Haematological and Histological Effects of Nanoplastics Released from Nonfood-grade and Nonwoven Polyethene Bags on Mice

ABSTRACT

Nonfood-grade and nonwoven polyethene (NFWP) bags which is frequently used in microwave cooking or carrying hot foods has been reported for releasing large amount of nanoplastics. Hence, the present study was designed to investigate the harmful effects of nanoplastics released from NFWP bags on mice. Water boiled with small pieces of NFWP bags for 5 minutes was used as nanoplastic-contaminated drinking water for treated mice for 50 days. Surprisingly, the body weights and organ weight were increased remarkably for treated mice than control mice.The total RBC count decreased remarkably while total WBC count increased significantly in treated mice than incontrol. The percentage of neutrophils decreased remarkably while percentage of Monocytes increased significantly in treated mice as compared with control. The consumption of nanoplastics caused histological damageto all studied organs. The layers of intestinal muscles in the villi of treated mice were disrupted and infiltrated with foam cells. Destruction of alveoli and fibrosis was observed in the lungs of treated mice. The heart muscles of treated mice were also disrupted and irregularly arranged with fibrosis. In the kidney of treated mouse, enhancement of renal spaces, shrinkage of glomeruli, eroded Bowman's capsule, deleted and congested glomeruli along with blood vessel were found. The liver of treated mice was affected by apoptosis, fibrosis, vacuole formation in hepatocyte, congested in hepatic tissue and dilation of blood vessel. Therefore, it can be concluded that consumption of nanoplastics released from hot NFWP bags has serious deleterious effect on animal health.

Keywords: Nanoplastics, Polythene bag,Haematology, Histology, Mouse

1. INTRODUCTION

The widespread use of plastics has drastically changed our modern environment, making it primarily plastic-centric [1]. Plastics pollution is a major threat to world health[2].While the entire effects of the pervasive plastic pollution are yet unknown, more focus is being placed on

particles with sizes between 1 µm and 5 mm, and especially on nanoplastics.According to Gigault*et al.* (2018), nanoplastics are plastic particles that range in size from 1 to 100 nm or 1-1,000 nm. Microplastics and nanoplastics are widely distributed throughout environmental media, such as aquatic environments, soils on land, biotic organisms, and airborne particles, resulting in emerging as the most notorious human-caused pollutants[3, 4].However, nanoparticles pose a heightened risk compared to microplastics due to their capacity to traverse biological barriers[5]. Primary plastic waste and secondary byproducts are the two main causes of nanoplastic contamination [6].Micromedicine, nanoimaging, nanosensors, and personal care products are some of the main sources of microplastics and nanoplastic emissions into the environment, with the emissions occurring at dimensions ranging from the microscale to the nanoscale [7]. Moreover, many other pollutants such as petroleum hydrocarbons, textile dye, industrial effluents can be degraded by microorganisms while plastics are very reluctant to microbial biodegradation causing their long-time existence in environment [8-15]. Hence, people are exposed to low quantities of nanoplastics for extended periods of time, often their whole lives, due to the widespread prevalence of plastic pollution [3]. Inhalation, ingestion, and skin contact are the three main ways that nanoplastics can enter the human body [16].Food-grade polypropylene (PP) nonwoven bags have long been thought to be safe and non-hazardous for the environment. They are frequently used for filtering food residue. However, because of their tendency to leach plastic when in contact with hot water, microplastics and nanoplastics (MPs/NPs) have emerged, and their usage in culinary applications must be reevaluated. When food-grade nonwoven bags are boiled once, they can release 0.12 to 0.33 million microplastics (>1 µm) and 17.6 to 30.6 billion nanoplastics (<1 µm), or 2.25 to 6.47 mg in mass[17]. It has been reported that tens of thousands of MPs/NPs can be released when objects like tea bags and disposable paper cups are exposed to hot water [18].Even though, plastic bags are widely used in everyday cooking operations in many countries like Bangladesh [19, 20].

Therefore, the use of food-grade plastics, such as nonwoven bags, in regular cooking should be done with caution since they emit a significant amount of microplastics and nanoplastics when heated and may be harmful to human health [17]. Moreover, microplastics and nanoplastics released from food-grade polythene bags may infiltrate into living organisms from their

surroundings and build up in a few organs, such as the kidney, liver, heart, and lung [21, 22]. Hence, it has been assumed that non-food grade non-oven polythene (NFWP) bags might be more hazardous to animal health when these are used in microwaves cooking, and hot food processing and/or transportation. But information on the effect ofnanoplastics released from NFWP bags on animal health is still inadequate. Hence, the present study was designed to investigate the harmful effects of nanoplastics released from NFWP bags on blood counts and histology of different organs *viz.* intestine, liver, heart, lung, and kidney in mice model.

