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RESEARCH **Open Access**

The Effect of Sub-Acute Inhalation Exposure to Polyethylene Micro-Nano Plastics on the Histopathological Features of the Mammary Glands in Female Wistar White Rats (Rattus Norvegicus)

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Abstract
The majority of household appliances are made of plastic derived from synthetic petroleum and the result of polymerization processes. One type of plastic is Polyethylene (PE). Polyethylene (PE) contains antimony trioxide compounds that are carcinogenic in the body if ingested in excessive amounts, triggering cancer and oxidative stress, which can be observed by measuring the levels of malondialdehyde (MDA) in the body. This study aims to determine the proliferation of abnormal cells in the lactiferous ducts and mammary gland acini as well as the increase in MDA levels. The research method used is a true experimental design with a Randomized Post Test Only Group Design. This study used the mammary organs and blood of female white rats that had been exposed to PE for 28 days. The number of samples used in this study was 12 female white rats. The results showed that there were significant differences in the histopathological features and MDA levels in the mammary glands. Based on the results of the Independent T-Test on the number of cell layers in the lactiferous ducts, acini, and the number of acini ($p<0.05$) and the Mann-Whitney test on MDA levels ($p<0.05$). There were significant changes in the histopathological features and MDA levels in the mammary glands exposed to Polyethylene (PE) plastic. Exposure to micro-nano plastics of PE type at a dose of 15 mg/m3 for 28 days differed significantly in the MDA levels of mammary glands, histopathological picture of mammary glands at cell proliferation events, both in the lactiferous ducts and acini cells. In addition, exposure to PE plastic has the potential to cause health problems in the breast organs if exposed for a long time and in excess doses.

Keywords: Polyethylene, Cell Mamae Proliferation, Breast Cancer.

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1. INTRODUCTION

Currently, the majority of household appliances are made from plastic, which is synthesized from petroleum and formed through polymerization processes (Anom & Lombok, 2020). Until now, plastic waste is still an unresolved problem, both in Indonesia and the world. Based on data from the Indonesian Ministry of Environment and Forestry in 2022, obtained from 171 districts/cities throughout Indonesia, the results of waste generation reached 20 million tons per year (Kementerian Lingkungan Hidup dan Kehutanan Republik Indonesia, 2022). Of the various types of waste generated, the largest type of waste comes from food waste as much as 40.6% and the second largest type of waste is plastic waste as much as 18.5% which is included in the category of non-renewable waste. Based on data obtained from Rudend, and Hermana, (2021), Indonesia is the second largest producer of plastic waste in the world with the amount of plastic waste reaching 85,000 tons per year. To deal with this, waste recycling is carried out, either used for crafts or used repeatedly, such as the use of plastic bottles.

Plastic comes in various types used as raw materials in the manufacture of household tools, one of which is Polyethylene (PE) (Sen & Raut, 2015). Polyethylene is a type of plastic composed of extended hydrocarbon chains originating from thermoplastic polymers and is divided into several types, commonly used ones being low-density polyethylene (LDPE) and high-density polyethylene (HDPE) (Sen & Raut, 2015). PE plastic is utilized in the production of plastic bags, disposable containers, bottles, packaging containers, and more (Ghatge et al. 2020). Polyethylene contains antimony trioxide, used as a catalyst in plastic production. Antimony trioxide can enter the body and is carcinogenic, potentially triggering cancer growth (Baharuddin, Asran & Ikhtiar, 2023; Tapiory et al., 2019).

Plastic particles can be categorized by diameter into microplastics (MP) and nano plastics (NP), with MPs measuring 1µm-5mm in diameter and NPs measuring less than 1µm (Shim & Thomposon, 2015). Airborne micro-nanoplastics are produced due to the degradation of larger plastics through UV rays. Once degraded, these particles can easily be blown into the atmosphere by air currents due to their small size and low density (Enyoh et al., 2019). There are three exposure routes for micro-nano plastics to enter the body, oral, respiratory (inhalation), and dermal exposure through cosmetic products (Chang et al., 2020; Vianello et al., 2019). Inhaled micro-nano plastics enter the respiratory system, are then ingested by macrophages, and transferred to the circulatory and lymphatic systems (Karlsson et al., 2017). Micro-nano plastics entering the blood induce free radicals (Campanale et al., 2020).

Cancer cells grow due to a failure to cease the proliferation process, which can be caused by exposure to chemicals that lead to an increase in Reactive Oxidative Species (ROS) (Ilmiawati et al., 2022). Elevated ROS can alter the expression of the p53 tumor suppressor gene, which is crucial for apoptosis. Therefore, oxidative stress can cause changes in gene expression, cell proliferation, and apoptosis, playing a significant role in the initiation, growth, and development of tumors (Jelic et al. 2021). The commonly used parameter to measure ROS in the body is Malondialdehyde (MDA) (Tirani & Haghjou, 2019). Increased ROS in the body will lead to genetic mutations in the nucleus, which can trigger breast cancer.

The entry of PE particles in the body can cause various kinds of adverse effects on the body, even direct exposure causes breast cancer in humans. Breast cancer remains the leading cause of cancer-related deaths in women worldwide. The Indonesian Ministry of Health (2022) reports that, based on data from the Global Burden of Cancer (GLOBOCAN) in 2020, the number of breast cancer cases reached 68,858 (16.6%) out of a total of 396,914 cancer cases in Indonesia, with more than 22,000 deaths (Kementerian Kesehatan Republik Indonesia, 2022). The exact cause of breast cancer is still not fully known. About 60% of all breast cancers are not due to family heredity, suggesting a possibility of breast cancer occurring due to exposure to chemicals and drugs in the body (Helm & Rudel, 2020). Research on the relationship between polyethylene microplastic exposure and breast cancer is still limited. However, some studies have found that exposure to microplastics can be at risk of triggering breast cancer. The purpose of this study is to determine the increase in MDA levels in the mammary organs and the occurrence of abnormal cell proliferation in the lactiferous ducts and mammary gland acini.

