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Subacute PVC Microplastic Inhalation Alters the Complete Blood Count Profile

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Abstract

Plastics are used in a wide range of items worldwide. With the increased usage of plastics, concerns about environmental contamination and the body's exposure to plastics have increased. Microplastics have been found in the atmosphere, and breathing them in might cause a risk to our health. One plastic polymer type commonly used is Polyvinyl Chloride (PVC). However, the harmful consequences of inhaling PVC microplastics are not well known. This study aims to determine the effects of inhaling PVC microplastics on female Wistar rats' blood cells. We exposed PVC microplastics to female Wistar rats via the whole-body inhalation method for 28 days. At the end of the study, we analyzed morphology and completed blood count tests such as erythrocyte count, erythrocyte index, leukocyte count, leukocyte differential count, and thrombocyte count. We discovered that sub-acute exposure to PVC microplastics increases the number of erythrocytes and hemoglobin levels without causing morphology alteration. PVC microplastics groups showed leukopenia consistent with an increment of inflammatory marker neutrophil/ leukocyte ratio. PVC microplastic groups also showed a rise in thrombocyte counts. These results could provide data for understanding Microplastics inhalation's toxicity and health risks, especially in the blood.

Keywords Polyvinyl chloride, microplastics, inhalation, blood

1.0 INTRODUCTION

Every year, the amount of plastic used in daily life rises dramatically [1]. There are several different types of plastic, including polyethylene terephthalate (PET), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), high-density polyethylene (HDPE), polystyrene (PS), and others [2]. Physical, chemical, and biological processes can disintegrate this material into tiny fragments. Microplastics are particles smaller than 5 mm, and nanoplastics are smaller than 1 µm [3]. There are four primary ways that plastic particles and their additives can enter and accumulate in the body: through skin pores, contact, oral ingestion, and inhalation [4]. There are four primary ways that plastic particles and their additives can enter and accumulate in the body: through skin pores, contact, oral ingestion, and inhalation [5]. This phenomenon has led to increased awareness of the dangers of exposure to microplastics on health [6]. It is well known that microplastics and chemical additions alter female reproductive hormones [7,8]. Moreover, progesterone and estrogen are widely acknowledged as essential blood regulators [9–11].

According to studies by Wu et al., microplastics may penetrate the bloodstream and raise free radicals [12]. Moreover, a spike in free radicals may raise the risk of cell function disturbances [13]. However, the effect of microplastic exposure on blood abnormality is not fully understood. In terms of erythrocyte, Iheanacho & Odo found that the number of erythrocytes increased, and the erythrocyte index was not changed when African catfish were given PVC microplastic combined with feed for 45 days [14]. On the other hand, a previous study discovered that mice exposed to microplastics had a reduction in erythrocyte count [15]. Cary et al. found that exposure to microplastics in the short term elevated IL-6 inflammatory markers [7]. Concerning thrombocytes, Wu et al. research on human thrombus revealed that exposure to microplastics in blood boosts thrombocytes counts, develops thromboses, and releases more particles, all of which may accelerate the onset of thrombosis [12]. In general, research on the effect of exposure to these microplastics on blood cells is still limited.

This study aimed to assess the effects of PVC microplastic exposure on blood profiles, a novel approach in microplastic research. PVC was chosen for this study due to its widespread use. Our findings, which represent the first evidence of whole-body PVC inhalation in a female laboratory animal used to evaluate systemic toxicities, provide a unique perspective on the potential health risks of microplastic inhalation.

2.0 EXPERIMENTAL

2.1 Experimental Design

This study employed a rigorous experimental design, including a randomized post-test-only control group design and a true experimental design. We compared complete blood counts from a control group and a group of rats exposed to PVC microplastics through inhalation. Our adherence to the research ethics protocol No. 254 /EC/ KEPK/ 08/ 2023 of the Faculty of Medicine at Brawijaya University in all aspects of this study ensures the reliability and validity of our results.

2.2 Animal Models

The experimental animals used were female Wistar white rats (Rattus norvegicus). Rats will be divided into a control group (n=5) and a treatment group exposed to PVC microplastics (n=6). The healthy rats utilized in the study were 12–15 weeks old, weighed 150–200 grams, and went through a regular oestrous cycle. Oestrous cycles were checked daily using visual assessment and vaginal cytology methods [16]. All rat care procedures during the study followed the research protocol and were carried out with efforts to minimize the suffering of the experimental animals.

