Evaluation of Hematotoxicity in Female Wistar Rats Following Sub-Acute Inhalation Exposure to Polyethylene Microplastic

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Abstract

Polyethylene (PE) becomes a source of microplastics that can be widely distributed through the digestive and respiratory systems. However, its effects on blood cells are still being investigated. This study aims to analyze the impact of Polyethylene Microplastic (PE-MPs) exposure on the blood of female rats, including erythrocytes, leukocytes, and platelets. This study used female Wistar rats, which were divided into control and PE-MP groups. PE-MP was administered via whole-body inhalation at a concentration of 15 mg/m\(^3\) for 4 hours daily for 28 days. The absorption of plastic particles detected in the human bloodstream is likely to occur through mucosal contact (either through ingestion or inhalation). After the exposure period, the rats were euthanized to collect blood samples through the heart. A complete blood count was performed using an automatic hematology analyzer, and blood morphology was analyzed using thin blood smears. This study used the Mann-Whitney test. PE-MP exposure increased erythrocyte and platelet counts without a corresponding rise in leukocytes. Erythrocyte showed abnormal morphology (12.73% with ovalocytes and tear-shaped cells). Erythrocyte indices (MCV, MCH, MCHC) showed no significant differences. Platelet count rose by 1.7% (p-value = 0.017). Leukocyte and neutrophil counts were lower (0.84 and 0.94 times lower, respectively), while lymphocytes and monocytes were higher (1.03 and 1.61 times higher, respectively) in the PE-MP group compared to controls. The neutrophil-to-lymphocyte ratio did not differ significantly. PE-MP exposure in rats disrupts blood parameters, altering erythrocyte morphology and increasing platelet counts. Potential causes include oxidative stress, immune responses, and compensatory mechanisms. Study limitations include a small sample size and exclusive focus on inhalation exposure. Integrating multiple exposure routes (inhalation, ingestion, dermal) could offer a broader view of microplastic impacts. Future research with larger samples, diverse doses and durations, and exploration of additional markers or organ-specific effects is crucial for understanding PE-MP toxicity in real-world scenarios.

Keywords: Microplastic, Polyethylene, Inhalation, Blood, Toxicity.

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1. INTRODUCTION

According to UN Comtrade data in 2018, the five major countries exporting plastic waste to Indonesia are the United States, Germany, the Marshall Islands, the Netherlands, and Australia. Approximately 9 million tons of plastic waste are produced annually in Indonesia, accounting for 15% of the national total. Plastic waste often ends up in rivers, comprising 20% to 38% of debris collected from water bodies in cities, with recycling efforts predominantly informal (15%). Plastic waste is frequently purchased by brokers or small traders. In East Java, Bangun and Tropodo villages in Sidoarjo Regency receive over 50 tons of plastic waste daily. In Tropodo, Sidoarjo Regency, East Java, about 50 tofu factories use plastic waste as fuel for their furnaces (Petrlik et al., 2019). Overall, this issue highlights serious challenges in plastic waste management in Indonesia, with significant environmental and public health impacts. Plastic waste inevitably releases into the environment, degrading mechanical and physicochemical characteristics, leading to the formation of plastic fragments. These fragments are termed microplastics (MPs) if their size is below 5 mm (Stock et al., 2021). MPs spread through air, water, and soil and can enter human bodies through ingestion and respiration. Exposure to MPs can cause toxicity in humans through inflammation and the accumulation of reactive oxygen species (ROS) (Enyoh et al., 2019).

Free radicals from MP exposure can cause damage to red blood cells and a decrease in erythrocytes (Franco et al., 2019). The high concentration of transition metal ions, oxyhemoglobin, and molecular oxygen in erythrocytes makes these cells very susceptible to oxidative damage (Qasim & Mahmood, 2015). The toxic effects of MP exposure also cause immune responses and reproductive and developmental toxicity (Li et al., 2023). Additionally, studies on MP exposure in fish cells in vitro reveal that MPs alter leukocyte and B cell activity (Zwollo et al., 2021; Espinosa et al., 2018). A recent study (Wu et al., 2023) showed that microplastics were found in thrombi and could increase the risk of hemostasis disorders. The impact of MP use on female mammals, whose blood is predominantly affected, remains uncertain. Microplastics can enter the fetus through the placenta, and babies can also ingest particles through breast milk.

