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RESEARCH **Open Access**

The Effect of Sub-Acute Inhalation Exposure to Polyethylene and Polyvinyl Chloride Micro-Nano Plastics on the Superoxide Dismutase (SOD) Level and Malondialdehyde (MDA) Level in Rat Ovary

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Abstract
Plastic is a synthetic or semi-synthetic organic polymer that is widely used in daily life and in industrial production. Microplastics are widespread contaminants and can enter the human body through the consumption of foods containing microplastics, inhalation of microplastics in the air, and skin contact with microplastic particles present in products. Microplastics can enter the ovaries as foreign bodies and can cause inflammation, oxidative stress, and even ovarian granulosa cell death. Polyethylene plastic shards generally have a higher ability to absorb environmental toxins than other types of plastic. Polyvinyl chloride (PVC) is one of the oldest thermoplastic polymers that is often used as water pipes. PVC has carcinogenic monomers and some harmful additives. This study aims to determine the effect of subacute exposure to micro-nanoplastics per inhalation on SOD and MDA levels in rat ovaries. The research method used is a true experimental design with a Randomize Post Test Only Group Design research design. This study used the ovarian organs of female white rats that had been exposed to PVC and PE for 28 days. The number of samples used in this study amounted to 18 female rats. Subacute exposure to micro-nanoplastics per inhalation can lower SOD levels and significantly increase MDA levels in rat ovaries. This study is expected to provide knowledge and an overview for future research on the mechanism of toxicity of micro-nanoplastic exposure that has an impact on female infertility through free radicals in the ovaries.

Keywords: Polyethylene, Polyvinyl Chloride, Oxidative Stress, Rat Ovary.

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1. INTRODUCTION

Infertility is a condition in which a couple has difficulty conceiving after having sex without using protection or contraception for a period of 12 months (WHO, 2023). In Indonesia, around 15% or as many as 4-6 million couples out of a total of 39.8 million couples of childbearing age experience infertility problems (Safitriana, 2022). Infertility in women was most commonly identified as being caused by ovulation disorders (25%), endometriosis (15%), pelvic attachment (12%), tubal obstruction (11%), other tube/uterine abnormalities (11%), hyperprolactinemia (7%) (Walker dan Tobler, 2022). Long-term exposure to a variety of endocrine disrupting chemicals in the form of industrial products, *pIasticizers* is associated with decreased fertility by disrupting various hormonal pathways (Bala, Singh and Rajender, 2021). Previous research has shown that reduced or impaired female reproductive function is caused by exposure to microplastics (Yang et al. 2023; Zhou et al. 2023). Continuous and prolonged exposure to microplastics can cause greater exposure to the toxic properties of plastics and interfere with reproduction and cause infertility (Zientika, Amin, and Yoswaty 2021).

Plastics are synthetic or semi-synthetic organic polymers that are widely used in daily life and in industrial production (Cheng et al. 2020). The total plastic waste in Indonesia reaches 3.22 million metric tons per year (Prokić et al. 2019). Plastic pollution has caused widespread concern in the world, especially microplastics (Z. Liu et al. 2022). Microplastics are widespread contaminants and can enter the human body through the consumption of microplastic-containing foods, inhalation of microplastics in the air, and skin contact with microplastic particles present in products (Prata et al. 2020). Microplastics can enter the ovaries as foreign bodies and can cause inflammation, oxidative stress, and even ovarian granulosa cell death (An et al. 2021). Research Wang et al. 2019, found that marine medaka (*Oryzias melastigma*) exposed to polystyrene may cause an increase in *Malondialdehyde* (MDA), a decrease in *superoxide dismutase* (SOD), *catalase* (CAT), *glutathione S-transferase* (GST) and *glutathione peroxidase* (GSH-PX) in the ovaries. This is related to the lack or disability of women's reproductive ability. The study also found that polystyrene can enter the ovarian in rat. In addition, exposure to polyvinyl chloride microplastics in *Daphnia magna* can affect reproductive parameters, such as reducing the number of offspring in the first brood, and interfering with the activity of enzymes such as SOD and CAT (Y. Liu et al. 2022).

