THE EFFECT OF PERORAL POLYVINYL CHLORIDE MICROPLASTIC ON THE VALUE OF PROTHROMBIN TIME AND ACTIVATED PARTIAL THROMBOPLASTIN TIME IN RATTUS NORVEGICUS WISTAR STRAIN

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ABSTRACT

Introduction: Microplastics can enter the human digestive system as polyvinyl chloride (PVC). Microplastics ingested by humans will accumulate in several organs. Microplastic accumulation in the liver causes inflammation, which damages hepatocyte cells, impairing liver synthesis function, one of which is the synthesis of blood clotting factors.
Purpose: The purpose of this study was to determine the effect of oral microplastic polyvinyl chloride on prothrombin time (PT) and activated partial thromboplastin time (APTT) in Rattus norvegicus strain Wistar (APTT).
Method: The experimental design incorporated a post-test-only control group. There were 12 rats randomly assigned to the control (K) or experimental (E) groups. For 28 days, Group E was exposed to microplastic type PVC at a concentration of up to 0.5 mg/day in 1 cc of Aquabidest via an oral probe. Blood samples were analyzed using a coagulation analyzer at BBLK Surabaya. The statistical test used an independent t-test.
Result: There was a significant difference in the mean PT value of group K (9.8 ± 0.99 seconds) compared to group E (14.23 ± 9 seconds) (p=0.024) and the mean APTT value of group K(18.32 ± 7.96 seconds) compared to group E(26.1 ± 18.15 seconds) (p=0.022).
Discussion: These findings confirm the theory that exposure to polyvinyl chloride microplastics in the liver can induce hepatocyte cell inflammation and impair the liver's ability to synthesize blood clotting factors, resulting in prolonged PT and APTT values.
Conclusion: Oral administration of PVC microplastic affects PT and APTT values.

Keyword: Microplastic, Polyvinyl Chloride, Blood Clotting Factors, Prothrombin Time, Activated Partial Thromboplastin Time.

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INTRODUCTION

The global consumption of plastic continues to increase. This is directly related to the increase in plastic production without a corresponding increase in waste management. According to 2016 data, global plastic production surpassed 335 million tons yearly [(1)]. Asia is the world's largest producer of plastic. With a plastic production capacity of 65.2 million tons annually, Indonesia is the world's second-largest producer [(2)].

This data will probably continue to increase as plastic is increasingly used by the public due to its low cost, ease of manufacture, resistance to water, durability, and corrosion. However, the benefits of plastic are not proportionate to the amount of time required for it to degrade again. This is the primary issue in plastic waste management [(3)].

Indonesia is currently ranked second behind China among the 20 countries that contribute the most plastic waste globally. According to data, the combined amount of plastic waste generated by these twenty countries exceeds 26.46 million metric tons annually and continues to increase. Indonesia generated 3.22 million metric tons of plastic waste, and between 0.48 and 1.29 million metric tons were discharged into the sea [(4)].

Plastic is a manufactured polymer that is extremely difficult to decompose in nature, taking nearly hundreds of years to decompose completely. Plastic degradation can occur due to the thermal oxidation process with UV light, temperature, and/or mechanical means. Microplastics are plastics that have degraded to less than 5 millimeters. According to some sources, microplastics are synthetic solid particles or polymer matrices with regular or irregular forms and sizes ranging from 1 micron to 5 millimeters. They include both water-soluble and insoluble plastics [(5)]. Microplastic particles are extremely difficult to collect and remove from the environment. Microplastics have been an issue since 1970, but they have been an interesting topic recently due to their significant influence on the environment and living creatures, particularly people [(3)].

A deficient waste management system leads to a large amount of waste being discharged into the sea, particularly degraded plastic waste. Microplastics are easily disseminated throughout the marine ecosystem due to their vast number and microscopic size. Data indicates that a range of marine organisms, including fish, seabirds, sperm whales, and other creatures, ingest plastic [(6)].

