IOP Conference Series: Earth and Environmental Science

PAPER • OPEN ACCESS

The Effect of Guava on the Improvement of Lipid Profile in Hypercholesterolemic Rats

To cite this article: Sugeng Maryanto and Yustinus Marsono 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **276** 012054

The Effect of Guava on the Improvement of Lipid Profile in Hypercholesterolemic Rats

Sugeng Maryanto[1,*] and Yustinus Marsono[2]

[1]Department of Nutrition, Faculty of Health Science, Universitas Ngudi Waluyo, Jl. Diponegoro 186, Semarang Regency-Indonesia
[2]Department of Food Science, Post Graduate Program, Gadjah Mada University, Jl. Flora No. 1, Bulaksumur, Yogyakarta-Indonesia

*Corresponding author: sugengmaryanto99@gmail.com; yustimar@ugm.ac.id

Abstract. Red guava is a fruit species with high vitamin C content and the highest fibers, in particular, soluble fiber (pectin) among tropical fruits and cereals. The soluble fiber is hypocholesterolemic in the body. Therefore, it has the potential to reduce cholesterol content. This study aimed to assess the potential of red guava in reducing cholesterol content in hypocholesterolemic rats. This study applied a pure experiment using a randomized pretest-posttest group design on hypocholesterolemic Sprague Dawley rats. The rats were fed with 0.72 g of red guava and pectin supplement as a comparison. The total cholesterol content, LDL, HDL and triglyceride were measured by CHOD-PAP and GPO-PAP methods. Data analysis used t-test and ANOVA with significance rate of 0.05. Red guava intake to the hypocholesterolemic rats significantly reduced the contents of: total cholesterol to 57.8 mg/dL (32%), LDL to 25.5 mg/dL (43%), triglyceride to 19.7 mg/dL (18%). Conversely, it significantly increased HDL content to 10.6 mg/dL (18%). The red guava has proven to give hypocholesterolemic effect by reducing the total cholesterol LDL and triglyceride; Conversely, it significantly increased HDL.

Keywords: Red guava fruit, hypercholesterolemia, Short Chain Fatty Acid, cholesterol caecum

1. Introduction

Red guava (*Psidium guajava* L.) is a tropical fruit plant originated from the American continent, especially Brazil and Antilles. There have been several varieties found to the date. The fruit has been spread to Southeast Asia, including Indonesia. The plant grows in the backyard and fields. Some genuses that become favorites among Indonesian people include Pasar Minggu, Bangkok, Palembang, sukun, apple, sari, and getas. Red guava has been an essential commodity for farmers and their family in Indonesia, especially Kendal Regency [1].

Traditionally, red guava is consumed as a fruit. Indeed, it has more benefits than as a fruit. The leaves are useful for traditional medicine healing various diseases. The fruit is believed to overcome several health disorders, such as hepatoprotection, antiallergy, antimicrobial, antitoxic, antiplasmodial, antispasmodic, cardioactive, anti-cough, antidiabetic, anti-inflammatory, diarrhea, as well as the source of antioxidant. Red guava can be used for healing dengue (DHF) [2]. It also contains high vitamin C up to twofold of orange. Vitamin C is useful for antioxidant. In addition to Vitamin C, the fruit is rich in fiber, in particular, soluble fiber (pectin). The fiber contents in the red guava are considered the highest among the tropical fruits and higher than that of cereals [3]. Dietary fiber, especially soluble fiber (pectin), is hypocholesterolemic in the body. It has an antibody effect on Cardiovascular Diseases (CVD) by reducing cholesterol [4]. Maryanto and Fatimah contended that white guava had been proven to lower serum lipids significantly [5]. Several mechanisms of cholesterol reduction by dietary fiber include inhibiting cholesterol absorption, reducing cholesterol availability so the transfer to the blood can be reduced, preventing cholesterol synthesis, reducing the density rate of dietary energy so that it reduces cholesterol synthesis and increases bile excretion. This mechanism can be assessed by examining short-chain fatty acid (SCFA) and digesta caecum cholesterol [6]. In doing so, this study examined hypocholemic *Sprague Dawley* rats.

