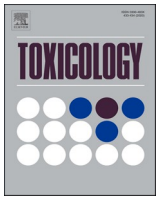
Toxicology 492 (2023) 153545

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol



Hematological consequences of polyethylene microplastics toxicity in male rats: Oxidative stress, genetic, and epigenetic links

Amina A. Farag [a], Heba S. Youssef [b], Rania E. Sliem [c], Walaa Bayoumie El Gazzar [d,e], Nashwa Nabil [f], Maha M. Mokhtar [a], Yasmin M. Marei [e], Nesma S. Ismail [a], Shaimaa E. Radwaan [c], Amira M. Badr [g,h], Alaa El-Din Hamid Sayed [i,j,*]

[a] Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Benha University, 13518, Egypt
[b] Department of Physiology, Faculty of Medicine, Benha University, 13518, Egypt
[c] Department of Zoology, Faculty of Science, Benha University, 13518, Egypt
[d] Department of Anatomy, Physiology and Biochemistry, Faculty of Medicine, The Hashemite University, Zarqa 13133, Jordan
[e] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Benha University, 13518, Egypt
[f] Department of Community, Environmental and Occupational Medicine, Faculty of Medicine, Benha University, 13518, Egypt
[g] Department of Pharmacology and Toxicology, Faculty of Pharmacy, King Saud University, Riyadh 11211, Saudi Arabia
[h] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt
[i] Department of Zoology, Faculty of Science, Assiut University, 71516, Egypt
[j] Molecular Biology Research & Studies Institute, Assiut University, 71516 Assiut, Egypt

ARTICLE INFO

Handling Editor: Dr. Mathieu Vinken

Keywords:
Polyethylene microplastics
Hematological
Red blood cells
Genotoxicity
DNA methylation
Epigenecity

ABSTRACT

Microplastics (MPs) pollution is a newly emerging environmental issue. MPs can accumulate within animals and humans, which can pose a serious health threat. Petroleum-based polyethylene (PE) is one of the most popular plastics. Accordingly, its exposure rates have steadily increased over the years. This study aimed to analyze the effects of PE-MPs on the hematological system of albino rats and the epigenetic effect. Five groups of adult male eight-weeks-old rats received either distilled water, corn oil, 3.75 mg/kg PE-MPs, 15 mg/kg PE-MPs, or 60 mg/kg of PE-MPs, daily by oral gavage for 35 days. PE-MPs significantly increased the body weights of the rats and lipid peroxidation, with concomitant reduction of superoxide dismutase activity and depletion of reduced glutathione, thus adversely affecting oxidants/antioxidants balance. Moreover, PE-MPs increased the % of abnormal RBCs, irregular cells, tear drop cells, Schistocyte cells, and folded cells. The genotoxic effects on DNA were evident by increased DNA damage, confirmed by the comet assay, in addition to increased DNA methylation. The effects of PE-MPs have been shown to be dose correlated. In conclusion, this study provides evidence of dose-related PE-MPs-induced hematological, genotoxic, and epigenetic effects in mammals, and thus emphasizes the potentially hazardous health effects of environmental PE-MPs.

1. Introduction

Plastics have encompassed a large part of our lives, with plastic production has risen significantly over the past decades. Plastics have been used as an alternative to other materials like glass, metals, paper, and wood (Geyer et al., 2017; Yao et al., 2022). There is a global production of millions of tons of plastic (ICH S2 (R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use - Scientific Guideline, 2022). It is suggested that by the year 2025, approximately 100–250 million tons will enter surface waters (Ali et al., 2021). However, with massive production comes more pollution and thus more potential hazardous health effects (Sayed et al., 2021; Abdel-Zaher et al., 2023).

Plastic wastes may be decomposed via hydrolysis, physical/mechanical forces, and ultraviolet light to form tiny particles, also known as microplastics (MPs) (Moore, 2008; Du et al., 2021). MPs are small plastic pieces ranging in size from 0.1 mm to 5 mm. Various MPs can be found within the environment. The main component of MPs includes polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene (PE), with PE being one of the most prevalent ones (Ali et al., 2021; Santacruz-Juárez et al., 2021). Although PE, PP, and PS-MPs were thought to be predominately found in the oceans (Wei et al., 2021), a

* Corresponding author at: Department of Zoology, Faculty of Science, Assiut University, 71516, Egypt.
E-mail address: alaasayed@aun.edu.eg (A.E.-D.H. Sayed).

https://doi.org/10.1016/j.tox.2023.153545
Received 6 April 2023; Received in revised form 2 May 2023; Accepted 9 May 2023
Available online 9 May 2023
0300-483X/© 2023 Elsevier B.V. All rights reserved.

recent study found that landfill plastic pollution was approximately 4–23 times that of plastic pollution found in the ocean (Rudolph et al., 2021).

Microplastics (MPs) are primarily produced by two sources: products containing plastic powders, such as cosmetics, detergents, sunscreens, and medicine delivery systems, in addition to those produced by break down of bigger plastic particles by ultraviolet radiation, mechanical abrasion, biological degradation, and other environmental factors (Abdel-Zaher et al. 2023). The predominate presence of MPs in food packaging, manufacturing, etc., increases the susceptibility of their ingestion, whether in animals, aquatic marine life, birds, mammals, and even humans (Auta et al., 2017; Ageel et al., 2022). MPs have the ability to accumulate in various organisms, including humans. Through many studies, it was confirmed that MPs can pass through body tissues, due to their non-degradable nature and minute size (Jin et al., 2021). MPs may enter the body of humans through three main routes: (1) oral intake of aquatic products (McNeish et al., 2018) and packaged food products (Waring et al., 2018), (2) dermal exposure (Hüffer et al., 2018), and (3) inhalation (Wright and Kelly, 2017; López et al., 2023). Oral route is considered the most common route of MPs exposure (Chang et al., 2020). Following exposure, MPs are absorbed via epithelial cells of the intestine, then subsequently enter the circulatory system and accumulate in many body cells and organs (Paul-Pont et al., 2018). MPs toxicity may be associated with Plastics themselves, or the additives found within them, as well as the adsorbed organic pollutants on the surfaces of MPs (Anderson et al., 2016; Kılıç et al., 2023).

MPs toxicity has been documented to affect many organs including liver, kidneys, brain, and reproductive ones (Kim et al., 2021). The pathophysiology of MPs toxicity in mammals is complex and is not yet extensively studied. MPs exposure is found to be associated with the induction of oxidative stress, cytotoxicity, and inflammation. They also interfere with energy and lipid metabolism and induce sub-cellular organ dysfunction (Llorca and Farré, 2021; Matthews et al., 2021). The toxicity of MPs may be enhanced by co-pollutants adsorbed on their surface (Zolotova et al., 2022). MPs beads' toxicity is determined by their size or type of plastic (Choi et al., 2020).

Blood and haematological parameters are an excellent means to determine toxic exposure of a substance and the overall subject health (Joshi et al., 2002; Mekkawy et al., 2011; Zbidah, 2014). The most abundant cells in the blood are the red blood cells (RBCs). In addition to anemia, impaired RBCs can cause hypoxia-related symptoms and various other health issues. When toxic xenobiotics enter the body, they will most likely affect RBCs. It has been reported that MPs cause apoptosis and necrosis in *Danio rerio* RBCs (Guimarães et al., 2021), and in amphibians, *Physalaemus cuvieri* tadpoles (da Costa Araújo and Malafaia, 2021). PS-MPs were also recently found to affect mice RBCs adversely (Wang et al., 2022).

One of the main concerns about MPs is how they can affect DNA and their role as mutagenic and epigenetic pollutants. There has been growing concern about possible genotoxicity to humans induced by MPs (Çobanoğlu et al., 2021). Since these substances are microscopic, they can pass through the cell membrane and reach DNA, causing DNA damage. Until now, little is known about the exact mechanism of MPs-associated genotoxicity but increased genetic defects have been linked to the increased reactive oxygen species (ROS). MPs exposure was found to reduce the antioxidant defenses of the cells, with a subsequent increase of ROS. ROS can induce DNA strand breakage, thereby increasing the risk of chronic disorders and cancer (Dusinska et al., 2017). Genotoxicity encompasses different forms of harm done to the genome, including mutagenic lesions, chromosomal rearrangements and/or breakage, and numerical chromosome aberrations. Gel electrophoresis, or comet assay, is one of the means used to analyze genotoxicity (Dusinska et al., 2017; Han et al., 2023). MPs genotoxicity was previously documented in fish (Guimarães et al., 2021; Pannetier et al., 2020), and recently in mice (Guimarães et al., 2023; Zheng et al., 2019).

Among the most intriguing research areas is epigenetic toxicology, which studies epigenetic changes induced by environmental exposures. Gene expression changes caused by epigenetic factors occur without alterations in DNA sequence. There is evidence that some environmental toxins influence the epigenome, by changing DNA methylation, modifying histone proteins, and affecting chromatin structure and miRNA expression. Evidence has proved that epigenetic alterations are involved in numerous pathologies, including, obesity, cancer, and neurological/psychological disorders (Marczylo et al., 2016).

The epigenetic alterations driven by MPs have rarely been studied. Evidence of MPs epigenetic effects have been recently reported in *Drosophila* (Zhang et al., 2020) and in mice (Li et al., 2022); however, the results are still preliminary, and the mechanism of action has not yet been fully elucidated. The epigenetic effect of PE-MPs in mammals was not previously studied, and no study about the effect of MPs on DNA methylation is yet available. Therefore, this study was conducted to help answer for the following questions: (1) Do PE-MPs have epigenetic effects in mammals, (2) If yes, are these effects correlated to the dose of PE-MPs. We also hypothesize that PE-MPs exposure can induce RBCs abnormalities and DNA damage in rats, which are dose-dependent and strongly correlated to PE-MPs- induced oxidative stress.

