestine, and large intestine. Park *et al.,* (2020) conducted research involving the exposure of albino mice to MPs over a period of 90 days. It was observed that the body weight of mice in the experimental groups experienced a significant decrease following exposure. Moreover, the study revealed an increase in the production of WBC's which notably affected the lymphocytes of mice which is similar to our studies. Hamed *et al.,* (2019) studied the effect of MPs on biomarkers in the blood of Nile Tilapia. The hematological indices including RBC's, Hb, HCT, MCHC, Platelets, WBCs, and monocytes exhibited a notable decrease following a 15-day exposure to MPs compared to the control group, whereas MCV and MCH displayed a significant increase during the same period of exposure. Our own research showed similar results, but the production of WBCs increased after MPs exposure for 35 days. However, based on our research, it seems that the oral administration of PS-MPs results in noticeable accumulation in the blood, kidney, spleen, liver, stomach, and intestine of albino mice, leading to increased hematological and histopathological factors.

Conclusion: In conclusion, our findings demonstrate that PS-MPs significantly harm *Mus musculus,* with doses of 0.4 and 0.8 mg/g causing notable toxicity in the gastrointestinal tract, liver, spleen, and kidneys. These adverse effects include blood and serum biochemistry alterations, inflammation, tissue structure changes, and potential disease onset, highlighting the critical need for mitigating microplastic pollution and further investigating its health impacts.

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