Pak. j. life soc. Sci. (2024), 22(2): 6621-6626 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences

www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2024-22.2.00500

RESEARCH ARTICLE

Effectiveness of Giving Moringa Oleifera and Folic Acid on Blood Cholesterol Levels in Rattus Novegicus

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ARTICLE INFO

Received: Jul 13, 2024

Accepted: Sep 24, 2024

Keywords

Hypercholesterolemia

Moringa Oleifera Capsules

Folic Acid

Female Rats

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ABSTRACT

Hypercholesterolemia is a disorder of lipid metabolism that is characterised by an increase or decrease in the lipid fraction in plasma which is marked by an increase in cholesterol in the blood. This study aims to measure the effectiveness of moringa (Moringa oleifera) and folic acid on blood cholesterol levels in female rats. True Experimental research method using pre and post-test control group design, samples used female rats, aged 8-12 weeks, with a body weight of 150-250 grams, the condition of the rats is not physically deformed which is intervened for 35 days in 5 groups of rats (n = 15), namely the positive group (healthy group), negative group (high-fat feeding), Moringa Oleifera capsule group (MO), Folic Acid group (AF) and Moringa Oleifera and Folic Acid combination group (MO + AF). Total blood cholesterol levels of rats were measured using a hematology analyzer. Statistical analysis was performed using a paired t-test, one-way ANOVA, and Krusskall-Wallis. The results showed a significant decrease in total blood cholesterol levels in the high-fat feeding group (P = 0.037). The decrease in total blood cholesterol levels in the moringa oleifera and folic acid combination treatment (P = 0.009) was significantly more effective, this is due to the content of moringa leaf powder and amino acids which are vitamins and minerals containing amino acids that work in metabolism in controlling blood lipids, preventing plaque formation in arteries, and lowering cholesterol levels. The combination of moringa leaf capsules and amino acids is more effective in improving lipid profile compared to moringa oleifera and folic acid alone.

INTRODUCTION

Cardiovascular disease caused by hypercholesterolemia increases by 30% with complications in *cardiovascular* disease and 50% is found in women [1]. Blockage of blood vessels with increased cholesterol levels in the circulatory system, causing high systolic blood pressure and high levels of *Low-density Lipoprotein* (LDL) cholesterol or fat will cause *foam cell* formation [2]. Hypercholesterolemia is a complex condition with multiple causes, including lifestyle and genetic aspects. It is also a risk factor for cardiovascular disease, which causes 172 million deaths/year [3]. Lifestyle and consumption of unhealthy and high-fat foods and lack of activity cause cholesterol to be in excessive amounts in the blood [4]. Hypercholesterolemia during pregnancy is caused by changes in sex steroid hormones, and liver and adipose metabolism. Increased maternal estrogen concentrations in pregnancy cause an increase in total cholesterol, LDL, and triglycerides which are atherogenic, small, and dense. Metabolism changes due to decreased lipoprotein lipase with

increased placental activity and activity in adipose tissue, changes in lipid metabolism in pregnancy are maternal fat reserves from early pregnancy to the second trimester of pregnancy [5].

Moringa is one of the plants that has potential as a medicinal plant, containing more than 90 and 539 nutrient compounds in the form of essential vitamins, minerals, amino acids, anti-aging, and anti-inflammatory [6]. Moringa leaves contain amino acid elements (essential) such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. In addition, moringa leaves contain protein, fat, beta carotene (Vit. A) thiamin (B1), riboflavin (B2), niacin (B3), vitamin C, calcium, calories, carbohydrates, copper, fiber, iron, magnesium, and phosphorus [7]. Moringa leaf powder contains 10 times the vitamin A found in carrots, 25 times the iron in spinach, 17 times the calcium in milk, 9 times the protein in yogurt, and 15 times the potassium in bananas (M (Maryani I and Suryadarma, I.G.P 2019) Moringa Oleifera extract is a choice of herbal or alternative medicine that can be used in the treatment of metabolic syndrome diseases [8]. It is expected that moringa leaf extract can stabilize blood cholesterol levels and increase serum *High-Density Lipoprotein (*HDL) cholesterol levels [9].

Liver and dark green vegetables such as spinach, mustard greens, and broccoli are some of the sources of folic acid which is rich in vitamins and minerals, the recommended amount of folic acid consumption is 400 mg/day [10]. According to WHO data, the incidence of physical congenital defects in the United States is 1.32 per 1000 births, due to folic acid deficiency, 75% of which are in developing countries, in Indonesia, around 24-60% of pregnant women do not know the amount of folic acid consumed in their daily diet [11]. Disability is one of the main impacts caused by stroke, stroke is the sudden death of brain cells due to hypoxia caused by blockage or rupture of arteries in the brain [12].

