Potency of Red Guava Fruitghurt on Total Cholesterol Levels

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Abstract. High total blood cholesterol levels are one of the factors that cause atherosclerosis and coronary heart disease. Fruitghurt is a probiotic product made from fruit juice. The content of potential ingredients in red guava and the role of LAB bacteria make fruitghurt a good and useful potential food. This study aims to determine the effect of red guava juice fruitghurt on total blood cholesterol in experimental animals induced by a high-fat diet. This research is a pure laboratory experiment. In this study, 5 different treatments were carried out on 5 groups of experimental animals. The data obtained were tested with several levels of statistical tests, to see the significance of the results. From the results obtained, it was concluded that the one that had a significant effect on reducing total blood cholesterol levels in rats was treatment group 3 given simvastatin and fruitghurt drugs with cholesterol levels reaching 49.54 mg/dl. The decrease in total blood cholesterol levels may be due to the mechanism of action of simvastatin and fruitghurt drugs. The mechanism of action of the drug simvastatin is to competitively inhibit *3-hydroxy-3-methylglutaroyl-coenzyme* A causing a decrease in cholesterol synthesis, causing a decrease in total cholesterol. Giving fruitghurt can reduce total cholesterol levels caused by the mechanism of action of probiotics which can increase the secretion of bile salt hydrolase enzymes and produce dehydrogenated cholesterol cofactors so that total cholesterol will decrease. The results of this study indicate that guava juice fruitghurt can reduce blood cholesterol levels in experimental animals.

Keywords: Fruitghurt · Probiotics · Cholesterol

1 Introduction

Hypercholesterolemia is defined as high total cholesterol in the blood that is ≥200 mg/dl [1]. High cholesterol levels in the blood can increase the risk of atherosclerosis, heart disease, diabetes mellitus, liver disease and kidney disease. Handling is needed immediately

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to control blood cholesterol levels in an effort to prevent further effects of hypercholesterolemia [2]. There are many ways that can be done to control cholesterol levels, including taking drugs that can lower cholesterol levels. The drug that is often used is simvastatin [3]. In the use of simvastatin, the activity of the enzyme *3-hydroxy-3-methylglutaroyl-coenzyme.* A reductase is inhibited, which causes mevalonic acid to not be formed and total cholesterol levels will decrease [4].

People with hypercholesterolemia can reduce their use of cholesterol-lowering drugs by taking probiotics. There are many forms of probiotics, one of which is fruitghurt. Fruitghurt is a drink that is shaped like yogurt and made from fruit juice by utilizing the help of lactic acid bacteria. Fruitghurt contains *Lactobacillus* which can inhibit the formation of cholesterol in the blood. In addition, fruitghurt can increase our body's immunity and can balance the bacteria in the body [5, 6]. Red guava is a fruit that is widely found in Indonesia and can be processed into various products. like fruitghurt [7].

Red guava contains a lot of pectin fiber, which is hypocholesterolemic, thereby lowering cholesterol levels [7]. In this study, experimental animals were white rats with a high-fat diet induced. Red guava juice fruitghurt was given as much as 1 ml to see changes in total cholesterol levels [8]. In this study also conducted a comparison between red guava juice fruitghurt with simvastatin in lowering cholesterol.

2 Subject and Methods

2.1 Research Subject

The research subjects were white rats of the wistar strain obtained from the Pharmacology Laboratory of the University of Padjadjaran. Before the study was conducted, the rats were adapted for 7 days. The 25 rats used were divided into 5 treatment groups.

2.2 Research Variable

The independent variable in this study was red guava juice fruitghurt. Meanwhile, the dependent variable in this study was the total cholesterol level of Wistar rats.

2.3 Research Material

The research material used was red guava from a guava plantation in Panyingkiran village, Majalengka which was fermented by *Lactobacillus acidophilus* bacteria and given 10% sucrose.

