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Kidney and Liver Disorders Due to Microplastic Exposure: Chronic Vivo Study in Male White Rats

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Abstract

The increasing use of plastics has caused severe environmental pollution, especially by microplastics with plastic particles of 5 mm or less in diameter. These particles are formed by environmental factors such as weathering and ultraviolet irradiation, thus making environmental pollution worse. This environmental pollution increases human exposure to microplastics through the food chain. Many studies have now reported the adverse effects of exposure to microplastics in food and aquatic organisms. However, relatively few studies have used white rats. the entry of microplastics into white rats can affect two important organs, namely the liver and kidneys, in this study we conducted an in-vivo experimental test of male white rats by giving acute and chronic exposure to pure microplastic PE (polyethylene) for 28 days respectively. repeated with an adjusted dose. Toxicological effects were comprehensively evaluated in white rats by looking at clinical signs, body weight, and kidney and liver activity through examination of blood parameters creatinine, SGOT, SGPT, and further kidney and liver abnormalities were analyzed through histopathological tests. Based on the results of the experiments conducted, it was found that PE-type microplastics had a significant impact on changes in body weight, increased functional activity of the kidneys and livers of white rats, and chronic histopathological abnormalities in male white rats, so it is hoped that this study can clarify that PE type microplastics greatly affect organs for those who consume it in the long term.

Introduction

In recent years, plastic particles with a diameter of <5mm have been increasingly recognized as a global environmental threat and health hazard to the human population (Katsnelson, 2015). Microplastics is a term for plastic particles for which there is no universal definition. In the literature, microplastics are often defined as plastic particles with dimensions up to 5 mm with no specified lower size limit (Leslie *et al*., 2022) Based on their origin, microplastics (MP) are classified into primary and secondary MP (Lee *et al*., 2022). MP primers are made for use in personal care products, cosmetics, toothpaste, detergents, sunscreens, and drug vectors (Chatterjee& Sharma, 2019) which, if discharged into the environment, can undergo UV oxidation,

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light degradation, and physical ablation (Dey *et al*., 2021). The presence of these harmful plastic fragments in ecosystems (terrestrial and aquatic) is due to different anthropogenic activities, which include domestic, industrial, and coastal activities.

The introduction of microplastics in aquatic ecosystems is mainly due to domestic runoff containing microbeads and microplastic fragments (used in cosmetics and other consumer products) and also from fragmentation of large plastic debris (Andrady, 2011). MPs encountered in the environment strongly represent a heterogeneous group, one of which is chemical composition (polypropylene, polyethylene, polystyrene, etc.). Polyethylene is a major source of microplastics and is widely present throughout the environment. Microplastics include plastic materials, such as polyethylene, polystyrene, and polypropylene. Polyethylene is a major source of microplastics and is widely and predominantly present throughout the environment (Uurasjärvi *et al*., 2021). as for the uses according to the type of MP, namely polyethylene (politen; PE) in the form of low density (LDPE; garbage bags, films) and high-density construction (HDPE; shopping bags, bottle caps) or as atetraphthalate (PET; bottles, food trays), polypropylene (PPL; kakubak, straws), polyvinyl chloride (PVC; pipes, door and window frames), and polystyrene, both rigid (PS; food pots, toys) and expanded (EPS; packaging, insulation) (Waring *et al*., 2018). Due to their small size and non-biodegradability, microplastics accumulate within aquatic organisms.

The food chain is the main pathway through which humans are exposed to microplastics (Mercogliano *et al*., 2020) so eventually it not only harms the environment but can easily transfer to marine biota and be consumed by humans through the food chain process (Lee *et al*., 2022). The accumulation of MP tissue can lead to various side effects, such as physical injuries (De Stephanis *et al*., 2013) Reduction of feeding activity, inhibition of growth and development (Yang *et al*., 2021), energy deficiency (Cole *et al*., 2015), immune response (Akhbarizadeh *et al*., 2020) oxidative stress (Kutralam-Muniasamy *et al*., 2020) neurotoxic response (Q. Li *et al*., 2020) and metabolic disorders (Lee *et al*., 2022). It is these releases to the environment that occur continuously and are now a global problem that requires urgent managerial strategies to reduce or avoid potential threats that worsen the survival of organisms, human health, and the aesthetic value of the environment (Barletta *et al*., 2019). These results are important clues to the accumulation and release of microplastics after exposure to the human body. Human feces were previously analyzed by Fourier Transform Infrared (FTIR) spectroscopy, providing evidence that micro-sized plastic particles can be excreted through the gastrointestinal tract (Zhang *et al*., 2021). Plastic particles were also detected in human colectomy specimens by FTIR (Ibrahim *et al*., 2021). Raman microspectroscopy was recently applied to the images and identified three polypropylene particles measuring between 5 and 10 μm in human placental tissue (Ragusa *et al*., 2021).

