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EFFECT OF ORAL INTAKE OF MICROPLASTIC ON THE CHANGES IN NEPHRON STRUCTURE AMONG MALE WISTAR RATS

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ABSTRACT

Introduction: Microplastic bioaccumulation in nephron tissue can cause high levels of nephron mitochondrial reactive oxygen species, endoplasmic stress, and inflammation-related proteins.
Purpose: to observe damage to the tubules and glomeruli structures in the experimental group exposed to microplastic particles
Methods: This study observed damage to experimental animals' tubular and glomerular structures due to oral intake of microplastics. 46 Wistar rats used were divided into one control (C) and five experimental groups (Xn). The experimental group was exposed to low-density polyethylene microplastic doses of 0.0375 mg, 0.075 mg, 0.15 mg, 0.3 mg, and 0.6 mg daily for 90 days with the probe, respectively. Damage to the tubular and glomerular structures was observed through microscopic examination of the preparations stained with hematoxylin-eosin.
Results: The results of the One-Way ANOVA test showed a significant difference between groups with tubular damage ($p < 0.05$). Meanwhile, the results of the Kruskal-Wallis test showed a significant difference between groups with glomerular damage ($p < 0.05$).
Conclusions: Multiple comparations indicated that exposure to microplastic started at 0.0375 mg daily had led to damage to the tubular and glomerular. Ingestion of microplastic particles causes renal tubular and glomerular damage in Wistar rats.

Keywords: Glomerular Damage, Low-Density Polyethylene, Microplastics, Renal Tubular Damage, Wistar Rats

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INTRODUCTION

Plastic is a material that provides many positive benefits to human life. However, it also hurts the sustainability of existing ecosystems. Exposure to ultraviolet radiation for a long time on plastic causes the macro-plastic to undergo catalysis into smaller sizes, namely 0.1-1000 μm^3 (1,2). Microplastics are plastic particles that are <5mm in size (3–5). Microplastics are divided into two types, namely primary and secondary microplastics (6,7). Primary microplastics are plastic particles deliberately formed with small sizes and used as raw materials for cosmetic and health products. This product contains both micro-exfoliate and micro-beads, for example, polyethylene. Meanwhile, secondary microplastics are formed from natural macro-plastic degradation processes, for example, macro-plastics exposed to ultraviolet light for a long time, hydrolysis processes, mechanical stress, biological processes, and climate change (3,8,9).

A study by Deng et al. in 2017 explains that the toxic effects of microplastic exposure in humans are still unclear. The reported toxicity of microplastics is in the form of oxidative stress and inflammation mechanisms. The emergence of this toxic effect of microplastics depends on the particles' size, the particles' surface area, the shape of the particles, the type of polymer, and the various chemical compositions contained in these microplastic particles (10,11). It is stated that all plastic particles contain reactive oxygen species (ROS) originating from polymerization and processing methods. If the concentration of ROS in the human body increases and continues continuously, it will cause symptoms of toxic effects in the body such as damage to organ structures (1,12).

Microplastics are distributed throughout the body's circulatory system and accumulate in the liver, kidneys, and digestive tract. This distribution occurs due to the small size of the microplastic particles that facilitate them to accumulate in such organs (4,13–15). Excessive bioaccumulation of microplastics in organ tissues can trigger mechanisms of oxidative stress, cytotoxicity, inflammatory reactions, neurotoxicity, carcinogenesis, and changes in the immune response (15–17). The mechanism of cytotoxicity due to microplastics involves stimulating oxidative stress via free radicals from ROS (13,16). Excessive ROS production that is not balanced with adequate antioxidant levels will initiate damage to cellular components. The study aims to observe damage to the tubules and glomeruli structures in the experimental group exposed to microplastic particles.

METHOD

Research Design

The current study applied a post-test-only control group design. Using experimental animals that were exposed to microplastics orally by the probe. After exposure, all rats were anesthetized using a mixture of ketamine and xylazine which were injected peritoneally, and the kidney organs were taken using a surgical technique. Then, the rats will be terminated by the cervical dislocation technique. The kidney organs were made histopathological preparations and examined under a microscope to assess damage to the kidney tubules and glomeruli. The research will be carried out starting from June 1st, 2022, to August 30th, 2022, in the animal laboratory of Widya Mandala Surabaya Catholic University. The results of the microscopic examination were carried out with statistical analysis. We put the rest of the rat's body in a wooden box and cremated it so it wouldn't pollute the environment.

