THE EFFECT OF LIME PEEL EXTRACT (*Citrus Aurantifolia*) TO TOTAL BLOOD CHOLESTEROL LEVELS OF MICE (*Mus musculus*) THAT GIVEN THE HIGH CHOLESTEROL DIET

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ABSTRAK

*Artikel ini untuk meninjau penelitian tentang efek dan potensi kulit jeruk nipis sebagai anti kanker. Kulit jeruk nipis mengandung flavonoid. Flavonoid adalah inhibitor ampuh sekresi apoB dan menghambat sintesis cholesterol dan sintesis triglycveride tanpa mempengaruhi aktivitas reseptor LDL. Selama 35 hari, penelitian yang menggunakan 30 tikus (Mus musculus) laki-laki sebagai hewan uji. 30 sampel dibagi menjadi lima group dengan enam tikus per group. Group pertama (kontrol), diberi diet biasa. Group kedua (kontrol negatif), hanya diberikan diet tinggi lemak. Group ketiga, group diberi diet tinggi lemak dengan ekstrak etanol kulit jeruk nipis 750 mg/kgBW dan pelarut CMC-Na 0,5%. Group keempat, group diberi diet tinggi lemak dengan ekstrak etanol kulit jeruk nipis 1500 mg/kgBW dan pelarut CMC–Na 0,5%. Group kelima, group diberi diet tinggi lemak dengan ekstrak etanol kulit jeruk nipis 2250 mg/kgBW dan pelarut CMC - Na 0,5%. Cholesterol total dalam group ketiga (diet tinggi lemak dengan ekstrak etanol kulit jeruk nipis 750 mg/kgBW dan 0,5% CMC - Na pelarut) lebih tinggi dibandingkan group keempat (diet tinggi lemak dengan dengan ekstrak etanol kulit jeruk nipis dan 1500 mg/kgBW 0,5% CMC - Na pelarut). Cholesterol total dalam group kelima (diet tinggi lemak dengan dengan ekstrak etanol kulit jeruk nipis 2250 mg/kgBW dan 0,5% CMC - Na pelarut) adalah group konsentrasi terendah. Menurut hasil penelitian dapat disimpulkan bahwa ekstrak etanol kapur (Citrus aurantifolia) kulit dari 750 mg/kgBW, 1500 mg/kgBW, dan 2250 mg/kgBW tidak dapat menurunkan kadar cholesterol total darah mencit (Mus musculus).**(FMI 2013;49:208-215)***

Keywords: *Kulit jeruk nipis, Ekstrak etanol, Cholesterol, Mice*

ABSTRACT

*This article is to review existing research on the effects and potential of lemon peel as anticarcinogenesis. Lime peel contain flavonoid. Flavonoids are potent inhibitor of apoB secretion and inhibits the synthesis of cholesterol and triglycveride synthesis without affecting LDL receptor activity. Over a period of 35 days, the research used 30 male of mice (Mus musculus) as animal-test. 30 samples were divided into five groups with the six miceeach. The first group (control), given regular diet. The second group (negative control), given only high-fat diet. The third group, high-fat diet with ethanolic extract of lime peel 750 mg/kgBW and 0,5% CMC-Na solvent group. The fourth group, high-fat diet with ethanolic extract of lime peel 1500 mg/kgBW and 0,5% CMC-Na solvent group. The fifth group, high-fat diet with ethanolic extract of lime peel 2250 mg/kgBW and 0,5% CMC-Na solvent group. Total cholesterol in third group (high-fat diet with ethanolic extract of lime peel 750 mg/kgBW and 0,5% CMC-Na solvent) was higher than fourth group (high-fat diet with with ethanolic extract of lime peel 1500 mg/kgBW and 0,5% CMC-Na solvent). Total cholesterol in fifth group (high-fat diet with with ethanolic extract of lime peel 2250 mg/kgBW and 0,5% CMC-Na solven) is the lowest concentration group. According the results of research it can be concluded that the ethanolic extract of lime (Citrus aurantifolia) peel of 750 mg/kgBW, 1500 mg/kgBW, and 2250 mg/kgBW can't reduce total blood cholesterol of mice (Mus musculu).**(FMI 2013;49:208-215)***

