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Research Article

The Effect of ethanol extract of Kaffir lime leaves on Lipid Profile, Aterogenetic Index and Blood Vessel Histopathology of Male White Rats Hyperlipidemia Model

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Abstract:

Kaffir lime leaf extract has the potential to be used as an antioxidant and anti-inflammatory, apart from that, the compounds contained in kaffir lime leaves have properties that can be used as medicine. This study used a laboratory experimental design on male white Wistar hyperlipidemic rats. The research method uses a post-test only controlled group design. Hypercholesterolemia induction uses a high-fat feed method, this feed contains quail egg yolk, duck egg yolk, 3 mL/300 mg/kgBW/day of used cooking oil. The statistical tests used are data normality and homogeneity tests. The results of phytochemical screening on kaffir lime leaves showed secondary metabolites such as flavonoids, phenols, saponins, tannins and alkaloids. In this study there was a significant difference in mean body weight ($p<0.05$) on the 14th day but there was no difference between groups on the 21st day. Apart from that, there were no significant differences in mean levels of total cholesterol, triglycerides, HDL (High-density lipoprotein), and LDL (Low-Density Lipoprotein) ($p<0.05$) between all groups. In eNOS levels there were no significant differences ($p<0.05$) between all groups. However, in HMGCOA levels, there were significant differences in levels ($p<0.05$) between all groups. Apart from that, the group with the ethanol extract of kaffir lime leaves at a dose of 35 mg/kgBW was better at controlling body weight, reducing total cholesterol, HDL, LDL and triglyceride levels, increasing eNOS levels and reducing HMGCOA levels.

1. Introduction

Hyperlipidemia is a condition where blood fat levels increase [1]. This is characterized by an increase in cholesterol, LDL (Low Density Lipoprotein) and triglyceride levels in the blood that exceed normal limits and a decrease in HDL (High Density Lipoprotein) [2, 3]. High cholesterol levels are a condition when the total blood cholesterol value increases above the normal value (>240mg/dL) (Dash & Saini, 2023). Where the limit for normal cholesterol levels in humans is <200mg/dL [4].

Overall, hyperlipidemia causes death in 4.4 million people in the world and causes 40.4 million Disability Adjusted Life Years (DALY) [5]. Hyperlipidemia itself can occur if disorders of lipid and cholesterol metabolism are found, consisting of total LDL (Low-Density Lipoprotein) and HDL (High-density lipoprotein) cholesterol [6]. This increase in total cholesterol levels can increase risk factors for Coronary Heart Disease (CHD) and other cardiovascular diseases [7]. High total cholesterol levels will form atherosclerosis [8]. Atherosclerosis can cause hypertension and blockages in the blood vessels of the brain, heart and leg blood vessels [9]. One of the pathogeneses that causes atherosclerosis in blood vessel walls is an increase in fat levels in blood plasma [10,11]. So, it can cause blockages in

the blood vessels of the heart or known as coronary heart disease (CHD) and stroke [12]. These two diseases are the biggest causes of death in the world [13].

Data from the World Health Organization (WHO) in 2014 shows that hyperlipidemia causes 18% of cardiovascular disease and 56% of ischemic heart disease worldwide [14, 5, 15]. Research results from the Health Research and Development Agency of the Ministry of the Republic of Indonesia in 2018 showed data that 1.5% or more than one million Indonesians had coronary heart disease [16]. Apart from that, 36.55% of Indonesians aged 15 years and over have cholesterol levels above normal (> 240 mg/dL), 24.9% have high LDL (Low Density Lipoprotein) levels (> 190 mg/dL), with HDL levels (High -density lipoprotein) below 40 mg/dl 24.3% and very high triglycerin levels (> 500 mg/dL) [17, 18].

In general, prevention of hyperlipidemia can be done by changing lifestyle for the better, such as maintaining diet and exercising [19, 20]. Meanwhile, pharmacological treatment of hyperlipidemia in most cases is conventional treatment [21]. Theoretically, preventive measures can be taken by administering medication [22]. However, continuous use of medication is not recommended [23]. Because several interventions originating from pharmacology are still doubtful about their results in reducing total cholesterol levels [24].

Apart from using conventional medicine, people also use traditional medicine as therapy for hyperlipidemia [25]. Research results in several health services in 7 provinces in Indonesia showed that 62.9% were given single herbal drug therapy and the rest were given a combination of herbal medicines and synthetic drugs [26]. It is hoped that the use of herbal medicines can reduce the use of synthetic drugs. Although the use of herbal medicines cannot completely replace the effects of synthetic drugs, herbal medicines are quite economical and have fewer side effects compared to synthetic drugs for treating hyperlipidemia in general [27].

Many medicinal plants have been proven to inhibit the formation and lower cholesterol, such as kaffir lime leaves [28]. Kaffir lime leaves contain flavonoid and phenolic compounds which act as antioxidants [29]. The flavonoid compounds contained work selectively on cholesterol metabolism in the liver in reducing cholesterol levels [30]. Flavonoids are able to increase bile acid excretion and reduce blood viscosity which causes cholesterol deposition in blood vessels [31]. This active ingredient has the potential to reduce total cholesterol levels in the blood [32].