This study aimed to assess the mechanism of cholesterol reduction in hypocholesterolemic rats fed with red guava. The study was expected to contribute to scientific information of the hypocholesterolemic effect of the red guava on rats with hypercholesterolemia.

2. Methods

This study applied a real experiment with a randomized pretest-posttest control group design [7]. This work used subjects of 28 individuals of 2 years old, 150-160 g of male *Sprague Dawley* rats provided by LPPT UGM. To determine the samples of each group, a Federer formula was used resulting in score 7 in each group [8].

The rats were adapted, fed with AIN 93 standard for three days, followed by high cholesterol AIN 93 standard by adding in 1% cholesterol crystal and sodium cholate for 14 days, followed by randomization and grouping at individual cages for 28 days. The rats were fed 15 g/day. For either high cholesterol or treatment groups, feed residuals were scaled daily [9].

The treatments were performed by feeding red guava dissolved in the water by *sonde*. In comparison to the soluble fibers in the red guava, pectin supplements were also dissolved in the water.

The making of red guava flour was performed by refining the dried fruits to produce flour. Fiber contents were analyzed by an Enzymatic-Gravimetric method (AOAC Official methods) [10]. The analysis took place at the Food and Nutrition Laboratory of the Soegijapranata Catholic University Semarang. The treatments referred the needs for dietary fiber for male adults recommended by ADA [11] and were converted for rats as recommended by Laurence and Bocharch at 0.72 g [12].

This study examined the parameters by blood sampling of the experiment rats by a sinus retro orbitalis concerning lipid profiles (total cholesterol, LDL, HDL, and triglyceride). In addition, the examination was also performed to SCFA and excretion of cholesterol digesta caecum. The examination of total cholesterol and LDL applied an Enzymatic Colorimetric Test CHOD-PAP method. The examination of triglyceride used a Precipitation of LDL, VLDL, and Chylomicrons method [13]. The examination of SCFA was performed by examining acetic, propionic and butyric acids contained within the digesta caecum aided by gas chromatography [14]. The examination of cholesterol excretion took place by examining cholesterol contained within the digesta caecum using Liebermann-Burchard method and spectrophotometer instrument [15]. The experimental animals were conserved and subjected to parameter examination at PAU UGM. The study took place pursuant to the Ethical Clearance issued by KEPK FK UNDIP/dr. Kariadi Hospital No. 174/EC/FK/RSDK/2011.

Data analysis performed was Saphiro Wilk data normality test, the analysis of change in lipid profiles before and after the treatments used a dependent t-test, different test on lipid profiles and the cholesterol digesta caecum after the treatments for all groups was analyzed using independent t-test and ANOVA. All analyses were performed at $\alpha = 0.05$.

3. Results

3.1. Lipid profiles

Lipid profiles examination was performed to find out the effect of red guava flour on total cholesterol, LDL, HDL, and triglyceride presented in Table 1 to 4.

Table 1. Total cholesterol contents by treatment group

Group	Mean (mg/dL)			T-test
	Initial	Final	Δ (%)	(*p-value*)
Normal feed	103.9	113.0	10.0 (10%)	< 0.001
High cholesterol feed	173.3	187.7	14.4 (8%)	< 0.001
Red guava treatment	179.7	122.0	-57.8 (32%)	< 0.001
Pectin treatment	176.4	120.0	-56.4 (32%)	< 0.001

Table 2. LDL contents by group

Group	Mean (mg/dL)			T-test
	Initial	Final	Δ (%)	(*p-value*)
Normal feed	24.3	31.5	7.2 (30%)	< 0.001
High cholesterol feed	57.5	62.3	4.8 (8)	< 0.001
Red guava treatment	60.0	34.5	-25.5 (43%)	< 0.001
Pectin treatment	56.3	29.8	-26.5 (47%)	< 0.001

