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Correlation of Microplastic Levels with Erythrocyte Profiles in Wistar Rats Orally Exposed to Polyethylene

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Keywords

Microplastics; erythrocytes; oxidative stress; polyethylene; Rattus norvegicus

Abstract

Polyethylene microplastics are widely used in single-use products and can enter the body through contaminated food or beverages. After ingestion, these particles may cross the intestinal barrier and reach systemic circulation. Their presence in blood is suspected to induce oxidative stress through increased reactive oxygen species, potentially affecting erythrocyte membrane integrity. Erythrocytes are essential for oxygen transport, and structural damage may reduce their flexibility and lead to morphological abnormalities. This study aimed to evaluate the correlation between microplastic counts in blood and erythrocyte profiles in Wistar rats exposed orally to polyethylene microplastics. A quantitative experimental study with a post-test only control group design was conducted. Male Wistar rats were assigned to one control group and three exposure groups receiving different microplastic doses for four weeks. Microplastics were identified using light microscopy and Fourier Transform Infrared spectroscopy, while erythrocyte profiles were assessed through peripheral blood smears and hematological examination. Microplastics were detected in exposed groups, accompanied by several abnormal erythrocyte forms. However, no significant differences or correlations were observed between microplastic counts and erythrocyte parameters. Oral exposure to polyethylene microplastics did not significantly alter erythrocyte profiles, although potential morphological effects cannot be excluded.

INTRODUCTION

The increase in Indonesia's population contributes to a rise in national waste volume, particularly plastic waste. According to data from the Ministry of Home Affairs (KEMENDAGRI) in 2020 (Badan Pusat Statistik, 2021), Indonesia's population reached 271 million people, generating a total of 67.8 million tons of waste (Husnalia et al., 2023). Globally, plastic production continues to increase and reached approximately 400 million tons per year in 2021. Between 1950 and 2017, around 9.2×10^9 metric tons of plastic were produced, with more than half generated after 2004, and approximately 8 million tons of plastic discarded into the oceans each year (Preda et al., 2024). Plastics contain various additives and are resistant to degradation, with decomposition times that can reach hundreds of years.

Plastic waste can fragment through hydrolysis, physical or mechanical forces, and exposure to ultraviolet radiation into small particles known as microplastics (MPs). Microplastics are defined as plastic particles measuring 0.1–5 mm, with main components including polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC), of which PE is the most widely used polymer (Farag et al., 2023). Plastic consumption in Indonesia, particularly packaging made from high-density polyethylene (HDPE), continues to increase, with total consumption reaching 6.2 million tons in 2020 and an annual growth rate of 6–7% (Khoironi et al., 2021).

Microplastics can enter the human body through various routes, primarily via the consumption of food and beverages (Candra, 2023). Microplastic contamination has been detected in seafood, salt, bottled drinking water, and tap water (Hartini & Dewi, 2021).

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The intake of microplastics from seafood, salt, and bottled beverages has been reported to reach thousands of particles per year, with bottled drinking water being the major contributor (Budiarti, 2021). In addition to the oral route, microplastic exposure may also occur through inhalation and dermal contact, particularly from indoor environments and the use of synthetic materials (Melindo et al., 2022). Microplastics have even been detected in human brain tissue, indicating a possible entry route via the olfactory system (Mardalisa et al., 2021).

Microplastic exposure, particularly low-density polyethylene (LDPE), has the potential to cause gastrointestinal irritation and inflammation and to act as a carrier for harmful environmental pollutants such as heavy metals and pesticides. These contaminants may accumulate in the body and contribute to various health disorders (Mardalisa et al., 2021; Ummah et al., 2023). Due to its widespread use in single-use plastic products, LDPE microplastics are among the most commonly encountered polymers in the environment and food chain, increasing the likelihood of chronic oral exposure.

Several studies have demonstrated that LDPE microplastics may exert adverse effects on erythrocytes. Erythrocytes possess a biconcave structure that is essential for efficient gas exchange and are highly vulnerable to oxidative stress induced by foreign particles (Aridya et al., 2023; Thiagarajan et al., 2021). Exposure to polyethylene-based microplastics has been associated with increased production of reactive oxygen species, which can trigger lipid peroxidation of the erythrocyte membrane, alter membrane fluidity, and compromise cellular integrity.