2. Materials and methods

2.1. Microplastics

The polyethylene microplastics (PE-MPs) were purchased from Micro Powders Inc (580 white Plains Rd, Tarrytown, NY 10591, United States, product name MPP-635XF, CAS number 9002-88-4). PE-MPs consisted of raw white powder with mean particles size ranging from 4.0 to 6.0 μm. A stock solution was prepared by dispersing PE-MPs in corn oil by using the magnetic stirrer according to the manufacturing procedures at room temperature. Prior to altering MPs structure or adding it to any substance, the structure of the MPs was documented using a scanning electron microscope at the transmission electron microscope unit (TEMU), Assiut University using JEOL JEM-1200 EX II (Massachusetts, USA). Fourier transform infrared spectroscopy (FTIR) was used to identify the microplastics composition. FTIR spectra were recorded in the range of 4000–400 cm^{-1} using (Thermo Scientific Nicolet iS10).

2.2. Animals

Thirty-five adults male Sprague Dawley rats, 8-week-old, weighting (150–180 g), were bought from the animal house, Veterinary Medicine College, Benha University, Egypt. The rats were placed in individualized, clean, environmentally controlled cages (3–4 animals/cage) (12 h light and dark cycle, 350–65 % humidity, 30–25 °C). Animals had free access to tap water as well as food ad libitum on commercial pellets and allowed to acclimate for one week prior to any experimentation. All procedures were approved and done according to the guidelines approved by the Ethics Committee of the Faculty of Medicine, Benha University, Egypt (approval no. RC: 31-11-2022), which is basically conform to the guide for the care and use of laboratory animals of the national institutes of health in the USA (NIH publication No.86-23, revised 1996). Furthermore, All methods are reported in accordance with ARRIVE guidelines.

2.3. Experimental scheme

Randomization of animals was done, based mainly on animal weight, to ensure initial animals weights don't show any significant difference. The rats were divided into five groups with seven rats per group. In this study, two separate negative control groups were used, one received distilled water and the other received corn oil; the vehicle. The three other groups were the experimental groups; PE-MPs groups; divided based on the amount of oral PE-MPs dosage as low-, medium-, and high-

dose groups. The low-dose group received 3.75 mg/kg body weight of PE-MPs daily, the medium-dose group received 15 mg/kg body weight of PE-MPs daily, and the high-dose group received 60 mg/kg of PE-MPs daily. Treatments were given using oral gavage for a total of 35 days (Park et al., 2020). Animal weights were recorded weekly.

2.3.1. Collection of blood samples

Following 24 h after the final dose, the rats were euthanized, rats were euthanized under the conventional protocol of inhalation anesthesia using isoflurane (El Amriya for pharmaceutical industries, Al Amyria, Alexandria). Blood samples were then obtained from the abdominal aorta. A portion of the blood collected was used to prepare blood smears and agarose microgels for the alkaline comet assay, another portion of blood was collected in the EDTA vacutainer tubes for DNA methylation analyses. The last portion of the blood sample was used for the preparation of serum, collected in uncoated tubes, and allowed to coagulate, it was then centrifuged at 2000 rpm for 30 min, serum was separated and stored at − 20°C until further biochemical processing.

2.3.2. Blood smear preparation

After the blood samples were collected, smears were done on a clean slide (non-heparinized blood sample). After the slides dried, they were fixed in absolute methanol for 10 min and subsequently stained with hematoxylin and eosin, rinsed in distilled water, air-dried, and mounted. Scores were assigned randomly to the slides based on the staining quality. In each group, 3000 cells (minimum of 100 cells per slide) were analyzed and were photographed at x400 magnification by using (OMAX with 14 Mp Camera, MN:A35140U3,China) for polymorphic erythrocytes according to (Al-Sabti and Metcalfe, 1995; Schmid, 1975). The marked morphological alterations of RBCs such as echinocyte cells, sickle cells, and acanthocyte cells were recorded.

2.3.3. Oxidative stress biomarkers

Serum malondialdehyde (MDA) levels were measured using commercial kit (Biodiagnostic, Egypt, Catalog number: MD 2529), according to the manufacturer's instructions. The principle is based on the reaction of MDA with thiobarbituric acid in acidic medium, to produce a thiobarbituric reactive compound with a pink color that can be measured spectrophotometrically at 534 nm. A method previously performed by Ohkawa et al. (1979).

The activity of serum superoxide dismutase (SOD) was measured spectrophotometrically at 560 nm, using commercial kit (Biodiagnostic, Egypt, Catalog number: SD 2521), according to the manufacturer's instructions. The kit methodology is based on the principle originally performed by Nishikimi et al. (1972).Serum reduced glutathion (GSH) was also determined using commercial kit (Biodiagnostic, Egypt, Catalog number: GR 2511, according to the manufacturer's instructions.

2.3.4. DNA damage detected by the alkaline comet assay

DNA breaks and alkali-labile sites can be detected using the Comet Assay (single cell gel electrophoresis). The blood samples were diluted using phosphate-buffered saline (PBS, 0.1 M) 1:2. After that, slides were coated with 100 μl of normal-melting-point agarose (0.7 %) as a first layer, once it dries, 75 μl of low-melting-point agarose and 15 μl of blood sample were applied, representing the second layer. These slides were putted in lysis buffer (1 % sodium sarcosinate, 2.5 M NaCl, 100 mM Na_2EDTA, 10 m M Tris-HCl, 1 % Triton X-100 and DMSO 10 %) for 2 h, after discarding the lysis buffer, slides were washed using cold distilled water for 15 min twice. Slides were then oriented on the horizontal gel box. Freshly prepared electrophoresis buffer (0.3 M NaOH, 1 mM Na_2EDTA, pH 13) was used to fill the buffer reservoirs, with the slides completely covered. The slides were left to sit in this buffer for 20 min at room temperature to allow for unwinding of DNA. A power supply producing 24 volts was attached for 30 min, and then the slides were stained with Ethidium bromide (20 ugh/ml), and left for a total of five minutes. Finally, the slides were dipped in cooled distilled water to remove any excess stain (Singh et al., 1988). The procedure was performed in the dark to avoid supernumerary damage of DNA. Analysis of 1000 nuclei (minimum of 100 nuclei per slide) were achieved by a fluorescent microscope (Olympus BX51) (under 400 x magnification). DNA damage measured using image analysis software TnTek Comet Score TM (AutoComet.com,Ver. 1.5).

2.3.5. Global DNA methylation

Using the EDTA blood collection tubes, blood samples were collected. DNA isolation from whole blood was performed using QlAamp Mini DNA Kit (Qiagen, USA), in accordance with the manufacturer's protocol. Thermo Scientific NanoDrop 2000c Spectrophotometer (Nanodrop Technologies) was used to analyze DNA quality. The starting DNA concentration for methylation analysis was 50 ng/μl. One microliters of DNA was used for the evaluation of the methylated fraction of DNA; 5-methyl-cytosine (5-mC), which was detected by using detection antibodies, and then colorimetrically quantified. Global DNA methylation was detected in the isolated blood DNA by MethylFlash™ Global DNA Methylation (5-mC), ELISA Easy Kit (Catalog#:P-12034, Epigentek, e, NY, USA) was performed as per the kit instructions. The percentage of 5- mC was calculated in 100 mg DNA extracted using the second order regression equation of the standard curve in experiment groups. The reactions were performed in duplicates and average values were used for statistical analysis.

2.3.6. Statistical analysis

The normality of distribution for the analyzed variables were tested using Kolmogorov-Smirnov and Shapiro tests assuming normality at $P > 0.05$. The collected data were summarized in terms of median and Inter Quartile Range (IQR) as appropriate for nonparametric data. The statistical significance of the difference between groups was evaluated using Kruskal Wallis test. Bonferroni correction for multiple tests was used for pairwise comparisons of the study group. Spearman correlation and simple linear regression were done for quantitative data to detect dose dependent effect (Statistical Package for the Social Sciences (SPSS) 28.0 for windows SPSS Inc., Chicago, IL, USA). P value < 0.05 was considered significant.

3. Results

3.1. Characterization of polyethylene micro-plastics (PE-MPs)

PE-MPs morphology was demonstrated using a scanning electron microscope (Fig. 1a &b). PE-MPs were found to have irregular fragmented shape particles with sharp edges. The FTIR analysis illustrated the significant peaks shown at 2854 and 2922 cm^{-1} for asymmetric -CH2. Peaks at 1463, 1159, and 722 cm^{-1} are related to bending -CH2, while the band of emulsion residue is observed at 1744 cm^{-1} (Fig. 1c). Peaks are consistent with PE characteristic ones (Zhang et al. 2021).

3.2. Effect of PE-MPs exposure on rat body weight

PE-MPs moderate and high dose induced a significant increase in body weight at the fifth week, compared to negative controls (distilled water and corn oil treated groups) ($P < 0.05$). No statistically significant difference was found between body weight among other studied groups during five weeks of treatments ($p > 0.05$) (Fig. 2).

3.3. Effect of PE-MPs exposure on oxidative stress related parameters

Both moderate and high dose PE-MPs induced a significant decrease of SOD and GSH depletion, with a correlated increase of lipid peroxidation, measured as MDA level, as compared to negative control groups ($P < 0.001$). Low dose PE-MPs didn't induce any significant changes in oxidative stress related parameters as compared to the control groups

