Jurnal Farmasi Galenika (*Galenika Journal of Pharmacy*) (e-Journal) 2021; 7 (2): 201 – 212
ISSN: 2442-8744 (electronic); 2442-7284 (printed)
Journal Homepage: https://bestjournal.untad.ac.id/index.php/Galenika
DOI: 10.22487/j24428744.2021.v7.i2.15535

The Effect of Ethanol Extract of Guava (*Psidium guajava* L) Leaf on Hypercholesterolemia-Diabetic Male White Rats Induced by High Fat Feed and Streptozotocin

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Article Info:
Received: 9 June 2021
in revised form: 13 July 2021
Accepted: 8 October 2021
Available Online: 10 October 2021

Keywords:
Hypercholesterolemia
Blood sugar levels
Ethanol extract
Guava leaf
Streptozotocin

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ABSTRACT

This study aims to determine the effect of guava (*Psidiium guajava* L) leaf extract on hypercholesterolemia-diabetic male white rats and the effective dose for reducing cholesterol and blood glucose levels. This study is a laboratory experimental study using 30 rats divided into 6 treatment groups where each group consisted of 5 rats. Group I was normal control, group II as negative control was given Na-CMC suspension, group III as positive control was given simvastatin, group IV was given dose 150 mg/kg BW, group V dose 250 mg/kg BW and group VI dose 350 mg/kg BW. The results showed that the ethanol extract of guava leaves influenced the reducing blood glucose levels of male white rats with an effective dose of 250mg/kg BW with an average reduction of 119 mg/dL and also effective to reduce the cholesterol levels at a dose of 150 mg/kg BW with an average reduction of 28,33 mg/dL.

How to cite (APA 6th Style):
Tandi, J., Pratiwi, F. D., Surisna. Dewi, N. P., Magfirah. (2021). *The Effect of Ethanol Extract of Guava (Psidium guajava L) Leaf on Hypercholesterolemia-Diabetic Male White Rats Induced by High Fat Feed and Streptozotocin*. *Jurnal Farmasi Galenika :Galenika Journal of Pharmacy (e-Journal), 7*(2), 201-212. doi:10.22487/j24428744.2021.v7.i2.15535

ABSTRAK

Penelitian ini bertujuan untuk mengetahui efek ekstrak daun jambu biji (*Psidium guajava* L) terhadap tikus hiperkolesterolemia-diabetes dan dosis ekstrak yang efektif terhadap penurunan kadar kolesterol dan glukosa darah. Penelitian ini merupakan penelitian eksperimen laboratorium dengan menggunakan hewan uji sebanyak 30 ekor tikus yang dibagi menjadi 6 kelompok perlakuan dimana tiap kelompok terdiri atas 5 ekor tikus. Kelompok I kontrol normal, kelompok II kontrol negatif yang diberi suspensi Na-CMC, kelompok III kontrol positif yang diberikan simvastatin, kelompok IV dosis ekstrak 150 mg/kg BB, kelompok V dosis ekstrak 250mg/kg BB dan kelompok VI dosis ekstrak 350 mg/kg BB. Hasil penelitian menunjukkan bahwa ekstrak etanol daun jambu biji memiliki efek terhadap penurunan kadar glukosa darah tikus putih jantan dengan dosis efektif 250mg/kg BB dengan rata-rata penurunan sebesar 119,2 mg/dL dan efektif menurunkan kadar kolesterol pada dosis 150 mg/kg BB dengan rata-rata penurunan sebesar 28,33 mg/dL.

Kata kunci: Hiperkolesterolemia, kadar gula darah, ekstrak etanol, daun jambu biji, Streptozotocin.

INTRODUCTION

Currently, health problems have shifted from infectious diseases to degenerative diseases. Degenerative diseases that affect quite a lot are diabetes mellitus and hypercholesterolemia. Elevated cholesterol levels are estimated to cause 2.6 million deaths and 29.7 million disabilities per year. According to the International Diabetes Federation (IDF), the number of people with diabetes mellitus from 425 million people in 2017, is expected to increase to 629 million by 2045. Half of this figure is in Asia such as India, China, and Indonesia. Indonesia is the 6th country with the most diabetes sufferers in the world (Tandi et al., 2020a).

