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Test of Stevia Leaf Ethanol Extract (*Stevia Rebaudiana*) Administered Orally to Female Wistar Rats (*Rattus Norvegicus*) on a High-Fat Diet to Prevent Dyslipidemia

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ARTICLE INFO	ABSTRACT

Keywords:
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This study aims to analyze and test the effect of Stevia Rebaudiana ethanol extract in preventing dyslipidemia in female Wistar rats (Rattus Norvegicus) fed a high-fat diet. The results showed that giving Stevia Rebaudiana ethanol extract at 40 mg / 200 gr BW of female Wistar rats given a high-fat diet orally prevented a decrease in HDL cholesterol levels. However, it will increase total cholesterol, LDL cholesterol, and triglycerides. This study demonstrated that the ethanol extract of Stevia Rebaudiana has beneficial properties in controlling hyperlipidemia, which is presumably due to the antioxidant and glycoside effects of the ethanol extract of Stevia Rebaudiana, which overall contributes to the development profile lipid rats were given a high-cholesterol diet.

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1. INTRODUCTION

Communities worldwide, including Indonesia, are currently experiencing changes in consumption patterns of foods high in fat. These changes are also accompanied by a sedentary lifestyle, namely the behavior of a person who does not move much and spends little energy, such as sitting, reading, watching television, studying, playing games, and using the computer. These lifestyle changes also occurred during the Covid-19 pandemic, which allegedly contributed to increased health problems, including dyslipidemia [1].

Dyslipidemia is a lipid metabolism disorder characterized by an increase or decrease in one or more lipid fractions in the blood. Some main lipid fraction abnormalities are increased total cholesterol, LDL cholesterol, triglycerides, and decreased HDL cholesterol. Increased total cholesterol and LDL blood levels can be caused by increased saturated fat and high cholesterol consumption. In the course of dyslipidemia, if cholesterol levels are not well controlled, it will increase the risk of acute and chronic complications [2].

Total cholesterol is a combination and description of the fat component in the blood, which consists of all lipoprotein parts, namely 60% -70% LDL, 20% -30% HDL, and 10% -15% VLDL. Human blood's normal total cholesterol level is <200 mg/dl. While in rats, the normal level of total cholesterol is 10-54 mg/dL, the normal level of triglycerides is 26-145 mg/dL. The increase in cholesterol is generally achieved within two weeks; it is said to be dyslipidemia if a weight gain of > 20% total serum cholesterol level > 240 mg/dL—abnormalities in lipid and lipoprotein metabolism cause dyslipidemia. Dyslipidemia is a major factor in atherosclerosis, which causes cardiovascular disease [3]. Cardiovascular disease deaths reach 17.8 million people worldwide and continue to increase [4]. The prevalence of dyslipidemia is also increasing in the Asia Pacific region, including Indonesia, due to changes in lifestyle and eating patterns of people who consume more and more foods high in fat [5].

The prevalence of dyslipidemia in Indonesia is still high, characterized by increased LDL and total cholesterol levels and an increase in the number of cases of coronary heart disease detected (Kemenkes RI, 2019). Based on the Indonesian Ministry of Health Basic Health Research

(RISKESDAS) report, it was recorded that 72.8% of Indonesia's population aged 15 years and over had LDL levels of more than 100 mg/dL and 28.8% had total cholesterol levels of than 200 mg/dL, where urban residents suffered more than rural residents [1].

Dyslipidemia risk factors include diet, stress, physical inactivity, and smoking. Dyslipidemia can be primary or genetic, and secondary, which is the effect of a certain condition or the impact of a drug that can increase plasma lipid levels. Clinical symptoms and complaints of dyslipidemia are generally absent. Clinical manifestations that arise are usually complications of dyslipidemia, such as stroke and coronary heart disease, namely narrowing or blockage of the coronary arteries. Very high triglyceride levels can cause acute pancreatitis, hepatosplenomegaly, paresthesia, shortness of breath, and impaired consciousness; it can also change the color of the retinal blood vessels to beige (*lipemia retinitis*) and change the color of blood plasma to milky white. In patients with very high LDL levels (familial hypercholesterolemia), corneal arches, xanthelasma on the eyelids, and xanthomas on the Achilles tendon, elbows, and knees may develop [6].