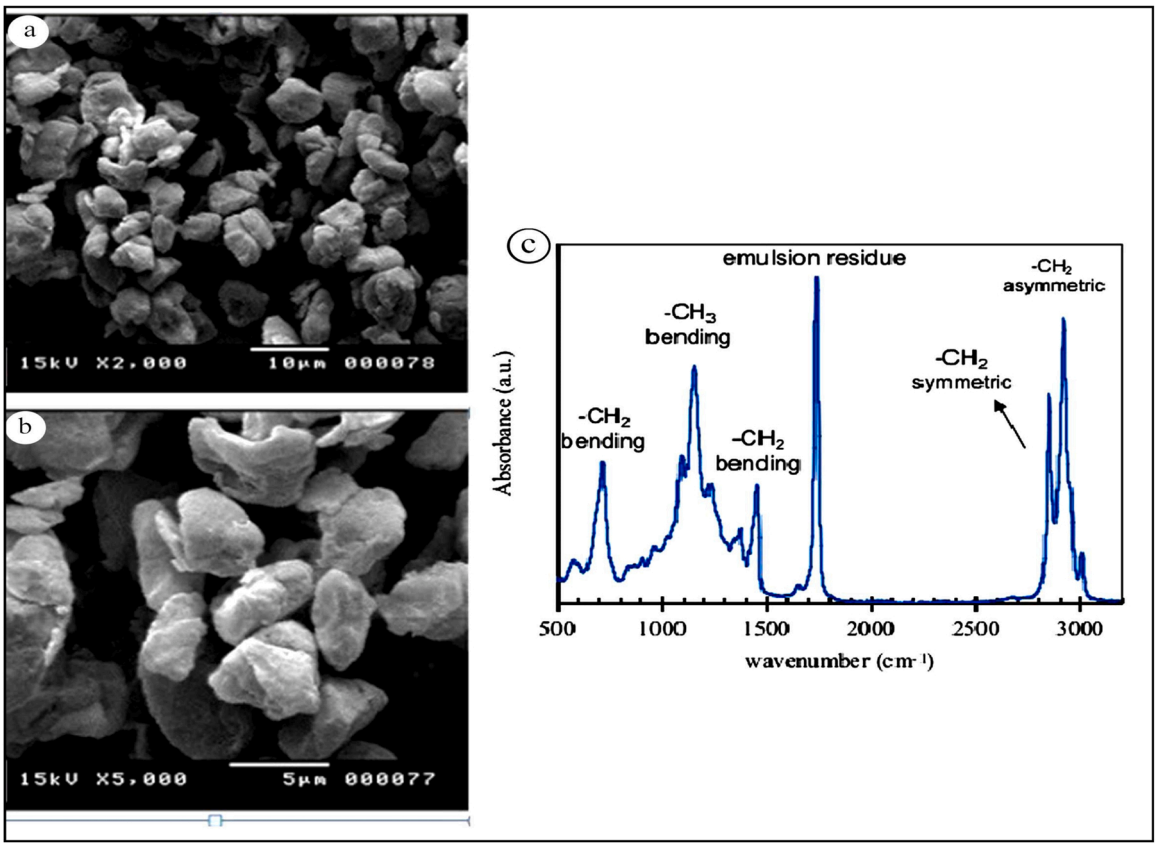


Fig. 1. Characterization of PE-MPs. (a) Low power and (b) high power image of PE-MPs under SEM. (c) FTIR spectroscopy of PE-MPs. PE-MPs: Polyethylene Microplastics. SEM: Scanning electron microscope. FITR: Fourier transform infrared spectroscopy.

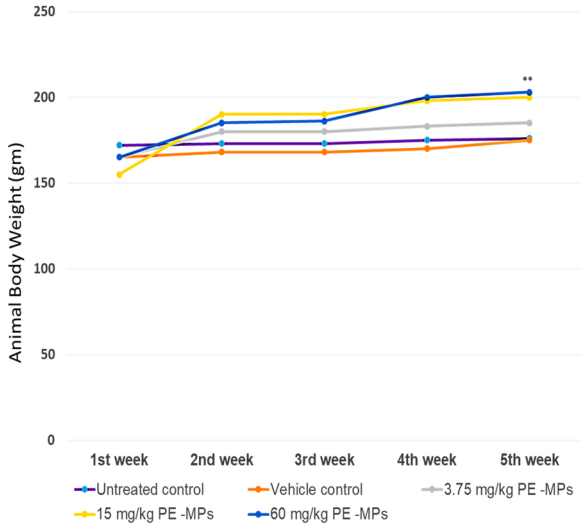


Fig. 2. The effect of high, medium, and low doses of polyethylene microplastics (PE-MPs) on changes in body weight in studied groups. Untreated control: Received only distilled water, Vehicle control: received corn oil (the vehicle), and the other groups received PE-MPs at three different doses. Results are represented as median. * *: Significantly different from untreated control and vehicle control groups, ($P < 0.05$) (Kruskall Wallis test=17.32 &17.89 respectively). n = 7.

($P > 0.05$) (Fig. 3a, b and c).

3.4. Effect of PE-MPs exposure on red blood cells

Both groups treated with 15 or 60 mg/kg body weight PE-MPs significantly increased RBCs abnormalities in comparison with other studied groups ($P < 0.001$). Low dose PE-MPs-induced RBCs abnormalities was not statistically significant compared to negative control groups ($p > 0.05$) (Fig. 4).

3.5. DNA damage induced by PE-MPs exposure

Fig. 5 depicts the PE-MPs-induced DNA damage, which was measured by the comet assay of the studied groups (Fig. 5). The intact nuclei can be observed in images representative of both control groups; distilled water and corn oil administered groups (Fig. 5a, and b) respectively. Group treated with 3.75 PE-MPs mg/kg showed mild tailing with slight damage in the nucleus (c). Group treated with 15 mg/kg showed a medium degree of damage (d). High dose, 60 mg/kg PE-MPs group, (e) showed a high degree of damage (long tail and small nucleus). There were high statistically significant differences between DNA changes by comet assay among high, moderate and low dose groups and distilled water groups and corn oil groups (Table 1). High dose of PE-MPs and moderate dose significantly increased percent of tail DNA and tail length in comparison to corn oil and distilled water groups ($P < 0.001$), in addition to a significant increase in tail length, tail and olive tail moments. There were no statistically significant differences between DNA changes by comet assay among low dose PE-MPs treated group and distilled water group and corn oil group ($p > 0.05$) (Table 1).

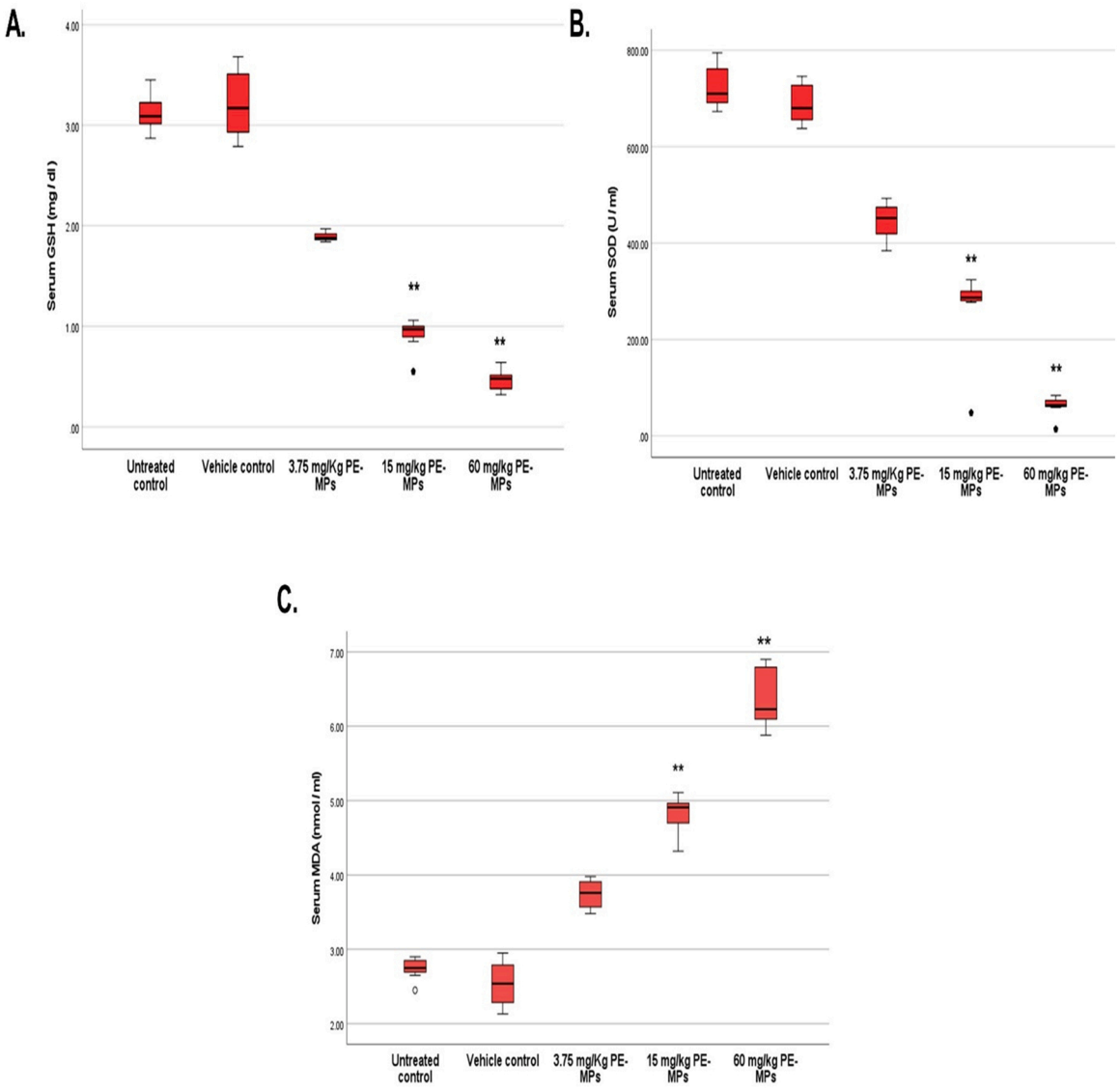


Fig. 3. The effect of high, medium, and low doses of polyethylene microplatics (PE-MPs) on oxidative stress markers. A: Serum level of glutathione (GSH), and B: Serum level of superoxide dismutase (SOD), A: Serum level of malondialdehyde (MDA). Untreated control: Received only distilled water, Vehicle control: received corn oil (the vehicle), and the other groups received PE-MPs at three different doses. * *: Significantly different from untreated control and vehicle control groups, ($P < 0.001$). $n = 7$.

3.6. Global DNA methylation induced by PE-MPs exposure

High dose of PE-MPs significantly increased DNA methylation in comparison to distilled water and corn oil among studied groups ($P < 0.05$). No statistically significant difference was recorded between DNA methylation level among other treated groups ($p > 0.05$) (Fig. 6).

3.7. Heat map correlation between different measured parameters

PE-MPs doses showed high negative correlation with GSH and SOD levels ($P < 0.001$), while showed positive correlation with other assessed parameters ($P < 0.001$). Significant negative correlation was found between MDA and both GSH and SOD, as reduced antioxidant defenses are associated with a rational increase of lipid peroxidation marker. RBCs abnormalities and DNA damage were negatively correlated with antioxidants, GSH and SOD, and positively correlated with increased lipid peroxidation and MDA, which emphasizes the role of oxidative stress in mediating RBCs and DNA damage. There were highly significant positive correlations between PE-MPs dose and RBCs abnormalities, DNA changes by comet assay, DNA methylation and body weight among studied among groups ($P < 0.001$), Fig. 7, Table 2.