The cause is thought to be due to lifestyle changes, environmental factors, lack of physical activity and diet. The number of fast-food restaurants sell foods that containing cholesterol. Cholesterol is a fat found in all parts of the body including the nervous system, skin, muscles, liver, intestines, and heart. Cholesterol is needed by the body in metabolic processes, for example as a cell wall-forming material, making bile acids, fat emulsifiers, forming vitamin D, as hormones and corticosteroids. (Tandi *et al.,* 2017).

Hypercholesterolemia is a condition in which the concentration of cholesterol in the blood is higher than normal. Hypercholesterolemia can cause insulin resistance. Insulin resistance is a disorder of the biological response to insulin and plays a role in increasing blood triglyceride levels and decreasing HDL. Insulin resistance can inhibit lipogenesis by decreasing glucose uptake in adipose tissue and increasing hepatic glucose production, causing diabetes mellitus. Diabetes mellitus that occurs can cause the formation of reactive oxygen (RO) compounds thereby increasing the modification of lipids, DNA, and proteins in various tissues. The reactive oxygen attack reaction of sepsis (ROS) on DNA molecules that causes damage to the DNA structure and affects its interior.

One of the biological parameters that can be used to identify DNA damage is the formation of 8-hydroxydeoxyguanosine (8-OHdG) (Tandi et al., 2020b).

People with diabetes, will have high blood glucose levels due to the reduced insulin. The glucose cannot be used by cells because it cannot be converted into glucose 6-phosphate (Ismawan, B., 2013)., so the energy obtained by the body comes from the breakdown of fat and protein metabolism which then increases the formation of acetyl coenzyme A or HMG-CoA that with mevalonate will produce cholesterol (Tandi *et al.*, 2019).

One of the plants commonly used for the treatment of hypercholesterolemia is the guava plant. The part used is the leaf because it contains chemical alkaloids, flavonoids, saponins, tannins, polyphenols, and steroids(Dewayani et al., 2019). In addition to treating hypercholesterolemia, guava leaves can also be used as a laxative, lowering blood sugar, antibacterial, and preventing cancer (Allo et al., 2013).

The purpose of this study was to determine the effect of guava (*Psidium guajava* L.) leaf ethanol extract on reducing cholesterol and blood glucose levels of male white rats (*Rattus norvegicus*) induced by high-fat diet and streptozotocin and to determine its effective dose as well.

MATERIAL AND METHODS

Materials

Guava leaves (*Psidium guajava* L) was obtained from Silae district at Palu City, aquadest, ammonia, hydrochloric acid (Merck®), hydrochloric acid p.a (Merck®), sulfuric acid p.a (Merck®), anhydrous acetic acid p.a (Merck®), citric acid, iron (iii) chloride (Merck®), ethanol 96% (Merck®), ether, ethyl acetate (Merck®), hematoxilin, chloroform, quail egg yolk, lard, standard feed, methanol (Sigma aldrich®), 0.5% Na CMC, n-hexane (Merck®), sodium chloride, sodium citrate, sodium carboxy methyl cellulose (Bioworld®), dragendorff reagent, magnesium powder, streptozotocin, metformin tablets and simvastatin tablets.

Extraction and Phytochemical Screening

Simplicia powder was extracted by maceration using 96% ethanol as solvent. The simplicia powder was weighed as much as 1000 grams which was divided into 3 maceration vessels using 2.5 liters of ethanol solvent in each vessel, closed, left for 3x24 hours, protected from light, and stirred occasionally. The extract was filtered using filter paper and then the residue and filtrate were

obtained. The filtrate was concentrated using a rotavapor at a temperature of 50˚C and continued with thickening carried out using a water bath at a temperature of 60˚C until it became a thick extract (Fadhilah et al., 2018). The extract was further analyzed for the phytochemical contents by using specific reagents such as dragendorf for alkaloid detection and $FeCl_3$ for polyphenol detection (Tandi et al., 2021).

