THE EFFECT OF PERORAL POLYVINYL CHLORIDE MICROPLASTIC ON ALKALINE PHOSPHATASE AND GAMMA-GLUTAMYL TRANSFERASE LEVELS IN RATTUS NORVEGICUS WISTAR STRAIN

Leonardo S Wicaksono[1)], Yudhiakuari Sincihu[2), 3)], Adi P Hendrata[4)]

Correspondent Email: leonardo.ado1320@gmail.com

ABSTRACT

Introduction: Human exposure to microplastics continues to rise. Microplastics are harmful and are suspected of contributing to various health problems in humans, including reduced liver function.
Purpose: This study aimed to determine the effect of oral polyvinyl chloride microplastic consumption on alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) levels in Rattus norvegicus Wistar strain.
Method: Male Rattus norvegicus Wistar strain experiments were separated into control and experimental groups, with the experimental group receiving a 0.5 mg/day dose of polyvinyl chloride microplastic. The experimental animals' blood was taken using the cardiac puncture technique, and the ALP and GGT levels were determined using the kinetic photometric test method.
Result and Discussion: The Shapiro-Wilk normality tests showed that the ALP variable from the control group (P=0.342) and the experimental group (P=0.727) was significantly different from the GGT variable from the control group (P=0.482) and experimental group (P=0.099). Thus, the data for the ALP and GGT variables are claimed to be normally distributed. The ALP variable (P = 0.237) and the GGT variable (P = 0.839) both passed the significant homogeneity test, indicating that the ALP and GGT variable data were homogeneous. The independent comparison test T-test findings indicated that the ALP variable (P=0.602) and the GGT variable (P=0.161) were not statistically significant.
Conclusion: The oral administration of polyvinyl chloride microplastic had no significant influence on the ALP and GGT levels in the blood of Rattus norvegicus Wistar strain in the experimental group compared to the control group.

Keyword: Microplastic, Alkaline Phosphatase, Gamma-glutamyl Transferase, Polyvinyl Chloride, Rattus norvegicus Wistar strain.

[1)] *Undergraduate Program Faculty of Medicine, Widya Mandala Surabaya Catholic University*
Email: leonardo.ado1320@gmail.com
[2)] *Department of Public Health, Faculty of Medicine, Widya Mandala Surabaya Catholic University*
[3)] *Student Doctoral Program of Public Health, Airlangga University*
[4)] *Department of Clinical Pathology, Faculty of Medicine, Widya Mandala Surabaya Catholic University*

INTRODUCTION

Plastic waste production continues to grow. Global plastic output hit 335 million tons in 2016, according to 2016 data. Asia produces the most plastic (50%), followed by Europe (19%), North America (18%), Africa (7%), and Latin America (4%). Additionally, it was stated that 20 of the 192 countries with coastlines generate more than 80% of the plastic garbage in the world's seas. Indonesia is the world's second greatest provider of plastic garbage, behind China, followed by the Philippines in third place. According to these statistics, we live in the Plastic Age (1). According to one projection, plastic trash production will exceed 33 trillion tons by 2050 (2).

Thompson et al. coined the word microplastic in 2004 to refer to the buildup of minute plastic fragments in marine sediments. Researchers have attempted to characterize microplastics in their terms, but there has been no clear consensus on this description. According to Frias and Nash, microplastics are insoluble in water solid synthetic particles or any polymeric matrix with regular or irregular forms and sizes ranging from 1 µm to 5 µm, including primary and secondary. Frias and Nash proposed this definition based on the statement of Arthur et al., who defined microplastics as plastic particles less than 5 µm in size (3), and Gigault et al.. The latter defined nano plastics as plastic particles of 1 nm in size up to 1 µm (4).

