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The Effect of LDPE Microplastics in the Blood on Lyedig Cell Damage, Sertoli Cell Damage, and Sperm Count in the Male Wistar

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Keywords

Microplastics; LDPE; Leydig cells; Sertoli cells; sperm count

Abstract

Low-density polyethylene (LDPE) microplastics are increasingly detected in the environment and can enter the human body through ingestion or inhalation. Microplastic exposure is associated with oxidative stress, inflammation, and cellular injury that may impair male reproductive health. Leydig and Sertoli cells play essential roles in spermatogenesis; therefore, damage to these cells can reduce sperm production and quality. However, evidence regarding the effects of LDPE microplastics on testicular morphology and spermatogenesis is still limited. This research aims to determine the effects of oral LDPE microplastic exposure on Leydig cell injury, Sertoli cell injury, and sperm count in male Wistar rats. This true experimental study used a post-test only control group design. Rats were divided into control and LDPE-treated groups. Testicular histopathology was evaluated, and data were analyzed using the Kruskal–Wallis test, Levene's test, and Spearman correlation. LDPE exposure caused degenerative changes in Leydig and Sertoli cells and significantly reduced sperm count ($p < 0.001$). Oral LDPE microplastic exposure induces testicular cell damage and decreases sperm count, indicating potential reproductive toxicity.

INTRODUCTION

Male infertility is defined as the inability of sperm to fertilize an ovum despite engaging in regular unprotected sexual intercourse for at least one year (Leslie et al., 2025). The prevalence of infertility in Indonesia is currently estimated to reach 12–15% of approximately 40 million reproductive-age couples experiencing fertility problems (Riskesdas, 2018). The testes are male reproductive glands located within the scrotum that play a crucial role in producing sperm and testosterone (Tiwana & Leslie, 2023). Male infertility may arise from several factors, including age, genetics, systemic diseases, disorders of sperm transport, abnormal testicular function, and other unidentified etiologies. Understanding these etiological factors is essential in reducing the incidence of infertility. Several studies have indicated that microplastics may contribute as a potential trigger of male infertility (Leslie et al., 2025). The widespread use of plastics in Indonesia is nearly universal due to their availability, affordability, and practicality in daily life. However, public awareness regarding the potential hazards of plastic exposure remains limited. Consequently, plastic production continues to increase in line with population growth. According to data from the National Waste Management Information System (SIPSN) in 2024, Indonesia generated approxi-mately 34.2 million tons of waste, of which 63.3% was managed, while the remaining 35.67% remained unmanaged (KLHK, 2024).

Microplastics are plastic fragments or particles smaller than 5 mm. Due to their minute size, humans are particularly susceptible to microplastic exposure, especially through ingestion. Microplastics can enter the human body through contaminated food and beverages

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(Jin et al., 2021). Once ingested, microplastics travel through the gastrointestinal tract and pass into the bloodstream and lymphatic system by crossing the intestinal villi, assisted by M cells in the Peyer's patches. After entering systemic circulation, microplastics may distribute to various organs and induce several biological responses, including inflammation, oxidative stress, and cytotoxicity (Rahman et al., 2021).

Microplastics have been shown to reduce antioxidant activity, leading to excessive production of Reactive Oxygen Species (ROS). Imbalances between ROS production and antioxidant defenses can result in sustained oxidative stress and inflammation, ultimately causing cellular injury. This cellular damage can occur in multiple organs, including the testes, contributing to reductions in Sertoli cell and Leydig cell populations (DAngelo & Meccariello, 2021).

Chronic microplastic exposure has also been reported to decrease levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone, all of which are essential for proper spermatogenesis. Each hormone has a specific function: FSH supports Sertoli cell proliferation, while LH stimulates Leydig cells to produce testosterone. FSH and testosterone work synergistically to promote spermatid maturation and the release of mature spermatozoa (DAngelo & Meccariello, 2021). Microplastics can disrupt the function of the Hypothalamic – Pituitary – Gonadal Axis (HPGA), thereby reducing the levels of these hormones. Hormonal disruption contributes to decreased Sertoli and Leydig cell populations, reduced sperm count, and abnormalities in sperm morphology (Ijaz et al., 2022).