2.3 Exposure to PVC Microplastics per Inhalation

The whole-body inhalation exposure method was carried out following the research of Trembley et al. (2022) with modifications to the duration of sub-acute administration [17]. Briefly, female rats in the oestrous phase were placed in a 50x50x50 cm inhalation box and exposed to technical grade PVC microplastic (CV. Subur Kimia Jaya®), which was exhaled via a blower for 4 hours per day for 28 days [18]. The PVC microplastic is examined under a stereo microscope (Nikon® SMZ1500) with a camera attached (Onglai Fixtool 51MP®). The microplastic size used in this study was 1,061$\pm$9,09 mm, and 92% of particles had a size below 600 mm. According to Occupational Safety and Health Administration (OSHA) recommendations, a dosage of 15 mg/m^3 of PVC microplastic is used [7]. After 28 days of treatment, the rats were euthanized using deep anaesthesia and their blood was taken from the heart for the examination.

2.4 Blood Analysis

Blood collection from the heart was carried out immediately after the rats were euthanized. Blood was collected in a vacutainer tube containing EDTA to prevent coagulation. A complete blood count was performed using an automated hematology machine (ABX Micros 60 Hematology Analyzer®), which provides accurate and reliable results. The complete blood examination includes erythrocyte count, hematocrit, hemoglobin, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW-SD), total leukocytes, neutrophils, lymphocytes, monocytes, and thrombocytes were analyzed.

2.5 Peripheral Blood Smear

Blood was dripped on one end of the slide and wiped onto the other, followed by Giemsa staining. The blood smear results were observed with an Olympus® CX-21 microscope.

2.6 Statistical Analysis

Data were analysed by Mann-Whitney using GraphPad Prism® 5. Data are presented in a whisker plot. The middle line in the box shows the mean, the bottom and top lines of the box show the minimum and maximum data, and the vertical line indicates the standard error. Asterisks (*) and (**) means significant variation at $p < 0.05$ and $p < 0.01$, respectively

3.0 RESULTS AND DISCUSSION

The detrimental effects of environmental pollutants on human health have been observed to have significantly increased in recent years [1]. Because of the large-scale manufacture of plastics and the negligent processing of plastic waste, microplastics are one of the main sources of air pollution [19]. According to a new study, microplastics damage the female reproductive system [7]. However, the impact of microplastics on the hematological system in mammals is poorly understood.

3.1 Exposure to PVC Microplastics per Inhalation Increases the Number of Erythrocytes and Hematocrit Level but not the Erythrocyte Index

We performed an erythrocyte count and hematocrit test to determine the effect of PVC microplastic exposure on the total number and proportion of erythrocytes in the blood. The PVC microplastic exposure group had 1.07 times more erythrocytes than the control group (p=0.02) (Figure 1A). In addition, in the PVC microplastic exposure group, the hematocrit level was 1.05 times higher than the control group (p=0.29) (Figure 1B). Details of these measurements are presented in Table 1.

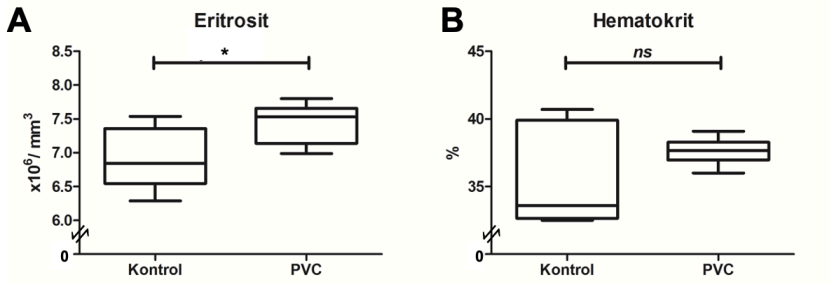

Figure 1 A. Average total erythrocytes number B. Average hematocrit level. Control group (n=5); PVC microplastics group (n=6). *ns:* not significant; *: $p < 0.05$.