Folic acid supplementation is an effective agent for improving endothelial function and is a prognostic factor for cardiovascular disease. Folic acid has an important role in the process of homocysteine metabolism, Decreased homocysteine levels are associated with improved neurological function. Folic acid as a substrate donor of methyl groups involved in the metabolic process of homocysteine can reduce total homocysteine levels by 20-25%, this is associated with a 19% reduction in the risk of stroke [13]. Poor nutritional intake during pregnancy and inadequate iron and folic acid supply in the body during pregnancy preparation can increase the risk of low birth weight (LBW) [14]. Folic acid can also reduce the risk of preeclampsia which functions as plasma homocysteine and one of the pathogenic agents to improve endothelial cell function in severe preeclampsia [15].

METHODS

Research design: This research is *True Experimental* using the *pre and post-test control group design* method which was carried out in the Biopharmaceutical Laboratory, Faculty of Pharmacy, Hasanuddin University, Makassar from May to July 2024.

Population and research samples: this study uses healthy female rat test animals (active movement, hair/fur is not dull, falling out or bald), no physical defects, 6-8 weeks of age, body weight 150-250 grams. The sample group was divided into 5 groups Group 1 Positive control; female rats were given standard feed and CMC 1% orally 150-200 ml/kg BW, Group 2 Negative control; female rats were given High-fat Feeding (HFD) 200 grams/kg BW / day, Treatment group 1 (P1); female rats were treated with oral administration of Moringa Oleifera at a dose of 150-200 mg/KgBW, Treatment group 2 (P2); female rats were treated with oral administration of Folic Acid at a dose of 150-200 mg/Kg BW and Treatment group 3 (P3): female rats were given moringa oleifera orally at a dose of 150-200 mg/Kg BW and Folic Acid orally at a dose of 150-200 mg/KgBW.

Data analysis

Data analysis starts from univariate analysis in the form of descriptive tests of data from the examination of total cholesterol levels, then the bivariate analysis is carried out using One Way Anova analysis to determine whether the data is normally distributed and or the data has the same variant (homogeneous), for hypothesis testing using the Kruskal-Wallis Nonparametric test to determine whether there are differences in sample groups that have received treatment with testing criteria

taken based on the probability value (sig) if> 0.05 then Ho is accepted and if the probability (sig) <0.05 then Ho is rejected.

RESULTS AND DISCUSSION

The results of the study include the value of blood cholesterol examination results of female rats before treatment, high-fat feeding (HFD), and examination results after treatment with moringa oleifera and folic acid.

Table 1: Distribution of blood cholesterol levels of female rats before treatment, PTL administration, and after treatment

	Group	Blood cholesterol levels Mean ± SD Jμ/L	P value
Pretest	Group 1 Positive (Na CMC 1%) Group 2 Negative (HFD) Cluster 3 MO administration Cluster 4 AF administration Cluster 5 MO +AF feeding	73.00 ± 5.292 93.33 ± 10.970 70.00 ±7.550 82.33 ± 9.866 74.67 ±1.528	0.110
PTL administration	Group 1 Positive (Na CMC 1%) Group 2 Negative (HFD) Cluster 3 MO administration Cluster 4 AF administration Cluster 5 MO +AF feeding	146.00 ± 7.937 172.00 ± 11.269 132.00 ± 6.000 158.00 ± 15.100 147.67 ± 11.060	0.562
Posttest	Group 1 Positive (Na CMC 1%) Group 2 Negative (HFD) Cluster 3 MO administration Cluster 4 AF administration Cluster 5 MO +AF feeding	145.00 ± 6.083 166.67 ± 6.110 75.00 ± 5.196 95.67 ± 6.028 67.33 ± 2.309	0.545

Table 1. shows the distribution of data on blood cholesterol levels of female rats in Pretest or before being given the highest cholesterol level value in group 2 negative (HFD) 93.33 ± 10.970, P-Value 0.110, indicating no significant decrease in blood cholesterol levels of female rats. In the administration of HFD, the highest value of blood cholesterol levels of female rats in group 2 was negative (HFD) 172.00 ± 11.269, P-Value 0.562 showing a significant increase in blood cholesterol levels of female rats and the posttest, the highest value in group 2 negative (HFD) 166.67 ± 6.110, P-Value 0.545.

The results showed significant cholesterol levels between the Pretest, HFD, and Posttest groups were negative (HFD), high blood cholesterol levels were influenced by the treatment given, namely the provision of high-fat feed purely due to the treatment, not because of the initial differences that already exist. Consumption of high-fat feed can occur because the fat that enters the body will be partially converted into cholesterol. Fat derived from endogenous synthesis and rations will be sent to the liver, endogenous in the form of free fatty acids while from the ration in the form of chylomicrons [16]. Foods derived from animal products such as fatty meats, butter, cheese, and milk cream, contain saturated fatty acids and cholesterol [17].