Flavonoids, especially quercetin, tannins and saponins, are compounds that can reduce cholesterol levels [33]. Quercetin reduces cholesterol levels by reducing the α-tocopherol content which is part of LDL, the reduction that occurs results in inhibited LDL oxidation [34. 35]. The results of research by Rustanti & Lathifah [36], stated that giving quercetin extract can reduce total cholesterol levels. The greater the concentration of quercetin, the higher the anti-cholesterol activity.

The quercetin contained in kaffir lime leaves is a source of antioxidants that can ward off free radicals [37]. Saponin can reduce cholesterol levels and increase the binding of cholesterol to fiber, causing cholesterol in the intestine to be blocked and form complex bonds that are not soluble in fat [38, 39]. Tannins reduce cholesterol levels by reacting with mucosal proteins and epithelial cells which causes fat absorption in the intestine to be hampered [40]. Based on the explanations, opinions and previous research that have been stated in the paragraph above, the researchers are interested in researching more deeply regarding the influence of the ethanol extract of Kaffir lime on the lipid profile, atherogenic index and blood vessel histopathology of male white mice in the hyperlipidemia model.

2. Material and Methods

This research used a laboratory experimental design on male white Wistar hyperlipidemic rats. The research method uses a post test only controlled group design. This design allows researchers to measure the effect of treatment (intervention) on the experimental group by comparing the experimental group with the control group. In this design the researcher does not determine how big the change occurs, because the test is carried out at the end of the treatment.

Hypercholesterolemia induction uses a high-fat feed method, this feed contains quail egg yolk, duck egg yolk, 3 mL/300 mg/kgBW/day of used cooking oil. The test animals were divided into 6 groups, namely the normal group (K1) which was not given treatment, (K2) which was given high fat feed 3 mL/200 mg/kgBW/day. (K3) were given high fat feed 3 mL/200 mg/kgBW/day + simvastatin 2.1 mg/kgBW/day. (K4) were given high-fat feed 3 mL/300 mg/kgBW/day + ethanol extract of kaffir lime leaves at a dose of 35 mg/200gBW. (K5) was given a high fat diet of 3 mL/300 mg/kgBW/day + ethanol extract of kaffir lime leaves at a dose of 70 mg/200gBW. (K6) was given high-fat feed 3 mL/300 mg/kgBW/day + ethanol extract of kaffir lime leaves at a dose of 140 mg/200gBW [41, 15].

The research was carried out at the Biomedical Science Master's Laboratory, Faculty of Medicine,

Indonesian Methodist University to produce ethanol extract from kaffir lime and the animal house as a place for surgical treatment of experimental animals. Faculty of Medicine, Master of Biomedical Sciences, Indonesian Methodist University, for examination of kaffir lime characterization and screening, examination related to lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and ratio of total cholesterol/HDL cholesterol), atherogenic index, and blood vessel histopathology. The statistical tests used are data normality and homogeneity tests. The data was found to be normally distributed so an ANOVA test was carried out. All data analysis was carried out using SPSS software. In this study, for statistical test decisions, a real level of 5% (p = 0.05) was taken which was considered meaningful or significant. If a p value <0.05 is obtained then a follow-up post hoc test is carried out.

3. Results and Discussions

Results

A. Total Flavonoid Test

The total flavonoid content was measured using a sample test with a concentration of 10 ppm, 0.5 mL was put into a 5 mL measuring flask, then 0.1 mL of 2% AlCl3, 0.1 mL of 1 M CH3COONa were added, then incubated for 30 minutes, absorbance of the sample solution was measured using a UV-Vis spectrophotometer with a maximum wavelength of 415 nm. The total flavonoid content was calculated using a linear regression equation from the obtained quercetin standard curve. The results of measuring the standard absorbance of quercetin can be seen in Figure 1 and the total flavonoids of the ethanol extract of kaffir lime leaves can be seen in Table 1.

