PROCEEDING SRIWIJAYA INTERNATIONAL CONFERENCE OF PUBLIC HEALTH 4th
"Health and Nutrition Innovation for Better Life Style in Digital Era"
September 21st, 2023 | The Zuri Hotel, Palembang

EFFECTIVENESS OF Moringa (Moringa oleifera) LEAVES ETHANOL EXTRACT ON REDUCING CHOLESTEROL LEVELS IN MALE WHITE RATS (Rattus norvegicus) INDUCED WITH QUAIL EGG YOLKS

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ABSTRACT

Cholesterol is a fatty substance contained in the body. Excessive cholesterol will accumulate in the walls of blood vessels, which can cause atherosclerosis (narrowing of the blood vessels) as a driving factor for coronary heart disease. The aim of this research was to determine the effectiveness of administering Moringa leaf extract on reducing cholesterol from Moringa oleifera leaf extract in male white rats Rattus norvegicus and to determine the concentration of Moringa leaf extract on the effect of reducing cholesterol in male white rats (Rattus norvegicus). This research is an experimental study which consists of several stages including: extract characterization and testing the effect of reducing cholesterol. The data obtained was analyzed by ANOVA (analysis of variance) and continued with the post hoc test. The samples in this study were male white rats, Rattus norvegicus, aged 8-12 weeks, weighing 200-300 gr. The research sample consisted of 30 male Rattus norvegicus rats. The results of the analysis found that the mean cholesterol of the rats before treatment was highest in the positive group (Simvastatin) of 155.8 mg/dl while the lowest was in the group of doses of 150 mh/kg BB, which was 132.2 mg/dl. The average cholesterol of rats after treatment, the highest was in the negative control group (CMC-Na) of 217.8 mg/dl while the lowest was at a dose level of 450 mg/kg BW which was 116.8 mg/dl.

Key Words : Cholesterol, Moringa Leaf Extract, male white rats

Introduction

Hypercholesterolemia is a condition where the amount of cholesterol in the blood exceeds normal limits. Cholesterol is an important element in the body that is needed to regulate chemical processes in the body, but high amounts of cholesterol cause atherosclerosis which ultimately results in coronary heart disease [1].

Cholesterol or fat levels in the blood can come from the food consumed. Increased cholesterol levels are caused by consuming foods that contain excessive fat. Cholesterol sufferers are usually fat people, but it is possible that thin people can also suffer from high cholesterol. Modern foods contain low fiber and high fat. High cholesterol can also be caused by hereditary factors, lack of exercise [2].

According to the World Health Organization (WHO), it is estimated that 17.9 million people die from cardiovascular disease, 85% of which are caused by heart attacks and strokes [3].

According [4] , The prevalence of heart disease has increased from the 2018 Riskesdas results from 0.5% to 1.5%.

Indonesia ranks first in coronary heart disease. According to the Sample Registration System survey, the death rate for coronary heart disease is 12.9% of all deaths [5] [6]. The incidence of coronary heart disease in South Sumatra is 1.2% or around 32,126 people [7]

Indonesia is number two with the most traditional plants after Berazilia. The community uses the existing plants for various concoctions from leaves, roots, fruit, wood and tubers, to cure various diseases. Traditional concoctions are often known as herbal medicine [8]

Moringa (Moringa oleifera Lamk.) is a type of plant that contains active compounds such as flavonoids in the form of quercetin and vitamin C (Lin et al., 2018). The antioxidants in Moringa leaves are able to reduce cholesterol levels through the process of increasing the activation of the enzyme cholesterol 7α-hydroxylase (CYP7A1), inhibiting the production of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA), and reducing levels of (Reactive Oxygen Species) ROS [10]

Based on the above background, research was conducted entitled "Effectiveness of Moringa oleifera leaf extract on reducing blood cholesterol levels in male white mice" to determine the reduction in blood cholesterol levels in mice by using Moringa leaf extract on cholesterol levels in mice. Male whites are induced with egg yolks.

Methods

This research is a pre and posttest laboratory experimental design with control group design. This study used 5 treatments, each treatment using male white rats (Rattus norvegicus) with a negative control design using CMC, positive control using Simvastatin, and treated with anti-inflammatory extract doses of 150 mg/kgBB, 300mg/kgBB, and 450 mg/kgBB. The research will be carried out in April 2023. The research location is in the STIK Siti Khadijah Pharmacology laboratory. The research data was analyzed using the SPSS program. Data analysis includes analysis of homogeneity of variance between groups with Levene's trsst. Then the dose suitability will be tested using the Post hoc Test.