Table 2: Results of Kruskal-Wallis test analysis of total blood cholesterol levels of female rats between groups

Kruskal-Wallis				
	Group	**N**	**Mean Rank**	**P Sig.**
Pretest	Group 1 Positive (Na CMC 1%) Group 2 Negative (HFD) Cluster 3 MO administration Cluster 4 AF administration Cluster 5 MO +AF feeding	3 3 3 3 3	6.00 13.33 4.67 9.50 6.50	0.123
HFD administration	Group 1 Positive (Na CMC 1%) Klp 2 Negative (HFD) Cluster 3 MO administration Cluster 4 AF administration Cluster 5 MO +AF feeding	3 3 3 3 3	7.00 13.33 2.33 10.33 7.00	0.037

Posttest	Group 1 Positive (Na CMC 1%)	3	11.00	0.009
	Group 2 Negative (HFD)	3	14.00	
	Cluster 3 MO administration	3	2.00	
	Cluster 4 AF administration	3	7.83	
	Cluster 5 MO +AF feeding	3	5.17	

Table 2 shows the results of the Kruskal-Wallis test in the Pretest group with the probability value Sig. 0.123 means the probability value of Sig.>0.05, then Ho is accepted, the group giving HFD probability value sig. 0.037 and the Postest group probability value sig. 0.009 means the probability value sig.<0.05, then Ho is rejected. So it can be concluded from the results of the Kruska-Wallis test in the pretest group, that there is no significant difference in effectiveness between each sample group. In the HFD administration group and the post-test group, there is a significant difference in effectiveness between each sample group that gets treatment. The high content of Moringa Minerals, vitamins such as beta carotene and vitamin A, Vitamin B, folic acid, Vitamin C, Vitamin D, and Vitamin E, and essential amino acids which are sterol bio centers in the body that can balance several vitamins and inhibit the appearance of chronic diseases such as coronary heart disease [18]. The content of flavonoids and polyphenols can significantly increase Superoxide Dismutase (SOD) and catalase and reduce lipid peroxidase levels to reduce cholesterol levels [19]. So giving moringa oleifera treatment to female rats can lower total blood cholesterol levels in female rats [20].

One ingredient in moringa oleifera is folic acid, also known as vitamin B9. Folic acid is an essential nutrient required for various bodily functions, including the formation of red blood cells and DNA synthesis. Folic acid also plays a role in lipid (fat) metabolism in the body. Several studies have shown that folic acid can affect enzyme activity in lipid synthesis and degradation. The form of this coenzyme is tetrahydrofolate (THF) [21]. By modulating the activity of these enzymes, folic acid can lead to decreased production or increased breakdown of cholesterol in the body [22]. The addition of folic acid during pregnancy is very important in addition to preventing disability in infants, it can also reduce various risks that occur, for example, preeclampsia, so 0.4 to 1.0 mg of folic acid is needed every day for fetal development [23].

The content of compounds called plant sterols, whose chemical structure is similar to animal cholesterol, can help absorption in the intestine, thereby reducing the amount of cholesterol entering the bloodstream, Moringa extract affects the activity of the enzyme HMG-CoA reductase and in the synthesis of cholesterol in the body, has the potential to modulate lipid metabolism, including lipid synthesis and degradation and Moringa has a positive effect on endothelial function in the inner lining of blood vessels that can contribute to cholesterol levels and vascular health. While folic acid can affect the activity of enzymes in lipid synthesis and degradation, by modulating the activity of these enzymes, folic acid can lead to decreased production or increased breakdown of cholesterol in the body, helping to prevent plaque formation and improve vascular health. Folic acid functions as an intestinal barrier, namely the absorption of cholesterol, thereby reducing cholesterol levels in the blood [24]–[26].

CONCLUSION

Giving each treatment of Moringa Oleifera and folic acid is significantly effective in reducing the total blood cholesterol levels of female rats conditioned by hypercholesterolemia. However, when compared to the combined treatment of Moringa Oleifera and Folic Acid, it is significantly more effective in reducing the total blood cholesterol levels of female rats conditioned by hypercholesterolemia.

Suggestions For further research, it is necessary to investigate the chemical mechanism and with a larger number of samples to reduce the variation in results and get a more definite conclusion regarding the effectiveness of Moringa Oleifera and Folic Acid and if possible, clinical trials in humans or other test animals are needed to see if similar effects occur.

Authors' contributions

CC and EW were involved in the conception and planning of the research, MA and SS performed the data acquisition/collection, CC and ANU calculated the experimental data and performed the

analysis, CC drafted the manuscript and designed the figures, and EW aided in interpreting the results. All authors took part in a critical revision of the manuscript.

Acknowledgments

The researchers are grateful to the Biopharmaceutical and Biopharmaca Laboratory of Hasanuddin University Makassar for providing the necessary facilities and resources to complete this study.

Thanks to the Pharmaceutical Research Ethics Committee of Hasanuddin University Makassar for ethical approval (No. 797/UN4.17.8/KP.06.07/2024) to conduct this research.

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