Keywords: *Lemon peel, Ethanolic extract, Cholesterol, Mice*

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INTRODUCTION

One of the major risk factors for atherosclerosis is dyslipidemia. In Indonesia, the prevalence of dyslipidemia increased. Cholesterol levels in the blood can be treated with traditional medicine using a variety of plants that many live in Indonesia. Lime (*Citrus aurantifolia*) are known to contain flavonoids have anti-inflammatory, antioxidant, hepatoprotective and anticarcinogenic. Flavonoids prevent LDL oxidation function and atherosclerosis that causes heart disease and stroke could be avoided (Anida 2010).

From the results study of polymethoxylated citrus flavonoid, nobiletin and tangeretin, potentially inhibits apoB secretion and simply inhibit the synthesis of cholesterol and triglyceride synthesis without affecting the activity of LDL receptors. Structure-activity analysis showed that the full ring of methoxylated flavonoids structure associated with strong inhibitory activity on the apoB secretion by the liver. This study using HepG2 cells showed that citrus flavonoids with a full-methoxylated ring A can lower blood cholesterol and triglyceride concentrations, especially with pressing apoB secretion by the liver (Lin et al 2011). In connection with the above, this will be an examination of the effects of lime extract (*Citrus aurantifolia*) to decrease blood cholesterol levels. This study will be conducted on a group of mice (*Mus musculus*) that has been given a high-fat diet and a group of mice given the extract of lime (*Citrus aurantifolia*).

MATERIALS AND METHODS

This study was an experimental study using a design of Randomized Controlled Post Test Design. Samples were taken by random sampling, a total of 30 mice (*Mus musculus*) sex of adult males, with about 3 months of age, 20 grams body weight, and with a healthy physical condition. Mice were obtained from the Laboratory of Biochemistry Faculty of Medicine, University of Airlangga. The main materials used include ethanolic extracts of lemon peel (*Citrus aurantifolia*). Lime used is healthy orange, green. Lime peel is dried and powdered. Lemon peel powder macerated using alcohol 70% with a ratio of 1:7,5. Results of maceration made into thick solvent extract by evaporation using a rotary vacuum evaporator (Pratiwi et al 2008). Mice were acclimatized for one week of habituation to the laboratory conditions for themselves during the study. During the study, mice were placed in cages with a size of 40cm x 30cm x 20 cm, each cage containing eight experimental animals. Enough of lighting, free entry and exit of air.

After the acclimatization period, the animals were divided randomly into five groups namely, group I, II, III, IV and V, each group consisting of 6 mice with group I (negative control) is the group given a regular diet. Group II is the group given a high-fat diet for 2 weeks with the addition of CMC-Na 0.5% solvent. Group III is the group given a high-fat diet for 2 weeks with the addition of ethanolic extract 750 mg/kgBW lemon peel (*Citrus aurantifolia*) with solvent of CMC-Na 0.5%. Group IV is the group given a high-fat diet for 2 weeks with the addition of ethanolic extract 1500 mg/kgBW lemon peel (*Citrus aurantifolia*) with CMC-Na 0.5% solvent. Group V is the group given a high-fat diet for 2 weeks with the addition of ethanolic extract 2250 mg/kgBW lemon peel (*Citrus aurantifolia*) with solvent of CMC-Na 0.5%. Measurement of total cholesterol serum levels in mice (*Mus musculus*) performed twice, after being given a high-fat diet (before given treatment) and after the research is done. Cholesterol measurements performed digitally using a NESCO. The process is a drop of blood with a diameter of approximately 5 mm were taken from mice placed on the end of sticks of cholesterol that has been linked with a digital check tool, then the result will come out after 150 seconds. Total colesterol data of the animal test from the control group and the treatment group were tested using descriptive statistics and Analysis of Variance (ANOVA). If significantly different results were obtained, followed by LSD test.

