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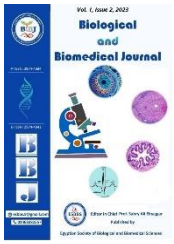

Long-term administrations of microplastics induces hepatorenal and intestinal tissues damages in experimental mice

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ABSTRACT

Microparticles (MPs) are well recognized as a global concern arising from plastic waste. MPs can cause various environmental and health problems. This study was conducted to evaluate the effect of the administrations with MPs for short and long terms on the pathophysiological and histological status of hepatorenal and intestinal tissues in mice. Forty (40) male CD-1 mice were divided into 4 groups (n = 10) as follows; the first group (Gp1) was served as a negative control, administered orally with 200 µl of dist. H_2O. Gp2 was administrated orally daily with MPs (66.4 mg/kg b. wt) for 28 days. Gp3 was administered as in Gp1 for 120 days and used as a negative control for Gp4. Gp4 was administrated with MPs as in Gp2 orally for 120 days. All groups were sacrificed, and blood samples, liver, kidney, and intestinal tissue samples were collected for haematological, biochemical, and histopathological analysis. The results showed that the short-term administration of MPs did not show significant changes on the haematological, biochemical parameters, histological investigations of the hepatorenal and intestinal tissues, however, administration for long term led to significant changes pathophysiological and histopathological alterations in the liver, kidney, and intestinal tissues. In conclusion, administration of MPs for long term could be harmful on the vital organs

Keywords:

Short-term, Long-term, Microplastics, Pathophysiology, Histopathological changes.

1. Introduction

Plastic waste is a major global concern. Microparticles (MPs), and nanoparticles affect different ecosystems and human health (Nor *et al*., 2021; Ray *et al*., 2022). MPs particles usually less than 5 mm in diameter. The great majority of MPs pollution originates from land, where MPs end up in wastewater treatment are not fully filtered out before they escape to the sea (Horton *et al*., 2017). MPs particles divided into two types, primary and secondary. Primary MPs are key ingredients in scrubs, handwashing soaps, toothpastes, and biomedical products. Secondary MPs are plastics originating from the fragmentation of larger plastic items. Examples include fibers from synthetic clothing, and fragments of items such as plastic bags and bottles (Burns and Boxall, 2018). Interests have shifted towards understanding which impacts MPs may have on the organisms they are found and assessing the physical impacts of MPs presence in the gastrointestinal tract (Kramm and Völker, 2018).

MPs pollution is an environmental concern that poses a risk to human health (Alfaro-Núñez *et al*., 2021). Human exposure to MPs is recognized as a global problem. Variability and lifetime accumulation of MPs are unresolved depending on their size, shape, and functional

group chemistry. Ingested MPs can cause various problems for human, animals and plants (Nor *et al*., 2021). Gastrointestinal dysmotility or obstruction were reported due to undigestible properties of MPs (Ray *et al*., 2022). Most MPs consumed by humans may found in food. The effects of MPs on human health were addressed (Chae and An, 2018). MPs less than 1.5 µm can damage cells directly and were able to penetrate tissues (Jiang *et al*., 2020; Yuan *et al*., 2022). From 1% to 4% of MPs in the intestine might migrate to the bloodstream and showed toxic effects. Human may experience oxidative stress, cytotoxicity, neurotoxicity, immune system disruption, and transfer of MPs to other tissues after being exposed to them (Bhuyan, 2022). MPs may cause mechanical damage to the digestive tract and malnutrition (Kramm and Völker, 2018), and found to inhibit food assimilation, reduce body weight, and negatively impact growth and thus reproductive fitness (Straub *et al*., 2017). This study was conducted to evaluate the pathophysiological consequences upon MPs administration on hepatorenal and intestinal tissues of mice after short- and long-term administration.

2. Materials and Methods

Chemicals

Liver transaminases (Aspartate transaminase, AST), (alanine transaminase, ALT), urea, creatinine, triacylglycerol (TG), total cholesterol (TC), alkaline phosphatase (ALP), alpha-fucosidase (FUCA), arginase (ARG), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) kits were purchased from the Bio-diagnostic Company in Egypt.

Experimental animals

Forty male CD1 mice (17 ± 4 g) allowed to acclimate for 1 week in the animal house conditions of the Faculty of Science, Tanta University, before being divided into groups. The institutional animal care committee at Zoology Department, Faculty of Science, Tanta University- Egypt, approved the experimental design (Number IACUC-SCI-TU-0220). Target values for temperature and relative humidity were about 22 ± 1ºC and 55 ± 5% respectively, light- dark (day/night) cycle was achieved. Mice were given drinking tap water and normal experimental pelleted animal food *ad libitium*.

Preparation of microplastics

To prepare the microplastics (MPs) particles, five empty clean autoclaved plastic bottles were used. Briefly, these bottles were cut down into small plastic pieces, then these pieces were further manipulated to produce tiny parts. These parts shredding into very tiny particles. These particles were sieved through a specific strainer to collect the MPs that have very small sizes.

Characterization of microplastics with dynamic light scattering (DLS) technique and scanning electron microscope (SEM)

For MPs characterization, dynamic light scattering (DLS), and scanning electron microscope (SEM) techniques were used to provide high sensitivity and high-resolution size. The Nicomp DLS system measures size and zeta potential of nano sized particles from 0.3 nm to 10 µm.

Samples were dried for SEM utilizing a critical point drying machine with SPI supplies. in an SPI-Module TM Vac/ Sputter, mounted on aluminum stubs, and coated with gold. Lastly, Tanta University in Japan used a scanning electron microscope, model JSM-5200LV, for photography.

Experimental protocols

Mice were divided into four groups (10 mice /group) according to body weights to minimize the standard errors between groups into two experiments as follows: Group 1 (Gp1): Negative control, mice had administrated orally by 200µl H_2O daily for 28 days. Gp2: Mice had administrated orally with (66.4 mg/kg b. wt) MPs in 200 µl H_2O for 28 days. Gp3: Negative control, mice had administrated orally by 200µl H_2O daily for 120 days. Gp4: Mice had

administrated with (66.4 mg/kg b. wt) MPs in 200 µl H_2O orally for 120 days.

Determination of the percentage of the body weight changes.

Initial body weight (I.B.Wt) and final body weight (F.B.Wt) were determined. The percentage of body weight changes (% B.wt) was calculated using Eq 1. B.wt (%) = {(F.B.Wt-I.B.Wt)/I.B.Wt} x100.

Hematological and Biochemical investigations

Complete blood count (CBC) was counted by the automated method using Dirui BCC-3600, MA, USA automated hematology analyzer.

