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The Effect of Oral Polyethylene Microplastic Doses on Femoral Bone Cell Profiles in Wistar Rats

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Keywords

Polyethylene; Osteocyte; Osteoblast; Osteoclast; Femur

Abstract

Microplastics pose a potential health risk because they can enter the body orally and accumulate in various organs, including bone, potentially disrupting bone homeostasis through oxidative stress and inflammation. This study aimed to analyze the effect of oral polyethylene microplastic exposure on the femoral bone cell profile of Wistar strain Rattus norvegicus. A laboratory experimental study with a post-test only control group design was conducted using 28 male Wistar rats divided into four groups: control (X0) and treatment groups receiving LDPE microplastics (≤20 µm) at doses of 1.25 mg/day (X1), 2.5 mg/day (X2), and 5 mg/day (X3) for 28 days. Femoral bones were examined histologically using HE staining, and osteocytes, osteoblasts, and osteoclasts were counted. Results showed a significant increase in osteocyte and osteoclast numbers in treatment groups (p=0.002), while osteoblast numbers were not significantly different (p=0.655). Increased osteoclasts indicate enhanced bone resorption that may impair bone homeostasis.

INTRODUCTION

Plastic has become one of the most widely used materials in daily life. One of the negative impacts of plastic use is exposure to microplastics. Microplastics are plastic particles smaller than 5 mm and have become a major threat to the environment and human health (Zhao & You, 2024). These particles are widely found in freshwater, marine, and airborne ecosystems and enter the food chain through organisms that ingest them (Campanale et al., 2020). Global plastic production has increased up to 240-fold in recent decades, exacerbating plastic pollution, particularly in rapidly industrializing countries. Poorly managed plastic waste degrades into microplastics that spread through water, air, and soil, contaminating food and drinking water sources (Giannandrea et al., 2024; Zhao & You, 2024).

Recent studies have shown that dietary and inhalational microplastic intake across 109 developing and industrialized countries increased more than sixfold between 1990 and 2018. Indonesia has been reported as the country with the highest per capita microplastic intake, reaching approximately 15 grams per month, largely due to high seafood consumption and severe aquatic plastic pollution (Zhao & You, 2024). In Asia, Africa, and the Americas, industrialization has been strongly associated with increased microplastic contamination of food and air.

Plastic is highly resistant to natural degradation and may persist in the environment for hundreds of years. In addition, plastics contain various hazardous substances that can adversely affect human health. Harmful additives such as bisphenol A (BPA) and phthalates are known endocrine-disrupting chemicals. Plastics may also contain carcinogenic compounds such as dichlorodiphenyltrichloroethane (DDT), chromium (Cr), and polycyclic aromatic

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hydrocarbons (PAHs), as well as heavy metals such as cadmium (Cd), which is highly toxic and can impair kidney function and bone health (Supit et al., 2022).

The study by Dzierżyński et al. (2024) demonstrated that polyethylene is more hazardous than polystyrene. Polyethylene is classified into two types, namely low-density polyethylene (LDPE) and high-density polyethylene (HDPE) (Dzierżyński et al., 2024). LDPE is one of the most widely recognized and commonly used types of plastic by the Indonesian population (Prasetya et al., 2024). Since the majority of plastics are produced, utilized, and discarded on land, plastic waste predominantly accumulates in terrestrial environments. These plastic particles can degrade into microplastics and persist in soil for up to 15 years. Furthermore, illegal waste disposal activities and direct dumping into the environment exacerbate the accumulation of plastic waste on land. Terrestrial environments can function as temporary sinks for plastic waste up to a certain capacity. This plastic debris may subsequently be transported through human activities, wind, and water. In addition to terrestrial environments, freshwater systems such as rivers, reservoirs, lakes, and urban drainage channels have also become heavily contaminated with plastic waste (Pilapitiya & Ratnayake, 2024).