2. MATERIALS AND METHODS

2.1 Collection of Polythene

Non-food grade non-oven polythene (NFWP) bags used in this study were collected from the neighborhood market located in Kazla, Rajshahi, Bangladesh.

2.2 Mice Rearing and Feeding

A total of six sexually mature female albino mice Mus musculus L. weighing 25-30g were collected from local market of Rajshahi. Then these mice, threefor control and another three for treatment,were reared in two separate cages(20×14.50×15.50 inch) with saw dust bedding in the laboratory under constant temperature (33± 40C) for 50 days. The rearing cages were labeled accordingly as control cage and treatment cage. Poultry feed which was available in the local market was used to feed for both the mice. Collected NFWP bags were cut into small pieces, then these pieces were boiledfor 5 minutes in mineral water in a microwave oven followed by cooling into room temperature and filtration with a stainless-steel sieve to prepare nanoplastics contaminated water. Control mice were reared with normal drinking water while treated mice were reared with nanoplastics contaminated water.The weight of all studied mice was measured at the Day 0 and the Day 50.In compliance with the standard animal ethical guidelines, the present investigation was carried out at the Laboratory of Genetics and Molecular Biology, Department of Zoology, University of Rajshahi, Bangladesh.

2.3 Histological Examination

At day 50, both control and treated mice were sacrificed for histological studies of the tissues of heart, liver, lung,intestine, and kidney. Then,the weight of dissected organs wasmeasured with electronic balance.The histological slides of those tissues were prepared and examined under light microscope as described previously [23, 24]. Briefly, the dissected tissues were immediately fixed in 10% bouin´s fluid and washed in distilled water, dehydrated in graded ethanol series (30%, 50%, 70%, 85% and 100%), infiltrated with xylene and embedded in paraffin wax at 56-60 °C. The tissues embedded in paraffin wax were sectioned using a rotator microtome (5 µm) sections were placed on glass slides and treated with xylene to remove paraffin and subsequently washed in 90%, 70%, 50% and 30% alcohol. Finally, paraffin-free sections were washed with distilled water, stained with haematoxylin for 3 min and washed in running tap water for 1 min. Finally, the tissues were stained in eosin for 45 Sec., examined under microscope (Labomed, California) and photographed. Finally,histopathological changes in the studied organs of treated mice were recorded and compared with those of control mice.

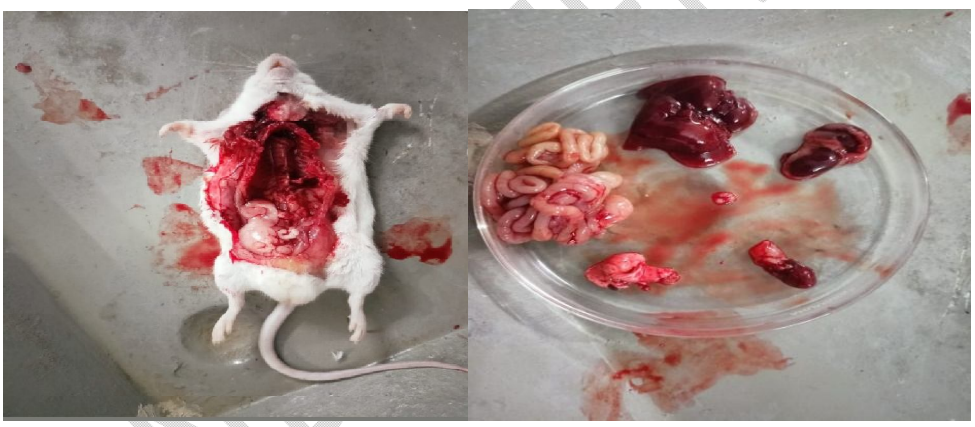

Fig.1.Different organs removedfrom sacrificed mice.