Dyslipidemia treatment plays an important role in the primary and secondary prevention of cardiovascular disease. The main goal of therapy for dyslipidemia is to prevent cardiovascular disease and its complications. The mainstay of treating dyslipidemia is the assessment of absolute risk factors, treating modifiable risk factors, and optimizing lifestyle, especially diet, and exercise. Handling or treating dyslipidemia can be done with non-pharmacological and pharmacological therapy. Non-pharmacological therapy includes lifestyle changes, including physical activity, medical, nutritional therapy, weight loss, and smoking cessation. While pharmacological treatment by giving anti-lipid drugs [6].

In addition to lifestyle changes and drug use, treating dyslipidemia can also use natural ingredients to reduce blood cholesterol levels and dyslipidemia, including stevia leaf (*Stevia Rebaudiana*). Stevia is one of the plants that can produce the sucrose the body needs. The sucrose produced by stevia leaves is low caloric and does not cause side effects on the body's health. Sucrose from stevia leaves is an alternative for someone who needs a strict diet for health or to reduce the body's sugar content. Reducing sugar from stevia leaves has non-carcinogenic properties, so it is safe and can be consumed long-term [7].

Stevia Rebaudiana is a natural sweetener with a sweetness level of 200-300 times sweeter than cane sugar. *Steviosides* in stevia leaves contain low to zero calories, so they are safe for people with diabetes or diet-conscious consumers. *Steviosides* are safe for consumption by the general public because they do not have teratogenic, mutagenic, or carcinogenic effects. Stevia leaves have antioxidant activity, so the immune system prevents disease-causing free radicals. The main constituents in stevia leaves are diterpenoid glycosides, which have the potential as sweeteners *stevioside, rebaudioside*, and *dulcoside* [8].

The largest phytochemical content in stevia leaves is glycosides, steroids, and tannins. The leaves of the stevia plant contain a mixture of diterpenes, triterpenes, tannins, stigmasterols, volatile oils, and eight sweet diterpene glycosides. Eight diterpene glycosides cause the leaves to taste sweet, namely *stevioside, steviolbioside, rebaudioside* A–E, and *dulcoside* A. Stevia also contains protein, carbohydrates, phosphorus, iron, calcium, potassium, sodium, flavonoids, zinc, vitamin C and vitamin A [9]. Other ingredients of Stevia leaf extract are flavonoids, alkaloids, chlorophyll, xanthophylls, hydroxycinnamic acid (*caffeine, chlorogenic*), water-soluble neutral oligosaccharides, free sugars, amino acids, fats, and essential oils. Critical bioactive components, including alkaloids, flavonoids, tannins, and phenolics with antioxidants, antimicrobials, and anti-inflammatories, cause the medicinal benefits of Stevia leaf extract [10].

Stevia leaves also contain an active compound, *stevioside* (approximately 4-15%). *Stevioside* compounds have the potential as an effective treatment for metabolic syndrome diseases such as hyperglycemia, hypertension, and dyslipidemia. *Steviosides* are included in the glycosides. Stevia leaves are also reported as natural antioxidants, which can bind free electron radicals and superoxides to inhibit oxidation, especially the oxidation of lipid compounds effectively [11]. In addition to its high level of sweetness, stevia leaves also contain compounds that are antioxidants, namely total phenolic compounds of 25.18 mg/g leaves (in dry weight), the flavonoid compound group of 21.73 mg/g (in dry weight), and the capacity of antioxidants by 39.86%. Several studies have shown that

stevia has several functional properties, such as antihyperglycemic and antidiabetic, which can significantly reduce blood glucose levels [12].

In Sulastri's study (2020), stevia leaf extract given to rats induced by streptozotocin-induced antihyperglycemic activity of stevia leaf extract powder which was observed after oral administration at a dose of 4 g/kg BW for three weeks can reduce blood glucose levels by 63%. Toxicity studies in mice with 70% ethanol extract had no adverse effect. The lethal dose (LD_{50}) can be greater than 5 g/kg body weight in rats. In addition, the 96% ethanol extract of stevia leaves can also inhibit the α-glucosidase enzyme involved in diabetes with a percent inhibition of 17.847 (IC_{50} 50.19 µg/ml) [13] .