Microplastics can enter the human body through oral exposure via contaminated food and beverages. Environmental plastic pollution increases the risk of microplastic ingestion "(Supit et al., 2022; Zhao & You, 2024). Once ingested, microplastics cannot be fully degraded in the gastrointestinal tract and may cross the intestinal epithelium through endocytosis and phagocytosis mechanisms. M cells in Peyer's patches transport microplastics into systemic circulation, while dendritic cells distribute them to organs such as bone, muscle, brain, and liver (Giannandrea et al., 2024; Zhang et al., 2024).

Microplastics increase the production of reactive oxygen species (ROS) due to their toxic chemical components, leading to oxidative stress (Zhang et al., 2024). Excessive ROS can induce apoptosis of bone cells and reduce the viability of osteocytes and osteoblasts, resulting in disruption of bone homeostasis '(Sibilia et al., 2021).

Microplastics are capable of penetrating protective barriers, including the blood–brain barrier, and may accumulate in the hypothalamus (Kaur et al., 2023). Chemicals contained in microplastics may mimic estrogen activity and activate the Receptor Activator of Nuclear Factor-κB (RANK) and Receptor Activator of Nuclear Factor-κB Ligand (RANKL), promoting osteoclastogenesis and bone resorption (Giannandrea et al., 2024; Zhang et al., 2024).

Several studies have reported that microplastic exposure negatively affects bone deposition by decreasing osteogenic proteins such as osteocalcin (OCN) and RUNX family transcription factor 2 (Runx2). Additionally, increased expression of inflammatory markers including interleukin-1 beta (IL-1β), matrix metalloproteinase-13 (MMP-13), and senescence-associated β-galactosidase (SA-βGal) has been observed in osteoblasts, indicating impaired musculoskeletal health (Pan et al., 2023).

Osteoporosis is characterized by decreased bone density and deterioration of bone microarchitecture, resulting in increased fracture risk (Clynes et al., 2020). The World Health Organization (WHO) classifies osteoporosis as the second most serious global health problem after cardiovascular disease. In Indonesia, osteoporosis prevalence reaches 32.3% in women and 28.8% in men, with most patients aged over 50 years (Anasulfalah et al., 2023).

Since research on microplastics is still relatively novel, this study aims to contribute to expanding knowledge regarding the potential health hazards of microplastic exposure in humans, particularly in relation to bone health.

Therefore, this study was conducted to investigate the effects of microplastics on bone density. Through histological examination, the numbers of osteocytes, osteoblasts, and osteoclasts in the femoral cortical bone tissue of *Rattus norvegicus* Wistar strain rats were compared. *Rattus norvegicus* is a species capable of adapting to human environments (Putro & Priyambodo, 2009). Moreover, rats share approximately 90% genetic similarity with humans, allowing various biological mechanisms, including gene regulation, immune responses, and disease development, to be studied with high relevance using rats as experimental models (Turner, 2013).

RESEARCH METHOD

This experimental study employed a post-test only control group design and was conducted from May to June 2024. Male Wistar strain Rattus norvegicus rats aged 6–8 weeks and weighing 150–200 grams were used. All animals were acclimatized for seven days and housed under controlled conditions with ad libitum access to food and water. A total of 28 rats were randomly assigned to four groups: control (X0) and treatment groups receiving oral LDPE microplastics at doses of 1.25 mg/day (X1), 2.5 mg/day (X2), and 5 mg/day (X3) for 28 consecutive days using oral gavage.

This study was conducted over a period of 37 days, from July 21 to August 26, 2025, at the Animal Laboratory, Faculty of Pharmacy, Widya Mandala Catholic University Surabaya. A total of 28 male Wistar strain (*Rattus norvegicus*) rats, aged 6–8 weeks and weighing 150–200 g, were randomly allocated into four groups: a control group (X0) and three treatment groups (X1, X2, and X3), with seven rats in each group. During the treatment period, two rats died: rat X2-3 on August 14, 2025, due to perforation caused by the oral gavage procedure, and rat X3-7 on August 20, 2025, which experienced a drastic decrease in body weight and reduced appetite one day prior to death. Consequently, the final number of experimental animals included in the analysis was 26 rats, with groups X0 and X1 consisting of seven rats each, while groups X2 and X3 consisted of six rats each.