2.4 Hematological Test

For count of WBC and RBC, the blood was collected from tail vein of control and treated mice at the Day 0 and the Day 50. For the total count of WBC and RBC, blood was diluted with WBC

and RBC diluting fluid respectively(Himedia, USA) and then dilutedblood was placed on haemocytometer and the number of blood cell was counted under light microscope and calculated the blood cell number per cummaccording toprotocol of reagent manufacturer (Himedia, USA). For differential count the collected blood was used to prepare a blood film on slide which was air dried rapidly. Then, freshly prepared, and rapidly air-dried blood film was covered with Leishman's Stain (HimediaS018, USA) and allowed to act for 1 minute. Methanol in Leishman's Stain fixed the preparation. Then, double the volume of Leishman's Stain on the slideby adding distilled water with a dropper and mixing slowly. The diluted stain was allowed to act for 10-12 minutes. After that, the film was washed with distilled water or phosphate buffer of pH 7.0, then drained and dried in air. Finally, blood film was examined under microscope by using oil immersion lens.

2.5 Statistical Analysis

Unless indicated otherwise, all experiments were independently conducted at least in three controlmice and three treated mice, and data were pooled for presentation as mean ± SEM. All data were analyzed with Prism software (GraphPad, La Jolla, CA, USA) using two-tailed unpaired Student's *t*-tests. *P*-values <0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Morphological Effects in Control and Treated Mice.

The result indicated that, in comparison to the control mouse, the treated mouse's body weight wasincreased considerably (Fig. 2).The body weight of three control micewere 27.4 g, 29.7 g, and 26.5 g at the Day 0 which were comparable to the body weights of three treated mice*viz*.28.4 g, 26.2 g, and 30.3 g on the Day 0 (Fig. 2). In contrast, the body weights of three control mice were 32.4 g, 31.5 g, and 35.1 g respectively on the Day 50 while body weights of treated mice were 39.9 g, 38 g, and 41 g respectively on the Day 50 indicating the significant effect of nanoplastics consumption on body weight of mice (Fig. 2).

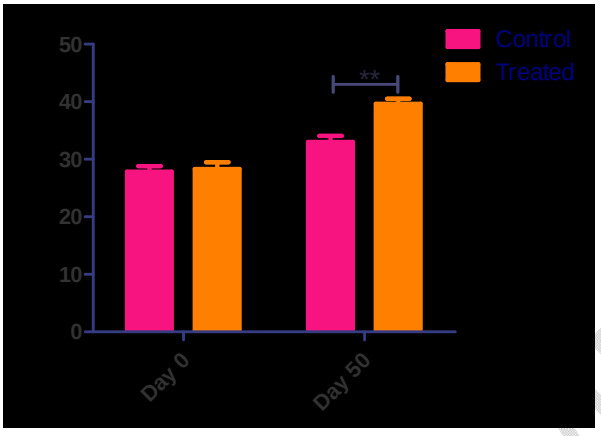

Fig. 2. Effect of nanoplastic on body weight of mice

At day 50 the control and treated mice were sacrificed. Then, the organs such as lung, heart, kidney, liver were removed from the body and the weight (gm) of the organs was measured.It was found that the weight of the organs was significantly higher in a treated mouse than a control mouse (Fig. 3). Moreover, the organs weight was normalized to body weight to confirm that the higher weight of organs of treated mice had not directly resulted from the increase of their body weight. Similarly,we found a significant increase of weight of all studied organs except lung (Fig. 3).

