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Effect Of Antiseptic on Male Mice (*Mus musculus* L.) Body Weight

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ABSTRACT

The safety of using liquid soap to wash fruit or vegetables is unclear. The purpose of this study was to determine the effect of liquid soap on the body weight of mice (*Mus musculus*). This research is pure experimental using a completely randomized design. The treatment given was soaking fruits and vegetables in liquid soap with a concentration of 20 mL/L for 30 and 60 seconds, each treatment had 10 replications. The mice used were male, aged 4 weeks, with a total of 30 mice selected by cluster random sampling. Fruit and vegetable extracts were administered once daily, alternating fruit and vegetable extracts for 8 weeks in addition to the main food. Mice's body weight was measured using a digital scale. The data were analyzed using One Way Anova and Duncan's advanced test. The results showed that liquid soap had a significant effect on the body weight of mice, but the average body weight of fruits and vegetables soaked in liquid soap for 30 seconds and 60 seconds was not significantly different. The conclusion of this study supports other research that the use of liquid soap containing surfactants for a long time continuously has a negative effect on body weight.

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1. INTRODUCTION.

To prevent transmission of the coronavirus during the COVID outbreak in 2019, people in various countries were forced to use antiseptics. This behaviour has become a habit until now, so there is an increase in the use of antiseptics, especially antiseptic soaps (Putri, *et al.*, 2022). Antiseptics are substances that can inhibit the growth and development of microorganisms without having to kill them in the living tissue so that they can prevent the occurrence of infection. (Gargi *et al*., 2017). The effectiveness of antiseptics in killing microorganisms depends on several factors, including concentration and length of exposure. (Dewi, 2016), During this time, research has focused more on the positive effects of antiseptics on killing bacteria.(Purbosari, 2021; Sharfina, *et al*., 2021), (Ujiani & Tuntun, 2019; Widyastuti, 2019). Some other studies examined the negative effects of detergents or their contents, i.e. surfactants in aquatic animals namely *Lutanus* sp (Fikri (2018) and *Oreochromis niloticus* (Solikhah, *et al*., 2015). Antiseptic studies on mammals or humans are still rarely carried out so it is necessary to start finding out its negative effects.

Antiseptic liquid soap is one type of cleanser that contains surfactants. This material is the main component of household detergents including clothes washing soap, other household cleaning agents such as floor cleaning to body cleaning components such as bath soaps, dishwashers, shampoos and hand cleaning fluids.(Yuan *et al*., 2014). The effects on animals and humans vary depending on the species. Surfacing studies of the cation type of *dodecyl dimethyl benzyl ammonium chloride* (DDBAC) doses of 1 µg/mL are toxic to the activity of fish larvae locomotion (*Zebrafish*) while the non-ionic fatty alcohol type of *polyoxyethylene ether* (AEO) results in the size of the head, smaller eyes, and shorter bodies (Wang *et al*., 2015).

One of the antiseptics that people often use is liquid soap to wash dishes as well as to wash fruits and vegetables. This liquid soap contains the active ingredients 20% *Linear alkylbenzene sulfonate sodium* (LAS-Na), *sodium laureth sulfate* (SLS), *sodium laureth ether sulfate* (SLES) and *cocamidopropyl betaine* (CAPB). (Solikhah, *et al*., 2015) showed that *Linear Alkyl Benzene Sulfonate* (LAS) disrupted the sense of smell in *Oreochromis niloticus*, as a result of which fish difficult to recognize environmental conditions, including food. LAS exposure with concentrations of 1-15 ppm in human intestinal Caco-2 cells has been shown to increase cell proliferation. (Bradai *et al*., 2016). SLS has the potential to irritate the eyes and skin, even though SLS is considered a sustainable material because it contains 100% natural ingredients, biodegradability and low potential for bioaccumulation. (Bondi *et al*., 2015).

People use liquid soap to wash fruits and vegetables with various doses, both volume and duration of washing. The dose applied is not in accordance with the instructions on the liquid soap packaging or does not comply with the WHO protocol (World Health Organisation). Therefore, it is necessary to evaluate its safety from chemical contamination because it is toxic and causes infection (Trevisani *et al*, 2019). This study aims to determine the effect of antiseptic liquid soap on the body weight of male mice (*Mus musculus*). Body weight is an indicator of physical growth (Husain, *et al*., 2015) and is influenced by several factors including environment, age, psychology, physical activity and diet (Ardiani, 2020). The results of this study will be used as a consideration for the community to be wiser in using liquid washing soap antiseptics for fruits and vegetables.