Moreover, the research by Untari & Pramukantoro (2020) shows that the ethanol extract of *Stevia rebaudiana* leaves has anti-hypercholesterolemia activity, as seen from the decrease in total blood cholesterol levels of rats that were given a high-fat diet. As well as the best dose of *Stevia rebaudiana* leaf ethanol extract, which reduces total blood cholesterol levels, is best at 30 mg/200 g BW [11]. Based on the previous studies described above, it is an important reason for researchers to examine more deeply using test animals of female white rats (*Rattus Norvegicus*) vistas strains through experimental laboratory research on administering stevia leaf ethanol extract (*Stevia rebaudiana*) orally to prevent the occurrence of dyslipidemia in female Wistar rats (*Rattus norvegicus*) that have been given a high-fat diet.

2. METHOD

This research is a pure laboratory experimental study with a research design using a post-test with a control group design. The posttest-only control group design is a research design in which there are at least two groups, one of which does not receive a treatment or intervention, and data are collected on the outcome measure after the treatment or intervention [14]. The research analyzes the effect of oral stevia (*Stevia rebaudiana*) leaf ethanol extract in preventing dyslipidemia in female Wistar rats (*Rattus norvegicus*) given a high-fat diet. Sampling is the selection of a subset of the population of interest in a research study [15]. The samples in this study were adult female Wistar rats (Rattus norvegicus) weighing 160-300 grams and 2-3 months old. The rat samples to be used in the study consisted of two groups: the control group, which was given a high-fat diet and distilled water, and the treatment group, which was given a high-fat diet and stevia leaf extract. The number of samples used was 20 rats.

Researchers can directly manipulate experimental independent variables. Researchers cannot manipulate subject variables, but they can be used to group research subjects categorically [16] . Research variables are everything that will become the object of research observation [17]. The variables in this study consist of independent variables and dependent variables. The independent variable in this study was the ethanol extract of stevia (*Stevia Rebaudiana*) leaves. In comparison, the dependent variable is dyslipidemia, including total cholesterol, LDL cholesterol, triglycerides, and HDL cholesterol levels.

Tools And Materials

The tools used in this study included: digital scales, sterile gloves, cannula, blenders, ovens, maceration vessels, jars, stir bars, rotary evaporators, vials, porcelain cups, Petri dishes, Pasteur pipettes, gastric sonde, masks, EDTA, label paper, rat cages, feed containers, stationery, markers, and rulers. In contrast, the materials used in this study included: stevia leaves (*Stevia Rebaudiana*), 96% ethanol, distilled water, foods high in fat (quail egg yolks and duck egg yolks), female white rats, rat feed, and drink.

Test Animal Acclimatization

Acclimatization is an adjustment (self) to a new climate, environment, conditions, or atmosphere. Before being given treatment, all white rats were first adapted to the Animal House Laboratory for one week. The white rat is adapted to a new place of residence, environment, food, and drink. Rats were given food and drink according to their needs (*ad libitum*).

Preparation of Stevia Leaf Ethanol Extract

Fresh stevia leaves are cleaned of dirt and washed with running water until clean and drained. Furthermore, the stevia leaves are cut into pieces and then dried using an oven at a temperature of 50-60 Celsius. After drying, the stevia leaves are blended to make Simplicia (powder). Stevia leaf extract

was prepared using the maceration method by soaking the stevia leaf Simplicia in a separate maceration vessel and then adding 96% ethanol until the simplicial was completely submerged. The maceration vessel was tightly closed and allowed to stand for ± 5 days while stirring once a day. The results were filtered and repeated three times, then accommodated in a bottle to be further concentrated using a rotary evaporator until a viscous ethanol extract of stevia leaves was obtained.

Treatment Procedure

All white rats acclimatized (adjusted) for seven days (one week) were randomly divided into two groups. Each group consisted of 10 rats, and each rat was labeled on its tail according to its group using a waterproof marker. Then given the following treatment:

a. The control group was given a high-fat diet (feed mixed with quail egg yolks and duck egg yolks) and distilled water every day for 28 days.
b. The treatment group was given a high-fat diet (feed mixed with quail egg yolk and duck egg yolk) and stevia leaf extract at 40 mg/200 g BW every day for 28 days.

