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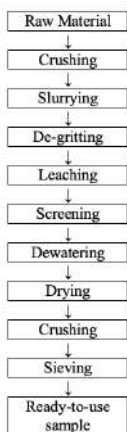


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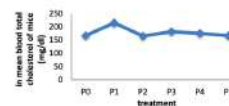
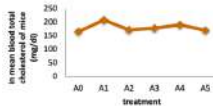
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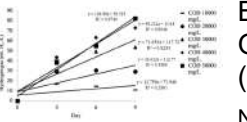
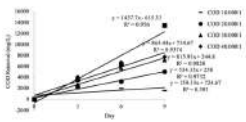


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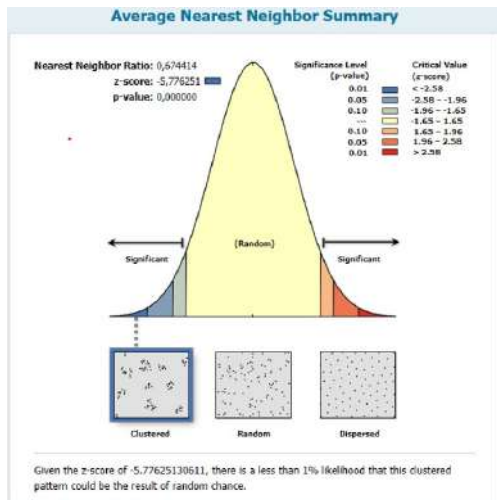


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Dr. Muhammad Dimiyati
NIP. 195912171984021001



Effect Of Pomegranate (*Punica granatum* L.) Fraction In Reducing Total Blood Cholesterol Levels And Triglyceride In Mice (*Mus musculus* L.)

Nurlaily Agustini^{1*}, Arum Setiawan², Salni²

¹Department Bioscience Program, Faculty of Science, Sriwijaya University, Jalan Padang Selasa 524, Palembang, Sumatera Selatan 30139, Indonesia.

²Department of Biology, Faculty of Mathematics & Natural Sciences, Sriwijaya University. Jalan Raya Palembang-Prabumulih km 32, Indralaya, Indonesia.

*Corresponding author e-mail: laily1708@yahoo.com

Abstract

Pomegranate (*Punica granatum* L.) will be examined by looking at the ability between pomegranate extracts or fractions, which are more effective in lowering total blood cholesterol and triglyceride levels in mice. This study aims to determine the effect pomegranate fraction of methanol water from several concentrate that can reduce the total cholesterol levels and triglyceride in mice. The research method used was an experimental method with a completely randomized design (CRD) pattern. The research used 6 treatment groups, and test animals divided into 6 treatment groups namely negative control given aquades, positive control fed a high-fat diet, given simvastatin, given 25 mg/grBB pomegranate fraction of methanol water, given 50 mg/grBB pomegranate fraction of methanol water, given 100 mg/grBB of pomegranate fraction of methanol water, each treatment group will be divided into 4 male mice. Based on the results of this study, the active fraction that has an effect on reducing blood cholesterol and triglyceride levels with a percentage reduction about 48% and the significance value of $0,000 < \alpha = 0.05$ is the treatment given 100mg/grBB pomegranate fraction of methanol water.

Keywords

pomegranate, total cholesterol, and triglyceride

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1. INTRODUCTION

Along with the development of times and changes in unhealthy lifestyles ranging from unbalanced eating patterns, consumption of foods high in fat and carbohydrates, to the lack of doing sports activities, many suffer from hypercholesterolemia. Increased levels of cholesterol and triglycerides in the blood. Increased levels of cholesterol and triglycerides in the blood cause the formation of free radicals. High cholesterol and triglyceride levels are also closely related to the increased incidence of cardiovascular disease (Budiyo and Candra, 2013; Roslizawaty et al., 2016).

Hypercholesterolemia is an increase in cholesterol levels in the blood that exceeds the limits needed by the body. Someone said to suffer from hypercholesterolemia when total plasma cholesterol levels exceed normal conditions, ie above 200 mg / dL. Cholesterol is one part of fat as one of the nutrients the body needs and the highest calorie producer (Wirawan, 2018). Hypercholesterolemia is a trigger for coronary heart disease because high cholesterol causes blockages in peripheral vessels that reduce blood supply to the heart. High cholesterol can also be a trigger for hypertension and

stroke (Soleha, 2012). Hypercholesterolemia is a trigger for coronary heart disease because of high cholesterol causes blockages in peripheral vessels that reduce blood supply to the heart. High cholesterol can also be a trigger for hypertension and stroke (Alwan et al., 2009).

Dietary management and diet modification is one way to help reduce blood lipid levels. The recommended dietary arrangement is by limiting the consumption of foods that contain high cholesterol and fat, especially saturated fat. In addition to limiting foods that are high in cholesterol and fat, consuming foods that have benefits for lowering cholesterol levels (Sundari and Dieny, 2013).

Medicinal plants have been used a lot by humans since ancient times. The development of the times is also increasing human knowledge about pharmacology and medical science, many people turn to chemical drugs that have been tested for laboratory efficacy. Over time with the development of public knowledge and awareness of the dangers posed by chemicals contained in medicines, people are encouraged to return to using traditional medicines with technological developments. More and more plants have been proven to have

laboratory efficacy and are guaranteed safe for consumption and cure diseases without causing side effect (Aseptianova, 2019).

Indonesia as a country that has developed modern health services but the number of people who use traditional medicine remains high. According to the 2001 national socioeconomic survey 57.7% of the population of Indonesia treated themselves without medical assistance, 31.2% of them used traditional medicinal plants and 9.8% chose other traditional methods of treatment (Novitasiah et al., 2012). WHO recommends the use of traditional medicines in the maintenance of public health, prevention and treatment of diseases, especially for chronic diseases, degenerative diseases and cancer. WHO also supports efforts to improve the safety and efficacy of traditional medicines (Bustanussalam, 2016).

Pomegranate is a fruit that comes from the Middle East. The potential for antioxidants in pomegranates is very high, which is around 92% (Nge et al., 2015). The function of antioxidants to inhibit the formation of degenerative diseases such as hypercholesterolemia (Mutia et al., 2018). Pomegranate has several active compounds namely alkaloids, flavonoids, saponins, tannins, and triterpenoids (Roswien, 2014). Based on some previous studies plants containing alkaloids and flavonoids have benefits in reducing cholesterol levels (Zulviana et al., 2017). So far there has not been much research on pomegranate fractions in reducing blood cholesterol and triglyceride levels. But there have been several studies on pomegranates, one of which was conducted by Wahyuni et al. (2013). In that study stated that pomegranate meat can reduce blood cholesterol levels. That is due to the presence of phytosterols and some other nutritional content found in pomegranates which are anticholesterol (Wahyuni et al., 2013). Based on the description above, this study aims to determine the effect of pomegranate fraction in reducing blood cholesterol levels and triglyceride in mice.

2. EXPERIMENTAL SECTION

2.1 Materials

The tools used in this study are blenders, filters, measuring cups, mortars, maceration containers, rotary evaporators, separating funnels, animal cages, wood chips, animal drinking bottles, analytical scales, oral needles, sonde, paper labels, blood vessels, surgical scissors, cotton swabs, masks, rubber gloves, blood cholesterol levels and the Nesco Multi-Check brand stripstest.

The materials used in this study were fresh pomegranate, 48 male strain mice, distilled water, 96% ethanol, n-hexane, ethyl acetate, used cooking oil, quail eggs, 1% CMC Na, simvastatin, 70% alcohol .

2.2 Experimental Animals

The experimental animals used were 48 male Swiss Webster strain mice. The mice used are around 2-3 months old and

weigh about 25-40 grams originating from livestock farming in the Bogor agriculture department. Rat cages are first sterilized by drying them in the sun, then given wood dust as the base of the cage. After the sterile cage, male rats were immediately placed and fed with pellets and drinking distilled water ad libitum (to taste). The cage was also given a woven wire as a cover and then the rats were acclimatized for 7 days. The cage is cleaned and the sawdust is replaced every 3 days.

2.3 Methods

2.3.1 Sampling

Samples to be used are pomegranates taken from pomegranate farmers weighing 10 kg in Situbondo, Central Java and then processed into extracts and fractions at the Genetic Laboratory of the Faculty of Mathematics and Natural Sciences, Sriwijaya University.

2.3.2 Making Pomegranate Ethanol Extract (*Punica granatum L.*)

This study used pomegranates, sorted and washed, drained and dried for 2-3 days. After drying, simplicia is then made into powder using a blender, and sieved to get the powder. The extraction process uses maceration method which is pomegranate powder weighed then immersed with 96% ethanol for 2x24 hours then filtered and obtained liquid extract. The liquid extract obtained was concentrated by distillation using arotary evaporator at a temperature of 80 C at a speed of 30 rpm so that it is free of ethanol and produces a thick concentrated extract. Then the ethanol that has been separated and then soaked again with pomegranate powder for 2 days then do it the previous way using a rotary evaporator until it gets the second concentrated extract. The second concentrated extract is then dried with a hairdryer until later the extract will be more concentrated like a paste.