2. RESEARCH METHOD

This research was a pure experimental study (true experiment) using a *Completely Randomized Design* (CRD) with 3 treatments (soaking time of liquid soap on fruits and vegetables) and 10 replications. The liquid laundry soap used is liquid laundry soap that is often used by the public and sold freely under certain brands. Based on the observations of LD_{50} (Lethal dose $_{50}$), the dose applied was 2mL of liquid soap in 1 liter of distilled water and used to soak fruits and vegetables with a soaking time of 30 seconds according to the label on the package and 60 seconds as recommended by WHO (2009).

Animals used in this study were male mice (*Mus musculus*) aged 4 weeks with an average weight of 10-11 g, healthy and active as many as 30 mice. The reason for using male animals was to avoid hormonal interference (bias) from the estrus cycle of female animals. The research sample was taken using the *Cluster Random Sampling* technique. Animals were kept in plastic tub cages measuring 35 cm x 20 cm x 17 cm which were given sawdust as a base and perforated wire as a cover. The food given was pellets and distilled water given ed *libitum*, animals ate and drank as desired, dosage was not limited. Each cage consisted of 5 mice and before being treated the mice were acclimatized for 7 days.

Manufacture of fruit and vegetable extracts. The fruits given are tomatoes and red grapes while the vegetables are mustard greens and spinach with the consideration that these foods are commonly consumed by the public. Before it is given, fruits or vegetables are soaked in liquid soap for 30 seconds and 60 seconds, then rinsed with running water and crushed using a mortar, to remove the extract. The amount of extract given to each animal according to its body weight is calculated using the Dosage Calculation Conversion Table from Nair & Jacob, (2016) and the specific gravity of each ingredient using a pycnometer (Septiani, 2019). For example, if the dose of spinach eaten by humans with a body weight of 50 kg = 250 g, according to the conversion table (body weight is determined as 70 kg) then the dose for humans is 70/50 x 250 g = 350 g. Convert the human dose to mice with a body weight of 20 g = 0.0026 (according to the table) x 350 g = 0.00091 g / g BW of mice. The specific gravity (BJ) of spinach was obtained from a calculation using a pycnometer. The pycnometer was weighed empty (PK) and contained ingredients, namely fruit or vegetable extracts (PB). Specific gravity of the material is the results of (PB – PK) / pycnometer volume. Thus, the dose given to mice weighing 25 g = 25 x 0.00091 /BJ of material.

Treatment of M. musculus. Before being treated, each mouse was weighed and then given fruit or vegetable extract according to the dose calculated based on body weight. The dosage calculation for each animal is as follows: (body weight x conversion dose): specific gravity of the substance. Fruit or vegetable extracts are given orally using a sonde alternately every day, once a day in the morning around 9 o'clock for 8 weeks.

Data analysis. Before being tested to determine whether there was a difference between the liquid soap treatments on the body weight of *M. musculus*, all data were analyzed for normality using the Kolmogorov-Smirnov test and homogeneity using the Levene test. The result of the analysis showed that the data were normally distributed ($p \geq 0.05$) and homogeneous ($p \geq 0.05$), so that they could be analyzed using the One-way Anova test using SPSS version 22. Because there were differences, the data was tested with Duncan's advanced test.

3. RESULT AND DISCUSSION

The results of observing the body weight of *M. musculus* every week after giving fruits and vegetables soaked in liquid soap for 8 weeks on various treatments are presented in Figure 1.