2.4 Research Procedure

2.4.1 Bacteria Suspension Manufacturing

Bacterial colonies that had been grown on MRSB media were incubated for 48 h at 37 °C. Then check for turbidity according to the standard *Mc Farland 0.5*. After that, prepare 25 ml of *Lactobacillus acidophilus* bacterial suspension from MRSB media for each red guava juice to which sucrose has been added.

2.4.2 Making Red Guava Juice

Red guava juice made by using a juicer with a speed of 47 rpm, then adding water in a ratio of 1:1, then filtered several times to obtain the last guava juice. Then put it in a *Schott Duran bottle* for sterilization. Each *bottle of Schott Duran* is filled with 225 ml of red guava juice with the addition of 22.5 g of sucrose.

2.4.3 Addition of Lactobacillus Acidophilus

The Schott Duran bottle containing guava juice was left to room temperature, then added 25 ml of *Lactobacillus acidophilus* suspension and homogenized. Then incubated at 37 °C for 24 h.

2.4.4 Effect of Anti Hypercholesterolemia

Experimental animals were adapted for 7 days, then given a high-fat diet using 1 ml quail egg yolk for 14 days. The experimental animals that had been given a high-fat diet were then drawn blood through the orbital vein and examined for total cholesterol using the CHOD-PAP method. Experimental animals were divided into 5 treatment groups, namely KN (negative control group, not given any treatment), KP (positive control group, only given a high-fat diet, and not given fruitghurt and simvastatin) P1 (treatment group 1, was given a high-fat diet and fruitghurt but not given simvastatin.), P2 (Treatment group 2 was given a high-fat diet and simvastatin, but not given fruitghurt.) P3 (Treatment group 3 was given a high-fat diet, fruitghurt, and simvastatin.). Fruitghurt and simvastatin were given to experimental animals for 7 days and then their total cholesterol levels were re-analyzed by examining their orbital venous blood.

2.5 Research Analysis

The data from the test results in the laboratory were tested for normality using the *Shapiro Wilk test*. To find out the difference before and after treatment, *paired T-test* was performed. *One way ANOVA test* was used to determine the difference in total blood cholesterol levels in each treatment group. And finally, the *Tukey post hoc test* to see differences between treatment groups.

This research was conducted in December at the Laboratory of Microbiology, Faculty of Medicine, Jenderal Achmad Yani University and at the Veterinary Laboratory, Faculty of Medicine, Jenderal Achmad Yani University. This research was conducted after obtaining an ethical permit approved by the Research Ethics Commission of the Faculty of Medicine, Universitas General Achmad Yani on December 1, 2021 with the number 023/UH1.11/2021 which has reviewed research proposals using experimental animal subjects in this study.

3 Results and Discussion

The effect of red guava fruitghurt on total blood cholesterol levels in experimental animals given a high-fat diet can be seen in Table 1.

Table 1. Total cholesterol levels after high-fat diet

Rats	KN	KP	P1	P2	P3
1	65.4	79.1	93.6	88.5	78.8
2	59.2	92.6	97.3	86.7	80.2
3	45.2	83.3	95.1	97.2	95.8
4	66.3	75.8	73.1	93.6	89.7
5	64.6	81.5	84.9	68.7	92.2

Information:
KN: Negative control group, not given any treatment.
KP: Positive control group, only given a high-fat diet, and not given fruitghurt and simvastatin
P1: Treatment group 1, was given a high-fat diet and fruitghurt but not given simvastatin
P2: Treatment group 2 was given a high-fat diet and simvastatin, but not given fruitghurt
P3: Treatment group 3 was given a high-fat diet, fruitghurt, and simvastatin.