2.3.3 Making a pomegranate fraction (*Punica granatum L.*)

Concentrated pomegranate extract was fractionated with 1 liter of n-hexane, shaken for 5 minutes. After that it is allowed to form 2 layers namely the n-hexane layer and the water layer. This treatment is repeated several times until the n-hexane layer looks clear so that the n-hexane fraction is obtained. The water layer is then fractionated with 1 liter of ethyl acetate, repeated several times as in the above treatment so that the water fraction and ethyl acetate fraction are obtained. Then the water fraction is mixed with 100 ml of methanol, shaken for 5 minutes and the water methanol fraction is obtained. All n-hexane, ethyl acetate and methanol water fractions were evaporated with a rotary evaporator until a thick fraction was obtained. After getting the concentrated fraction, dry it using a hairdryer until a concentrated fraction of paste is obtained.

2.3.4 Classification and Induction of Experimental Animals

The animals used in this study were 48 male Swiss Webster strain mice aged around 2-3 months and weighing around 25-40 grams. The experimental animals will be divided into 6 groups for preliminary tests namely negative control treatment (A0), positive control (A1), pomegranate extract (A2), pomegranate n-hexane fraction (A3), pomegranate acetate fraction (A4), water methanol fraction pomegranate (A5) and 6 groups for further tests namely negative control treatment (P0), positive control (P1), pomegranate water methanol fraction (P3), pomegranate water methanol fraction (P4), pomegranate water methanol fraction (P5). Each group consists of 4 mice each. In the positive control group quail yolk was induced by 1% body weight of mice and high-fat diet foods were added.

2.3.5 Dosage Planning

The dosages used in the preliminary test were 25 mg/grBB each in pomegranate extract, n-hexane fraction, ethyl acetate fraction, and pomegranate water methanol fraction. The treatment between the extract and the most effective fraction will be carried out further tests on the fraction that is most effective in reducing blood cholesterol levels and mice triglycerides.

The dose of simvastatin for humans is 20 mg/day so the dose must be converted to mice. The dose of simvastatin for mice is $0.0026 \times 20\text{mg} / x / \text{tail} = 0.052 \text{ mg} / x / \text{tail}$. Two 10 mg simvastatin tablets were crushed until smooth and parchment paper was given 1% CMC Na, pitch and measured to obtain 1 g CMC Na. Then give CMC Na 1 g of warm water then crushed with crushed simvastatin and add 100 ml of distilled water (Artha et al., 2017).

2.3.6 Research Test Stage

In the preliminary test conducted to determine the effective fraction in reducing blood cholesterol levels in mice. Provision of treatment in this test will be carried out for 7 days, then only found the most effective fraction in reducing total blood cholesterol levels and the most effective fraction is what will be used for further testing stages in reducing blood total cholesterol levels and mice triglycerides. Animal mice will be divided into 6 groups. Each group will be divided into 4 mice and given the treatment as in Table 1.

The follow-up test used was the active fraction in reducing blood cholesterol levels and mice triglycerides. The active fraction used will be increased to 1, 2, and 4 times the initial test dose (25, 50, 100 mg/grBB). The treatment group in this follow-up test is the same as the treatment group in the preliminary test, which is 6 groups each divided by 4 mice. Further testing was carried out for 14 days. On the 14th day the mice measured blood cholesterol levels, and on the 14th day the triglycerides were measured. Further grouping of tests as in Table 2.

2.3.7 Measurement of Total Blood Cholesterol Levels and Triglycerides

Measurements were made using the Nesco MultiCheck gauge. This tool will automatically turn on when the strip is inserted and will die when the strip is removed. After the blood enters the strip, the reaction from the strip container will automatically absorb blood into the strip through the capillaries. When the strip test is filled with blood, the device will process the blood for 150 seconds to get the results of measurements of the body's blood cholesterol levels.

Triglycerides measurements will be performed at the Center Laboratory Kesehatan (BBLK) Palembang with sampel blood of mice. The method used is the CHOD-PAP method. Previously, the blood of mice was collected first in a test tube, then let stand for 20 minutes. After that the blood is centrifuged for 20 minutes at 3000 rpm then the serum is taken as much as 10 μl and then put into a test tube, added as much as 1000 triglyceride reaction solution and mix well using vortex. Let stand for 20 minutes at room temperature and measure the absorption at a wavelength of 500 nm against the blank. Standard absorption measurements are carried out in the same way as sample absorption measurements.

2.3.8 Data analysis

This study used a Completely Randomized Design (CRD) divided into 6 treatment groups with 4 replications. The data obtained will then be tested for normality with the Kolmogorov-Smirnov Test, homogeneity test with Levene's, and quantitative tests for total blood cholesterol and triglyceride levels obtained by the One Way Anova test, if there are significant differences between treatments followed by the Duncan Multiple Area test (WBD).

3. RESULTS AND DISCUSSION

3.1 Normality Test and Homogeneity Test

Based on the results of the normality test using the Kolmogorov-Smirnov test and homogeneity test using the Leven's test that the results of the study reduced blood cholesterol levels in mice in the preliminary test that is normally distributed and homogeneous data ($P > 0.05$). In further tests the decrease in blood cholesterol and triglyceride levels in mice also had normal distribution and homogeneous data ($P > 0.05$). The results of the normality test and homogeneity test are presented in Table 3 and Table 4.

3.2 Effectiveness of active fractions on Pomegranate (*Punica granatum L.*) in reducing Total Blood Cholesterol levels in mice (*Mus musculus L.*)

Based on the preliminary test results, it can be seen that the effect of treatment on extracts and fractions with the average in the decrease in total blood cholesterol levels of mice is A0 = 165.65 mg / dl, A1 = 208.30 mg / dl, A2 = 170.91 mg / dl, A3 = 177.58 mg / dl, A4 = 189.75 mg / dl, A5 = 169.65 mg / dl. Then he analyzed the significance of

Table 1. Dose of Treatment in the Preliminary Test

| Group Treatment | Treatment | |
|-----------------|---------------------------------|---|
| | Days 1-5 | Days 6-10 |
| A0 | Distilled water 0.1ml/10grBB | Distilled water 0.1ml/10grBB |
| A1 | MDLT 0.1ml/10grBB | Distilled water 0.1ml/10grBB |
| A2 | MDLT 0.1ml/10grBB | 25 mg/grBB of pomegranate extract |
| A3 | MDLT 0.1ml/10grBB | 25 mg/grBB of n-hexane pomegranate fraction |
| A4 | MDLT 0.1ml/10grBB | 25 mg/kg body weight of pomegranate ethyl acetate fraction |
| A5 | MDLT 0.1ml/10grBB | 25 mg/kgBB of pomegranate water methanol fraction |

Table 2. Dose of Treatment in Advanced Tests

| Group Treatment | Treatment | |
|-----------------|-----------------------------------|---|
| | Days 1-7 | Days 8-14 |
| P0 | Distilled water 0.1ml / 10grBB | Distilled water 0.1ml / 10grBB |
| P1 | MDLT 0.1ml / 10grBB | Distilled water 0.1ml / 10grBB |
| P2 | MDLT 0.1ml / 10grBB | Simvastatin drug |
| P3 | MDLT 0.1ml / 10grBB | 25 mg/kgBB of pomegranate water methanol fraction |
| P4 | MDLT 0.1ml / 10grBB | 50 mg/kgBB of pomegranate water methanol fraction |
| P5 | MDLT 0.1ml / 10grBB | 100 mg / kgBB of pomegranate water methanol fraction |

Table 3. Normality test result of total blood cholesterol levels and triglyceride in the preliminary and advanced test

| Research variable | n | p | Distribution |
|-------------------|----|-------|--------------|
| Total cholesterol | | | |
| Preliminary Test | 24 | 0.18 | Normal |
| Advanced Test | 24 | 0.363 | Normal |
| Triglycerides | | | |
| Advanced Test | 24 | 0.209 | Normal |

Table 4. Homogeneity test result of total blood cholesterol levels and triglyceride in the preliminary and advanced test

| Research variable | F | p | Information |
|-------------------|-------|-------|-------------|
| Total cholesterol | | | |
| Preliminary Test | 1,508 | 0.237 | Homogeneous |
| Advanced Test | 0.521 | 0.757 | Homogeneous |
| Triglycerides | | | |
| Advanced Test | 0.284 | 0.916 | Homogeneous |

the data with the One Way Anova test which showed that the value of $F = 26.316$ and the value of $p = 0,000$. This means that the mean total cholesterol in the blood group after treatment was highly significant ($p < 0.05$). Data on the results of the study of the differences in the average reduction in total blood cholesterol levels of mice and their graphs in the preliminary test are shown in Table 5 and

Figure 1.