Aspartate aminotransferase (AST) activity was assayed by using commercial kit according to the method of Reitman and Frankel (1957). Urea in serum was assayed according to the method of Thomas, (1998). Creatinine was assayed according to the method of Lamb *et al*. (2006). Triacylglycerol (TG) was determined according to the method of Fossati and prencipe, (1982). Total cholesterol (TC) was determined according to the method of Allain *et al*., (1974). CAT was assayed according to the method of Aebi, (1984). SOD was assayed according to the method of Nishikimi *et al*. (1972). ALP activity was determined according to Belfield and Goldberg, (1971). Arg activity was determined according to Corraliza *et al*. (1994). The activity α–FU was determined according to Zielke *et al*. (1972).

Preparation of liver, kidney, and intestine sections for histological investigation

Small parts from the liver, kidney, small, and large intestine were preserved in 10% phosphate-buffered formalin, dehydrated in graded alcohol series, cleared in xylene, and embedded in paraffin blocks. 4-5 µm sections of the collected sections were stained with hematoxylin and eosin for histopathological examination under a light microscope (Optica light microscope, B-350) (Bancroft and Gamble, 2008).

Immunohistochemical procedure for PCNA and Bcl-2

Paraffin sections of small and large intestine were prepared for staining with monoclonal antibodies against Bcl-2 PCNA or Bcl-2 (Thermo Fisher Scientific Industries). These markers are developed in small and large intestine by avidin biotinylated horseradish peroxidase complex ABC technique and expressed as a color reaction that was developed by using diamino–benzidine (DAB) and gave a brown color. Hematoxylin was used for counterstaining **(Hsu *et al*., 1981).**

Statistical analysis

All data are the means of 3 replicates. The data were expressed as mean ± SD. Comparison between groups was carried out using one-way ANOVA. If there a significant difference between means, Tukey post hoc comparisons among different groups were performed. For all statistical tests P values < 0.05 was statistically significant. Data and statistical analysis were performed using Excel 2013 (Microsoft Corporation, USA), and Minitab version 18

3. Results

Characterization of the microplastics (MPs)

As shown in the materials and methods section, the microplastics (MPs) were prepared by shredding the clean empty into very tiny particles. These particles were sieved through a specific strainer to collect the MPs that have very small size (Fig. 1A). The dynamic light scattering (DLS) analysis showed that the MPs size was approximately 0.9 µm (Fig. 1B). Furthermore, the scanning electron microscope (SEM) investigation showed that the MPs size was 0.5 µm (Fig. 1C).

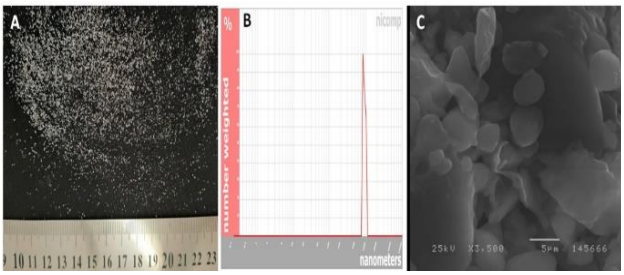

Fig. (1A-C). Photomicrographs shows the size of MPs that were used in the study.

The body weight changes

The results showed that as compared to control groups, mice that were administered with MPs daily for 28-days (Short-term) did not show a

significant alteration ($p > 0.05$) in the relative body weigh changes (% B. wt). Compared to the control groups, mice that were administered with MPs for 120 days (Long-term) showed a significant increase ($p < 0.05$) in the % B. wt changes (Fig. 2).

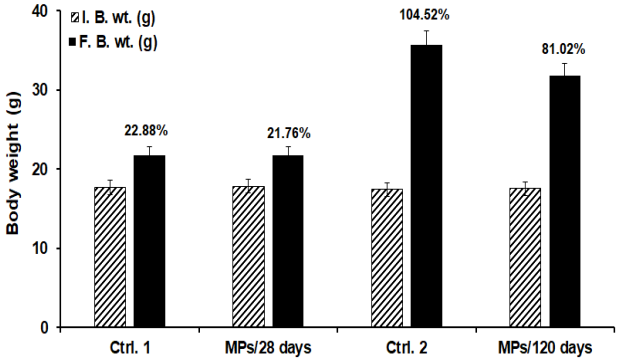

Fig. 2. Initial, final body weights, and the percentages of body weight changes in the different groups under the study. The values represented as means ± S.D; Ctrl. 1: Control of 28 days; MPs/28 days: sub-acute administrations of microplastics for 28 days; Ctrl. 2: Control of 120 days; MPs/120 days: Chronic administration of microplastics for 120 days.

Effect of administration with MPs on the hematological parameters

The total red blood cell (R.B.Cs) count, hemoglobin (Hb) level, and hematocrit (Hct) value were determined in all the groups under the study. As compared to the control group, the administration of the MPs for 28 or 120 days daily did not show any significant changes in the previous parameters. Also, the number of the total platelets count was significantly decreased ($p < 0.05$) in the group of mice that were administered the MPs for 120 days when compared to the control group (Table 1a). The results showed that there were no significant changes in the total white blood cells (W.B.Cs) count in the group of mice that were administered with MPs for 28 days when compared to their control ($p > 0.05$).

However, the percentage of neutrophiles and monocytes were significantly increased in the group of mice that were administered with MPs for 28 days when compared to their control ($p < 0.05$). The results showed that there was a significant change in the total W.B.Cs count in the group of mice that were administered with MPs for 120 days when compared to their control ($p < 0.05$). In addition, the percentage of lymphocytes was decreased and neutrophiles percentage was increased in the group of mice that were administered with MPs for 120 days when compared to their control ($p < 0.05$) (Table 1b).

Liver transaminases levels increased after short- and long-term administrations of microplastics.

The results showed that the administration of MPs for 28 days did not increase the level of the liver transaminases, AST and ALT significantly ($p > 0.05$) when compared to their control levels. The administration of mice with MPs for 120 days, however, increased the level of AST and ALT, significantly ($p < 0.05$) when compared to their control values (Fig. 3).

The results showed that the administration of MPs for 28 days did not increase urea, and creatinine when compared to their control levels. The administration with MPs for 120 days, however, increased the level of the urea and creatinine significantly ($p < 0.05$) when compared to their control values (Fig. 4).