RESULTS

Research on the effects of ethanolic extract of lime peel (*Citrus aurantifolia*) could lower blood cholesterol levels in mice (*Mus musculus*) that were suffering hypercholesterolemia conducted in the Laboratory of Biochemistry Faculty of Medicine, University of Airlangga. Samples from 30 individuals were divided into five groups by six mice each. There were two dead mice in group I and group V. This study was conducted for 35 days from November 1 2011-5 January 2012.The taking of blood samples held on January 5, 2012. Based on the results of blood samples from 30 mice (*Mus musculus*), the data obtained in total blood cholesterol levels of mice as in Table 1.

Tabel 1. Total Cholesterol Level

Sample Replication	Total Blood Cholesterol (mg/dL)				
	Group I	Group II	Group III	Group IV	Group V
1	136	105	120	122	112
2	137	180	165	153	105
3	101	144	152	117	105
4	166	129	152	152	127
5	136	144	144	137	115
6	-*)	129	107	137	-*)

*) Dead Mice

Normal distribution test

To determine whether the analyzed data were normally distributed or not, it tested for normality with the Kolmogorov Smirnov test. If the Kolmogorov Smirnov test value was greater than 0.05, means there was no significant difference between the data tested with normal data that it could be interpreted that the raw data tested was normal. From the data obtained, each group respectively tested the normality of blood cholesterol using Kolmogorov-Smirnov. In the control group (group I), p-value = 0.708, the value of p> 0.05 that the data were normally distributed. Furthermore, in group II Kolomogorov Smirnov test gave the value of 0.862 (p > 0.05) that the data of the group was normal. In group III normal data with Kolomogorv-Smirnov test results was 0.883 (p > 0.05). In group IV normality test value was 0.984 (p > 0.05) that the data of the group was normal. In group V normality test value was 0.984 (p > 0.05) that the data of the group was normal. Kolomogorov Smirnov normality test results could be seen in the table below.

Table 2. Normality Test (p) Kolmogorov Smirnov

Group	Cholesterol Level
Group I	0.708
Group II	0.862
Group III	0.883
Group IV	0.984
Group V	0.984

Descriptive statistics test

Descriptive statistics were based on test variables of each group. In the descriptive statistical tests, obtained an average value, standard deviation, standard error, confidence interval (CI), the minimum and maximum of each group. The average value of group I (135.2 mg/dl) and in group II (138.5 mg/dl) but no significant differences. Average of group I (135.2 mg/dl) compared to group III (140 mg/dl) there were significant differences, however, it expected to decrease, while the result found in the third group was an improvement. The average value of group I (135.2 mg/dl) and in group IV (136.33 mg/dl) did not seem to have significant differences, and compared to group I (135.2 mg /dl), group IV (136.33 mg/dl) had higher value. The average value of group I (135.2 mg/dl) and in group V (112.8 mg/dl), it was found significant differences.

The average value of group II (138.5 mg/dl) and in group III (140 mg/dl), it was not found significant differences, however as group III result expected to decrease, the result found was an improvement. Average group II (138.5 mg/dl) compared to group IV (136.33 mg/dl) but no significant differences found. The average value of group II (138.5 mg/dl) and in group V (112.8 mg/dl) that it was found significant differences. Average group III (140 mg/dl) compared to group IV (136.33 mg/dl) but no significant differences found. Average group III (140 mg/dl) compared to group V (112.8 mg/dl) in the result was found significant differences. Average group IV (136.33 mg/dl) compared to group V (112.8 mg/dl) in the result was found significant differences.

