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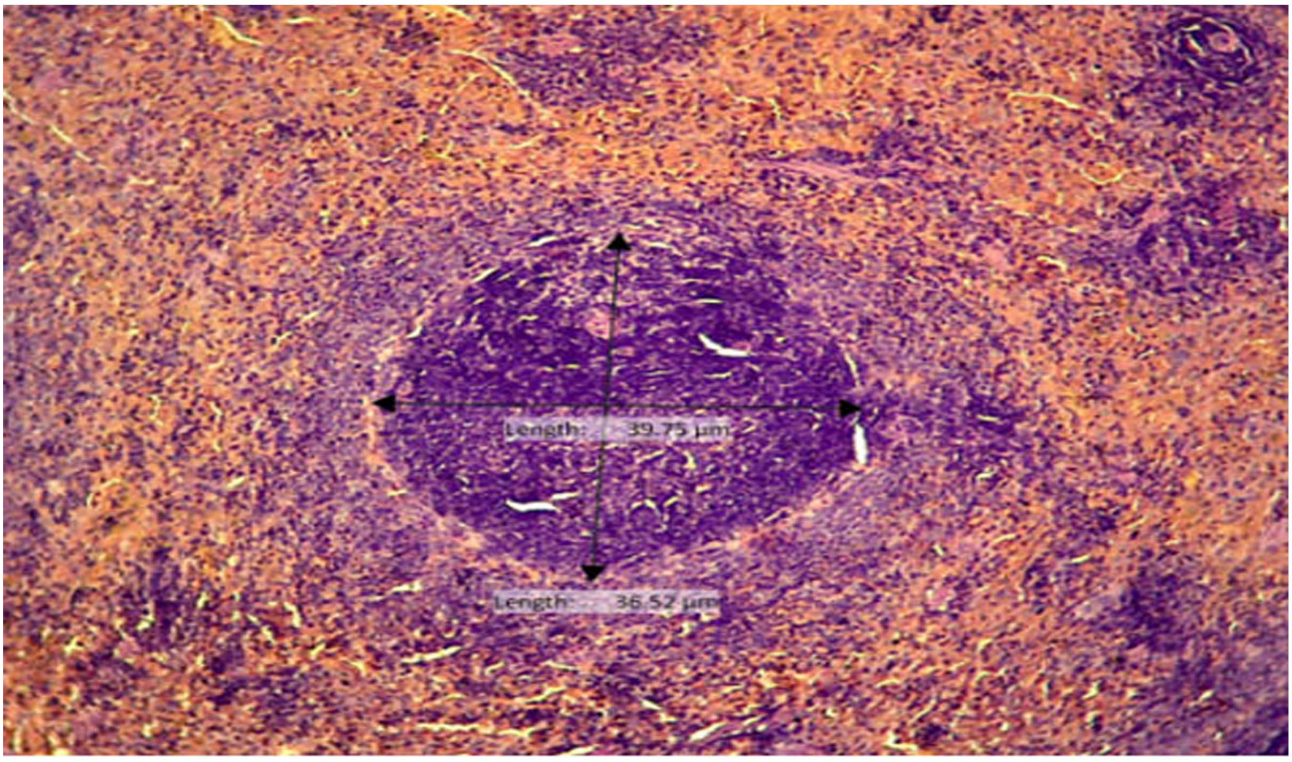

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LIPJPHKI

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THE EFFECT OF POLYPROPYLENE PLASTIC RESIDUE ON HEATED PALM OIL AGAINST THE HISTOPATHOLOGICAL CHANGES OF KIDNEY ON MALE RATS (*Rattus norvegicus*)

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ABSTRACT

This research was conducted to observe the effect of polypropylene plastic residue on heated palm oil against the histopathological changes on rat's kidney. There were 18 male rats (*Rattus norvegicus*) that used as the examination animal and were divided into three groups and were given the mixture of heated palm oil and polypropylene plastic per oral with intubation needle. The treatment was done for five weeks. The treatment for P0 group was 84 g of palm oil, for P1 was 84 g of palm oil with 2.3 g of plastic, and for the P2 was 84 g of palm oil with 6.8 g of plastic. The histopathological changes of the kidney were observed using microscope with 400x magnification and the data was analysed using Kruskal-Wallis and continued with Mann Whitney U test. The result showed that there were significant differences between three groups of treatment ($P<0.05$). There were effects of polypropylene plastic residue on heated palm oil against the histopathological changes of kidney in this research such as cast, epithelial sloughing and necrosis in the mild scale.