Table 3. HDL contents by group

Group	Mean (mg/dL)			T-test
	Initial	Final	Δ (%)	(*p-value*)
Normal feed	72.1	67.3	-4.8 (7%)	< 0.001
High cholesterol feed	57.7	50.6	-7.1 (12%)	< 0.001
Red guava treatment	59.2	69.7	10.6 (18%)	< 0.001
Pectin treatment	62.3	74.6	12.3 (20%)	< 0.001

Table 4. Triglycride contents by treatment group

Group	Mean (mg/dL)			T-test
	Initial	Final	Δ (%)	(*p-value*)
Normal feed	72.4	82.0	13.0 (12%)	< 0.001
High cholesterol feed	110.4	127.5	17.1 (15%)	< 0.001
Red guava treatment	108.3	88.6	-19.7 (18%)	< 0.001
Pectin treatment	104.9	85.3	-19.6 (19%)	< 0.001

The red guava intake was proven to reduce the contents of total cholesterol (32%), LDL (43%), and triglyceride (19%). Such treatment also increased HDL content (18%). The t-test result reported that the red guava group reduced total cholesterol, LDL, and triglyceride in the rats with hypercholesterolemia significance at p<0.001. It appeared that red guava was capable of repairing the lipid profiles of the rats with hypercholesterolemia.

The different test on groups concerning total cholesterol, LDL and triglyceride resulted in no difference between red guava and pectin groups (Table 5). It proved that the red guava had a similar capacity to pectin in reducing total cholesterol, LDL, and triglyceride of the rats with hypercholesterolemia.

Table 5. Different test rates on lipid profiles by treatment group

Parameter	Group	(Δ) Mean	± SD *
1.Total cholesterol	Normal feed	10.0	± 2.30[a]
	High chol.	14.4	± 3.10[a]
	Guava	-57.8	± 5.33[b]
	Pectin	-56.4	± 2.25[b]
2. LDL	Normal feed	7.2	± 1.70[a]
	High chol.	4.8	± 1.20[a]
	Guava	-25.5	± 1.60[b]
	Pectin	-26.5	± 2.09[b]
3. HDL	Normal feed	-4.8	± 1.10[a]
	High chol.	-7.1	± 1.1[a]
	Guava	10.6	± 1.83[b]
	Pectin	12.3	± 1.45[b c]
4. Triglyceride	Normal feed	13.0	± 2.80[a]
	High chol.	17.1	± 3.3[a]
	Guava	-19.7	± 2.19[b]
	Pectin	-19.7	± 2.29[b]

*Different superscript notations showed that there were significant differences in treatment effects

3.2. SCFA (short chain fatty acid)

The SCFA examination (Table 6) aimed to find out the fermentation products of soluble fibers contained within the red guava in the colon, which included acetic, propionic, and butyric acids.

Table 6. SCFA digesta caecum contents by treatment group

Group	Average SCFA content (mg/g)		
	Acetic	Propionic	Butyric
Normal feed	10.2	4.3	1.0
High cholesterol feed	8.2	4.6	1.0
Red guava treatment	18.9	7.8	2.4
Pectin treatment	8.5	4.6	1.1

Table 7 shows that acetic, propionic, and butyric acids in the red guava group had higher contents than that of pectin group. Meanwhile, differential test between groups showed a significant difference between the red guava and pectin groups. This result proved that the red guava had a considerable capability of producing the SCFA.

Table 7. Different test on SCFA digesta caecum by treatment group

Parameter	Group	Mean	± SD *
1.SCFA : acetic	Normal feed	10.20	± 1.42 [a]
	High chol.	8.23	± 0.58[a]
	Guava	18.90	± 1.83[b]
	Pectin	8.49	± 1.01[a]
2.SCFA : propionic	Normal feed	4.34	± 0.32 [a]
	High chol.	4.60	± 0.26[a]
	Guava	7.77	± 0.56[b]
	Pectin	4.55	± 0.54[a]
3.SCFA : butyric	Normal feed	1.00	± 0.16 [a]
	High chol.	0.96	± 0.14[a]
	Guava	2.34	± 0.22[b]
	Pectin	1.06	± 0.27[a]

* Different superscript notations show that there are significant differences in treatment effects

3.3. *Cholesterol digesta caecum*
The examination of cholesterol digesta caecum aimed to measure the cholesterol contents excreted from the body by binding of soluble fibers contained in the red guava (Table 8).