The largest standard deviation was in group II (24.84) and the smallest standard deviation was in the group V (9.07). Standard deviation of each group was quite large when compared to the average difference of each group, showed a large dispersion of the average counting value. The important of descriptive statistics test was for the study of variables description before moving on the next test.

Homogeneity of variance test

Homogeneity of variance test is a test to determine the variance of the data. The parameter used was the value of significance (p). Significance value (p) of the homogeneity test variants derived from this study was 0.554. Since the significance value (p) was more than 0.05, it means there were no differences between groups of data variance, or in other words homogeneous variant. Homogeneous variant was one of the requirements for ANOVA test.

Table 3 Average value and standard deviation for each group Cholesterol

Groups	N	Mean	SD	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
I	5	135.2000	23.03693	10.30243	106.5959	163.8041
II	6	138.5000	24.84150	10.14150	112.4304	164.5696
III	6	140.0000	21.99091	8.97775	116.9220	163.0780
IV	6	136.3333	14.85485	6.06447	120.7441	151.9225
V	5	112.8000	9.06642	4.05463	101.5426	124.0574
Total	28	133.1786	20.78471	3.92794	125.1191	141.2380

Table 4. Homogenity of Variant test cholesterol

Levene Statistic	df1	df2	Sig.
.789	4	23	.544

ANOVA Test

ANOVA test can be performed if two requirements have been met, the data are normally distributed and homogeneous variant. In this study, data were normally distributed and homogeneous variants, so the ANOVA test was valid. The parameter used was the value of significance (p). Significance value (p) in the ANOVA test obtained from this study was the 0.195, F = 0.051. Since the significance value (p) was over 0.05 then it showed that there was no significant difference in ethanolic extract of lime peel (*Citrus aurantifolia*) on blood cholesterol levels of mice (*Mus musculus*).

Tabel 5. ANOVA Test Result

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	29.700	1	29.700	.051	.826
Within Groups	5208.300	9	578.700		
Total	5238.000	10			

The results of this study were not in accordance with the initial hypothesis, because the significance value (p) was over 0.05 on ANOVA test that it didn't need to proceed with LSD. The analysis was performed based on descriptive statistical tests, by comparing the average value and standard deviation of each group. Total cholesterol levels in mice (*Mus musculus*) was 77.7 to 111.7 mg/dL (Gentil & Perret 2012).

The average value of cholesterol levels of mice in the control group (normal diet) was 135.2 mg/dl. The average value of cholesterol levels of mice in group II (high-cholesterol diet for 2 weeks) was 138.50 mg/dl compared to the average control value (135.2 mg/dl), it could be proven that high-cholesterol diet for 2 weeks in group II able to raise cholesterol levels of mice, but statistically non-significant increase in the group of mice, from that result it could not be said the mice was undergoing hypercholesterolemia. The average value of the group III cholesterol (high-cholesterol diet +0.5% ethanolic extract + 750 mg/kgBW CMC-Na Solvent) was 140 mg/dl, the value was compared to the control group (135.2 mg/dl) showed increasement. The average value of cholesterol group IV (high-cholesterol diet + 0.5% ethanolic extract+ 1500 mg/kgBW CMC-Na solvent) was 136.33 mg/dl, the value was compared with the control group (135.2 mg/dl) did not show a lowering. The average value of the group V cholesterol (high-cholesterol diet + 0.5% ethanolic extract + 2250 mg/kgBW CMC-Na solvent) was 112.8 mg/dl, the value when compared with the control group (135.2 mg/dl) showed significant differences but because of the wide standard deviation, this difference did not give significant meaning.