Animal

The experimental animals used in this study were male Wistar rats with an age of about 2-3 months. Wistar rats were purchased from Rat Farm, which had a certificate of animal ethics. There were 46 experimental animals used in this study. Wistar rats were assigned into one control group and five experimental groups, with 7-

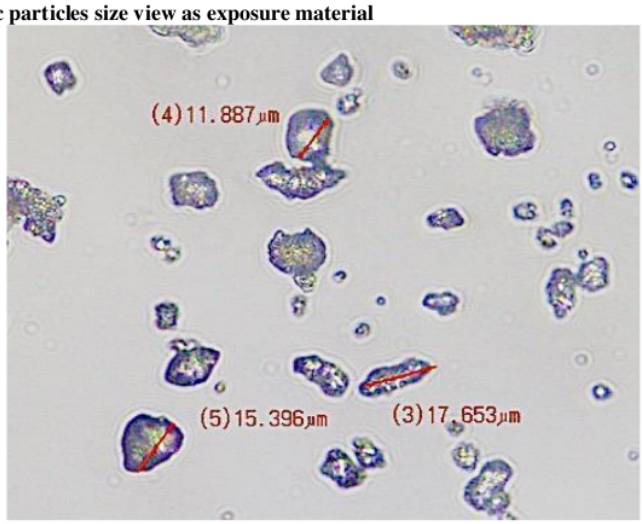
8 animals in each group. The adaptation period of this study was implemented by keeping all rats for one week with the same treatment. The rat nursery area was cleaned daily, the rats were given food and drink ad libitum, air circulation was stable, the room temperature was 18-26ºC, and humidity was 40-70%. We weighed the rats for each group at the beginning of the experiment. The mean body weight in the control group (C) was 151.44±7.986g, experimental group 1 (X1) was 166.67±8.602g, experimental group 2 (X2) was 158.67±7.071g, experimental group 3 (X3) was 162.44±14.258g, experimental group 4 (X4) was

192.56±17.565g, and experimental group 5 (X5) was 191.22± 11.344g. One-Way ANOVA analysis showed that the rats' body weight variations were homogeneous.

Exposure Material

This study used plastics obtained from degraded plastic food packaging. Then, the macro-plastics were mechanically crushed to the size of ≤20 µm using the Fomac-FCT Z100 machine in the laboratory. The obtained microplastic powder was filtered using an 800 mesh sieve with 18-micron pores.

Fig 1. Microplastic particles size view as exposure material



In Figure 1, the microplastic particles used in the study were observed using a microscope Nikon Eclipse with a magnification of 40x10 to ensure that the size of the microplastic particles was correct ≤20 µm (Fig. 1).

Microplastic Dosing

This study used microplastic doses in the form of dry powder of 0.0375 mg/day, 0.075 mg/day, 0.15 mg/day, 0.3 mg/day, and 0.6 mg/day. According to the dosage, the powder was mixed with two cc of distilled water to become a suspension solution. This solution was shaken before being given to rats orally using a probe for 90 days. The control group was only given a distillate water probe without containing microplastics. The determination of the dose of microplastic dry powder referred to a study conducted by Deng et al., which found that the minimum exposure dose for causing damage to biological tissue was five mg/L or equivalent to 0.15 mg of microplastic dry powder.

Kidney histopathology preparation and examination

Histopathology preparations were made from rat kidney samples at the Anatomic Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University. Histopathology slides were stained with hematoxylin-eosin. Furthermore, the examination of histopathology slides by an anatomical pathologist at the Anatomical

Pathology Laboratory, Faculty of Medicine, Widya Mandala Surabaya Catholic University. In renal tubular damage, observations were made on 20 proximal convoluted tubule visual fields using a microscope with 400x magnification. Damage to the proximal convoluted tubule included loss of the brush border, dilatation of the tubular lumen (>60 μm in diameter), presence of debris, and vacuolization of the tubular cells. Observations were made on 50 glomeruli in renal glomerular damage by counting the number of mesangial cell nuclei using 400x magnification. Damage to the glomerulus was determined based on the difference in the number of nuclei of mesangial cells in the glomerulus between the control and experimental groups.

Statistical Analysis

Statistical data analysis was carried out independently using Statistical Package for the Social Sciences version 21 at least three times. The Shapiro-Wilk test showed a p-value>0.05 in both tubular damage variables, but not on variable renal glomerular damage. Levene's method of analysis also showed a p-value >0.05. This means that the data for renal tubular damage was normally distributed and homogeneous, while the glomerular damage data were not normally distributed. Statistical analysis on renal tubule damage data used the One-Way ANOVA test, while data on renal glomerular damage used the Kruskal Wallis test. If a significance value of <0.05 was obtained, then the null hypothesis (H0) was rejected. This study has a 95% confidence interval. Multiple comparative analysis is used to determine the exposure dose that affects renal tubular and glomerular damage. The Post Hoc LSD (Least Square Difference) test was used for tubular damage variables because the data requirements must be homogeneously distributed to be met. In contrast, for the glomerular damage variable, the Mann-Whitney test was used.