Preliminary test results showed that the treatment given 25 mg/grBB of pomegranate fraction of methanol water was more effective in reducing total blood cholesterol levels in mice compared to other treatments. Therefore, the study continued with increasing the dose of active fraction that is the pomegranate fraction of water methanol to 25, 50 and

Table 5. Preliminary test results of total blood cholesterol levels in mice

| Treatment group | N | Average ± SD |
|---|---|---------------|
| A0 (negative control) | 4 | 165.65 ± 5.70 |
| A1 (positive control) | 4 | 208.30 ± 7.70 |
| A2 (Pomegranate Extract 25 mg / grBB) | 4 | 170.91 ± 4.95 |
| A3 (fraction of n-hexane 25 mg / grBB) | 4 | 177.58 ± 4.02 |
| A4 (25 mg / grBB ethyl acetate fraction) | 4 | 189.75 ± 9.41 |
| A5 (25 mg / grBB water methanol fraction) | 4 | 169.65 ± 3.72 |

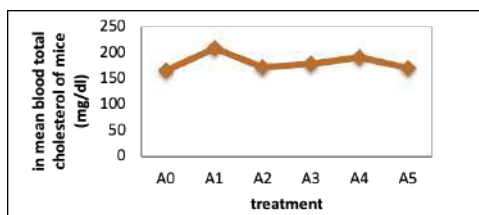


Figure 1. Graph of average differences blood total cholesterol levels of mice after being treated in a preliminary test

Note: A0; negative control (not treated), A1; positive control (MDLT-induced), A2; MDLT + pomegranate extract 25 mg / grBB, A3; MDLT + n-hexane fraction 25 mg / grBB, A4; MDLT + ethyl acetate fraction 25 mg / grBB, A5; MDLT + methanol water fraction 25 mg / grBB.

100 mg / grBB then compared with the antihypercholesterol drugs namely simvastatin.

3.3 Effects of Pomegranate (*Punica granatum .L*) Active Fraction in Lowering Total Blood Cholesterol Levels and Triglycerides in Mice (*Mus musculus L.*)

3.3.1 Total Blood Cholesterol Levels

The results of the study after the administration of the treatment with various concentrations in the treatment groups P0, P1, P2, P3, P4, and P5 showed a difference in the average amount of total cholesterol in the blood of mice after treatment. The average total cholesterol level of men cit blood is P0 = 167.69 mg / dl, P1 = 215.48 mg / dl, P2 = 166.12 mg / dl, P3 = 182.15 mg / dl, P4 = 175, 82 mg / dl, P5 = 166.71 mg / dl. The avaragetotal cholesterol level in blood of mice can be seen in Table 6 and the graph in Figure 2.

After he analyzed the significance with the One Way Anova test showed that the value of F = 26.825 and the value of p = 0.000. This means that the total cholesterol in the blood of the six groups after treatment was significantly

Table 6. Result of total blood cholesterol levels of mice after being given treatment

| Treatment group | N | Average ± SD |
|--|---|----------------|
| P0 (negative control) | 4 | 167.69 ± 5.87 |
| P1 (positive control) | 4 | 215.48 ± 10.98 |
| P2 (simvastatin) | 4 | 166.12 ± 5.14 |
| P3 (25 mg / grBB water methanol fraction) | 4 | 182.15 ± 6.85 |
| P4 (50 mg / grBB water methanol fraction) | 4 | 175.82 ± 5.11 |
| P5 (100 mg / grBB water methanol fraction) | 4 | 166.71 ± 8.14 |

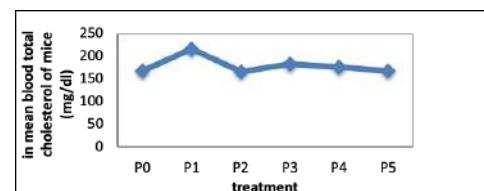


Figure 2. Graph of differences in mean levels total cholesterol of the blood of mice after treatment

Note: P0; negative control, P1; positive control (MDLT-induced), P2; MDLT + simvastatin, P3; MDLT + methanol water fraction 25 mg / grBB, P4; MDLT + mehanol water fraction 50 mg / grBB, P5; MDLT + methanol water fraction 100 mg / grBB

different (p<0.05). In the Post Hoc Duncan test, it was shown that the P2 treatment given simvastatin when compared with P5, P0, and P4 was not significantly different in reducing total blood cholesterol levels in mice. Whereas P2, P5, PO, and P4 were significantly different from the decrease in total cholesterol levels in mice. In the treatment of P5 given 100 mg/grBB of methanol waterfraction, almost comparable to the treatment of P2 given antihypercholesterolemia drugs simvastatin in giving the effect of reducing total cholesterol levels in blood of mice. Pos Hoc Duncan's test can be seen in Table 7.

Based on the results above shows that the mice given 100 mg/grBB of pomegranate water methanol fraction has the effect of reducing total blood cholesterol levels which is almost comparable to the blood given simvastatin. This is because 92% of pomegranates have antioxidant content (Nge et al., 2015). The presence of antioxidant compounds such as flavonoids, alkaloids, and saponins. In other treatment groups such as at doses of 25 and 50 mg / grBB the pomegranate water methanol fraction also had a decrease but this was not significant. The treatment group gave a dose of 100 mg / grBB of pomegranate water methanol fraction was able to reduce the maximum and is a dose which is appropriate compared to other doses, this reduction is almost comparable to the decrease in the treatment

Table 7. Post Hoc Duncan Test effectiveness of pomegranate fraction (*Punica granatum* L.) againsts decreased total blood cholesterol levels in mice (*Mus musculus* L.)

| Treatment group | n | Subset for alpha = 0.05 | | |
|--|---|-------------------------|---------------------|---------------------|
| | | 1 | 2 | 3 |
| P2(simvastatin) | 4 | 166.12 _a | | |
| P5(100mg/grBB water methanol fraction) | 4 | 166.71 _a | | |
| P0(positive control) | 4 | 167.69 _a | | |
| P4(50mg/grBB water methanol fraction) | 4 | 175.82 _{a,b} | 175.82 _b | |
| P3(25mg/grBB water methanol fraction) | 4 | | 182.12 _b | |
| P1 (negative control) | 4 | | | 215.48 _c |
| Sig. | | 0.101 | 0.237 | 1,000 |

group given simvastatin which if consumed continuously has adverse side effects for the body. Mechanism of flavonoid compounds can reduce total cholesterol levels by inhibiting 3-Hydroxy-3-Methyl-Glutaryl-CoenzymeA (HMG-CoA) reductase that causes a decrease in cholesterol synthesis and increases the number of LDL receptors contained in the liver cell membranes and extrahepatic tissue so that levels total cholesterol will decrease, with a decrease in total cholesterol levels, LDL which functions as a means of transporting lipids in the blood will decrease in levels (Nge et al., 2015). Saponins form a complex that is insoluble with cholesterol, thereby preventing the absorption of cholesterol in the small intestine. In addition, saponins reduce the absorption of bile sap by forming micelle complexes that cannot be absorbed because the molecular weight is too large (Matsui et al., 2009). Tannins will bind to the body's protein and will coat the intestinal wall, so that fat absorption is inhibited. Tannins in plants inhibit the absorption of cholesterol in the surface of the small intestine by reacting with mucosal protein and intestinal epithelial cells and increase the formation of gallbladder acid from cholesterol and then excreted through feces so that it will reduce cholesterol levels (Mu'nisa et al., 2014). Pomegranates also contain fiber and vitamin C (Wahyuni et al., 2013). Fiber can inhibit the absorption of cholesterol in the small intestine and ultimately will reduce the concentration of cholesterol in plasma and increase cholesterol synthesis by the liver, bile synthesis, and excretion of cholesterol through feces. Therefore, food fiber has been widely used and recommended to maintain blood cholesterol concentrations to remain normal while vitamin C is mentioned that plants that have vitamin C have the potential to reduce cholesterol (Hernawati, 2015; Wahyuni et al., 2013). Besides flavonoids, saponins, and tannins, in some literature pomegranates also contain alkaloids, and triterpenoids (Roswien, 2014).

Giving 100 mg/grBB of pomegranate water methanol

fraction in mice is almost comparable to hypercholesterol mice given antihypercholesterol drugs in the form of simvastatin. Simvastatin is a statin antihypercholesterol drug which causes side effects such as diarrhea, nausea, vomiting, headaches and body aches. While pomegranates are an alternative in reducing total blood cholesterol levels because the nutritional content of pomegranates is quite adequate as a source of vitamins. So that white pomegranate meat is safer to be used as a blood cholesterol-lowering drug compared to simvastatin drug (Wahyuni et al., 2013).

3.4 Triglycerides

The results of the study after the administration of treatments with various concentrations in the treatment groups P0, P1, P2, P3, P4, and P5 showed that there were differences in the average amount of mice triglycerides after treatment. The average triglyceride men cit are P0 = 101.63 mg/dl, P1 = 153.02 mg/dl, P2 = 90 mg/dl, P3 = 82.48 mg/dl, P4 = 77.08 mg/dl, P5 = 74.61 mg/dl. The average of mice triglycerides can be seen in Table 8 and the graph in Figure 3.