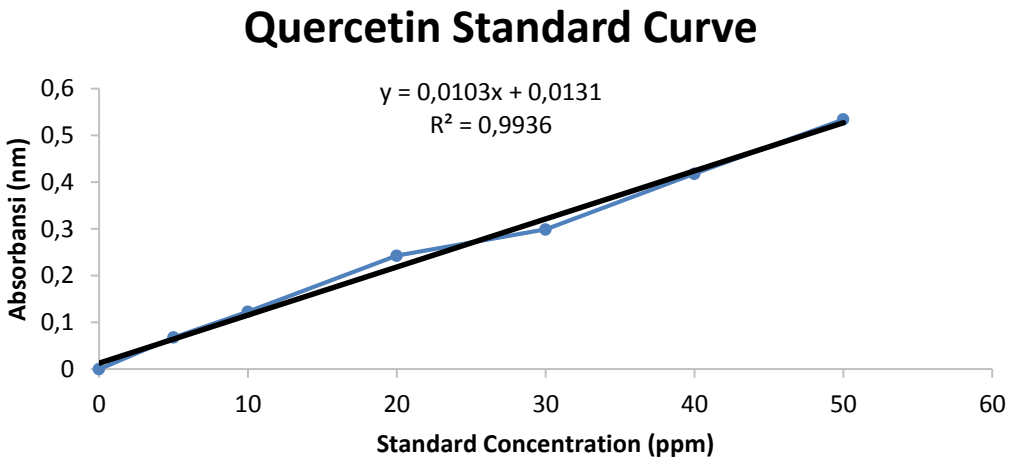

Figure 1. *Quercetin standard curve*

Table 1. *Total Flavonoids from Ethanol Extract of Kaffir Lime Leaves*

Sample	Extract Concentration (ppm)	Replication	Absorbance (nm)	Total Flavonoid Level (mg/L)	Average Total Flavonoid Levels (mg/L)
Kaffir Lime Leaf Ethanol Extract	10	1	0.2773	25.650	26.417
		2	0.2874	26.631	
		3	0.2909	26.971	

Table 2. *Total Phenolics Ethanol Extract of Kaffir Lime Leaves*

Sample	Extract Concentration (ppm)	Replication	Absorbance (nm)	Total Phenolic Content (mg/L)	Average Total Phenolic Content (mg/L)
Kaffir Lime Leaf Ethanol Extract	110	1	0.342	484.957	503.429
		2	0.356	504.857	
		3	0.367	520.571	

B. Total Phenolic Test

The total phenolic content was measured using a sample test with a concentration of 110 ppm, 0.5 mL was put into a 5 mL measuring flask, then 0.1 mL of Follin-Ciocalteau reagent was added, shaken and left for 4-8 minutes. Then 0.1 mL of 2% Na2CO3 was added and filled with aquaset to the limit mark. After incubation for 30 minutes, the absorbance of the sample solution was measured with a UV-Vis spectrophotometer at a maximum wavelength of 765

nm. The total phenolic content was calculated using a linear regression equation from the obtained gallic acid standard curve. The results of measuring the standard absorbance of gallic acid can be seen in Figure 2 and the total phenolics of the ethanol extract of kaffir lime leaves can be seen in Table 2.

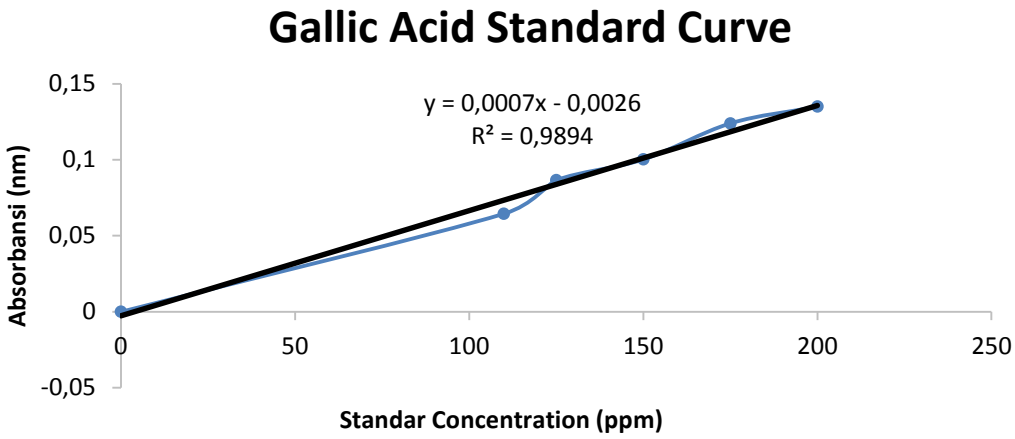

Figure 2. *Gallic acid standard curve*

C. Phytochemical Screening Analysis of Ethanol Extract of Kaffir Lime Leaves

The results of the phytochemical screening examination of the ethanol extract of kaffir lime leaves showed secondary metabolites such as flavonoids, alkaloids, saponins, tannins and phenols. The results of the screening examination can be seen in Table 3.

Table 3. *Phytochemical Screening of Ethanol Extract of Kaffir Lime Leaves*

Test	Flavonoids	Alkaloids	Saponins	Tannin	Phenol
Picture					
Results	(+)	(+)	(+)	(+)	(+)

D. Mean Value of Differences in Body Weight After Giving Ethanol Extract of Kaffir Lime Leaves

The results of the analysis of differences in body weight levels H-0, H-7 and H-14 in a group of male Wistar white rats (Rattus norvegicus) with a hyperlipidemia model after administration of kaffir lime leaf ethanol extract can be seen in Table 4.