The average value of cholesterol levels in group II (high cholesterol diet 2 weeks) was 138.5 mg/dl compared to the average value of the group III cholesterol (high-cholesterol diet + 0.5% ethanolic extract + 750 mg/kgBW CMC-Na solvent) (140 mg/dl) showed increasment of cholesterol levels. The average value of cholesterol levels in group II (138.5 mg/dl) compared with group IV (high-cholesterol diet + 0.5% ethanolic extract + 1500 mg/kgBW CMC-Na solvent) (136.33 mg/dl) showed differences but due to the wide standart deviation this differences gave no significant meaning. The average value of cholesterol levels in group II (138.5 mg/dl) compared with the value of the group V (high-cholesterol diet + 0.5% ethanolic extract + 2250 mg/kgBW CMC-Na solvent) (112.8 mg/dl) showed differences but due to the wide standart deviation this differences gave no significant meaning.

Table 6. Comparison of Average, Standard Deviation and Significance Control Group with Group II, III, IV, and V

Group	Cholesterol	Group	Cholesterol	Standard deviation	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
I	135.2	II	138.5	24.84150	.786	-28.1592	21.5592
		III	140	21.99091	.693	-29.6592	20.0592
		IV	136.33	14.85485	.926	-25.9925	23.7259
		V	112.8	9.06642	.088	-3.5646	48.3646

Table 7. Comparison of Group II to Group III, IV, and V

Group	Cholesterol	Group	Cholesterol	Standart deviation	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
II	138.5	III	140	21.99091	.897	-25.2023	22.2023
		IV	136.33	14.85485	.852	-21.5356	25.8690
		V	112.8	9.06642	.043	.8408	50.5592

Table 8. Comparison of Group III to Group IV, and V

Group	Cholesterol	Group	Cholesterol	Standard deviation	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
III	140	IV	136.33	14.85485	.752	-20.0356	27.3690
		V	112.8	9.06642	.033	2.3408	52.0592

Table 9. Comparison of Group IV to Group V

Group	Cholesterol	Group	Cholesterol	Standard deviation	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
IV	136.33	V	112.8	9.06642	.062	-1.3259	48.3925

The average value of cholesterol levels in group III (high-cholesterol diet + 0.5% ethanolic extract + 750 mg/kgBW CMC-Na solvent) (140 mg/dl) compared with the value of group IV (high-cholesterol diet + 0.5% ethanolic extract + 1500 mg/kgBW CMC-Na solvent) (136.33 mg/dl) appears to differences but these differences but was not significant. The average value of cholesterol levels in group III (140 mg/dl) compared to the value of the group V (high-cholesterol diet + 0.5% ethanolic extract + 2250 mg/kgBW CMC-Na solvent) (136.33 mg/dl) (112.8 mg/dl) showed differences but due to the wide standart deviation this differences gave no significant meaning.

The average value of group IV (high-cholesterol diet + 0.5% ethanolic extract + 1500 mg/kgBW CMC-Na solvent) (136.33 mg/dl) compared with group V (high-cholesterol diet + 0.5% ethanolic extract + 2250 mg/kgBW CMC-Na solvent (112.8 mg/dl) showed differences but due to the wide standart deviation this differences gave no significant meaning.

DISCUSSION

This study aimed to determine the effect of ethanolic extract of lime peel (*Citrus aurantifolia*) on total blood cholesterol of mice (*Mus musculus*) underwent hypercholesterolemia. Mice used a same-sex male mice, aged 2-3 months, and in a healthy physical condition. Mice divided into 5 group with 6 mice each. This study was conducted over 35 days from November 1, 2011-January 5, 2012 in the Laboratory of the Department of Biochemistry Faculty of Medicine, University of Airlangga. Treatment of lemon peel ethanolic extract given respectively in treatment group III, IV, and V were conducted using oral feeding tube. Group of mice were tube feed every day using intragastric feed as 0.01 ml/GBB. Feeding tube was conducted for 2 weeks after 2 weeks previously given high-cholesterol diet. Measurement of total blood cholesterol levels of mice conducted at the Laboratory of the Department of biochemistry Faculty of Medicine, University of Airlangga. Results of data from measurement of serum total cholesterol levels of mice were then calculated by using Analysis of Variance (ANOVA) with an error rate of 5%, which, if it found any significant differences followed test using LSD (Least Significant Difference) with a significance level of $p < 0.05$.