Table 8. Result of triglycerides in mice after being treated

| Treatment group | N | Average ± SD |
|--|---|---------------|
| P0 (negative control) | 4 | 101.63 ± 5.32 |
| P1 (positive control) | 4 | 153.02 ± 6.36 |
| P2 (simvastatin) | 4 | 90.00 ± 8.82 |
| P3 (25 mg / grBB water methanol fraction) | 4 | 82.48 ± 10.29 |
| P4 (50 mg / grBB water methanol fraction) | 4 | 77.08 ± 8.77 |
| P5 (100 mg / grBB water methanol fraction) | 4 | 74.61 ± 8.95 |

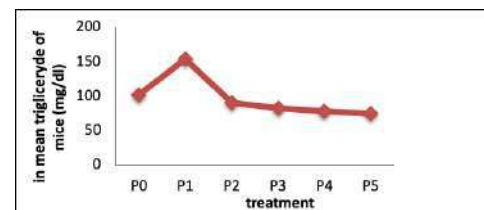


Figure 3. Graph of differences in mean triglyceride of mice after treatment

Note: P0; negative control, P1; positive control (MDLT-induced), P2; MDLT + simvastatin, P3; MDLT + methanol water fraction 25 mg / grBB, P4; MDLT + mehanol water fraction 50 mg / grBB, P5; MDLT + methanol water fraction 100 mg / grBB.

After he analyzed the significance with the One Way Anova test, it showed that the value of F = 50.522 and p = 0.000. This means that the mean total cholesterol in the

Table 9. Post Hoc Duncan Test effectiveness of pomegranate fraction (*Punica granatum* L.) against decreased triglyceride in mice (*Mus musculus* L.)

| Treatment group | n | Subset for alpha = 0.05 | | | |
|---|---|-------------------------|-----------------------|----------------------|---------------------|
| | | 1 | 2 | 3 | 4 |
| P5 (100mg / grBB water methanol fraction) | 4 | 74 6175 _a | | | |
| P4 (50mg / grBB water methanol fraction) | 4 | 77 0825 _a | | | |
| P3 (25mg / grBB water methanol fraction) | 4 | 82 4875 _{ab} | 82 4875 _b | | |
| P2 (simvastatin) | 4 | | 90 0000 _{bc} | 90 0000 _c | |
| P0 (positive control) | 4 | | | 101 63 _c | |
| P1 (negative control) | 4 | | | | 153 02 _d |
| Sig. | | 0.218 | 0.215 | 0.62 | 1,000 |

blood of the six groups after treatment was significantly different ($p < 0.05$). In the Post Hoc Duncan test, it was shown that the P2 treatment given simvastatin when compared with P5, P0, and P4 was not significantly different in reducing total blood cholesterol levels in mice. Whereas P2, P5, P0, and P4 were significantly different from the decrease in total cholesterol levels in mice. Post Hoc Duncan test results showed that the treatment of P5 given a pomegranate fraction of methanol water 100 mg/grBB when compared with the treatment of P4, and P3 were not significantly different to the decrease in triglyceride in mice. Whereas P5, P4, and P3 were significantly different compared to treatments P2 and P0. In the treatment given pomegranate water methanol fraction from several concentrations is comparable to the decrease in the treatment given simvastatin can be seen in Table 9.

Based on the results of the above study, it is known that 92% of pomegranates contain powerful antioxidants (Nge et al., 2015). The benefits of pomegranates in the fight against free radicals that can cause degenerative diseases have been widely studied by scientists (Muffihunna et al., 2014). An increase in cholesterol levels in the blood can cause formation. Free radicals are compounds that are very reactive and have unpaired electrons in their outer skin. Free radicals can come from pollution, dust and can be produced from metabolic processes that can be bad (Yuliani and Dinina, 2015). Free radicals will complement their unpaired electrons by attracting nearby macromolecular electrons such as proteins, nucleic acids and DNA. Macromolecules in cells if oxidized and degraded can cause damage to cells (Astuti, 2012). Therefore, the formation of free radicals due to oxidation of triglycerides and cholesterol can damage endothelial cells causing atherosclerosis (Jufri et al., 2015). Chemical content of pomegranates, namely flavonoids, p-phenol and catechins as well as vitamin A and vitamin C. Where compounds -This compound is a powerful antioxidant that is useful to prevent the development of free radicals in the body and repair damaged body cells (Muffihunna et al., 2014).

From the results of research that has been done shows

that mice given 100 mg / grBB are more effective in reducing triglycerides. This is caused by the presence of antioxidant compounds such as flavonoids, alkaloids, and saponins. In other treatment groups such as at doses of 25 and 50 mg / grBB the pomegranate water methanol fraction also had a decrease but this was not significant because the treatment group gave a dose of 100 mg / grBB of pomegranate water methanol fraction was able to reduce the maximum and is a dose which is appropriate compared to other doses, this reduction is almost comparable to the decrease in the treatment group given simvastatin which if consumed continuously has adverse side effects for the body. The mechanism of flavonoid compounds can reduce triglyceride levels through increased activity of the LPL enzyme, with the increase in the enzyme VLDL that carries triglycerides will hydrolyse into fatty acids and glycerol. The released fatty acids will be absorbed by muscles and other tissues, then oxidized to produce energy and adipose tissue will store them as energy reserves (Mu'nisa et al., 2014). The flavonoids can inhibit Fatty Acid Synthase (FAS), which is a very important enzyme in the metabolism of fat. Barriers to FAS can directly reduce the formation of fatty acids, thereby reducing the formation of triglycerides (Zhang et al., 2009). Saponin mechanism can reduce triglyceride levels, namely by inhibiting the absorption of cholesterol and triglycerides in the intestine and increasing the reaction of bile acid formation from cholesterol then excreted through feces (Arauna and Aulanni'am, 2016). Saponins bind with bile acids and cholesterol (from food), then form micelles that cannot be absorbed by the intestine and also inhibit the work of the LPL enzyme (Rahmawati et al., 2016). Saponins can inhibit the absorption of cholesterol and triglycerides in the intestine by forming insoluble complex bonds in cholesterol, binds to bile acids to form micelles and increases binding of cholesterol and triglycerides by fiber (Ekananda, 2015). While tannin compounds can reduce triglyceride levels by reducing the absorption of cholesterol and triglycerides in the small intestine and increasing the excretion of bile acids (Cahaya and Ayu, 2017).

4. CONCLUSIONS

The conclusion from the results of this study is that mice given pomegranate fractions are effective in reducing total cholesterol and triglyceride levels due to the presence of antioxidant compounds as antihypercholesterol. The active dose that can reduce blood cholesterol and triglyceride levels in mice is in the treatment group given 100 mg / grBB and that was very significantly different to other treatment groups given doses of 25 and 50 mg / grBB of pomegranate water methanol fraction.

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Effect Of Pomegranate (*Punica granatum* L.) Fraction In Reducing Total Blood Cholesterol Levels and Triglyceride in Mice (*Mus musculus* L.)

By Arum Setiawan

Effect Of Pomegranate (*Punica granatum* L.) Fraction In Reducing Total Blood Cholesterol Levels And Triglyceride In Mice (*Mus musculus* L.)

Nurlaily Agustini^{1*}, Arum Setiawan², Salni²

¹Department Bioscience Program, Faculty of Science, Sriwijaya University, Jalan Padang Selasa 524, Palembang, Sumatera Selatan 30139, Indonesia.

²Department of Biology, Faculty of Mathematics & Natural Sciences, Sriwijaya University, Jalan Raya Palembang-Prabumulih km 32, Indralaya, Indonesia.

*Corresponding author e-mail: laily1708@yahoo.com

Abstract

Pomegranate (*Punica granatum* L.) will be examined by looking at the ability between pomegranate extracts or fractions, which are more effective in lowering total blood cholesterol and triglyceride levels in mice. This study aims to determine the effect pomegranate fraction of methanol water from several concentrate that can reduce the total cholesterol levels and triglyceride in mice. The research method used was an experimental method with a completely randomized design (CRD) pattern. The research used 6 treatment groups, and test animals divided into 6 treatment groups namely negative control given aquades, positive control fed a high-fat diet, given simvastatin, given 25 mg/grBB pomegranate fraction of methanol water, given 50 mg/grBB pomegranate fraction of methanol water, given 100 mg/grBB of pomegranate fraction of methanol water, each treatment group will be divided into 4 male mice. Based on the results of this study, the active fraction that has an effect on reducing blood cholesterol and triglyceride levels with a percentage reduction about 48% and the significance value of $0,000 < \alpha = 0.05$ is the treatment given 100mg/grBB pomegranate fraction of methanol water.

Keywords

pomegranate, total cholesterol, and triglyceride

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1. INTRODUCTION

Along with the development of times and changes in unhealthy lifestyles ranging from unbalanced eating patterns, consumption of foods high in fat and carbohydrates, to the lack of doing sports activities, many suffer from hypercholesterolemia. Increased levels of cholesterol and triglycerides in the blood cause the formation of free radicals. High cholesterol and triglyceride levels are also closely related to the increased incidence of cardiovascular disease (Budiyono and Candra, 2013; Roslizawaty et al., 2016).

Hypercholesterolemia is an increase in cholesterol levels in the blood that exceeds the limits needed by the body. Someone said to suffer from hypercholesterolemia when total plasma cholesterol levels exceed normal conditions, ie above 200 mg / dL. Cholesterol is one part of fat as one of the nutrients the body needs and the highest calorie producer (Wirawan, 2018). Hypercholesterolemia is a trigger for coronary heart disease because high cholesterol causes blockages in peripheral vessels that reduce blood supply to the heart. High cholesterol can also be a trigger for hypertension and

stroke (Soleha, 2012). Hypercholesterolemia is a trigger for coronary heart disease because of high cholesterol causes blockages in peripheral vessels that reduce blood supply to the heart. High cholesterol can also be a trigger for hypertension and stroke (Alwan et al., 2009).