To determine the size, variety, shape, and quality of erythrocytes, we performed peripheral blood smear analysis (Figure 2) and erythrocyte index measurements consisting of Hemoglobin, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and Red Cell Distribution Width (RDW-SD) (Table 1). The size, shape, and arrangement of the blood cells in the peripheral blood smear did not differ between groups. Hematology machine analysis results supported these findings, showing no significant changes ($p>0.05$) across groups (Figure 3A) but a trend toward a modest rise in hemoglobin and a slight decrease in MCV, MCH, and MCHC (Figure 3B-D). This indicates that the erythrocyte volume and size in the PVC microplastic exposure treatment group is microcytic. In this study, a significantly low level of RDW-SD was obtained in the PVC microplastic exposure treatment group (p=0.002) (Figure 3E). This indicates that the variations in shape in this group are low. Microcytic erythrocytes accompanied with low RDW-SD show similar characteristics as thalassemia traits, a genetic condition where a person carries a mutation in globin protein genes, which is responsible for producing hemoglobin [20]. The PVC microplastic may be toxic to bone marrow.

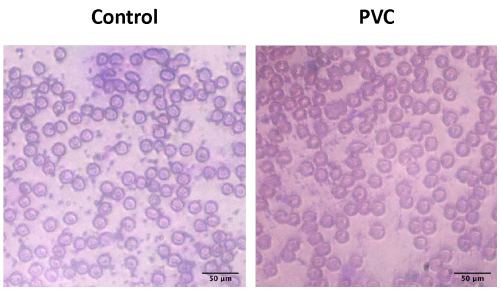

Figure 2 Representative images of peripheral blood smear. Scale bar: 50 µm

Studies in African catfish after 45 days of PVC microplastics showed that oral exposure might not alter the number of erythrocytes [14]. Another study by Abdel-Zaher et al., 2023 found that exposure to high-dose polyethylene microplastics for 15 days decreased the erythrocyte and altered its morphology. Experiments using polystyrene nano plastics in rats also showed abnormal quantity and quality of the erythrocyte [20]

From those studies, the researchers believed that oxidative stress plays a pivotal role in interfering with the erythrocyte. Surprisingly, in this study, we found that the number of erythrocytes was slightly increased after the rats were exposed to PVC microplastics per inhalation. We speculate that the shorter exposure may not trigger erythrocyte damage, but may decrease plasma volume, which causes the concentration of red blood cells to seem higher than usual. This phenomenon is called stress polycythemia [21]. Another possible mechanism is that microplastics decrease the estrogen hormone [7]. Studies on chronic hypoxic conditions of the Andean population showed excessive erythrocytosis, especially in post-menopausal females [9]. The discrepancy between this study and the previous study might come from dose-dependent effects, species-specific mechanisms, and types of microplastics.