These oxidative alterations may reduce erythrocyte deformability, disrupt ion homeostasis, and increase the risk of hemolysis, ultimately leading to morphological abnormalities such as poikilocytosis and anisocytosis, which may impair oxygen delivery to tissues (Abdel-Zaher et al., 2023; Rajendran & Chandrasekaran, 2023).

Previous studies have shown that exposure to polystyrene microplastics at a dose of 0.5 mg/day for 28 days can affect the hematological system. Although these findings were obtained using polystyrene microplastics, similar biological responses may also occur following exposure to low-density polyethylene (LDPE) microplastics, given their comparable particle size and potential to induce oxidative stress. Considering that the lifespan of rat erythrocytes is relatively short (approximately 45–60 days), a 28-day exposure period is considered sufficient to observe early changes in erythrocyte profiles. Male *Rattus norvegicus* Wistar strain rats were selected as the experimental model due to their hematological and genetic similarities to humans. Therefore, this study aimed to analyze the effects of oral exposure to LDPE microplastics on erythrocyte profiles in male Wistar rats (Pan et al., 2023).

RESEARCH METHOD

This study was a quantitative experimental study with a post-test only control group design using male *Rattus norvegicus* Wistar strain rats as experimental animals. The study population consisted of all *Rattus norvegicus* Wistar strain rats that met the research criteria. The samples were healthy male rats aged 6–8 weeks with body weights of 150–200 g, selected using a random allocation technique.

At the initial stage, the animals underwent a 7-day acclimatization period under controlled environmental conditions. Sample size was determined using the Lemeshow formula with a minimum of five rats per group and adjusted using the Higgins & Sellers (1994) correction with an estimated 30% dropout rate, resulting in seven rats per group or a total of 28 rats. The rats were then randomly divided into four groups: one control group without microplastic exposure and three treatment groups

Table 1
Hematology Analyzer Results and Peripheral Blood Smear Reading

Variable	Control Group	Experimental Group		
		X1	X2	X3
	(mean ± SD)			
Erythrocyte Count	7,42±2,22	6,86±1,17	7,06±0,87	8,41±4,68
Erythrocyte Size	73,61±22,22	76,27±21,18	96,68±41,84	70,71±46,16
Erythrocyte Morphology	9,71±2,62	9,11±1,30	14,77 ± 7,77	11,22±7,21

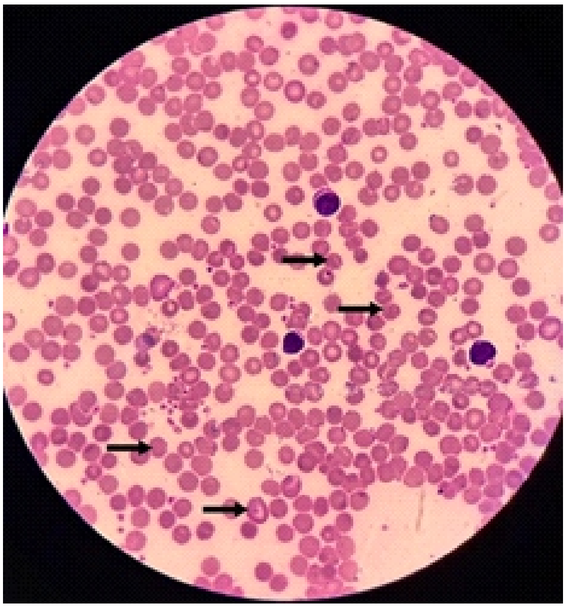
Source: Processed Primary Data, 2025

receiving oral low-density polyethylene (LDPE) microplastics with a particle size of ≤20 μm at doses of 1.25 mg/day, 2.5 mg/day, and 5 mg/day for 28 days.