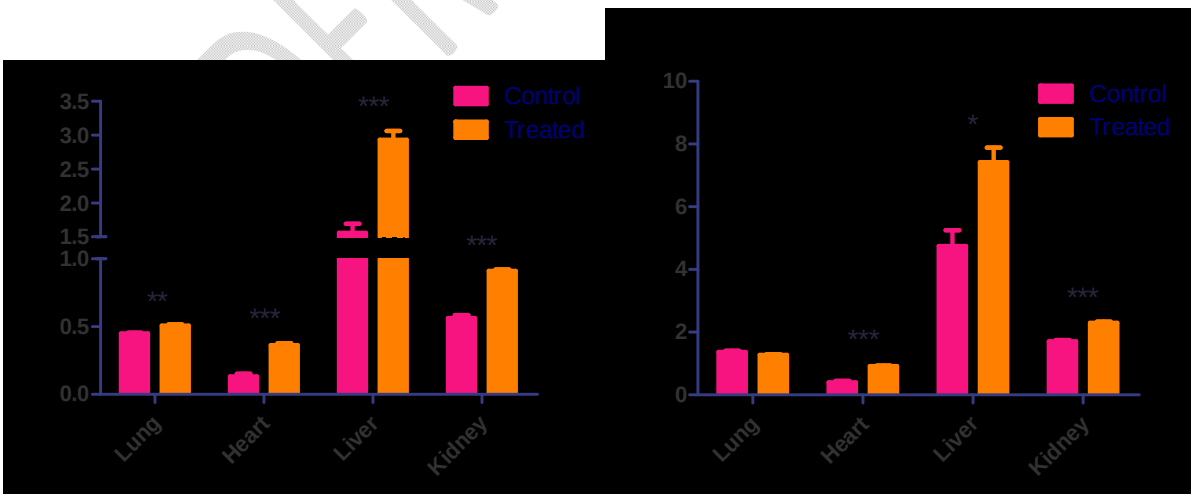

Fig. 3. Effect of nanoplastic on weight of different organs of mice.

Thus, the present study revealed that drinking of nanoplastic contaminated water caused weight gain of the mice significantly as compared with the mice reared with normal drinking water. Similarly, there was a noticeable increase in the weight of heart, liver and kidney of the treated mice than the control mice. As reported by other studies, the weight gain of the treated mice might be resulted from chronic inflammation related metabolic syndrome caused by deposition of nanoplastics in different organs [25, 26].

3.2 Evaluation of Hematological Effects in Controlled and Treated Mice.

The total counts of RBC and WBC, and differential counts of WBC were assessed on the Day 0 and on the Day 50. The results of the hematological investigation demonstrated that there was no appreciable variation in the total counts of RBC and WBC between Days O and 50 for mice. In contrary, the total count of RBC decreased remarkably while the total count of WBC increased significantly in treated mice as compared with those of control mice (Fig. 4 and 5) indicating the adverse effects of nanoplastics on hematology. Decline of RBC in the treated mice might be resulted from increased rate of hemolysis and genotoxicity induced by nanoplastics infiltration [27-29]. Contrary, increase of total number of WBC in treated mice might be related to chronic inflammation [27-29].

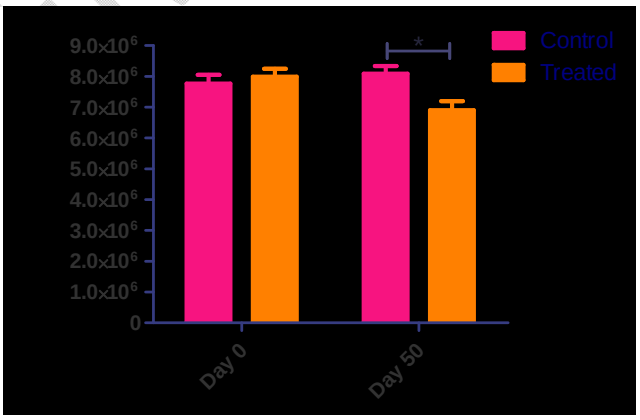

Fig. 4. Total counts of red blood cells in controlled and treated mice.

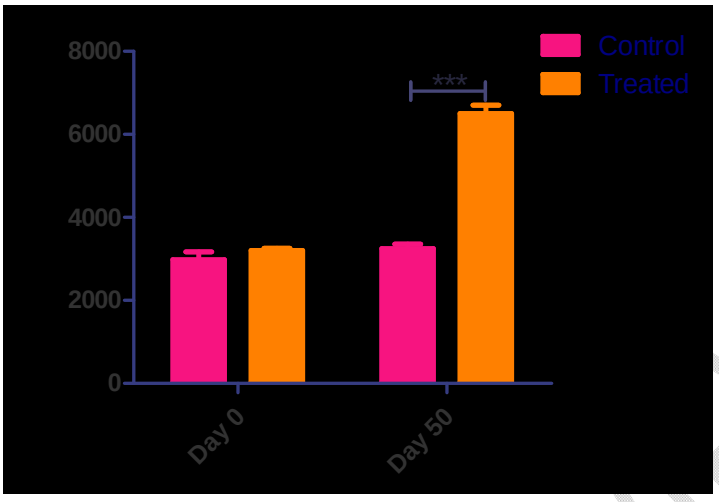

Fig. 5. Total counts of white blood cells in controlled and treated mice.