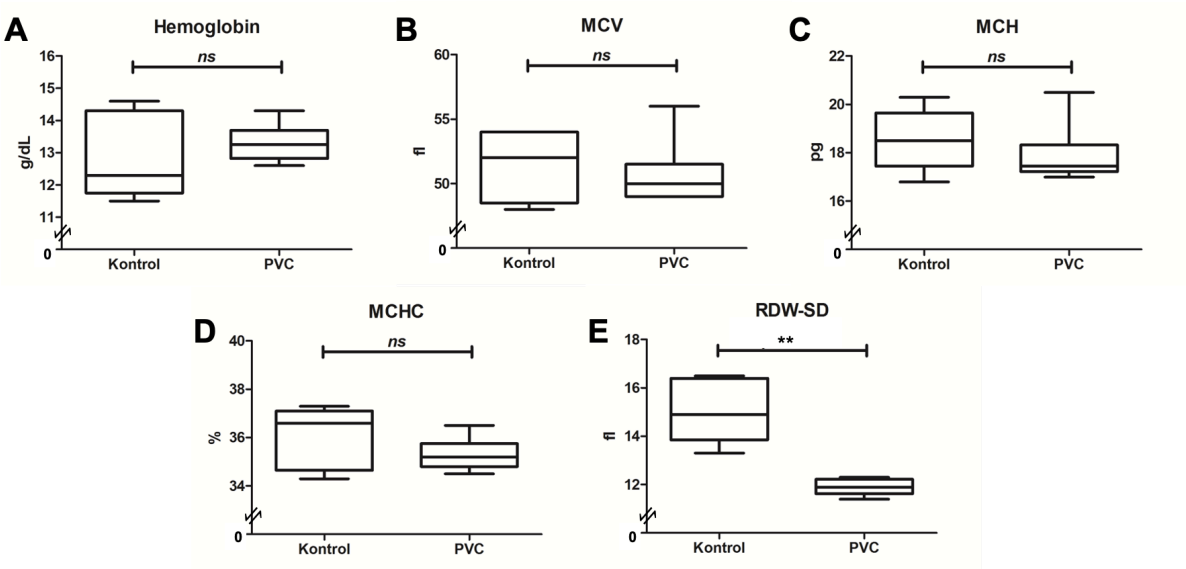

Figure 3 A. Average hemoglobin levels B. Average Mean corpuscular volume (MCV) levels C. Average Mean Corpuscular Hemoglobin (MCH) levels D. Average Mean Corpuscular Hemoglobin Concentration (MCHC) levels E. Average Red Cell Distribution Width (RDW-SD). Control group (n=5); PVC microplastics group (n=6). *ns:* not significant; **: $p < 0.01$

3.2 Exposure to PVC Microplastics per Inhalation Decreases Number of Leukocytes

Total and differential leukocyte counts were carried out to determine the total number and the percentages of each type of leukocyte (Table 1). The PVC microplastic exposure group showed 26% lower total leukocytes than the control group (p=0.02) (Figure 4A). The PVC microplastic exposure group's differential leukocyte counts revealed a decline in polymorphonuclear and mononuclear leukocyte counts (Figure 4B-D). Next, predictive markers of inflammation were examined by comparing the number of neutrophils and lymphocytes. In this study, there was no increase in the neutrophil-lymphocyte ratio in the PVC microplastic exposure group, indicating that no inflammation or at least exposure to PVC microplastics did not activate the immune system (Figure 4E). The neutrophil-to-lymphocyte ratio, is a biomarker that represents the equilibrium between two components of the immune system—acute and chronic inflammation (represented by the neutrophil count) and adaptive immunity (represented by the lymphocyte count) [22]. Our study underwent in sub-acute that the acute inflammation biomarker might have already decreased but the chronic inflammation marker has not risen yet. Looking at our results in more detail, we discovered that the ratio of neutrophils and lymphocytes in the PVC microplastics exposure group is slightly higher than in the control.

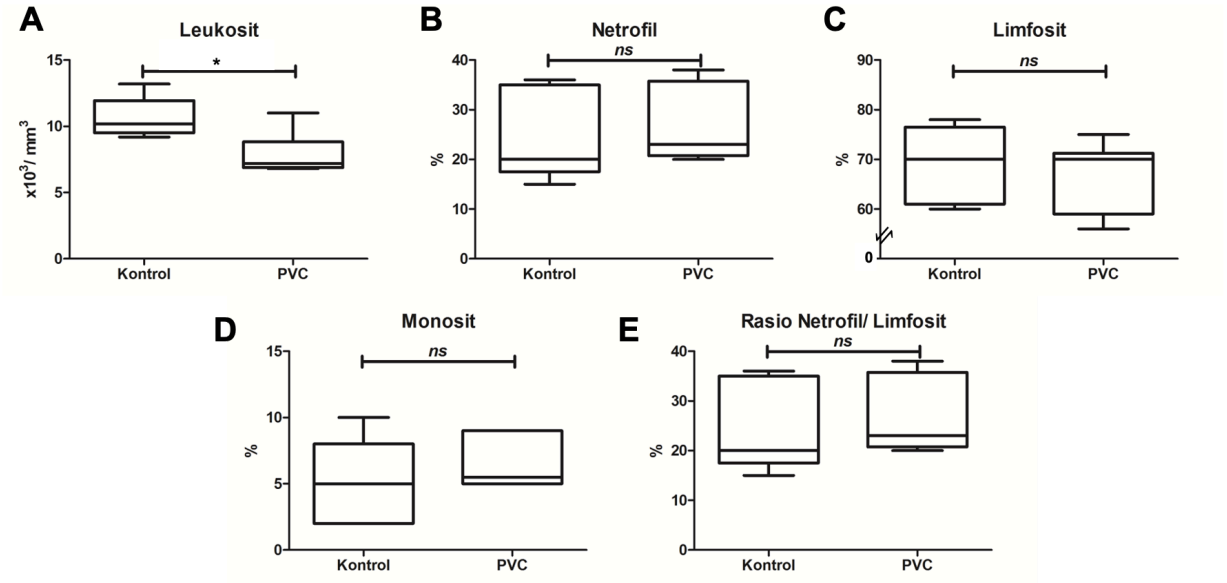

Figure 4 A. Average total leukocytes number B. Average neutrophils number C. Average lymphocytes number D. Average monocytes number E. Average ratio of neutrophils per lymphocyte. Control group (n=5); PVC microplastics group (n=6). *ns:* not significant; *: $p < 0.05$