Microplastics in the environment can be classified into two categories based on the process by which they are formed, namely primary and secondary microplastics (5). Primary microplastics are plastic particles smaller than 5 mm in diameter that is purposefully created for use in a product (6). Primary microplastics are mostly created due to the daily use of plastic products. Microbeads in cosmetics (7), toothpaste, various baby products, abrasives in cleaning products (8), microfibers from clothing, microplastics discharged while painting, and artificial plants are all examples of primary microplastics (9). Secondary microplastics are plastic particles smaller than 5 mm in diameter created in the environment due to degradation and fragmentation processes (3). Mechanical abrasion, ultraviolet light, thermooxidation, and microbiological degradation all contribute to the process (6,10). Due to the intense UV radiation, mechanical abrasion by waves, oxygen availability, and turbulence, beaches are the most effective location for plastic fragmentation into microplastics (8).

Microplastics enter the oceans from a variety of sources. The primary source of microplastics in the ocean is wastewater treatment. Wind, gutters, and various other currents and fluxes all contribute to the production of microplastics that end up in the oceans (8).

Various aquatic organisms can consume microplastics in the oceans, including shellfish, echinoderms, amphipods, and zooplankton (11,12). The diversity of organisms capable of consuming microplastics is inversely proportionate to their size. The smaller the particles, the greater the variety of creatures capable of ingesting microplastics (8). At lower trophic levels, microplastics become more accessible to creatures. At a lower level, organisms are less picky and will consume anything of the appropriate size. Microplastics can be transferred between trophic levels, according to research. Higher trophic level creatures, such as humans, can consume microplastics indirectly by preying on polluted organisms (11). Microplastics can be discarded and negatively affect the species that consume them. Microplastics are known to produce liver toxicity and inflammation, one of their effects (8).

Microplastics contain a variety of organic pollutants, some of which are introduced during the manufacturing process, such as Diethylhexyl phthalate (DEHP) and others that are absorbed from seawater. Microplastics are a transport medium or carrier for a variety of toxic elements, including organic pesticides

(*dichlorodiphenyltrichloroethane, hexachlorobenzene*, and *polycyclic aromatic hydrocarbons*), persistent organic pollutants, synthetic dyes (safranin, brilliant green, and crystal violet), and various heavy metals (aluminum, lead, silver, copper, iron, zinc, and cadmium). Accumulating these substances in the body has been shown to stimulate the formation of reactive oxygen species (ROS). It is associated with various negative impacts, including homeostatic disorders, endocrine disorders, immune system problems, malignancies, and developmental problems in children (5,8,12).

Microplastics are ingested by humans mostly through food and beverages. This is corroborated by the discovery of high amounts of microplastics in various human food and beverages. After ingestion by humans, microplastics can be absorbed into the bloodstream and transported to multiple organs due to their small size (13). Microplastic particles can enter the intestine by endocytosis with the assistance of M cells from Peyer's Patch. Microplastics are absorbed and transferred from the gastrointestinal tract lumen to the lymphoid tissue mucosa via a paracellular perspiration mechanism. Paracellular perspiration is a mechanical mechanism that allows solid particles to flow through pores in the single-layered epithelium at the end of the digestive tract's villi (desquamation zone) and into the circulatory system (14,15). According to Deng et al. 2017 research, microplastics with a diameter of 5 µm and 20 µm can accumulate in the livers of rats after 28 days of exposure to a dose of 0.5 mg/day (16). Microplastics that collect in various organs have the potential to release toxic substances from the inside to the surface via a concentration gradient process and eventually spread to adjacent tissues, triggering an immune response (14). Numerous investigations have demonstrated that microplastics can disrupt the metabolism of amino acids, bile acids, and liver lipids (9).

Research on mammalian cells revealed that microplastics could cause oxidative stress, disruption of cell membranes, and activation of inflammatory cells and apoptotic pathways. Research by isolating rat hepatocyte cells aged 3 months indicated activation of Reactive Oxygen Species (ROS) and DNA damage due to exposure to microplastics (17). The accumulation of microplastics appears to be in conjunction with the increase in oxidative stress in the liver. Oxidative stress and alterations in the metabolic profile generated by microplastics eventually cause inflammation and decreased liver function (18). Inflammation generated by microplastics can cause injury or trauma to the bile ducts, which can encourage the proliferation and fibrosis of bile duct endothelial cells (19). This can lead to obstruction of the bile ducts. ALP levels will increase in response to the liver for obstruction of the bile ducts, and ALP will enter the blood circulation. Examination of ALP levels is sensitive but not specific. In contrast, examination of GGT levels is specific to signal liver problems so that the presence of bile duct obstruction can be detected by raising serum Alkaline Phosphatase (ALP) and Gamma-glutamyl Transferase (GGT). Both indicators have been accepted and used clinically to detect the existence of bile duct obstruction (20,21).