Micro-nanoplastics can be classified into several types based on the polymers that make them up such as polyethylene (PE) and polyvinyl chloride (PVC) (Mardiyana and Kristiningsih 2020). PE and PVC are the most widely produced plastics and are potentially hazardous to health (Plastics Europe, 2019). Polyethylene plastic shards generally have a higher ability to absorb environmental toxins than other types of plastic (Prokić et al. 2019). Exposure to polyethylene that occurs continuously for a long time and concentrations that exceed the threshold or overuse can cause adverse side effects because polyethylene can release antimony trioxide compounds that are harmful if they enter the body because they can cause disruption of the reproductive system in women (Dhaka et al. 2022). Polyvinyl chloride (PVC) is one of the oldest thermoplastic polymers that is often used as water pipes. This type of plastic can degrade at relatively low temperatures when exposed to light, releasing hydrogen chloride. PVC has high chemical resistance, as well as resistance to water and various weather conditions (Trivantira, Fitriyah and Ahmad, 2023). PVC has carcinogenic monomers and some harmful additives (Blackburn and Green, 2022). Phthalate and bisphenol A (BPA) are the main raw materials used for PVC synthesis. When BPA is released into the environment and ingested, it destroys amino acid metabolism, steroid metabolis, and energy metabolism pathways and induces oxidative stress (Yang et al. 2022).

The purpose of this study was to determine the effect of subacute exposure to PE and PVC micro-nanoplastics per inhalation on SOD levels and MDA levels in the ovaries of rats of the wistar strain (*Rattus norvegicus*).

2. RESEARCH METHOD

This study is a laboratory experimental research with *a Post Test Only Control Group Design* research design. This study used 18 female wistar rats, confined at a temperature of ±25°C, humidity 40-70%, and fed standard feed and clean water. The mice were divided into three groups consisting of 6 rats each: Group 1, control (not exposed to microplastics); Group 2, PE group (exposed to 15 mg/m3 of PE for 28 days); Group 3, PVC group (exposed to 15 mg/m3 of polyvinyl chloride (PVC) for 28 days). In this study, micro-nanoplastics were exposed per inhalation with an exposure dose based on OSHA *(Occupational Safety and Health Administration)* which is 15 mg/m3. The exposure method refers to the research of Cary et al (2023), namely *the whole body inhalation* method because the exposure is similar to the reality of exposure to microplastics to humans today.

After 28 days of exposure to microplastics, the animals were then dissected in the proestrus phase and the ovarian organs were isolated. Fresh ovarian organs were homogenized using *phosphate buffer saline* (PBS), then crushed using mortar until smooth and added with 1 ml of Tris KCl 1.17% pH 7.6 buffer. Then, the sample is centrifuged at 4°C, 4000 rpm, for 15 minutes. For every 500 µL of sample, 200 µL of EDTA 100 mM, 100 µL units of NBT 25, 100 µL of xanthin 25 mM, and 100 µL of xanthin oxidase 1 unit are added. The mixture is vortexed and incubated at 37°C for 30 minutes. Then, centrifuge using 4000 rpm for 5 minutes at 4°C and filter. Next, the aquabidest is added before reading using a spectrophotometer with λ580 nm.

This research was conducted at the Biochemistry Laboratory and Pharmacology Laboratory, Faculty of Medicine, Brawijaya University. This research was carried out with the approval of the Health Research Ethics Committee of the Health Research Ethics Committee, Faculty of Medicine, Brawijaya University, number 254/EC/KEPK/08/2023. The data of this study was analyzed by One Way Anova statistics using SPSS. The significance data was shown by $p<0.05$ and further analysis using the Tukey HSD post-hoc test.