Previous research had suggested that lime peel contains flavonoids (Pratiwi et al 2008). The results of previous study proved that citrus flavonoids polymethoxylated, tangeretin and nobiletin, potentially inhibits apoB secretion in a simple way and inhibit the synthesis of cholesterol and triglyceride synthesis without affecting the activity of LDL receptors (Lin et al 2011). Lime peel extract (*Citrus aurantifolia*) feed to mice orally

using intragastric tube. Lime peel extract (*Citrus aurantifolia*) was expected to lower blood cholesterol levels of mice. In vitro studies indicate that the flavonoid components in citrus essential oils given the effect of lowering cholesterol in liver cells kultur dan on recent experiments studying the effect of dyslipidemia in both animals and humans (Yaghmaie et al 2011).

Average group of a given regular diet (135.20 mg/dL) lower than the average group given high dietary cholesterol (138.50 mg/dL). However, this research had not been successful in making the mice underwent hypercholesterolemia due to rising cholesterol levels which was not significant. Average group given high-cholesterol diet with the addition of extract 750 mg/kgBW (140 mg/dl) higher than the group with the group given high dietary cholesterol (138.50 mg/dL) and the addition of 1500 mg/kgBW extract (136.33 mg/dL). The group with the high-cholesterol diet and the addition of 2250 mg/kgBW extract (112.80 mg/dL) had an average value of the lowest among all groups.

Comparison of Total Blood Cholesterol Levels in Mice (*Mus musculus*) between Group Diet High Cholesterol Diet Ordinary and without treatment

In this study, there was an average difference between the control group and the high-cholesterol diet group, ie 135.20 mg/dl and 138.50 mg/dl. This showed the average total blood cholesterol levels of mice was lower than the control group high-cholesterol diet group. High-cholesterol diet used in this study was not yet able to make the circumstances hypercholesterolemic in the mice. Although normal cholesterol levels in mice (*Mus musculus*) was 77.7 to 111.7 mg/dL (Gentil & Perret 2012), the increase in cholesterol in the regular diet group and high-cholesterol diet showed a large standard deviation and statistical differences with the control group diet group however the high cholesterol did not significantly increase most likely because of improper way of the treatment giving or period of high-cholesterol diet that was too short, the giving of high-cholesterol diet was not careful, the condition of the environment could affect the health of the mice cages, cholesterol check tool was invalid, and intrinsic factor from mice that could not be controlled by researchers such as hormonal conditions, malabsorption symptoms and stress could also be a factor that coused no lowering of cholesterol level happened.

Comparison of Total Blood Cholesterol Levels in Mice (*Mus musculus*) between the group and the Ordinary Diet High Cholesterol Diet Group were given ethanolic extract Leather Lime (*Citrus aurantifolia*) 750 mg/kgBW dose, 1500 mg/kgBW, and 2250 mg/kgBW.

Normal values of blood cholesterol in mice (*Mus musculus*) was 77.7 to 111.7 mg/dL (Gentil & Perret 2012). While in this study the average total blood cholesterol levels control group was 135.2 mg/dl. This suggests that the mice used as controls had an increasement of cholesterol. Average total cholesterol levels in the group of mice given a high-cholesterol diet and ethanolic extract of 750 mg/kgBW dose was 140 mg/dl. Groups of mice given a high-cholesterol diet and ethanolic extract of 750 mg/kgBW dose in this study was expected to be lower than the control group, but the results showed an increasement, the dose of 750 mg/kgBW ethanolic extract was not able to lower total blood cholesterol levels of mice. This could happen due to the giving of high-cholesterol diet was not careful that the number of diet each day was unstable, the dose had not been able to give effect, the cholesterol tool checks invalid, and cholesterol intake higher than the amount of extract given. Intrinsic factors of mice that could not be controlled by researchers such as hormonal conditions, malabsorption symptoms and stress could also be a factor caused no lowering in cholesterol levels.