Results

Results of Testing the Effect of Reducing Cholesterol Levels

Mice that had been given quail egg yolks for 7 days had their cholesterol checked using Easy Touch brand test strips. Cholesterol examination results data can be seen in the following table:

Table.1 Cholesterol levels before and after Moringa leaf extract treatment

Group Treatment	H0	H1	H3	H7	Selisih H1- H7
	160	222	155	109	113
Simvastatiin	147	246	179	126	120
	150	217	152	103	114
	164	235	164	126	109
	158	230	168	121	109
Average	779 : 5 = **155,8**	1.150 : 5 = **230**	818 : 5 = **163,6**	585 : 5 = **117**	230-117 : 230 X 100% **=49,13**
	140	214	210	210	4
	137	209	203	201	8
CMC Na	158	219	215	212	7
0,5%	138	246	241	238	8
	140	234	230	228	6
Average	713 : 5 = **142,6**	1.122 : 5 = **224,4**	1.099 : 5 = **219,8**	1.087 : 5 = **217,8**	224,4-217,8 : 224,4 X 100% **=2,9 %**
	148	206	184	147	59
Dosis	139	268	341	192	76
150 Mg/KgBB	124	228	206	164	64
	132	208	188	150	58
	118	246	221	176	70
Average	661 : 5 = **132,2**	1.150 : 5 = **230**	1.140 : 5 = **228**	829 : 5 = **165,8**	230-165,8 : 230 X 100 % = **27,9%**
	170	280	267	198	82
Dosis	150	218	186	148	70
300 Mg/KgBB	137	209	178	133	76
	114	218	185	138	80
	171	294	250	193	101
Average	742 : 5 = **148,4**	1.219 : 5 = **243,8**	1.066 : 5 = **213,2**	810 : 5 = **162**	243,8-162 : 243,8 X 100% **= 33,5 %**
	142	250	178	145	105
Dosis	150	225	156	110	115
450 Mg/KgBB	147	220	152	113	107
	150	218	163	114	104
	138	211	151	101	101
Average	727 : 5 = **145,4**	1.124 : 5 = **224,8**	800 : 5 = **160**	583 : 5 = **116,8**	224,8 – 116,8 : 224,8 X 100 % **=48,0 %**

Anova Test Analysis

The One Way Anova test which was carried out aimed to determine whether there were differences between the experimental groups before and after treatment. If the pvalue is ≤ 0.05 then there is a difference, if the pvalue is > 0.05 then there is no difference. The results obtained are as follows:

Table 2. One Way Anova

Time to observe	Sig.
H0	0,000
H1	0,000
H3	0,000
H7	0,000

POST HOC TEST

The post hoc test is used to compare the results of several treatment groups. If the ρ value is ≤0.05 then there is a difference, if the ρ value is ≥0.05 then there is no difference. The results of the post hoc test are as follows:

Table 3. Bonferroni Post Hoc

Treatment group	Sig. H7
Dosis 150 mg/kg BB	0,000
Dosis 300 mg/Kg BB	0,000
Dosis 450 mg/kg BB	0,199
Simvastatin	0,201

Discussion

Mean cholesterol before treatment

Table 1 shows that on day 0, the highest mean score for simvastatin cholesterol levels was obtained 155,8 while the lowest mean was found at the dose level 150 mg/kg BB that is the amount 132.3. On day 1, the highest mean score was obtained for dose cholesterol levels 300mg/kgBB with the amount 243, while the lowest mean was found at the dose level CMC-Na, y sebesar 224,4. Pada hari ke-3 diperoleh rerata skor tertinggi pada kadar kolesterol dosis 150mg/kgBB with the amount 228,0 while the lowest mean was at dose level 450 mg/kg BB, with the amount 160.0. On the 7th day, the highest average cholesterol levels were obtained CMC-Na with the amount 219,8 while the lowest mean was found in dose 450 mg/kg BB, with the amount 116,8, While administering the dose 300 mg/kg BB is in normal condition.

Research conducted by [12] Regarding the effectiveness test of Moringa oleifera leaf extract on reducing blood cholesterol levels in male white mice, the results of all concentrations showed the effect of reducing cholesterol levels significantly based on comparison with the negative control. The best effect is given by a concentration of 350 mg/ml. The results of the cholesterol test after treatment showed that on the 3rd day the highest average score was obtained for the dose cholesterol level 150mg/kgBB with the amount 228,0 while the lowest is the dose level 450 mg/kgBB with the amount 160,0. On the 7th day, the highest average score for cholesterol levels was obtained CMC-Na with the amount 217,8 while the lowest is the dose level 450 mg/kg BB with the amount 116.8.

