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Effect of Combination of Averrhoa Bilimbi Extract and Curcuma Longa Extract on Low Density Lipoprotein Levels in Rats Fed a High Fat Diet

Pengaruh Kombinasi Ekstrak Belimbing Wuluh (Averrhoa Bilimbi L.) dan Ekstrak Kunyit (Curcuma Longa L.) terhadap Kadar Low Density Lipoprotein

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ABSTRACT

Background: Hypercholesterolemia is a state of total blood cholesterol levels transported by LDL exceeding normal limits. As a result, it can clog blood vessels, and this condition is a risk factor for CHD. Alternative medicine includes flavonoids in Averrhoa bilimbi (belimbing wuluh) known to reduce cholesterol and LDL levels, and curcumin in turmeric known to reduce fat absorption and increase fat excretion.
Objectives: The aim of this study was to analyze the combined effect of bilimbi fruit and turmeric extracts on LDL levels.
Methods: This experimental research used post-test only with a control group design involving male Wistar rats divided into 6 (six) random groups. Group KN (standard feed, aquadest for 28 days), K- (high-fat feed for 14 days, followed by standard feed and aquadest), K+ (high-fat feed for 14 days, followed by standard feed, aquadest, and simvastatin 0.18mg/200 g BW/day), P1 (high-fat feed for 14 days, followed by standard feed, aquadest and extracts of bilimbi fruit and turmeric extract at a dose of 240: 270 mg/200 g BW/day), P2 (high-fat diet for 14 days, followed by standard feed, distilled water and bilimbi fruit and turmeric extract at a dose of 360: 175 mg/200 g BW/day), P3 (high-fat diet for 14 days, followed by standard feed, distilled water and extracts of bilimbi fruit and turmeric at a dose of 120: 405 mg/200 g BW/day). The determination of LDL levels was done through a direct method using a spectrophotometer with a wavelength of 550 nm.
Results: The mean LDL levels were KN (53.60 ± 10.69) mg/dL, K- (193.40 ± 16.86) mg/dL, K+ (100.20 ± 42.48) mg/dL, P1 (118.00 ± 64.71) mg/dL, P2 (104.00 ± 45.28) mg/dL, P3 (78.00 ± 4.69) mg/dL. The data received was analyzed using the Kruskal-Wallis test. Then, the results obtained differences in LDL levels between various groups ($p<0.001$). Subsequently, it was continued with the Mann-Whitney test on KN compared to K-, K+, P1, P2, P3 indicating $p=0.009$. As a result, it was found a significant difference in LDL levels between KN and K-, K+, P1, P2, and P3.
Conclusions: This research concludes that providing a mixture of bilimbi fruit extract and turmeric extract can reduce LDL levels.

INTRODUCTION

Hypercholesterolemia is a condition in which the level of total blood cholesterol transported by LDL exceeds normal limits, which can block blood vessels and is a risk factor for CHD[1]. The current treatment for hypercholesterolemia, namely statin drugs, has side effects including rhabdomyolysis, myopathy, gastrointestinal disorders, muscle pain, irritation of the gastric mucosa, impaired hepatic function, creation of gallstones, and kidney damage if used in the long term[1,2,3]. As an alternative treatment for patients with hypercholesterolemia with statin complications, flavonoids in Averrhoa bilimbi are needed to reduce cholesterol and LDL levels, and curcumin in turmeric to reduce fat absorption and increase fat excretion through feses[4,5,1]. The extracts of bilimbi fruit and turmeric have been proven to reduce the level of LDL in the blood. As a result, by combining these two natural ingredients, LDL in the blood is expected to be reduced more significantly than by using only one of the natural ingredients. LDL was

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chosen as a parameter of hypercholesterolemia condition because LDL functions to transport the most cholesterol in the blood and tends to stick to the walls of blood vessels. As a result, it can form cholesterol deposits and cause narrowing to blockage of the blood artery, increasing the risk of coronary heart disease[6].

The prevalence of hypercholesterolemia is still relatively high, according to WHO data, which is 39% in the world and 29% in Southeast Asia[7]. It was recorded that 34.820 or 28.8% of the Indonesian population, suffered from hypercholesterolemia in 2018, and there were 1.5% of CHD patients caused by hypercholesterolemia[9]. Basic health research data in 2018 indicated that heart disease and stroke patients in Central Java were 1.6% and 11.8%, respectively[10]. This is in line with the statement that high cholesterol levels also cause heart disease, where the incidence of coronary heart disease (CHD) in Semarang City reached 53%[11].

Pratiwi (2016) and Doja (2020) state that bilimbi fruit and turmeric can reduce cholesterol levels and LDL levels[12,13]. Research related to the effect of bilimbi fruit and turmeric extract mixture on LDL levels is still limited. Research on bilimbi fruit extract containing active substances flavonoids, pectins, saponins, and tannins at a dose of 480 mg/200 g BW/day proved to reduce total cholesterol levels in male Wistar rats[13]. Another research reveals turmeric extract containing the active substance curcumin at a dose of 2.7 g/kg body weight can reduce LDL levels by 59.55% in rats[12]. Based on the above background, further research should be carried out to recognize whether the effect of giving a mixture of bilimbi fruit and turmeric extracts to rats induced by high-fat food can reduce the level of Low-Density Lipo-protein (LDL) more significantly than giving one extract merely.