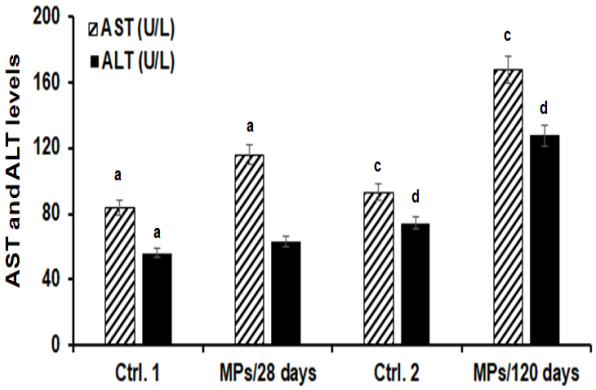

Fig. 3. Sera aspartate transaminases (AST) and alanine transaminases (ALT) in the different groups under the study. The values represented as means ± S.D.; Ctrl. 1: Control of 28 days; MPs/28 days: sub-acute administrations of microplastics for 28 days; Ctrl. 2: Control of 120 days; MPs/120 days: Chronic administration of microplastics for 120 days. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Table 1 a. The effect of sub-acute and chronic administrations of microplastics on R.B.Cs count, Hb, Hct percentage, and platelets count

Groups	R.B.Cs ($\times 10^6$/µL)	Hb (g/dL)	Hct (%)	Platelets ($\times 10^3$/µL)
Ctrl. 1	7.9 ± 0.6 [a]	12.2 ± 1.07 [a]	42.3 ± 2.9 [a]	1080 ± 62.6 [a]
MPs/28 days	7.5 ± 0.4 [a]	11 ± 1.38 [a]	38.1 ± 1.9 [a]	1052.5 ± 59.2 [a]
Ctrl. 2	8.5 ± 0.45 [a]	14.7 ± 1.35 [c]	48.2 ± 1.8 [a]	960.5 ± 70.5 [a]
MPs/120 days	9.3 ± 0.5 [d]	13.1 ± 1.1 [a,c]	46.2 ± 2.8 [a]	671.25 ± 49.2 [d]

The values represented as means ± S.D.; Ctrl. 1: Control of 28 days; MPs/28 days: sub-acute administrations of microplastics for 28 days; Ctrl. 2: Control of 120 days; MPs/120 days: Chronic administrations of microplastics for 120 days; RBCs: Red blood cells; Hb: Hemoglobin; Hct: Hematocrit. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Table 1b. The effect of sub-acute and chronic administrations of microplastics on W.B.Cs count, and its differential percentages

Group	W.B.Cs ($\times 10^3$/µL)	Lymph (%)	Neut. (%)	Mono. (%)
Ctrl. 1	4.4 ± 0.56 [a]	81 ± 0.89 [a]	16.03 ± 0.47 [a]	2.97 ± 0.42 [a]
MPs/28 days	5.18 ± 0.59 [a]	80.3 ± 0.73 [a]	14.65 ± 0.41 [b]	5.27 ± 1.2 [b]
Ctrl. 2	5.13 ± 1.96 [a]	79.00 ± 9.27 [a]	15.00 ± 6.38 [a]	6.00 ± 3.6 [a]
MPs/120 days	11.15 ± 1.2 [b]	71.50 ± 9.15 [b]	23.75 ± 4.27 [b]	5.75 ± 5.56 [a]

The values represented as means ± S.D.; Ctrl. 1: Control of 28 days; MPs/28 days: sub-acute administrations of microplastics for 28 days; Ctrl. 2: Control of 120 days; MPs/120 days: Chronic administrations of microplastics for 120 days; WBCs: White blood cells; Lymph: Lymphocytes; Neut: Neutrophiles; Mono: Monocytes. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Effect of the sub-acute and chronic administrations of microplastics on the SOD, CAT activities and MDA level

The results showed that the administration of MPs for 28 days did not change the SOD, CAT activities or MDA level when compared to their control values. The administration with MPs for 120 days, however, decreased the activities of SOD and CAT significantly ($p < 0.05$) when compared to their control values. In addition, the administration with MPs for 120 days, increased the level of MDA significantly ($p < 0.05$) when compared to their control values (Table 2).

Effect of the short- and long-term administrations of MPs on ALP, Arg, and α-FUC activities

The results showed that the administration of MPs for 28 days did not increase the ALP, Arg. and α- FUC activities significantly ($p > 0.05$) when compared to their control values. The administration of mice with MPs for 120 days, however, the activities of these enzymes were significantly increased ($p < 0.05$) when compared to their control values (Table 3).

3.6. Effect of the sub-acute and chronic administration of MPs on glucose, total cholesterol, and triglycerides levels

The results showed that the administration of MPs for 28 days did not alter the levels of glucose, TC, and TG significantly ($p > 0.05$) when compared to their control levels. However, the administration of mice with MPs for 120 days led to a slight increase in the level of glucose, TC, and TG when compared to their control values (Table 4).

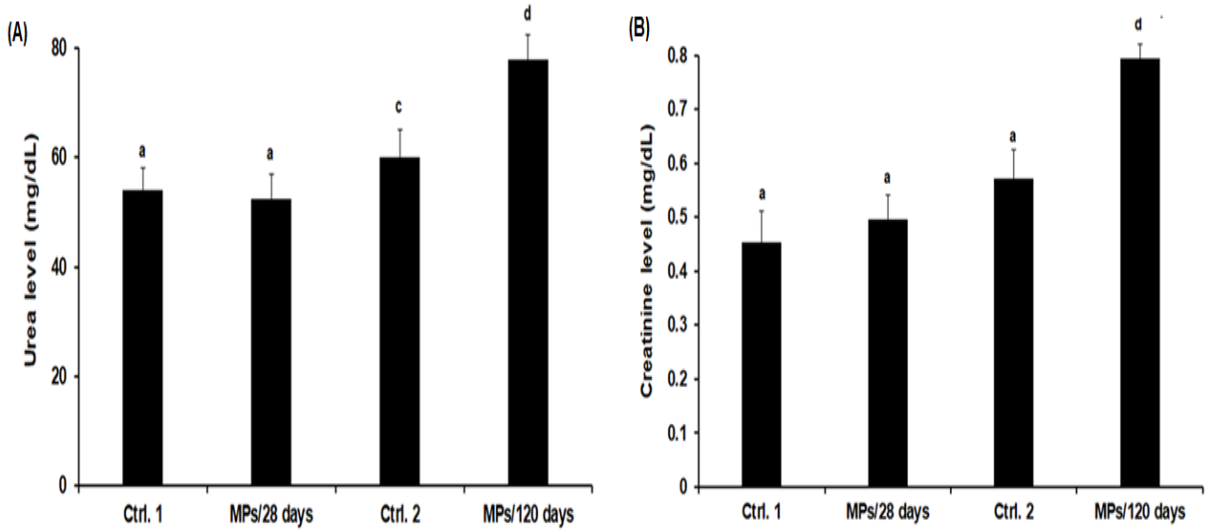

Fig. 4. Urea (A) and creatinine (B) levels in the different groups under the study. The values represented as means ± S.D.; Ctrl. 1: Control of 28 days; MPs/28 days: sub-acute administrations of microplastics for 28 days; Ctrl. 2: Control of 120 days; MPs/120 days: Chronic administration of microplastics for 120 days. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Table 2. Hepatic SOD, CAT activities and MDA level after sub-acute and chronic administrations of microplastics.