For differential count of WBC in control and treated mice, the stained blood film was observed under complex microscope in order to identify the different types of white blood cells viz. Neutrophil, Lymphocyte, Eosinophil, Basophil and monocyte. It was observed that Neutrophil had a multi-lobed nucleus while Eosinophil had bi-lobed nucleus. Grey blue colouredsegmented nucleus is seen in mature monocyte while lymphocyte had round purple nucleus. Basophils were largest in size and had many cytoplasmic blue granules (Fig. 6).

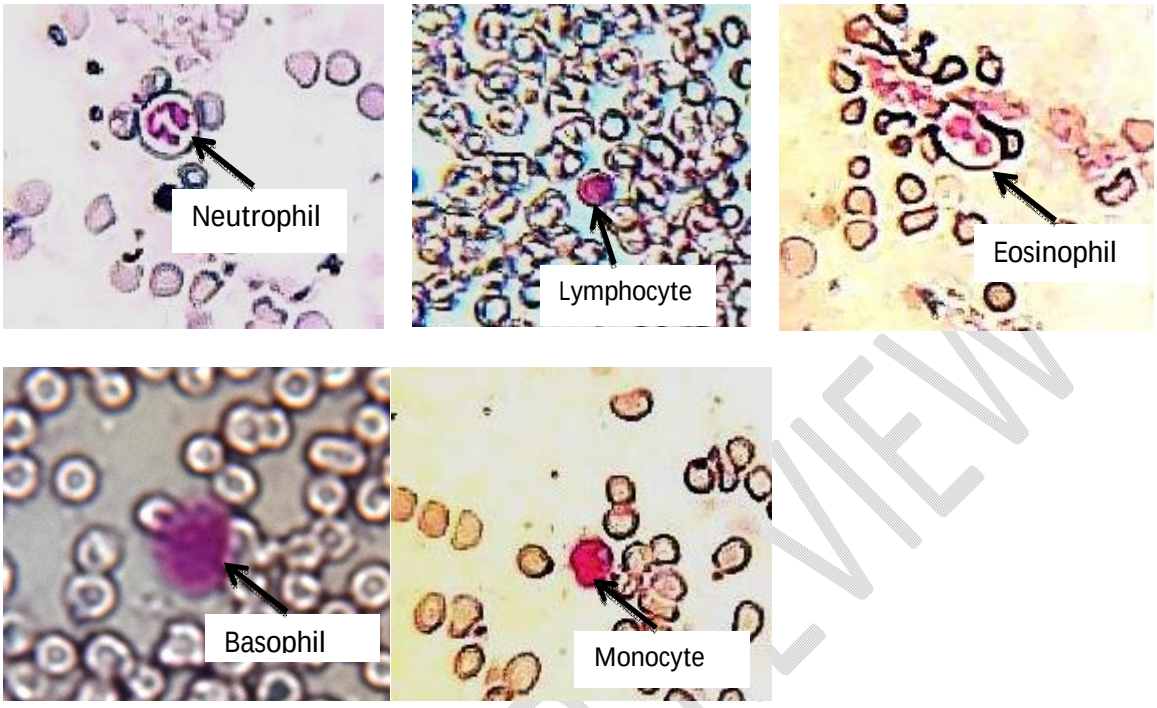

Fig. 6. Representative images of different types of White Blood Cells (WBC) observed under light microscope for differential count of WBC in control and treated mice.

According to the results of the differential count of white blood cells in control mice, the percentage of neutrophils (53.33±3.51) was the highest, followed by lymphocytes (20.66±1.52), basophils (9.33±1.52), monocytes (11.66±1.52) and eosinophils (about 5.00±2.00) on Day 0 (the first day of treatment) (Fig. 7). The proportion of various WBC counts between Day 0 and Day 50 did not significantly change (it varied by just 2-3%), indicating that the control mice were in good health after being raised in the lab. Every mouse displayed comparable outcomes.