3. RESULTS AND DISCUSSION

Microplastic exposure caused a decrease in SOD levels in the treatment group compared to the control group ($p < 0.05$). Tukey's HSD test showed that the PE treatment group showed a significant difference compared to the control group ($p < 0.05$), while there was no significant difference in the PVC group compared to the control ($p > 0.05$) and PVC with PE ($p > 0.05$).

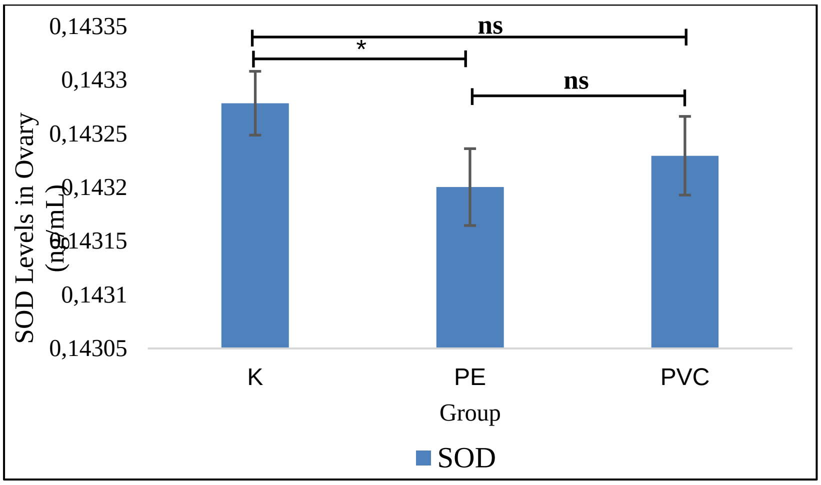
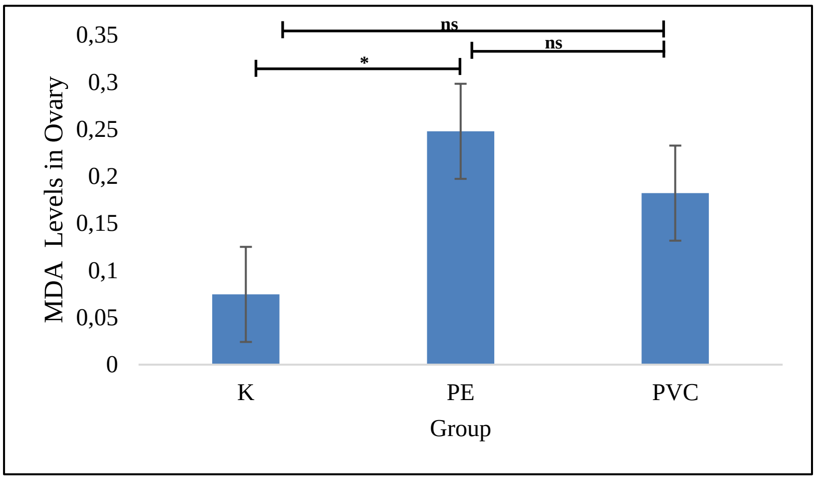


Figure 1. Graph of Average SOD Levels in Rat Ovary

Microplastic exposure caused an increase in MDA levels in the treatment group compared to the control group ($p < 0.05$). Tukey's HSD test showed that the PE treatment group showed a significant difference compared to the control group ($p < 0.05$), while there was no significant difference in the PVC group compared to the control ($p > 0.05$) and PVC with PE ($p > 0.05$).