Groups	SOD (U/mg tissue)	CAT (µM/min/mg tissue)	MDA (nmol/g tissue)
Ctrl. 1	5.106 ± 0.708 [a]	81.06 ± 3.167 [a]	39.37 ± 2.19 [a]
MPs/28 days	4.023 ± 0.207 [b]	74.293 ± 0.977 [b]	41.816 ± 1.35 [a]
Ctrl. 2	4.27 ± 0.413 [b]	75.62 ± 2.222 [b]	45.90 ± 3.24 [a]
MPs/120 days	3.156 ± 0.096 [c]	61.74 ± 2.77 [c]	58.02 ± 3.58 [b]

The values represented as means ± S.D.; **Ctrl. 1:** Control of 28 days; **MPs/28 days:** sub-acute administrations of microplastics for 28 days; **Ctrl. 2:** Control of 120 days; **MPs/120 days:** Chronic administrations of microplastics for 120 days; **SOD:** Superoxide dismutase; **CAT:** Catalase; **MDA:** Malondialdehyde. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Table 3. Hepatic alkaline phosphatase, arginase, and α-fucosidase activities in the groups of mice

Groups	ALP (U/g tissue)	Arg. (U/g tissue)	α-fuc. (U/g tissue)
Ctrl. 1	9.096 ± 1.44 [a]	289.66 ± 10.08 [a]	2.47 ± 0.298 [a]
MPs/28 days	10.58 ± 0.42 [a]	299.33 ± 12.85 [a]	2.88 ± 0.303 [a]
Ctrl. 2	13.39 ± 1.20 [c]	276 ± 13.93 [c]	3.84 ± 0.283 [c]
MPs/120 days	18.85 ± 1.84 [d]	327.33 ± 13.05 [d]	4.66 ± 0.348 [d]

The values represented as means ± S.D.; **Ctrl. 1:** Control of 28 days; **MPs/28 days:** sub-acute administrations of microplastics for 28 days; **Ctrl. 2:** Control of 120 days; **MPs/120 days:** Chronic administrations of microplastics for 120 days; **ALP:** Alkaline phosphatase; **Arg:** Arginase; **α-fuc:** Fucosidase. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Table 4. Sera glucose, total cholesterol, and triglycerides level in the groups of mice.

Group	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
Ctrl. 1	131.4 ± 8.61	126.58 ± 10.69	121.97 ± 8.13
MPs/28 days	134.33 ± 10.85	123.25 ± 6.82	124.88 ± 8.21
Ctrl. 2	139.51 ± 9.54	129.42 ± 9.33	129.41 ± 8.59
MPs/120 days	144.24 ± 8.43	137.47 ± 7.73	134.88 ± 7.67

The values represented as means ± S.D.; **Ctrl. 1:** Control of 28 days; **MPs/28 days:** sub-acute administrations of microplastics for 28 days; **Ctrl. 2:** Control of 120 days; **MPs/120 days:** Chronic administrations of microplastics for 120 days. Means that do not share a letter in each column are significantly different ($p < 0.05$).

Histopathological investigations of liver and kidney sections

The results showed that the section of liver of control group showing normal architecture of hepatic tubules with polyhedral hepatocytes, with central nuclei arranged in strands separated by blood sinusoids (bs) around the central vein (cv) with normal arrangement of Kupffer cells (arow) hematoxylin eosin (Figure 5). Sections of the liver tissues of mice group that were administered with MPs for 28 days, showing approximately normal of most hepatocytes (HC) with homogeneous cytoplasm and usual nuclei radiating from the central vein (CV), few cytoplasmic vacuolations are noted (V), regular blood sinusoids (BS) lined with Kupffer cells (KC) and inflammatory cells are also demonstrated beside the portal vein (I) (Figure 5). Sections of the liver tissues of mice group that were administered with MPs for 120 days, showing changed of many hepatocytes (HC), vacuolations of the cytoplasm (V), karyolitic of nuclei (K), dilation of the central vein (CV)and dilation of blood sinusoids (BS) with hypertrophied Kupffer cells (KC) (Figure 5). Renal cortex of control mice showed normal appearance with normal glomerulus surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes (Figure 5). Sections of kidneys of mice group that were administered with MPs for 28 days showing little widen Bowmen space of glomerulus (G), few changes in the cells of proximal convoluted tubules (PCT) and distal convoluted tubules (DCT), hemorrhage in between the renal tissue (H) and few inflammatory cells (I) (Figure 5). Sections of kidney tissues of mice group that were administered with MPs for 120 days showing an obvious change in the glomerulus (G) demonstrated with widen in Bowman's space (blue arrow), vacuolation and degeneration of the cells of proximal convoluted tubules (PCT) and distal convoluted tubules (DCT), and few hemorrhage in interstitial tissue (Figure 5).

Histopathological investigations of small and large intestine

Sections of small intestines of mice group that were administered with MPs for 28 days showing vacuolation of the cytoplasm (V) of many intestinal cells, Karyolitic nuclei (black arrow), an obvious reduction of many goblet cells (blue arrow), accumulation of inflammatory cells (I), and appearance necrotic area (N) (Figure 6). Sections of small intestine in mice group that were administered with MPs for 120 days showing inflamed villi with very few goblet cells (blue arrow), desquamation of the columnar cells and fusion of most villi are observed (F), inflammatory cells (I) are seen, shrinkage in some villi (red arrow) (Figure 6). Sections of large intestine of mice group that were administered with MPs for 28 days showing vacuolation of cytoplasm (V), desquamation of the absorptive columnar cells, reduction in number of superficial goblet cells (blue arrow), distortion of crypts and necrotic area are observed (N), inflammatory cells (I) are seen (Figure 6). Sections of large intestine of mice group that were administered with MPs for 120 days showing desquamation of the absorptive columnar cells absence of superficial

goblet cells, distortion of crypts and necrotic area (N) are observed, inflammatory cells (I) are seen, and huge macrophages (red arrow) (Figure 6).