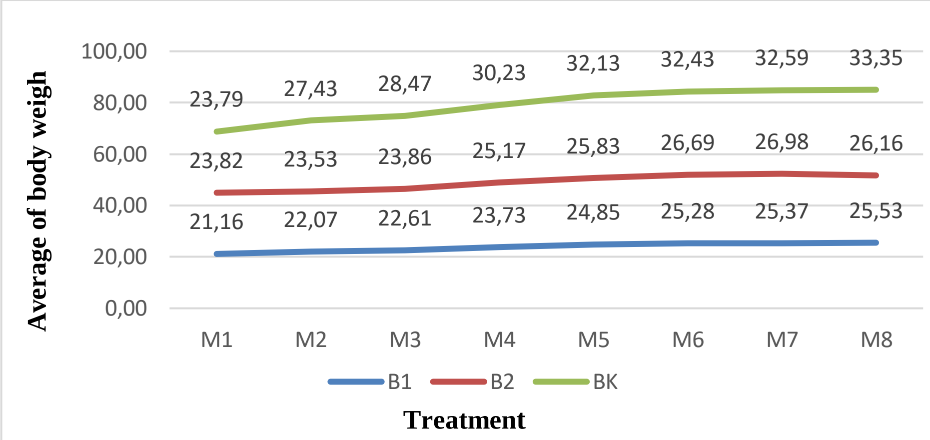

Figure 1. Average Body Weight of *M. musculus* After Treatment
B1= 30 seconds; B2= 60 seconds; BK = control.

The results of data analysis using the one-way Anova test showed that there was a difference in the body weight of *M. musculus* between the treatment and control groups ($p \leq 0.05$). Furthermore, the results of Duncan's further test (Table 1) confirmed that the average body weight of *M. musculus* in the 30-second immersion treatment with liquid soap (B1) was not different from the 60-second immersion (B2) but both were significantly different from the control (BK). The data also showed that the average weight of the control group had the highest body weight, compared to other treatments.

Table 1. Duncan test results for male *M. musculus* weight

The Treatment	The Weight Average
B1	23,30 ^{a}
B2	24,70 ^{a}
Bk	29,60 ^{b}

Information:
a, b: Different letters indicate significant differences in each treatment group.
B1: Antiseptic application of liquid soap 2ml/30 seconds of immersion time.
B2: Antiseptic application of liquid soap 2ml/60 seconds of immersion time.
BK: Control.

Based on the results of the ANOVA analysis, it proved that the administration of antiseptics had a negative effect on the body weight of *M. musculus*, even though the average body weight for the treatment of soaking the ingredients in liquid soap for 30 seconds did not differ from soaking for 60 seconds. The effect of liquid soap on body weight in this study is in accordance with several previous studies. about the ingredients contained in liquid soap, namely surfactants. According to the dominant content in the liquid soap used in this study, *Linear Alkyl Benzene Sulfonate* (LAS) is the type of surfactant most commonly used in various studies. Exposure to LAS in *Oreochromis niloticus* not only caused damage to the gill tissue but also affected weight loss (Solikhah, *et al.*, 2015). Gill damage such as gill tissue congestion is a decrease in network performance due to network load that exceeds network capacity. Weight loss is caused by a series of surfactant effects, namely causing disturbances in the sense of smell so that animals have difficulty recognizing environmental conditions. One of the environmental conditions that is closely related to body weight is food. Animals that find it difficult to recognize food have an impact on loss of appetite and will subsequently lose weight. Weight loss occurs because there is no weight gain as a response to the animal's lack of appetite and the use of stored energy in the body. As explained in the method, apart from treatment, animals were given food in the form of pellets and distilled water.

The same condition is also shown in the study results of the detergent effect on several fish species. Detergents which also contain surfactants have a negative effect on the weight growth and survival of *Oreochromis niloticus* (Rachmi, 2020). While in *Platy* sp, detergents damage the gill epithelium and inhibit growth. It appears that there is a linear relationship between the death of *Platy* sp and exposure to detergent concentrations, the increase in mortality is in line with the increase in detergent concentration (Kamiswari, *et al.*, 2013). LAS toxicity to *Lutanus* sp is also in the form of gill histological damage with the highest level of severity, namely 99.98 ppm, causing necrosis of cells or tissues (Fikri, 2018). Necrosis is cell death due to lack of food or oxygen. Based on the results of research on aquatic animals, the severity of surfactant effects ranging from damage to gill tissue to death corresponds to the concentration given. In contrast to the type of surfactant used in previous studies, exposure to *alkyl benzene sulfonate* (ABS) surfactant in *Oreochromis niloticus* caused acute toxicity to gill tissue and liver at a concentration of 13 ppm with LC50 = 7.56 ppm (Hardini, *et al.*, 2012). The cationic surfactant *dodecyl dimethyl benzyl ammonium chloride* (DDBAC) and *non-ionic fatty alcohol polyoxyethylene ether* (AEO) at a dose of 1 μg/mL are toxic to the locomotion activity of fish larvae (*Zebrafish*), even AEO results in smaller head size, eyes, and shorter body compared to DDBAC (Wang *et al.*, 2015).