4. Discussion

Recent global attention has been focused on MPs' environmental contamination. MPs can adversely affect human health because plastics are highly resistant to degradation and can endure in the environment for a long time. Air, food, drinks, packaging, and even packaging materials expose humans to MPs. In living organisms, MPs accumulate in their cells and tissues, potentially causing chronic biological effects, such as gastrointestinal disorders, immunity, respiratory problems, cancer, infertility, and chromosome modifications. The health effects of MPs and mechanisms of toxicity must be extensively studied because of the threat they pose to human health (Haindongo et al., 2023; Mamun et al., 2023).

MPs serve as carriers for a variety of elements and different toxicants that result in many hazardous effects on the physiology of humans and other animals. Up till now, there is little known regarding the impact of

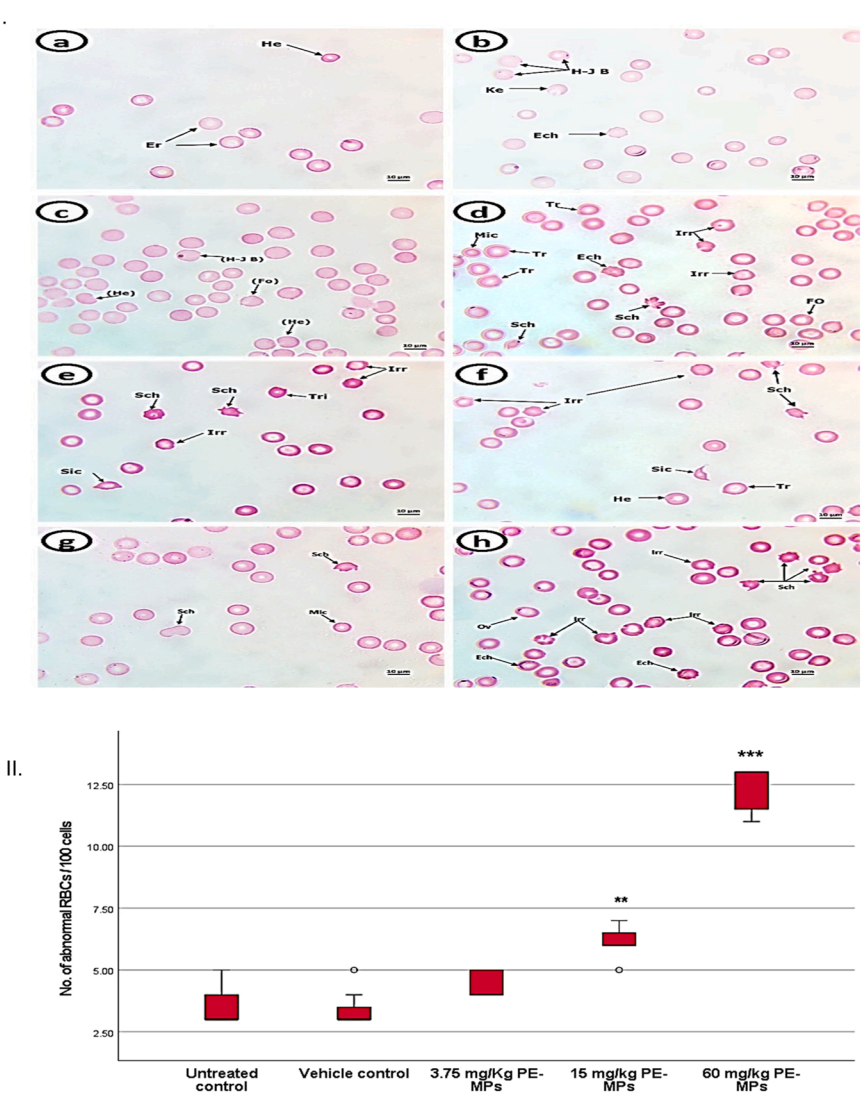


Fig. 4. RBCs abnormality caused by high, medium and low doses of polyethylene microplatics (PE-MPs) in studied groups. (I) Representative photographs of H& E. (a) Untreated control: Received only distilled water, (b)Vehicle control: received corn oil (the vehicle), (c and d) rats exposed to low dose (3.75 mg/kg PE-MPs),1 (e and f) rats exposed to medium dose (15 mg/kg PE-MPs), and (g and h) rats exposed to high dose (60 mg/kg PE-MPs). (Ach) Achanthocyte cell, (Ech) Echinocyte cell, (Er) Erythrocyte cell, (Fo) Folded cell, (He) Helmet Cell, (H-J B) Howell-Jolly Bodies, (Irr) Irregular shape, (Ke) Keratocyte Cell, (Ke) Keratocyte Cell, (Mi) Microcytes, (Mic) Microcyte, (Ov) Ovalocyte cell, (Sch) Schistocyte cell, (Sic) Sickle Cell, (Tr) Tear drop cell. Scale bar = 20 μm. (II) Number of abnormal RBCs/100 cells caused by high, medium and low doses of PE-MPs in studied groups, 5 different samples per group were counted. * ** significant difference from untreated control, vehicle control and low dose PE-MPs-treated groups ($P < 0.001$); * * significant difference from untreated and vehicle control groups ($P < 0.001$); Kruskall wallis test= 28.63, n = 5. Magnification= 400x.

MPs on human health (Kim et al., 2021). Animal models have proved useful in determining MPs exposure risks, which can aid in determining its effect on the human body as well (da Silva Brito et al., 2022). PE is the main kind of MPs in the environment (Sun et al., 2021).

Body weight is a common sensitive indicator used in the field of toxicology (Xie et al., 2022). The current study demonstrated an increase in body weight in high dose PE-MPs treated rats when compared with the control group. The same observation was noted by others (da Costa Araújo and Malafaia, 2021; Han et al., 2021; Xie et al., 2022). This increase in body weight has been found to be related to PE-MPs induction of oxidative stress and associated alteration of energy and fatty acid metabolism. Accumulation of MPs in the liver and kidney has also been shown to boost the growth and accumulation of fat cells and disrupt energy balance, which ultimately increases body weight (Sun et al., 2021). Furthermore, the obesogenic effect of MPs might be due to affection of the gut-liver axis as gut microbiota dysbiosis is a common effect of MPs. Changes in gut microbiota can perturb physiological homeostasis, leading to an alteration of body weight (Shi et al., 2022). Besides, a connection between exposure to MPs and induction of insulin resistance has been suggested, which may further explain increased body weight (Huang et al., 2022). Our result was in contrast to Deng et al., 2021; Park and Kim, 2022 who found a regression in body weight on short-term administration of PE-MPs.

Evidence suggest that PE-MPs have pro-oxidative properties. PE-MPs alter the oxidative–antioxidative system within mouse serum, as demonstrated by various studies, and these imbalanced alterations result in increased ROS production and subsequent oxidative stress on the cells (Dusinska et al., 2017). In the same context, in our study we recorded reduced serum levels of cellular key antioxidant players; SOD and GSH; which may be attributed to MPs-induced oxidative stress as mentioned earlier. The oxidative stress could lead to depletion of circulating antioxidant markers, as well as induction of mitochondrial dysfunction through ROS production, which was demonstrated by other studies. One of the main organelles responsible for the antioxidant defense systems is the mitochondria, which also produces SOD and GSH (Surai et al, 2019).

Reduced antioxidant defenses result in increased ROS, that oxidize cellular macromolecules, including proteins, lipids, and DNA. Our study demonstrated significantly increased levels of MDA (lipid peroxidation byproduct), when animals were sub-chronically exposed to PE-MPs. These increased MDA levels caused by the accumulation of ROS like hydrogen peroxide, superoxide anion, and hydroxyl radicals. With increased oxidative stress, hemoglobin itself undergoes an auto-oxidation reaction which results in the formation of methemoglobin ($Hb–Fe^{3+}$), and if not converted back to its original state, $Hb–Fe^{3+}$ will degrade leading to even more ROS production (Barbarino et al., 2021).

In our study, PE-MPs induced oxidative stress was found to be correlated to the dose. Rats exposed to higher doses of PE-MPs