Despite these reports, studies on mammals concerning the toxicological effects of microplastics are still limited. In particular, rodent toxicity tests to evaluate the risk assessment of microplastics in humans currently lack consideration of their risk or severity. Therefore, the need and interest in in vivo toxicity and accumulation evaluation needs to be carried out in this study. We tested the in vivo effects of polyethylene (PE) microplastics at a size of 5 μm2, the study was conducted by orally administering polyethylene microplastics (MP-PE) at repeated doses chronically for 28 days and evaluating toxicity in male white rats. Evaluation of renal and hepatic impairment was carried out by taking blood before and after exposure to creatinine, SGOT, and SGPT parameters. We also more fully identified whether MP-PE can cause damage to kidney and liver organs through histopathological tests. Our findings provide insight into the in vivo toxicity of MP-PE and its potential bioaccumulation in organs with graded doses for 28 days in a complete manner.

Method

Pure MP-PE particles purchased at local industry provider of pure polyethylene type microplastic raw materials in powder form which is then measured first using light and

transmission electron microscopy at the Animal Laboratory, Faculty of Medicine, Diponegoro University and identified the size of MP-PE which is 5 μg^2 and this MP-PE is sterilized first with aquades liquid and then mixed with 0.5% CMC Na solution so that it is easy to dissolve and orally in white rats, The preparation of 0.5% CMC Na solution is made by weighing 500 mg of CMC Na into 10 ml of hot aquades then left for approximately 15 minutes until clear and shaped like jelly. Next, it is stirred to a homogeneous mass and diluted in a measuring flask with aquades up to a volume of 100 ml. Thirty-six mice were assigned to six groups each: group 1, which was considered a control group; group 2, which gets 200 µg/kg MP-PE, group 3, which gets 400 µg/kg MP-PE; group 4, which gets 600 µg/kg MP-PE, group 5, which gets 800 µg/kg MP-PE; and group 6, who got 1000 µg/kg MP-PE for 28 days.

Animal observations, the presence of near-dead or dead animals, and animal weight measurements were performed once a day, twice a day, and once a week, respectively, for 28-day repeated dose toxicity studies. In addition, food and drinking water consumption was measured daily for repeated dose-toxicity studies over 28 days. For quantitative in vitro determination of creatinine concentration in rats, plasma, or urine in Konelab analyzer using an enzymatic method. All test results are interpreted with a clinical context in mind and examined using Indiko Thermo scientific: Photometer (End point &; Colorimetric) Auto Analyzer. For quantitative determination of alanine aminotransferase in vitro (L-Alanine: Activity of 2-Oxoglutarate Aminotransferase (ALT), EC 2.6.1.2) in serum or human plasma on Konellab analysis. All test results must be interpreted concerning the clinical context and examined using the Indiko Thermo scientific Auto Analyzer: Photometer (End point &; Colorimetric). For in vitro quantitative determination of aspartate aminotransferase (L-Aspartate: 2-Oxoglutarate Aminotransferase (AST) activity, EC 2.6.1.1) in serum or rat plasma on Konelab Analysis. All test results must be interpreted concerning the clinical context and examined using the Indiko Thermo scientific Auto Analyzer: Photometer (End point &; Colorimetric). All hematology, serum biochemistry, weight, and organ data are presented as minimum, maximum, mean ± standard deviation (SD). The abnormally distributed data were analyzed in a nonparametric manner by the unpaired Mann-Whitney test. Comparison of several groups was done using analysis of variance with post-test. According to Bonferroni. A P value of <0.05 is considered statistically significant.