Increased production and degradation of plastic waste indicate a constant increase in human exposure to microplastics (13). Exposure to microplastics in humans produces oxidative stress, inflammation, immune system dysfunction, neurotoxicity, neoplasia, and metabolic and homeostatic problems (17). This has become a concern among the scientific community and the public (1). However, thorough research on the effects of microplastics on humans is considered very important until now. No research/clinical study can be carried out to evaluate the health risks due to exposure to microplastics in humans, so various

assumptions regarding the effects of microplastics on humans have not been proven and clearly understood. This is because knowledge of trustworthy biomarkers is considered insufficient, technological improvements are inadequate, and ethical considerations are considered (13,17). Currently, to quantify microplastics' effects on humans, research is carried out either in vivo or in vitro in mammalian models (13). Mammals are a standard model in toxicological research, so the potential health effects of microplastics in humans indicated by mouse studies should not be neglected (22). This study seeks to examine the effect of oral polyvinyl chloride microplastic intake on alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) levels in Rattus norvegicus Wistar strain.

METHOD

This is an experimental study. This study used a post-test-only control group design. The population used in this investigation was a 12-week-old male Rattus norvegicus Wistar strain.

All samples/tested animals were modified in the veterinary laboratory for seven days. The experimental animals were then randomly separated into two groups, one control and one experimental, each containing six experimental animals. The control group was not exposed to microplastics. In contrast, the experimental group received a dose of 0.5 mg/day of microplastic type PVC measuring ≤20µm in the form of a solution via a probe for 28 days.

After 28 days, the experimental animals were anesthetized with Diethyl Ether, and blood samples were taken through cardiac puncture. The animal's blood is sent to the examination site in less than two hours. The experimental animals were terminated via cervical dislocation and gathered in one spot to be burned.

In this study, the dependent variable was the concentrations of ALP and GGT in the blood of experimental animals, which were represented in Units per Liter (U/L). The Central Surabaya Health Laboratory evaluated the blood samples.

The independent variable in this study was a ≤20µm length of microplastic PVC. Microplastics were obtained by crushing PVC-type plastic with a Fomac FCT Z100 machine and screening with a mesh size of 625 to ensure that the microplastic employed was ≤20µm in size. A total of 0.5 mg of microplastic was dissolved in water and administered via a probe to experimental animals.

Statistical analysis of the blood sample data was performed using the Statistical Package For The Social Sciences (SPSS) tool. To begin, normality and homogeneity tests were conducted. After establishing that the data were normally distributed and homogeneous, a T-test comparison test was used to validate the hypothesis in this study using two free samples.

RESULTS

Results of Examination of ALP and GGT Levels in the Control and Experiment Group

Based on the data processing results with the help of SPSS presented in table 1, the average ALP variable for the control group is 203.5 with a standard deviation of ± 22.863. The experimental group is 215 with a standard deviation of ± 58.371. For the control group, the mean variable GGT is 17 .33 with a standard deviation of ± 1.211 and the experimental group 16.5 with a standard deviation of ± 1.378.

Table 1. Result of Examination ALP and GGT Levels in the Control and Experiment Group

Variabel	Kelompok Kontrol (U/L)	Kelompok Eksperimen (U/L)
ALP	203,5 ± 22,863	215 ± 58,371
GGT	17,33 ± 1,211	16,5 ± 1,378

ALP and GGT Variable Data Normality Test

According to the results of the normality test performed using SPSS, the significance value for the control group ALP variable is 0.342 (P>0.05) and the experimental group is 0.727 (P>0.05), while the significance value for the control group GGT variable is 0.482 (P> 0.05) and the experimental group is 0.099 (P> 0.05). Thus, the data for the ALP and GGT variables were normally distributed (P> 0.05), met the requirements of the T-test, and could be continued with the homogeneity test.