Average cholesterol levels in groups of mice given a high cholesterol diet and fed lime peel ethanolic extract of 1500 mg/kgBW dose was 136.33 mg/dl. This average was higher than the average in the regular diet group (135.2 mg/dl). The results of this study indicatethat cholesterol in the group of 1500 mg/kgBW doses ethanolic extract of lime peel higher than the levels of cholesterol in the regular diet group. These results indicate 1500 mg/kgBW doses ethanolic extract of lime peel had not been ableto reduce levels of total blood cholesterol mice compared with mice in regular diet group. These results could be affected by many factors such as the giving of high-cholesterol diet was not careful, the given dose of the extract had not been able to give the effect, the cholesterol tool checks was invalid, cholesterol intake higher than the amount of extract given, and factors such as mice intristic factor like hormonal conditions, malabsorption and symptoms of stress could also be a factor caused no lowering in cholesterol levels.

Average cholesterol levels in groups of mice given a high cholesterol diet and fed the lime peel ethanolic extract of 2250 mg/kgBW dose was 112.80 mg/dl. This average was lower than the average in the regular diet group (135.20 mg/dl). In both groups the results statistically significant difference, but this lowering was not significant because cholesterol had not been able to restore to normal levels also because of the wide of deviation value. These results could be affected by many factors such as the giving of high-cholesterol diet was not careful, the given dose of the extract had not been able to give the effect, the cholesterol tool checks

was invalid, cholesterol intake higher than the amount of extract given, and factors such as mice intristic factor like hormonal conditions, malabsorption and symptoms of stress could also be a factor caused no lowering in cholesterol levels. From the results of the study group with ethanolic extract of 1500 mg/kgBW and 750 mg/kgBW was not able to lower cholesterol levels compared to the usual diet group, in the group with the 250 mg/kgBW found the lowering but did not decrease significantly. This showed that the ethanolic extract of lime peel could not lower the total blood cholesterol levels of mice.

Comparison of Total Blood Cholesterol Levels in Mice (*Mus musculus*) between Group of High Cholesterol Diet High Cholesterol Diet and groups are given lime peel extract (*Citrus aurantifolia*) 750 mg/kgBW dose, 1500 mg/kgBW, and 2250 mg/kgBW

Normal values of blood cholesterol in mice (*Mus musculus*) was 77.7 to 111.7 mg/dL (Gentil & Perret 2012). In this study, the average of total blood cholesterol levels in high cholesterol diet group was 138.50 mg/dL. However, these mice could not be said to be in a state of hypercholesterolemia due to an increase in cholesterol was not statistically significant when compared to the control group.

Average total cholesterol levels in the group of mice with a high-cholesterol diet given 0.01 ml/GBB lime peel ethanolic extract of 750 mg/kgBW dose was 140 mg/dl. The average value was higher compared to the values in the group given high dietary cholesterol (138.5 mg/dl). In this study, cholesterol levels in the group given 750 mg/kgBW ethanolic extract did not decrease could be due to several factors such as the giving of high-cholesterol diet was not careful, the given dose of the extract had not been able to give the effect, the cholesterol tool checks was invalid, cholesterol intake higher than the amount of extract given, and factors such as mice intristic factor like hormonal conditions, malabsorption and symptoms of stress could also be a factor caused no lowering in cholesterol levels.

