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**RESEARCH ARTICLE**

# The Effect of Used Cooking Oil on Total Blood Cholesterol Levels in Mice (*Mus Musculus L*)

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**Abstract**: Waste cooking oil is leftover cooking oil, used for frying. Cooking oil that is constantly heated and used repeatedly (>2 times) will accelerate oxidative processes that can cause the formation of fatty acids. This research was conducted to determine the effect of waste cooking oil on the total blood cholesterol levels of mice (*Mus Musculus L*) with different heating frequencies, namely 4, 8, and 12-time heating. This study used True Experimental or laboratory with the post-test only with a control group design method, a purposive random sampling technique of 28 selected mice divided into 4 groups each consisting of 7 mice. The results of this study found that the total cholesterol level without heating was 176.67 mg/dl, the total cholesterol level with 4-time heating was 119 mg/dl, the total cholesterol level with 8-time heating was 127.67 mg/dl, and the total cholesterol level with 12-time heating of 146.17 mg/dl. The results of this study found that the average total cholesterol level without heating was 176.67 mg/dl, the total cholesterol level with 4 times heating was 119 mg/dl, the total cholesterol level with 8 heating times was 127.67 mg/dl and the cholesterol level total with 12 times heating of 146.17. The results of the study showed a p-value of 0.006 ($p < 0.05$). The conclusion is that there is a significant difference between the control group and the group induced by cooking oil by heating 4 times and heating 8 times, The mean total cholesterol levels in the control group cooking oil without heating and the group induced by cooking oil by heating 12 times exceeds the normal limit.

**Keywords:** Cholesterol LDL, oxidative stress, waste cooking oil

## INTRODUCTION

Used cooking oil is used cooking oil that is heated several times.[1,] The use of used cooking oil causes diseases, such as CHD (coronary heart disease), sore throat, dyslipidaemias, obesity, and Atheroskerosis.[1.2]

One of the main needs of the community to meet their daily needs, especially as a source of fat, is cooking oil.[1] The Food and Agriculture Organization (FAO) in 2015 explained that the consumption of vegetable oil as a food ingredient reached 19 kg per person.[1]

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According to the Indonesian Vegetable Oil Industry Association (GIMNI), there are 8.144 million tons of vegetable oil producers and consumers.[1] The most widely used type of cooking oil is palm cooking oil, which is around >70%.[1] The estimated consumption of palm cooking oil is 11.09 litres/capita/year in 2019 and 11.38 litres/capita/year in 2020.[3] This shows that palm cooking oil consumption has increased every year.[3] Vegetable oils function as a medium of heat transfer, and flavourings, and add nutritional value and calories to food, so they are widely used in the processing of various food ingredients.[4]

Fried noodles that are heated repeatedly are not good for health, and the price of cooking oil is quite high for some people.[4] Indonesian society still prioritizes economic values over health values that are currently generally ignored.[4] Cooking oil during heating will undergo oxidation which can cause the formation of fatty acids.[4] In addition, longer temperatures and heating duration can affect the antioxidant activity of vegetable oils.[4]

Heating oil for cooking causes the release of fatty acids from triglycerides so that free fatty acids are easily oxidized to aldehydes, ketones, and alcohols that cause rancidity.[4.] The greatest free fatty acids are produced when oil is fried repeatedly up to 4 times at temperatures above 1000 C causing auto-oxidation, oxidation and thermal polymerization as well as the formation of free radicals.[4]

Used cooking oil contains saturated fatty acid bonds that are not easily broken down and carried by the bloodstream.[5] Fat can settle in the blood vessels of the heart so clogging blood flow can increase blood cholesterol.[5] The effect of high levels of cholesterol in the blood caused by saturated fatty acids that cause endothelial damage.[5] This endothelial cell damage is due to the formation of superoxide radicals which then result in LDL oxidation.[6] Oxidized LDL can inactivate Nitric Oxide (NO). oxidized LDL cholesterol causes a state of oxidative stress.[6] Endothelial cells are associated with several cardiovascular disorders, such as hypertension, dyslipidaemias, and diabetes mellitus, associated with endothelial dysfunction and can be the basis or complication of the disease.[6]