Information: ns : *not significant*
(*) : p<0.05
K : Control
PE : Polyethylene
PVC: Polyvinyl chloride

Figure 2. Graph of Average MDA Levels in Rat Ovaries

DISCUSSION

Exposure of Micro-nanoplastics to SOD Levels in the Ovary. This study found that inhalation exposure to polyethylene (PE) and polyvinyl chloride (PVC) micro-nanoplastics at a dose of 15 mg/m³ for 28 days significantly reduced the levels of the antioxidant enzyme superoxide dismutase (SOD) compared to the control group. This result is supported by the research of Hou et al. (2021) and An et al. (2021), which showed a decrease in SOD enzyme activity and an increase in malondialdehyde (MDA) in rat ovaries due to exposure to microplastics. Previous research by Farag et al (2023) of exposure to polyethylene micro-plastics using various doses from low to high is 3.75 mg/kg, PE-MP, 15 mg/kg, and 60 mg/kg for 35 days, showing that there is a significant difference in the reduction of superoxide dismutase (SOD) activity at the highest dose (60mg/kg), while for the lowest dose there are no significant results.

Microplastics are known to have pro-oxidative properties, disrupt the balance of the oxidative-antioxidant system, and increase the production of reactive oxygen species (ROS), causing oxidative stress. This oxidative stress is characterized by an imbalance between oxidative and antioxidant defenses in the body, reducing antioxidant enzymes such as SOD causing mitochondrial dysfunction (Farag et al. 2023). Research conducted by (De Guzman, Chua, and Sedano (2020) on zebrafish causes embriottoxicity and teratogenicity to increase as the concentration of polyethylene is given. Continuous exposure to polyethylene for a long time and concentrations that exceed the threshold can cause adverse side effects because polyethylene can release antimony trioxide compounds that risk disrupting the reproductive system in women (Dhaka et al., 2022).

The results of a study from Ma et al. (2024) show that polyethylene and polyvinyl chloride micro-nanoplastics induce oxidative stress in liver cells triggered by increased intracellular ROS levels and cause negative impacts such as apoptosis, metabolic disorders and mitochondrial dysfunction. SOD activity has an antioxidant capacity that plays an important role in regulating biochemical reactions such as redox reactions and warding off free radicals in the human body (Zheng et al. 2023). The antioxidant system has been activated in the body to fight oxidative damage caused by excessive ROS production. When the impact caused by micro-nanoplastics exceeds the modulating capacity of antioxidant enzymes, an increase in ROS will lead to a decrease in SOD level activity. Increased ROS (*Reactive Oxygen Species*) in the ovaries can cause apoptosis in granulosa cells and cause follicular atresia in rat which can be a factor in infertility (An et al. 2021).

Exposure of Micro-nanoplastics to MDA Levels in the Ovary. The results of this study showed that subacute exposure to PE micro-nanoplastics per inhalation for 28 days resulted in a significant increase in MDA levels in the ovaries compared to the control group. This proves that exposure to PE micro-nanoplastics can increase ovarian MDA levels which is one of the markers of oxidative stress. Exposure to PVC micro-nanoplastics did not significantly increase ovarian MDA levels compared to the control group. Other studies using PE at higher doses (40 mg/kg/day) for 30 days can cause DNA damage, apoptosis, oxidative stress, and mitochondrial dysfunction in Kunming rat oocytes (Hong, Wu, and Wei 2023). Polyethylene consists of long chains formed by ethylene monomers. It is a stable polymer and an excellent electrical isolator characterized by high strength and flexibility (Baj et al. 2022).

In the study of Sincihu (2022) who used microplastic exposure to LDPE-type PE administered orally for 90 days, showed that the mean expression of MDA and 8-dihydro-2-deoxyguanosine (8-OHdG) metabolites was higher in the microplastic-exposed group. Hippocampal neurons of Wistar rats responded to microplastic by decreasing the synthesis of intracellular antioxidant enzymes. This was due to the consumption of Wistar rats which significantly increased the amount of particles in the blood. Statistically, the large number of microplastics in the blood resulted in lower expression of SOD enzyme. The low level of SOD expression causes membrane damage (seen from the expression of MDA metabolites) and deoxyribonucleic acid (seen from the expression of 8-OHdG metabolites) in hippocampal neurons. Oxidative stress occurs after antioxidants decrease and are no longer able to perform the reduction process. This is one of the body's biological responses to microplastic exposure due to high levels of free radicals. Another study by Cheng et al. (2020) using plastic mulsa film made from polyethylene as exposure to *Eisenia fetida* showed an increase in oxidative stress with a decrease in SOD and CAT gene expression and an increase in MDA and 8-OHdG metabolites. Likewise, the results of this study in rats, which showed a significant increase in ovarian MDA levels following exposure to PE-type microplastics.