The manufacture of histological preparations of organs begins with the stage of fixation of organs. Organ tissues are put in a 10% neutral formalin-buffered fixation solution for at least 24 hours. Fixation is used to prevent post-mortem degeneration, kill microorganisms that may still be present, and harden tissue so that it is easily cut. The finished fixed organ is cut 5 mm thick and inserted in a tissue cassette. Following the dehydration stage, pieces of tissue in the cassette tissue are put into a stratified concentration of alcohol (70% alcohol, 80% alcohol, 96% alcohol, I absolute alcohol, II absolute alcohol, III absolute alcohol for 30 minutes each) to remove the water content in the tissue. Furthermore, in the clearing stage, pieces of tissue in the cassette tissue are then inserted in xylol solution for 2 x 30 minutes, to remove the alcohol content in the tissue, the goal is that the tissue becomes clearer and transparent so that it can be filled with liquid paraffin. Next to the impregnation stage, pieces of tissue are put into liquid paraffin for 2 x 2 hours. Next to the embedding stage, pieces of tissue in cassette tissue are planted into paraffin which has a melting point of 56-580C, and waited until the paraffin becomes solid. The tissue already embedded in solid paraffin is cut 4 µm thick with a microtome. Pieces of tissue are affixed to glass objects. The tissue on the glass object is heated to a temperature of 56-580C to dilute and remove residual paraffin between the tissues, then rinsed with aquades. Sequentially the tissue on the glass object is inserted in: Xylol for 1 minute, Xylol for 2 minutes, Xylol for 3 minutes, Alcohol 100% for 2 minutes, Alcohol 96% for 2 minutes, Alcohol 70% for 1 minute, Aquades for 1 minute, Alcohol 100% for 2 minutes, Hematoxyllin for 2 minutes, Aquades for 2 minutes, Eosin for 2 minutes, Aquades for 15 seconds, Alcohol 80% for 15 seconds, Alcohol 96% for 30 seconds, Alcohol 100%

for 30 seconds, Xylol for 1 minute, Xylol for 1 minute, Tissue that has been stained on the glass object is covered with a glass cover, which was previously dripped with entellan which is a transparent adhesive. Scoring of disorders in the kidney organs and liver of male white rats was carried out in this study. Animal studies have been reviewed and approved by the Health Research Ethics Committee of the Faculty of Public Health, Universitas Airlangga with No: 57/EA/KEPK/2021.

Result and Discussion

Chronic administration of MP-PE doses with repeated doses here is presented based on differences in rat body weight over time to see at what dose of weight loss based on the graph (figure 1) and also more fully presented the average ± SD to see the value.

In Table 1, it can be seen that most of the rats given exposure to MP-PE doses experienced a decrease in body weight per dose according to the length of exposure time, but the focus here is on the difference in body weight between controls and graded treatment doses, namely doses of 400 mg/kgBW, 600 mg/kgBW; 800 mg/kg to 1000 mg/kgBW where the decrease occurred at a dose of 400 mg/kgBW on day 7 (198.00 ± 9.192) to (178.50 ± 50.205) on day 28; a dose of 600 mg/kgBW on day 7 (207.33 ± 7.202) to (204.50 ± 18.912) and the final dose or the highest dose of 1000 mg/kgBW on day 7 (231.00 ± 9.201) to (223.33 ± 25.580) on day 28 (Table 1). When compared with the control dose, it is very different, not experiencing weight loss but weight gain, because the control was only given food and drink during this chronic exposure study, where the body weight of rats on day 7 (180.00 ± 4.427) becomes (203.50 ± 4.370). Complete body weight tends to decrease when rats are exposed to graded doses starting from a low dose of 200 mg/kgBW to a high dose of 1000 mg/kgBB. If you look at the table presented (Table 1), it can be seen that most rats given exposure to MP-PE doses will experience significant changes based on the length of time the rats are exposed. Looking at the results of the one-way anova analysis conducted between the treatment dose and body weight based on days 7-28, it was found that there was a significant relationship between the dose of MP-PE and the body weight of rats based on the length of exposure, namely on days 7 ($p = 0.000$); day 14 ($p = 0.001$); day 21 ($p = 0.001$) and day 28 ($p = 0.022$). In administering MP-PE doses, we also monitor the value of kidney activity by checking creatinine levels to ensure that the exposure given is also detected in the blood so that it can represent a single cause that can enter the body of mice. The renal function

Table 1. Changes in Body Weight of Male White Rats Given MP-PE Doses at Various Doses