After treatment, rats were euthanized using ketamine and xylazine. The right femur was harvested and processed for histological examination, including fixation in 10% formalin, decalcification, dehydration, clearing, paraffin embedding, sectioning, and Hematoxylin–Eosin staining.

Histological examination was performed using a light microscope at 400× magnification. Osteocytes, osteoblasts, and osteoclasts were counted in five fields of view per sample. Polymer identity was confirmed using Fourier Transform Infrared (FTIR) spectroscopy. Data analysis was performed using SPSS version 25.

RESULTS AND DISCUSSION

Prior to evaluating the effects of the experimental treatments, it was essential to assess the baseline characteristics of the experimental animals to ensure comparability among the study groups. Initial body weight was selected as an important baseline parameter because substantial differences could potentially influence physiological responses and affect the interpretation of treatment outcomes. In addition, statistical analyses were performed to examine the distribution of the data, the homogeneity of variances, and the presence of differences among groups. The results of these baseline assessments are presented in Table 1.

Based on the data presented in Table 1, the mean initial body weight of the experimental animals in the control group was 164.43 ± 21.555 g, while those in the treatment groups X1, X2, and X3 were 190.57 ± 31.516 g, 181.86 ± 11.553 g, and 171.29 ± 27.128 g, respectively. The Shapiro–Wilk test yielded a p-value of 0.378, and Levene's test resulted in a p-value of 0.252, indicating that the data

Table 1
Results of Body Weight Examination of Experimental Animals

Group	Mean± SD	Normality Test	Homogeneity Test	Comparison Test	Conclusion
Control	164.43± 21.555	*Saphiro-Wilk* Method	*Levene* Method	*One Way Anova*	Variance homogenouse
Experiment:					
X1	190.57± 31.516				
X2	181.86± 11.553				
X3	171.29± 27.128				
P		0.378	0.252	0.001	

Source: Primary Data Processed, 2025

were normally distributed and exhibited homogeneity of variances. Although the One-Way ANOVA analysis produced a p-value of 0.001, suggesting a statistically significant difference among groups, the variation in body weight across groups was considered comparable. Therefore, the initial body weight conditions of the experimental animals were deemed unlikely to introduce substantial bias into the study outcomes.

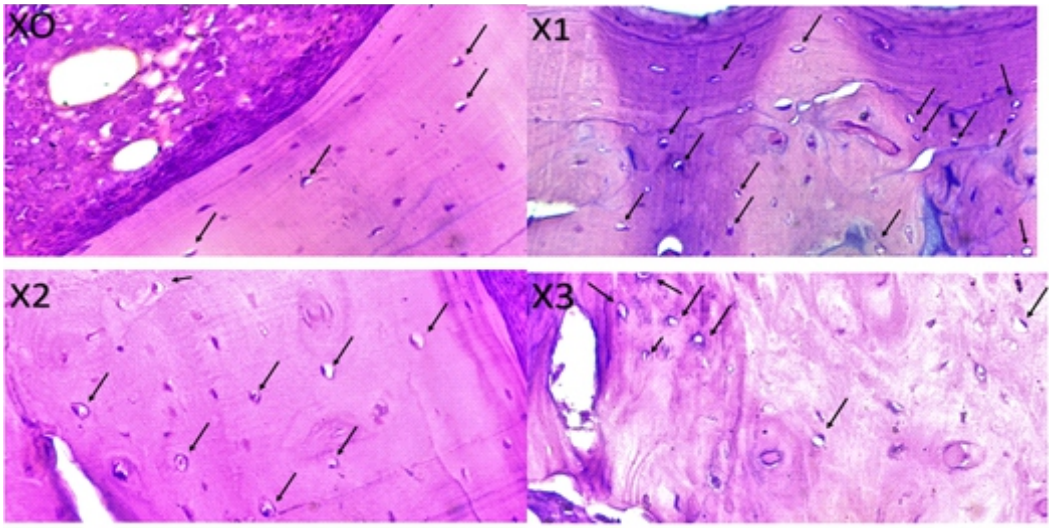
Bone remodeling is regulated through the coordinated activities of osteocytes, osteoblasts, and osteoclasts, each of which plays a distinct role in maintaining bone integrity and metabolic balance. Therefore, histological evaluation of these cellular components provides valuable insight into the effects of the experimental treatment on bone remodeling dynamics. Quantitative analysis was performed by determining the mean number of osteocytes, osteoblasts, and osteoclasts in each experimental group. The results of these measurements are summarized in Table 2.