Preparation of Streptozotocin (STZ) Solution

Streptozotocin was weighed as much as 0.32 grams and then dissolved using citrate-buffered saline at pH 4.5 and then induced in test animals' male white rats via intraperitoneal. The dose of streptozotocin is 30 mg/kg BW.

Preparation of simvastatin suspension

Simvastatin tablet powder was weighed as 1.8 mg, crushed in a mortar by adding 0.5% Na CMC suspension and grinding until homogeneous. Then it was put in a 100 mL measuring cup. The volume was made up with 0.5% Na CMC to 100 mL.

Preparation of Metformin Suspension

The dose of metformin in adult humans is 500 mg per day, if converted to rats weighing 200 g is 0.018 mg, then the dose of metformin for rats is 45 mg/kg BW, weighed the metformin tablet powder which is equivalent to 90 mg then suspended in Na CMC 0 .5% to 25 ml.

Preparation of High Fat Feed

The high-fat feed used was Pig oil and quail egg yolk with a ratio of 50: 50. The feed was prepared in the following way: pig oil was melted by heating until the pig oil became oil. Then, the quail eggs were separated from the yolk and egg white, the yolk was taken and mixed with pig oil until homogeneous and then given orally for 14 days.

Test animal treatment

30 male white rats were divided into 6 groups and adapted for 2 weeks in the laboratory. On day 0, the rats were measured the initial cholesterol levels and initial glucose levels, then fed high-fat diet was given orally for 14 days. On day 14, the rats were then measured for cholesterol levels after being fed a high-fat diet. On the same day, rats were induced by streptozotocin 30 mg/kg BW.

Glucose levels were measured after giving STZ. After cholesterol levels and glucose levels increased, it was given treatment orally for 14 days. Data for measuring glucose levels and blood cholesterol levels before and after treatment were recorded and analyzed. This study was performed on ethical committee permission issued by the Ethics Committee for Medical and Health Research, Medicine Faculty, Tadulako University no. 7317/UN28.1.30/KL/2020.

Determination of Blood Glucose Levels

The blood samples of male white rats were taken from the tail vein and their blood glucose levels were measured using a glucometer to ensure that all male white rats had normal blood glucose levels before being given treatment. Normal fasting blood glucose levels in rats in the range between 50 – 135 mg/dl. Then the glucometer is turned on and the glucose stick is inserted into the glucometer, blood is taken through the tail end of the rat that has been cleaned with 70% alcohol, then sorted slowly then the tail end is pierced with a small needle. The blood that comes out is then dripped on the glucometer stick, within 10 seconds Blood glucose levels will be measured automatically and the results can be read on the glucometer monitor. This glucometer tool works enzymatically involving the glucose oxidase reaction where this reaction produces a color intensity that will be detected by this tool.

Determination of Cholesterol Levels

Analysis of cholesterol levels using the evolution 201 UV-Visible Spectrophotometer with a wavelength of 500 nm. Cholesterol reagent composition consists of enzyme reagent (phosphate buffer 100 mmol/L, 4-amonophenazone 0.25 mmol/L, phenol 5 mmol/L, peroxidase >150 L, cholesterol esterase >100 L and 0.05 % sodium azide). The amount of serum needed is 10 L, then 2000 L of reagent is added, incubated at 25˚C for 10 minutes, and then measured using UV spectrophotometry.

Data analysis

The data obtained in the form of total blood cholesterol and glucose levels obtained in the study were calculated and all data were statistically analyzed using the One-way Anova test at a 95% confidence level. The Least Significant Differences (LSD) follow-up test was used to see the significant differences between groups.

RESULTS AND DISCUSSION

Table 1. Phytochemical Test Results of Guava Leaf Extract.