Environmental exposure to microplastics was sufficient to compromise immune systems, leading to autoimmune diseases or immunosuppression [23]. Long-term microplastic exposure damages the cells and disrupts the immunomodulator systems. This series of events would result in the production of autoantibodies [24]. In the current study, we discovered that exposure to PVC microplastics reduced the quantity of leukocytes. This result is consistent with the study of PVC-instilled mice that show lower inflammatory cell count [25]. According to earlier studies, employees in PVC industries may get pneumoconiosis after ten years of exposure to PVC. Additionally, a rise in the concentration of PVC in the air, combined with prolonged exposure and inhalation, may cause fibrosis or carcinogenesis through chronic inflammatory stimulation [26]. Inhalation microplastics study using polyamide, polypropylene, and polyvinyl chloride trigger systemic inflammation characterized by increased cytokines [7,25].

3.3 Exposure to PVC Microplastics per Inhalation Increases the Number of Thrombocytes

A thrombocyte count is conducted to ascertain the quantity of thrombocytes in the blood (Figure 5). The thrombocytes count was 1.08 times greater in the PVC microplastic exposure treatment group than in the control group (Table 1), though the difference was not statistically significant (p=0.33).

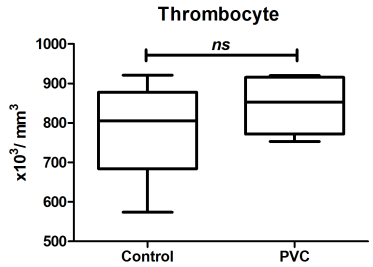

Figure 5 Average thrombocyte count. Control group (n=5); PVC microplastics group (n=6). *ns:* not significant.

Compared to the control group, this study demonstrated that the thrombocytes had a greater exposure to PVC microplastics. A study in zebrafish, which is exposed to non-functionalized Polystyrene microbeads, showed an increment of genes encoding thrombocytes [27]. Studies in rodents and humans showed a correlation between microplastics and clot formation [20,28]. Possibly, microplastics triggered the clotting cascade by increasing fibrin polymerization rates and enhancing clot strength in a size and concentration-dependent way [29].

Table 1. Average of complete blood count

Parameter	Control (n=5)		PVC (n=6)		Higher/ lower than control	p-value
	Mean	S.E.	Mean	S.E.		
Erythrocyte (x10^6/mm^3)	6,93	0,209	7,44	0,122	□	0,026*
Hematocrit (%)	35,74	1,726	37,62	0,413	□	0,292
Hemoglobin (g/dL)	12,88	0,601	13,30	0,240	□	0,268
RDW-SD (fl)	15,08	0,599	11,90	0,137	□	0,002**
MCV (fl)	51,40	1,249	50,67	1,085	□	0,463
MCH (pg)	18,54	0,572	17,88	0,530	□	0,214
MCHC (%)	36,02	0,581	35,30	0,282	□	0,180
Leukocyte (x10^3/mm^3)	10,62	0,690	7,87	0,655	□	0,026*
Neutrophil (%)	25,00	4,195	26,67	3,211	□	0,203
Lymphocyte (%)	69,00	3,521	66,83	2,949	□	0,287
Monocyte (%)	5,00	1,483	6,50	0,806	□	0,255
Neutrophil/Lymphocyte ratio (%)	25,00	4,195	26,67	3,211	□	0,203
Thrombocyte (x10^3/mm^3)	785,80	57,440	845,20	27,990	□	0,331

S.E.= standard error; * means significant

4.0 CONCLUSION

To our knowledge, this is the first whole-body PVC microplastic inhalation study in female rats that examines the effects on the blood. According to this study, exposure to PVC microplastics raised erythrocyte and thrombocyte counts but decreased leukocyte counts. Factors that may underlie the phenomenon are bone marrow toxicity, stress polycythemia, hormone disruption, and compensatory mechanisms. Plastics are widely used, and this study's findings about their negative impacts emphasize the need for a deeper comprehension of microplastic toxicities to support regulatory policy and ecological restoration.

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