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Research OPEN ACCESS

Acute toxicity test of avocado (*Persea americana*) oil in mice

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Abstract

Avocado (*Persea americana*) fruit has a high oil content, so it is widely used in the pharmaceutical and cosmetic industries. This study aims to determine the toxicity of avocado oil in mice using the lethal dose (LD_{50}) method so that it can be used as a reference for further testing. This study used a total of 20 DDY strain female mice, which were divided into 5 groups: one control group and four treatment groups that were fed with avocado oil with 5, 10, 15, and 20 g/kg BW doses orally. The mortalities of experimental mice were observed for 14 days after treatment. Other parameters observed in this study were physiological response, body weight, absolute organ weight, and relative organ weight. There was a change in behavior, and the obtained LD_{50} value was 25.4 g/kg BW. Observation of physiological responses, body weight, absolute organ weights, and relative organ weights showed no significant differences. It was concluded that avocado oil is considered relatively harmless and safe to use.

Keywords: acute toxicity test | avocado oil | LD_{50} | mice | mortality

Introduction

Avocado (*Persea americana*) is one of the well-known fruits in Indonesia and is widely consumed because it has many benefits. This fruit is rich in protein and contains more fat-soluble vitamins compared to the other fruits. Avocado fruit has a high oil content, so it is widely used in the pharmaceutical and cosmetic industries, with its fatty acid composition similar to olives. This fruit has also been recognized for its health benefits, mainly because of the compounds present in the lipid fraction, such as omega fatty acids, phytosterols, tocopherols, and squalene (Duarte *et al.*, 2016).

Avocado oil is obtained from a series of avocado extraction processes. The potentials of avocado oil as a food ingredient, medicine, and cosmetic are widely known, but specific information regarding the safety and toxic potential of avocado oil is still very limited (Pham *et al.*, 2020). One of the safety tests that can be carried out to obtain this information is the acute toxicity test, which produces the value of lethal dose 50 (LD_{50}). LD_{50} acute toxicity testing is carried out to determine the effect of giving a single dose of a compound to animals and to assess the safety of a drug or substance to be used acutely (Ayun *et al.*, 2021). LD_{50} is defined as a benchmark in the administration of a single dose of substance that can cause the death of 50% of the test animals. In general, the smaller the LD_{50} value, the more toxic the compound is (Umboro *et al.*, 2020).

Methods

Experimental design

This research was conducted from November 2022 to March 2023 at the Laboratory Animal Management Unit (UPHL) and Toxicology Laboratory, Division

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of Pharmacology and Toxicology, School of Veterinary Medicine and Biomedical Sciences, IPB University. This study used 20 adult females of *Deutschland Denken Yoken* (DDY) strain mice (*Mus musculus*) with a body weight range of 20–35 g and commercial avocado oil. This research procedure has received approval from the Animal Ethics Commission of the School of Veterinary Medicine and Biomedical Sciences, IPB University, with the number 023/KEH/SKE/III/2023.

This study used the method of determining the Lethal Dose 50 (LD_{50}) value referring to the regulation of the Indonesian Food and Drug Authority (BPOM, 2014). A total of 20 acclimatized female mice were divided into five groups consisting of 4 mice per group. Group 1 was the group of mice that were given corn oil at a dose of 20 g/kg BW as a negative control. Treatment groups 2 to 5 were groups of mice given avocado oil with different doses of 5, 10, 15, and 20 g/kg BW, respectively.

Avocado oil was administered once orally on day 0 using a gastric tube. Any symptoms of toxicity and death that occurred in the experimental mice were observed every day for 14 days. The body weights of the experimental mice were measured on days 0, 7, and 14. The mice were given food and drinking water ad libitum during the study. Mice that had been observed for 14 days were then euthanized by cervical dislocation technique. Mice that had been euthanized were then necropsied, and each organ was weighed, and their relative weights were calculated.

The observation parameter for the LD_{50} test is mice mortality for 14 days. Data on mice mortality in the acute toxicity test were then calculated using probit analysis to determine the LD_{50} value. The other parameters observed include clinical symptoms, animal physiology, body weight, and absolute and relative organ weights. Organs, including the heart, lungs, liver, kidneys, intestines, and stomach, were separated, removed from the visceral site, weighed, and macroscopic changes were observed.