Dietary management and diet modification is one way to help reduce blood lipid levels. The recommended dietary arrangement is by limiting the consumption of foods that contain high cholesterol and fat, especially saturated fat. In addition to limiting foods that are high in cholesterol and fat, consuming foods that have benefits for lowering cholesterol levels (Sundari and Dieny, 2013).

Medicinal plants have been used a lot by humans since ancient times. The development of the times is also increasing human knowledge about pharmacology and medical science, many people turn to chemical drugs that have been tested for laboratory efficacy. Over time with the development of public knowledge and awareness of the dangers posed by chemicals contained in medicines, people are encouraged to return to using traditional medicines with technological developments. More and more plants have been proven to have

laboratory efficacy and are guaranteed safe for consumption and cure diseases without causing side effect (Aseptianova, 2019).

Indonesia as a country that has developed modern health services but the number of people who use traditional medicine remains high. According to the 2001 national socioeconomic survey 57.7% of the population of Indonesia treated themselves without medical assistance, 31.2% of them used traditional medicinal plants and 9.8% chose other traditional methods of treatment (Novitasiah et al., 2012). WHO recommends the use of traditional medicines in the maintenance of public health, prevention and treatment of diseases, especially for chronic diseases, degenerative diseases and cancer. WHO also supports efforts to improve the safety and efficacy of traditional medicines (Bustanussalam, 2016).

Pomegranate is a fruit that comes from the Middle East. The potential for antioxidants in pomegranates is very high, which is around 92% (Nge et al., 2015). The function of antioxidants to inhibit the formation of degenerative diseases such as hypercholesterolemia (Mutia et al., 2018). Pomegranate has several active compounds namely alkaloids, flavonoids, saponins, tannins, and triterpenoids (Roswien, 2014). Based on some previous studies plants containing alkaloids and flavonoids have benefits in reducing cholesterol levels (Zulviana et al., 2017). So far there has not been much research on pomegranate fractions in reducing blood cholesterol and triglyceride levels. But there have been several studies on pomegranates, one of which was conducted by Wahyuni et al. (2013). In that study stated that pomegranate meat can reduce blood cholesterol levels. That is due to the presence of phytosterols and some other nutritional content found in pomegranates which are anticholesterol (Wahyuni et al., 2013). Based on the description above, this study aims to determine the effect of pomegranate fraction in reducing blood cholesterol levels and triglyceride in mice.

2. EXPERIMENTAL SECTION

2.1 Materials

The tools used in this study are blenders, filters, measuring cups, mortars, maceration containers, rotary evaporators, separating funnels, animal cages, wood chips, animal drinking bottles, analytical scales, oral needles, sonde, paper labels, blood vessels, surgical scissors, cotton swabs, masks, rubber gloves, blood cholesterol levels and the Nesco Multi-Check brand stripstest.

The materials used in this study were fresh pomegranate, 48 male strain mice, distilled water, 96% ethanol, n-hexane, ethyl acetate, used cooking oil, quail eggs, 1% CMC Na, simvastatin, 70% alcohol .

2.2 Experimental Animals

The experimental animals used were 48 male Swiss Webster strain mice. The mice used are around 2-3 months old and

weigh about 25-40 grams originating from livestock farming in the Bogor agriculture department. Rat cages are first sterilized by drying them in the sun, then given wood dust as the base of the cage. After the sterile cage, male rats were immediately placed and fed with pellets and drinking distilled water ad libitum (to taste). The cage was also given a woven wire as a cover and then the rats were acclimatized for 7 days. The cage is cleaned and the sawdust is replaced every 3 days.

2.3 Methods

2.3.1 Sampling

Samples to be used are pomegranates taken from pomegranate farmers weighing 10 kg in Situbondo, Central Java and then processed into extracts and fractions at the Genetic Laboratory of the Faculty of Mathematics and Natural Sciences, Sriwijaya University.

2.3.2 Making Pomegranate Ethanol Extract (*Punica granatum L.*)

This study used pomegranates, sorted and washed, drained and dried for 2-3 days. After drying, simplicia is then made into powder using a blender, and sieved to get the powder. The extraction process uses maceration method which is pomegranate powder weighed then immersed with 96% ethanol for 2x24 hours then filtered and obtained liquid extract. The liquid extract obtained was concentrated by distillation using arotary evaporator at a temperature of 80 C at a speed of 30 rpm so that it is free of ethanol and produces a thick concentrated extract. Then the ethanol that has been separated and then soaked again with pomegranate powder for 2 days then do it the previous way using a rotary evaporator until it gets the second concentrated extract. The second concentrated extract is then dried with a hairdryer until later the extract will be more concentrated like a paste.

2.3.3 Making a pomegranate fraction (*Punica granatum L.*)

Concentrated pomegranate extract was fractionated with 1 liter of n-hexane, shaken for 5 minutes. After that it is allowed to form 2 layers namely the n-hexane layer and the water layer. This treatment is repeated several times until the n-hexane layer looks clear so that the n-hexane fraction is obtained. The water layer is then fractionated with 1 liter of ethyl acetate, repeated several times as in the above treatment so that the water fraction and ethyl acetate fraction are obtained. Then the water fraction is mixed with 100 ml of methanol, shaken for 5 minutes and the water methanol fraction is obtained. All n-hexane, ethyl acetate and methanol water fractions were evaporated with a rotary evaporator until a thick fraction was obtained. After getting the concentrated fraction, dry it using a hairdryer until a concentrated fraction of paste is obtained.

2.3.4 Classification and Induction of Experimental Animals

The animals used in this study were 48 male Swiss Webster strain mice aged around 2-3 months and weighing around 25-40 grams. The experimental animals will be divided into 6 groups for preliminary tests namely negative control treatment (A0), positive control (A1), pomegranate extract (A2), pomegranate n-hexane fraction (A3), pomegranate acetate fraction (A4), water methanol fraction pomegranate (A5) and 6 groups for further tests namely negative control treatment (P0), positive control (P1), pomegranate water methanol fraction (P3), pomegranate water methanol fraction (P4), pomegranate water methanol fraction (P5). Each group consists of 4 mice each. In the positive control group quail yolk was induced by 1% body weight of mice and high-fat diet foods were added.

2.3.5 Dosage Planning

The dosages used in the preliminary test were 25 mg/grBB each in pomegranate extract, n-hexane fraction, ethyl acetate fraction, and pomegranate water methanol fraction. The treatment between the extract and the most effective fraction will be carried out further tests on the fraction that is most effective in reducing blood cholesterol levels and mice triglycerides.

The dose of simvastatin for humans is 20 mg/day so the dose must be converted to mice. The dose of simvastatin for mice is $0.0026 \times 20\text{mg} / \text{x} / \text{tail} = 0.052 \text{ mg} / \text{x} / \text{tail}$. Two 10 mg simvastatin tablets were crushed until smooth and parchment paper was given 1% CMC Na, pitch and measured to obtain 1 g CMC Na. Then give CMC Na 1 g of warm water then crushed with crushed simvastatin and add 100 ml of distilled water (Artha et al., 2017).

2.3.6 Research Test Stage

In the preliminary test conducted to determine the effective fraction in reducing blood cholesterol levels in mice. Provision of treatment in this test will be carried out for 7 days, then only found the most effective fraction in reducing total blood cholesterol levels and the most effective fraction is what will be used for further testing stages in reducing blood total cholesterol levels and mice triglycerides. Animal mice will be divided into 6 groups. Each group will be divided into 4 mice and given the treatment as in Table 1.

The follow-up test used was the active fraction in reducing blood cholesterol levels and mice triglycerides. The active fraction used will be increased to 1, 2, and 4 times the initial test dose (25, 50, 100 mg/grBB). The treatment group in this follow-up test is the same as the treatment group in the preliminary test, which is 6 groups each divided by 4 mice. Further testing was carried out for 14 days. On the 14th day the mice measured blood cholesterol levels, and on the 14th day the triglycerides were measured. Further grouping of tests as in Table 2.

2.3.7 Measurement of Total Blood Cholesterol Levels and Triglycerides

Measurements were made using the Nesco MultiCheck gauge. This tool will automatically turn on when the strip is inserted and will die when the strip is removed. After the blood enters the strip, the reaction from the strip container will automatically absorb blood into the strip through the capillaries. When the strip test is filled with blood, the device will process the blood for 150 seconds to get the results of measurements of the body's blood cholesterol levels.

Triglycerides measurements will be performed at the Center Laboratory Kesehatan (BBLK) Palembang with sampel blood of mice. The method used is the CHOD-PAP method. Previously, the blood of mice was collected first in a test tube, then let stand for 20 minutes. After that the blood is centrifuged for 20 minutes at 3000 rpm then the serum is taken as much as 10 μl and then put into a test tube, added as much as 1000 triglyceride reaction solution and mix well using vortex. Let stand for 20 minutes at room temperature and measure the absorption at a wavelength of 500 nm against the blank. Standard absorption measurements are carried out in the same way as sample absorption measurements.