Dosage Group (mg/kgBW)	Mean± std. deviation (day-)			
	7	14	21	28
Control	180.00±4.427	187.00±4.899	194.50±5.010	203.50±4.370
200	188.33±4.412	196.17±6.795	202.67±10.558	206.33±13.967
400	198.00±9.192	184.00±16.823	182.33±25.968	178.50±50.205
600	207.33±7.202	201.40±20.305	206.50±16.783	204.50±18.912
800	209.00±6.595	215.50±5.802	236.25±10.720	236.25±10.720
1000	231.00±9.201	220.50±15.588	223.00±19.053	223.33±25.580
Average±SD	200.41±17.061	200.07±17.125	206.54±20.984	209.96±22.404
Max	231.00	220.00	236.00	236.00
Min	180.00	187.00	182.00	178.00
p-value	0.000*	0.001*	0.001*	0.022*

(*: significant ($p<0.05$)

Note: All groups were treated for 28 days. The data is expressed as the average ± SEM. high dose (KP5) = 1000 mg/kg/day; Medium dose (KP2-KP4) = 400-800 mg/kgBW/day); Low dose (KP2) = 200 mg/kg/day and control (KK) supplemented with rat food pellets.

Table 2. Effect of MP-PE on Kidney (Creatinine Levels) and Liver (SGOT Levels, SGPT) Activity in Male White Rats

Group	Creatinine (mean±SD)		SGOT (mean±SD)		SGPT (mean±SD)	
	Pre	Post	Pre	Post	Pre	Post
Control	0.250±0.0548	0.333±0.0516	179.50±200.839	268.00±97.724	70.50±6.156	116.50±58.092
Dosage 200	0.300±0.0000	0.400±0.0000	294.83±252.166	233.67±119.823	184.33±225.713	297.17±407.604
Dosage 400	0.267±.00516	0.333±0.0816	191.67±84.327	236.83±236.83	90.00±30.013	103.67±18.408
Dosage 600	0.283±0.0408	0.367±0.0516	167.17±53.056	304.33±304.33	88.00±20.159	135.83±11.441
Dosage 800	0.300±0.0000	0.383±0.0753	145.33±43.944	278.17±109.077	84.00±17.123	110.83±11.906
Dosage 1000	0.300±0.0632	0.300±0.0632	203.67±84.282	206.83±79.668	116.83±52.186	112.83±35.997
Ave±SD	0.283±0.0447	0.353±0.0654	197,03±140,964	254,64±91,055	105,61±96,806	146,14±172,389
Max.	0.300	0.400	294.83	278.17	184,33	297,17
Min	0.250	0.333	145,33	206,83	70.50	103,67
P-value	0.000*		0.007*		0.012*	

(*: significant (p<0.05)

Table 3. Analysis of the Value of Damage to Kidney Tissue with Repeated Doses for 28 Days (200, 400, 600, 800, and 1000 mg/kg body weight/day)

Network	Group	Disorders					
		Normal	Swelling	Inflammation	Necrosis	Hyaline cast	Fibrosis
Kidney	Control						
	200		+	+			
	400		++	++			
	600		++	++			
	800		++	++			
	1000		+++	+++		+	