2. RESEARCH METHOD

This study is a true experimental research design with random sample selection using a Randomized Post Test Only Group Design. It involves comparing and analyzing the results obtained at the end of the study after the treatment (post-test) with a control group that did not receive the treatment. The samples used in this study were female Wistar rats (Rattus norvegicus) aged 12-15 weeks and weighing 150-200 grams, which had been exposed to Polyethylene (PE) micro-nano plastics at a concentration of 15 mg/m3 by inhalation for 28 days (Cary et al., 2023).In this study, micro-nano plastics 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 in humans today.

The study used 2 groups: 1 control group and 1 Polyethylene (PE) exposure group, each consisting of 6 rats, randomly selected, with a total of 12 rats required. The number of samples in this study refers to the Frommlet & Heinze, (2020) study, which explains that research using experimental animals with a sample size that is too small will not produce significant conclusions, but using a sample that is too large will cause more animals to suffer. This will be contrary to research ethics, namely the 3R (Replacement, Reduction, Refinement). The study showed that the number of repetitions for each treatment group between (3,4,6,8) had almost the same effectiveness and was not much different. The research samples observed were mammary organ preparations and MDA levels in the mammary organs.

The study was conducted from December 2023 to January 2024. The research was carried out at the Faculty of Medicine, Universitas Brawijaya, specifically in the Pharmacology Laboratory, Biochemistry-Biomedicine Laboratory, and Anatomical Pathology Laboratory of FK UB. The data were statistically analyzed with the help of SPSS 27.0 with a level of significance 0,05 ($p<0,05$). Data analysis employed the parametric Unpaired Independent T-Test and the Mann-Whitney test. This study received ethical approval from the ethics committee of Universitas Brawijaya with the number: 254/EC/KEPK/08/2023.

3. RESULTS AND DISCUSSION

MDA Levels in Mammary Glands

Measurement of MDA levels in the mammary glands of female white rats was conducted to determine the concentration of MDA (nmol/mL) in each group. In the control group, the average MDA level was 57.6 ± 12 (nmol/mL). Meanwhile, in the treatment group, the average MDA level in the mammary organs was 92 ± 43 (nmol/mL). From these results, it can be concluded that the PE (plastic exposure) group has MDA levels 1.6 times higher than the control group, as presented in the graph in Figure 1.

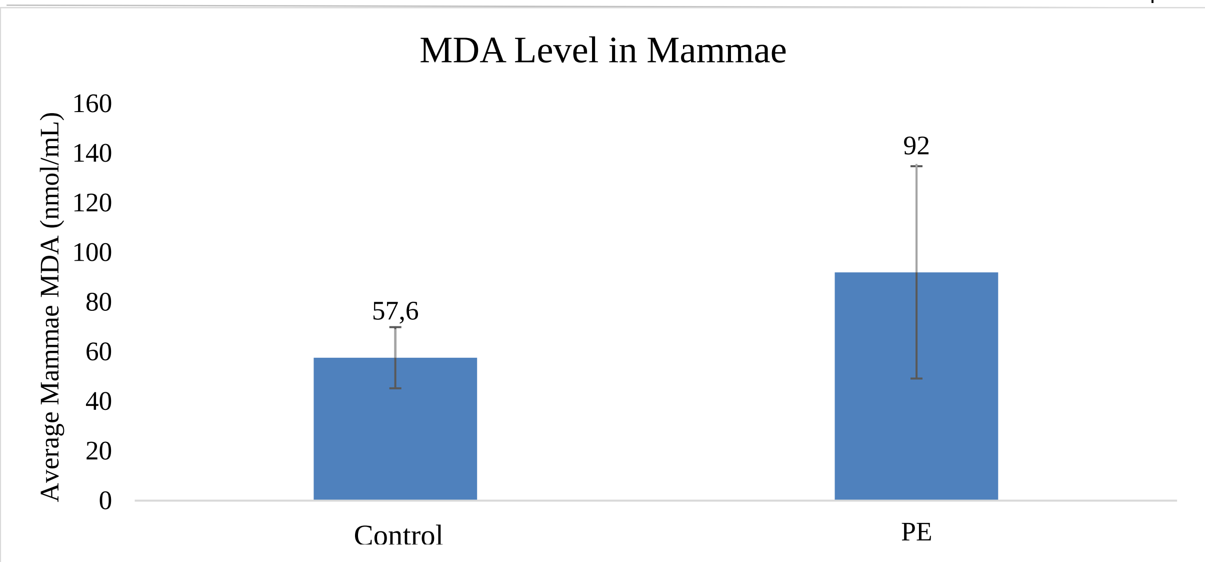
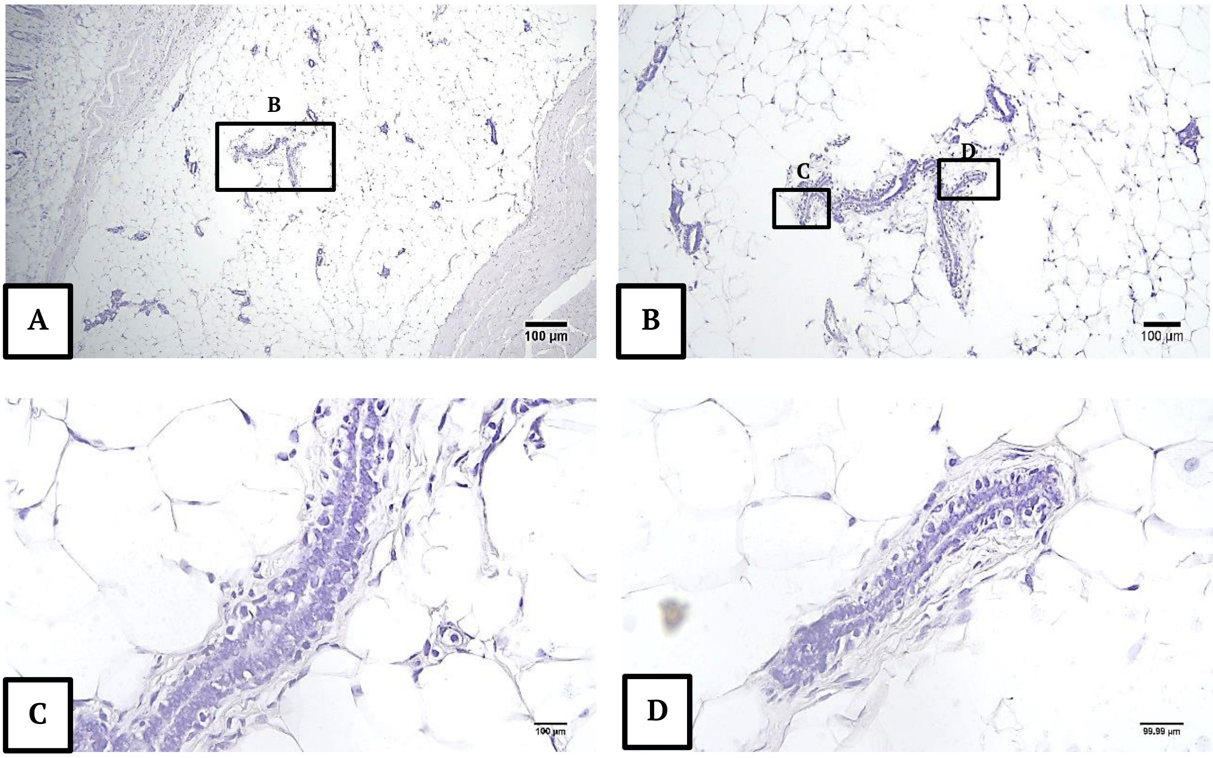