After the treatment period, the rats were anesthetized using a combination of ketamine and xylazine, then euthanized by cervical dislocation, followed by blood sample collection. Blood samples were used for microplastic analysis using chemical digestion, membrane filtration, and microscopic observation methods, as well as for erythrocyte profile examination through Wright-stained peripheral blood smears and assessment of erythrocyte count and size using a hematology analyzer. The collected data were processed and analyzed using the Statistical Package for the Social Sciences (SPSS) version 27, with Shapiro–Wilk and Levene tests as prerequisite analyses, followed by One Way ANOVA or Kruskal–Wallis tests and appropriate post hoc analyses as needed, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

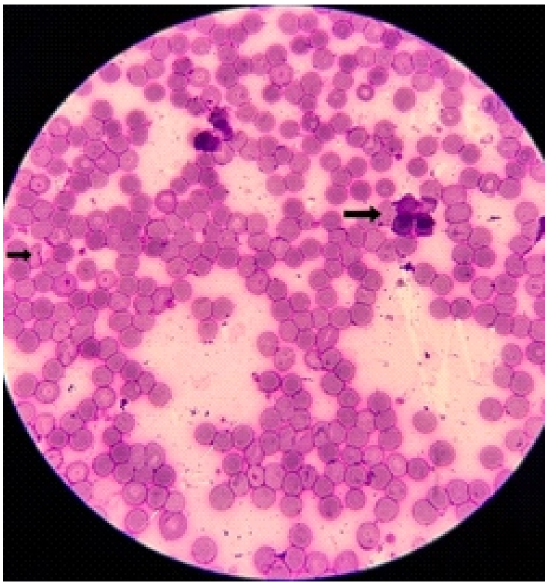
Based on Table 1, the results show varying values among the groups. The mean erythrocyte count in the control group was 7.42 ± 2.22, while the treatment groups showed values of 6.86 ± 1.17 (X1), 7.06 ± 0.87 (X2), and 8.41 ± 4.68 (X3). Group X3 had the highest mean and standard deviation, indicating greater data variability compared to the other groups. Erythrocyte size in the control group was recorded at 73.61 ± 22.22. Group X1 showed a mean size of 76.27 ± 21.18, group X2 of 96.68 ± 41.84, and group X3 of 70.71 ± 46.16. The greatest variability in erythrocyte size was observed in groups X2 and X3, as reflected by their high standard deviation values. The erythrocyte shape variable showed a mean of 9.71 ± 2.62 in the control group, 9.11 ± 1.30 in X1, 14.77 ± 7.77 in X2, and 11.22 ± 7.21 in X3. Group X2 had the highest mean and the greatest variability, indicating that exposure to microplastics at a dose of 2.5 mg/day had the most pronounced effect on erythrocyte morphological changes compared to the other doses.



Source: Processed Primary Data, (2025)
Figure 1
Microscopic Appearance of Erythrocyte Morphology in the Control Group

In the control group, erythrocytes predominantly exhibited normal morphology. The red blood cells showed a typical biconcave shape with relatively uniform size and a well-defined central pallor. No morphological abnormalities, such as schistocytes, acanthocytes, or poikilocytosis, were observed. Small fragments or staining deposits indicated by arrows were identified as preparation artifacts and did not represent abnormal erythrocytes.

These findings indicate that, in the absence of microplastic exposure, erythrocyte membrane integrity and structural characteristics were well maintained. The normal erythrocyte morphology observed in the control group confirms the physiological condition of the animals and provides a reliable baseline for comparison with the experimental groups exposed to microplastics.



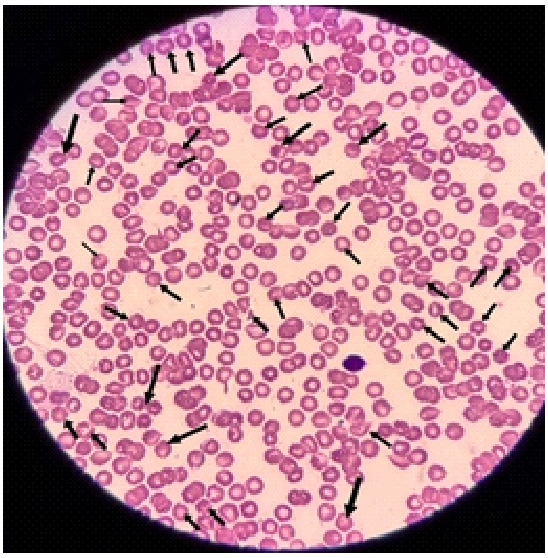
Source: Processed Primary Data, (2025)

Figure 2

Microscopic Appearance of Erythrocyte Morphology in Experimental Group 1

In Experimental Group 1, erythrocytes generally maintained a predominantly normal appearance; however, subtle morphological changes began to emerge when compared with the control group. Most erythrocytes retained their biconcave shape and relatively uniform size, although slight variations in cell contour and reduced clarity of central pallor were observed in a small proportion of cells. These findings suggest the early onset of cellular stress associated with low-dose microplastic exposure. Areas indicated by arrows correspond to debris or staining precipitates and were identified as technical artifacts rather than true cellular abnormalities. No significant poikilocytosis, anisocytosis, or erythrocyte fragmentation indicative of overt hemolysis was observed.