Microplastics are absorbed into our bodies via the digestive and respiratory systems through food consumption or inhalation of contaminated air. Even more recently, a study discovered that sugar contains 0.44 MPs/g, salt has 0.11 MPs/g, alcohol contains 0.03 MPs/g, and bottled water contains 0.09 MPs/g. Humans are also predicted to consume approximately 80 MPs/g per day via microplastic-infected fruits and vegetables absorbed from contaminated soil [(7)]. Additionally, due to the characteristics of many microplastics and their small size, microplastics are more easily absorbed into our respiratory tract. This is certainly hazardous for workers, particularly those employed in synthetic textile industries [(8)].

According to research, annual increases in fish consumption constitute a potential concern to human health, as ingestion of some marine species may result in the transfer of microplastics and associated chemicals. Even after the fish's internal organs are removed, particles smaller than 150 microns can flow via the intestines to the lymph nodes and into the circulatory system [(9)]. In humans, the same thing occurs. Microplastics are absorbed in the human digestive tract by paracellular perspiration, which allows them to flow through gaps in the single-layered epithelium at the end of the villi and into the circulatory system, where they are transported to organs and all body tissues [(10)].

Deng et al. showed that microplastics with a size of 5 µm accumulated substantially over 20 µm in the liver, kidney, and intestines after 28 days of exposure [11]. Additionally, research shows that microplastics can function as a stimulus to create Reactive Oxygen Species (ROS) at specific concentrations. Increased ROS production has the potential to cause genotoxic stress and DNA damage. Microplastics can also activate pro-inflammatory cells, resulting in cell death (apoptosis)[12].

Other research shows that microplastic accumulation can result in oxidative stress and alterations in metabolic profiles, resulting in disturbances in fat and energy metabolism [13]. This is shown by a significant decrease in acetylcholinesterase activity (aCHE). Along with aCHE, a reduction in triglyceride and total cholesterol levels was observed.

Microplastic buildup in the liver, which increases ROS production, gradually causes liver damage via cell necrosis. Additionally, there will be variations in the production of anticoagulant and thrombotic components in the liver throughout time. There was a decrease in the synthesis of coagulation factors (II, V, VII, X, XI, XII, and XIII) and fibrinogen, as well as thrombocytopenia, reduced platelet function, and vitamin K shortage in anticoagulant factors. In thrombotic factors, there is a rise in factor XIII synthesis, a decrease in von Willebrand factor synthesis, and a decrease in liver protein C, protein S, and anti-thrombin 3 synthesis. This results in patients with liver disease-producing fewer clotting factors. They do, however, create fewer clotting-inhibiting factors. Hemostasis is highly dependent on the liver's synthesizing activity and the interaction of the pro-coagulation and anticoagulation pathways. The PT, INR, and APTT coagulation profiles are the most often used in clinical laboratories [14].

Microplastics are not a minor matter that we can ignore, especially considering the health risks they represent to animals, particularly rats. We must be alerted to prevent this from spreading further down the human food chain. According to the researchers, no research journal has been identified examining the effect of microplastic accumulation in the liver on the hemostasis process. As a result, researchers conducted microplastic exposure studies to ascertain the influence of oral polyvinyl chloride microplastics on the PT and APTT values in Rattus norvegicus Wistar strains.

METHOD

This is an experimental study with a single post-test control group. Male Rattus norvegicus Wistar strain rats aged ±12 weeks with a weight of 180-200 grams in good health were used in this study. A total of 12 rats were randomly assigned to the control (K) or experimental (E) groups. For seven days, the animals were adapted in the Animal Laboratory of the Faculty of Pharmacy, Widya Mandala Catholic University, Surabaya.

For 28 days, the experimental group was exposed to microplastic PVC measuring ≤20 µm as 0.5 mg/day in 1 cc of aquabidest via an oral probe. All rats were sedated with diethyl ether, and blood was obtained using the heart puncture procedure after 28 days. The drawn blood is placed in a 3 ml plain Vaculab blood tube that has been treated with 3.8 percent sodium citrate in a 1:9 ratio (0.1 ml sodium citrate: 0.9 ml blood plasma). The animal's blood is sent to the examination site in less than two hours. The experimental animals were killed via cervical dislocation and collected in one location to be burned.