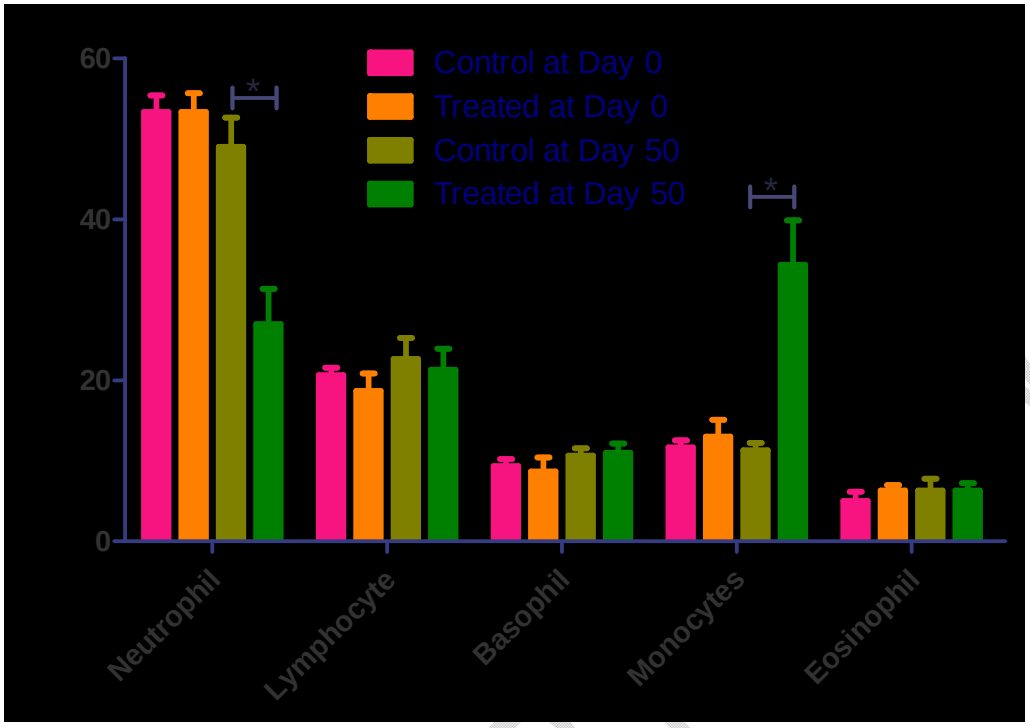

Fig. 7. Differential counts of white blood cells in controlled and treated mice.

The differential count of WBC in the treated mice showed that the highest proportion of WBC at Day 0 was found in neutrophils (53.33±4.04), lymphocytes (18.66±3.78), and monocytes (13.00±3.60).On the other hand, on the Day 50, there was a sharp rise in the percentage of monocytes (34.33±9.60) and lymphocytes (21.33±4.50) in the treated mice (Fig. 7). On the other hand, the percentage of neutrophils (27.00±7.54) dropped down significantly in the treated mice. Altogether, the count of Neutrophil and Monocyte was significantly affected by the consumption of nanoplastics released from NFWP bags (Fig. 7).

Neutrophils and Monocytes are phagocytic cells. Neutrophils make up more than half of all leukocytes in the bloodstream of healthy individuals. The results of differential count of WBC has been supported by other studies reporting that nanoplastics are phagocytosed by Neutrophils and induce their degranulation [27, 30, 31]. Moreover, the number of Monocyte can be modulated by nanoplastics induced cytokine production [27, 28, 32].

3.3 Evaluation of Histological Effect in Control and Treated Mice

The histological effect of consumption of nanopalastics on the heart, lung, kidney, liver, intestine of the mice was studied. The result revealed that consumption of nanoplastics disrupted the histological structure of different organs of mice. The histological slides of intestine of the Control mouseshowed normal pattern of villi whereas significant changes have been observed in the intestine of treated mouse(Fig. 8). Tissues have been disrupted and infiltrated with foam cells (Fig. 8). Similar results have been reported by others for consumption of nanoplastics by different types of fishes and mammals [33-36].In the case of control mouse, the histology of lung showed regular structure with veins.On the other hand,pathological features were observed in histology of the lung of treated mouse. It was found that the alveoli were disrupted in lung in treated mouse. Major fibrosis was also observed (Fig. 9).Destruction of alveoli and fibrosis was observed in the lungs of treated mice in this study which was also supported by other studies [20, 37-39].The histological slides of heartof the Control mouse showed a compact, well-organized heart muscle, proving that the mouse was healthy on theDay 50.On the contrary, heart muscles of treated mouse were disrupted and irregularly arranged with some pathological features. It was found that muscles became compact in some areas because of fibrosis (Fig. 10). Alteration cardiac muscles and myocardial inflammation resulted from nanoplastics pollution in different animals has been reported in many other studies [40-42].Additionally, ingestion of nanoplastics affected the histological structure of kidney of mouse.The control mouse's kidney histology was regular in structure with renal tubules, Bowman's capsule, and glomerulus.However, these structures were altered in the treated mouse. In the kidney of treated mouse, enhancement of renal spaces, shrinkage of glomeruli, eroded Bowman’s capsule, deleted and congested glomeruli along with blood vessel were found (Fig. 11).The liver of treated mice was affected by apoptosis, fibrosis, vacuole formation in hepatocyte, congested in hepatic tissue and dilation of blood vessel(Fig. 12).Similar effects of nanoplastics consumption on kidney and liver has been observed in human and other animals [43-47].