Table 8. Cholesterol digesta caecum contents by treatment group

Group	Average cholesterol digesta caecum content (mg/g)
Normal feed	0,28
High cholesterol feed	0,45
Red guava treatment	0,54
Pectin treatment	0,46

Table 9. different test on cholesterol digesta caecum by treatment group

Parameter	Group	Mean	± SD *
Cholesterol caecum	Normal feed	0.28	± 0.04[a]
	High chol.	0.45	± 0.08[a]
	Guava	0.54	± 0.04[b]
	Pectin	0.46	± 0.04[a]

* Different superscript notations showed that there were significant differences in treatment effects

4. Discussion
Red guava was capable of repairing the lipid profiles because its dominant content was soluble fiber (pectin). Based on the test before and after treatment, there was a decrease in the level of total cholesterol (32%), LDL (43%), and triglyceride (18%), for HDL levels there was a significant increase (18%). This result was not different compared to the administration of pectin. Thus, it was proven that red guava fiber had the same ability as pectin in reducing cholesterol levels. This case indicated that the administration of guava fruit improved the lipid profile of hypercholesterolemic rats [16]. In theory, dietary fibers can reduce cholesterol rate through several mechanisms, including (i) inhibiting cholesterol absorption with the presence of dietary fiber, (ii) reducing cholesterol availability so that

the transfer rate to the blood decreases, (iii) preventing cholesterol synthesis with the presence of dietary fiber, (iv) reducing dietary energy density, and increasing bile excretion [17].

The explanation of the mechanism of blood cholesterol reduction in this experimental study was given by SCFA synthesis examination (acetic, propionic, and butyric). SCFA was the output of the soluble fiber fermentation from red guava by bacteria living in the colon. The examination of the cholesterol excretion aimed to explain the mechanism of cholesterol excretion from the body because of the cholesterol-binding from diet and bile secretion by soluble fiber within the red guava. The SCFA examination and cholesterol digesta caecum were intended to explain the hypocholesterolemic effect of the red guava on rats with hypercholesterolemia [18].

According to Brownlee *et al*., short chain fatty acid in the colon plays a significant role in various events. Acetic is useful for the primary energy source within the colon, whereas propionic is capable of suppressing cholesterol synthesis [18]. A study conducted by Demigne *et al.* concluded that propionic effectively inhibits cholesterol synthesis in the isolated hepar cells [19]. The current study showed that cholesterol reduction happened due to the inhibition of cholesterol synthesis by propionic. The cholesterol reduction by preventing cholesterol synthesis occurred at the stage of HMG-co A reductase enzymatic activity inhibition. This enzyme played a pivotal role in the formation of mevalonate, which was the main product of cholesterol formation. The inhibition of HMG-co A reductase enzymatic activity prevents the mevalonate as well as cholesterol formation [19]. The results of the current study showed that red guava was useful for an alternative agent to reduce cholesterol rate by prevention using natural and safe materials.

The mechanism of the cholesterol reduction in the body occurs by the mechanism of the increasing cholesterol excretion. This event took place by an increase in bile secretion as well as the increase in cholesterol excretion from dietary intake. The experiment showed that the average rate of cholesterol caecum in guava group was 0.54 mg/g and in pectin group was 0.45 mg/g (Table 8). The different test (Table 9) resulted in a significant difference between red guava group and pectin group [19].