Based on the results presented in Table 2, the osteocyte count in the control group demonstrated a regular and evenly distributed arrangement of osteocytes within the bone matrix lacunae, with a mean value of 23.00 ± 3.742 cells per field of view. The treatment groups exhibited a substantial increase in osteocyte numbers, with mean values of 56.43 ± 22.788 (X1), 39.83 ± 8.060 (X2), and 44.83 ± 17.927 (X3). The X1 group, which received the lowest dose, showed the highest increase,

Table 2
Results of Femoral Bone Cell Profile Counts in Rattus Norvegicus Strain Wistar

Variable	Control Group	Experiment Group		
		X1	X2	X3
		Mean ± SD		
Osteocyte Cell Count	23.00±3.742	56.43±22.788	39.83±8.060	44.83±17.927
Osteoblast Cell Count	58.57±8.715	52.57±25.612	56.67±19.367	65.83±12.983
Osteoclast Cell Count	12.29±4.990	24.00±1.915	20.83±5.307	20.83±4.309

Source: Primary Data Processed, 2025



Source: Primary Data Processed, 2025

Figure 1
Microscopic Appearance of Osteocyte Cells in All Experimental Groups Using Hematoxylin–Eosin (HE) Staining

followed by groups X3 and X2. Morphologically, Osteocytes in all groups exhibited an oval to round morphology, with nuclei stained dark purple by hematoxylin. However, the number of osteocytes was markedly higher in the treatment groups.

Osteocyte

The results of the study demonstrated a statistically significant difference in osteocyte numbers between the control and treatment groups ($p = 0.002$). The control group showed a mean osteocyte count of 23.00 ± 3.742, whereas the treatment groups exhibited a substantial increase, with mean values of 56.43 ± 22.788 (X1), 39.83 ± 8.060 (X2), and 44.83 ± 17.927 (X3). The increased osteocyte numbers observed in the microplastic-exposed groups suggest disrupted bone homeostasis, which may be attributed to several underlying pathophysiological mechanisms.

First, microplastic exposure may induce excessive production of reactive oxygen species (ROS), leading to oxidative stress at the cellular level (Sibilia et al., 2021; Zhang et al., 2024). Oxidative stress can alter the expression of genes and proteins involved in the regulation of osteocyte apoptosis. Under normal conditions, aged or damaged osteocytes undergo apoptosis as part of the regulated bone remodeling process (Al-Bari & Mamun, 2020). However, excessive ROS may activate cellular survival pathways such as PI3K/Akt, which inhibit apoptosis, thereby resulting in the accumulation of osteocytes within the bone matrix (Sibilia et al., 2021).

Second, enhanced differentiation of osteoblasts into osteocytes may occur as a compensatory response. A study by Pan et al. (2023) demonstrated that microplastic exposure can induce osteoblast senescence, characterized by increased expression of SA-βGal and the Senescence-Associated Secretory Phenotype (SASP). As a compensatory mechanism to maintain bone formation, the body may enhance the recruitment and differentiation of mesenchymal stem cells into new osteoblasts, which subsequently differentiate into osteocytes at a higher rate (Al-Bari & Mamun, 2020).