Table 4. *Results of Analysis of Differences in Body Weight Levels on H-0, D-7 and D-14 in Groups After Giving Ethanol Extract of Kaffir Lime Leaves*

PostHoc		BB H-0	BB H-7	BB H-14
		P	P	P
Normal Group	Negative Group	<0.001*	<0.001*	0.002*
	Positive Group	<0.001*	<0.001*	0.330*
	Group I	<0.001*	0.005*	0.534*
	Group II	<0.001*	<0.001*	0.289*
	Group III	<0.001*	0.027*	0.964*
Negative Group	Normal Group	<0.001*	<0.001*	0.002*
	Positive Group	0.751	0.076*	0.026*
	Group I	0.313	0.002*	0.011*
	Group II	0.657	0.030*	0.032*
	Group III	0.150	<0.001*	0.003*
Positive Group	Normal Group	<0.001*	<0.001*	0.330*
	Negative Group	0.751	0.076*	0.026*
	Group I	0.468	0.121*	0.721*
	Group II	0.447	0.667*	0.929

PostHoc		BB H-0	BB H-7	BB H-14
		P	P	P
	Group III	0.082	0.030*	0.352
Group I	Normal Group	<0.001*	0.005*	0.534*
	Negative Group	0.313	0.002*	0.011*
	Positive Group	0.468	0.121	0.721*
	Group II	0.150	0.255	0.656*
	Group III	0.018	0.504	0.563*
Group II	Normal Group	<0.001*	<0.001*	0.289*
	Negative Group	0.657	0.030*	0.032*
	Positive Group	0.447	0.667*	0.929*
	Group I	0.150	0.255*	0.656*
	Group III	0.313	0.076*	0.309*
Group III	Normal Group	**<0.001***	0.027*	0.964*
	Negative Group	0.150	<0.001*	0.003*
	Positive Group	0.082	0.030*	0.352*
	Group I	0.018	0.504*	0.563*
	Group II	0.313	0.076*	0.309*

*Post Hoc (Significant <0.05)

E. Mean Value of Differences in Total Cholesterol Levels After Giving Ethanol Extract of Kaffir Lime Leaves

The results of the analysis of differences in total cholesterol levels on H-0, H-7, H-14 and H-21 in a group of male white Wistar rats (Rattus norvegicus) with a hyperlipidemia model after administration of kaffir lime leaf ethanol extract can be seen in Table 5.

Table 5. *Results of Analysis of Differences in Total Cholesterol Levels on H-0, H-7, H-14 and H-21 in Groups After Giving Ethanol Extract of Kaffir Lime Leaves*

Total Cholesterol Levels		H-0	D-7	H-14	H-17
		P	P	P	P
Normal Group	Negative Group	<0.001*	<0.001*	<0.001*	<0.001*
	Positive Group	<0.001*	<0.001*	<0.001*	<0.001*
	Group I	<0.001*	<0.001*	<0.001*	<0.001*
	Group II	<0.001*	<0.001*	<0.001*	<0.001*
	Group III	<0.001*	<0.001*	<0.001*	<0.001*
Negative Group	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Positive Group	0.002	0.015*	<0.001*	0.228*
	Group I	0.062	0.238*	0.013*	0.001*
	Group II	0.314	0.575*	0.532*	<0.001*
	Group III	0.500	0.916*	0.814*	<0.001*
Positive Group	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Negative Group	0.002*	0.015*	<0.001*	0.228*
	Group I	0.182*	0.177*	0.024*	0.018*
	Group II	0.030*	0.052*	<0.001*	0.005*
	Group III	0.014*	0.011*	<0.001*	0.002*
Group I	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Negative Group	0.062*	0.238*	0.013*	0.001*
	Positive Group	0.182*	0.177*	0.024*	0.018*
	Group II	0.370*	0.528*	0.053*	0.606*
	Group III	0.220*	0.200*	0.022*	0.376*
Group II	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Negative Group	0.314*	0.575*	0.532*	<0.001*
	Positive Group	0.030*	0.052*	<0.001*	0.005*
	Group I	0.370*	0.528*	0.053*	0.606*
	Group III	0.735*	0.506*	0.695*	0.708*
Group III	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Negative Group	0.500*	0.916*	0.093	<0.001*
	Positive Group	0.014*	0.011*	0.415	0.002*
	Group I	0.220*	0.200*	0.038	0.376*
	Group II	0.735*	0.506*	0.181	0.708*

*Post Hoc (Significant <0.05)

F. Mean Values of Differences in Plasma Atherogenic Index (IAP) Levels After Administration of Kaffir Lime Leaf Ethanol Extract

The results of the analysis of differences in plasma atherogenic index (IAP) levels between groups of male Wistar white rats (Rattus norvegicus) with a hyperlipidemia model after administration of kaffir lime leaf ethanol extract can be seen in Table 6.