Table 2. Total cholesterol levels after administration of fruitghurt and simvastatin

Rats	KN	KP	P1	P2	P3
1	64.2	81.2	68.3	56.3	40.5
2	62.1	93.6	69.6	58.5	43.1
3	48.9	84.8	71.3	72.8	53.5
4	68.5	77.9	48.6	66.9	57.3
5	59.9	85.7	63.3	44.8	53.3

Information:
KN: Negative control group, not given any treatment
KP: Positive control group, only given a high-fat diet, and not given fruitghurt and simvastatin
P1: Treatment group 1, was given a high-fat diet and fruitghurt but not given simvastatin
P2: Treatment group 2 was given a high-fat diet and simvastatin, but not given fruitghurt
P3: Treatment group 3 was given a high-fat diet, fruitghurt, and simvastatin.

In Table 1, it can be seen that the total cholesterol levels of rats exceeded normal cholesterol levels for rats in the KP, P1, P2, P3 groups.

Rats were categorized as suffering from hypercholesterolemia after total cholesterol examination was carried out with results of 54 mg/dl.

Total cholesterol levels of wistar rats after administration of fruitghurt and simvastatin can be seen in Table 2.

In the Table 2, it can be seen that there was a decrease in blood cholesterol levels in groups P1, P2, and P3. In the P3 group, the decrease in blood cholesterol levels in experimental animals was greater than in the P1 and P2 treatment groups.

Table 3. Effect of fruitghurt on cholesterol levels

Group	Cholesterol level (mg/dl) ± SD		*p-value*
	Before	After	
KN	60.14 ± 8.79	60.72 ± 7.33	0.729
KP	82.46 ± 6.32	84.64 ± 5.88	0.016
P1	88.80 ± 9.95	64.18 ± 9.23	0.000
P2	86.94 ± 11.00	59.86 ± 10.70	0.000
P3	87.34 ± 7.49	49.54 ± 7.30	0.000

Information:
KN: Negative control group, not given any treatment
KP: Positive control group, only given a high-fat diet, and not given fruitghurt and simvastatin
P1: Treatment group 1, was given a high-fat diet and fruitghurt but not given simvastatin
P2: Treatment group 2 was given a high-fat diet and simvastatin, but not given fruitghurt
P3: Treatment group 3 was given a high-fat diet, fruitghurt, and simvastatin

3.1 Effect of Fruitghurt on Total Blood Cholesterol Levels

In Table 3, the P1 group there was a significant difference in blood cholesterol levels before and after the fruitghurt treatment (p-value 0.000). The results of calculations in the P2 group using the T-dependent test showed a significant difference in blood cholesterol levels before and after treatment with simvastatin (p-value 0.000).

In the P3 group, with the T-dependent test, there were significant differences in blood cholesterol levels before and after the fruitghurt and simvastatin treatment (p-value of 0.000). Meanwhile, in the KN and KP groups, there was no difference in blood cholesterol levels before and after treatment (p-value > 0.05).

3.2 Differences in Decrease in Total Blood Cholesterol Levels After Giving Fruitghurt

In Table 4, it can be seen that the one way ANOVA test results obtained an ANOVA value of $f = 12.129$ (p-value 0.000). The results of this test showed that there were significant differences in cholesterol levels in the 5 treatment groups in the study. This proves that red guava fruitghurt can reduce blood cholesterol levels in experimental animals.

3.3 Differences in Decrease in Total Blood Cholesterol Levels After Giving Fruitghurt and Simvastatin

In Table 5, the *Post Hoc Tukey test* above shows that there are significant differences between all treatment groups (P1, P2, and P3) and the KP group ($p < 0.05$). This shows that giving fruitghurt can reduce blood cholesterol levels in experimental animals that are given a high-fat diet. The decrease in blood cholesterol levels that reached normal levels was only found in the P3 group, namely the treatment group that received fruitghurt

Table 4. The effect of giving red guava juice fruitghurt on total cholesterol levels of the five groups

Variable (total cholesterol (mg/dl))	n	*Mean*	SD	*F(Anova)*	*p*
KN	5	60.72	7.33	12.129	0.000
KP	5	84.64	5.88		
P1	5	64.18	9.22		
P2	5	59.86	10.70		
P3	5	49.54	7.30		

* $p \leq 0.05$ there is a significant difference.
Information:
KN: Negative control group, not given any treatment
KP: Positive control group, only given a high-fat diet, and not given fruitghurt and simvastatin
P1: Treatment group 1, was given a high-fat diet and fruitghurt but not given simvastatin
P2: Treatment group 2 was given a high-fat diet and simvastatin, but not given fruitghurt
P3: Treatment group 3 was given a high-fat diet, fruitghurt, and simvastatin.