Immunohistochemical investigations of small and large intestine

Section of small intestine of the control normal mouse stained with PCNA immunostain showing very few immunoexpression of PCNA (arrows) demonstrated as abrown color in the nuclei. Sections of the small intestine acute mice group brown color in the nuclei. Sections of small intestines the chronic mice group stained with PCNA immunostain showing strong positive immunoexpression of PCNA (arrows) demonstrated as brown color in nuclei. Sections of the large intestines the control normal mouse stained with PCNA immunostain showing approximately negative immunoexpression of PCNA as a brown color in the nuclei (Figure 7). Sections of large intestines of the acute mouse stained with PCNA immunostain showing positive immunoexpression of PCNA as demonstrated with brown color in nuclei (arrows). Sections of the large intestines of the chronic mouse stained with PCNA immunostain showing strong positive immunoexpression of PCNA as a brown color in nuclei (arrows) (Figure 7). Sections of the small intestines in control normal mice group stained with BCl-2 immunostain showing strong positive immunoexpression of BCl-2 as a brown color in stained with PCNA immunostain showing positive moderate to strong immunoexpression of PCNA demonstrated a the cytoplasm. Section of small intestine acute mouse stained with BCl-2 immunostain showing moderate positive immunoexpression of BCl-2 as a brown color. Section of small intestine achronic mouse stained with BCl-2 immunostain showing rare or approximately no immunoexpression of BCl-2 as a brown color (Figure 8). Sections of a large intestine control normal mouse stained with BCl-2 immunostain showing strong positive immunoexpression of BCl-2 in many intestinal cytoplasmic cells as a brown color. Section of alarge intestine the acute mouse stained with BCl-2 immunostain showing few positive immunoexpression of BCl-2 as a brown color. Section of a large intestine the chronic mouse stained with BCl-2 immunostain showing approximately negative immunoexpression of BCl-2 as a brown color (Figure 8).

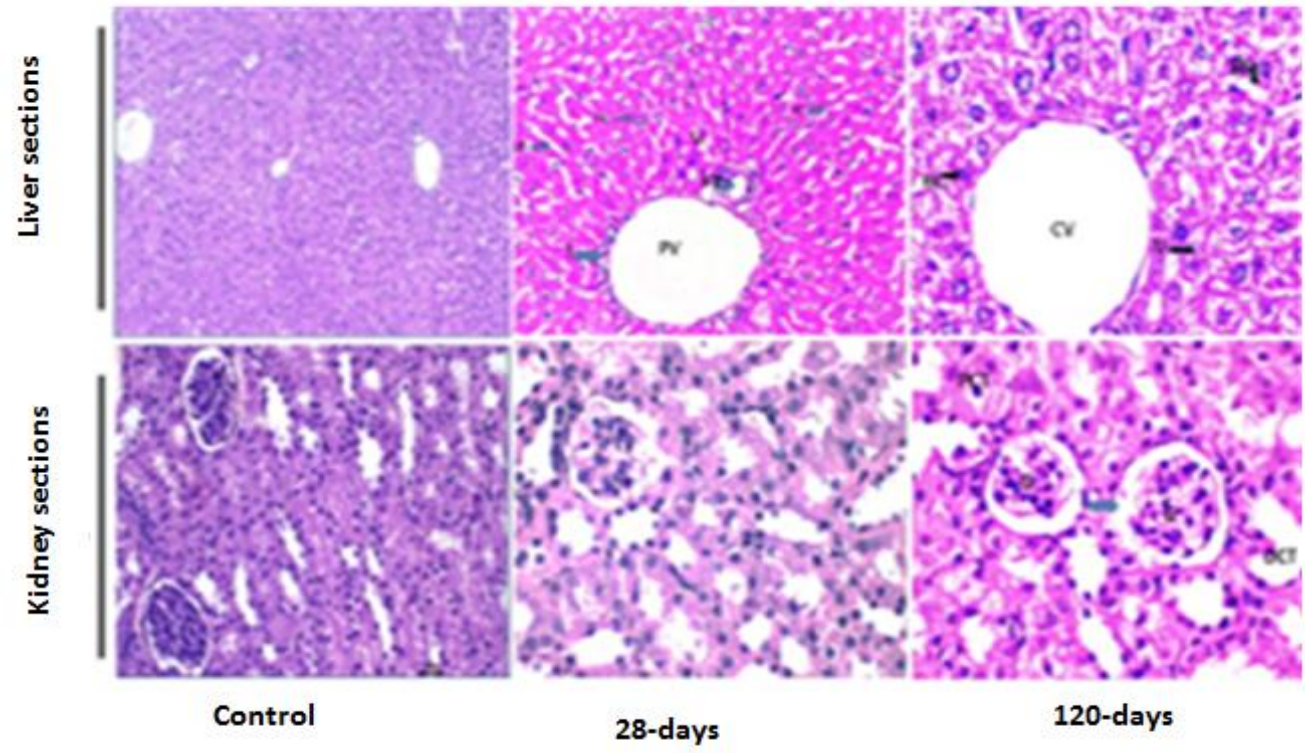

Fig. 5. Photomicrographs of liver and kidney sections of normal, subacute, and chronic groups administered with MPs.