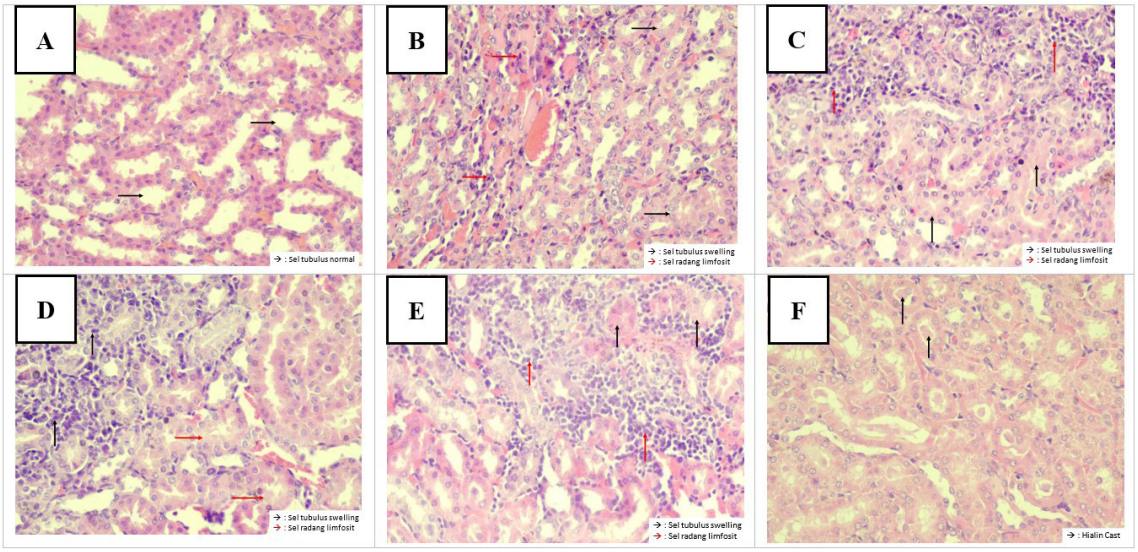

Figure 1. Histopathology in Kidney Tissue A) Control Group (0 mg/kg MP-PE), B) Given with MP-PE Dose (200 mg/kg), C) Given with MP-PE Dose (400 mg/kg), D) Given with MP-PE Dose (600 mg/kg)). E) Given with a Dose of MP-PE (800 mg/kg), F) Given with a Dose of MP-PE (1000 mg/kg). Evaluation of Kidney Tissue Reveals the Normal Structure of Each Dose has Significant Changes, Namely Abnormalities Swelling Tabule Cells, and Lymphocyte Inflammation

activity between before treatment and after treatment at each dose has been presented in the form of average±SD so that quantitative values can be seen in Table 2.

The results of testing kidney function activity through examination of serum creatinine levels in male white rats showed increased activity in each dose given during the sub-chronic period of 28 days, white rat serum stratified concentrations in the MP-PE group doses of 200 mg/kg, 400 mg/kg, 600 mg/kg, 800 mg/kg and 1000 mg/kg compared to the control group, i.e., serum urea levels increased compared to the control group. In addition, there was a significant decrease ($p < 0.05$) which showed a significant difference between serum urea concentrations in normal doses (mean ± SEM) compared to the low-dose group.

It was found that the overall dose showed abnormalities in the kidney and liver organs through several categories of damage observed to cause death, the higher the dose, the higher the damage. In the histopathological picture of rat kidney organs starting with a swelling disorders score of 2 in all groups with the presence of tubule cells that experience degeneration/swelling of the cytoplasm in < 25% of tubule cells, which then also experience inflammatory disorders with a score of 2, namely found a moderate distribution of inflammatory lymphocyte cells/plasma cells in the interstitial area even at the highest dose found Hyaline cast abnormalities with a score of 1 found hyaline cast in the lumen of the tubule, All these findings conclude that microplastics have an impact on the function, activity and also kidney

Table 4. Analysis of the Value of Damage to Liver Tissue with Repeated Doses 28 Days (200, 400, 600, 800, and 1000 mg/kg body weight/day)

Organ	Group	Disorders			
		Normal	Ballooning	Inflammation	Steatosis
Liver	Control				
	200		++	+	
	400		++	++	
	600		++	++	
	800		++	++	
	1000		+++	+++	+