From a pathological perspective, the mild morphological variations observed in Experimental Group 1 may reflect an initial adaptive response of erythrocytes to oxidative stress induced by microplastic exposure. At low doses, microplastics may increase reactive oxygen species production without exceeding the antioxidant defense capacity of erythrocytes, thereby limiting structural damage. Consequently, erythrocyte integrity remains largely preserved, and morphological alterations are minimal. These findings indicate that early-stage exposure to microplastics may not immediately result in significant erythrocyte damage but could represent a preliminary phase preceding more pronounced alterations at higher exposure levels.



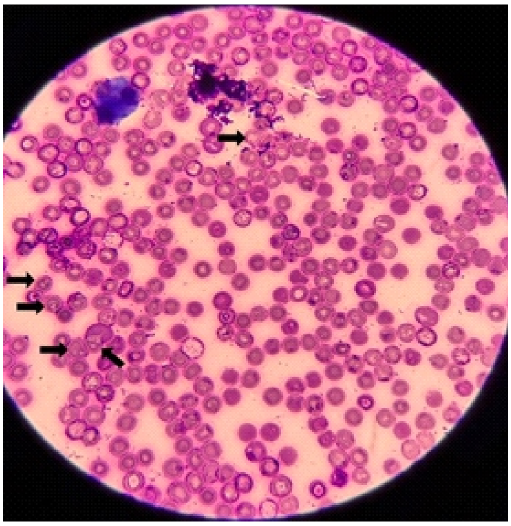
Source: Processed Primary Data, (2025)

Figure 3

Microscopic Appearance of Erythrocyte Morphology in Experimental Group 2

In Experimental Group 2, erythrocyte morphological alterations were more pronounced compared with both the control group and Experimental Group 1. A considerable number of erythrocytes exhibited poikilocytosis and anisocytosis, characterized by irregular, elongated, and distorted cell shapes. Several cells showed enlarged or irregular central pallor, while others appeared shrunken or had lost their typical biconcave configuration, indicating substantial membrane stress and structural instability.

These morphological changes are consistent with increased oxidative stress induced by microplastic exposure, which may lead to lipid peroxidation of the erythrocyte membrane and compromise membrane integrity. Damage to membrane lipids can reduce erythrocyte deformability and increase cellular fragility, making the cells more susceptible to structural abnormalities. Although staining artifacts were still present in some areas, the predominance of abnormal erythrocytes suggests that the observed alterations represent a true biological response rather than technical error. Overall, Experimental Group 2 demonstrated the most evident degree of erythrocyte damage, indicating a stronger toxic response at a microplastic dose of 2.5 mg/day.



Source: Processed Primary Data, (2025)

Figure 4

Microscopic Appearance of Erythrocyte Morphology in Experimental Group 3

In Experimental Group 3, erythrocyte morphological alterations were less prominent when compared with both the control group and the other experimental groups. The majority of erythrocytes retained a normal biconcave shape with relatively homogeneous cell size and a clearly defined central pallor. The minor irregularities observed were more likely attributable to staining artifacts or variations in smear preparation rather than true pathological changes.

In contrast to Experimental Group 2, which demonstrated more pronounced poikilocytosis and anisocytosis, Experimental Group 3 exhibited a comparatively stable erythrocyte morphology. This pattern suggests that the erythrocyte response to microplastic exposure does not necessarily follow a linear dose–response relationship. At higher exposure levels, adaptive or compensatory mechanisms, such as enhanced antioxidant activity or increased clearance of damaged erythrocytes, may mitigate observable morphological damage. Therefore, the variability observed in this group may reflect both biological adaptation and technical factors related to sample preparation rather than a direct toxic effect of microplastics on erythrocyte morphology.