Data analysis

Data were analyzed quantitatively using *Microsoft Excel*® 2018 and *Minitab* 21® software, and using the probit analysis method, LD_{50} was calculated. Qualitative data were displayed descriptively. Quantitative data were analyzed using the *one-way analysis of variance* (ANOVA) test followed by the *Tukey-HSD post-hoc* test.

Results

Mortality

Mortality observation was carried out after administration of avocado oil. The analysis result of the LD_{50} value of avocado oil obtained using the probit analysis method was 25.4 g/kg BW. The number of dead mice at various doses of avocado oil are presented in **Table 1**.

Table 1 Effects of giving various doses of avocado oil on mice mortalities

Avocado oil dosages (g/kg BW)	Number of mice	Number of dead mice	Death day	Death percentage (%)
0	4	0	-	0
5	4	1	10	25
10	4	0	-	0
15	4	2	2, 10	50
20	4	1	5	25

Clinical symptoms

Observations of clinical symptoms in mice were carried out for 14 days after administration of avocado oil, and daily observations were carried out at the same hour. Based on the results of qualitative test observations, several clinical symptoms appeared in mice administered with avogado oil at doses of 5, 15, and 20 g/kg BW. Clinical symptoms that appeared through observation were the decreased locomotor activity, decreased reflexes, lethargy, piloerection, and death.

Body weight

The experimental mice were weighed on days 0, 7, and 14 after treatment and the body weights of

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Table 2 Effect of giving various doses of avocado oil on the averages body weights of mice

Day	Avocado oil dosages (g/kg BW)				
	0	5	10	15	20
0	28.00 ± 2.97[a]	25.60 ± 1.60[a]	28.45 ± 3.50[a]	27.96 ± 1.00[a]	28.53 ± 2.93[a]
7	29.38 ± 3.93[a]	25.38 ± 4.79[a]	29.95 ± 3.79[a]	27.23 ± 3.10[a]	29.37 ± 2.86[a]
14	29.98 ± 3.93[a]	27.50 ± 1.06[a]	30.28 ± 4.60[a]	28.50 ± 3.11[a]	29.73 ± 2.86[a]

The same superscript letter on the same row showed no significant difference in all treatments (p>0.05).

experimental mice were presented in **Table 2**. Based on **Table 2**, the results of the analysis of the average weights of experimental mice treated with different doses of avocado oil did not show significantly different results (p>0.05) at the 95% confidence level. The result proves that avocado oil does not affect the body weights of experimental mice.

Physiological response

Physiological response parameters also observed in the toxicity test for avocado oil were body temperature, respiratory rate, and heart rate. The results of measuring the body temperature of experimental mice for 14 days are presented in **Figure 1A**. The body temperatures of experimental mice after avocado oil administration remained within the normal range. The average body temperatures of the treatment groups were not significantly different compared to that of the control group.

The averages respiratory rates of experimental mice after giving avocado oil were not significantly different from that of the control group. The result means that giving avocado oil to mice does not affect the respiratory rate. The results of measuring the respiratory rates of experimental mice for 14 days are presented in **Figure 1B**.

The last physiological observation made on the experimental mice was heart rate frequency that were presented in Figure 1C. Based on **Figure 1C**, the averages heart rate of experimental mice after administration of avocado oil at doses of 5, 10, 15, and 20 g/kg body weight were not significantly different compared to the control group, which means that giving avocado oil to mice does not affect heart rate. The result proves that avocado oil does not affect the body temperatures, respiratory rates, and heart rates of experimental mice.

Organ weights

The results of the analysis of the averages relative organ weights of the experimental mice did not show any significant differences in the observed organs (p>0.05) at the 95% confidence level at various doses of avocado oil. The average relative organ weights of mice are presented in **Table 3**.