Key words: Kidney, Palm Oil, Polypropylene Plastic, *Rattus norvegicus*

INTRODUCTION

Plastic become widely used materials in daily necessities thus it is hard to be separated from society because plastic is considered more convenient and quite strong. One of the utilisation of plastic is for food packaging. In the past two decades, plastic packaging has seized the world's packaging market share, replacing the packaging from cans and glasses (Sulchan *et. al.*, 2007).

However, aside from the favourable features, plastic also has negative traits which can be detrimental to people. Plastics are polymeric materials that generally obtained from petroleum derivatives which indicate that plastics fabrics come from non-renewable and non-biodegradable sources. In addition for being one factor of environmental damage, plastic also has its own risk of health. One of the health problem caused by plastics such as stated on previous research that the reckless use of plastic was able to release carcinogenic compounds (causes and triggers cancer), which able to stimulate the growth of cancer cells (Damanik, 2012). In several occasions, plastic is often used for packing the food, either it is in the low or in the high temperature.

Although giving a convenient impression, using a food packaging like that also have negative impact because the wrapped food especially ones with high temperature will react with the chemical substance which contained in the plastic (Setyowati *et. al.*, 2017). On the same study from Setyowati *et. al.* (2017), it was explained that the elements on plastic will easily

contaminating food if the food itself is on high temperature.

In the past few years were known the fraud that done by street vendors where they mix the plastic to the heated palm oil which used for frying the fried food they offer. By frying the plastic all at once together with the palm oil they used, the street vendors believe that the food would be more crispy and durable.

Based on screening that conducted in Jati Padang, Padang, West Sumatera by Sari *et. al.* (2014), showed that 9 of 10 samples of heated palm oil from several street vendors contained *isopropyl* compound from *polyethylene* and *polypropylene* plastic. The other screening was conducted by *Ryosa et. al.* (2017) on 5 samples that taken from the street vendors at Jati Padang, Padang, West Sumatera. Based on the screening result, it found that 3 of 5 samples that taken form the street vendors contained plastic derivate up to 15,65%, which was cyclopentene. Most derivate that can be found on plastic are benzene (phthalate), acrylic, and cyclopentene (Ryosa *et. al.*, 2017).

Lithner (2011) in his thesis stated that in the process of manufactured polymer plastic were also used several of chemical substances as additives to support its function. Many additives that used are hazardous for both health and the environment. Many of the chemicals that are used to make plastic polymers and plastic products have been tested for toxicity to a varying extent, and the assembled data have provided the basis or harmonised environmental and/or health classification for these substance (Lithner, 2011). In the same study, Lithner also stated that the knowledge of effects from exposure to the mixture of substance that may be released from complexly composed plastic products is very limited.

Based on the previous research, foreign substances such as microplastics which were absorbed by intestine were detectable in the lumen of blood and lymph vessel and were eventually eliminated in the urine. This also verifies there is translocation of foreign substance from the intestine to other body fluids is possible.

The rate of renal blood flow of approximately 400 ml/100 mg of tissue per minute is much greater than that observed in other well perfused vascular beds such as heart, liver, and brain (Matovinović, 2009). Kidney with its function to eliminate waste products and foreign substances in the body will eventually be affected by polypropylene plastic contamination which presents on heated palm oil. Renal damage can impair renal perfusion as well as glomerular and/or tubular functions (Lang *et. al.*, 2000). Additionally, the glomerulus is equipped with its own clearance mechanism, a function of mesangial cell (Newman *et. al.*, 2007). Continued or severe injury can result in chronic changes characterized at first by atrophy and fibrosis of the glomerular tuft and secondarily by atrophy of renal tubules (Newman *et. al.*, 2007).

Renal tubular epithelial cells can response to injury by undergoing degeneration, necrosis, apoptosis and/or atrophy (Newman *et. al.*, 2007).

RESEARCH MATERIALS AND METHOD

Materials and Equipment

The experimental animal that used in this research were 18 male rats (*Rattus norvegicus*) Wistar strain with average body weight around 150-200 g. Materials that used in this research were polypropylene plastic, palm oil, feeds, and drinking water. The materials that used to make histology slides were formaldehyde 10% for fixation, alcohol 70%, 80%, 90%, and 96% for dehydration process, paraffin for embedding process, xylol for clearing process, and dissolving paraffin and haematoxylin eosin for staining.

The equipment that used in this research were scale to measure the weight of the rats, three units of cage which made from plastic and equipped by wire, feed container and drink, intubation needle for rat, tuberculin syringe, surgical scissor, forceps, scalpel, plastic pots, object glass, cover glass, microscope, optilab camera, and a camera for documentation. The equipment that used for making the histological slides are oven, microtome, water bath, hot plate, platening table, staining jar, staining rack, and paraffin dispenser.