2.3.8 Data analysis

This study used a Completely Randomized Design (CRD) divided into 6 treatment groups with 4 replications. The data obtained will then be tested for normality with the Kolmogorov-Smirnov Test, homogeneity test with Levene's, and quantitative tests for total blood cholesterol and triglyceride levels obtained by the One Way Anova test, if there are significant differences between treatments followed by the Duncan Multiple Area test (WBD).

3. RESULTS AND DISCUSSION

3.1 Normality Test and Homogeneity Test

Based on the results of the normality test using the Kolmogorov-Smirnov test and homogeneity test using the Leven's test that the results of the study reduced blood cholesterol levels in mice in the preliminary test that is normally distributed and homogeneous data ($P > 0.05$). In further tests the decrease in blood cholesterol and triglyceride levels in mice also had normal distribution and homogeneous data ($P > 0.05$). The results of the normality test and homogeneity test are presented in Table 3 and Table 4.

3.2 Effectiveness of active fractions on Pomegranate (*Punica granatum L.*) in reducing Total Blood Cholesterol levels in mice (*Mus musculus L.*)

Based on the preliminary test results, it can be seen that the effect of treatment on extracts and fractions with the average in the decrease in total blood cholesterol levels of mice is $A0 = 165.65 \text{ mg} / \text{dl}$, $A1 = 208.30 \text{ mg} / \text{dl}$, $A2 = 170.91 \text{ mg} / \text{dl}$, $A3 = 177.58 \text{ mg} / \text{dl}$, $A4 = 189.75 \text{ mg} / \text{dl}$, $A5 = 169.65 \text{ mg} / \text{dl}$. Then he analyzed the significance of

Table 1. Dose of Treatment in the Preliminary Test

| Group Treatment | Treatment | |
|-----------------|---------------------------------|---|
| | Days 1-5 | Days 6-10 |
| A0 | Distilled water 0.1ml/10grBB | Distilled water 0.1ml/10grBB |
| A1 | MDLT 0.1ml/10grBB | Distilled water 0.1ml/10grBB |
| A2 | MDLT 0.1ml/10grBB | 25 mg/grBB of pomegranate extract |
| A3 | MDLT 0.1ml/10grBB | 25 mg/grBB of n-hexane pomegranate fraction |
| A4 | MDLT 0.1ml/10grBB | 25 mg/kg body weight of pomegranate ethyl acetate fraction |
| A5 | MDLT 0.1ml/10grBB | 25 mg/kgBB of pomegranate water methanol fraction |

Table 2. Dose of Treatment in Advanced Tests

| Group Treatment | Treatment | |
|-----------------|-----------------------------------|---|
| | Days 1-7 | Days 8-14 |
| P0 | Distilled water 0.1ml / 10grBB | Distilled water 0.1ml / 10grBB |
| P1 | MDLT 0.1ml / 10grBB | Distilled water 0.1ml / 10grBB |
| P2 | MDLT 0.1ml / 10grBB | Simvastatin drug |
| P3 | MDLT 0.1ml / 10grBB | 25 mg/kgBB of pomegranate water methanol fraction |
| P4 | MDLT 0.1ml / 10grBB | 50 mg/kgBB of pomegranate water methanol fraction |
| P5 | MDLT 0.1ml / 10grBB | 100 mg / kgBB of pomegranate water methanol fraction |

Table 3. Normality test result of total blood cholesterol levels and triglyceride in the preliminary and advanced test

| Research variable | n | p | Distribution |
|-------------------|----|-------|--------------|
| Total cholesterol | | | |
| Preliminary Test | 24 | 0.18 | Normal |
| Advanced Test | 24 | 0.363 | Normal |
| Triglycerides | | | |
| Advanced Test | 24 | 0.209 | Normal |

Table 4. Homogeneity test result of total blood cholesterol levels and triglyceride in the preliminary and advanced test

| Research variable | F | p | Information |
|-------------------|-------|-------|-------------|
| Total cholesterol | | | |
| Preliminary Test | 1,508 | 0.237 | Homogeneous |
| Advanced Test | 0.521 | 0.757 | Homogeneous |
| Triglycerides | | | |
| Advanced Test | 0.284 | 0.916 | Homogeneous |

the data with the One Way Anova test which showed that the value of $F = 26.316$ and the value of $p = 0,000$. This means that the mean total cholesterol in the blood group after treatment was highly significant ($p < 0.05$). Data on the results of the study of the differences in the average reduction in total blood cholesterol levels of mice and their graphs in the preliminary test are shown in Table 5 and

Figure 1. Preliminary test results showed that the treatment given 25 mg/grBB of pomegranate fraction of methanol water was more effective in reducing total blood cholesterol levels in mice compared to other treatments. Therefore, the study continued with increasing the dose of active fraction that is the pomegranate fraction of water methanol to 2 5, 50 and

Table 5. Preliminary test results of total blood cholesterol levels in mice

| Treatment group | N | Average ± SD |
|---|---|---------------|
| A0 (negative control) | 4 | 165.65 ± 5.70 |
| A1 (positive control) | 4 | 208.30 ± 7.70 |
| A2 (Pomegranate Extract 25 mg / grBB) | 4 | 170.91 ± 4.95 |
| A3 (fraction of n-hexane 25 mg / grBB) | 4 | 177.58 ± 4.02 |
| A4 (25 mg / grBB ethyl acetate fraction) | 4 | 189.75 ± 9.41 |
| A5 (25 mg / grBB water methanol fraction) | 4 | 169.65 ± 3.72 |

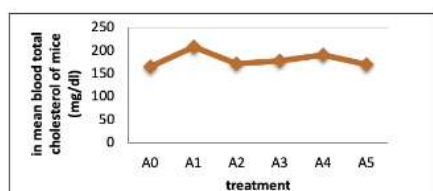


Figure 1. Graph of average differences blood total cholesterol levels of mice after being treated in a preliminary test

Note: A0; negative control (not treated), A1; positive control (MDLT-induced), A2; MDLT + pomegranate extract 25 mg / grBB, A3; MDLT + n-hexane fraction 25 mg / grBB, A4; MDLT + ethyl acetate fraction 25 mg / grBB, A5; MDLT + methanol water fraction 25 mg / grBB.

100 mg / grBB then compared with the antihypercholesterol drugs namely simvastatin.

3.3 Effects of Pomegranate (*Punica granatum* L.) Active Fraction in Lowering Total Blood Cholesterol Levels and Triglycerides in Mice (*Mus musculus* L.)

3.3.1 Total Blood Cholesterol Levels

The results of the study after the administration of the treatment with various concentrations in the treatment groups P0, P1, P2, P3, P4, and P5 showed a difference in the average amount of total cholesterol in the blood of mice after treatment. The average total cholesterol level of men cit blood is P0 = 167.69 mg / dl, P1 = 215.48 mg / dl, P2 = 166.12 mg / dl, P3 = 182.15 mg / dl, P4 = 175, 82 mg / dl, P5 = 166.71 mg / dl. The avaragetotal cholesterol level in blood of mice can be seen in Table 6 and the graph in Figure 2.

After he analyzed the significance with the One Way Anova test showed that the value of F = 26.825 and the value of p = 0.000. This means that the total cholesterol in the blood of the six groups after treatment was significantly

Table 6. Result of total blood cholesterol levels of mice after being given treatment

| Treatment group | N | Average ± SD |
|--|---|----------------|
| P0 (negative control) | 4 | 167.69 ± 5.87 |
| P1 (positive control) | 4 | 215.48 ± 10.98 |
| P2 (simvastatin) | 4 | 166.12 ± 5.14 |
| P3 (25 mg / grBB water methanol fraction) | 4 | 182.15 ± 6.85 |
| P4 (50 mg / grBB water methanol fraction) | 4 | 175.82 ± 5.11 |
| P5 (100 mg / grBB water methanol fraction) | 4 | 166.71 ± 8.14 |

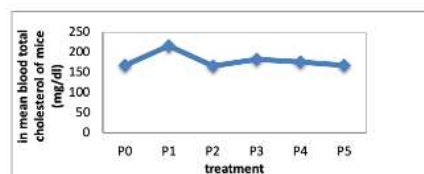


Figure 2. Graph of differences in mean levels total cholesterol of the blood of mice after treatment

Note: P0; negative control, P1; positive control (MDLT-induced), P2; MDLT + simvastatin, P3; MDLT + methanol water fraction 25 mg / grBB, P4; MDLT + mehanol water fraction 50 mg / grBB, P5; MDLT + methanol water fraction 100 mg / grBB

different (p<0.05). In the Post Hoc Duncan test, it was shown that the P2 treatment given simvastatin when compared with P5, P0, and P4 was not significantly different in reducing total blood cholesterol levels in mice. Whereas P2, P5, PO, and P4 were significantly different from the decrease in total cholesterol levels in mice. In the treatment of P5 given 100 mg/grBB of methanol waterfraction, almost comparable to the treatment of P2 given antihypercholesterolemia drugs simvastatin in giving the effect of reducing total cholesterol levels in blood of mice. Pos Hoc Duncan's test can be seen in Table 7.