Discussion

The main parameter of the acute toxicity test used to determine the level of toxicity of a compound is mortality or death rate (Ayun *et al.*, 2021). The mortality value was used to analyze the LD_{50} value of avocado oil, which was obtained using the probit analysis method. This acute toxicity test on avocado oil was carried out to determine the value of the LD_{50} and the use of the right dosage of avocado oil. The LD_{50} value of avocado oil was obtained at 25.4 g/kg BW. Based on the acute toxicity category of Hodge & Sterner (2005), this value is classified as practically non-toxic because the LD_{50} value obtained is more than 15 g/kg BW. Although relatively harmless, giving various doses of avocado oil to mice was able to cause toxic symptoms and death in the mice tested.

Avocado oil has active ingredients such as saponins, alkaloids, flavonoids, tannins, tocopherols, phytosterols, carotenoids, and polyphenols (Tan & Ghazali, 2019). According to Marlinda *et al.* (2012), the active compounds contained in medicinal plant preparations will cause toxic symptoms when given

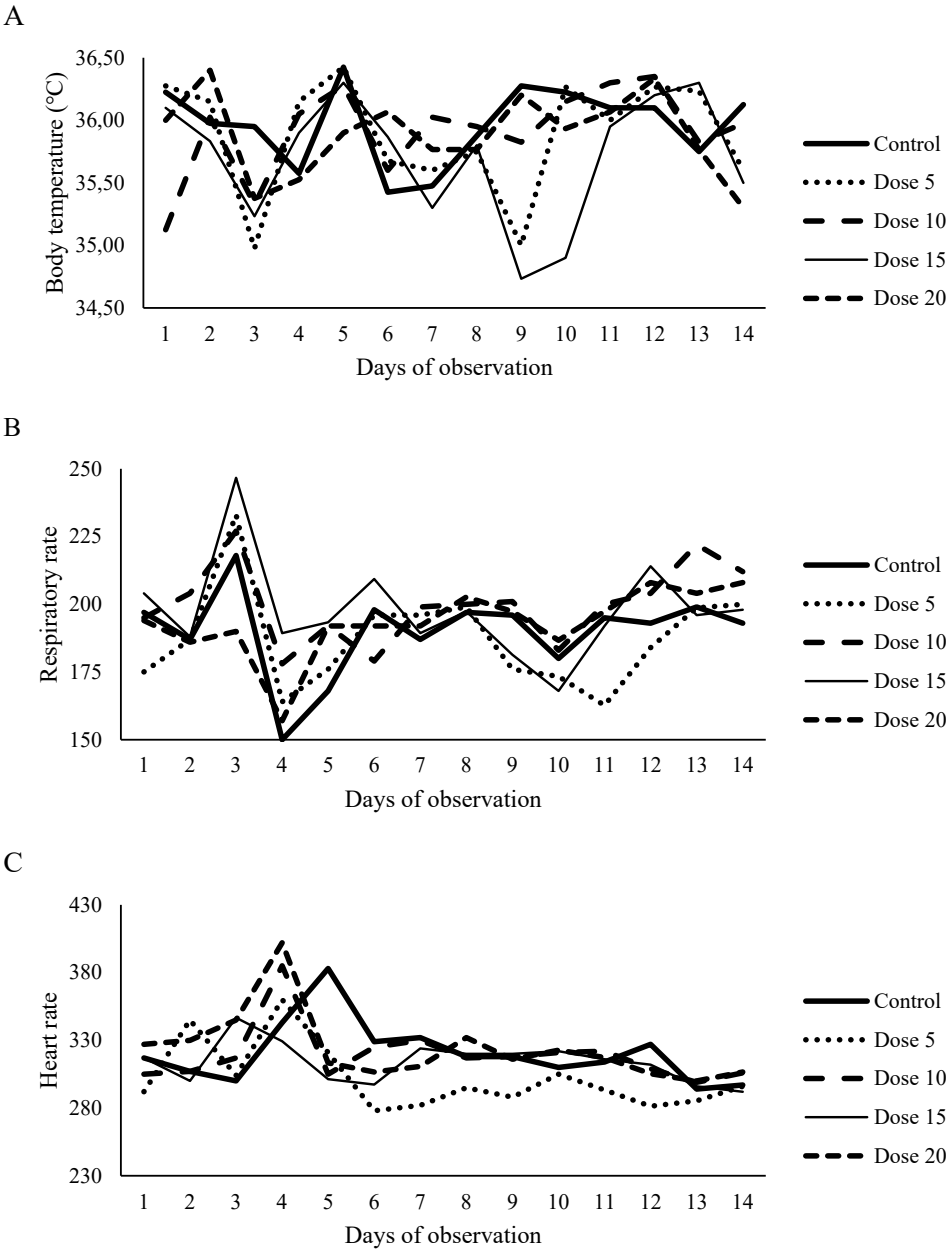

Figure 1 Body temperatures (A), respiratory rates (B), and heart rates (C) of experimental mice after administration of avocado oil at different doses.

in high doses. All toxic symptoms occur due to reactions between toxic substances and receptors in the body.