Some studies related to used cooking oil, namely free fatty acids formed cooking oil 1x changes and the peroxide number is almost the same as oil without cooking.[7] According to Mustika's research, repeated use of used cooking oil increases desquamation villi small intestine 3x, 6x and 9x.[7] Based on research on the peroxide content of cooking oil affects the frying of cooking oil enriched with vitamin A.[7] The lowest average peroxide level is found in a zero fryer and increases to fourth.[7] Frying can increase total cholesterol, LDL, VLDL, triglycerides, and total cholesterol ratios as well as decrease HDL in the blood plasma of rats given cooking oil higher.[7] Although still below the maximum limit compared to other controls and treatments (coconut oil, bulk oil and lard oil), it is necessary to be aware of its use.[7]

Therefore, researchers are interested in directly examining the effect of used cooking oil on the total blood cholesterol of mice (*Mus Musculus L*).

BULETIN FARMATERA

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## METHODS

This study used True Experimental or laboratory research methods with the post-test only with the control group design method. This research was conducted from April 2022 to August 2022 at the Veterinary Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh. The population of this study was male mice (*Mus Musculus L*).

The sampling technique in this study was a purposive random sampling technique, as many as 28 rats were randomly selected and divided into 4 groups of 7 mice each. Frederer's formula, 1991 is the formula used in determining the sample size for experimental tests, namely.[8]

$$(n-1)(t-1)\geq 15$$

Information:
n = number of samples per treatment group
t = number of treatment groups
Based on the above formula, a sample size calculation with t = 4 is performed, then it gets:
(n-1) (5-1) ≥ 15

$$(n - 1) \times (t - 1) \geq 15$$
$$(n - 1) \times (4 - 1) \geq 15$$
$$(n - 1)\ (3) \geq 15$$
$$(3n - 3) \geq 15$$
$$3n = 15 + 3$$
$$3n = 18$$
$$n = 18/3$$
$$n = 6$$

Therefore, the number of mice used in this study was 24 heads and the number of samples in each experimental group was 6 mice and 4 groups, each group added one mouse, so the number of groups used was 28 mice, divided into 4 groups.

Used cooking oil uses cooking oil that is repeatedly heated four times to fry eight times, and twelve times to fry sweet potatoes, tempeh, and tofu. The frying process begins by putting fresh cooking oil into the frying pan using ± 1 litre of bulk cooking oil, then heating to a temperature of 180°C or 200°C (SNI-013741-2013) For 10 minutes then add food ingredients such as sweet potatoes, tofu and tempeh fried until cooked.

The dose of used cooking oil in mice refers to Bonita's research in 2017 for the dose of used cooking oil according to body weight, which is 0.1 -0.3 ml/day with a body weight of mice 10-30 grams. Used cooking oil is given orally using mouse gastric sonde and syringe 3 ml, given 0.2 ml/day and taking into account the remaining volume of the mouse stomach for eating and drinking and avoiding gastric perforation of mice given for 21 consecutive days

This study used 26 mice as experimental animals induced cooking oil with different heating and divided into 4 treatment groups with a dose of 0.1 ml to see the total cholesterol levels of the sample. The dose was given which was originally 0.2 ml to 0.1 ml because, after treatment with a dose of 0.2 ml, many mice had diarrhoea which resulted in many mice dying, so optimization of mice was carried out with a decrease in the dose of 0.1 ml. At the time of optimization with a dose of 0.1 ml, mice did not experience diarrhoea or death, so the next study used a dose of 0.1 ml with new mice.

Acclimatization was carried out at the beginning of the study for seven days to adjust the conditions of experimental

BULETIN FARMATERA

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animals to the same state of stress and adjustment to the environment. Blood tests begin with the examination of total cholesterol levels in all groups (K1, K2, K3 and K4) carried out after the adaptation period. Furthermore, used cooking oil was given to the K1 and P2 groups as much as 0.1 ml BB orally per day for 21 days then re-checked total cholesterol levels after using used cooking oil.