The negative effects of surfactant on mammals are shown by Perfluorooctane sulfonate (PFOS), an organic surfactant ingredient as an exogenous chemical, which affects the body's immune response. Mice infected with 5 mg/kg PFOS orally for 60 days caused inhibition of the body's immune function. Even higher doses of PFOS exert direct toxic effect on cells. (Zheng *et al*., 2017). Study of surfactant in human is obtained from the clinical field, mesenchymal stem cells exposed to surfactant for 15 and 60 minutes experienced a significant decrease in viability and mitochondrial membrane potential. Mitochondria are important organelle in cell life. The permeability of the outer membrane of mytochondria and the release of cytochrome c and other proapoptotic particles are the initial signals that activate the apoptotic pathway. This proves that surfactants reduce in vitro viability and in vivo mesenchymal stem cell function through mitochondrial dysfunction (Chen *et al*., 2017). Another study on surfactan in human is the cationic surfactant hexadecyltrimethyl–ammoniumbromide (CTAB) containing nanosystems resulting in the most severe inflammatory response of neutrophil cells in the form of cell death and changes in cell morphology. Surfactants will also cause health problems in humans if they consume food contaminated with surfactants. This is because surfactant will react with liver and serum proteins, resulting in long-term metabolic effects and endocrine disorders (Badmus *et al*., 2021).

If the results of antiseptic studies on aquatic animals and mammals are compared, the same pattern appears, namely that they cause negative effects and higher concentrations trigger more severe effects. In aquatic animals, various types of antiseptic content cause damage to organs ranging from gills, liver, weight loss, movement disorders, growth retardation to death. The antiseptic effect, especially surfactants, on mammals namely mice and humans, also shows suitability for aquatic animals. The negative responses that occured are decrease in immune function including changes in morphology and death of neutrophil cells, decreased cell viability and endocrine disorders. Slightly different from the results of the study above, although the results of this study show a negative effect, the increase in exposure time is not linear with the severity level. The body weight of animals consuming fruit and vegetables with 30 and 60 second immersion treatment did not show a significant difference. This is caused by differences in several factors including the species used, the surfactant exposure method and the concentration given. Some studies used aquatic animals namely fish, surfactants are dissolved in the water where the fish are so that the surfactants come into contact and enter directly into the gills. The method of surfactant exposure in this study was by soaking the food in a liquid soap solution, and then washing the fresh food with running water before being given to *M. musculus.* Thus, the dose of surfactant received by fish is greater than the dose received by mice because the amount of surfactant is reduced through the washing process with running water. Other studies use cells in vitro while this study uses animals in vivo. Studies with the same animals but the type of surfactant used is different, namely PFOS which is organic.

In this study, surfactant entered through the digestive tract of *M. musculus* because it received fruit and vegetable foods that had been soaked in liquid laundry soap, which predominantly contained LAS at a concentration of 2 mL/L (2 ppm) every day for 8 weeks. After being absorbed, the results of this digestion will be distributed throughout the body and it can accumulate. Exposure to surfactants for a long time has a negative effect because it can leave residues that can be detrimental to health (Wastiti *et al*., 2017). When compared to the treatment group, the control group had the highest average body weight of mice. This condition was caused by the control treatment only receiving the main feed without additional food (vegetables and fruit) soaked in liquid soap. Thus, this group is not exposed to foods containing surfactants so that weight gain is not hampered. Under these conditions, the sense of smell of mice is not disturbed or able to recognize the surrounding environment, including food. Therefore, their appetite is also normal and the function of the food they eat is also to gain weight. This is supported by the opinion of Yulianti, *et al.* (2018) that the increase and decrease in body weight is influenced by environmental factors and one of these factors is the food consumed including the amount of food and activities carried out. In addition, another factor that must also be met is food safety, namely the food consumed is not chemically, biologically or physically contaminated (Megasari, 2011). If the food that enters the body is contaminated with chemicals or bacteriological substances, it can result in intoxication (food poisoning) or infection (Trevisani *et al*., 2019)

4. CONCLUSION

This research proves that antiseptic liquid soap used on fresh food has a negative effect on health, especially on body weight parameter in the form of growth retardation. Although this effect is not linear with the length of exposure, public should be careful when using liquid soap on food for a long period. Therefore, further research is needed on the effect of the same liquid soap on other health parameters.

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