Based on the results above shows that the mice given 100 mg/grBB of pomegranate water methanol fraction has the effect of reducing total blood cholesterol levels which is almost comparable to the blood given simvastatin. This is because 92% of pomegranates have antioxidant content (Nge et al., 2015). The presence of antioxidant compounds such as flavonoids, alkaloids, and saponins. In other treatment groups such as at doses of 25 and 50 mg / grBB the pomegranate water methanol fraction also had a decrease but this was not significant. The treatment group gave a dose of 100 mg / grBB of pomegranate water methanol fraction was able to reduce the maximum and is a dose which is appropriate compared to other doses, this reduction is almost comparable to the decrease in the treatment

Table 7. Post Hoc Duncan Test effectiveness of pomegranate fraction (*Punica granatum L.*) against decreased total blood cholesterol levels in mice (*Mus musculus L.*)

| Treatment group | n | Subset for alpha = 0.05 | | |
|--|---|-------------------------|---------------------|---------------------|
| | | 1 | 2 | 3 |
| P2(simvastatin) | 4 | 166 12 _a | | |
| P5(100mg/grBB water methanol fraction) | 4 | 166 71 _a | | |
| P0(positive control) | 4 | 167 69 _a | | |
| P4(50mg/grBB water methanol fraction) | 4 | 175 82 _{a,b} | 175 82 _b | |
| P3(25mg/grBB water methanol fraction) | 4 | | 182 12 _b | |
| P1 (negative control) | 4 | | | 215 48 _c |
| Sig. | | 0.101 | 0.237 | 1,000 |

group given simvastatin which if consumed continuously has adverse side effects for the body. Mechanism of flavonoid compounds can reduce total cholesterol levels by inhibiting 3- Hydroxy -3- Methyl- Glutaryl-CoenzymeA (HMG-CoA) reductase that causes a decrease in cholesterol synthesis and increases the number of LDL receptors contained in the liver cell membranes and extrahepatic tissue so that levels total cholesterol will decrease, with a decrease in total cholesterol levels, LDL which functions as a means of transporting lipids in the blood will decrease in levels (Nge et al., 2015). Saponins form a complex that is insoluble with cholesterol, thereby preventing the absorption of cholesterol in the small intestine. In addition, saponins reduce the absorption of bile sap by forming micelle complexes that cannot be absorbed because the molecular weight is too large (Matsui et al., 2009). Tannins will bind to the body's protein and will coat the intestinal wall, so that fat absorption is inhibited. Tannins in plants inhibit the absorption of cholesterol in the surface of the small intestine by reacting with mucosal protein and intestinal epithelial cells and increase the formation of gallbladder acid from cholesterol and then excreted through feces so that it will reduce cholesterol levels (Mu'nisa et al., 2014). Pomegranates also contain fiber and vitamin C (Wahyuni et al., 2013). Fiber can inhibit the absorption of cholesterol in the small intestine and ultimately will reduce the concentration of cholesterol in plasma and increase cholesterol synthesis by the liver, bile synthesis, and excretion of cholesterol through feces. Therefore, food fiber has been widely used and recommended to maintain blood cholesterol concentrations to remain normal while vitamin C is mentioned that plants that have vitamin C have the potential to reduce cholesterol (Hernawati, 2015; Wahyuni et al., 2013). Besides flavonoids, saponins, and tannins, in some literature pomegranates also contain alkaloids, and triterpenoids (Roswien, 2014).

Giving 100 mg/grBB of pomegranate water methanol

fraction in mice is almost comparable to hypercholesterol mice given antihypercholesterol drugs in the form of simvastatin. Simvastatin is a statin antihypercholesterol drug which causes side effects such as diarrhea, nausea, vomiting, headaches and body aches. While pomegranates are an alternative in reducing total blood cholesterol levels because the nutritional content of pomegranates is quite adequate as a source of vitamins. So that white pomegranate meat is safer to be used as a blood cholesterol-lowering drug compared to simvastatin drug (Wahyuni et al., 2013).

3.4 Triglycerides

The results of the study after the administration of treatments with various concentrations in the treatment groups P0, P1, P2, P3, P4, and P5 showed that there were differences in the average amount of mice triglycerides after treatment. The average triglyceride men cit are P0 = 101.63 mg/dl, P1 = 153.02 mg/dl, P2 = 90 mg/dl, P3 = 82.48 mg/dl, P4 = 77.08 mg/dl, P5 = 74.61 mg/dl. The average of mice triglycerides can be seen in Table 8 and the graph in Figure 3.

Table 8. Result of triglycerides in mice after being treated

| Treatment group | N | Average ± SD |
|--|---|---------------|
| P0 (negative control) | 4 | 101.63 ± 5.32 |
| P1 (positive control) | 4 | 153.02 ± 6.36 |
| P2 (simvastatin) | 4 | 90.00 ± 8.82 |
| P3 (25 mg / grBB water methanol fraction) | 4 | 82.48 ± 10.29 |
| P4 (50 mg / grBB water methanol fraction) | 4 | 77.08 ± 8.77 |
| P5 (100 mg / grBB water methanol fraction) | 4 | 74.61 ± 8.95 |

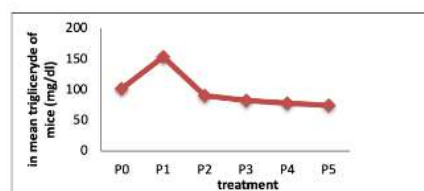


Figure 3. Graph of differences in mean triglyceride of mice after treatment

Note: P0; negative control, P1; positive control (MDLT-induced), P2; MDLT + simvastatin, P3; MDLT + methanol water fraction 25 mg / grBB, P4; MDLT + methanol water fraction 50 mg / grBB, P5; MDLT + methanol water fraction 100 mg / grBB.

After he analyzed the significance with the One Way Anova test, it showed that the value of $F = 50.522$ and $p = 0.000$. This means that the mean total cholesterol in the

Table 9. Post Hoc Duncan Test effectiveness of pomegranate fraction (*Punica granatum* L.) against decreased triglyceride in mice (*Mus musculus* L.)

| Treatment group | n | Subset for alpha = 0.05 | | | |
|---|---|-------------------------|-----------------------|----------------------|---------------------|
| | | 1 | 2 | 3 | 4 |
| P5 (100mg / grBB water methanol fraction) | 4 | 74 6175 _a | | | |
| P4 (50mg / grBB water methanol fraction) | 4 | 77 0825 _a | | | |
| P3 (25mg / grBB water methanol fraction) | 4 | 82 4875 _{ab} | 82 4875 _b | | |
| P2 (simvastatin) | 4 | | 90 0000 _{bc} | 90 0000 _c | |
| P0 (positive control) | 4 | | | 101 63 _c | |
| P1 (negative control) | 4 | | | | 153 02 _d |
| Sig. | | 0.218 | 0.215 | 0.62 | 1,000 |

blood of the six groups after treatment was significantly different ($p < 0.05$). In the Post Hoc Duncan test, it was shown that the P2 treatment given simvastatin when compared with P5, P0, and P4 was not significantly different in reducing total blood cholesterol levels in mice. Whereas P2, P5, P0, and P4 were significantly different from the decrease in total cholesterol levels in mice. Post Hoc Duncan test results showed that the treatment of P5 given a pomegranate fraction of methanol water 100 mg/grBB when compared with the treatment of P4, and P3 were not significantly different to the decrease in triglyceride in mice. Whereas P5, P4, and P3 were significantly different compared to treatments P2 and P0. In the treatment given pomegranate water methanol fraction from several concentrations is comparable to the decrease in the treatment given simvastatin can be seen in Table 9.

Based on the results of the above study, it is known that 92% of pomegranates contain powerful antioxidants (Nge et al., 2015). The benefits of pomegranates in the fight against free radicals that can cause degenerative diseases have been widely studied by scientists (Mufihunna et al., 2014). An increase in cholesterol levels in the blood can cause formation. Free radicals are compounds that are very reactive and have unpaired electrons in their outer skin. Free radicals can come from pollution, dust and can be produced from metabolic processes that can be bad (Yuliani and Dinina, 2015). Free radicals will complement their unpaired electrons by attracting nearby macromolecular electrons such as proteins, nucleic acids and DNA. Macromolecules in cells if oxidized and degraded can cause damage to cells (Astuti, 2012). Therefore, the formation of free radicals due to oxidation of triglycerides and cholesterol can damage endothelial cells causing atherosclerosis (Jufri et al., 2015). Chemical content of pomegranates, namely flavonoids, p-phenol and catechins as well as vitamin A and vitamin C. Where compounds -This compound is a powerful antioxidant that is useful to prevent the development of free radicals in the body and repair damaged body cells (Mufihunna et al., 2014).