Table 6. *Results of Analysis of Differences in Plasma Atherogenic Index (IAP) Levels Between Groups After Administration of Kaffir Lime Leaf Ethanol Extract*

Test	Group	IAP	
		Mean ± SD	P
Pre	Normal Group	0.430±0.259	<0.001*
	Negative Group	0.220±0.038	
	Positive Group	0.236±0.025	
	Group I (100)	0.220±0.018	
	Group II (200)	0.220±0.015	
	Group III (300)	0.220±0.015	
Post	Normal Group	0.065±0.037	<0.001*
	Negative Group	0.241±0.021	
	Positive Group	0.151±0.027	
	Group I (100)	0.219±0.020	
	Group II (200)	0.199±0.034	
	Group III (300)	0.187±0.028	

*Anova Test (Significant <0.05)

G. Mean Value of Differences in Total Cholesterol Levels Pre and Post Triglyceride Levels After Administration of Kaffir Lime Leaf Ethanol Extract

The results of the analysis of differences in total cholesterol levels and pre and post triglyceride levels in a group of male Wistar white rats (Rattus norvegicus) with a hyperlipidemia model after administration of kaffir lime leaf ethanol extract can be seen in Table 7.

Table 7. *Results of Analysis of Differences in Total Cholesterol Levels and Triglyceride Levels Pre and Post in Groups After Giving Ethanol Extract of Kaffir Lime Leaves*

Group		Total cholesterol		Triglyceride Levels	
		Pre	Post	Pre	Post
		P	P	P	P
Normal Group	Negative Group	<0.001*	<0.001*	<0.001*	<0.001*
	Positive Group	<0.001*	<0.001*	<0.001*	<0.001*
	Group I	<0.001*	<0.001*	<0.001*	<0.001*
	Group II	<0.001*	<0.001*	<0.001*	<0.001*
	Group III	<0.001*	<0.001*	<0.001*	<0.001*
Negative Group	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Positive Group	0.004*	<0.001*	0.033*	0.015*
	Group I	0.080*	0.004*	0.791*	0.107*
	Group II	0.345*	0.014*	0.285*	0.333*
	Group III	0.500	0.033*	0.413*	0.563*
Positive Group	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Negative Group	0.004*	<0.001*	0.033*	0.015*
	Group I	0.210*	0.015*	0.072*	0.364*
	Group II	0.041*	0.004*	0.002*	0.120*
	Group III	0.020*	0.001*	0.005*	0.055*
Group I	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Negative Group	0.080*	0.004*	0.791*	0.107*
	Positive Group	0.210*	0.015*	0.072*	0.364*
	Group II	0.400*	0.597*	0.157*	0.504*
	Group III	0.250*	0.363*	0.242*	0.290*
Group II	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*

Group		Total cholesterol		Triglyceride Levels	
		Pre	Post	Pre	Post
		P	P	P	P
	Negative Group	0.345*	0.014*	0.285*	0.333*
	Positive Group	0.041*	0.004*	0.002*	0.120*
	Group I	0.400*	0.597*	0.157*	0.504*
	Group III	0.751*	0.700*	0.797*	0.692*
Group III	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Negative Group	0.527*	0.033*	0.413*	0.563*
	Positive Group	0.020*	0.001*	0.005*	0.055*
	Group I	0.250*	0.363*	0.242*	0.290*
	Group II	0.751*	0.700*	0.797*	0.692*

*Anova Test (Significant <0.05)

H. Mean Value of Differences in HDL Levels and LDL Levels Pre and Post After Giving Ethanol Extract of Kaffir Lime Leaves

The results of the analysis of differences in HDL levels and pre and post LDL levels in a group of male Wistar white rats (Rattus norvegicus) with a hyperlipidemia model after administration of kaffir lime leaf ethanol extract can be seen in Table 8.

Table 8. *Results of Analysis of Differences in HDL and LDL Levels Pre and Post in Groups After Giving Ethanol Extract of Kaffir Lime Leaves*

Group		HDL levels		LDL levels	
		Pre	Post	Pre	Post
		P	P	P	P
Normal Group	Negative Group	<0.001*	0.026*	<0.001*	<0.001*
	Positive Group	<0.001*	<0.001*	<0.001*	0.048*
	Group I	<0.001*	0.011*	<0.001*	<0.001*
	Group II	<0.001*	<0.001*	<0.001*	<0.001*
	Group III	<0.001*	<0.001*	<0.001*	<0.001*
Negative Group	Normal Group	<0.001*	0.026*	<0.001*	<0.001*
	Positive Group	0.568*	<0.001*	0.064*	<0.001*
	Group I	0.927*	0.731*	0.320*	0.011*
	Group II	0.525*	0.017*	0.142*	0.001*
	Group III	0.649*	0.001*	0.317*	<0.001*
Positive Group	Normal Group	<0.001*	<0.001*	<0.001*	0.048*
	Negative Group	0.586*	<0.001*	0.064*	<0.001*
	Group I	0.525*	<0.001*	0.367*	<0.001*
	Group II	0.927*	0.011*	0.679*	<0.001*
	Group III	0.927*	0.175*	0.371*	0.002*
Group I	Normal Group	<0.001*	0.011*	<0.001*	<0.001*
	Negative Group	0.927*	0.731*	0.142*	0.011*
	Positive Group	0.525*	<0.001*	0.679*	<0.001*
	Group II	0.468*	0.038*	0.622*	0.399*
	Group III	0.586*	0.002*	0.627*	0.203*
Group II	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Negative Group	0.525*	0.017*	0.142*	0.001*
	Positive Group	0.927*	0.011*	0.679*	<0.001*
	Group I	0.468*	0.038*	0.622*	0.399*
	Group III	0.856*	0.202*	0.627*	0.659*
Group III	Normal Group	<0.001*	<0.001*	<0.001*	<0.001*
	Negative Group	0.649*	0.001*	0.317*	<0.001*
	Positive Group	0.927*	0.175*	0.371*	0.002*
	Group I	0.586*	0.002*	0.994*	0.203*
	Group II	0.856*	0.202*	0.627*	0.659*