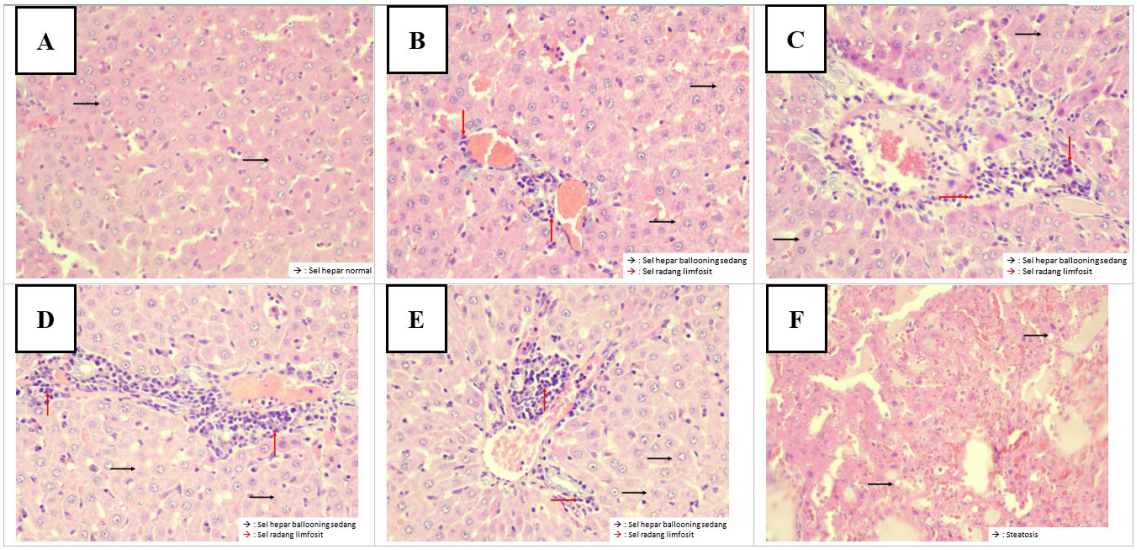

Figure 2. Histopathology in Liver Tissue A) Control Group, B) Given with MP-PE Dose (200 mg/kg), C) Given with MP-PE Dose (400 mg/kg), D) Given MP-PE Dose (600 mg/kg)). E) Given with a Dose of MP-PE (800 mg/kg), F) Given with a Dose of MP-PE (1000 mg/kg). Evaluation of Kidney Tissue Reveals the Normal Structure of Each Dose Has Significant Changes, Namely Abnormalities Namely Sweeling Tabule Cells, and Lymphocyte Inflammation

organ damage of white mice.

Based on histopathological features in the liver, by looking at the activity of SGOT and SGPT in previous rats which increased their activity, then in line with organ damage, ballooning damage was found at each dose with a score value of 2 where the distribution of hepatocyte cells that experienced moderate hydropic degeneration was found and a score of 3 at a high dose of 1000 mg/kg where the distribution of hepatocyte cells that experienced severe hydropic degeneration or hepatocyte cells were found. The necrosis is even worse with steatosis damage at high doses with a score of 1 where hepatocyte cells experience intracellular fat accumulation. If you look at it as a whole, this is very dangerous. Due to sub-chronic stratified exposure, much damage is found to the liver.

As the production of plastic products increases, plastic waste also increases. Microplastics are formed due to weathering and environmental exposures, and plastic waste collects in the oceans. Microplastics, which are environmental pollutants, have recently attracted the attention of the wider community. In marine environments, aquatic organisms can ingest microplastics, leading to human exposure based on the food chain. A study noted the presence and type of microplastics found in human feces (Zhang *et al*., 2021). As a result, there is increasing interest in studying the prevalence and effects of environmental microplastics (Deng *et al*., 2017). The impact of microplastics was evaluated using aquatic (Zhu *et al*., 2020), mouse (Yang *et al*., 2019), and human cells (Schirinzi *et al*., 2017), and there was a significant increase in the negative impact of environmental pollution on human health (Inhorn and Patrizio, 2014).

In this study, using standard toxicity evaluation methods (OECD guidelines 408, 423, and BPOM Indonesia), 5 concentrations of MP-PE (200, 400, 600, 800, 1000, mg/kgBW/day) were administered to male white rats with single and repeated doses for 28 days. In addition, we determined whether the microplastics administered could enter the blood of white rats and affect the condition of kidney and liver organs through histopathological tests. The polyethylene microplastic (PE-MP) used was pure type with an average size of 5.21 μm^2. Many studies are using spheroidal microplastics. (Au *et al*., 2015) consider that this type of microplastic is very common in plastic products discharged into the environment, so it is very risky to degrade in the environment and can enter the human body. Therefore, microplastics reflecting these characteristics were prepared and used in this study.

To confirm the sub-chronic dose that triggers clinical disorders to death from MP-PE, repeated oral dose toxicity studies were conducted by looking at the consequences in the form of clinical symptoms, changes in body weight, mortality or death, and histopathological evaluation in the kidney and liver organs. From the results of the administration of graded doses carried out, significant differences were found in the treatment of graded doses with groups that were not exposed to MP-PE, from the aspect of weight gain, changes were found that showed the greater the dose, the lower the body weight of white rats (Figure 1). From the significance test, it was found that each dose given affected the weight at each week during the treatment period on day 7 ($p=0.000$), day 14 ($p=0.001$), day 21 ($p=0.001$), and day 28 ($p=0.022$). So it can be concluded that as a result of repeated oral doses of PE-MP, we determined that exposure to MP-PE greatly affected the metabolic system of rats so that in graded doses the ability of rats to eat and drink normally was impaired.