Figure 1. Graph of Average MDA Levels in Mammary Glands of Female White Rats

Observation of Lactiferous Ducts

Observation of epithelial cell proliferation in lactiferous ducts in histopathological images was conducted using an Olympus CX23 microscope at magnifications of 40x, 100x, and 400x. In each field of view, the number of layers of epithelial cells surrounding the lactiferous ducts was counted. The counted epithelial cells were the number of cuboidal epithelial cells from the inner surface to the edge of the lactiferous ducts. Histopathological images were taken in the lactiferous duct area in 10 different fields of view for each group, as presented in Figure 2.

Control Group



PE Group

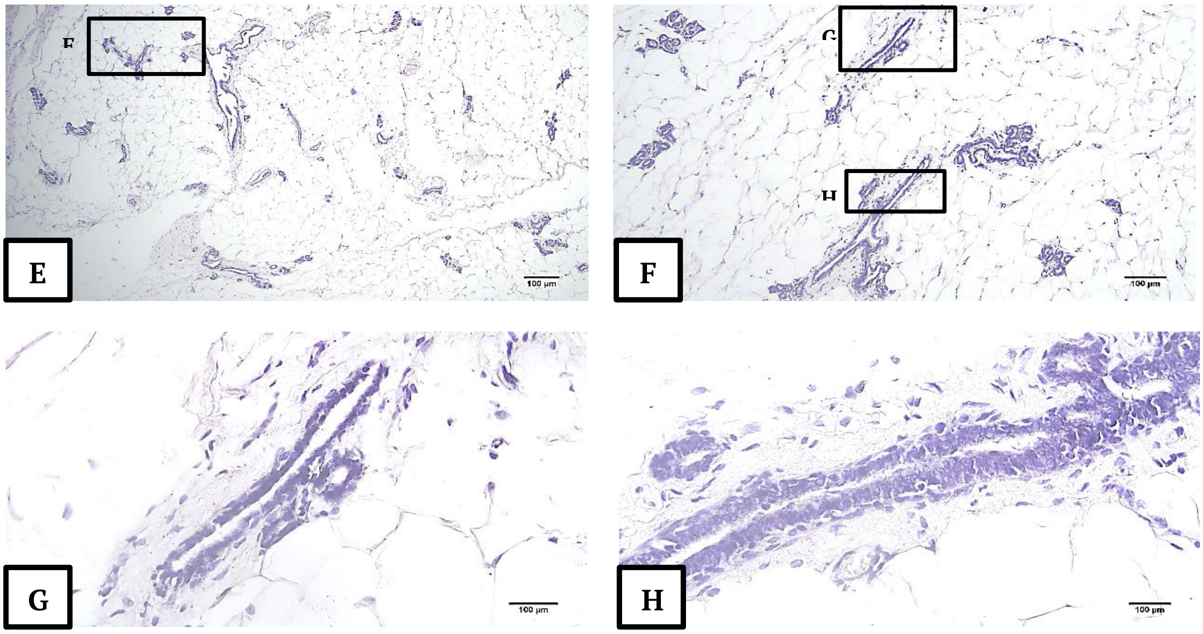


Figure 2. Histopathological Images of Lactiferous Ducts in Mammary Glands with HE Staining in the Control Group (A-D) at magnifications of 40x (A), 100x (B), 400x (C and D), and Treatment Group PE (E-H) at magnifications of 40x (E), 100x (F), 400x (G and H), scale bar: 100 µm.