In this study, we investigated the impact of polyethylene MP in vivo. Polyethylene Microplastics (PE-MPs) were used as a representative plastic test material because it is the most common type of plastic in the world (Rodrigues et al., 2019). Polyethylene plastic is often used as single-use plastics (SUP), such as plastic bags, plastic bottles, houseware, and other items (Mentes et al., 2023). PE has the ability to change easily during processing, providing relatively longer chain length, density, and crystallinity, allowing PE products to have properties tailored for various applications. High-density polyethylene (HDPE) and low-density polyethylene (LDPE) are some types of PE plastics. HDPE is lightweight and has good tensile strength, while LDPE has good chemical resistance (Kumar et al., 2022). Research shows that PE MPs are more easily absorbed and accumulated in the body (Yang et al., 2022).

In this study, PE-MPs were administered via whole-body inhalation to female rats. Female rats were chosen because they have a distinctive estrous cycle, making them ideal for studying the impact of environmental toxins on reproductive and endocrine systems. Additionally, this choice helps avoid bias in the study. Microplastics were administered at multiple doses for 28 days to assess their toxicity in the blood, particularly on red blood cells/erythrocytes, white blood cells/leukocytes, and platelet/thrombocyte profiles. The broader implications of our research findings highlight significant public health concerns. Since polyethylene microplastics are prevalent in the environment and commonly used in single-use plastics, our study suggests that chronic exposure to these particles could adversely affect blood health. This raises concerns about similar impacts on human health, especially for populations with high exposure to...
airborne microplastics. Understanding the toxicological effects of PE-MPs can inform regulatory policies and promote the development of safer plastic alternatives, ultimately contributing to improved public health outcomes by mitigating the risks associated with microplastic exposure.

2. **RESEARCH METHOD**

This study is a true experimental research with a post-test-only control group design conducted in 2023 at the Pharmacology and Biochemistry Laboratory of the Faculty of Medicine, Brawijaya University. The experimental animals used in this study were female Wistar rats (Rattus norvegicus) aged 12-15 weeks. The rats were housed and fed standard food and water. They were divided into Control and Polyethylene Microplastics (PE-MPs) groups, consisting of 5 rats in the Control group and 6 rats in the PE-MP group, with the difference in sample size due to dropouts. The determination of sample size in this study followed the "Resource Equation Approach" described in the journal (Arifin and Zahiruddin, 2017). All rats were housed in standard animal facilities at Brawijaya University, and every effort was made to minimize the number of animals used and their suffering. Only rats with regular estrous cycles were included in the study. These animals were synchronized to the same estrous cycle phase. The estrous cycle influences various physiological processes, including immune function and metabolism. By selecting rats with regular cycles and exposing them at the same phase, this study controlled hormonal fluctuations, ensuring that any observed effects on blood toxicity could be directly attributed to PE-MP exposure rather than hormonal variations, thus reducing potential confounding variables related to hormonal changes. The entire experimental protocol was approved by the Animal Care and Use Committee of Brawijaya University (Approval No. 254/EC/KEPK/08/2023).

Polyethylene Microplastics (PE-MP) exposure was conducted on rats in the estrous phase. PE-MP exposure was done using a blower containing PE powder. The average size of PE-MPs in this study was 252.76 ± 4.401 micrometers, obtained from a fine powder form of PE plastic purchased from CV. Subur Kimia Jaya (lot number 2110052) and analyzed at the Ecoton Laboratory in Gresik. The exposure was conducted via whole-body inhalation for 4 hours daily for 28 days with a PE-MP concentration of 15 mg/m³ in a 60x60x60 cm inhalation chamber. The exposure dose and duration followed the limits allowed by the Occupational Safety and Health Administration (OSHA) (Cary, et al., 2022).

Blood Collection and Hematology Analysis. Blood collection was done after 28 days of PE-MP exposure by extracting 0.5 ml of blood from the rat's heart. The rats were euthanized via deep anesthesia using diethyl ether for 1 minute to help minimize the stress response in the animals. Stress can significantly alter blood parameters, such as increasing cortisol levels and affecting white blood cell counts. By reducing stress, the anesthetic approach helps maintain the validity of the measured blood parameters. Blood was then collected in vacutainer tubes containing EDTA (Ethylene Diamine Tetraacetic Acid). The following parameters were analyzed using an automatic hematology analyzer (ABX Micros 60 Hematology Analyzer): total red blood cell/erythrocyte count, erythrocyte indices, platelet count, and leukocyte count.

Blood was thinly smeared on glass slides, and its morphology was analyzed to identify changes in erythrocyte shape. To determine the impact of PE-MP exposure as a xenobiotic agent triggering the immune system, leukocyte analysis was performed using an Olympus® CX-21 microscope with 400x magnification for each group.