Third, disturbances in bone mineralization and extracellular matrix quality may alter osteocyte formation. Zhang et al. (2024) reported that microplastic exposure can disrupt bone mineralization by affecting hydroxyapatite deposition and collagen fiber organization. A poorly mineralized matrix may allow more osteoblasts to become embedded and survive during the embedment process due to improved

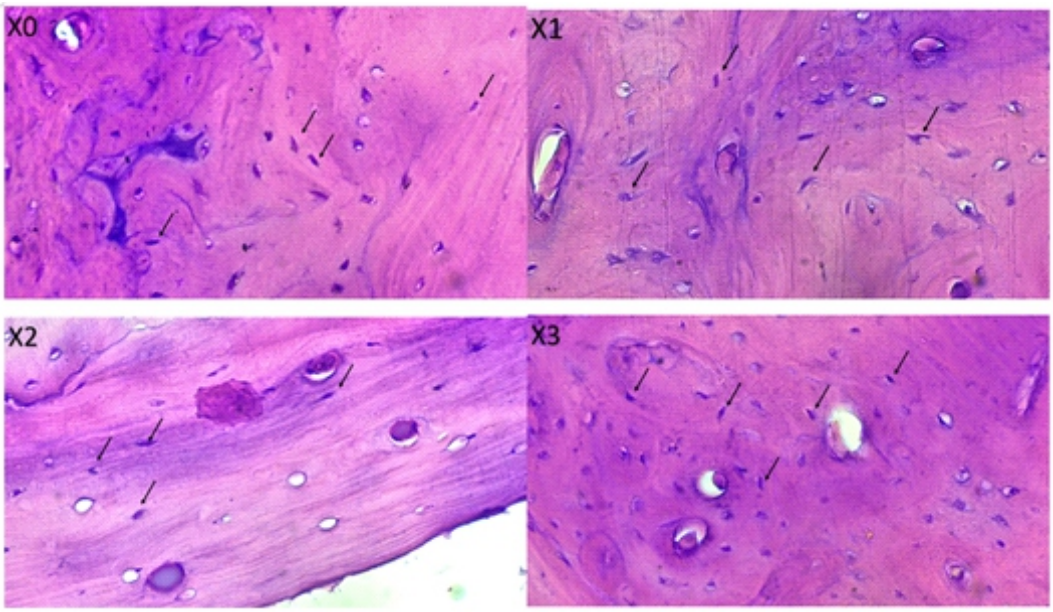
diffusion of nutrients and oxygen, resulting in an increased number of osteocytes. Fourth, the concurrent increase in osteocytes and osteoclasts may indicate a bone injury response. Physiologically, osteocytes may signal the body's attempt to reinforce bone tissue or respond to mechanical loading. However, when accompanied by an increase in osteoclasts, a net loss of bone mass typically occurs as bone resorption exceeds bone formation (Liu et al., 2024).

A non-linear response pattern to microplastic dosage represents an interesting finding. Group X1, which received the lowest dose (1.25 mg/day), exhibited the highest increase in osteocyte count (56.43 ± 22.788), followed by group X3 (44.83 ± 17.927) and group X2 (39.83 ± 8.060). This phenomenon may be explained by the concept of hormesis, a biphasic response to stressors in which low doses elicit effects distinct from those of higher doses (Sun et al., 2021). At low doses (X1), microplastics may induce adaptive stress responses that stimulate cellular activity without causing significant damage. At moderate doses (X2), toxic effects may begin to counterbalance adaptive responses and induce apoptosis in some osteocytes. At higher doses (X3), a combination of toxic effects and stronger compensatory mechanisms may occur (Fusagawa et al., 2025; Sun et al., 2021).

These findings have important public health implications given the widespread prevalence of microplastics in the environment and food chain (Supit et al., 2022; Zhao & You, 2024). Zhao and You (2024) reported that per capita microplastic intake increased more than sixfold between 1990 and 2018, with Indonesia recorded as having the highest intake. If significant changes in osteocyte numbers can occur within a 28-day exposure period, chronic lifetime exposure in humans may result in more severe disturbances in bone structure and function.