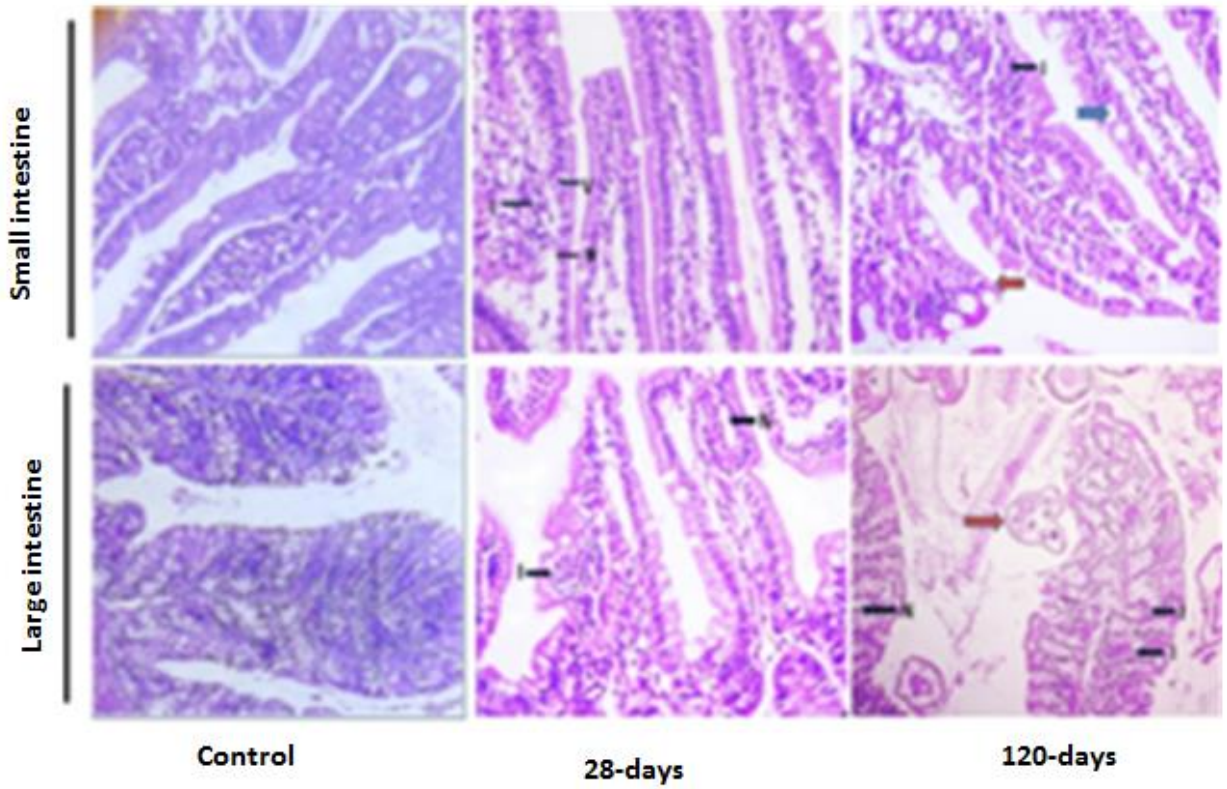

Fig. 6. Photomicrographs of small and large intestine sections of normal, subacute, and chronic groups administered with MPs.

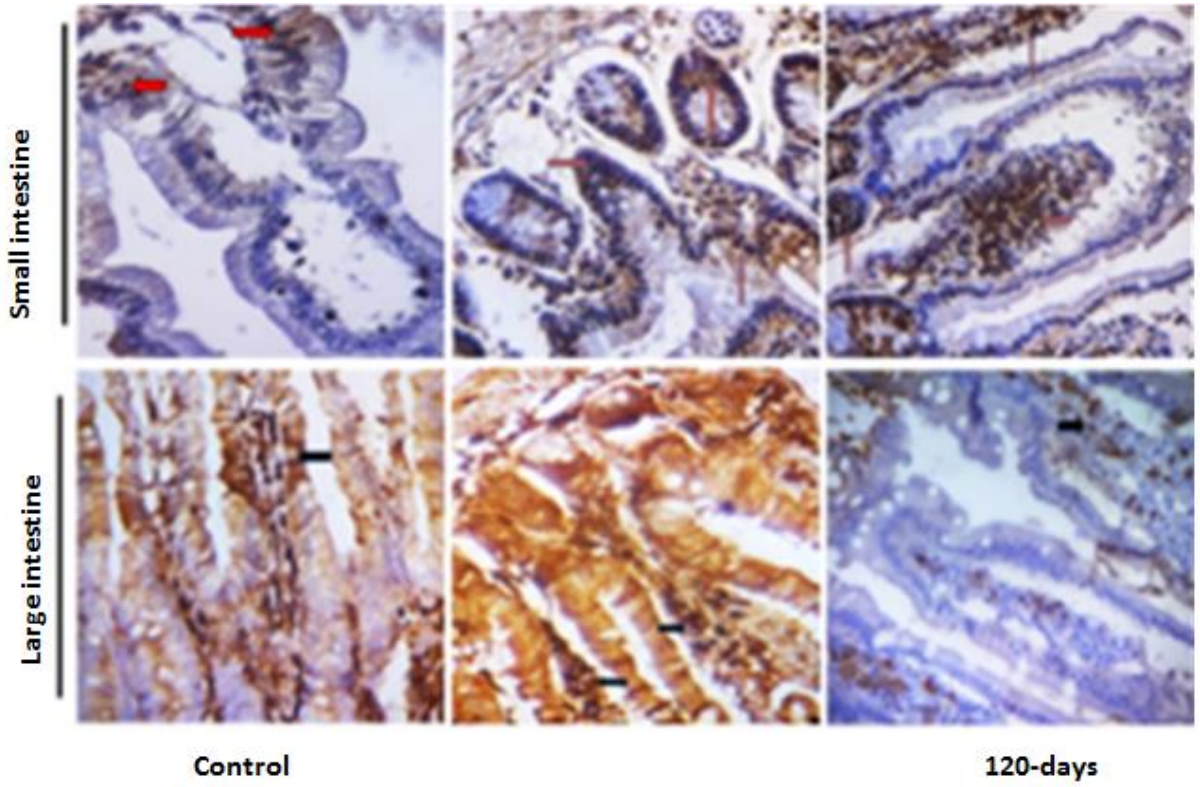

Fig. 7. Photomicrographs of small and large intestines sections immunostained with PCNA of normal, subacute, and chronic groups administered with MPs.