Compounds	Reagents	Results	Interpretation
Flavonoids	Magnesium and HCl	Formation of red orange color	+
Alkaloids	Dragendorff's reagent	Formation of a brick red precipitate	+
Polyphenol	Addition of FeCl3	Formation of dark green color	+
Tannins	Addition of FeCl3	Formation of dark blue color	+
Saponins	Foam formation test	The presence of foam as high as ± 1 cm and remains stable for 5 minutes after vigorous shaking	+

Table 2. Mean and Standard Deviation of Blood Glucose Level Measurement.

Days to-	Mean± SD Blood Glucose Level (mg/dl)						
	Normal Control	Negative control	Positive control	Dose 150 mg/kg BW	Dose 250 mg/kg BW	Dose 350 mg/kg BW	P*
0	90,8 ± 18,00	96 ± 20,83	89,8 ± 6,04	86,4 ± 14,86	100,4 ± 18,93	105,6 ± 19,18	0,61
21	97,4 ± 11,90	281,2± 45,33	161,6 ± 14,11	196 ± 47,80	189,2 ± 7,08	186,8 ± 7,49	0,000
28	104,4± 4,61	260,4 ± 6,65	108,2± 3,27	126,8 ± 5,84	124,8 ± 7,08	126,6 ± 2,68	0,000
35	117± 5,14	282,6 ± 33,04	110,4± 9,52	122,2 ± 13,82	119,2 ± 7,59	129,4 ± 11,52	0,000

*P>0.05: Not Significantly Different, P<0.05: Significantly different

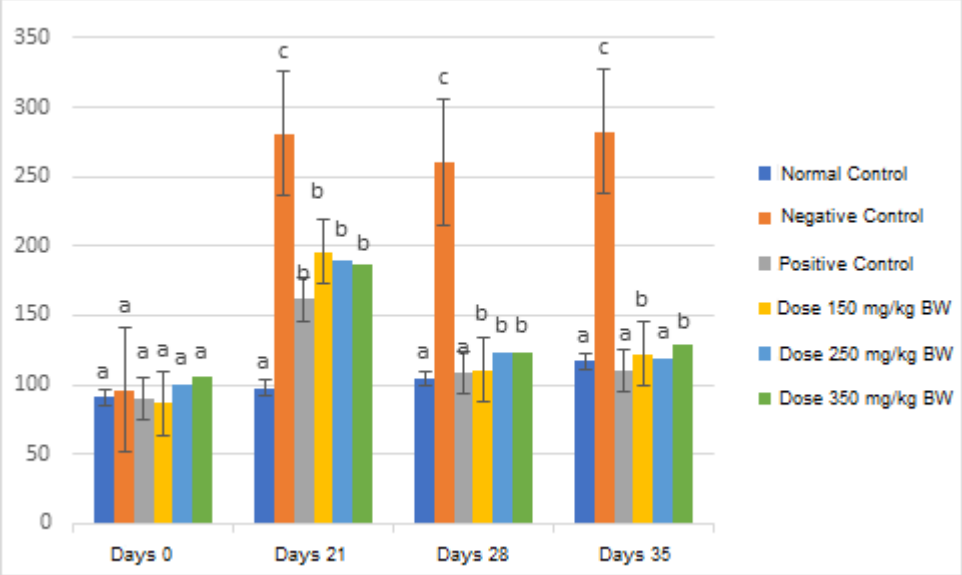

Figure 1. Graph of blood glucose level measurement. Different letter showed significantly different.

Measurement of initial blood glucose levels obtained the mean data for the normal control group, positive control, negative control, extract dose 150 mg/kg BW, extract dose 250 mg/kg BW and extract dose 350 mg/kg BW which is 90.8 mg/dL, 96 mg/dL 89.8 mg/dL, 86.4 mg/dL, 100.4 mg/dL and 105.6 mg/dL (Table 2), respectively. Based on the results of the One-way Anova statistical test, it showed that there was an insignificant difference between all treatment groups which was indicated by a P value > 0.05 (p value = 0.61). This shows that the blood glucose levels of the test animals are homogeneous. On day 7 to day 14, the test animals were given a high-fat diet and continued with streptozotocin. Measurement of blood glucose levels on day 21 obtained the average data for the normal control group, positive control, negative control, extract dose 150 mg/kg BW, extract dose 250 mg/kg BW and extract dose 350 mg/kg BW which is 97.4 mg /dL, 281.2 mg/dL, 161.6 mg/dL, 196 mg/dL, 189.2 mg/dL and 186.8 mg/dL, respectively.