Osteoblast

The results of osteoblast counts showed that the control group had a mean osteoblast number of 58.57 ± 8.715 cells per field of view, with osteoblasts arranged orderly along the bone surface in a cuboidal to columnar pattern. The treatment groups showed mean values of 52.57 ± 25.612 (X1), 56.67 ± 19.367 (X2), and 65.83 ± 12.983 (X3). Groups X1 and X2 tended to show a decrease in osteoblast numbers compared to the control group, whereas group X3 exhibited an increase.



Source: Primary Data Processed, 2025

Figure 2
Microscopic Appearance of Osteoblast Cells in All Experimental Groups Using Hematoxylin–Eosin (HE) Staining

Despite variations in osteoblast counts among groups, their distribution and morphology remained within normal characteristics, with nuclei stained dark purple.

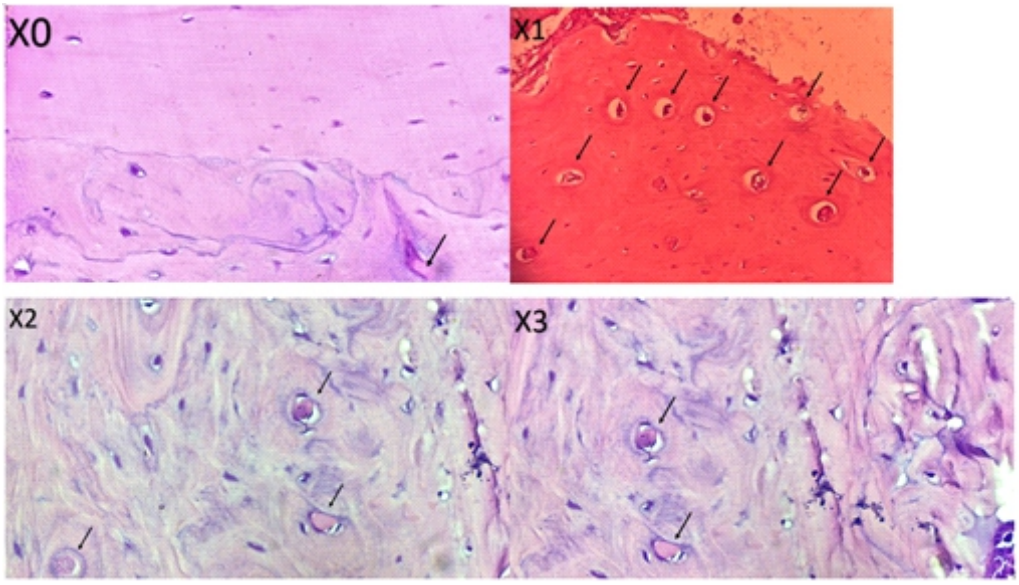
Statistical analysis showed no significant difference in osteoblast numbers between the control and treatment groups ($p = 0.655$). The control group exhibited a mean osteoblast count of 58.57 ± 8.715, while the treatment groups showed values of 52.57 ± 25.612 (X1), 56.67 ± 19.367 (X2), and 65.83 ± 12.983 (X3). Post-hoc Mann–Whitney tests confirmed the absence of statistically significant differences: X1 ($p = 0.898$), X2 ($p = 1.000$), and X3 ($p = 0.151$). Although not statistically significant, the observed fluctuation pattern showed a decrease in groups X1 and X2, but a substantial increase in group X3. The decrease observed at low-to-moderate doses may be explained by several mechanisms. First, microplastic exposure induces osteoblast senescence. Pan et al. (2023) demonstrated a significant increase in Senescence-Associated β-Galactosidase (SA-βGal) expression in osteoblasts exposed to polystyrene microplastics, with cells exhibiting a Senescence-Associated Secretory Phenotype (SASP) characterized by excessive secretion of pro-inflammatory cytokines such as IL-1β, TNF-α, and MMP-13. Second, microplastic-induced oxidative stress promotes osteoblast apoptosis through excessive ROS production, activating apoptotic pathways via caspase-3 and inhibiting the Wnt/β-catenin pathway, which is critical for osteoblast differentiation (Sibilia et al., 2021; Zhang et al., 2024). Third, reduced expression of osteogenic transcription factors such as Runx2 and osteocalcin (OCN) indicates disruption of osteogenic pathways at the molecular level (Pan et al., 2023).