*Anova Test (Significant <0.05)

İ. Mean Value of Differences in HMGCOA Levels Pre and Post After Giving Kaffir Lime Leaf Ethanol Extract

The results of the analysis of differences in pre and post HMGCOA levels in a group of male Wistar white rats (Rattus norvegicus) with a hyperlipidemia model after administration of kaffir lime leaf ethanol extract can be seen in Table 9.

Table 9. *Results of Analysis of Differences in Pre and Post HMGCOA Levels in Groups After Administration of Kaffir Lime Leaf Ethanol Extract*

HMGCOA levels		Pre	Post
		P	P
Normal Group	Negative Group	**<0.001***	**<0.001***
	Positive Group	**<0.001***	**0.048***
	Group I	**<0.001***	**<0.001***
	Group II	**<0.001***	**<0.001***
	Group III	**<0.001***	**<0.001***
Negative Group	Normal Group	**<0.001***	**<0.001***
	Positive Group	**<0.001***	**<0.001***
	Group I	**0.022***	**0.011***
	Group II	**0.029***	**0.001***
	Group III	**0.029***	**<0.001***
Positive Group	Normal Group	**<0.001***	**0.048***
	Negative Group	**<0.001***	**<0.001***
	Group I	**0.004***	**<0.001***
	Group II	**0.003***	**<0.001***
	Group III	**0.003***	**0.002***
Group I	Normal Group	**<0.001***	**<0.001***
	Negative Group	**0.022***	0.011*
	Positive Group	**0.004***	**<0.001***
	Group II	0.902*	0.399*
	Group III	0.899*	0.203*
Group II	Normal Group	**<0.001***	**<0.001***
	Negative Group	**0.029***	**0.001***
	Positive Group	**0.003***	**<0.001***
	Group I	0.902*	0.399*
	Group III	0.998*	0.659*
Group III	Normal Group	**<0.001***	**<0.001***
	Negative Group	**0.029***	**<0.001***
	Positive Group	**0.003***	**0.002***
	Group I	0.899*	0.203*
	Group II	0.988*	0.659*

*Anova Test (Significant <0.05)

Histopathological Picture of the Abdominal Aorta of a Group of Male White Wistar Rats (Rattus norvegicus) Hyperlipidemia Model After Administration of Kaffir Lime Leaf Ethanol Extract

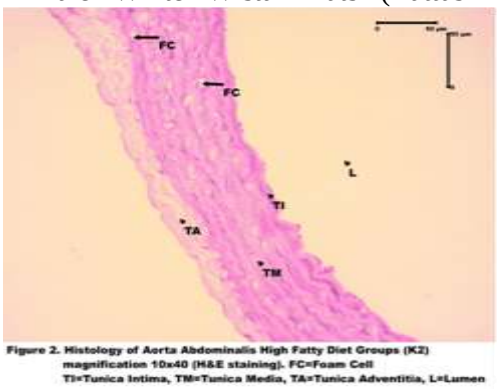

Figure 2. Histology of Aorta Abdominalis High Fatty Diet Groups (K2) magnification 10x40 (H&E staining). FC=Foam Cell TI=Tunica Intima, TM=Tunica Media, TA=Tunica Adventitia, L=Lumen

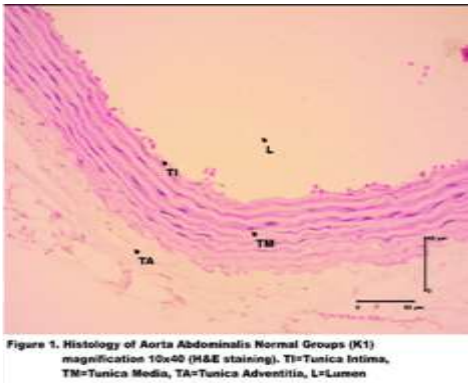

Figure 1. Histology of Aorta Abdominalis Normal Groups (K1) magnification 10x40 (H&E staining). TI=Tunica Intima, TM=Tunica Media, TA=Tunica Adventitia, L=Lumen