Based on the results of the One-way Anova statistical test, there was a significant difference which was indicated by the P value < 0.05 (p value = 0.000), so a post hoc LSD test was carried out. Fisher's Least Significant Difference (LSD) or real difference to find out which pairs of averages are the most different among the existing pairs. The results of the post hoc LSD test showed that the extract at a dose of 150 mg/kg BW, extract at a dose of 250 mg/kg BW and extract at a dose of 350 mg/kg BW were not significantly different from the negative control and positive control, but significantly different from the normal control (Figure 1). Measurement of blood glucose levels on day 28 obtained

the average data for the normal control group, negative control, positive control, extract dose 150 mg/kg BW, extract dose 250 mg/kg BW and extract dose 350 mg/kg BW which is 104.4 mg /dL, 260.4 mg/dL, 108.2 mg/dL, 126.8 mg/dL, 124.8 mg/dL and 126.6 mg/dL, respectively. The results of the One-way Anova statistic showed that there was a significant difference which was indicated by the P value < 0.05 (p value = 0.000) so that a post hoc LSD test was carried out. The results of the post hoc LSD test showed that the 150 mg/kg BW, 250 mg/kg BW and 350 mg/kg BW dose groups were significantly different from the positive control group and the normal control group, but when compared between the 150 mg/kg BW group, 250 mg/kg BW and 350 mg/kg BW were not significantly different from the negative control group. This indicated that the three groups of guava leaf extract at doses of 150 mg/kg BW, 250 mg/kg BW and 350 mg/kg BW had an effect in lowering blood glucose levels, but not close to the normal and positive control group.

Measurement of blood glucose levels on day 35 obtained the average data for the normal control group, negative control, positive control, extract dose 150 mg/kg BW, extract dose 250 mg/kg BW and extract dose 350 mg/kg BW which is 117 mg/dL, 282.6 mg/dL, 110.4 mg/dL, 122.2 mg/dL, 119.2 mg/dL and 129.4 mg/dL, respectively. The results of the One-way Anova statistic showed that there was a significant difference which was indicated by the P value < 0.05 (p value = 0.000) so that a post hoc LSD test was carried out (Tandi et al., 2020d). The results of the post hoc LSD test showed that the dose of 150 mg/kg BW was significantly different from normal, positive, and negative controls. This shows that a dose of 150 mg/kg BW has a lowering effect on blood glucose levels but it is not equivalent to normal and positive controls. This is due to a lack of dose that has not been able to reach the target cells so that it does not provide the maximum effect. Meanwhile, the dose of 250 mg/kg BW was significantly different from the negative control, but when compared with normal control and positive control, the dose of 250 mg/kg BW was not significantly different. This shows that guava leaf extract at a dose of 250 is effective in reducing blood glucose levels in rats. This is because guava leaves contain flavonoids. In previous studies it was stated that guava leaves contain very high flavonoid content, especially quarcetin. Quarcetin is thought to induce glucose uptake by liver cells so that it can reduce the blood glucose levels (Guspratiwi *et al.*, 2019).

The three treatment groups of guava leaf ethanol extract had the potential to reduce blood glucose levels with an average decreasing of 122.2 mg/dL for 150 mg/kg BW and 129.4 mg/dL for 350 mg/kg BW. Meanwhile, at a dose of 250 mg/kg BW (119.2mg/dL), it was close to normal control. This is because guava leaves have secondary metabolites, namely alkaloids, flavonoids, saponins, polyphenols and tannins (Table 1). Based on previous research, compounds that have potential as antioxidants are flavonoids. Flavonoids containing flavone, flavanone, catechin, and anthocyanin groups in their molecular structure that possessing antioxidant activity. This is supported by previous

research that these antioxidants can bind to hydroxyl radicals that damage the cells of the pancreatic islets of Langerhans so that insulin production will be maximized (Tandi et al., 2020c).