Total cholesterol measurement in mice was done after being satisfied for 6 hours. The mice are then cleaned with alcohol cotton on the tail so that the dirt contained in the rat's tail can be cleaned (sterilization of the mouse's tail). Next, blood is taken with a few drops on the tail that has been injured and measured using a multi-check cholesterol tool brand *easy touch.*[9] Before being given the treatment, rats were adapted for seven days easy touch oak cholesterol brand easy touch

Data were statistically analysed to see normality and homogeneity using the *Saphiro-Will* test and *the Levene test*. The results of the normality and homogeneity test show normally distributed data characterized by p values > 0.05. Furthermore, *the One-Way ANOVA* test was carried out to find out if there were significant differences in all groups. If you get p > 0.05 it shows that there is a significant difference. There are differences between the four groups. Then proceed with post-hoc test data analysis using LSD.

## RESULTS

The research was conducted on total cholesterol levels to determine the effect of cooking oil given to male mice (*Mus Musculus L*) with 4x, 8x and 12x heating. Total cholesterol levels in each group sample had varying cholesterol levels. The measurement results of each sample in the group are shown in the form of the following Table 1:

**Table 1: Average Values of Total Blood LDL Cholesterol Levels1 in All Study Groups (mg/dL).**

| Mice | Control | P1 | P2 | P3 |
|---|---|---|---|---|
| 1 | 209 | 108 | 112 | 127 |
| 2 | 216 | 100 | 137 | 167 |
| 3 | 105 | 109 | 121 | 137 |
| 4 | 180 | 116 | 145 | 133 |
| 5 | 150 | 138 | 101 | 149 |
| 6 | 200 | 143 | 150 | 164 |

The difference in average total cholesterol levels after treatment in all groups. for each treatment group can be seen in Table 1.

The total cholesterol levels below are shown in Table 2:

**Table 2: Results of total LDL cholesterol levels in mice 6 Hours after treatment in all study groups (mg/dL).**

| Group | Average Total LDL Cholesterol Levels 6 Hours after treatment (mg/dl) ±SD$\bar{X}$ |
|---|---|
| Control Group Unheated cooking oil | 176,6 ± 42,4 |
| Treatment Group I Cooking Oil With 4X Heating | 119,0 ± 17,4 |
| Treatment Group II Cooking Oil With 8X Heating | 127,6 ± 19,4 |
| Treatment Group III Cooking Oil With 12X Heating | 146,1 ± 16,6 |

BULETIN FARMATERA

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The above data shows that there are differences in cholesterol levels in each group. The average values of the four groups, namely the control group, treatment I, treatment II, and treatment III, were 176.6 mg/dl, 119 mg/dl, 127.6 mg/dl, and 146.1 mg/dl after treatment. Elevated cholesterol levels occurred in the control group and treatment group. Treatment group I and treatment II total cholesterol levels are still within normal limits of 40-130 mg / dL.

## DISCUSSION

Based on the results of research that the authors tested related to the effect of total cholesterol levels on mice (*Mus Musculus L*) induced using cooking oil, it was obtained that each variant group had differences that were not much different.

Used cooking oil is a low-quality cooking oil because of its simple filtering so that the colour is not clear.[9] Bulk cooking oil contains many fatty acids, namely saturated fatty acids including myristic 1-5%, palmitic acid 5-15%, stearate 5-10%, unsaturated acids namely oleic 70-80%, linoleic 3-11%, palmitoleic 0.8-1.4%.[10] In general, cooking oil is very susceptible to oxidation damage due to the repeated frying process used.[9] This reaction will cause rancidity and deterioration in the quality of cooking oil and foodstuffs.[10] The high content of unsaturated fatty acids can make the oil perishable during frying since the oil is constantly heated to a high temperature during frying, which causes contact with oxygen in the outside air, which forms an oxidation reaction in the oil.[9]