Average Number of Lactiferous Ducts

Microscopic examination of the mammary glands of female white rats reveals several parts, namely lactiferous ducts and acinar cells. Each sample of mammary tissue from female white rats has a variable number of ducts. The observation of duct numbers in each sample of mammary tissue from female white rats was conducted in 10 fields of view for each sample, and the total number, as well as the average number of ducts for each group, were counted and presented in graphical form in Figure 3 below.

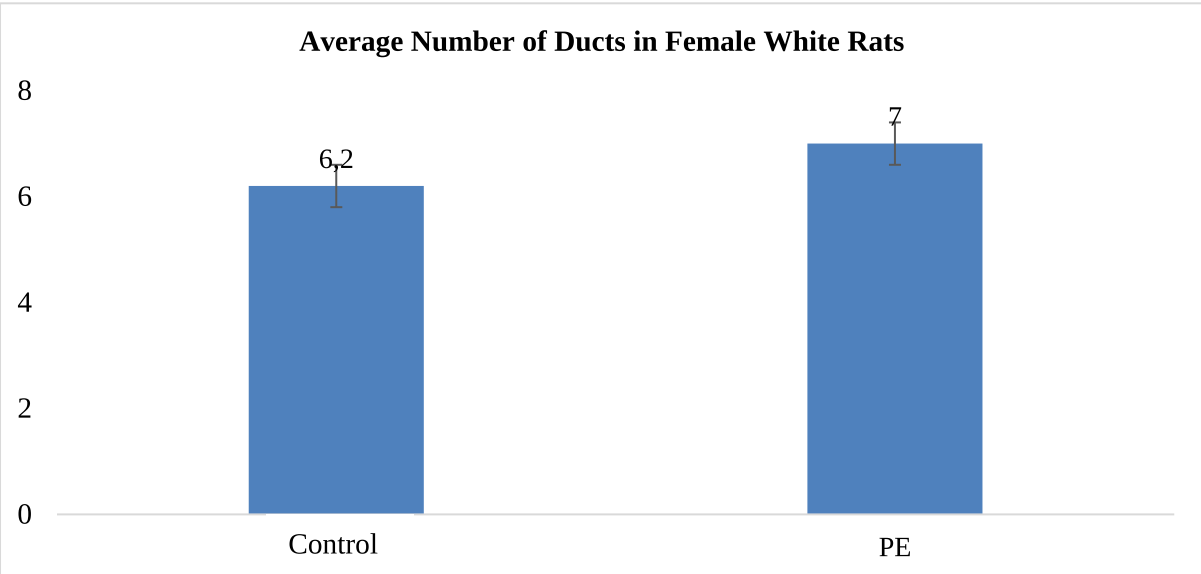


Figure 3. Graph of Average Number of Ducts in Female White Rats

Upon calculation, it was found that in the control group, the average number of ducts was 6 ± 3.42, while in the PE (plastic exposure) group, the average number of ducts was 7 ± 2.09. It can be concluded that the total average duct count in the control group and the treatment group did not differ significantly. The normality test results for the number of ducts showed a result of 0.464, indicating a normal data distribution. The Independent T-test resulted in a significance value of 0.644 (t > 0.05), hence it can be concluded that exposure to micro-nano plastics did not differ significantly between the two groups.

Number of Layers of Cuboidal Epithelial Cells in Lactiferous Ducts

Microscopic examination of the mammary glands of female white rats in the control group showed normal lactiferous ducts, which were surrounded by an average of 1 layer of slightly elongated square-shaped cuboidal epithelial cells, surrounded by normal connective tissue and fat tissue, with fine chromatin nuclei, and no inflammatory cells were found. In the treatment group, the lactiferous ducts were surrounded by an average of 2 layers of cuboidal epithelial cells that had changed shape to become round, appeared thicker, with coarse chromatin nuclei, and no inflammatory cells were found. Histopathological images of lactiferous ducts in mammary glands for each group are presented in Figure 4.

Control **PE**

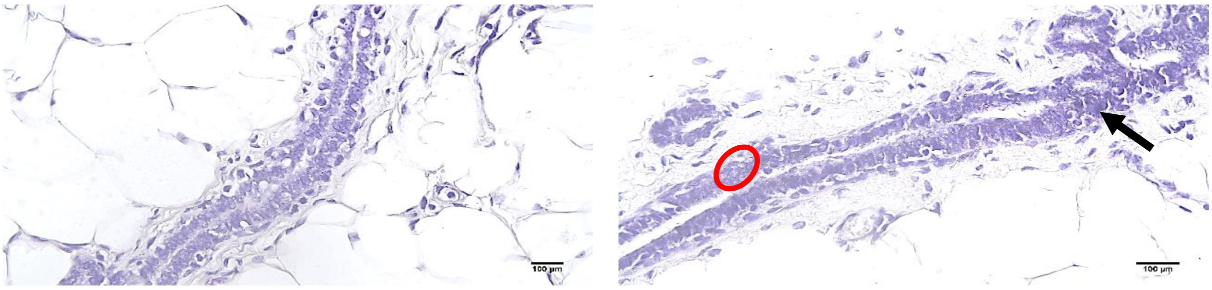


Figure 4. Comparison of Histopathological Images of Lactiferous Ducts in the Control Group and PE Group, red circles indicate the number of layers of cuboidal epithelial cells, which are 1 layer and round in shape, arrows indicate coarse chromatin nuclei, scale bar: 100 µm.