Table 5. Differences in mean total cholesterol between treatment groups

Group		*Post Hoc Tukey Test*	
		P-value	Conclusion
(KN)	- KP	0,002	significant difference
	- P1	0,962	No significant difference
	- P2	1.000	No significant difference
	- P3	0,243	No significant difference
(KP)	- P1	0,007	significant difference
	- P2	0,001	significant difference
	- P3	0,000	significant difference
(KP1)	- P2	0,919	No significant difference
	- P3	0,074	No significant difference
(KP2)	- P3	0.313	No significant difference

Information:
KN: Negative control group, not given any treatment
KP: Positive control group, only given a high-fat diet, and not given fruitghurt and simvastatin
P1: Treatment group 1, was given a high-fat diet and fruitghurt but not given simvastatin
P2: Treatment group 2 was given a high-fat diet and simvastatin, but not given fruitghurt
P3: Treatment group 3 was given a high-fat diet, fruitghurt, and simvastatin.

and simvastatin, while the P1 and P2 groups were close to normal levels of total blood cholesterol.

The decrease in blood cholesterol levels in the P3 group could be caused by the cooperation of the drugs simvastatin and fruitghurt given. The mechanism of action of simvastatin is by competitively inhibiting the HMG CoA reductase enzyme, an enzyme that plays a role in cholesterol synthesis, causing a decrease in cholesterol synthesis and increasing the number of LDL receptors, especially in the liver and extrahepatic tissue, causing a decrease in total cholesterol because the lactic acid bacteria contained in fruitghurt are able to increase the secretion of bile salt hydrolase enzymes which can increase bile acid excretion, causing the need for total cholesterol in the body to increase so that total blood cholesterol levels can be reduced. In addition, lactic acid bacteria can also produce dehydrogenated cholesterol cofactors which function to activate the cholesterol reductase enzyme so that sterols cannot be absorbed by the intestines, so total cholesterol will decrease better than giving fruitghurt or simvastatin alone.

In addition, the decrease in cholesterol levels can also be influenced by the presence of pectin fiber found in red guava which can reduce total cholesterol levels. Pectin fiber can lower cholesterol levels, because it can cause slow gastric emptying, so it can limit the number of calories that enter. Pectin fiber will also cause an increase in the thickness of the intestinal lining which functions as a place for lipid absorption, so that the amount of lipid absorbed is getting smaller. Pectin fiber can also cause hypomotility, so it can slow down the process of digestion and absorption of nutrients. Pectin fiber can also bind bile acids through the enterohepatic cycle which is the end product of cholesterol. As a result, this bile will continue to be carried to the large intestine and secreted with feces. In the digestive tract, fiber also functions as a prebiotic which will be fermented by bacteria, and produces propionic and butyric acetic acid which functions to inhibit cholesterol synthesis. This can cause a decrease in cholesterol levels in the body [9].

In a previous study, papaya juice probiotic drink with a dose of 1 ml had antihyperolesterolemic activity which had an effect on lowering cholesterol levels in white rats given a high-fat diet. This antihypercholesterolemic effect is caused by lactic acid bacteria contained in probiotic drinks [10].

4 Conclusion

Red guava juice fruitghurt can reduce cholesterol levels in experimental animals given a high-fat diet. The combination of red guava juice fruitghurt and simvastatin had a higher cholesterol-lowering effect than only fruitghurt or simvastatin.

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