This study aims to analyze the effects of oral LDPE microplastic exposure on Leydig cell damage, Sertoli cell damage, and sperm count in male Wistar rats. The findings are expected to serve as a scientific warning that microplastic exposure may adversely affect human health, particularly reproductive function.

RESEARCH METHOD

This study was a quantitative experimental investigation employing a *post-test only control group design* to evaluate the effects of oral exposure to Low-Density Polyethylene (LDPE) microplastics on Leydig cell damage, Sertoli cell damage, and sperm count in male Wistar rats (*Rattus norvegicus*). The animals used were healthy, mature male rats aged approximately 4–8 weeks and weighing 150–200 grams(Sudakov et al., 2021). Prior to treatment, the rats underwent an acclimatization period of eight days in standard laboratory cages maintained at a temperature of 22–25°C, with food and water provided *ad libitum (Mutiarahmi et al., 2021).*

LDPE microplastics were derived from degraded low-density polyethylene pipes, which were processed into micro-sized fragments and subsequently suspended in sterile distilled water (Lovina et al., 2024). The suspension was administered orally using an orogastric sonde according to predeter-mined doses and exposure duration. The rats were divided into three groups: a control group without microplastic exposure, and two treatment groups receiving LDPE microplastics at doses of 1.25 mg and 2.5 mg, respectively. Microplastic exposure was administered daily for 28 days.

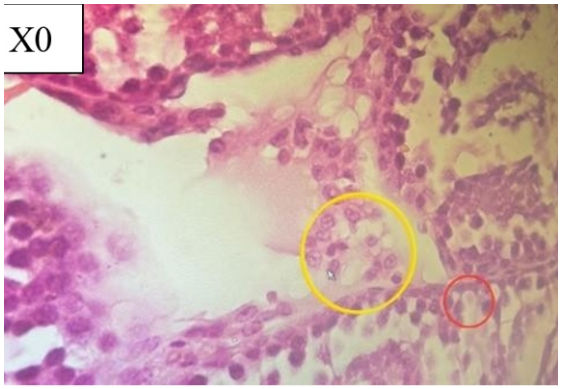
At the end of the exposure period, the animals were euthanized, and testicular tissues wereharvested for histological examination. Hematoxylin – Eosin (H&E) staining was performed to evaluate the morphological integrity of Leydig cells, Sertoli cells, and sperm count. Histopathological assessments included indicators such as cytoplasmic vacuolization, nuclear pyknosis, degenerative alterations, and disorganization of the seminiferous epithelium. Data were tested for normality and homogeneity, followed by statistical analysis using the Kruskal–Wallis test, Levene's test, and Spearman correlation. All experimental procedures received ethical approval and adhered to established principles of laboratory animal welfare.

Table 1
Testicular Histopathology and Sperm Count Results

Variable	Control Group	Experiment Group	
		X1	X2
	(Mean ± SD)		
Leydig Cell Damage	4.19 ±0.24	4.65±0.31	4.34±0.29
Sertoli Cell Damage	5.28±0.47	7.12±0.54	8.27±0.52
Sperm Count	9.31±1.77	6.93± 0.77	6.48±1.17