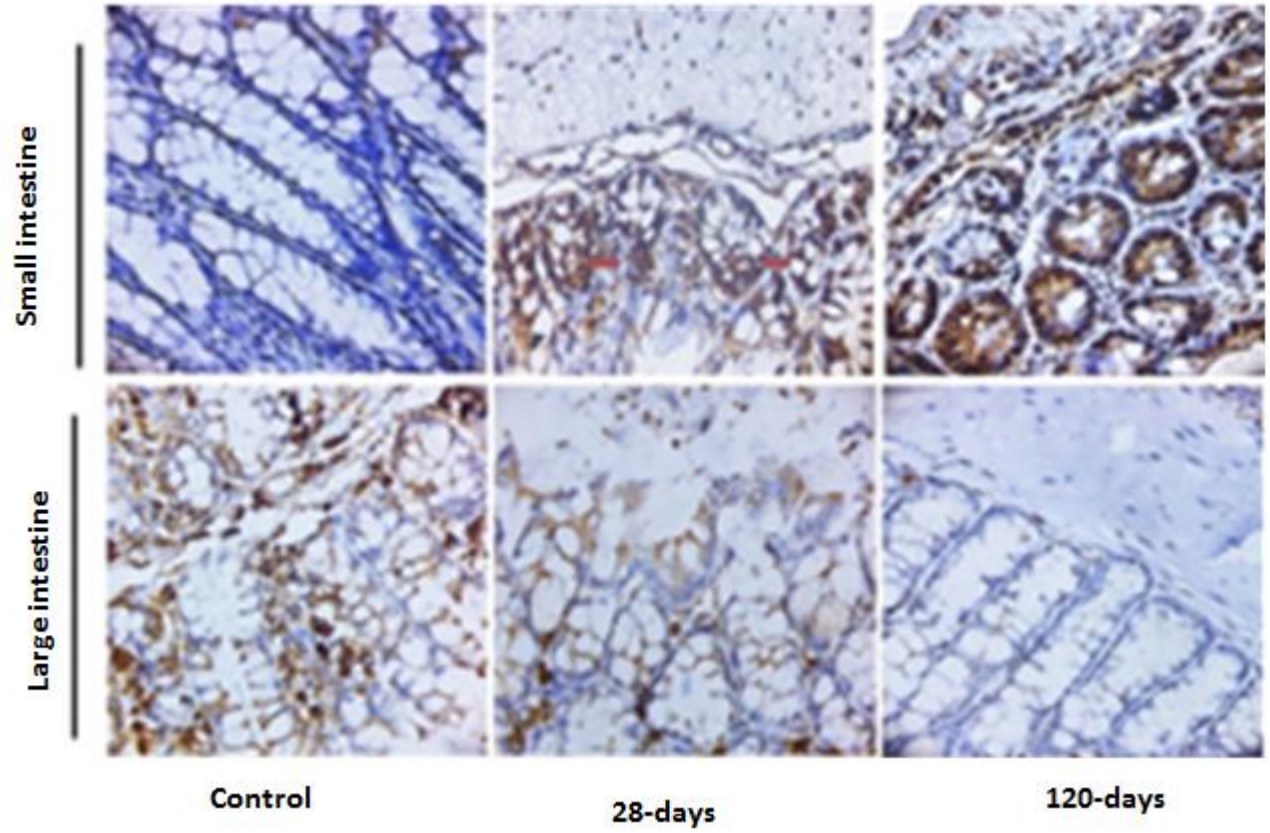

Fig. 8. Photomicrographs of small and large intestines sections immunostained with BCl-2 of normal, subacute, and chronic groups administered with MPs.

4. Discussion

Microplastics (MPs) pollution is an environmental concern that poses a risk to human health (Alfaro-Núñez et al., 2021). Due to the abundance of MPs in the environment, exposure may occur via consumption, inhalation, and skin contact. Exposure to MPs has also been linked to oxidative stress, cytotoxicity, neurotoxicity, and immune system disruption (Bhuyan, 2022). MPs can induce oxidative stress by producing oxidizing substances adsorbing to their surface, as well as reactive oxygen radicals created by the host during the inflammation (Anbumani and Kakkar, 2018). MPs accelerated hemolysis and contributed to the production of a pro-inflammatory molecule, according to Hwang et al. (2019).

The present study investigated the pathophysiological consequences upon MPs administration on some vital organs of mice after short- and long-term administrations. In addition, quantitative analysis of size and concentration of MPs is a crucial step for having a better understanding of plastic pollution and toxicity, the dynamic light scattering (DLS), scanning electron microscopy (SEM), and fourier-transform infrared spectroscopy (FTIR) are the most advanced methods for MPs analysis and characterization (Huang et al., 2023). The present results showed that DLS analysis of MPs size was approximately 0.9 µm. Furthermore, SEM investigation showed that the MPs size was 0.5 µm. A previous study reported the physicochemical properties of environmentally relevant MPs using DLS and SEM, which characterize and quantify the size range

distribution between single-digit micrometer particles, DLS displayed a hydrodynamic diameter of 2733 nm, a high polydispersity index of 0.97, and provided more detailed information regarding particle size and shape (Paul et al., 2022). Previous study analyzed the size and the concentration of MPs in water using static light scattering, furthermore, analysis of MPs in water using multi-angle static light scattering have been reported (Choobbari et al., 2023) .

The present results showed that as compared to control group, mice that were administered with MPs daily for 28-days as sub-acute dose (short-term) did not show a significant alteration in the % B. wt changes. The ingestion of MPs may cause damage to the digestive tract and malnutrition (Kramm and Völker, 2018), and found to inhibit food assimilation, reduce body weight, and negatively impact growth and thus reproductive fitness (Straub et al., 2017). However, mice that were administered with MPs for 120 days led to a decrease in body weight with a significant increase in the % B. wt changes. Other studies reported that ingestion of MPs promotes adiposity and obesity in experimental mice (Liu et al., 2022; Zhao et al., 2022; Okamura et al., 2023). Ingestion of MPs led to immune system depression. More recently, mice models have been used to investigate the potential impacts of MPs on host cellular and metabolic damages of mammalian tissues (Yong et al., 2020; Osman et al., 2023) .

The current study showed that the total platelets count was significantly decreased in the group of mice administered the MPs for 120 days, furthermore, there was a non-significant change in the total WBCs count in the group of mice administered with MPs for 28 days, however, a significant change in the total WBCs count in the group of mice administered with MPs for 120 days was reported. The percentage of neutrophiles and monocytes were significantly increased in the group of mice administered with MPs for 28 days. A previous study showed the hemotoxic effects of MPs on mice (Abdel-Zaher et al., 2023). Another study showed the impacts of MPs on the hematological system and gene expression in bone marrow cells of mice (Sun et al., 2023).

The present study registered that the administration of MPs to mice for 120 days significantly increased the level of AST, ALT, ALP, arginase, and α-fucosidase. These findings were in accordance with previous study indicated that the prolonged oral ingestion of MPs induced inflammation in the liver tissues of experimental mice through the increases of liver weight, general liver index as well as expression of serum, liver function-related indicators (Abdel-Zaher et al., 2023). Ingestion of MPs led to alterations in gastrointestinal tract physiology, oxidative stress, and differential gene expression (Osman et al., 2023). Previous study indicated that MPs accumulated in liver with a tissue-accumulation kinetics and distribution pattern that was strongly dependent on the MPs particle size (Deng et al., 2017) .

The present study recorded that the administration of MPs for 120 days increased the level of the urea and creatinine when compared to their control values. The previous study by Xiong et al. (2023) reported that the effects of MPs on kidney injury and fibrosis under long-term accumulation in mice. Furthermore, a previous study investigated the uptake, bioaccumulation, and the toxic effects of MPs in the kidneys of mice (Meng et al., 2022). Additionally, polystyrene MPs induced nephrotoxicity associated with oxidative stress, inflammation, and endoplasmic reticulum stress in juvenile rats (Wang et al., 2023). Furthermore, it has been reported that MPs induced kidney injury through enhancing oxidative stress, autophagy, apoptosis, and fibrosis (Zou et al., 2022).