Rats were chosen randomly and divided into three groups P0, P1, and P2. Each group of treatment consist of 6 rats and before given the treatment, rats were adapted for one week and only provided with feed and drink.

Research Plan

In this research there was only one source of variability which was the random effect of treatment to the rats, therefore the different result of the treatment only caused by the treatment's effect and random effect. This research used three groups and six replicates for each group.

Treatments

Rats (*Rattus norvegicus*) were captived in the cage placed in Laboratory of Experimental Animal at the Faculty of Veterinary Medicine Universitas Airlangga, and were divided into three groups. After one week of the adaptation period, the treatment groups were given with heated palm oil that contained polypropylene plastic residue per oral for five weeks.

The treatment explained as follows:

a. P0 = Control, 84 g of palm oil;
b. P1 = 84 g of palm oil with 2.3 g of plastic;
c. P2 = 84 g of palm oil with 6.8 g of plastic.

After 24 hours from the last treatment, the rats were euthanized by giving chloroform and the kidney of the rats collected.

Microscopic Examination

Microscopic examination was including scoring of the damage that happened in the renal tubules such as cast, epithelial sloughing, and necrosis. The examination was conducted by observing five fields of view with 400 x magnifications and the determination score was modified from Arsad *et. al.* (2014). The scores that were collected from cast, epithelial sloughing, and necrosis were summed to get the total score.

Data Analysis

The data that collected from this research was analysed using Kruskal-Wallis test then continued with Mann-Whitnney U test to see the significant differences ($p<0.05$) of the comparison on each treatment group. The data analysis used Statistical Package for the Social Sciences (SPSS) 2.1 program.

Research Result

The histopathological slides were stained using Haematoxylin and Eosin (H&E). The microscopic examination was done by observing the 5 different fields of view of each histopathology slide using 100x magnifications and then continued with 400x magnifications.

The comprehensive results are displayed on table 1 wherein this table can be known the average or mean value of damages that observed. From the same table also can be known the average value of the total score of each damage on each treatment group. On the P0 group has the mean score of the total damage as much as 0.36. While in

the P1 group was 0.76 and in the P2 group was 1.43. Meanwhile in the table 2 showed the comparison result of each treatment group. The comparison difference between P0 group and P1 group has significant differences (p=0,018) and the comparison between P1 and P2 also has the same value (p= 0,018). There was a significant difference as well between the P0 and P2 (p= 0,005). The result of the tubules damage score were in the following table:

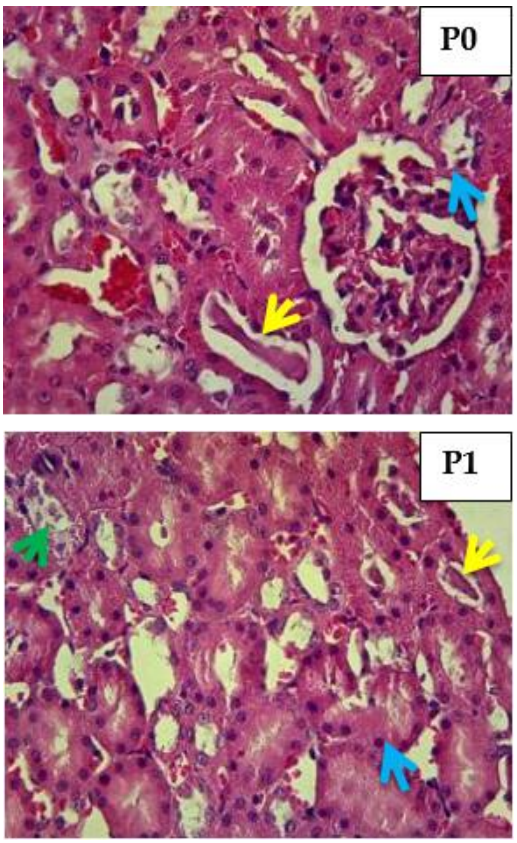
Table 1. The Average Value of Damages in the Tubules

Treatment	Cast	Epithelial Sloughing	Tubular Necrosis	Mean Total Score
P0	0,26	0	0,1	$0,36^{a} \pm 0,26$
P1	0,06	0,3	0,4	$0,76^{b} \pm 0,19$
P2	0,23	0,5	0,7	$1,43^{c} \pm 0,46$

Table 2. The Result of Mann-Whitney U test for Comparison Between Groups

Comparison Between Group	Asymptotic Significant (p<0.05)
P0 and P1	$0,018^{a}$
P0 and P2	$0,005^{b}$
P1 and P2	$0,018^{c}$

Different superscript indicated significant differences (p<0.05)