Symptoms of death in mice are thought to be caused by the active ingredient of avocado oil, which causes hypoglycemia. Acute hypoglycemia has been shown to cause depressive-like behaviors in mice (Park *et al.*, 2012), which is shown in observation. According to Rahman *et al.* (2022), avocado peel extract significantly reduced blood glucose levels in mice. The effectiveness of avocados in lowering blood glucose levels can be influenced by the active ingredients contained in avocados. The active ingredients are the high contents of saponins, flavonoids, and polyphenols. A similar result is also supported by previous research (Masyruroh, 2021), which the three active ingredients can suppress the increases in blood glucose levels and stimulate

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Table 3 Average absolute and relative organ weights of experimental mice after administration of various doses of avocado oil

Organ	Avocado oil dosages (g/kg BW)				
	0	5	10	15	20
Absolute weight (g)					
Heart	0.17 ± 0.03[a]	0.17 ± 0.04[a]	0.14 ± 0.02[a]	0.15 ± 0.06[a]	0.17 ± 0.03[a]
Lungs	0.49 ± 0.16[a]	0.42 0.27[a]	0.41 ± 0.09[a]	0.50 ± 0.28[a]	0.32 ± 0.08[a]
Liver	1.72 ± 0.45[a]	1.73 0.55[a]	2.02 ± 0.26[a]	1.84 ± 0.43[a]	1.82 ± 0.28[a]
Kidney	0.44 ± 0.11[a]	0.34 ± 0.04[a]	0.41 ± 0.04[a]	0.38 ± 0.11[a]	0.41 ± 0.06[a]
Stomach and intestines	4.27 ± 0.58[a]	3.97 ± 1.31[a]	4.17 ± 0.97[a]	4.07 ± 1.06[a]	3.87 ± 1.22[a]
Relative weight (%)					
Heart	0.58 ± 0.04[a]	0.69 ± 0.14[a]	0.44 ± 0.02[a]	0.57 ± 0.23[a]	0.60 ± 0.10[a]
Lungs	1.70 ± 0.51[a]	2.02 2.05[a]	1.33 ± 0.37[a]	2.06 ± 1.61[a]	1.12 ± 0.40[a]
Liver	5.93 ± 0.90[a]	6.75 0.88[a]	6.56 ± 0.38[a]	7.02 ± 1.25[a]	6.33 ± 0.89[a]
Kidney	1.45 ± 0.34[a]	1.38 0.29[a]	1.33 ± 0.15[a]	1.46 ± 0.39[a]	1.48 ± 0.32[a]
Stomach and intestines	14.90 ± 1.00[a]	15.33 ± 2.05[a]	13.41 ± 1.27[a]	15.35 ± 2.26[a]	13.21 ± 3.15[a]

The same superscript letters on the same row showed no significant differences in all treatments ($p>0.05$).

pancreatic β cells to produce more insulin and increase the deposit of glucose reserves in the liver, resulting in a decrease in blood glucose levels.

Changes in organ weights indicate the effect of the contents of the preparation on the organs. Relative organ weight can be obtained by calculating the ratio of absolute organ weight to body weight. According to Lazic *et al*. (2020), the value of relative organ weight is used to interpret the effect of a compound directly on the organs, not indirectly on the changes in body weight.

Conclusion

The acute toxicity test of avocado oil in mice resulted in a lethal dose of 50 (LD_{50}) of 25.4 g/kg BW. This value is classified as practically non-toxic and safe to use.

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Author contributions: ASB performed the experiments, analyzed the data, and wrote the paper; AA and BFP designed the experiments and wrote the paper.

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