In the research of An et al. (2021) who used polystyrene-type microplastics orally at a dose of 1.5 mg/kg/bb for 90 days, MDA levels increased while CAT, SOD, and GSH-PX levels decreased in the exposure group compared to the control group in rat ovaries. The level of ROS increased significantly in granulosa sel given exposure so these results indicate that polystyrene microplastics can induce oxidative stress in the ovaries. Likewise the research of Wang et al. (2019), exposure to polystyrene microplastics in sea bream (*Oryzias melastigma*) can cause an increase in MDA, a decrease in SOD, CAT, glutathione S-transferase (GST) and glutathione peroxidase (GSH-PX) in the ovaries.

Exposure to 4 mm PVC microplastics of 1 gram given to lotus (*Nelumbo nucifera*) after 7 days caused a significant increase in CAT activity. Exposure to PVC caused CAT activity in lotus seedlings to increase by 63% compared to exposure to PET and HDPE PE. GST activity also increased significantly by 82.6% with PVC and 50.3% with PET (Esterhuizen and Kim 2022). In this study, exposure to PVC microplastics increased MDA levels but not significantly.

Plastics are considered to be biochemically inert. In the environment, polymers tend to degrade through biotic and abiotic processes releasing harmful additives. These compounds can penetrate cell membranes and disrupt biochemical reactions resulting in toxic effects PET (Esterhuizen and Kim 2022). Microplastics can enter by inhalation. In the lungs, a very thin tissue barrier smaller than 1 µm separates the alveoli lumen from the bloodstream. Nano-sized particles have the potential to penetrate the blood vessel system and be distributed throughout the human body. In vitro studies have shown that nanoplastic particles are absorbed by the alveolar epithelium. Nanoplastic particles induce a stronger antioxidant response compared to microplastics. In addition to particle toxicity, micro-nanoplastics can also pose chemical and biologic risks. Plastic has many additives that enhance its properties. There are about 144 hazardous chemicals used as additives. These substances can be released from the plastic matrix inside the organism. The most widely investigated substances are bisphenol A (BPA), vinyl chloride (VC), and benzyl butyl phthalate (BBP) (Baj et al. 2022).

In this study showed a significant difference in MDA activity in rat ovary organs. Malondialdehyde (MDA) is an oxidant or free radical as the final result of lipid peroxide due to the breakage of fatty acid chains which become toxic compounds to cell. Lipid peroxides are formed due to excess reactive oxygen species (ROS) products that attack sel components (lipid membranes and proteins) by involving double fatty acid residues of phospholipids that are very sensitive to oxygen. The imbalance between oxidants in this case ROS and antioxidants will lead to oxidative stress. High MDA concentration indicates the oxidation process in the membrane (Triananda, Primadiamanti, and Angin 2023). Oxidative stress induction is one of the toxic mechanisms of microplastics. An imbalance between ROS products and the anti-oxidation system causes oxidative stress (An et al. 2021). Oxidative stress can induce apoptosis which is considered to be the main route of micro- and nanoplastic toxicity. The source of oxidative stress may come from the large surface area of plastic particles, metals deposited on their surface, and the induction of inflammatory responses. Plastic particles have various functional groups and chemical bonds (such as fenil groups, amino groups) that may be associated with oxidative stress (Baj et al. 2022).

4. CONCLUSION

Subacute exposure to micro-nanoplastics per inhalation can lower SOD levels and significantly increase MDA levels in rat ovaries. Exposure to micro-nanoplastics per inhalation of polyethylene (PE) type further lowered SOD levels and increased MDA levels compared to polyvinyl chloride, but the difference was not significant.

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