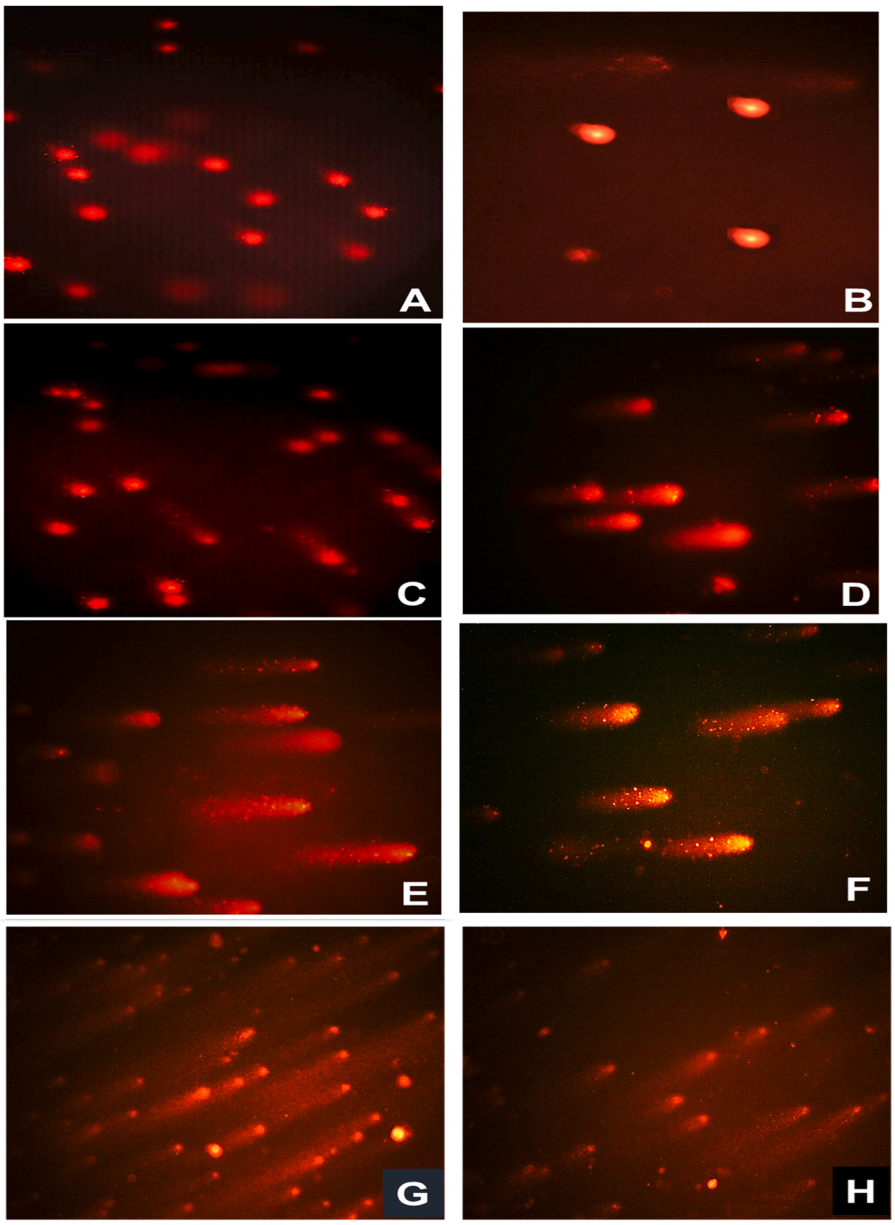


Fig. 5. DNA damage induced by high, medium, and low doses of polyethylene microplatics (PE-MPs) conducted by using comets assay. (A) Untreated control: Received only distilled water, (B) Vehicle control: received corn oil (the vehicle), (C and D) rats exposed to low dose (3.75 mg/kg PE-MPs), (E and F) rats exposed to medium dose (15 mg/kg PE-MPs), and (G and H) rats exposed to high dose (60 mg/kg PE-MPs).

Table 1
Differences of PE-MPs induced DNA damage between studied groups.

DNA Damage	Study groups						
	Untreated control Median (IQR)	Vehicle control Median (IQR)	3.75 mg/Kg PE-MPs Median (IQR)	15 mg/kg PE-MPs Median (IQR)	60 mg/kg PE-MPs Median (IQR)	Kruskall wallis test	P-value
Tail Intensity (TI)	5.87 (5.33–6.33)	5.98 (5.39–6.41)	11.34 (11.13–2.13)	15.03** (14.33–5.33)	18.62** (17.33–9.73)	31.04	<0.001
Tail length (PX)	6.33 (5.39–6.51)	6.25 (5.87–6.59)	8.96 (8.33–9.44)	11.13** (10.86–1.25)	12.88** (12.25–13.0)	31.03	<0.001
Tail DNA (% TDNA)	2.69 (2.17–3.23)	3.18 (2.85–3.81)	7.01 (6.95–7.12)	8.80** (8.40–9.71)	10.76** (10.57–1.34)	31.53	<0.001
Tail moment	0.21 (0.17–0.33)	0.20 (0.18–0.31)	0.59 (0.58–0.66)	0.94** (0.91–0.98)	1.06** (1.02–1.12)	31.11	<0.001
Olive tail moment	0.49 (0.31–0.53)	0.42 (0.40–0.50)	0.81 (0.72–0.85)	1.27** (1.21–1.37)	1.75** (1.69–1.83)	30.75	<0.001

* * Statistically significant from untreated control (received only distilled water) and vehicle control (received only corn oil). n = 7.

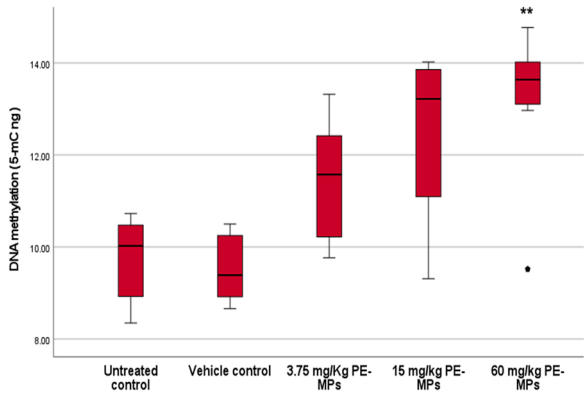


Fig. 6. DNA methylation induced by high, medium, and low doses of polyethylene microplatics (PE-MPs). Untreated control: Received only distilled water, Vehicle control: received corn oil (the vehicle), and the other groups received PE-MPs at three different doses. * *: Significantly different from untreated control and vehicle control groups, ($P < 0.001$); Kruskall Wallis test = 15.564. n = 7.

demonstrated a marked increase in ROS generation and impairment in antioxidant defense pathways when compared to animals with moderate dose exposure. Low-dose exposure, on the other hand, resulted in no significant change in these parameters. Our Fundings coincide with other studies (Ali et al., 2021; Dong et al., 2022; Ijaz et al., 2022; Sincihu et al., 2023; Sun et al., 2021; Xie et al., 2022). The results of (Li et al., 2019), were not comparable to our results. They found that polystyrene nanoparticles decreased ROS and shutdown ferroptosis via triggering lysosome stress. These findings can be due to the fact that, different types of plastics and different sized may lead to different toxic effects.

MPs can enter into the circulatory system and reach other organ systems causing lethal reactions depending on the amount of exposure, and various studies approved the accumulation of MPs in blood cells, which is why hematological parameters are suggestive to be a useful method for determining the substance toxicity and general levels within the body (Ma et al., 2020; Scanes et al., 2019). Erythron profiles (poikilocytosis and nuclear abnormalities) have proved useful in determining MPs cytotoxicity and are therefore essential biomarkers.

In the current study, PE-MPs caused an imminent increase in the percentage of poikilocytosis cells in RBCs in medium and high dose when compared to the control group. According to our current knowledge, it is the first one to show the effect of PE-MPs on rat RBCs poikilocytosis. Our findings revealed that the percentage of poikilocytosis is dose related. PE-MPs showed Howell-Jolly bodies, keratocyte cells, and echinocyte cells in low doses. Howell-Jolly Bodies are cytopathologically identified as basophilic nuclear remnants (DNA clusters) in circulating erythrocytes (Tong et al., 2019). It is caused by an erythropoiesis defect. Generally, RBCs nuclear fragments are removed mainly by the spleen, it acts by removing the inclusions without destroying the cells

Table 2
Simple linear regression between PE-MPs dose and hematological parameters.

PE -MPs dose	B	P value	95% CI	
			Lower	Upper
MDA (nmol/ml)	0.058	<0.001	0.049	0.067
SOD (U/ml)	-9.667	<0.001	-11.757	-7.577
GSH (mg/dl)	-0.040	<0.001	-0.050	-0.029
RBCs abnormalities	0.145	<0.001	0.133	0.156
Tail intensity (TI)	0.187	<0.001	0.146	0.228
Tail length(PX)	0.097	<0.001	0.074	0.120
Tail DNA (% TDNA)	0.117	<0.001	0.088	0.146
Tail moment	0.012	<0.001	0.009	0.015
Olive tail moment	0.020	<0.001	0.017	0.024
DNA methylation	0.051	<0.001	0.027	0.076
Body weight	0.365	<0.001	0.195	0.536

PE-MPs dose is a highly significant predictor of oxidative stress markers level, RBCs abnormalities, DNA changes by comet assay, DNA methylation and body weight. GSH: Reduced glutathione, MDA: Malondialdehyde, PE-MPs: polyethylene microplatics, RBCs: Red blood cells, SOD: Superoxide dismutase.

Spearman correlation coefficient	PE-MPs dose	GSH	SOD	MDA	RBCs abnormalities	% tailed	Tail length	% DNA in tail	Tail moment	Olive tail moment	DNA methylation	ody weight
PE-MPs dose	1											
GSH	-0.953	1										
SOD	-0.943	0.941	1									
MDA	0.955	-0.881	-0.886	1								
RBCs abnormalities	0.91	-0.906	-0.874	0.841	1							
% tailed	0.956	-0.871	-0.883	0.93	0.829	1						
Tail length	0.955	-0.897	-0.877	0.916	0.865	0.917	1					
% DNA in tail	0.956	-0.922	-0.925	0.916	0.838	0.893	0.895	1				
Tail moment	0.956	-0.92	-0.899	0.922	0.874	0.895	0.927	0.92	1			
Olive tail moment	0.95	-0.894	-0.881	0.911	0.858	0.911	0.878	0.924	0.9	1		
DNA methylation	0.67	-0.711	-0.633	0.622	0.622	0.585	0.633	0.924	0.71	0.597	1	
Body weight	0.724	-0.675	-0.598	0.71	0.701	0.696	0.684	0.7	0.69	0.733	0.524	1

Fig. 7. Heat map correlation matrix. There were highly significant correlations between PE-MPs dose and oxidative stress marker, RBCs abnormalities, DNA changes by comet assay, DNA methylation and body weight among studied groups ($P < 0.001$). There were highly significant negative correlations between PE-MPs dose and GSH and SOD levels among studied group ($P < 0.001$). There was highly significant positive correlation between PE-MPs dose and MDA level among studied groups ($P < 0.001$). There were highly significant positive correlations between PE-MPs dose and RBCs abnormalities, DNA changes by comet assay, DNA methylation and body weight among studied among groups ($P < 0.001$). GSH: Reduced glutathione, MDA: Malondialdehyde, PE-MPs: polyethylene microplastics, RBCs: Red blood cells, SOD: Superoxide dismutase. $P < 0.001$.

that contain them, which can happen due to cell fragmentation. Nuclear fragments are also removed by the help of the bone marrow, that takes place as the normoblast leaves the bone marrow via the endothelial pores. With these theories in mind, Holly-Jolly bodies are associated with nuclear maturation pathologies (Scafidi et al., 2022). So, MPs-induced spleen and bone marrow damage could result in these bodies (Zwollo et al., 2021). Furthermore, keratocyte cells are fragments of RBCs known as bite cells due to a bite-like defect in their membrane. These are caused by phagocytosis of a Heinz body, which leaves a bite in the cell. Echinocyte is thus a type of RBCs characterized by an abnormal cell membrane with small, evenly spaced thorn-like projections(Barger, 2022). These abnormal cells could be the result of a blood vessel wall disease that causes the membrane of some RBCs to rupture. In this regard, MPs-induced toxicity with microcirculation could result in keratocyte and echinocyte production (Park and Kim, 2022).