Ethical Statement

This research has followed the Helsinki ethical procedures and received an ethical certificate from the Health Research Ethics Committee of Widya Mandala Surabaya Catholic University, reference number 0268/WM12/KEPK/MHS/T/2022. This ethical review was submitted where the research was conducted.

RESULT

Analysis of the effect of microplastics on damage to the renal tubular

Table 1. One-Way ANOVA test on renal tubular damage

Group	Mean ±Standard deviation	p-value One-Way ANOVA	Conclusion
C	313,78 ±13,122	0,001	significant difference
X1	364,63 ±15,445		
X2	440,13 ±7,717		
X3	468,86 ±8,934		
X4	493,00 ±8,062		
X5	518,86 ±14,803		

In Table 1, tubular damage was more common in the group exposed to higher doses of microplastics. There was also renal tubular damage in the control group (C). The highest average renal tubular damage was in experimental group X5, which was exposed to microplastics at 0.6 mg/day. The One-Way ANOVA test obtained a significance value <0.05, indicating a significant difference.

Tabel 2. Significance post hoc LSD test on renal tubular damage

Group	C	X1	X2	X3	X4	X5
C	-	0.001	0.001	0.001	0.001	0.001
X1	0.001	-	0.001	0.001	0.001	0.001
X2	0.001	0.001	-	0.001	0.001	0.001
X3	0.001	0.001	0.001	-	0.001	0.001
X4	0.001	0.001	0.001	0.001	-	0.001
X5	0.001	0.001	0.001	0.001	0.001	-

Table 2 shows that the microplastic exposure dose of 0.0375 mg/day was effective in causing damage to the renal tubules of Wistar rats.

Analysis of the effect of microplastics on renal glomerular damage

Tabel 3. Kruskal-Wallis on renal glomerular damage

Group	Mean ±Standard deviation	P value Kruskal-Wallis	Conclusion
C	1,36889±0,171462	0,005	Significant difference
X1	1,16256±0,085072		
X2	1,08700±0,040574		

X3	1,10471±0,052677
X4	1,16029±0,056738
X5	1,14729±0,083338

In Table 3, all experimental groups (X1, X2, X3, X4, X5) had lower mean glomerular damage than the control group (C). The control group had the highest average renal glomerular damage, which was not exposed to microplastics. The Kruskal-Wallis obtained a significance value <0.05, which indicated that there was a significant difference.

Tabel 4. Significance Mann-Whitney test on renal glomerular damage

Group	C	X1	X2	X3	X4	X5
C	-	0.027	0.003	0.010	0.017	0.023
X1	0.027	-	0.083	0.118	0.862	0.728
X2	0.003	0.083	-	0.418	0.028	0.132
X3	0.010	0.118	0.418	-	0.085	0.338
X4	0.017	0.862	0.028	0.085	-	0.848
X5	0.023	0.728	0.132	0.338	0.848	-

Table 4 shows that the microplastic exposure dose of 0.0375 mg/day was effective in causing damage to the renal glomerular of Wistar rats.

Fig 2. Microplastic view of nephron structure

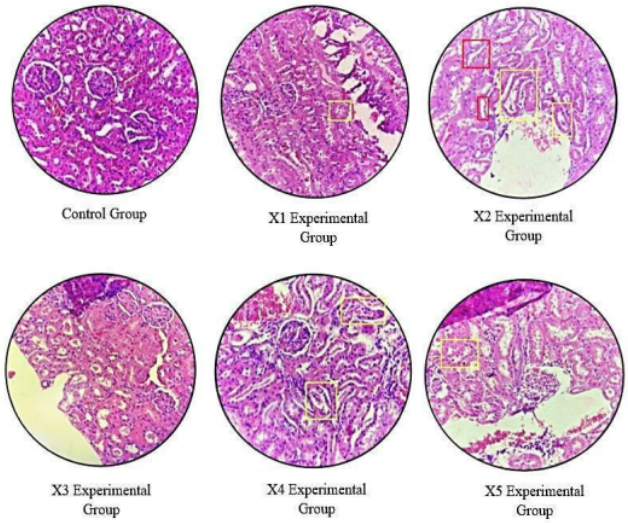


Figure 2 shows an overview of the damage to the tubular and glomerular structures of the Wistar rat's kidney exposed to microplastics orally. Histopathological slide images stained with Hematoxylin-eosin were observed using a light microscope. Based on the observations, there was sloughing of tubular epithelial cells (yellow box) which left debris in the lumen. Then, the tubular epithelium was flattened (red box). Meanwhile, the glomerular structure was still similar to the image of a normal glomerular structure and only changed in the number of glomerular cell nuclei.