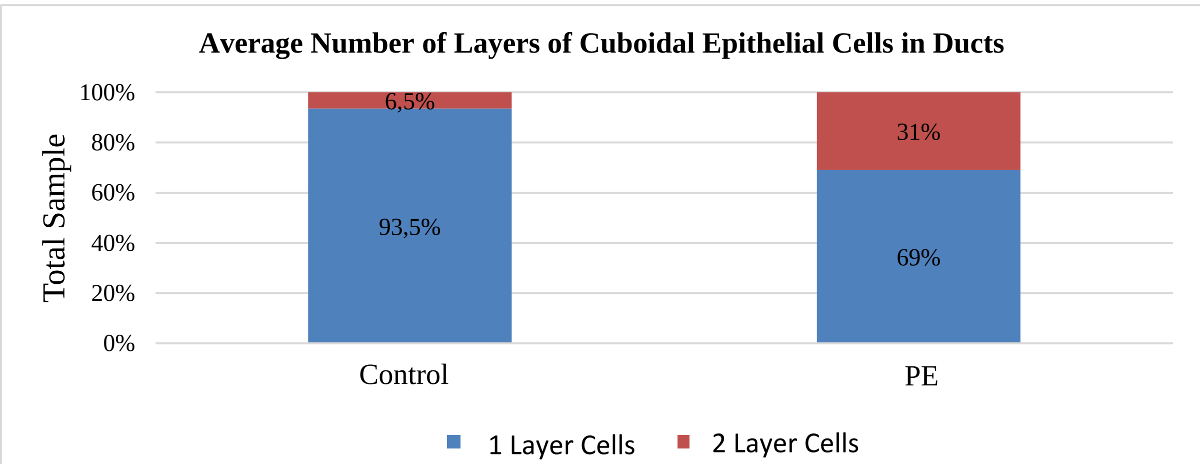


Figure 5. Graph of Average Number of Layers of Cuboidal Epithelial Cells in Ducts

Figure 5 shows the total number of layers of ductal epithelial cells counted per sample with 10 fields of view. It can be observed that in the 2-layer sample, the treatment group has a higher percentage (31%) compared to the control group (6.5%), indicating that the PE group experienced a 4.7-fold increase in proliferation compared to the control group.

Data analysis was performed on the 2-layer ductal epithelial cell samples in the control group and PE group. The normality test results showed a result of 0.02, indicating that the data distribution was not normal. The Mann Whitney test resulted in a significance value of 0.013 ($t < 0.05$), concluding that exposure to micro-nano plastics significantly differed between the two groups.

Observation of Acinar Cells

Observation of epithelial cell proliferation surrounding the acini in histopathological images was conducted using an Olympus CX23 microscope at magnifications of 40x, 100x, and 400x. Histopathological images were taken in the acini area in 10 different fields of view, as presented in Figure 6. In each field of view, the number of layers of secretory epithelial cells surrounding the mammary acini was counted. The counted epithelial cells were the number of cuboidal epithelial cells from the inner surface to the outer surface of the mammary acini.