Data obtained in this study were statistically analyzed using the Mann-Whitney test with GraphPad Prism® 5 software at a significance level of 0.05 (p<0.05). Data are presented as mean ± S.E.
3. RESULTS AND DISCUSSION

Polyethylene Microplastic (PE-MP) Exposure may increase the number of erythrocytes. We analyzed erythrocyte count, hematocrit, and hemoglobin levels to determine the effect of PE-MP exposure on the number and oxygen-binding ability of erythrocytes. As shown in Figure 1, the number of erythrocytes in the PE-MPs group is 1.07 times higher than control. Hematocrit and hemoglobin levels in the PE-MP group also show 1.08 and 1.09 times higher than the control, respectively, even though not statistically different. Subsequently, we were interested in learning the size and volume of the variance of erythrocytes. Then, we performed The Red Cell Distribution Width (RDW) test. In this study, the PE-MP group shows no different variations compared to the control group.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Polyethylene microplastic (PE-MP) exposure may increase erythrocyte counts, hematocrit, and hemoglobin level but not red cell distribution width. (A) Erythrocyte count (B) Hematocrit test (C) Hemoglobin level (D) Red cell distribution width - standard deviation. *ns*, insignificant.

Polyethylene Microplastic (PE-MP) Exposure alters the morphology of erythrocytes. To identify alterations in the form of erythrocytes, we underwent a blood smear. The control group displays erythrocytes with a round form shaped like a disc. Conversely, PE-MP group displays 12.73% of abnormal erythrocyte such as ovalocytes and teardrop-like cells (Figure 2).

![Figure 2](https://example.com/figure2.png)

**Figure 2.** A blood smear of the PE-MPs group reveals a large number of erythrocytes with abnormal morphology. Asterisk (*) means cells with abnormal morphology.
Polyethylene Microplastic (PE-MP) Exposure does not affect the measurement of the erythrocyte index. To re-check the volume and concentration amount of hemoglobin in erythrocytes, we performed an erythrocyte index analysis consisting of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin, and Mean Corpuscular Hemoglobin Concentration (MCHC). There are no significant differences between groups (Figure 3).

![Figure 3](image-url) Polyethylene Microplastic Exposure may result in a slight increment of erythrocyte index. (A) Mean Corpuscular Volume (B) Mean Corpuscular Hemoglobin (C) Mean Corpuscular Hemoglobin Concentration. *ns*, insignificant.

Polyethylene Microplastic (PE-MP) Exposure enhances the number of thrombocytes. To identify the blood clotting risk, we examine the thrombocyte count. As shown in Figure 4, the number of thrombocytes increases by 1.7% after exposure to PE-MPs (p-value= 0.017).

![Figure 4](image-url) Polyethylene microplastics increase thrombocyte count.

Polyethylene Microplastic (PE-MP) Exposure does not affect the leukocytes. To know about the effect of PE-MP exposure as a xenobiotic agent triggers the immune system, we underwent leukocyte analysis (Figure 5). Leukocyte and Polymorphonuclear leukocyte/neutrophil counts in the PE-MP group tend to be lower than in control groups (0.84 and 0.94 times lower, respectively). In contrast, mononuclear leukocyte count, such as lymphocyte and monocyte in the PE-MP group, show a higher amount than the control. More specifically, the mononuclear leukocyte count of the PE-MP group displays 1.03 and 1.61 times greater than the control. The neutrophil-lymphocyte ratio is conducted to evaluate inflammatory responses. We found that the ratio between groups was quite similar.
The studies on how microplastics, especially PE-MPs, affect the human body are still in the exploratory stages. Plastic particles can enter the human body through the consumption of contaminated food and water supplies or by inhaling plastic particles in the air originating from synthetic textiles and polluted outdoor air. Although the skin membrane is too fine to be penetrated by plastic particles, nanoplastics can penetrate wounds and weaken the skin barrier, either directly or indirectly (Yee et al., 2021). This research demonstrates PE-MP exposure to blood cell parameters using a female rat model. The initial analysis found a significant increase in erythrocytes, commonly referred to as red blood cell count, after exposure to microplastics. Additionally, the results indicate that although hematocrit and hemoglobin levels did not significantly differ between groups, it is noteworthy that PE-MP treatment resulted in higher hematocrit and hemoglobin concentrations compared to the control. The high erythrocyte count in this study may be related to the model's compensatory ability to exposure. Microplastics can impact erythrocyte deformability and oxygenation, leading to low oxygen status in the body (Nader et al., 2019). Perceived oxygen deficiency can trigger the release of erythropoietin from the kidneys, stimulating the bone marrow to produce more erythrocytes for adequate reoxygenation processes (Arias et al., 2024; Lee et al., 2019). On the other hand, the high hemoglobin concentration observed may be due to microplastic-mediated ROS-induced erythrocyte hemolysis.