Source: Processed Primary Data, 2025

Based on Table 1, histological evaluation of testicular tissue demonstrated varying degrees of Leydig cell damage, Sertoli cell damage, and differences in sperm count among the groups. The mean Leydig cell damage score in the control group was 4.19 ± 0.24, while the X1 and X2 treatment groups showed increased scores of 4.65 ± 0.31 and 4.34 ± 0.29, respectively. This pattern suggests a dose-related trend toward increased Leydig cell injury following microplastic exposure. Sertoli cell damage exhibited a similar pattern. The control group showed a mean score of 5.28 ± 0.47, whereas the X1 and X2 groups displayed markedly higher scores of 7.12 ± 0.54 and 8.27 ± 0.52, with the highest degree of damage observed in group X2. Conversely, sperm count declined in both treatment groups. The control group had a mean sperm count of 9.31 ± 1.77, while X1 and X2 groups demonstrated reductions to 6.93 ± 0.77 and 6.48 ± 1.17, respectively. The most pronounced decrease occurred in the X2 group, indicating that microplastic exposure contributes to compromised spermatogenesis.



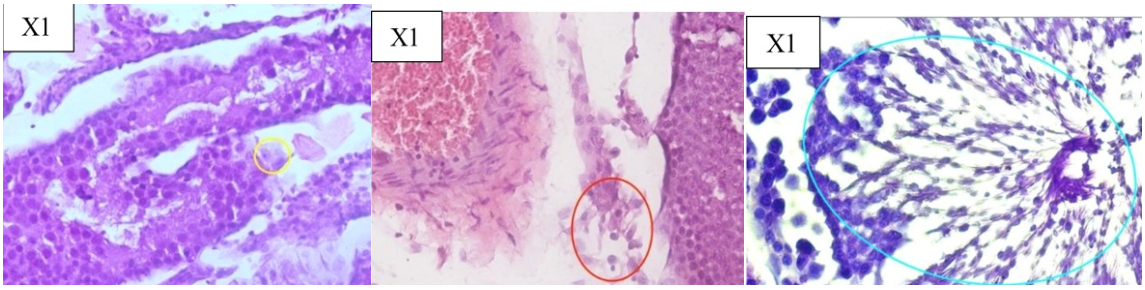
Source: Processed Primary Data, (2025)
Figure 1
Leydig Cell Damage, Sertoli Cell Damage, and Sperm Count Group X0

In the control group (X0) , testicular tissue showed normal morphology. Sertoli cells appeared intact with well-defined oval nuclei (yellow circle) (França et al., 2016)and Leydig cells (red circle) were adequate in number without evidence of vacuolization or degeneration (Aladamat & Prasanna, 2022). Sperm density within the lumen (blue circle) was abundant indicating physiologically normal spermatogenesis(Wald et al., 2021).

In group X1, reduced sperm density within the lumen (blue circle) was observed, accompanied by mild vacuolization in some Sertoli cells (yellow circle). Leydig cells (red circle) were still present but showed signs of early damage, including decreased cellularity and pyknotic nuclei, indicating the onset of pathological changes due to low-dose microplastic exposure(Liu et al., 2024).

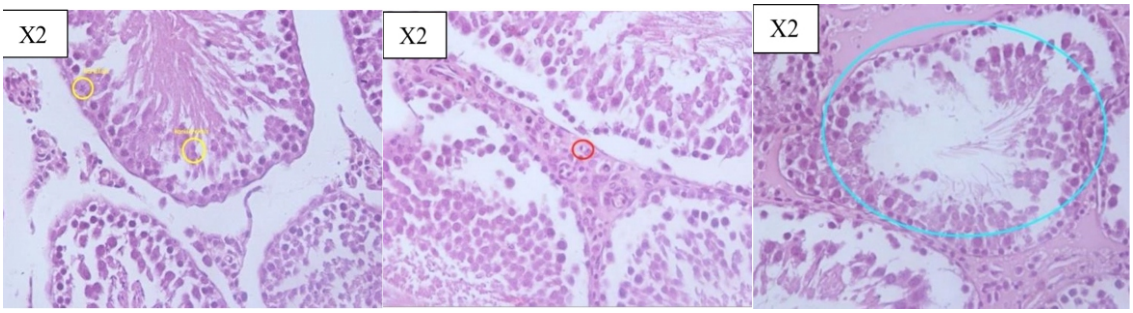
Group X2 displayed more extensive histopathological alterations. Sertoli cells exhibited karyorrhexis and karyolysis (yellow circle), consistent with nuclear degeneration (Li et al., 2021). Leydig cells showed nuclear pyknosis and cytoplasmic vacuolization (red circle), indicating significant interstitial cell damage. The lumen of the seminiferous tubules contained markedly reduced spermatozoa (blue circle), confirming impaired spermatogenesis at higher LDPE doses.