The increase in osteoblast numbers observed in group X3 represents a paradoxical phenomenon that may be explained by compensatory response mechanisms. First, increased bone resorption activity by osteoclasts in group X3 (20.83 ± 4.309) triggers coupling mechanisms between bone resorption and formation. Enhanced osteoclast activity releases growth factors from the degraded bone matrix, including TGF-β and BMPs, which recruit and stimulate mesenchymal stem cell differentiation into osteoblasts to replace lost bone (Al-Bari & Mamun, 2020; Gartner & Hiatt, 2007). Second, mechanical stress and bone microdamage activate osteocytes to reduce sclerostin production, a natural inhibitor of the Wnt/β-catenin pathway, thereby enhancing osteoblastic activity (Al-Bari & Mamun, 2020). Third, high-dose exposure may induce hormetic effects, in which the body activates stronger cellular survival pathways, including increased expression of heat shock proteins and endogenous antioxidant enzymes (Sun et al., 2021).

The effects of microplastics on osteoblasts must be understood within the context of complex interactions among cells within the Basic Multicellular Unit (BMU). The significant increase in osteocytes and osteoclasts suggests that microplastics affect the entire bone cellular ecosystem. Increased osteocytes may influence osteoblast activity through regulation of sclerostin and RANKL, creating complex feedback cycles in bone remodeling regulation (Al-Bari & Mamun, 2020). If the increase in osteoblast numbers in group X3 is not accompanied by a proportional increase in functional activity, for example due to microplastic-induced senescence or dysfunction, a net imbalance detrimental to bone homeostasis may occur.

Osteoclast

The results of osteoclast counts indicated that the control group had a mean osteoclast number of 12.29 ± 4.990 cells per field of view. Osteoclasts were identified as large, multinucleated cells with dark purple hematoxylin-stained nuclei. The treatment groups showed a substantial increase, with mean values of 24.00 ± 1.915 (X1), 20.83 ± 5.307 (X2), and 20.83 ± 4.309 (X3). All treatment groups demonstrated increased osteoclast numbers compared to the control group, with group X1



Source: Primary Data Processed, 2025

Figure 3
Microscopic Appearance of Osteoclast Cells in All Experimental Groups Using Hematoxylin–Eosin (HE) Stainingclast

exhibiting the highest increase.

The study results demonstrated a statistically significant difference in osteoclast numbers between the control and treatment groups ($p = 0.002$). The control group showed a mean osteoclast count of 12.29 ± 4.990, where as the treatment groups exhibited substantial increases, with mean values of 24.00 ± 1.915 (X1), 20.83 ± 5.307 (X2), and 20.83 ± 4.309 (X3). Post-hoc Mann–Whitney tests revealed significant differences between the control group and all treatment groups: X1 ($p = 0.002$), X2 ($p = 0.018$), and X3 ($p = 0.010$). The increased osteoclast numbers observed in all microplastic-exposed groups represent a critical and concerning finding, as they indicate enhanced bone resorption activity.

The mechanisms underlying increased osteoclast numbers following microplastic exposure may be explained through several molecular pathways. First, microplastics can induce the production of RANKL, a key factor in osteoclastogenesis. Zhang et al. (2024) demonstrated that microplastic exposure activates the RANK/RANKL /OPG pathway, in which an increased RANKL-to-osteoprotegerin (OPG) ratio promotes osteoclast differentiation and activation. RANKL binds to the RANK receptor on osteoclast precursor surfaces, activating intracellular signaling pathways involving TNF Receptor-Associated Factor 6 (TRAF6) and Nuclear Factor-κB (NF-κB), ultimately inducing expression of Nuclear Factor of Activated T-cells, cytoplasmic 1 (NFATc1), the master transcription factor required for osteoclast differentiation (Al-Bari & Mamun, 2020).