High and medium doses of PE-MPs, on the other hand, revealed the achanthocyte cell, echinocyte cell, erythrocyte cell, folded cell, helmet cell, howell-jolly bodies, irregular shape, keratocyte cell, microcytes, ovalocyte cell, schistocyte cell, sickle cell, and tear drop cell. It could be explained that PE-MPs interacts with erythrocytes, limiting the dehydrogenase of delta-aminolevulinic acid and causing RBCs cytoskeleton disruption through the affection of spectrin and ankyrin fibrils that are responsible for its normal biconcavity, resulting in poikilocytosis (Barbarino et al., 2021). Although, the increased production of ROS in RBCs may provide a plausible explanation for RBCs abnormalities, it may also be caused by direct interaction between MPs and their plasma membranes(da Costa Araújo and Malafaia, 2021). These data are corroborated by a previous study reported by Hamed et al., 2021, who noted the presence of MPs-induced poikilocytosis and eryptosis in early juvenile Nile tilapia (Oreochromis niloticus).

It has been recommended that comet assay is a highly sensitive and efficient method for assessing DNA damage and detecting DNA strand breakage in ICH S2 (R1) guidelines. In the current study, PE-MPs exposure resulted in significant DNA damage, which was indicated by increased tail length, increased DNA percentage within the tail, the tail moment, as well as the olive tail moment parameters. The genotoxic effects of PE-MPs may be attributed to increased ROS which can induce oxidation-mediated DNA damage. Roursgaard et al., 2022 did not detect any direct cytotoxicity in their study, but they did confirm that exposure to nanoplastics from PET and PP resulted in DNA strand breaks, ROS production, and altered cell cycle distribution, which confirms that MPs are a potential genotoxic substance that induces genomic damage. They attributed the genotoxic mechanism of action is due to direct physical interaction between nanoplastic particles and DNA, rather than oxidative stress-induced one. In harmony with the current study, Ballesteros et al., (2020) found significantly elevated levels of DNA damage in monocytes and polymorphoneuclear (PMN) cells following PS nanoparticles exposure. Malinowska et al. (2022), also noted increased PS-NPs single/double-strand break formation, elevated 8-oxo-2′-deoxyguanosine (8-oxodG) levels, and oxidized purines and pyrimidines, which was completely repaired in the case of the larger PS-NPs. They concluded that, genotoxic changes in peripheral blood mononuclear cells (PBMCs) were dependent on the size of particles tested. Studies with contradicting results can be attributed to the differences in plastic particles, such as its type, functionalized status, and size (Malinowska et al., 2022).

To elucidate the underlying mechanisms of MPs epigenetic toxicity, we investigated the MPs exposure effect on global DNA methylation. DNA function can be altered by environmental agents with no change in its sequence, by simply changing the DNA methylation status. Surprisingly, we demonstrated that MPs exposure is linked to DNA hypermethylation and that the degree of hypermethylation increased with higher doses exposure. This finding contradicts what previously reported by Im et al., 2022, who demonstrated a significant DNA hypomethylation in zebrafish exposed to PS-MPs. Our results are inconsistent also with the hypothesis that the oxidative stress can affect DNA compatibility to DNA methyltransferases (DNMTs), leading to DNA hypomethylation (Udomsinprasert et al., 2016; Ziech et al., 2011). Lind et al. (2013) results were supportive to some extent to the results of this study as they demonstrated that persistent organic pollutants are associated with changes in DNA methylation. In the study conducted by Jiang et al., 2020, they suggested that DNA oxidation, especially in the repeated sequences, can induce methylation through the polymerase β-DNMTs 3b during base excision repair, inducing hypermethylation in repeated sequences which further support our findings.

The present study is the first to elucidate that PE-MPs exposure is linked to global DNA hypermethylation in albino rats. Interesting, several studies have revealed the association between DNA methylation alteration and obesity, with evidence of implication of epigenetic mechanisms involving the alteration of methylation of genes related to metabolism (Mahmoud, 2022; Na et al., 2014; Samblas et al., 2019). This may to some extent explains the increased body weights of animals received PE-MPs in the current study1. It is of interest to elucidate the exact role of altered DNA methylation status triggered by toxic environmental agents on genes implicated in obesity in future studies.

This study emphasizes the toxicity associated with MPs and that their effect can be extended to DNA epigenetic damage in mammals, which may be linked to many of their hazardous effects on the biological systems. This supports the need for limiting the use of MPs, to minimize their potential risks. However, our study has some limitations, as only the effect on global DNA methylation was studied, however, the elucidation of the link between DNA methylation status and different DNA methyltransferases activity. In addition, only one type of MPs and one species of mammals was used, and the exact level of MPs in the blood was not measured. Therefore, further research using the same techniques for detecting DNA methyltranaferases activity using the same research model and the same MPs type and size are needed. More studies are still required to extensively elucidate MPs epigenetic mechanisms, and extend the study to other species, tissues using different types and sizes of MPs.

5. Conclusion

The current study showed that ingested MPs have negative effects on the hematological antioxidative statuses and elevated poikilocytosis cells percent in RBCs of rats. The study also provides extra evidence of PE-PMs-induced genotoxicity in mammals and is the first one to document their epigenetic toxicity in mammals. It also confirms that the toxicity of MPs is highly dose-related, and that the effects of PE-MPs can be minimized by reducing exposure.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval

Experimental conditions were carried out in compliance with the guidelines of the Medical Research Ethics Committee of the faculty of medicine, Benha University, Egypt (Rc: 31-11-2022). All methods were carried out following the relevant regulations and ARRIVE guidelines.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Code availability

Not applicable.

CRediT authorship contribution statement

Experimental design: **AF** and **AHS**. Experiment and analysis: **AF, RS, SH.R, MM, NS, YM, AH, T.KH** , and **NN**. Data interpretation: **AF, HY, NN, WB,** and **AB**. Writing and revision: **AF, HY, WB, RS, SH.R, AHS**, and **AB**. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated or analyzed during this study are included in the research article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tox.2023.153545.

References

Abdel-Zaher, S., Mohamed, M.S., Sayed, A.H., 2023. Hemotoxic effects of polyethylene microplastics on mice. Front. Physiol. 14, 141072797.

Ageel H.K., Harrad S., Abdallah M.A.-E. Occurrence, human exposure, and risk of microplastics in the indoor environment. Environ. Sci. Process. Impacts (Internet). The Royal Society of Chemistry; 2022, 24(1):17–31. ⟨https://pubs.rsc.org/en/content/articlelanding/2022/em/d1em00301a⟩. (Accessed 2 May 2023).

Ali, I., Cheng, Q., Ding, T., Yiguang, Q., Yuechao, Z., Sun, H., Peng, C., Naz, I., Li, J., Liu, J., 2021. Micro- and nanoplastics in the environment: occurrence, detection, characterization and toxicity – a critical review. J. Clean. Prod. 313, 127863 https://doi.org/10.1016/j.jclepro.2021.127863.

Al-Sabti, K., Metcalfe, C.D., 1995. Fish micronuclei for assessing genotoxicity in water. Mutat. Res. Genet. Toxicol. 343, 121–135. https://doi.org/10.1016/0165-1218(95)90078-0.

Anderson, J.C., Park, B.J., Palace, V.P., 2016. Microplastics in aquatic environments: implications for Canadian ecosystems. Environ. Pollut. 218, 269–280. https://doi.org/10.1016/j.envpol.2016.06.074.

Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. Environ. Int 102, 165–176. https://doi.org/10.1016/j.envint.2017.02.013.

Ballesteros, S., Domenech, J., Barguilla, I., Cortés, C., Marcos, R., Hernández, A., 2020. Genotoxic and immunomodulatory effects in human white blood cells after *ex vivo* exposure to polystyrene nanoplastics. Environ. Sci. Nano 7, 3431–3446. https://doi.org/10.1039/D0EN00748J.

Barbarino, F., Wäschenbach, L., Cavalho-Lemos, V., Dillenberger, M., Becker, K., Gohlke, H., Cortese-Krott, M.M., 2021. Targeting spectrin redox switches to regulate the mechanoproperties of red blood cells. Biol. Chem. 402, 317–331. https://doi.org/10.1515/hsz-2020-0293.

Barger, A.M., 2022. Erythrocyte morphology. In: In: Schalm's Veterinary Hematology. Wiley, pp. 188–197. https://doi.org/10.1002/9781119500537.ch24.

Chang, X., Xue, Y., Li, J., Zou, L., Tang, M., 2020. Potential health impact of environmental micro- and nanoplastics pollution. J. Appl. Toxicol. 40, 4–15. https://doi.org/10.1002/jat.3915.

Choi, D., Bang, J., Kim, T., Oh, Y., Hwang, Y., Hong, J., 2020. In vitro chemical and physical toxicities of polystyrene microfragments in human-derived cells. J. Hazard Mater. 400, 123308 https://doi.org/10.1016/j.jhazmat.2020.123308.

Çobanoğlu, H., Belivermiş, M., Sıkdokur, E., Kılıç, Ö., Çayır, A., 2021. Genotoxic and cytotoxic effects of polyethylene microplastics on human peripheral blood lymphocytes. Chemosphere 272, 129805. https://doi.org/10.1016/j.chemosphere.2021.129805.

da Costa Araújo, A.P., Malafaia, G., 2021. Microplastic ingestion induces behavioral disorders in mice: a preliminary study on the trophic transfer effects via tadpoles and fish. J. Hazard Mater. 401, 123263 https://doi.org/10.1016/j.jhazmat.2020.123263.

Deng, Y., Yan, Z., Shen, R., Huang, Y., Ren, H., Zhang, Y., 2021. Enhanced reproductive toxicities induced by phthalates contaminated microplastics in male mice (Mus musculus). J. Hazard Mater. 406, 124644 https://doi.org/10.1016/j.jhazmat.2020.124644.