In this study, the dependent variable was the PT and APTT concentrations in the blood plasma of experimental animals expressed in seconds. The Surabaya Health Laboratory Center evaluated the blood samples.

In this study, the independent variable was microplastic polyvinyl chloride with a size of ≤ 20µm. Microplastics are created

from the collection of plastic waste at Kenjeran beach. The chosen plastic is a PVC polymer that has degraded naturally in nature through biological, chemical, or mechanical degradation. After that, the plastic is crushed to a particle size of less than 20 µm. The crushed plastic was then filtered to guarantee that the resulting particles were ≤20 µm in diameter.

Statistical analysis of the blood sample data was performed using the Statistical Package For The Social Sciences (SPSS) application. 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 PT and APTT Values in Control and Experimental Groups

Table 1 and Table 2 show the average value of PT control group is 9.8 seconds with a standard deviation of 0.99 seconds and the experimental group PT has a mean value of 14.23 seconds with a standard deviation of 9 seconds. For the results of the average value of APTT the control group is 18.32 seconds with a standard deviation of 7.96 seconds, and the APTT experimental group has a mean value of 26.1 seconds with a standard deviation of 18.15 seconds.

Table 1. Mean Value and Standard Deviation of PT

Treatment Group	Mean PT Value	Standard Deviation
Control	9.8	± 0.99
Experiment	14.23	± 9

Table 2. Mean Value and Standard Deviation of APTT

Treatment Group	Mean APTT Value	Standard Deviation
Control	18.32	± 7.96
Experiment	21.1	± 18.15

Normality Test of PT and APTT Variable Data

The significance value of the PT variable in the control group is 0.140 (P>0.05) and in the experimental group is 0.165 (P>0.05) based on the results of the normality test with SPSS presented in Table 3. The significance value of the APTT variable is 0.165 (P>0.05) based on the results of the normality test with SPSS presented in table 4. The control group had a mean of 0.130 (P>0.05), and the experimental group had a mean of 0.589 (P>0.05), indicating that the PT and APTT variable data were normally distributed (P>0.05) and met the T-test requirements, allowing the study to proceed using the homogeneity test.

Table 3. PT Data Normality Test using Shapiro-Wilk

Group	P (>0,05)	Note
Control	0,140	Normal distributed data
Experiment	0.165	Normal distributed data

Table 4. APTT Data Normality Test using Shapiro-Wilk

Group	P (>0,05)	Note
Control	0,130	Normal Distribution
Experiment	0,589	Normal Distribution

Homogeneity Test of PT and APTT Variable Data

Based on the results of the homogeneity test with the help of SPSS presented in table 5, the significance value of the PT variable is 0.070 (P> 0.05), and the APTT variable was 0.168 (P> 0.05), so it is stated that the PT and APTT variable data were homogeneous (P> 0.05) and the parametric test requirements have been met. They can be continued with a T-test comparison test of 2 free samples.

Table 5. Table of Homogeneity Test of Research Result Data

	P (>0,05)	Note
PT Data Processing Value	0,070	Homogeneous
APPT Data Processing Value	0,168	Homogeneous

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 PT variable was 0.024 (P0.05), and the APTT variable was 0.022 (P0.05), indicating that there was a significant difference between the control and experimental groups (P0.05) in both variables.

DISCUSSION

The prothrombin time and activated partial thromboplastin time values in the blood of Rattus norvegicus Wistar strain were compared between groups exposed to and not exposed to microplastics. The experimental group received treatment from oral exposure to 20 m of microplastic type PVC at a dose of 0.5 mg/day.

The results of this investigation indicated that oral microplastic exposure altered the prothrombin time and activated partial thromboplastin time values in rats of the Rattus norvegicus Wistar strain in both the control and experimental groups. According to the sample assessment results, the average value for the PT control group was 9.8 seconds. This is less than the average of 18.32 seconds for the treatment group. APTT has a mean value of 14.23 seconds in the control group. This result is also less than the average for the APTT experimental group, 26.1 seconds. These two findings corroborate Deng et al. that microplastics with a diameter of 20 µm may accumulate in the liver after 28 days of exposure, causing several changes in liver function, most notably coagulation factor synthesis.