Based on previous research that has shown that changes in body weight due to exposure to toxic substances are the most visible indicator and an early indicator of the toxic effects of the test sample given, experimental animals that receive high doses generally lose weight due to physiological changes in rats and decreased food intake and metabolic state (Sireeratawon *et al*., 2010). In another study of a different type of MP-PS conducted on zebrafish animals, it was found that Polystyrene (PS) caused a decrease in body weight, body length, and body mass index as well as an increase in inflammatory cytokine and chemokine gene expression in zebrafish. (B. Li *et al*., 2020), research conducted with polyethylene (PE) exposure given to rats led to the conclusion

that MP-PE affects growth by causing undesirable things namely satiety by inflaming the digestive tract, changing the intestinal barrier, and decreasing lender secretion (B. Li *et al*., 2020), also in other studies, it has been shown that the inclusion of MP (PE-PS) can cause localized effects on the immune system, increase intestinal inflammation, and affect diet resulting in weight loss (Wright and Kelly, 2017).

In addition to MP-PE affecting the body weight of rats, it cannot be denied that MP particles are very small particles that can enter the organs. In this case, based on the identification results, MP-PE entered the body and affected kidney and liver function. Creatinine can be used as a biomarker for kidney disorders and as an indicator of glomerular filtration rate (Lien *et al*., 2006) and enzymes (AST and ALT) are found in the cells of several organs throughout the body and the release of these enzymes and elevated blood levels are signs of cell membrane damage (Lenaerts *et al*., 2005). The results of kidney function activity tests with creatinine level parameters in the serum of male white rats showed that there was increased activity in each dose given during the sub-chronic period of 28 days. The dose of MP-PE 200-1000 mg/kg between pre and post-treatment was found to increase compared to the control group with a mean + SD creatinine value of pre (0.283 ± 0.0447) and post (0.353 ± 0.0654), and for liver activity SGOT and SGPT also found differences between pre and post serum tests, namely pre SGOT (197.03±140.9640); post SGOT (254.64±91.055) and Pre SGPT (105.61±96.806); Post SGPT (146.14±172.389).

When referring to the dose and the increase in SGOT, SGPT, and creatinine activity, it should be noted that the treatment group members with high doses had lower hematrocytes (HT) or red blood cells, and also had high doses of MP. Hemoglobin (Hb) levels were much lower, which is considered an indication of sickle cell disease (anemia) (Magrì *et al*., 2018). Research by Wang *et al*. showed the induction of polystyrene microplastics in rats showed a decrease in rat body weight, an increase in creatinine levels, and an increase in pro-inflammatory mediators such as IL-1β, IL-6, and TNF-α. The study also showed that the occurrence of lesions in the histopathological picture of rat kidneys was due to inflammation caused by microplastics.(Wang *et al*., 2023) Palaniappan also showed the results of their research in vitro on the effect of exposure to polyethylene microplastics on renal epithelial cells, showing that the cells experienced damage characterized by an increase in TNF-α levels, which is a pro-inflammatory mediator so that inflammation occurs (Palaniappan *et al*., 2022).

Based on previous studies, MP-PS can be distributed to the liver, kidney, and gastrointestinal tract and affect energy metabolism, lipid metabolism, oxidative stress, and neurological functions (Deng *et al*., 2017). When viewed further with the ANOVA test, it was found that there was a significant relationship between MP-PS dosing and kidney function activity, namely p=0.000 (p< 0.05) and liver function SGOT and SGPT have a significant relationship, namely p=0.007 (p<0.05) and p=0.012 (p<0.05). Based on the histopathological results, abnormalities were found in the kidney and liver organs (Figures 1 and 2); this followed the high kidney and liver activity found in the serum of rats with creatinine, SGOT, and SGPT parameters (Table 2). If seen in more detail, the damage that appears in the kidneys consists of swelling, inflammation, and even hyaline cast damage.

In Table 4, exposure to microplastics at a low dose of 200 mg/kg found swelling and inflammatory damage with a score of 1, where tubule cells were found to have degeneration or cytoplasmic swelling in 25% of tubule cells. The higher the dose (400, 600, or 800 mg/kg), the more the disorder increases with a score of 2, where a moderate distribution of inflammatory cells (lymphocytes and plasma cells) is found in the interstitial area. A score of 3 occurs at a dose of 1000 mg/kg where a hard or diffuse distribution of inflammatory cells, lymphocytes, and even hyalin cast damage with a score of 1 is found in the area of fibrosis in the kidney parenchyma. Liver damage (Figure 2) shows that ballooning damage is found at each dose with a score of 2 where there is a distribution of hepatocyte cells that experience moderate hydropic degeneration, and at a high dose of 1000 mg/kg, there is increased damage, namely steatosis damage with a score of 1