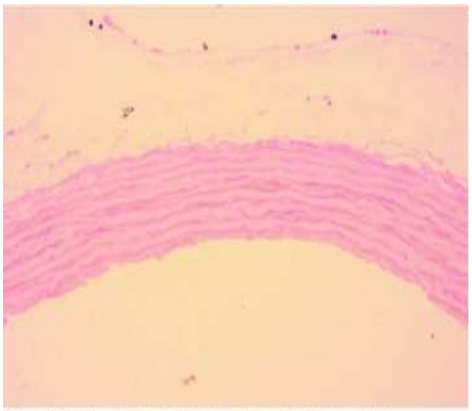

Figure 3. Histology of Aorta Abdominalis Simvastatin Groups (K3) magnification 10x40 (H&E staining).

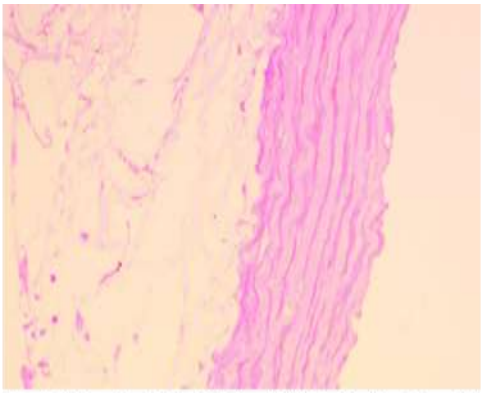

Figure 4. Histology of Aorta Abdominalis 35 mg/kg BW Extract Jeruk Purut Groups (K4) magnification 10x40 (H&E staining).

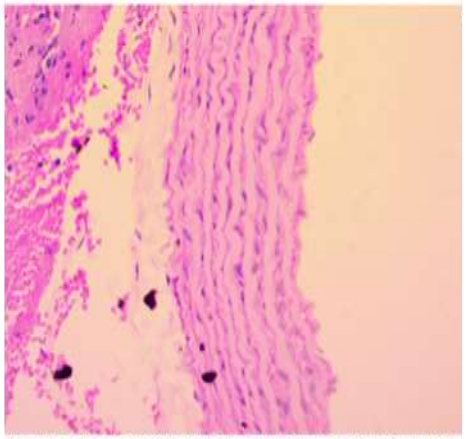

Figure 5. Histology of Aorta Abdominalis 70 mg/kg BW Extract Jeruk Purut Groups (K5) magnification 10x40 (H&E staining).

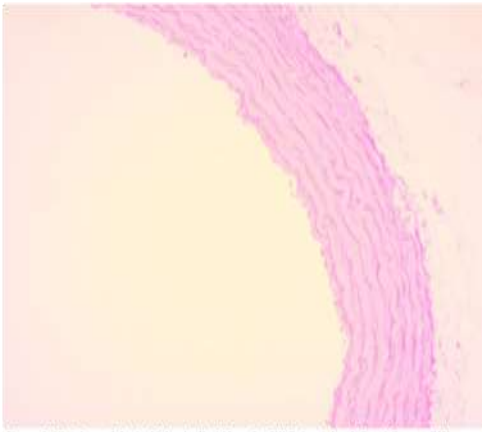

Figure 6. Histology of Aorta Abdominalis 140 mg/kg BW Extract Jeruk Purut Groups (K6) magnification 10x40 (H&E staining).

Discussion

In this study, a phytochemical screening examination of kaffir lime leaf ethanol was carried out and secondary metabolites such as flavonoids, alkaloids, saponins, tannins and phenols were obtained. The results of this examination show that kaffir lime leaves have potential as an antioxidant and anti-inflammatory. The compounds contained in kaffir lime leaves have properties that can be used as traditional medicine [42]. Flavonoid compounds have hydroxy groups that can react with boric acid, producing intense yellow fluorescence at a UV wavelength of 366 nanometers. The results of the alkaloid test showed the formation of an orange precipitate after adding Dragendorff's reagent and a yellow precipitate after adding Mayer's reagent. The presence of saponin can be confirmed by the formation of stable foam after warm water is added. Tannin, on the other hand, is a phenolic compound containing hydroxyl groups, which when reacted with FeCl3 produces a greenish black color.

The results of the analysis of differences in body weight showed that there were significant differences between groups on the 7th and 14th days ($p<0.001$), but there were no significant differences on the 21st day. These results show that the ethanol extract of kaffir lime leaves can reduce the blood weight of male white Wistar rats with a model of hyperlipidemia. This can happen because flavonoids can reduce cholesterol levels in the blood by inhibiting the action of the enzyme 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMG Co-A reductase) [43]. Reducing cholesterol levels in the bloodstream can reduce fat accumulation in the body's organs and reduce the risk of obesity [44]. By reducing cholesterol levels, the risk of cholesterol buildup in the body's organs decreases, so the risk of obesity is also reduced [45]. Apart from that, compounds such as alkaloids, tannins, saponins, triterpenoids, quinones, steroids, stilbenes, phenolic acids, anthrax, panclin, and hydroxylic acids also play an important role as compounds that can inhibit lipase activity in the body [46].