Average cholesterol levels in groups of mice given a high cholesterol diet and fed lime peel ethanolic extract of 1500 mg/kgBW dose was 136.33 mg/dl. This average was lower than the average in the group with the higher dietary cholesterol (138.50 mg/dL). This suggested lime peel ethanolic extract of 1500 mg/kgBW dose able to lower blood total cholesterol levels compared with the group of mice a high-cholesterol diet. However, because of the lowering was not statistically significants that the effect of the loweing in this study was not significant. These results could be affected by many factors such as the giving of high-cholesterol diet was not careful, the given dose of the extract had not been able to give the effect, the cholesterol tool checks was invalid, cholesterol intake higher than the amount of extract given, and factors such as mice intristic factor like hormonal conditions, malabsorption and symptoms of stress could also be a factor caused no lowering in cholesterol levels

Average cholesterol levels in groups of mice given a high cholesterol diet and fed lime peel ethanolic extract of 2250 mg/kgBW dose was 112.80 mg/dl. This average was lower than the average in the group with high dietary cholesterol (138.50 mg/dl). The average value in the group given high-cholesterol diet and fed ethanolic extract 2250 mg/kgBW lower than the group with a higher cholesterol diet, based on the loweing was statistically significant but due to the wide standard deviation this was not giving significant meaning. These results suggested that lime peel ethanolic extract of 2250 mg/kgBW dose potentially inhibit the secretion of apoB and simply inhibiting the synthesis of cholesterol and triglyceride synthesis.

Comparison of Total Blood Cholesterol Levels in Mice (*Mus musculus*) between

High Cholesterol Diet groups were given ethanolic extract Leather Lime (*Citrus aurantifolia*) 750 mg/kgBW dose, 1500 mg/kgBW, and 2250 mg/kgBW. Previous research had suggested that lime peel contains flavonoids (Pratiwi et al 2008). The results of previous study proved that citrus flavonoids polymethoxylated, tangeretin and nobiletin, potentially inhibits apoB secretion in a simple way and inhibit the synthesis of cholesterol and triglyceride synthesis without affecting the activity of LDL receptors (Lin et al 2011).

In this study, there were differences in mean total of blood cholesterol levels between high-cholesterol diet group and the group given ethanolic extract of lime peel of 750 mg/kgBW, 1500 mg/kgBW, and 2250 mg/kgBW. The average value of cholesterol levels in the group of mice given a high cholesterol diet and fed lime peel ethanolic extract of 1500 mg/kgBW was 136.33 mg/dl lower than the group of mice fed high-cholesterol diet and lime peel ethanolic extract of 750 mg/kgBW (140 mg/dl), but based on the data that was not statistically significant difference in value so the difference in value was not meaningful. This showed that 1500 mg/kgBW ethanolic extract did not inhibit the secretion of apoB and potentially inhibit the synthesis of cholesterol and triglycerides.

The average value of a group of mice given a high cholesterol diet and fed lime peel ethanolic extract of 2250 mg/kgBW (112.8 mg/dl) lower than the group

mice fed high-cholesterol diet and lime peel ethanolic extract of 750 mg/kgBW (140 mg/dl), and this difference was statistically significant. This proves that the ethanolic lime peel extract of 2250 mg/kgBW dose given a total cholesterol-lowering effect bigger than the group given ethanolic extract of 750 mg/kgBW, but due to the wide standard deviation, this difference was not significant. These results indicated that ethanolic extract of 2250 mg/kgBW dose had the potential to lower cholesterol levels compared to the group given ethanolic extract of 750 mg/kgBW dose.

The average value of cholesterol levels in the group of mice given a high-cholesterol diet and fed 2250 mg/kgBW ethanolic extract (112.8 mg/dl) value was lower than the group of mice given a high-cholesterol diet and 500 mg/kgBW ethanolic extract (136.33 mg/dl) but based n the statistic different value, the different was not significant. These results indicated that ethanolic extract of 2250 mg/kgBW dose had not been able to provide a significant lowering compared to the group given ethanolic extract of 1500 mg/kgBW dose.

CONCLUSION

From the research that has been carried out, it can be concluded that the study for ethanolic extract of lime peel (*Citrus aurantifolia*) 750 mg/kgBW, 1500 mg/kgBW, and 2250 mg/kgBW not be able to lower total blood cholesterol levels of mice (*Mus musculus*).

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