Dong, R., Zhou, C., Wang, S., Yan, Y., Jiang, Q., 2022. Probiotics ameliorate polyethylene microplastics-induced liver injury by inhibition of oxidative stress in Nile tilapia (Oreochromis niloticus). Fish. Shellfish Immunol. 130, 261–272. https://doi.org/10.1016/j.fsi.2022.09.022.

Du H., Xie Y., Wang J. Microplastic degradation methods and corresponding degradation mechanism: research status and future perspectives. J. Hazard. Mater. (Internet). 2021. 4218,1264377. ⟨https://www.sciencedirect.com/science/article/pii/S0304389421013418⟩. (Accessed 2 May 2023).

Dusinska, M., Tulinska, J., El Yamani, N., Kuricova, M., Liskova, A., Rollerova, E., Rundén-Pran, E., Smolkova, B., 2017. Immunotoxicity, genotoxicity and epigenetic toxicity of nanomaterials: New strategies for toxicity testing? Food Chem. Toxicol. 109, 797–811. https://doi.org/10.1016/j.fct.2017.08.030.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3. https://doi.org/10.1126/sciadv.1700782.

Guimarães, A.T.B., Charlie-Silva, I., Malafaia, G., 2021. Toxic effects of naturally-aged microplastics on zebrafish juveniles: a more realistic approach to plastic pollution in freshwater ecosystems. J. Hazard Mater. 407, 124833 https://doi.org/10.1016/j.jhazmat.2020.124833.

Guimarães, A.T.B., Freitas, Í.N., Mubarak, N.M., Rahman, Md.M., Rodrigues, F.P., Rodrigues, A.S., de, L., Barceló, D., Islam, A.R., Md, T., Malafaia, G., 2023. Exposure to polystyrene nanoplastics induces an anxiolytic-like effect, changes in antipredator defensive response, and DNA damage in Swiss mice. J. Hazard. Mater. 442, 130004 https://doi.org/10.1016/j.jhazmat.2022.130004.

Haindongo, N.N.K., Breen, C.J., Neretin, L., 2023. Emerging contaminants related to plastic and microplastic pollution. In: In: Present Knowledge in Food Safety. Elsevier, pp. 270–280. https://doi.org/10.1016/B978-0-12-819470-6.00050-0.

Hamed, M., Osman, A.G.M., Badrey, A.E.A., Soliman, H.A.M., Sayed, A.H., 2021. Microplastics-induced eryptosis and poikilocytosis in early-juvenile Nile Tilapia (Oreochromis niloticus). Front. Physiol. 12. https://doi.org/10.3389/fphys.2021.742922.

Han, M., Zhang, Z., Liu, S., Sheng, Y., Waigi, M.G., Hu, X., Qin, C., Ling, W., 2023. Genotoxicity of organic contaminants in the soil: a review based on bibliometric analysis and methodological progress. Chemosphere 313, 137318.

Han, Y., Song, Y., Kim, G.W., Ha, C., Lee, J., Kim, M., Son, H., Lee, G., Gautam, R., Heo, Y., 2021. No prominent toxicity of polyethylene microplastics observed in neonatal mice following intratracheal instillation to dams during gestational and neonatal period. Toxicol. Res. 37, 443–450. https://doi.org/10.1007/s43188-020-00086-7.

Huang, D., Zhang, Y., Long, J., Yang, X., Bao, L., Yang, Z., Wu, B., Si, R., Zhao, W., Peng, C., Wang, A., Yan, D., 2022. Polystyrene microplastic exposure induces insulin resistance in mice via dysbacteriosis and pro-inflammation. Sci. Total Environ. 838, 155937 https://doi.org/10.1016/j.scitotenv.2022.155937.

Hüffer, T., Weniger, A.-K., Hofmann, T., 2018. Sorption of organic compounds by aged polystyrene microplastic particles. Environ. Pollut. 236, 218–225. https://doi.org/10.1016/j.envpol.2018.01.022.

ICH S2 (R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use - Scientific Guideline, 2022. European Medicines Agency, Science Medicines Health.

Ijaz, M.U., Ayaz, F., Mustafa, S., Ashraf, A., Albeshr, M.F., Riaz, M.N., Mahboob, S., 2022. Toxic effect of polyethylene microplastic on testicles and ameliorative effect of luteolin in adult rats: environmental challenge. J. King Saud. Univ. Sci. 34, 102064 https://doi.org/10.1016/j.jksus.2022.102064.

Im, J., Eom, H.-J., Choi, J., 2022. Effect of early-life exposure of polystyrene microplastics on behavior and DNA methylation in later life stage of zebrafish. Arch. Environ. Contam. Toxicol. 82, 558–568. https://doi.org/10.1007/s00244-022-00924-9.

Jiang, Z., Lai, Y., Beaver, J.M., Tsegay, P.S., Zhao, M.-L., Horton, J.K., Zamora, M., Rein, H.L., Miralles, F., Shaver, M., Hutcheson, J.D., Agoulnik, I., Wilson, S.H., Liu, Y., 2020. Oxidative DNA damage modulates DNA methylation pattern in human breast cancer 1 (BRCA1) gene via the crosstalk between DNA polymerase β and a de novo DNA methyltransferase. Cells 9, 225. https://doi.org/10.3390/cells9010225.

Jin, H., Ma, T., Sha, X., Liu, Z., Zhou, Y., Meng, X., Chen, Y., Han, X., Ding, J., 2021. Polystyrene microplastics induced male reproductive toxicity in mice. J. Hazard Mater. 401, 123430 https://doi.org/10.1016/j.jhazmat.2020.123430.

Joshi, P.K., Bose, M., Harish, D., 2002. Changes in certain haematological parameters in a siluroid cat fish Clarias batrachus (Linn) exposed to cadmium chloride. Pollut. Res. 2.

Kılıç, Ö., Belivermiş, M., Sıkdokur, E., Sezer, N., Aksüt, Y., Pekmez, M., Kösesakal, T., Gerçek, Y.C., 2023. The combined effects of polyethylene microplastics and benzoanthracene on Manila clam Ruditapes philippinarum. Chemosphere 329, 138664.

Kim, J.-H., Yu, Y.-B., Choi, J.-H., 2021. Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: a review. J. Hazard. Mater. 413, 125423 https://doi.org/10.1016/j.jhazmat.2021.125423.

Li, L., Sun, S., Tan, L., Wang, Y., Wang, L., Zhang, Z., Zhang, L., 2019. Polystyrene nanoparticles reduced ROS and inhibited ferroptosis by triggering lysosome stress and TFEB nucleus translocation in a size-dependent manner. Nano Letters 19 (11), 7781–7792.

Li, Y., Shi, T., Li, X., Sun, H., Xia, X., Ji, X., Zhang, J., Liu, M., Lin, Y., Zhang, R., Zheng, Y., Tang, J., 2022. Inhaled tire-wear microplastic particles induced pulmonary fibrotic injury via epithelial cytoskeleton rearrangement. Environ. Int. 164, 107257 https://doi.org/10.1016/j.envint.2022.107257.

Lind, L., Penell, J., Luttropp, K., Nordfors, L., Syvänen, A.-C., Axelsson, T., Salihovic, S., van Bavel, B., Fall, T., Ingelsson, E., Lind, P.M., 2013. Global DNA hypermethylation is associated with high serum levels of persistent organic pollutants in an elderly