The comparative analysis using the Kruskal–Wallis test showed a significance value of $p = 0.762$ ($p > 0.05$) for the erythrocyte count variable, indicating no statistically significant differences among the control group (X0) and treatment groups X1, X2, and X3. For the erythrocyte size variable, the Kruskal–Wallis test also yielded a p value of 0.605 ($p > 0.05$), suggesting no significant differences among the groups. Analysis of the erythrocyte morphology variable resulted in a p value of 0.209, indicating that microplastic exposure at doses of 1.25 mg, 2.5 mg, and 5 mg did not produce statistically significant differences in erythrocyte morphology when all groups were analyzed simultaneously.

Tabel 2
Results of Comparative Analysis of Erythrocyte Profile

Variable	Statistical Analysis Test	P
Erythrocyte Count		0,762
Erythrocyte Size	*Kruskal -Wallis test*	0,605
Erythrocyte Morphology		0,209

Source: Processed Primary Data, 2025

Tabel 3
Results of Spearman Correlation Analysis Between the Number of Microplastic Particles in Blood and Study Variables

Variable	Statistical Analysis Test	P	R
Number of Microplastics in Blood & Erythrocyte Count		0,708	-0,077
Number of Microplastics in Blood & Erythrocyte Size	Spearman Correlation Test	0,692	-0,081
Number of Microplastics in Blood & Number of Abnormal Erythrocyte Forms		0,781	0,057

Source: Processed Primary Data, 2025

Based on Table 3, the Spearman correlation analysis showed that the number of microplastic particles in the blood had no significant relationship with any of the hematological parameters tested. The correlation between microplastic count and erythrocyte count demonstrated a very weak and non-significant association (r = −0.077; p = 0.708). The relationship between microplastic count and erythrocyte size was also very weak with a negative direction (r = −0.081; p = 0.692). The correlation between microplastic count and the number of abnormal erythrocytes showed a very weak and non-significant association (r = 0.057; p = 0.781). Overall, these findings indicate that the number of microplastic particles in the blood is not significantly correlated with erythrocyte count, erythrocyte size, or the degree of erythrocyte morphological abnormalities.

Oral exposure to LDPE for 28 days is presumed not to have directly affected the hematopoietic system, thereby maintaining erythrocyte production within physiological limits. Microplastics that enter the bloodstream tend to interact at the level of the cell membrane through their hydrophobic properties and surface charge, without reaching the bone marrow as the primary site of erythropoiesis. Such interactions may induce mild structural changes in the erythrocyte membrane without a concomitant decrease in the overall red blood cell count (Hamed et al., 2021).

The stability of erythrocyte size indicates that cellular volume regulation mechanisms remain optimal. Erythrocytes are capable of maintaining intracellular ion and water balance through osmotic regulation; therefore, microplastic exposure does not directly trigger changes in erythrocyte size. This condition is consistent with previous reports stating that microplastics may induce localized oxidative stress on the erythrocyte membrane without causing significant alterations in cell size (Abdel-Zaher et al., 2023; Hamed et al., 2021).

The observed changes in erythrocyte morphology were mild and varied among individuals, with a tendency toward an increased number of abnormal erythrocytes at the intermediate dose. This pattern indicates that the erythrocyte response to microplastic exposure is not linear with increasing dose, but rather is influenced by individual adaptive capacity and biological responses. Microplastic-induced oxidative stress may enhance membrane lipid peroxidation and affect erythrocyte flexibility; however, these effects were not sufficiently strong to produce consistent morphological changes at the population level (Kadac-Czapska et al., 2024; Yong et al., 2020).

The absence of a relationship between the number of microplastic particles in the blood and erythrocyte parameters indicates that the presence of microplastics in the circulation has not yet become a primary determinant of quantitative or morphological changes in red blood cells. This condition reflects the presence of effective cellular compensatory mechanisms that maintain erythrocyte integrity during short- to medium-term microplastic exposure (Deng et al., 2017).

CONCLUSION

Based on the results of this study, oral exposure to LDPE microplastics showed no significant correlation with the erythrocyte profile in *Rattus norvegicus* Wistar strain rats. The observed variations in erythrocyte morphology did not demonstrate a consistent dose-dependent pattern and therefore cannot be interpreted as a direct effect of microplastic exposure. Furthermore, LDPE exposure did not significantly affect erythrocyte count or size, indicating that the hematological response of the rats at the administered doses remained within physiological compensatory limits. Overall, these findings suggest that oral exposure to LDPE microplastics under the conditions of this study did not induce measurable alterations in erythrocyte parameters

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