The Spearman correlation analysis indicated no significant relationship between microplastic concentration in blood and Leydig cell damage ($r = 0.154$; $p = 0.518$). In contrast, a significant positive correlation was found between microplastic levels and Sertoli cell damage ($r = 0.813$; $p < 0.001$). For sperm count, microplastic levels demonstrated a



Source: Processed Primary Data, (2025)

Figure 2
Leydig Cell Damage, Sertoli Cell Damage, and Sperm Count Group X1



Source: Processed Primary Data, (2025)

Figure 3
Leydig Cell Damage, Sertoli Cell Damage, and Sperm Count Group X2

significant correlation ($r = 0.751$; $p < 0.001$), consistent with the findings of decreased sperm count in exposed groups.

Exposure to LDPE microplastics for 28 days was shown to increase Leydig cell and Sertoli cell damage and to reduce sperm count in male Wistar rats. Significant Leydig cell injury, characterized by cytoplasmic vacuolization and pyknotic nuclei, indicates disrupted steroidogenesis, which may subsequently decrease testosterone production (Deng et al., 2017). Because testosterone plays a central role in regulating spermatogenesis, impaired Leydig cell function directly contributes to the decline in sperm count observed in the treatment groups.

Sertoli cell damage was markedly characterized by nuclear alterations, including karyorrhexis and karyolysis. Although the increase in Sertoli cell damage was not statistically significant in some analyses, the observed structural deterioration indicates compromised support for germ cells. As Sertoli cells form a key component of the blood–testis barrier, their degeneration weakens the barrier's protective function and disrupts the microenvironment required for

Tabel 2
Spearman Correlation Between Microplastic Levels in Blood and Histological Parameters

Variable	Analysis Test	p	R
Amount of microplastics in the blood & Leydig Cell Damage	Spearman Test	0,518	0,154
Amount of microplastics in the blood & Sertoli Cell Damage		<0,001	0,813
Amount of microplastics in the blood & Sperm Count		<0,001	0,751

Source: Processed Primary Data, 2025

optimal spermatogenesis (Bardaweel et al., 2018; Hu & Palić, 2020).

The reduction in sperm count across all treatment groups reflects a consistent physiological impact of LDPE microplastic exposure. The primary mechanism responsible for this decline is likely the increase in oxidative stress and inflammatory responses. Excessive production of reactive oxygen species (ROS) can damage DNA, cellular membranes, and organelles within spermatogenic cells, ultimately impairing sperm quantity and quality (Bardaweel et al., 2018).

Overall, these findings demonstrate that microplastics are capable of reaching reproductive tissues and inducing cellular injury within the testes (Gao et al., 2023). The results underscore the potential for LDPE microplastics to exert harmful effects on male reproductive health primarily through oxidative stress, inflammation, and disruption of testicular cell function (Araújo et al., 2025).

CONCLUSION

Based on the data analysis and discussion of this study, it can be concluded that microplastic particles were detected in the blood of male Wistar rats following oral administration of LDPE. Oral exposure to LDPE microplastics caused histopathological damage to testicular cells, specifically resulting in damage to both Leydig cells and Sertoli cells in male Wistar rats. In addition, LDPE microplastic exposure led to a decrease in sperm count. Further analysis showed that the number of microplastic particles present in the blood was not significantly associated with Leydig cell damage; however, a significant association was observed between blood microplastic levels and Sertoli cell damage as well as sperm count in male Wistar rats exposed orally to LDPE microplastics.

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