Table 3. Average and Standard Deviation of Total Cholesterol Levels

Group	0	14	21	28	35
Normal Control	49,02±6,64 [a]	46,91±5,47 [a]	44,72±13,79 [a]	37,36±9,44 [a]	26,87±11,53 [a]
Negative Control	45,77±9,68 [a]	140,2±10,96 [b]	134,31±4,45 [c]	134,59±14,09 [c]	136,22±8,31 [b]
Positive Control	48,33±2,84 [a]	135,77±8,06 [b]	120,32±1,62 [b]	71,34±7,75 [b]	24,47±8,98 [a]
Extract 150 mg/kg BW	47,58±3,63 [a]	132,05±1,38[b]	120,2±1,2 [b]	81,564±2,50 [b]	28,33±5,39 [a]
Extract 250 mg/kg BW	45,2±7,30 [a]	132,97±10,314 [b]	110,49±9,27 [b]	77,64±9,02 [b]	21,26±3,43 [a]
Extract 350 mg/kg BW	45.45 ±3.87[a]	133.09 ± 15.26[b]	118.09 ± 10.89 [b]	78.76 ± 0.52[b]	21.5 ± 6.15[a]

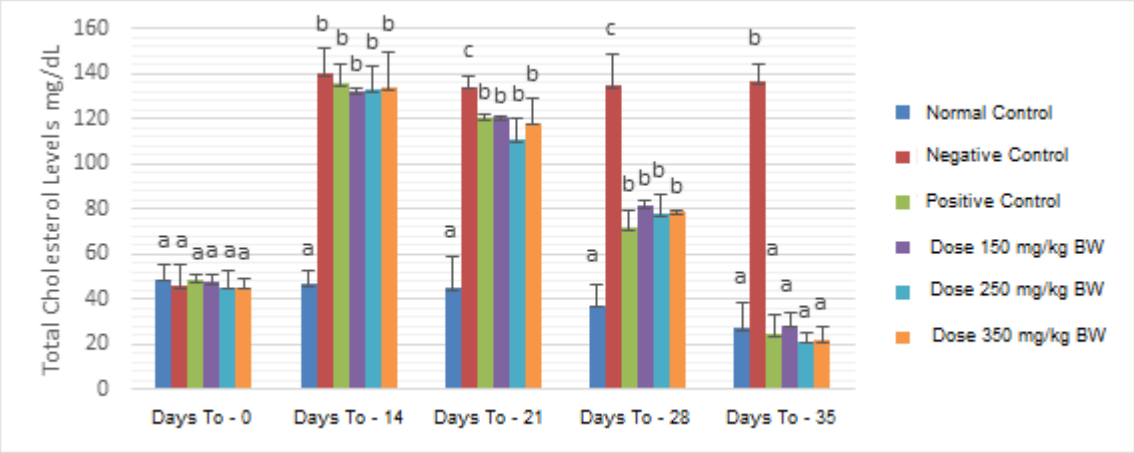

Figure 2. Graph of total blood cholesterol levels measurement. Different letter showed significantly different.

Based on the results of the One-way Anova statistical test on day 0 the data of cholesterol levels obtained was 45.77 mg/dL-49.02 mg/dL indicating that there was no significant difference between all treatment groups. This can be seen at the P value of 0.876 (> 0.05) indicating that all test animals before treatment had normal total cholesterol levels because they were in the range of 10-54 mg/dL (Tandi et al., 2017). The data from the One-way Anova statistical test showed that the results of measuring total cholesterol levels in male white rats on day 14 had a significant difference in the value of P 0.000 (P <0.05) against all treatment groups. The results of the LSD further test showed that the normal group was significantly different from all other control groups which showed that there was an effect of high-fat and streptozotocin feeding on male white rats. The increase in cholesterol reached

130 mg/dL-140 mg/dL with an average cholesterol level in each group, namely the negative group 140.02 mg/dL, the positive group 135.77 mg/dL, the treatment group at a dose of 150 mg/kg BW. was 132.05 mg/dL, the treatment group with a dose of 250 mg/kg BW was 132,97 mg/dL and the treatment group at a dose of 350 mg/kg BW was 133.09 mg/dL (Table 3). This difference in cholesterol levels was caused by the provision of high cholesterol feed consisting of quail egg yolk and pig oil which can contain 25% saturated fat which can increase the cholesterol levels of rats.