Polyethylene Microplastic (PE-MP) Exposure may increase the number of erythrocytes, the morphology of erythrocytes, the measurement of the erythrocyte index, and the number of thrombocytes, affect the leukocytes. Intoxicated erythrocytes showing abnormal morphology may reflect pre-apoptotic erythrocyte death, as seen in fish experiments (Hamed et al., 2021). These findings are partially confounded by previous studies stating that microplastic exposure...
for over a month can inhibit hematopoiesis, causing erythrocyte morphological changes (poikilocytosis) and inducing eryptosis through cellular reactive oxidative stress and inflammation, which, through these mechanisms, are expected to decrease erythrocyte lifespan and count (Abdel-Zaher et al., 2023; Hamed et al., 2021; Rajendran & Chandrasekaran, 2023).

This study also shows that platelets experienced a significant increase in PE-MP exposure compared to the control. This somewhat aligns with other research where microplastics increase the risk of thrombosis, which cannot occur without an increase in platelet count (Lett et al., 2021). Inflammation and response to chemicals can trigger thrombocytosis (Schafer, 2001).

Plastic particles have various excretion pathways from the human body, including elimination through the kidneys, bile, or accumulation in organs like the liver, spleen, or other organs. Various factors such as particle size, shape, surface, chemistry, and charge influence their interaction with biological systems, including the formation of a protein layer on the particle surface. Blood, as the primary transport medium in the body, allows direct sampling without contact with plastic materials, making it an ideal matrix for monitoring plastic particles in the human body (Leslie et al., 2022).

No significant statistical differences between groups were found in leukocyte analysis. Interestingly, polymorphonuclear leukocytes (PMNs) showed a decreasing trend while mononuclear leukocytes (MN) showed the opposite trend. Lead poisoning shows a similar “right shift” leukocyte analysis (Chwalba et al., 2018). One indicator of inflammation is the neutrophil-to-lymphocyte ratio. However, the overall estimated group ratio is still less than 1, indicating a decrease in neutrophil count in the blood, thus reducing the ability for an appropriate immune response (Buonacera et al., 2022).

The potential internalization and clearance mechanisms of microplastics in the lungs involve lung lining fluid (surfactant and mucus) reducing the likelihood of microplastic transfer. Particles <10 μm are cleared by mucociliary action, while particles <1 μm are absorbed through the epithelium and can also penetrate the thin lung lining fluid and contact the epithelium, circulating and metastasizing via diffusion or active cellular uptake. Surface charge and molecular surface interactions of different microplastics affect immune cell clearance. For macrophages, positively charged MPs and coated MPs (combined with proteins or other substances) are more likely to interact with cell membranes. Other immune cells are also involved in defense against MPs, such as antigen presentation by dendritic cells and trapping and phagocytosis of MPs through NET release by neutrophils, leading to immune activation (Yang et al., 2022).

The differences in results compared to previous studies are thought to be due to the relatively low microplastic exposure concentration, which is 15 mg/m3. As suggested by many studies, differences in size, dose, and exposure time to MPs can produce different defense mechanisms and toxicity effects for different organs and systems (Farag et al., 2023; Iheanacho & Odo, 2020; Wang et al., 2022). Subacute inhalation toxicity studies often aim to determine concentration limits to assess risks for workers in occupational environments. This study was conducted to assess the impact of repeated daily inhalation exposure to chemicals over a 28-day period (OECD, 2018). Further research needs to address these issues using higher concentrations and longer microplastic exposure times to obtain more conclusive results.

4. CONCLUSION

This research shows that exposure to PE-MPs increases the number of erythrocytes and thrombocytes without increasing leukocyte response. Potential causes include oxidative stress, immune system reactions, and compensatory mechanisms. Limitations of this study include a relatively small sample size of rats and focusing only on inhalation exposure. A combination of exposure routes (inhalation, ingestion, dermal) may provide a more comprehensive
understanding of the overall impacts of microplastics. The study primarily focused on hematological parameters. Other biological markers or specific organ effects were not assessed, which could provide additional insights into PE-MP toxicity. Further research is needed with larger sample sizes, varying concentrations and durations of exposure, and combining different exposure routes (inhalation, ingestion, dermal) to better mimic real-world scenarios and gain a holistic understanding of microplastic effects. Recommendations for tofu factory workers, scavengers, and sanitation workers include using personal protective equipment such as gloves, masks, and coveralls to reduce direct contact with plastic waste. Supporting policies that promote safer working conditions, reduce environmental pollution from plastic waste, and enhance waste management practices and recycling initiatives are also crucial.

REFERENCES