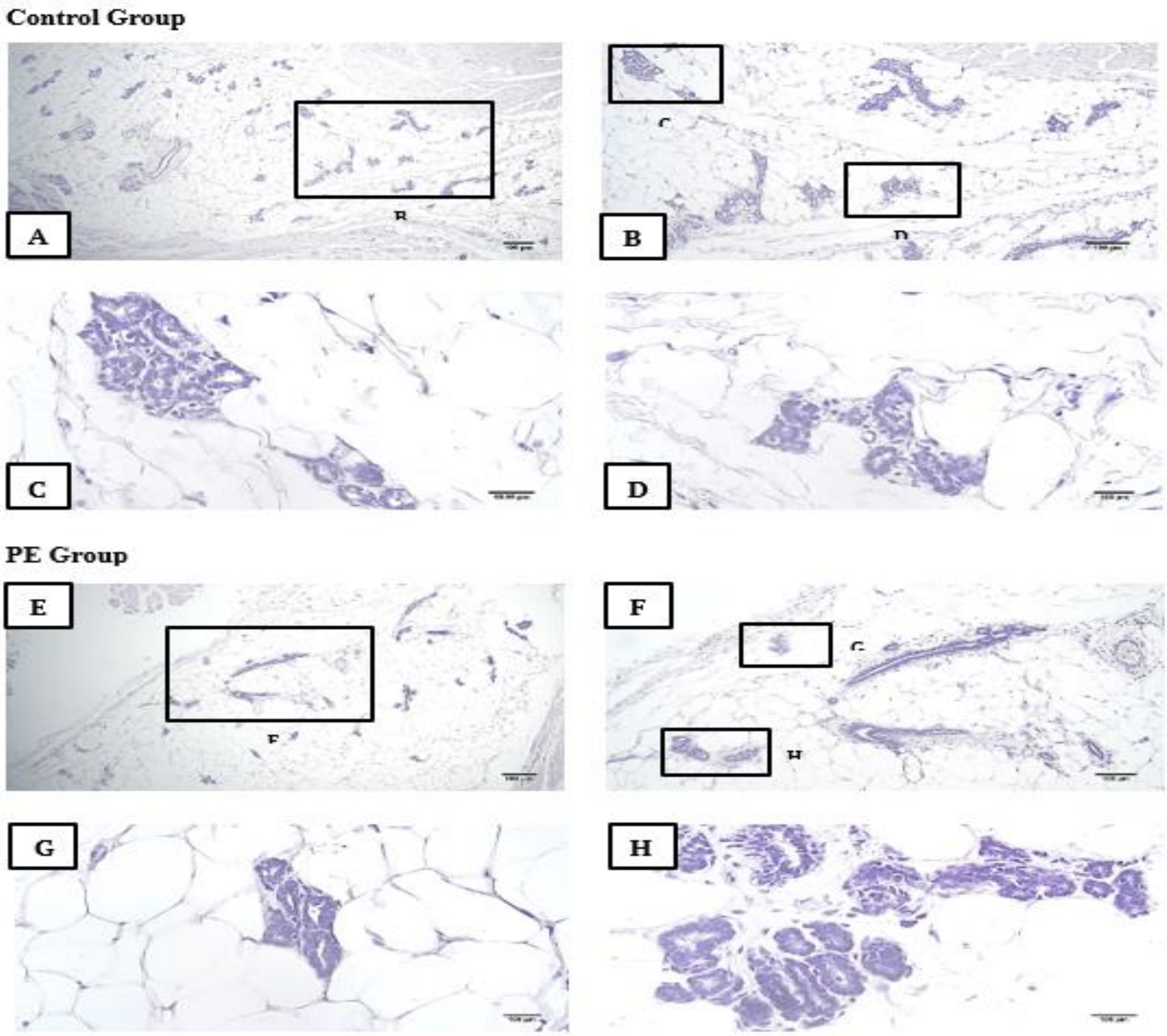


Figure 6. Histopathological Images of Mammary Gland Acini with HE Staining in the Control Group (K) at magnifications of 40x (A), 100x (B), 400x (C and D), and Treatment Group PE (P) at magnifications of 40x (E), 100x (F), 400x (G and H), scale bar: 100 µm

Average Number of Acinar Cells

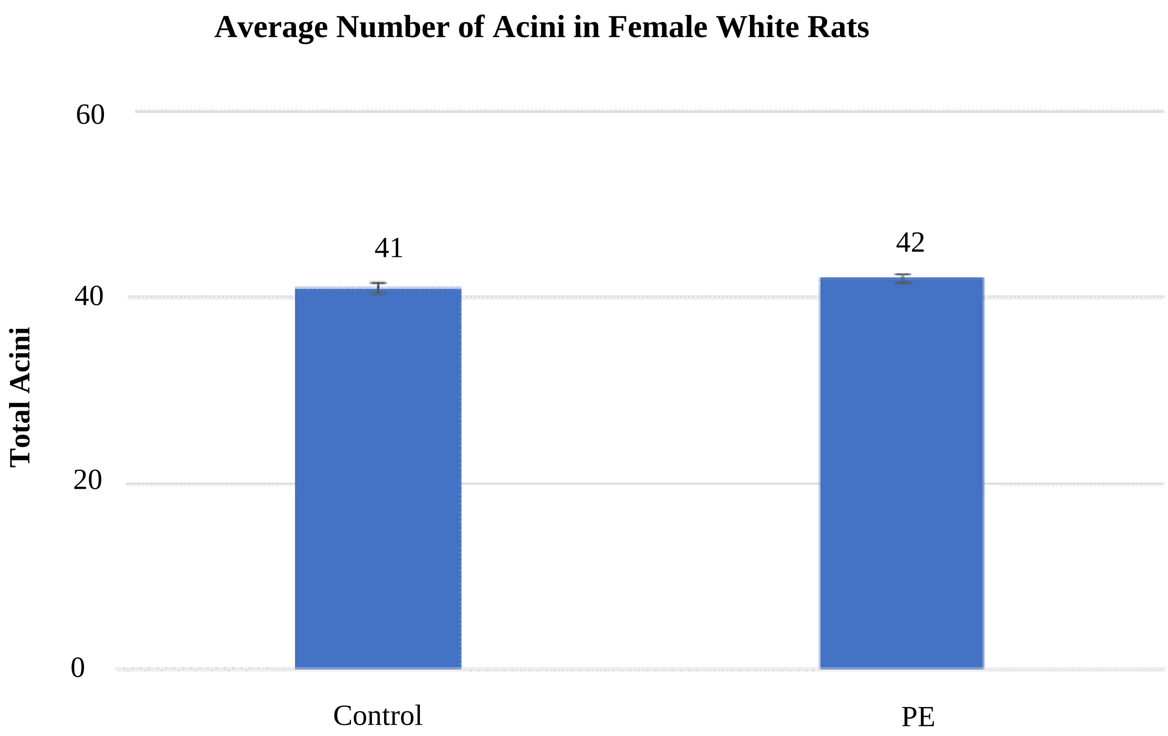


Figure 7. Graph of Average Number of Acini in Female White Rats

Microscopic examination of the mammary glands of female white rats reveals several parts, namely lactiferous ducts and acinar cells. Each sample of mammary tissue from female white rats has a variable number of acinar cells. Observation of acinar cell counts in each sample of mammary tissue from female white rats was conducted in 10 fields of view, and the total number as well as the average number of acinar cells for each group were counted and presented in graphical form, as shown in Figure 7.

Upon calculation, it was found that in the control group, the average number of acinar cells was 41 ± 24.37, whereas in the PE (plastic exposure) group, the average number of acinar cells was 42 ± 16.46. These results indicate a significant difference in the total average acini between the control and treatment groups.

The normality test results for the number of mammary acini showed a result of 0.951, indicating a normal data distribution. The Independent T-test resulted in a significance value of 0.960 ($t > 0.05$), hence it can be concluded that exposure to micro-nano plastics did not significantly differ between the two groups.

Number of Layers of Cuboidal Epithelial Cells in Acini

Microscopic examination of the mammary glands of female white rats in the control group revealed acini surrounded by an average of 1 layer of normal cuboidal epithelial cells, slightly elongated in a square shape, surrounded by normal connective tissue and fat tissue, with fine chromatin nuclei.