The data from the measurement of total cholesterol levels in male white rats on day 21 based on the results of the One-way Anova statistical test showed significantly different results with a value of P 0.000 (P <0.05) for all treatment groups, so it was continued with the LSD test. The results of the LSD further test showed that there was a significant difference between normal controls and other controls. The normal group and all groups were difference because of the normal controls were not given streptozotocin induction. Meanwhile, the negative controls were significantly different from the positive controls, and the dose group was 150 mg/kg. BW, the dose group of 250 mg/kg BW and the dose group of 350 mg/kg BW (Figure 2). This was due to the administration of streptozotocin.

The administration of streptozotocin causes insulin resistance so that it raises the blood glucose levels, as a result, the test animals experience diabetes and metabolic disorders that occur in lipids and hormone sensitive lipase is activated. This causes the increasing fat levels in the blood circulation and decreasing in adipose tissue, besides that the active hormone sensitive lipase causes the increasing free fatty acids in plasma (Tandi et al., 2017).

The data on the measurement of total cholesterol levels in male white rats on day 28 based on the results of the One-way Anova test showed significantly different results with a P value 0.000 (P <0.05) for all treatment groups, so it was continued with the LSD test. The LSD test showed that there was a significant difference between normal control and all treatment groups. Negative control was significantly different from positive control, the dose group of 150 mg/kg BW, the dose group of 250 mg/kg BW, and the dose group of 350 mg/kg BW. The extract group at a dose of 150 mg/kg BW, 250 mg/kg BW and 350 mg/kg BW and positive control had not been able to statistically reduce the cholesterol levels after 7 days but descriptively decrease the total cholesterol levels. Simvastatin has not yet given optimal effect because the work of the pancreas of test animals has been disrupted, so it takes longer for treatment. Several factors that influence the measurement results of cholesterol levels obtained are the environment, stress, and changes in diet (Dwianita,2017).

The data from the measurement of total cholesterol levels in male white rats on the 35th day based on the results of the One-way Anova test showed significantly different results with P value 0.000 (P <0.05) against all treatment groups, so that the LSD test was continued. The LSD test showed that

there was no insignificant difference between normal control and positive control and 150 mg/kg BW, 250 mg/kg BW, and the 350 mg/kg BW dose groups. This shows that on the 35th day, total cholesterol levels in male white rats decreased with an average of 28.33 mg/dL for 150 mg/kg BW, 21.26 mg/dL for 250 mg/kg BW, 21.26 mg/dL for 250 mg/kg BW and 21.5 mg/dL for 350 mg/kg BW. It means that the effect of giving simvastatin and guava leaf extract is able to reduce the total cholesterol levels that close to normal levels. Simvastatin is a class of HMG Co-A inhibitor drugs with competitive inhibition that most effective for lowering cholesterol levels. The flavonoid, tannins and polyphenols compounds contained in the ethanol extract of guava leaves which act as antioxidants have been able to reduce he LDL (low density lipoprotein) in blood vessel and can inhibit the work of the HMG Co-A reductase enzyme (Tandi et al., 2017).

CONCLUSION

Ethanol extract of guava leaves (*Psidium guajava* L) that contains alkaloids, flavonoids, saponins, polyphenols and tannins, influenced the reducing of total cholesterol levels in male white rats (*Rattus norvegicus*) in graded doses. Ethanol extract of guava leaves (*Psidium guajava* L) at a dose of 150 mg/kg BW is an effective dose for lowering cholesterol levels and at a dose of 250 mg/kg BW is effective in reducing blood glucose levels in white rats.

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