The results of the analysis of differences in total cholesterol, triglyceride, HDL and LDL levels in male white mice after being given kaffir lime leaf extract showed the effect of disiplidemia. In this study it was seen that there was a decrease in cholesterol levels. This is because kaffir lime leaves contain flavonoid, saponin and tannin compounds which have anti-cholesterol effectiveness (Buathong & Duangsrisai, [47]. Flavonoids can reduce cholesterol levels by inhibiting cholesterol synthesis, increasing LDL receptor expression, and improving overall cholesterol metabolism [48]. As antioxidants, flavonoids can also overcome free

radicals and prevent lipid peroxidation which is an early stage in the formation of atherosclerosis [49]. LDL, as well as increasing the activity of lipoprotein lipase and lecithin cholesterol acyl transferase [50].

The results of the analysis of differences in ENOS levels in this study showed no significant differences between groups in ENOS levels with $p>0.05$. These results suggest that flavonoids can stimulate eNOS production and increase eNOS expression in the vascular endothelium, which in turn increases NO production. In addition, flavonoids also have antioxidant properties that can protect eNOS and NO from damage by free radicals, maintaining optimal eNOS activity [51]. In the analysis of HMGCOA levels, there was a significant difference between groups in HMGCOA levels in mice ($p>0.05$). This shows that the compounds contained in the ethanol extract of kaffir lime leaves can inhibit the HMGCOA enzyme [52]. Excessive cholesterol production can be overcome by inhibiting HMG-CoA reductase which mediates the formation of acetyl-CoA into mevalonate; precursor for making cholesterol [53]. Statin drugs have been known to be strong HMG-CoA reductase inhibitors with several undesirable side effects. Therefore, we are looking for safer HMG-CoA reductase inhibitors, especially from kaffir lime leaves [54].

The flavonoids in orange leaf extract are effective in significantly reducing cholesterol and triglyceride levels by inhibiting the enzymes HMG-CoA reductase and acetyl-coenzyme A acetyl-transferase (ACAT) [55]. In their role in reducing blood cholesterol levels in hyperlipidemia conditions, flavonoids can reduce cholesterol synthesis by inhibiting cholesterol esterification in the intestines and liver through inhibiting the activity of the acyl-CoA enzyme cholesterol acyl transferase (ACAT) in HepG2 cells. Apart from that, flavonoids can also inhibit cholesterol synthesis by reducing the activity of the enzyme 3-hydroxyl-3-metal-glutaryl-CoA (HMG-CoA) reductase, thereby reducing cholesterol levels in the liver [56].

Flavonoids function as inhibitors of the HMG-CoA reductase enzyme, thereby causing a decrease in cholesterol synthesis [57]. When cholesterol is transported from the intestine to the liver, the activity of HMG-CoA reductase, which is responsible for converting acetyl-CoA to mevalonate in the process of cholesterol synthesis, is inhibited. As a result, cholesterol production by the liver will be reduced. Alkaloids act as antioxidants by releasing hydrogen ions similar to flavonoids [58]. In addition, this compound can inhibit the activity of the pancreatic lipase enzyme, thereby increasing fat excretion through feces. As a result, fat absorption by the liver is hampered, so fat cannot be converted into cholesterol. Reduced activity of the pancreatic lipase enzyme can reduce the accumulation of triglycerides entering from the small intestine because this enzyme converts triglycerides into two monoglycerides and two free fatty acids which can be absorbed by the blood vessels. In addition, tannins can inhibit fat absorption in the intestine by interacting with mucosal proteins and intestinal epithelial cells [59]. In addition, tannins can accumulate on the protein mucosa on the surface of the small intestine, resulting in a decrease in the effectiveness of cholesterol and fat absorption [60].

4. Conclusions

The results of phytochemical screening on kaffir lime leaves showed secondary metabolites such as flavonoids, phenols, saponins, tannins and alkaloids. In this study there was a significant difference in mean body weight ($p<0.05$) on the 14th day but there was no difference between groups on the 21st day. Apart from that, there were no significant differences in mean levels of total cholesterol, triglycerides, HDL and LDL ($p<0.05$) between all groups. In eNOS levels there were no significant differences ($p<0.05$) between all groups. However, in HMGCOA levels, there were significant differences in levels ($p<0.05$) between all groups. Apart from that, the group with the ethanol extract of kaffir lime leaves at a dose of 35 mg/kgBW was better at controlling body weight, reducing total cholesterol, HDL, LDL and triglyceride levels, increasing eNOS levels and reducing HMGCOA levels.

Author Statements:

- **Ethical approval:** The conducted research is not related to either human or animal use.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper
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- **Data availability statement:** The data that support the findings of this study are available on

request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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