Meanwhile, in the treatment group, the mammary acini were surrounded by an average of 2 layers of round-shaped cuboidal epithelial cells, thickened, with chromatin nuclei appearing coarse, and no inflammatory cells were found.

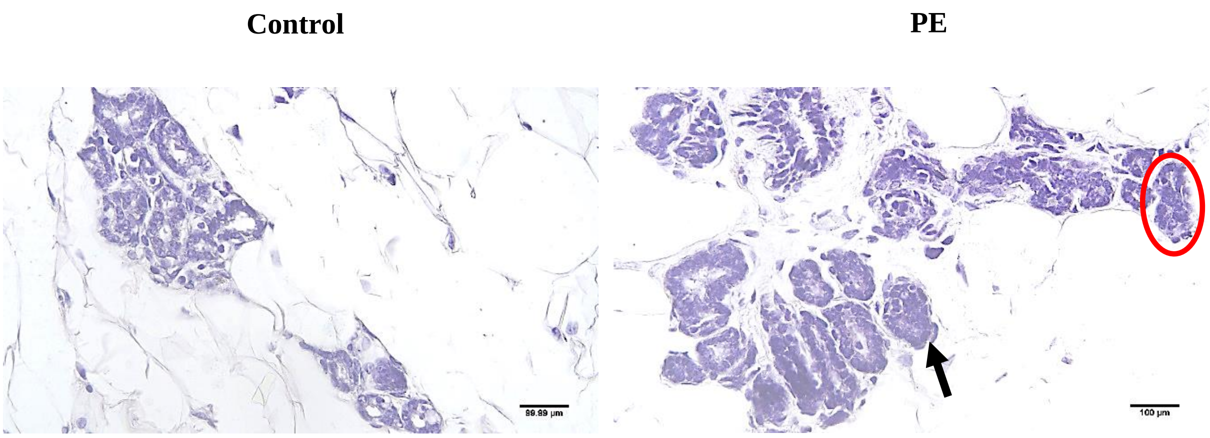


Figure 8. Comparison of Histopathological Images of Acini in Control Group and PE Group, red circles indicate the number of layers of cuboidal epithelial cells which are 1 layer in round shape, arrows indicate coarse chromatin nucleus, scale bar: 100 µm.

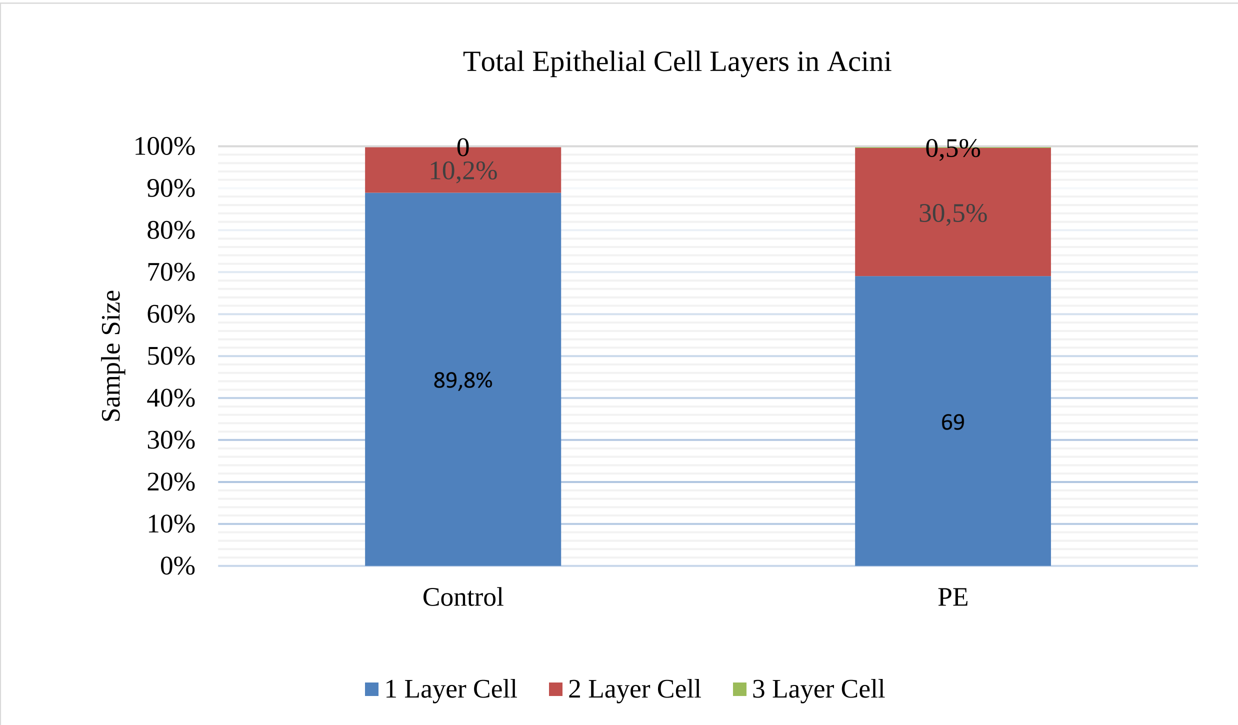


Figure 9. Total Epithelial Cell Layers in Acini

Figure 9 shows the total number of epithelial cell layers in the acini, counted per sample with 10 fields of view. In the control group, acini with a single layer of epithelial cells accounted for 89.8%, two layers for 10.2%, and three layers for 0%. In the PE group, acini with a single layer of epithelial cells accounted for 69%, two layers for 30.5%, and three layers for 0.5%. It can be seen that the percentage of two-layer epithelial cells in the treatment group is higher than in the control group, indicating that the PE group experienced a threefold increase in proliferation compared to the control group.