From the results of research that has been done shows

that mice given 100 mg / grBB are more effective in reducing triglycerides. This is caused by the presence of antioxidant compounds such as flavonoids, alkaloids, and saponins. In other treatment groups such as at doses of 25 and 50 mg / grBB the pomegranate water methanol fraction also had a decrease but this was not significant because the treatment group gave a dose of 100 mg / grBB of pomegranate water methanol fraction was able to reduce the maximum and is a dose which is appropriate compared to other doses, this reduction is almost comparable to the decrease in the treatment group given simvastatin which if consumed continuously has adverse side effects for the body. The mechanism of flavonoid compounds can reduce triglyceride levels through increased activity of the LPL enzyme, with the increase in the enzyme VLDL that carries triglycerides will hydrolyse into fatty acids and glycerol. The released fatty acids will be absorbed by muscles and other tissues, then oxidized to produce energy and adipose tissue will store them as energy reserves (Mu'nisa et al., 2014). The flavonoids can inhibit Fatty Acid Synthase (FAS), which is a very important enzyme in the metabolism of fat. Barriers to FAS can directly reduce the formation of fatty acids, thereby reducing the formation of triglycerides (Zhang et al., 2009). Saponin mechanism can reduce triglyceride levels, namely by inhibiting the absorption of cholesterol and triglycerides in the intestine and increasing the reaction of bile acid formation from cholesterol then excreted through feces (Arauna and Aulanni'am, 2016). Saponins bind with bile acids and cholesterol (from food), then form micelles that cannot be absorbed by the intestine and also inhibit the work of the LPL enzyme (Rahmawati et al., 2016). Saponins can inhibit the absorption of cholesterol and triglycerides in the intestine by forming insoluble complex bonds in cholesterol, binds to bile acids to form micelles and increases binding of cholesterol and triglycerides by fiber (Ekananda, 2015). While tannin compounds can reduce triglyceride levels by reducing the absorption of cholesterol and triglycerides in the small intestine and increasing the excretion of bile acids (Cahaya and Ayu, 2017).

4. CONCLUSIONS

The conclusion from the results of this study is that mice given pomegranate fractions are effective in reducing total cholesterol and triglyceride levels due to the presence of antioxidant compounds as antihypercholesterol. The active dose that can reduce blood cholesterol and triglyceride levels in mice is in the treatment group given 100 mg / grBB and that was very significantly different to other treatment groups given doses of 25 and 50 mg / grBB of pomegranate water methanol fraction.

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Effect Of Pomegranate (Punica granatum L.) Fraction In Reducing Total Blood Cholesterol Levels and Triglyceride in Mice (Mus musculus L.)

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|-----|-------------------|--|
| 1. | Indikasi Plagiasi | 3 % |
| 2. | Linearitas | Topik tidak linier dengan bidang keilmuan biologi konservasi |

II. Hasil Penilaian Peer Review :

| Komponen Yang Dinilai | Nilai Maksimal Jurnal Ilmiah (isikan di kolom yang sesuai) | | | | | Nilai Akhir Yang Diperoleh |
|--|--|-------------------------|---|---|--|----------------------------|
| | Internasional Bereputasi (Maks 40) | Internasional (Maks 20) | Nasional Terakreditasi S1, S2 (Maks 25) | Nasional Terakreditasi S3, S4 (Maks 20) | Nasional tidak Terakreditasi (maks 10) | |
| Kelengkapan dan Kesesuaian unsur isi jurnal (10%) | | | | 2 | | 0 |
| Ruang lingkup dan kedalaman pembahasan (30%) | | | | 6 | | 4 |
| Kecukupan dan Kemutakhiran data/informasi dan metodologi (30%) | | | | 6 | | 6 |
| Kelengkapan unsur dan kualitas penerbit (30%) | | | | 6 | | 6 |
| Total = (100%) | | | | 20 | | 16 |
| Kontribusi Pengusul (Penulis Pertama /Anggota Utama) | Anggota Utama = $(0,4 \times 16) / 2 = 3,2$ | | | | | 3,2 |

KOMENTAR/ULASAN PEER REVIEW

| | |
|---|--|
| • Kelengkapan dan Kesesuaian Unsur: | Paper terkait pengaruh fraksi <i>Punica granatum</i> untuk mengurangi kadar kolesterol darah. Isi paper sudah memenuhi kaidah-kaidah karya ilmiah, namun tidak sesuai dengan bidang biologi konservasi. |
| • Ruang Lingkup dan Kedalaman Pembahasan: | Hasil penelitian dibahas cukup komprehensif dengan penyampaian perbandingan dari temuan-temuan penelitian lainnya dan teori terkait. Referensi yang diacu dalam pembahasan sudah cukup update untuk bidang kajian ini. |
| • Kecukupan & Kemutakhiran Data & Metodologi: | Data-data hasil penelitian cukup baik dan didukung penjelasan dan gambar yang ditampilkan cukup menarik. Data didapatkan dengan menggunakan metode yang standard. |
| • Kelengkapan Unsur & Kualitas Penerbit: | Penerbit Magister Biologi Universitas Sriwijaya berkualitas baik, dan jurnal terindeks di SINTA 3. |

Surabaya, 18 Mei 2020
Penilai 1



Prof. Hery Purnobasuki, M.Si., Ph.D.
NIP 196705071991021001
Unit Kerja : Jurusan Biologi FST Unair
Bidang Ilmu : Biologi
Jabatan/Pangkat : Guru Besar/ Pembina Utama Madya

FORMAT PENILAIAN (VALIDASI & PEER REVIEW)

LEMBAR

HASIL PENILAIAN SEJAWAT SEBIDANG ATAU PEER REVIEW

KARYA ILMIAH : JURNAL ILMIAH

Jurnal Artikel Ilmiah : Effect Of Pomegranate (Punica granatum L.) Fraction In Reducing Total Blood Cholesterol Levels and Triglyceride in Mice (Mus musculus L.)

Penulis Artikel Ilmiah : Arum Setiawan

Identitas Jurnal Artikel Ilmiah : a. Nama Jurnal : Indonesian Journal of Environmental Management and Sustainability (IJEMS)
 b. Nomor/Volume/Hal : 1/4/14-22
 c. Edisi (bulan/tahun) : Maret/2020
 d. Penerbit : Magister Biologi Universitas Sriwijaya
 e. Jumlah Halaman : 9

Kategori Publikasi Jurnal Ilmiah (beri \surd pada kategori yang tepat) : Jurnal Ilmiah Internasional Bereputasi
 Jurnal Ilmiah Internasional
 Jurnal Ilmiah Nasional Terakreditasi Sinta 1, Sinta 2
 Jurnal Ilmiah Nasional Terakreditasi **Sinta 3**, Sinta 4
 Jurnal Ilmiah Nasional Tidak Terakreditasi


I. Hasil Penilaian Validasi :

| No. | ASPEK | URAIAN/KOMENTAR PENILAIAN |
|-----|-------------------|---------------------------|
| 1. | Indikasi Plagiasi | 3 % |
| 2. | Linearitas | |

II. Hasil Penilaian Peer Review :

| Komponen Yang Dinilai | Nilai Maksimal Jurnal Ilmiah (isikan di kolom yang sesuai) | | | | | Nilai Akhir Yang Diperoleh |
|--|---|-------------------------|---|---|--|----------------------------|
| | Internasional Bereputasi (Maks 40) | Internasional (Maks 20) | Nasional Terakreditasi S1, S2 (Maks 25) | Nasional Terakreditasi S3, S4 (Maks 20) | Nasional tidak Terakreditasi (maks 10) | |
| Kelengkapan dan Kesesuaian unsur isi jurnal (10%) | | | | 2 | | 0 |
| Ruang lingkup dan kedalaman pembahasan (30%) | | | | 6 | | 4 |
| Kecukupan dan Kemutakhiran data/informasi dan metodologi (30%) | | | | 6 | | 4 |
| Kelengkapan unsur dan kualitas penerbit (30%) | | | | 6 | | 5 |
| Total = (100%) | | | | 20 | | 13 |
| Kontribusi Pengusul (Penulis Pertama /Anggota Utama) | Dalam artikel tidak ada judul pada Jurnal IJEM. Narasi diblok di 3 lokasi. Penulis ke 2 dari 3. Nilai maksimal 65%. Nilai pengusul: $(0,4 \times 0,65 \times 20)/2 = 2,6$. | | | | | 2,6 |
| KOMENTAR/ULASAN PEER REVIEW | | | | | | |
| • Kelengkapan dan Kesesuaian Unsur: | Judul diblok (= tidak ada/tidak bisa dinilai). Pustaka 27 (ada yg tidak urut tahun publikasi). Ada abstrak, pembahasan, kesimpulan. | | | | | |
| • Ruang Lingkup dan Kedalaman Pembahasan: | Masih dalam ruang lingkup bidangnya. Apa kepanjangan BB, pada 100mg/gBB?. Hipotesis? | | | | | |
| • Kecukupan & Kemutakhiran Data & Metodologi: | Pada metode, sampel dari Situbondo (dimana Situbondo Jawa Tengah?). Populasi sampel masih dianggap kurang sehingga kurang data. | | | | | |
| • Kelengkapan Unsur & Kualitas Penerbit: | Cukup sebagai penerbit. | | | | | |

Yogyakarta, 12 Juni 2020

tanda tangan

 Prof. Dr. Suwarno Hadisusanto
 NIP 195411161983031002
 Unit Kerja : Fakultas Biologi UGM
 Bidang Ilmu : Biologi/Ekologi
 Jabatan/Pangkat : Guru Besar/ Pembina Utama Madya