Second, microplastic-induced oxidative stress plays a crucial role in enhancing osteoclastogenesis. Excessive ROS can increase RANKL expression in osteoblasts and osteocytes and enhance the sensitivity of osteoclast precursors to RANKL (Sibilia et al., 2021). Sibilia et al. '(2021) reported that oxidative stress can directly activate the NF-κB pathway in osteoclast precursors, accelerating their differentiation into mature osteoclasts without requiring substantial increases in RANKL. Additionally, ROS may increase the expression of Macrophage Colony-Stimulating Factor (M-CSF), a growth factor essential for osteoclast precursor proliferation and survival.

Third, inflammation triggered by microplastic exposure contributes to increased osteoclastogenesis. Pro-inflammatory cytokines such as Tumor

Necrosis Factor-alpha (TNF-α), Interleukin-1 beta (IL-1β), and Interleukin-6 (IL-6) are known to enhance osteoclast formation through multiple mechanisms: increasing RANKL expression in bone marrow stromal cells, enhancing osteoclast precursor sensitivity to RANKL, and prolonging mature osteoclast survival by inhibiting apoptosis. Pan et al. (2023) reported significant increases in IL-1β and TNF-α expression in the femoral bone of rats exposed to polystyrene microplastics, correlating with increased osteoclast numbers(Pan et al., 2023).

Fourth, microplastics may disrupt hormonal balance, particularly estrogen, which exerts protective effects on bone by inhibiting osteoclastogenesis. Chemical substances contained in microplastics, such as Bisphenol A (BPA) and phthalates, are known endocrine-disrupting chemicals (EDCs) that interfere with steroid hormone function "(Supit et al., 2022; Zhao & You, 2024). Zhang et al. (2024) explained that microplastics can accumulate in the hypothalamus and disrupt reproductive hormone production, which in turn may affect bone homeostasis. Estrogen deficiency or impaired estrogen signaling increases pro-inflammatory cytokine and RANKL production while reducing OPG production, shifting the balance toward increased bone resorption.

The consistent response pattern observed across all treatment groups (X1, X2, and X3) indicates that the effects of microplastics on osteoclast number are not linearly dose-dependent. Although group X1, which received the lowest dose (1.25 mg/day), exhibited the highest increase (24.00 ± 1.915), groups X2 and X3 also showed significant and nearly equivalent increases (20.83 ± 5.307 and 20.83 ± 4.309, respectively). This phenomenon may be explained by a threshold effect, in which microplastic exposure at a certain dose is sufficient to maximally activate osteoclastogenic pathways, such that further dose increases do not produce proportional responses.

At higher doses, compensatory or adaptive mechanisms may attenuate stimulatory effects on osteoclastogenesis. For instance, excessive oxidative stress at high doses may induce apoptosis not only in osteocytes and osteoblasts but also in osteoclast precursors or mature osteoclasts themselves, thereby limiting the observable increase in osteoclast numbers. The effects of microplastics on the musculoskeletal system often exhibit complex and non-linear dose–response relationships (Fusagawa et al., 2025).

The observed increase in osteoclast numbers has significant clinical implications. Excessive osteoclast activity enhances bone resorption, resulting in the formation of more resorption cavities (Howship's lacunae) on bone surfaces. If this increased resorption is not counter-balanced by equivalent bone formation by osteoblasts, net bone loss will occur. Over time, this process may lead to decreased bone mineral density (BMD), deterioration of bone microarchitecture, and an increased risk of osteoporotic fractures (Anasulfalah et al., 2023; Clynes et al., 2020).

CONCLUSION

The findings of this study demonstrate that oral administration of microplastics at all tested doses significantly increased the numbers of osteocytes and osteoclasts. However, no significant increase or decrease was observed in the number of osteoblasts in the cortical bone tissue of the femur in male Rattus norvegicus Wistar strain rats. These findings contribute to a broader understanding of bone health disorders, which commonly affect populations over 50 years of age.

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