Table 2. Normality Test of ALP and GGT Variable Data

Variabel	Kelompok	Uji Normalitas	P	Kesimpulan
ALP	Kontrol	*Shapiro-Wilk*	0,342	Data berdistribusi normal
	Eksperimen		0,727	Data berdistribusi normal
GGT	Kontrol		0,482	Data berdistribusi normal
	Eksperimen		0,099	Data berdistribusi normal

ALP and GGT Variable Data Homogeneity Test

According to the results of the homogeneity test conducted using SPSS, the significance value for the ALP variable is 0.237 (P> 0.05) and for the GGT variable is 0.839 (P> 0.05), indicating that the data for the ALP and GGT variables are homogeneous (P> 0.05) and that the parametric test requirements have been met and can be continued with a T-test comparison test of 2 free samples.

Table 3. Homogeneity Test of ALP and GGT Variable Data

Variabel	Uji Homogenitas	P	Kesimpulan
ALP	*One-Way Anova*	0,237	Data homogen
GGT		0,839	Data homogen

Comparative Test T-test 2 free samples

According to the results of the T-test comparison test performed on two independent samples using SPSS, the significance value for the ALP variable is 0.602 (P>0.05). The significance value for the GGT variable is 0.161 (P>0.05), indicating that there is no significant difference in the two variables (P>0.05) between the control and experimental groups.

Table 4. Comparative T-test of 2 Free Samples

Variabel	Uji Komparasi	P	Kesimpulan
ALP	Independen *T-test*	0,602	Tidak ada perbedaan yang bermakna
GGT		0,161	Tidak ada perbedaan yang bermakna

DISCUSSION

The levels of alkaline phosphatase and gamma-glutamyl transferase were compared between the control and experimental groups of male Rattus norvegicus Wistar strains. Microplastics were not present in the control group. For 28 days, the experimental group was given oral exposure to microplastic type PVC measuring 20 m at a dose of 0.5 mg/day. The inclusion criteria for all samples and reserves used were met.

This study was conducted in response to Deng et al. (2017) findings that rats exposed to 20 m microplastic at a dose of 0.5 mg/day for 28 days developed accumulation, inflammation, and necrosis in the liver (16). This inflammation is subsequently associated with bile duct damage/trauma, which can result in obstruction, impairing the liver's excretory function, namely bile acid excretion (2,19,20).

In line with Deng et al. findings, a Conti et al. (2021) study showed that vinyl chloride induces liver and central nervous system organ damage. Vinyl chloride relates to liver cancers such as angiosarcoma and hepatocellular carcinoma (23). This theory appears to be consistent with a recent case study on angiosarcoma in workers exposed to vinyl chloride by Guido et al. (2016). (24). Several more research, including those conducted by Karbalaei et al. (2018), Sharma et al. (2017), and Lam et al. (2018), have established that microplastic PVC causes endocrine system issues, liver function abnormalities, and delayed fetal development (27). There were no studies on the influence of microplastics on the excretory function of hepatic bile before the researchers conducted this investigation, which is why this study was conducted.

PVC microplastic is the most harmful type of plastic due to its composition of carcinogenic materials, some of which can cause mutations (23). Microplastics have been discovered in the digestive systems of a variety of marine species, including shellfish, crustaceans, and fish (25). According to Gabriel Enrique De-la-Torre (2020), only microplastic particles with a size ≤20 μm can reach specific organs, while particles of smaller size can reach all organs and tissues (28). Microplastics have been discovered to collect in the liver, spleen, and kidney and have been shown to translocate to other tissues and organs, particularly during times of inflammation (6). PVC has the potential to leak toxic monomers into the environment, causing cytotoxicity, chronic inflammation, and oxidative stress in nearby organs and tissues (25,28). The notion of microplastics and their hazardous effects corroborates the findings of the studies conducted by various researchers.