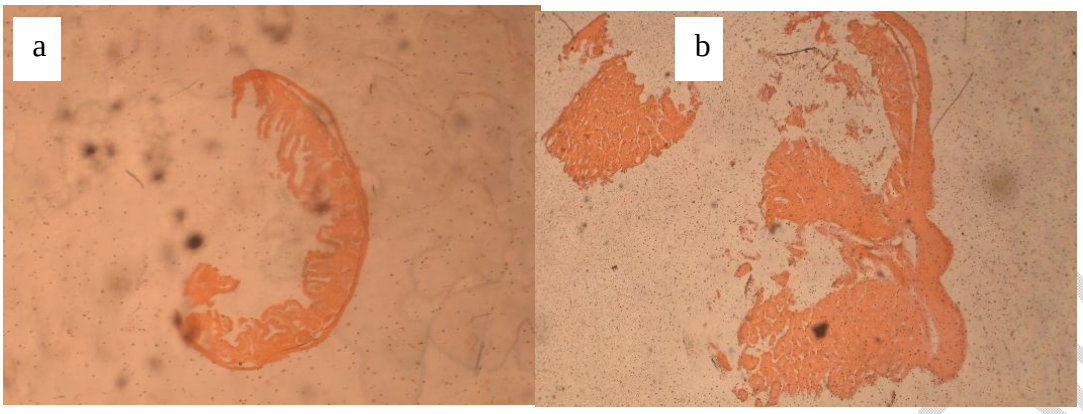

Fig. 8. Histological slide of Intestine. (a) control mouse, (b) treated mouse

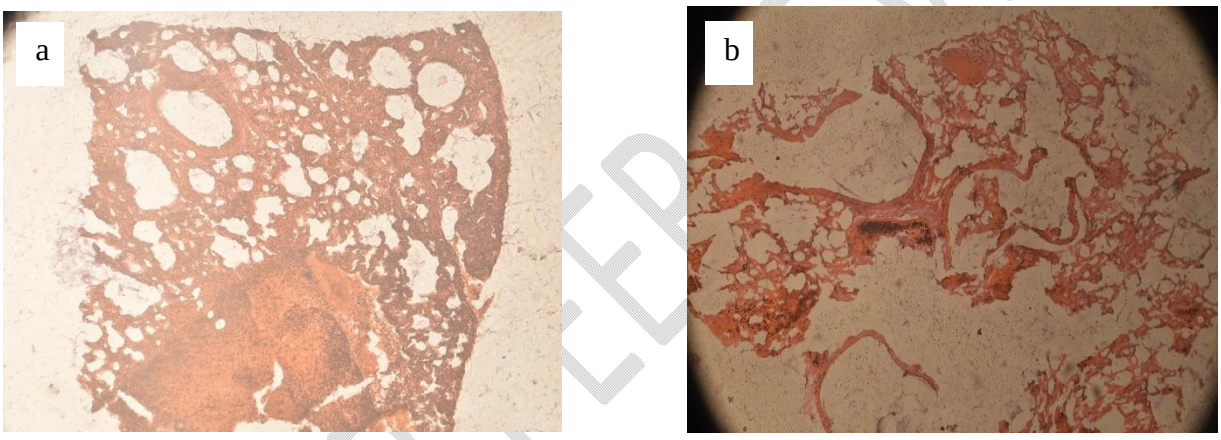

Fig.9. Histological slide of Lung. (a) control mouse, (b) treated mouse

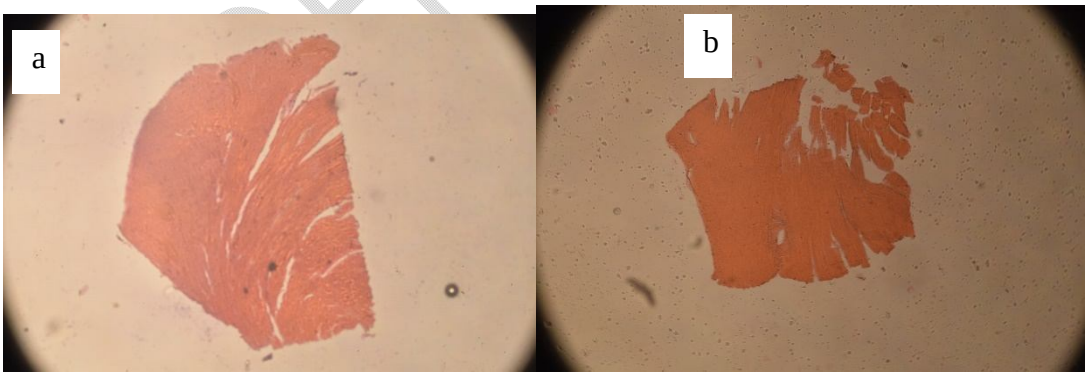

Fig.10. Histological slide of Heart. (a) control mouse, (b) treated mouse

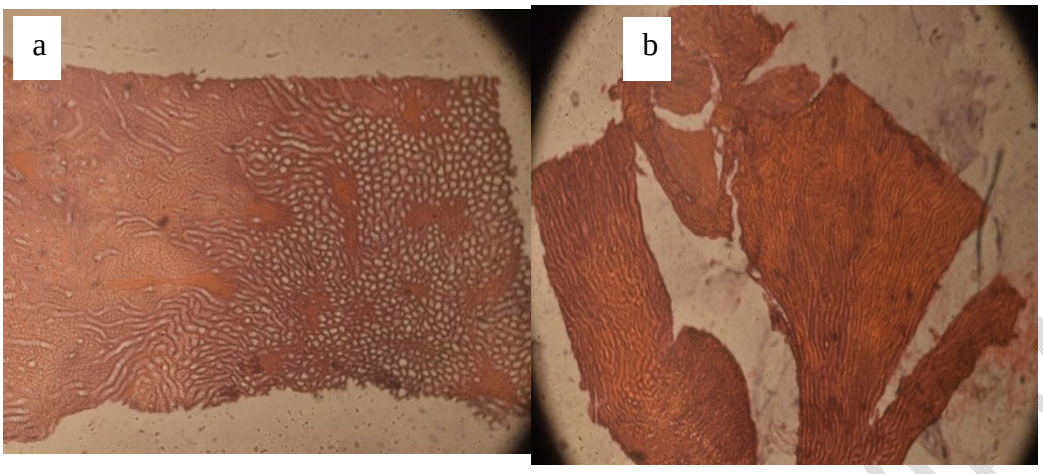

Fig. 11. Histological slide of Kidney. (a) control mouse, (b) treated mouse