Based on the research conducted, it is known that on average each treatment has different total cholesterol levels.[10] Differences in total cholesterol levels were observed in the treatment group induced with used cooking oil by heating 4, 8 and 12 times.[10] The increase in total cholesterol levels in each treatment induced by used cooking oil, namely treatments I, II and III has a significant difference, although the treatment I and II total cholesterol levels are still within normal limits and those who experience a significant increase in total cholesterol levels are treatment group III, namely the treatment group with 12 times heating used cooking oil.[10]

This suggests that warming leads to the formation of reactive oxygen species (ROS) and reduces antioxidants.[10] Heating can change the fat composition of cis-isomer fatty acids into trans-isomers, peroxides and free radical components, increasing the risk of cancer.[10] These saturated fatty acid bonds are difficult to break down in the body and be absorbed into the bloodstream.[10] These fats accumulate slowly in the blood vessels of the heart and increase blood cholesterol levels.[10]

Used cooking oil can cause changes in taste, colour and odour as a result of continuous heating.[10] Rancid odour is also caused by improper storage over a long period resulting in the breaking of triglyceride bonds into glycerol and FFA (free fatty acid) or saturated fatty acids.[10] Repeated use of used cooking oil contains carcinogenic free radicals such as peroxides, epoxides, and others.[11] Used cooking oil contains a lot of free saturated and radial fats produced by per-fat oxidation.[10] Antioxidants can prevent these free radicals.[10] Consumption of saturated fat can increase cholesterol in the blood as a cause

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of cardiovascular disease, namely atherosclerosis and coronary heart disease.[10]

The results showed that total cholesterol levels increased in the control group, and the results obtained showed that total cholesterol levels in the group of mice given unheated cooking oil (control group) increased at different frequencies of 4.8 and 12 times.[11]

Total cholesterol levels in the control group should not have increased[11], However, in this study, it was precisely the control group that experienced an increase in total cholesterol levels and the cause of the increase in total cholesterol levels in the cooking oil group without heating is still not obtained because further research is needed on the content of substances in cooking oil that can increase cholesterol levels.[11]

The same thing also happened to Bonita's (2017) research which found that cholesterol levels in the rat group after reheating were lower than in the group of rats given palm oil.[12] Repeated heating is less than that of palm oil without heating.[12.13] Palm oil and used cooking oil had relatively similar effects on blood lipid profile concentrations. Palm oil and used cooking oil had relatively similar effects on blood lipid profile concentrations.[12] However, used cooking oil significantly lowers HDL levels, and improves LDL-cholesterol, and LDL ratio.[12] LDL is one indicator of a disorder in fat metabolism in the body of experimental mice, namely dyslipidemia.[12]

In this study, it was found that the used cooking oil in the control group was higher than the treatment group with heating 4x, 8x and 12x. Repeated heating of cooking oil has no real effect on total cholesterol levels because palmitic acid, a fatty acid present in palm oil and unsaturated fatty acids in reheated oil, has approximately the same effect and does not differ significantly in increasing total cholesterol levels.[13]

Based on this study, cooking oil that is not heated and heated repeatedly has an impact on total blood cholesterol levels in experimental mice so food processed using cooking oil and cooking oil with repeated heating should be avoided and other food alternatives can be avoided.[14] This is following Bonita's research (2017) that giving palm cooking oil for 60 days without being heated or heated repeatedly can increase the absorption of saturated, unsaturated, and trans fats contained in it, increasing serum cholesterol levels in the blood of mice in the cooking oil group without being heated or heated repeatedly.[13,14]

## CONCLUSION

The average cholesterol levels in each group had varying differences, namely the control group and the treatment group with heating 4, 8 and 12 times. Although the administration of cooking oil with heating 4 times and 8 times has cholesterol levels still within normal limits significant differences were found in the control group that induced cooking oil without heating with the used cooking oil treatment group with 4x and 8x heating.

## SUGGESTION

Based on the results of research on the effect of used cooking oil on total cholesterol levels. The researchers

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conducted further research with histology to determine the effect of treatment on the level of organ damage.

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