where there are hepatocyte cells that experience intracellular fat accumulation. When viewed as a whole, this is very dangerous. Due to subchronic graded exposure, a lot of damage is found in the liver. The damage caused by MP-PE exposure is also based on research that found that it is very true that the accumulation of microplastics in rat kidneys is the cause of histopathological damage, increased levels of endoplasmic reticulum stress markers, inflammatory markers, and nephrotoxicity (Kuhlman, 2022). Furthermore, Goodman *et al*.'s research on the effect of microplastics in vitro on embryonic kidney and liver cells showed that microplastics produce toxicity effects on cell metabolism and cell interactions, one of which is a decrease in gene expression of antioxidant enzymes such as superoxide dismutase 2 (SOD2) and catalase (CAT) which reduces the ability of SOD2 and CAT enzymes to detoxify reactive oxygen species (ROS) and cause oxidative stress on cells so that cells are damaged (Goodman *et al*., 2022).

Abdel-Zaher *et al*. mentioned that exposure to microplastics in mice affects the morphology of red blood cells. Red blood cells have a variety of shapes due to exposure to microplastics. This morphological change affects oxygen transportation to organs so that it can affect organ function, one of which is the kidney. The study also showed an increase in creatinine, AST (SGOT), and ALT (SGPT) levels in the group of rats exposed to microplastics compared to the control. AST and ALT enzymes are found in the cells of several organs. High levels of AST and ALT in the blood indicate the presence of damaged cells, so these enzymes that should be in the cells become present in the bloodstream (Abdel-Zaher *et al*., 2023). The accumulation of MP in the liver can be considered as a consequence of chronic liver disease. For example, the possibility of having hypertension (a major cause of clinical complications of liver cirrhosis), causing impaired bowel function (also known as "leaky gut), and allowing MP particles to migrate through the intestinal wall, and be transported to the liver (Camilleri, 2019).

Even in studies with human respondents, the liver identification of MPs was found Overall, this proof-of-concept case series assessed the presence of MPs in human liver tissue. We observed that MPs were found in the livers of individuals with liver cirrhosis, but not in those who did not carry congenital liver disease, and from six microplastics of different sizes Polymers ranging from 4 to 30 μm suggest that chronic liver disease appears to be a major driver in MP accumulation in humans (Horvatits *et al*., 2022). Also, toxicity in mammals is conducted by many studies that show the entry of particles into the lymphatic system occurs in humans (particle size 0.2-150 μm) rodents (30-40 μm), rabbits (0.1-10 μm) and dogs (3-100 μm), via the intestine. For dogs, PVC particles (5-110 μm) were found in the portal vein and were found to reach the liver. Also, toxicity in mammals conducted by many studies showed the entry of particles into the lymphatic system occurred in humans (particle size 0.2-150 μm) rodents (30-40 μm), rabbits (0.1-10 μm), and dogs (3-100 μm), via the gut. For dogs, PVC particles (5-110 μm) are found in the portal vein and are found to reach the liver (Wright and Kelly, 2017).

This study provides new insights to improve our understanding of the toxicity effects of MP-PE and the biological safety of microplastics to male white rats which can be a preclinical depiction of the impact of MP-PE on human health after exposure. In this investigation, we found that the accumulation of MP particles is mainly dose-dependent in the digestive system. Their particle size strongly influenced their distribution and tissue accumulation kinetics, and accumulated in the liver, kidney, and intestine (Deng *et al*., 2017). To overcome some of the limitations of this study, it is necessary to evaluate the toxicity of repeated microplastic administration for more than 28 days. In addition, studies on the mechanism of microplastic toxicity should be conducted simultaneously. Because humans and other organisms are continuously exposed to microplastics through food intake.

Conclusion

In male white rats, polyethylene microplastics produced several toxins and caused impacts on the digestive system, kidney and liver organ abnormalities, and histopathological abnormalities. These

biochemical parameters can cause severe toxic effects on all organs at concentrations ranging from low doses to higher doses and for a long time with repeated exposure. The findings showed that the microplastic dose groups had damaging effects on kidney and liver organ cells, reflecting the harmful impact of these dose groups on human health. The current study may initiate future comprehensive studies to determine the hazardous doses of microplastic exposure, especially the polyethylene type.

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