Data analysis was conducted on samples with two layers of epithelial cells in the ducts from both the control and PE groups. The normality test results for the number of epithelial cell layers surrounding the mammary acini showed a result of 0.928 (p-value > 0.05), indicating that the data distribution is normal and suitable for parametric testing with the Independent T-test. The Independent T-test results showed a significance of 0.007 ($t < 0.05$), indicating that the exposure to micro-nano plastics resulted in a significant difference between the two groups.

DISCUSSION

Effect on MDA Levels in Mammary Glands

Malondialdehyde (MDA) is the most commonly used biomarker for measuring oxidative stress in the human body in various health issues, especially cancer (Maurya et al., 2021). Oxidative stress is caused by an increase in the production of ROS or free radicals in the body (Nakai & Tsuruta, 2021). Oxidative stress can be caused by various factors, one of which is exposure to chemicals that enter the body which can cause genetic mutations and hormonal dysfunction that can initiate the development of cancer cells in the breast organ (Miao et al., 2021).

In this study, MDA levels in the breast were measured to determine the increase in free radicals in the body after female white rats were exposed to PE. The results showed that MDA levels in the PE group were 1.6 times higher than in the control group. This indicates the presence of excessive free radicals in the body in the PE group, potentially leading to genetic mutations and abnormal cell proliferation (Jelic et al., 2021).

These results are consistent with the study by Ijaz et al., (2022), which investigated the effects of PE exposure on reproductive toxicity in male rats. One of the findings of this study was a significant increase in MDA levels in the treatment rats compared to the control rats. This can occur due to the accumulation of free radicals in the body, which increases ROS levels, thus raising MDA levels (Nakai & Tsuruta, 2021).

To date, no exact dose has been determined for the minimum and maximum toxicity of PE plastic exposure in either rat or human studies. However, using PE-type equipment exposed to sunlight or heat for 12 days or more can release antimony compounds beyond normal limits, which can be carcinogenic if they enter the body (Tapiory et al., 2019). This is similar to the use of PE plastic containers used to store water or warm or even hot food, which results in the continuous release of antimony compounds (Diningsih & Rangkuti, 2020).

Effect on Histopathological Features of Mammary Glands

Microscopically, the breast consists of ducts, lobules, acinar cells, fat glands, connective tissue, plasma cells, blood vessels, and muscles (Krisdianilo, Sumantri, & Sidabutar 2021). When the body is exposed to micro-nano plastics, it can cause various toxic effects, including on the breast. Exposure to MNPs can lead to excessive free radical exposure, causing oxidative stress. Oxidative stress will initiate cell cycle development, leading to abnormal cell proliferation and resulting in breast cancer (Chen et al., 2022; Park et al., 2023). Histologically, ducts and acini are lined by a single layer of cuboidal epithelial cells. If the cell layer proliferates into two or more layers or if there are changes in the shape of the epithelial cells, this may indicate abnormal cell proliferation activity (Rinjaya & Mardhia, 2022).

In this study, the number of ducts and acini did not differ significantly, indicating no abnormalities in these parts. However, the average number of epithelial cell layers in each group showed significant results. In the control group, both ducts and acini were lined by a single layer of slightly elongated cuboidal epithelial cells, with smooth chromatin and no inflammatory cells. In the PE group, ducts and acini were lined by two layers of rounded cuboidal epithelial cells, which were slightly thickened, had coarse chromatin, and showed no inflammatory cells, with a 4.7-fold increase in duct proliferation and a 3-fold increase in acini compared to the control group.

This study aligns with the findings of Nansi et al., (2015), who administered the chemical benzo(α)pyrene around the cancer area, and with the study by Rinjaya & Mardhia, (2022), who injected 7,12-dimethylbenz (α)anthracene (DMBA) as a cancer-inducing chemical in a breast cancer rat model. These chemicals can be metabolized by the body, causing gene mutations in DNA and having carcinogenic properties that can lead to cancer (Rinjaya & Mardhia, 2022). Both studies support the basic hypothesis in this study that exposure to certain carcinogens can cause significant genetic mutations and have strong carcinogenic properties. These findings provide a strong scientific basis for the mechanism of action of the carcinogens used in this study, although the types of carcinogens used are different.

Another study by (Kim et al., 2021) exposed MNPs of Polypropylene at a dose of 25 mg/kg body weight/day for 4 weeks and showed no significant differences, suggesting that MNP exposure at this dose does not have adverse effects on humans. However, the study by Ijaz et al., (2022), which exposed 50 mg/kg body weight of PE MNPs for 56 days in experimental animals, showed significant results, indicating that high doses and long exposure times can cause damage to various organ systems in the body.

Antimony trioxide contained in PE plays a significant role in the carcinogenesis process in the mammary glands. Continuous exposure to antimony trioxide can cause disturbances in the body's systems, especially the reproductive system in women, as it can interfere with pregnancy and cause abortion (Baharuddin, Asran & Ikhtiar 2023; Tapiory et al., 2019). Additionally, antimony trioxide is potentially a cancer-causing agent in the human body (Dhaka et al., 2022).

4. CONCLUSION

Exposure to micro-nano plastics of PE type at a dose of 15 mg/m3 for 28 days differed significantly in the MDA levels of mammary glands, histopathological picture of mammary glands at cell proliferation events, both in the lactiferous ducts and acini cells. In addition, exposure to PE plastic has the potential to cause health problems in the breast organs if exposed for a long time and in excess doses. For further research, it is better to use inhalation and oral exposure methods with various types of microplastic combinations to better resemble the actual event.

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