The current study indicated that the administration of mice with MPs for 120 days led to a slight increase in the level of glucose, total cholesterol (TC) and triglycerides (TG). The impacts of MPs exposure on lipid profile and oxidative stress status of experimental animals have been documented (Kannan and Vimalkumar, 2021). It has been reported that oral

exposure to MPs caused dyslipidemia, decreased high density lipoprotein cholesterol (HDL-C) and increased low density lipoprotein cholesterol (LDL-C) (Nnoruka et al., 2022). These findings agreed with previous studies reported the that the oral exposure to MPs led to disturbance in the lipids profile of the experimental animals (da Silva Brito et al., 2022; Okamura et al., 2023). Moreover, a recent study reported that the exposure of rats to MPs exhibited an increase in the levels of TG, TC, and LDL and decrease in HDL (Saeed et al., 2023).

The current study showed that the administration of MPs to mice for 120 days decreased the activities of SOD and CAT significantly, the administration with MPs for 120 days, increased the level of MDA significantly when compared to their control values. In agreement with previous studies, MPs administration in the experimental animals led to a significant decrease in the activity of SOD and CAT (Anbumani and Kakkar, 2018; Bhuyan, 2022; Saeed et al., 2023). The short- and long-term MPs exposure promotes oxidative stress through Wnt/beta-catenin signaling (Schmidt et al., 2022). Moreover, MPs induced oxidative stress in the hepatocytes of mice (Zou et al., 2022). MPs can accumulate in the liver and gut. The results of organ toxicity assessment indicated that MPs could induce inflammation and lipid accumulation in the liver. Meanwhile, changes of oxidative stress and lipid energy metabolism were noted by analyzing the increase or decrease of some enzyme activities (Lu et al., 2016) .

The present results showed the liver architecture of acute mice group that given MPs for 28 days, approximately normal of most hepatocytes were seen with homogeneous cytoplasm, intact nuclei, regular blood sinusoids lined with normal Kupffer cells. Liver sections of the chronic mice group that administered with MPs showed changed of many hepatocytes, vacuolations of the cytoplasm, karyolitic of nuclei, dilation of the central vein and widen the blood sinusoids with hypertrophied Kupffer cells .In agreement with previous histological studies that indicated the effect of MPs exposure in mice hepatocytes was observed by using H&E staining. In the MPs-exposed group, the hepatocytes had dilated central venous pores, bruising, swollen cells with indistinct margins, cloudy cytoplasm, and granular degeneration with massive inflammatory cell infiltration (Luo et al., 2022; Zou et al., 2022). Although MPs considered an inert substance, there is a wide range of properties of MPs that characterize it as their shape, chemical composition, size, and hydrophobicity, that may cause damage and effect the particles cytotoxicity to cells and tissues (Wright and Kelly, 2017). It has been found that mice exposed to MPs with diameters at 5 and 20μm for 28 days showed the presence of MPs in the liver, kidney, and gut (Deng et al., 2017). Polystyrene particles of the size 50 nm have cytotoxic and genotoxic impacts on pulmonary epithelial cells and macrophages (Paget et al., 2015) .

In the current study, the kidney sections of the acute mice group showed little widen Bowmen space of glomerulus, few changes in the cells of proximal and distal convoluted tubules, hemorrhage in between the renal tissue and infiltrations of few inflammatory cells. Sections of the kidney tissues of chronic mice group showed obvious changes in the glomerulus, more widen in Bowman's space, abundant of the vacuolation and degeneration of the cells of proximal, distal convoluted tubules, and hemorrhage in interstitial tissue. In agreement, the previous studies of many authors showed the effect of MPs exposure on the histopathology of renal tissues in the experimental animals (Wang et al., 2021; Zou et al., 2022; Xiong et al., 2023). MPs exposure can induce an inflammatory response, oxidative stress, and cell apoptosis in the kidney and induce kidney injury, which ultimately promotes kidney fibrosis (Xiong et al., 2023).

The present results indicated that the histological studies of small intestine of acute mice group showed few changes as vacuolation of the cytoplasm of many intestinal cells, Karyolitic nuclei, an obvious reduction of many goblet cells,

increment of the inflammatory cells, and appearance of the necrotic area. Significant changes were seen in the small intestine in chronic mice group showed inflamed villi with very few goblet cells, desquamation of the columnar cells and fusion of most villi, inflammatory cells were observed, shrinkage in some villi. Furthermore, the large intestine sections of acute mice group showed desquamation of the absorptive columnar cells, reduction in number of superficial goblet cells, distortion of crypts and necrotic area were observed. Sections of large intestine of chronic mice group showed desquamation of the absorptive columnar cell's absence of superficial goblet cells, distortion of crypts and necrotic area were observed, and huge macrophages. These results were in agreement with previous studies that showed the effect of MPs exposure on the histopathology of renal tissues in experimental animals (Deng et al., 2017; Xie et al., 2022; Jia et al., 2023; Okamura et al., 2023).

Concerning with IHC in the current study, the small intestine section of acute mice group expressed PCNA immunostain as positive moderate to strong immunoreaction. The intensity of immunostain to PCNA of small intestine in the chronic mice group was expressed as strong positive immunoreaction in the nuclei. The large intestine sections of mice taken MPs orally for 28 days stained with BCL-2 showing moderate positive immunoexpression of BCL-2 as a brown color. Section of small intestine achronic mouse stained with BCL-2 immunostain showing rare or approximately no immunoexpression of BCL-2 as a brown color. Immunohistochemical analysis in a previous study by Jia et al. (2023) reported that exposure to MPs via oral ingestion induces colonic apoptosis and intestinal barrier damage through oxidative stress and inflammation in mice.

5. Conclusion

The results recorded that there were no significant effects on each of the blood, biochemical and histological markers of each of the liver, kidneys, small and large intestine after dosing the mice with microplastic particles for a continuous period of 28 days. The results concluded that dosing mice with microplastic particles for a continuous period of 120 days led to significant changes in several blood indicators, an increase in liver enzymes and vital signs of kidney function, and significant changes in the activity of antioxidant enzymes, and significant changes in lipids. Significant changes in tissues. Both the liver, kidneys, small and large intestine.

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Declarations

Ethics approval and consent to participate All experimental procedures were conducted in accordance with the ethical standards and were approved by the Institutional Animal Care and Use Committee (IACUC) at National Organization for Drug Control and Research (NODCAR) (approval no. NODCAR/III/41/2019).

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Conflict of interest

All authors declared that there was no conflict of interest.

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Author contributions

SR conceptualized the study, performed the experiments, analysis date and wrote the draft. The author read and approved the final manuscript

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