One of the traditional medicines used to lower blood cholesterol levels is Moringa leaves *(Moringa oleifera).* Various studies conducted show that Moringa leaves *(Moringa oleifera)* It has been used by Indians as a hypocholesterolaemic agent in obese patients. They tested the crude extract of Moringa oleifera leaves and showed that it had hypocholesterolaemic activity[13].

Research conducted by [16] regarding the administration of Moringa oleifera leaf extract on cholesterol levels in male white rats (Rattus norvegicus) which was induced by alloxan and it was found that there was a significant effect of giving Moringa leaf extract on cholesterol levels in mice. ($P<0.05$). Giving Moringa leaf extract at doses 150 mg/kgBB can reduce cholesterol levels in mice significantly.

There are research results and supporting theories and research, so the author assumes that giving quail egg yolk induction food can actually increase the cholesterol levels of male white rats, Rattus norvegicus. Cholesterol is declared high if the cholesterol level is above 200 mg/dl. So all male white rats (Rattus norvegicus) can be used as test animals in testing to reduce cholesterol levels.

Average Cholesterol in Rats After Treatment

The results of the cholesterol test after treatment showed that on the 3rd day the highest average score was obtained for the dose cholesterol level 150mg/kgBB with the amount 228,0 while the lowest is the dose level 450 mg/kgBB with the amount 160,0. On the 7th day, the highest average score for cholesterol levels was obtained CMC-Na with the amount 217,8 while the lowest is the dose level 450 mg/kg BB with the amount 116.8.

Moringa leaves (Moringa oleifera) are a traditional medicine used to lower cholesterol levels in the blood. Various studies conducted show that Moringa leaves (Moringa oleifera) have been used by Indians as a hypocholesterolaemic agent in obese patients. They tested the crude extract of Moringa oleifera leaves and showed that it had hypocholesterolaemic activity[13].

Research conducted by [14], regarding the administration of Moringa oleifera leaf extract on cholesterol levels in male white rats (Rattus norvegicus) which was induced by alloxan and it was found that there was a significant effect of giving Moringa leaf extract on cholesterol levels in mice. ($P<0.05$). Giving Moringa leaf extract at doses 150 mg/kgBB can reduce cholesterol levels in mice significantly.

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Analisis One Way Anova

The results of the analysis of the effectiveness test for reducing cholesterol from the ethanol extract of Moringa oleifera leaves are ρ volume = 0.00 which is compared with the value α = 0.199, so the hypothesis (Ho) is rejected, the hypothesis (Ha) is accepted. This shows that there is a difference in the average cholesterol in the 5 test groups, which means Moringa leaf extract has been proven to reduce cholesterol levels with a difference in the average of the 3rd test of 27,9%, 33,5%,48,0%.

Moringa leaves contain active compounds, namely alkaloids and flavonoids. These two compounds are effective in reducing pain caused by rheumatic diseases. Alkaloid and flavonoid compounds can also reduce cholesterol levels in the blood. Moringa leaves as a traditional medicine are not yet optimally used by the community. The active compounds contained in Moringa leaves are alkaloids, flavonoids, tannins, saponins. These four compounds are effective in reducing pain due to rheumatism, are antimicrobial, maintain healthy production and reduce cholesterol levels [15].

Research conducted by [16], regarding the effect of giving moringa leaf extract (Moringa oleifera) on reducing cholesterol in white mice *(Rattus norvegicus strain wistar),* one-way test results were obtained ANOVA showed that there were differences between groups of mice (ρ<0.05). The post hoc test results show the dose 600mg/kgBB the most significant in reducing cholesterol. Moringa leaf extract (Moringa oleifera) has an effect of 78,5% on cholesterol in the blood, due to the presence of flavonoids.

With the research results and supporting theories and research, the author assumes that there is a decrease in cholesterol levels in male white rats (Ratus norgevicus), because Moringa leaf extract combines with bile acids produced by the liver, to break down fat in the small intestine. Most of the bile acids will be excreted as waste material and will not be absorbed again. Cholesterol is the basic ingredient in the formation of bile acids which are used to replace lost bile acids. Cholesterol is removed from the blood circulation, so that in this process cholesterol levels in the blood decrease. Moringa leaf extract in the intestine binds fatty acids, bile salts and cholesterol from food, thereby inhibiting the absorption of these substances and carrying them out with the feces.

Acknowledgement

The author would like to thank the management and colleagues at STIK Siti Khadijah Palembang who have helped and supported during this research.

Funding

Our have no funding for the reasearch

Conflict of Interest

The authors declare that they have no conflict of interest.

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