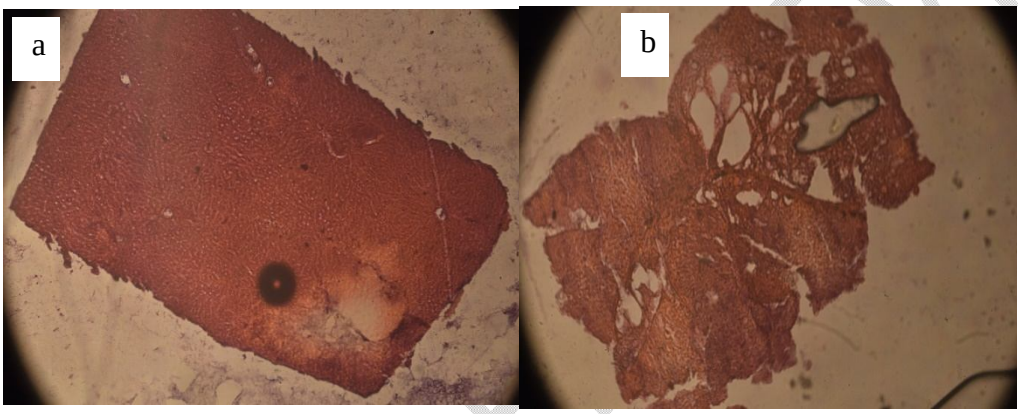

Fig. 12. Histological slide of Liver. (a) control mouse, (b) treated mouse

4. CONCLUSION

Our findings demonstrate that nanoplastics released from hot nonfood-grade and nonwoven polyethylene bags can adversely affect total and differential count of RBC and WBC as well as morphology and histology of mice intestine, liver,heart,lung andkidney,leading to alterations of their body weight.

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