A high-fat diet is given for 28 days to ensure that blood cholesterol increases compared to normal. After being given treatment for 28 days, total cholesterol, LDL cholesterol, triglyceride, and HDL cholesterol levels were examined as a posttest. Rat blood was collected by all rats being given an anesthetic injection with ketamine and xylazine to reduce the rat's consciousness. Blood was taken after being fasted for 12 hours (still given a drink) through the tail vein with the medial canthus sinus orbitalis using a 1.2 ml microcapillary tube.

A blood sample is put into EDTA. Blood examination after treatment, centrifugation was carried out at 3500 rpm for 5 minutes on blood samples, and blood serum ± 0.6 ml was obtained. Furthermore, the GOD PAP method examined the total cholesterol, LDL cholesterol, triglyceride, and HDL cholesterol levels.

Data analysis

The research data were analyzed using the SPSS 26 for Windows program. The data normality test used the Shapiro-Wilk test ($p>0.05$). Homogeneity test with Levene's test ($p> 0.05$). To test the effect of giving stevia leaf ethanol extract (*Stevia Rebaudiana*), it was analyzed using the t-test or the Independent Sample T-Test approach ($p <0.05$) [18].

3. RESULTS AND DISCUSSION

This study was an experimental study using a post-test-only control group design using 20 female Wistar rats (Rattus norvegicus) aged 2-3 months, weighing 160-300 grams, given a high cholesterol diet, divided into two each group consisted of 10 rats, namely the control group (K) which was given a high cholesterol diet and placebo in the form of distilled water and the treatment group (P) which was given a high cholesterol diet and *Stevia Rebaudiana* extract (dose 40 mg/200 g body weight of rats). The results of this study were then analyzed and presented using descriptive analysis, data normality, data homogeneity, and comparability test.

Table 1. Phytochemical Test Results of Stevia Leaf Extract

Compound	Procedure	Lab Test	Result
Tannins	5 ml sample + ethanol + iron (III) chloride	Blackish green color	+
Saponins	0.05-gram sample + hot distilled water and shake vigorously	Foam formed	+
Alkaloids	Sample + 5 ml of distilled water + 2 ml of HCL until sour, filtered. Filtrate + 1 ml of Dragendorff reagent	Orange precipitate	+
Triterpenoids	Sampel + Liebermann Bourchard	Red-orange color	+
Flavonoids	Sample + 5 ml of distilled water + 0.1 mg powder + 1 ml of alcohol solution + concentrated hydrochloric acid + amyl alcohol	Red-orange color on the amyl alcohol layer	+

Stevia leaves contain antioxidants with different biochemical roles, ascorbic acid, and phenolic compounds, including flavonoids and tannins. Antioxidant activity and prevention of oxidative DNA damage were reported in vitro by methanol and ethyl acetate extracts of stevia leaves. Methods based

on stable radical scavenging 1,1 -diphenyl-2-picrylhydrazyl (DPPH) have been widely used to predict plant antioxidant activity. According to Jahan, DPPH activity, total phenol, and total flavonoids from stevia leaf extract at different concentrations and it was found that stevia leaf extract in 80% ethanol had significant antioxidant activity so that it had the potential as a natural antioxidant [19].

Figure 1. Compound Content in Stevia Leaf Extract

The antihyperlipidemic action of stevia leaf extract is thought to be related to its constituents, including flavonoids, saponins, tannins, triterpenes, and alkaloids. Flavonoids have been known to have a wide range of biological activities, including hypolipidemic activity from their antioxidant activity. The lipid-lowering capacity of this plant is thought to originate from its constituents which work to inhibit the hydroxyl-methyl-glutaryl-CoA reductase enzyme, which plays a role in de novo cholesterol biosynthesis.

The results of the descriptive analysis of total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol levels after 28 days of treatment (post-test) in each group are presented.

Table 2. Results of Lipid Profile Descriptive Analysis.