The statistical analysis of the sample examination data using SPSS revealed that the data were normally distributed (Table 5.2) and homogeneous (Table 5.3), but no significant results were obtained for the independent comparison test T-test (table 5.4), indicating that there is no significant difference between experimental animals in the control group and experimental animals exposed to microplastics (experimental group). This contradicts the presented hypothesis, which stated that the male Rattus norvegicus Wistar strain group exposed to microplastics would have higher ALP and GGT levels than the control group. The research hypothesis is rejected based on the graphic description and the results of statistical tests.

The study's insignificant findings were influenced by five factors: the time of microplastic exposure, the size of the microplastics exposed, antioxidant levels, the type of cell death that occurred, and the potential of microplastic contamination. The experimental mice were exposed for only 28 days, even though inflammation can result in proliferation, fibrosis, and obstruction of the bile ducts over a longer/chronic period. Shrestha et al. (2016) reported fibrosis in the livers of rats after administering CCL4 intraperitoneally for 10 weeks, while Gracia-Sancho et al. (2019) found fibrosis in the livers of rats after administering CCL4 orally for 12 to 14 weeks (29,30).

The size factor of the specified microplastic; in this study, ≤20 μm microplastics were used, indicating that the actual size fluctuates between nano plastic and 20 μm. According to Deng et al. (2017)'s study, which served as the basis for determining the size of microplastics in this study, microplastics measuring 20 μm accumulated more in the liver, whereas microplastics measuring 5 μm accumulated more in the digestive tract and kidneys (16). Microplastics with a size closer to 20 μm will be stopped in the liver, but those with a size 5 μm will continue to circulate and be filtered and stopped in the kidneys. The size of microplastics is one of the aspects that could affect the study's results because if the microplastics utilized in this study are closer to 5 μm in size than those

measuring 20 µm, then some of the microplastics will collect in the digestive system and kidneys in an insufficient amount of liver of an experimental animal. Suppose there is insufficient microplastic formation in the liver. In that case, there will be no situation/condition of oxidative stress, preventing hepatocyte cell death and maintaining the liver in a functionally normal state. As a result of this assertion, the microplastics employed in this study may be closer to the nano plastic limit (5 µm) than the exact size of 20 µm.

Oxidative stress occurs when reactive oxygen species (ROS) levels exceed antioxidant levels. Antioxidants such as SOD, GSH, and CAT contribute to the body's ROS degradation. If the number of antioxidants available is sufficient to break down ROS, there will be no ROS excess, or what is referred to as oxidative stress. There will be no inflammation in the liver of the experimental animal if there is no oxidative stress (31,32). This is also related to the duration of the exposure, with the researchers suspecting that due to the short duration of the exposure, the body was still strong enough and capable of maintaining homeostatic circumstances. Because neither ROS nor antioxidant levels were tested in this investigation, this must be considered a factor is impacting the study's outcomes.

While it is intended that this study would result in cell death via the necrosis route, it should also be acknowledged that what is occurring is apoptosis. In contrast to necrosis, produced by acute cell destruction, apoptosis is a process in which cells die on a regular/programmed basis. Necrosis results in the formation of a necrotic body, which upon lysis or rupture activates an immunological response, one of which is macrophages, which can activate hepatic stellate cells and produce more severe inflammation. Apoptosis is a process of programmed cell death that does not induce inflammation and hence has no adverse effect on liver tissue. According to the description above, it is vital to consider that the apoptotic process of cell death remains the predominant pathway in this study, as opposed to the necrosis pathway.

Food and drink offered to experimental animals was an uncontrolled variable in this investigation. Food and beverages in the control group were not screened in advance for the presence of microplastics. According to the theory described in the literature review part of this study, it is possible that the food and beverages given to experimental animals in the control group were contaminated with microplastics. However, this had no significant impact on the study's results.

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CONCLUSION

There were no significant impacts between the experimental and control groups regarding alkaline phosphatase and gamma-glutamyl transferase levels in the blood of Rattus norvegicus Wistar male strains.

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