DISCUSSION

Dense microplastic particles and rough edges play a role in the pathophysiology of microplastic toxicity in the kidney (Figure 1). The body's defense mechanisms, such as cellular and adaptive immunity system activation, cannot destroy microplastic particles. Renal tubular and glomerular damage might occur since microplastics increased reactive oxygen species (ROS) production in the tubules, resulting in oxidative stress. The toxic effect of microplastics that enter the kidneys can cause excessive production of ROS. This statement and study findings align with Wang et al. (2021) and Goodman et al. (2022) research. Excessive levels of ROS that are not balanced with adequate antioxidant levels will initiate damage to cellular components, such as deoxyribonucleic acid, ribonucleic acid, carbohydrates, lipids, and protein. A

previous study conducted by Wang et al. (2021) found the occurrence of inflammatory conditions and accumulation of ROS in the kidneys of rats exposed to 2 μm microplastics at a dose of 0.2 mg/day and 0.4 mg/day orally for 32 days.

Inflammatory conditions and accumulation of ROS could occur due to renal damage caused by toxic agents from microplastics. Microplastic particles may cause damage to the nephron structures, namely the tubules, and glomeruli. The findings of this study indicate that kidney tubule damage increases along with higher doses of microplastic exposure (Table 1). The doses that caused harm were smaller than the study by Wang et al. (2021), but the exposure time in this study was 90 days. The dose that caused tubular damage was 0.0375 mg/day (Table 2). In addition, based on a study conducted by Goodman et al. (2022) which administered exposure to microplastics with doses of 5 and 100 μm for 72 hours, there was an increase in ROS production in the kidneys for each dose and every 24 hours of examination. Furthermore, a study conducted by Goodman et. (2022) revealed that microplastic exposure caused a decrease in glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase, and antioxidant enzymes (superoxide dismutase and catalase) which play a role in detoxifying ROS.

ROS was defined as a free radical originating from oxygen, which contributes to cell injury (15,18). Continuously produced ROS will further produce oxidative stress by inhibiting the body's antioxidant performance. The antioxidants catalase and superoxide dismutase act as the first line of defense against ROS (5). The superoxide dismutase enzyme plays a role in converting O2 to H2O2. Meanwhile, the catalase enzyme plays a role in reducing H2O2 to water. In addition, the glutathione peroxidase enzyme plays a role in detoxifying H2O2 or organic hydroperoxides into water (5). Oxidative stress will cause the activation of the immune system response and the release of pro-inflammatory cytokines (19,20). This process will occur continuously and cause apoptosis of tubular cells. Thus, there is damage to the tubules, which can be characterized by changes in the structure of the tubules, namely sloughing and flattening of the tubular epithelium, dilatation of the tubular lumen, and the presence of necrotic cells in the tubular lumen (Fig. 2).

Table 3 shows that damage to the glomerulus structure did occur, which can be observed from the decrease in the mean number of glomerular cell nuclei in the experimental group compared to the control group. The control group was not exposed to microplastics, so a higher mean number was obtained, which indicated that the control group had more healthy cell nuclei. The decrease in the mean number of glomerular cell nuclei in the experimental group could occur due to the ROS mechanism, as described above. Moreover, there are nephron structures in the kidney, one of which is the glomerulus which plays a role in the blood filtration process to form urine. Therefore, any damage to the glomerulus will interfere with the filtration process for urine formation.

This study had a limitation, namely, the levels of cellular antioxidants and kidney inflammation biomarkers needed to be examined. However, this biomarker has been confirmed by various other studies. This study also cannot explain the cause of Wistar rat's kidney damage, whether from microplastic particles or other chemical compounds contained in microplastic particles. As per our recommendation, identifying chemical compounds contained in microplastic is necessary to clarify the pathophysiology of microplastic in the kidney.[21,22]

CONCLUSION

There was a significant effect of oral intake of low-density polyethylene microplastic with a size of < 20 μm starting with a dose of 0.0375 mg/day for 90 days

on damage to the tubular and glomerular structures among male Wistar rats.

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