Variable	Subject Group	n	Mean (mg/dl)	SD	Min (mg/dl)	Max (mg/dl)
Total Cholesterol	Control (K)	10	219.87	9.25	204.56	231.18
	Treatment (P)	10	114.09	5.91	104.87	123.14
Triglycerides	Control (K)	10	150.76	8.48	135.92	162.81
	Treatment (P)	10	99.93	4.93	91.73	110.33
HDL Cholesterol	Control (K)	10	26,91	2,54	23.82	30.35
	Treatment (P)	10	38,77	1,70	36.12	42.11
LDL Cholesterol	Control (K)	10	68.71	2.07	64.46	71.77
	Treatment (P)	10	40.32	2.63	35.06	43.79

Table 3. Data Normality Test Results

Test of Normally							
Variable	**Group**	**Kolmogorov-Smirnov**^{a}			**Shapiro-Wilk**		
		Statistic	**Df**	**Sig.**	**Statistic**	**Df**	**Sig.**
Total Cholesterol	Control	.222	10	.179	.890	10	.171
	Treatment	.176	10	.200*	.961	10	.793
Triglycerides	Control	.213	10	.200*	.942	10	.576
	Treatment	.159	10	.200*	.957	10	.754
HDL Cholesterol	Control	.197	10	.200*	.881	10	.133
	Treatment	.271	10	.037	.926	10	.411
LDL Cholesterol	Control	.138	10	.200*	.968	10	.876
	Treatment	.244	10	.093	.921	10	.368

*. This is a lower bound of the truly significant

a. Lilliefors Significant Correction

The levels of total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol after 28 days of treatment in each group were tested for normality with the Shapiro-Wilk test (because the sample was less than 50). The results show that the data is normally distributed (p>0.05), as in Table 3. The levels of total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol after 28 days of treatment in each group were tested for homogeneity using Levene's Test. The results showed that the

variance of the research data for the total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol levels was homogeneous (p>0.05). The data is presented in Table 4.

Table 4. Data Homogeneity Test Results

Test of Homogeneity of Variances					
	Base on	**Levene Statistic**	**df1**	**df2**	**Sig,**
Total Cholesterol	Mean	.676	1	18	.422
	Median	.675	1	18	.422
Triglycerides	Mean	1.771	1	18	.200
	Median	1.760	1	18	.201
HDL Cholesterol	Mean	2.191	1	18	.156
	Median	2.290	1	18	.148
LDL Cholesterol	Mean	.375	1	18	.548
	Median	.120	1	18	.733

Table 5. T-test results

Independent Samples Test								
Independent Samples Test			**t-test for Equality of Means**					
Variances	F	Sig.	t	df	Mean Diff.	Std. Error Diff.	95% Confidence Interval of the Difference	
							Low	Up
Total Cholesterol	.676	.422	30.464	18	105.777	3.472	98.482	113.07
Triglycerides	1.771	.200	16.380	18	50.834	3.103	44.313	57.35
HDL Cholesterol	2.191	.156	-12.290	18	-11.863	.9652	-13.89	-9.835
LDL Cholesterol	.375	.548	26.805	18	28.394	1.059	26.168	30.619

Sig. (2-tailed) = 0.0000

Table 6. Mean Value of Variables between Groups After Treatment

Variable	**Group**	**n**	**Avg (mg/dl)**	**SD**	**t**	**p**
Total Cholesterol	Control	10	219.87	9.25	30.464	0.000
	Treatment	10	114.09	5.91		
Triglycerides	Control	10	150.76	8.48	16.380	0.000
	Treatment	10	99.93	4.93		
HDL Cholesterol	Control	10	26.91	2.54	-12.290	0.000
	Treatment	10	38.77	1.70		
LDL Cholesterol	Control	10	68.71	2.07	26.805	0.000
	Treatment	10	40.32	2.63		

Table 6 shows the results of the comparability analysis of comparing the mean total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol levels between groups after 28 days of treatment. The results of the significance analysis were tested using an independent t-test, and all tests showed very significant (p<0.01).

Discussion

The mean total cholesterol level after 28 days of treatment for the control group was 219.87 ± 9.25 mg/dl, and the treatment group was 114.09 ± 5.91 mg/dl. Analysis of significance with the independent t-test showed that the value of t = 30,464 and the value of p = 0.000. The mean triglyceride levels in the control group after 28 days of treatment were 150.76 ± 8.48 mg/dl; in the treatment group, it was 99.93 ± 4.93 mg/dl. Analysis of significance with the independent t-test showed that the value of t = 16,380 and the value of p = 0.000. The mean HDL cholesterol level in the control group after 28 days of treatment was 26.91 ± 2.54 mg/dl and 38.77 ± 1.70 mg/dl in the treatment group. Analysis of significance with the independent t-test showed that the value of t = -12,290 and the value of p = 0.000. The mean LDL cholesterol level in the control group was 68.71±2.07 mg/dl and 40.32±2.63 mg/dl in the treatment group. Analysis of significance by independent t-test showed that the value of t = 17.041 and the value of p = 0.000. This means the

mean total cholesterol, triglyceride, HDL, and LDL cholesterol levels differed significantly after 28 days of treatment between the control and treatment groups (p<001).