METHODS

This research was a laboratory experiment with a post-test only with a control group design. The sample in this research amounted to 30 male Wistar rats which were randomly divided into 6 (six) categories. Inclusion criteria in this research were male Wistar rats weighing 150-200 grams, aged 2-3 months, healthy, and active. Male rats were selected as research samples because aggressive behavior is relatively minimal. Moreover, they do not experience menstruation, which affects the speed of the adaptation period. Exclusion criteria in this research were dead rats after being induced with high-cholesterol food during treatment. Rats were adapted for 7 (seven) days. Then, randomization was held, and 6 (six) categories were obtained, namely KN (normal control), rats without treatment, only given standard feed in the form of pellets and aquadest for 28 days, K- (negative control), namely rats induced with high-cholesterol feed for 14 days followed by standard feed and aquadest without being treated, K+ (positive control) the same induction includes K-, followed by simvastatin 0.18 mg/200 g BW/day for 14 days, P1 (treatment 1) induction included the same as K- plus a mixture of bilimbi fruit extract at 240 mg/200 g BW/day and turmeric extract at 270 mg/200 g BW/day for 14 days, P2 (treatment 2) induction included K- plus a mixture of bilimbi fruit extract dosed at 360 mg/200 g BB/day and turmeric extract dosed at 135 mg/200 g BB/day for 14 days, P3 (treatment 3) induction included K- plus a mixture of bilimbi fruit extract dosed at 120 mg/200 g BB/day and turmeric extract dosed at 405 mg/200 g BB/day for 14 days.

Bilimbi fruit and turmeric extracts were obtained using a maceration method using 70% ethanol extractor. High-fat feed induction was conducted by adding 1.5% chicken egg yolk, 10% goat fat, and 1% coconut oil to the standard feed (pellets). The high-fat diet was given ad libitum for 14 days. All rats except KN were confirmed to be hypercholesterolemic before treatment by measuring their cholesterol levels after 14 days of high-fat feed using a cholesterol checker (GCU) and exceeded normal cholesterol values. Category K+ was treated with simvastatin 0.18 mg/200 g BW/day dissolved in water with 1% CMC suspending agent as much as 2 mL given orally. The P1, P2, and P3 categories were given a mixture of bilimbi fruit extract and turmeric extract dissolved in water with 1% CMC suspending agent as much as 2 mL given orally. After 7 (seven) days of the adaptation period, 14 days of the high-fat feed induction period, and 14 days of the treatment period, on day 36, blood serum samples were taken. To determine the LDL level in the blood of rats, 200 µL of blood serums were taken from the retro-orbital plexus and mixed with 500 µL of LDL reagent kit. After that, the centrifuge was used for 10 minutes at 3000 rpm, and the LDL level was measured by direct method using a spectrophotometer at a wavelength of 550 nm[12]. Data were processed using the SPSS 16.0 program. The normality test used Shapiro-Wilk because the number of samples <50 and test homogeneity used Levene's Test. The data obtained were not normal and not homogeneous, as a result, non-parametric tests were carried out by testing Kruskal-Wallis and continued with the Mann-Whitney test. The Ethics Committee of Health and Medical Studies of Faculty of Medicine Universitas Islam Sultan Agung Semarang has permitted to study the effect of bilimbi fruit extract (*Averrhoa Bilimbi L.*) and turmeric extract (*Curcuma Longa L.*) on cholesterol readings with registration number 370/IX/2022/Bioethics Commission.

RESULTS AND DISCUSSIONS

There were no rats died after the induction of the high-fat diet or during the treatment, as a result, no rats were included in the exclusion criteria. Since the Shapiro-Wilk test for normality resulted in abnormal data distribution ($p<0.05$) and Levene's-Test for homogeneity resulted in inhomogeneous/unequal data variation ($p<0.05$), the requirement of parametric One Way Annova test was waived, and instead non-parametric Kruskal-Wallis test was conducted, followed by Mann-Whitney test. With a $p=0.001$ (p-value <0.05), the Kruskal-Wallis test indicated a statistically significant difference in LDL values between the two categories. The Mann-Whitney U test was then used to assess the statistical significance between treatment groups.