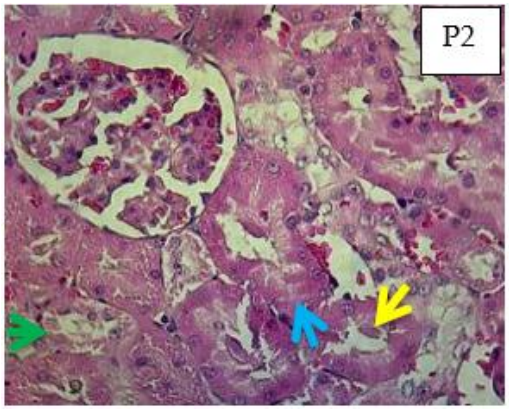


Figure 1. The histopathological changes of rat's kidney with H&E staining and 400x magnification using Optilab viewer. The yellow arrow shows cast in tubules; the green arrow shows tubular epithelial sloughing; and the blue arrow shows necrosis.

DISCUSSION

Based on the research result, there were significant differences on each treatment group. On the P0 group that only given heated palm oil encountering significant differences compared to the P1 and P2 group that each group was given 2.3 g and 6.8 g of plastic.

The damages that occur in the tubules in this research are the effect of plastic residue that contained in the heated palm oil. However, the actual exposure does not come from only on product but from a whole array of different plastic products, and exposure to a mixture of chemicals will often be continuous (Lithner, 2011). Additives are not bound to the polymer matrix and because of their low molecular weight, these substances can leach out of the plastic polymer into the surrounding environment, including into air, water, food or body tissues (Galloway, 2015). Volkheimer (1977) on Galloway (2015) explained that persorption of particles can occur as a passive process in areas of the gut where the intestinal mucosa is covered by a single layer of epithelium and persoberd particles were detectable in the lumen of blood and lymph vessels within minutes and were eventually eliminated in the urine. This also confirming that translocation of particles from the gut to the other body fluid is possible.

The surface properties of nanopolymer are predicted to adsorb macromolecules such as proteins and lipids from the surrounding body fluids onto their surface, in a process influenced by surface energy, charge and specific affinity for certain biomolecules (Galloway, 2015). Fruijter-Polloth (2012) on Galloway (2015) stated that translocation is dependent on interactions with the cell membrane and is most likely to proceed, as for uptake by enterocytes in the gut, through pinocytic, phagocytic, and receptor-mediated endocytosis.

The damage that can be seen in this research, particularly in the P1 and P2 group, were the changes on the tubules that experienced necrosis. Necrosis is a form of a cell injury that result the death of cells in tissue. Necrosis may occur as a direct adverse effect of a metabolite or xenobiotic on the tubules or it may occur secondary to ischemia, but the morphologic picture and sequelae are generally similar (Frazier *et. al.*, 2012).

The necrotic tissue will cause inflammation and it will be destroyed

and removed with the purpose to pave the way for the repair process to replace the necrotic tissue (Arimbi *et. al.*, 2015). Some common causes of tubular necrosis are (1) severe ischemia and inadequate supply of oxygen and nutrients to the tubular epithelial cells and (2) poisons, toxins, or medication that destroy the tubular epithelial cells (Guyton, 2006).

From the samples that were collected and observed, the tubules encountered necrosis. Moreover, some of the tubules appeared to be scattered and hard to distinguish between each other as the borders hardly visible. The renal tubules have epithelial cells that have high metabolic activity so that it makes the renal tubules are more susceptible towards any pathological changes compared to glomerulus, intrarenal blood vessels, and interstitial.

The urinary cast also presents and can be seen from this research, especially in the P0 group. Casts are a structure that mostly composed of mucoprotein and may form in the presence or absence of cells in the tubular lumen. A low number of casts may not indicate any renal pathology because cats have been identified in the urine of animal that didn't encounter any tubular injury. However, large amounts of protein within tubules can induce damage to the surrounding tubular epithelium as well (Frazier *et. al.*, 2012).

Each nephron contains (1) a tuft of glomerulus capillaries called the glomerulus, through which large amounts of fluid are filtered from the blood, and (2) a long tubule in which the filtered fluid is converted into urine on its way to the pelvis of the kidney (Guyton, 2006).

When nephrons are lost because of injury, remaining tubules can undergo compensatory hypertrophy in an attempt to maintain overall renal function, but there is no regeneration of nephrons (Newman, 2007).

CONCLUSION

Based on the result of this research, it can be concluded that there were effect of polypropylene plastic residue on heated palm oil against the histopathological changes of kidney on male rats. The histopathological damages that showed on this research were in the mild scale.

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