population. Environ. Int. 59, 456–461. https://doi.org/10.1016/j.envint.2013.07.008.
Llorca, M., Farré, M., 2021. Current insights into potential effects of micro-nanoplastics on human health by in-vitro tests. Front. Toxicol. 3. https://doi.org/10.3389/ftox.2021.752140.
López, A.P.A, Trilleras, J., Arana, V.A., Garcia-Alzate, L.S., Grande-Tovar, C.D., 2023. Atmospheric microplastics: exposure, toxicity, and detrimental health effects. RSC Adv. 13, 7468–7489.
Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., Xing, B., 2020. Microplastics in aquatic environments: toxicity to trigger ecological consequences. Environ. Pollut. 261, 114089 https://doi.org/10.1016/j.envpol.2020.114089.
Mahmoud, A.M., 2022. An overview of epigenetics in obesity: the role of lifestyle and therapeutic interventions. Int J. Mol. Sci. 23, 1341. https://doi.org/10.3390/ijms23031341.
Malinowska, K., Bukowska, B., Piwoński, I., Foksiński, M., Kisielewska, A., Zarakowska, E., Gackowski, D., Sicińska, P., 2022. Polystyrene nanoparticles: the mechanism of their genotoxicity in human peripheral blood mononuclear cells. Nanotoxicology 1–21. https://doi.org/10.1080/17435390.2022.2149360.
Mamun, A.Al, Prasetya, T.A.E., Dewi, I.R., Ahmad, M., 2023. Microplastics in human food chains: food becoming a threat to health safety. Sci. Total Environ. 858, 159834 https://doi.org/10.1016/j.scitotenv.2022.159834.
Marczylo, E.L., Jacobs, M.N., Gant, T.W., 2016. Environmentally induced epigenetic toxicity: potential public health concerns. Crit. Rev. Toxicol. 46, 676–700. https://doi.org/10.1080/10408444.2016.1175417.
Matthews, S., Mai, L., Jeong, C.-B., Lee, J.-S., Zeng, E.Y., Xu, E.G., 2021. Key mechanisms of micro- and nanoplastic (MNP) toxicity across taxonomic groups. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 247, 109056 https://doi.org/10.1016/j.cbpc.2021.109056.
McNeish, R.E., Kim, L.H., Barrett, H.A., Mason, S.A., Kelly, J.J., Hoellein, T.J., 2018. Microplastic in riverine fish is connected to species traits. Sci. Rep. 8, 11639. https://doi.org/10.1038/s41598-018-29980-9.
Mekkawy, I.A., Mahmoud, U.M., Sayed, A.H., 2011. Effects of 4-nonylphenol on blood cells of the African catfish Clarias gariepinus (Burchell, 1822). Tissue Cell 43, 223–229. https://doi.org/10.1016/j.tice.2011.03.006.
Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ. Res 108, 131–139. https://doi.org/10.1016/j.envres.2008.07.025.
Na, Y.K., Hong, H.S., Lee, D.H., Lee, W.K., Kim, D.S., 2014. Effect of body mass index on global DNA methylation in healthy Korean women. Mol. Cells 37, 467–472. https://doi.org/10.14348/molcells.2014.0073.
Nishikimi, M., Appaji Rao, N., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 46, 849–854. https://doi.org/10.1016/S0006-291X(72)80218-3.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.
Pannetier, P., Morin, B., Le Bihanic, F., Dubreil, L., Clérandeau, C., Chouvellon, F., Van Arkel, K., Danion, M., Cachot, J., 2020. Environmental samples of microplastics induce significant toxic effects in fish larvae. Environ. Int. 134, 105047 https://doi.org/10.1016/j.envint.2019.105047.
Park, E.J., Han, J.S., Park, E.J., Seong, E., Lee, G.H., Kim, D.W., Son, H.Y., Han, H.Y., Lee, B.S., 2020. Repeated-oral dose toxicity of polyethylene microplastics and the possible implications on reproduction and development of the next generation. Toxicology letters 324, 75–85.
Park, S.H., Kim, K., 2022. Microplastics induced developmental toxicity with microcirculation dysfunction in zebrafish embryos. Chemosphere 286, 131868. https://doi.org/10.1016/j.chemosphere.2021.131868.
Paul-Pont, I., Tallec, K., Gonzalez-Fernandez, C., Lambert, C., Vincent, D., Mazurais, D., Zambonino-Infante, J.-L., Brotons, G., Lagarde, F., Fabioux, C., Soudant, P., Huvet, A., 2018. Constraints and priorities for conducting experimental exposures of marine organisms to microplastics. Front Mar. Sci. 5. https://doi.org/10.3389/fmars.2018.00252.
Roursgaard, M., Hezareh Rothmann, M., Schulte, J., Karadimou, I., Marinelli, E., Møller, P., 2022. Genotoxicity of particles from grinded plastic items in Caco-2 and HepG2 Cells. Front Public Health 10. https://doi.org/10.3389/fpubh.2022.906430.
Rudolph, J., Völkl, M., Jérôme, V., Scheibel, T., Freitag, R., 2021. Noxic effects of polystyrene microparticles on murine macrophages and epithelial cells. Sci. Rep. 11, 15702. https://doi.org/10.1038/s41598-021-95073-9.
Samblas, M., Milagro, F.I., Martínez, A., 2019. DNA methylation markers in obesity, metabolic syndrome, and weight loss. Epigenetics 14, 421–444. https://doi.org/10.1080/15592294.2019.1595297.
Santacruz-Juárez, E., Buendia-Corona, R.E., Ramírez, R.E., Sánchez, C., 2021. Fungal enzymes for the degradation of polyethylene: molecular docking simulation and biodegradation pathway proposal. J. Hazard Mater. 411, 125118 https://doi.org/10.1016/j.jhazmat.2021.125118.
Sayed, A.H., Hamed, M., Badrey, A.E.A., Soliman, H.A.M., 2021. Bioremediation of hemotoxic and oxidative stress induced by polyethylene microplastic in Clarias gariepinus using lycopene, citric acid, and chlorella. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 250, 109189 https://doi.org/10.1016/j.cbpc.2021.109189.
Scafidi M, J., Amraei, R., Gupta, V., 2022. Histology, Howell Jolly Bodies. Treasure Island (FL): StatPearls Publishing.
Scanes, E., Wood, H., Ross, P., 2019. Microplastics detected in haemolymph of the Sydney rock oyster Saccostrea glomerata. Mar. Pollut. Bull. 149, 110537 https://doi.org/10.1016/j.marpolbul.2019.110537.
Schmid, W., 1975. The micronucleus test. Mutat. Res. Environ. Mutagen. Relat. Subj. 31, 9–15. https://doi.org/10.1016/0165-1161(75)90058-8.
Shi, C., Han, X., Guo, W., Wu, Q., Yang, X., Wang, Y., Tang, G., Wang, S., Wang, Z., Liu, Y., Li, M., Lv, M., Guo, Y., Li, Z., Li, J., Shi, J., Qu, G., Jiang, G., 2022. Disturbed Gut-Liver axis indicating oral exposure to polystyrene microplastic potentially increases the risk of insulin resistance. Environ. Int 164, 107273. https://doi.org/10.1016/j.envint.2022.107273.
da Silva Brito, W.A., Mutter, F., Wende, K., Cecchini, A.L., Schmidt, A., Bekeschus, S., 2022. Consequences of nano and microplastic exposure in rodent models: the known and unknown. Part Fibre Toxicol. 19, 28. https://doi.org/10.1186/s12989-022-00473-y.
Sincihu, Y., Lusno, M.F.D., Mulyasari, T.M., Elias, S.M., Sudiana, I.K., Kusumastuti, K., Sulistyorini, L., Keman, S., 2023. Wistar rats hippocampal neurons response to blood low-density polyethylene microplastics: a pathway analysis of SOD, CAT, MDA, 8-OHdG expression in hippocampal neurons and blood serum Aβ42 levels. Neuropsychiatr. Dis. Treat. 19, 73–83. https://doi.org/10.2147/NDT.S396556.
Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 184–191. https://doi.org/10.1016/0014-4827(88)90265-0.
Sun, H., Chen, N., Yang, X., Xia, Y., Wu, D., 2021. Effects induced by polyethylene microplastics oral exposure on colon mucin release, inflammation, gut microflora composition and metabolism in mice. Ecotoxicol. Environ. Saf. 220, 112340 https://doi.org/10.1016/j.ecoenv.2021.112340.
Surai, Kochish, Fisinin, Kidd, 2019. Antioxidant defence systems and oxidative stress in poultry biology: an update. Antioxidants 8, 235. https://doi.org/10.3390/antiox8070235.
Tong, Y.T., Nguyen, N.D., Wahed, A., 2019. Howell-Jolly body–like inclusions in neutrophils of patients with myelodysplastic syndrome: a novel correlation. Arch. Pathol. Lab Med 143, 112–114. https://doi.org/10.5858/arpa.2017-0328-OA.
Udomsinprasert, W., Kitkumthorn, N., Mutirangura, A., Chongsrisawat, V., Poovorawan, Y., Honsawek, S., 2016. Global methylation, oxidative stress and relative telomere length in biliary atresia patients. Sci. Rep. 6, 26969. https://doi.org/10.1038/srep26969.
Wang, J., Wang, X., Zhang, C., Zhou, X., 2022. Microplastics induce immune suppression via S100A8 downregulation. Ecotoxicol. Environ. Saf. 242, 113905 https://doi.org/10.1016/j.ecoenv.2022.113905.
Waring, R.H., Harris, R.M., Mitchell, S.C., 2018. Plastic contamination of the food chain: a threat to human health? Maturitas 115, 64–68. https://doi.org/10.1016/j.maturitas.2018.06.010.
Wei, Y., Zhou, Y., Long, C., Wu, H., Hong, Y., Fu, Y., Wang, J., Wu, Y., Shen, L., Wei, G., 2021. Polystyrene microplastics disrupt the blood-testis barrier integrity through ROS-Mediated imbalance of mTORC1 and mTORC2. Environ. Pollut. 289, 117904 https://doi.org/10.1016/j.envpol.2021.117904.
Wright, S.L., Kelly, F.J., 2017. Plastic and human health: a micro issue? Environ. Sci. Technol. 51, 6634–6647. https://doi.org/10.1021/acs.est.7b00423.
Xie, L., Chen, T., Liu, J., Hou, Y., Tan, Q., Zhang, X., Li, Z., Farooq, T.H., Yan, W., Li, Y., 2022. Intestinal flora variation reflects the short-term damage of microplastic to the intestinal tract in mice. Ecotoxicol. Environ. Saf. 246, 114194 https://doi.org/10.1016/j.ecoenv.2022.114194.
Yao, Z., Seong, H.J., Jang, Y.S., 2022. Environmental toxicity and decomposition of polyethylene. Ecotoxicol. Environ. Saf. 242, 113933.
Zbidah, M., 2014. Mechanisms of Apoptotic Death of Red Blood Cells Induced by Environmental Contaminants and Natural Products.
Zhang X., Li S., Liu Y., Yu K., Zhang H., Yu H., et al., Neglected microplastics pollution in the nearshore surface waters derived from coastal fishery activities in Weihai, China. Sci. Total Environ. (Internet). 2021 May 10 [cited];768:144484. Available from: ⟨https://www.sciencedirect.com/science/article/pii/S0048969720380153⟩. (Accessed 2 March 2023).
Zhang, Y., Wolosker, M.B., Zhao, Y., Ren, H., Lemos, B., 2020. Exposure to microplastics cause gut damage, locomotor dysfunction, epigenetic silencing, and aggravate cadmium (Cd) toxicity in Drosophila. Sci. Total Environ. 744, 140979 https://doi.org/10.1016/j.scitotenv.2020.140979.
Zheng, T., Yuan, D., Liu, C., 2019. Molecular toxicity of nanoplastics involving in oxidative stress and desoxyribonucleic acid damage. J. Mol. Recognit. 32. https://doi.org/10.1002/jmr.2804.
Ziech, D., Franco, R., Pappa, A., Panayiotidis, M.I., 2011. Reactive oxygen species (ROS)—induced genetic and epigenetic alterations in human carcinogenesis. Mutat. Res. /Fundam. Mol. Mech. Mutagen. 711, 167–173. https://doi.org/10.1016/j.mrfmmm.2011.02.015.
Zolotova, N., Kosyreva, A., Dzhalilova, D., Fokichev, N., Makarova, O., 2022. Harmful effects of the microplastic pollution on animal health: a literature review. PeerJ 10, e13503. https://doi.org/10.7717/peerj.13503.
Zwollo, P., Quddos, F., Bagdassarian, C., Seeley, M.E., Hale, R.C., Abderhalden, L., 2021. Polystyrene microplastics reduce abundance of developing B cells in rainbow trout (Oncorhynchus mykiss) primary cultures. Fish. Shellfish Immunol. 114, 102–111. https://doi.org/10.1016/j.fsi.2021.04.014.