Based on the results of this study, the treatment group showed better lipid profile results than the control group. Total cholesterol levels between the two groups showed a very significant difference; the treatment group showed results 48.11% (p <0.01) lower than the control group. LDL cholesterol levels between the two groups showed a very significant difference; namely, the treatment group showed results 41.32% (p <0.01) lower than the control group. It was also found that the triglyceride level was similar; namely, the treatment group showed results 33.72% (p<0.01) lower than the control group. Likewise, the HDL cholesterol level between the two groups showed a significant difference; the treatment group showed 44.07% (p <0.01) higher than the control group.

In the control group, the average total cholesterol was 219.87 ± 9.25 mg/dl, which means that the mice in the control group suffered from dyslipidemia (total cholesterol > 200 mg/dL). In contrast, the rats in the treatment group had an average total cholesterol within normal limits (114.09 ± 5.91 mg/dL). So, it can be concluded that the ethanol extract of Stevia Rebaudiana leaves prevented dyslipidemia in female Wistar rats (Rattus norvegicus) given a high-cholesterol diet.

The results of this analysis cannot show the composition of the extract as a whole due to the limitations of laboratory reference materials for material research. The anti-hyperlipidemic activity of stevia rebaudiana is thought to be due to its constituents, including glycosides and various antioxidants. The ingredients in stevia rebaudiana leave that can lower cholesterol levels and prevent dyslipidemia are stevioside, isosteviol, flavonoids, saponins, tannins, and phenolic compounds. Flavonoids are known to have broad biological activities, including hypolipidemic action from their antioxidant work, thought to originate from their constituents that work to inhibit the enzyme hydroxyl-methyl-glutaryl-CoA reductase which plays a role in de novo cholesterol biosynthesis. Inhibition of this enzyme will lead to inhibiting cholesterol synthesis and ultimately decreasing cholesterol production. Flavonoids can increase the body's resistance to oxidation of LDL cholesterol, which is believed to inhibit atherosclerosis.

Flavonoids also increase the ratio of HDL cholesterol/LDL cholesterol, thereby accelerating the removal of cholesterol from peripheral tissues to the liver for catabolism and excretion. Flavonoids prevented the inhibition of adenosine monophosphate-activated protein kinase (AMPK) and even doubled the phosphorylation of AMPK and ACC in diabetic rat livers; the same thing was found in human liver cells (HepG2 Hepatocytes) exposed to high glucose. This will cause a decrease in acetyl-CoA carboxylase (ACC) and hepatic lipids, and finally, AMPK activation will reduce lipid synthesis and increase fatty acid oxidation [20]; [21]. This study shows that the ethanol extract of Stevia Rebaudiana has beneficial properties in controlling hyperlipidemia, which is thought to be due to the antioxidant and glycoside effects of the ethanol extract of Stevia Rebaudiana, which overall contribute to the development of improving the lipid profile of rats given a high cholesterol diet.

However, the results of this study have yet to be able to describe the effect on humans and the exact mechanism of protection of the Stevia Rebaudiana ethanol extract against blood lipid profiles, so further research is needed. This study also could not show whether one of each of the active components contained in the ethanol extract of Stevia Rebaudiana leaves played a major role in the repair of the lipid profile that occurred. Thus, it is hoped that the ethanol extract of *Stevia Rebaudiana* can be used later as a preventive agent. Furthermore, the active components can be extracted for later use as adjuvant therapy against dyslipidemia.

4. CONCLUSION

The results of this study were analysis of blood lipid profile data in both groups, namely the control group and the treatment group, which showed that the data were normally distributed (normality test with Shapiro Wilk) and homogeneous (homogeneity test with Levene's test) with a p > 0.05. The mean comparability test of lipid profile levels between the control group and the treatment group was carried out by the independent t-test.

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