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Table 1. Analysis of mean of LDL* levels between treatment groups and results of normality and homogeneity tests

Group	Mean ± SD LDL* (mg/dL)	Shapiro-Wilk	Levene's Test	Kruskal-Wallis
KN	53.60 ± 10.69	0.042	0.002	0.001*
K-	193.40 ± 16.86	0.736		
K+	100.20 ± 42.48	0.012		
P1	118.00 ± 64.71	0.165		
P2	104.00 ± 45.28	0.000		
P3	78.00 ± 4.69	0.154		

LDL: Low-Density Lipoprotein, KN: Normal Category, K-: Negative Category, K+: Positive Category, P1: Treatment 1, P2: Treatment 2, P3: Treatment 3, *) Significant difference found, p value<0.05

Table 2. Mann-Whitney test results

Mann Whitney Test		P-value
KN	K-	0.009*
	K+	0.009*
	P1	0.009*
	P2	0.009*
	P3	0.009*
K-	K+	0.012*
	P1	0.076
	P2	0.021*
	P3	0.009*
K+	P1	0.834
	P2	0.459
	P3	0.346
P1	P2	0.597
	P3	0.753
P2	P3	0.035*

KN: Normal Category, K-: Negative Category, K+: Positive Category, P1: Treatment 1, P2: Treatment 2, P3: Treatment 3, *) Significant difference found, p value<0.05

Category K- has the highest LDL levels due to high cholesterol intake without receiving treatment. Based on previous researchers' findings, feeding quail egg yolk and beef fat has been shown to increase cholesterol levels in male Wistar rats because quail egg yolk and beef fat contain cholesterol and saturated fat[5]. K+ category has experienced a decrease in LDL. After all, this treatment category received simvastatin treatment at a dose of 0.18 mg/200 g BW/day for 14 days. Simvastatin can reduce LDL levels because statins will reduce cholesterol formation in the liver by inhibiting the performance of the HMG-CoA reductase enzyme as a precursor for cholesterol synthesis. As a result, the level of cholesterol in the blood is expected to decrease[1].

The percentage decrease in LDL levels in P1 is 18% lower when compared to the K+ category. LDL levels in P1 decrease but are not yet comparable to K+, presumably because the flavonoids, pectins, tannins, and saponins in Averrhoa bilimbi and curcumin in turmeric at this dose did not contain enough bioactive substances to be able to reduce LDL levels to cover the effect of a decrease in K+, as a result, the mixture of extracts in P1 is still not as good as the activity of simvastatin to reduce LDL levels. This is in line with research[1] revealing that the provision of bilimbi fruit extract at a dose of 250 mg/kg BW and 500 mg/kg BW was not proven to reduce LDL levels significantly. The percentage decrease in LDL levels in P1 is 4% lower when compared to the K+ category. LDL levels in P2 decrease almost equivalent to the average LDL levels in K+, presumably because the content of flavonoids, pectins, tannins, and saponins in bilimbi fruit and curcumin in turmeric at this dose can work synergistically as a result that can reduce LDL levels to reach almost equivalent to the effect of reducing K+. This is in line with research[14] revealing that the administration of bilimbi fruit extract at a dose of 320 mg/200 g BW is the most effective dose to reduce LDL levels to reach 51.540 mg/dL. The percentage decrease in LDL levels in P3 is 22% higher when compared to the K+ category. LDL levels at P3 decrease higher when compared to K+, presumably because the content of flavonoids, pectins, tannins, and saponins in Averrhoa bilimbi and curcumin in turmeric at this dose already contains enough bioactive substances to be able to reduce LDL levels beyond the reducing effect on K+. This is in line with the previous study, which revealed that when giving bilimbi fruit extract at a dose of 160 mg/200 g BW there was a decrease in cholesterol levels reaching 53.62 mg/dL[13]. No rats died or experienced behavioral and physical changes when the rats were observed after oral sonde treatment of bilimbi fruit extract at a dose of 2000 mg/kg BW and 5000 mg/kg BW[15]. This research is in line with previous findings mentioning that flavonoid compounds are proven to reduce cholesterol by inhibiting the performance of the enzyme HMG-CoA reductase as a precursor for cholesterol synthesis by forming hydrogen bonds with histidine, one of the amino acids that make up HMG-CoA reductase, so that it does not bind to HMG-CoA as a result mevalonate is not created. If mevalonate is not created, the stages of cholesterol formation

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including scualen, lanosterol, and isoprenoids will be inhibited and cholesterol levels will decrease[13]. This research is also in line with previous research revealing that by giving the highest dose of turmeric extract from their research, namely a dose of 2.7 g/kg BW, it was proven that it could reduce LDL levels the most until the LDL levels of rats reached an average of 13.27 ± 1.42 with a percentage reduction in LDL as much as 59.55%[12]. No changes were found in rats given a dose of curcumin 20 mg/kg BW for 28 days. As a result, it can be concluded that this dose did not cause toxic effects on the experiment rats[16].

CONCLUSIONS

A mixture of bilimbi fruit extract (*Averrhoa Bilimbi L.*) and turmeric extract (*Curcuma Longa L.*) is proven to reduce LDL levels in rats induced by high cholesterol feed. The results of the analysis indicate that the P2 category can reduce LDL levels almost equivalent to K+, and P3 is the most effective dose to reduce LDL levels to reach (78.00 ± 4.69 mg/dL). For researchers who will conduct further research, they can measure other fat levels, including total cholesterol, HDL, and triglycerides must be carried out testing the toxicity of the mixture between the two extracts in long-term use must be carried out testing the levels of active substances in the extract and be carried out research with more varied doses to obtain the most effective dose in reducing LDL levels.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

All authors have no conflict of interest in this article.

AUTHOR CONTRIBUTIONS

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