Soultati's research on coagulation function abnormalities in liver disease established that the liver plays a critical role in the blood clotting process because the liver serves as a site for the production of most blood clotting factors and their inhibitors [(15)]. This is proven by research findings that uncontrolled bleeding accounts for 60% of deaths during invasive procedures performed on patients with a history of liver cirrhosis [(15)]. Soultati revealed that PT prolongation of more than 1.5 seconds and 2.5 seconds resulted in a 47% and 87% mortality rate, respectively, compared to a 7% mortality rate in patients with normal PT values [(15)]. Additionally, another research showed that bleeding occurred in 11% of patients with acute liver failure, with the most of bleeding occurring in the upper gastrointestinal tract, indicating that extension of time on PT examination is a hallmark and marker of coagulopathy [(16)].

The hypothesis was tested using an independent T-test comparison between the control and experimental groups. The results obtained from this test were P 0.05. This demonstrates a significant difference in the PT and APTT values between the control and MP-exposed groups. These findings support the hypothesis that the buildup of microplastics in the liver is one of the reasons for inflammation in the liver, which can damage hepatocytes and impair blood clotting function, both of which are primarily produced by liver cells. These factors included factors I, II, V, VII, VIII, IX, X, XI, XII, and XIII. The prevalence of intrinsic and extrinsic mechanisms is largely determined by factors synthesized by the liver (17). Additionally, PVC is classified as plastic with the triangle logo number three. That is, it is biologically classified as a foreign object that is hard for the body's immune system to remove and can bioaccumulate in the liver. Meanwhile, the components of PVC microplastics include various

contaminating substances such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and DDT. This component is lipolytic, a carcinogen, and readily absorbs other hazardous substances, causing harm to the liver's synthetic function [58].

Table 6. Hypothesis Testing Table using the Independent T-Test

Variable	Mean Control ± SD	Mean Experiment ± SD	P (<0,05)	Note
PT	9.8 ± 0.99	14.23 ± 9	0,024	Significant
APTT	18.32 ± 7.96	21.1 ± 18.15	0,022	Significant

The results of this investigation indicated that exposure to microplastic type polyvinyl chloride measuring ≤20 µm orally at a dose of 0.5 mg/day for 28 days resulted in a difference in the PT and APTT values between the control and experimental groups in the form of decreased liver synthesis function. This conclusion is based on the fact that there is a statistically significant difference in the PT and APTT test values between the control and experimental groups. These findings corroborate Deng et al. that microplastics with a size of ≤20 µm can impair liver function. However, the control group in this study cannot be used as a reference for normal values because the researchers used a post-test-only control group design, which prevented researchers from knowing what the normal or initial values for PT and APTT were before microplastic exposure. This is because the technique used to collect blood is the cardiac puncture technique. Apart from failing to obtain ethical approval, the heart puncture procedure cannot be repeated on experimental animals (pre and post). Microplastic research has its own set of difficulties in preventing contamination of experimental animal food and beverages, air, and cage care, among others.

The findings of this study may be due to the possibility that microplastics act as a catalyst for the generation of oxidative stress. Increased oxidative stress stimulates the release of pro-inflammatory cells, which stimulates the production of reactive oxygen species (ROS). If this repeatedly occurs during exposure, acute inflammation can progress to chronic inflammation, resulting in hepatocyte cell damage or death (18). Cell death will continue indefinitely until the liver cannot compensate and its function is disrupted. One of the liver functions noticed here is blood clotting, as the liver synthesizes the majority of coagulation factors.

CONCLUSIONS

There was a significant effect on the value of prothrombin time and activated partial thromboplastin time in the blood plasma of Rattus norvegicus Wistar strain due to oral intake of polyvinyl chloride microplastic in the experimental group compared to the control group.

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