The examination reports proved that red guava was capable of excreting cholesterol significantly. The cholesterol is excreted from the body by bile secretion in unesterified form and converted as bile acid derived from the liver. Primary bile acids (cholic and kenodeoxicholic acids) in the bile join glycine and taurine to form bile salt. At normal stance, the primary bile acids excreted by the bile to intestinum will be absorbed into the liver through enterohepatic path/circulation. After being excreted into the colon by bacteria, secondary bile acids (deoxycholic and lithocholic acids) existed. Furthermore, both primary and secondary bile acids were unified in the colon in the forms of sterol/cholesterol feces namely coprostano [20]. The binding of bile liquids by dietary fibers in the colon and simultaneous excretion through the feces, the liver will continue to absorb cholesterol from the blood to be metabolized and excreted to the bile. This process will reduce cholesterol content in the blood. The increasing excretion of the cholesterol due to the cholesterol binding from the dietary intake occurs because of the fundamental characteristic of the dietary fibers. Dietary fibers directly bound cholesterol from the diet to be excreted along with feces, reducing the absorbed cholesterol. Kristensen *et al.* supported this finding by concluding that flaxseed fiber intake for seven days increased fat excretion [20].

The above discussion has provided a further explanation that red guava possessed a hypocholesterolemic effect. The impact was found by the inhibition of the cholesterol synthesis by propionic SCFA derived from the soluble fiber fermentation in the red guava by bacteria in the colon. Another hypocholesterolemic effect occurred in the increase in the cholesterol excretion from the binding of the soluble fiber in the red guava. This experiment recommended treatment with red guava for the human with hypercholesterolemia to reduce blood cholesterol.

5. Conclusion

The red guava has been proven to possess hypocholesterolemic effect through an inhibition of the cholesterol synthesis by propionic SCFA derived from the fermentation of the soluble fiber by microbial in the colon and the increase in cholesterol excretion. Further studies on the importance of red guava for human medical treatment might be focused on the variances of the treatment, e.g., red guava-sourced tablets.

Acknowledgments
The author is indebted to the Ngudi Waluyo Foundation and Universitas Ngudi Waluyo that have provided financial support for this study.

References
[1] Gayatri S, Sumarjono D, and Satmoko S 2018 Understanding of social capital condition among red guava farmers in Tambahrejo Village, Pageruyung District, Kendal Regency IOP Conf. Series: Earth and Environmental Science
[2] Gutiérrez RM, Mitchell S and Solis RV 2008 A review of its traditional uses, phytochemistry and pharmacology J Ethnopharmacol; 117(1):1-27
[3] Moreno MA, Zampini IC, Costamagna M, Sayago JE, Ordoñez RM, and Isla MI 2014 Food and Nutrition Sciences 5: 725-732
[4] Mirmiran P, Bahadoran Z, Moghadam SK, Vakili AZ,and Azizi F 2016 Tehran Lipid and Glucose Study Nutrients 8: 686; doi:10.3390/nu8110686
[5] Maryanto S dan Fatimah S 2004 Jurnal M Med Indon 39(2):10-16
[6] Timm, DA and Slavin, JL 2008 American Journal Of Lifestyle Medicine; 2: 233
[7] Campbell DT and Stanley JC 1966 Experimental and quasi-experimental designs for research Boston: Houghton Mifflin Company: 13-24
[8] Mason RL, Gunst RF, and Hess JL 2003 Statistical Design and Analysis of Experiments John Wiley & Sons, Inc., Hoboken, New Jersey
[9] Reeves PG 1997 J Nutr; 127: 838S–841S
[10] AOAC International 2012 Official Method 2011.25 Insoluble, Soluble, and Total Dietary Fiber in Foods
[11] Clemens ME 2015 Dietary Fiber: Production Challenges, Food Sources And Health Benefits New York Nova Science Publishers, Inc
[12] Shurtleff W and Aoyagi A 2015 History Of Soy Fiber And Dietary Fiber Soyinfo Center, USA
[13] Wilson DD 2008 Manual of laboratory and diagnostic test Mc Graw Hill
[14] Kitson FG, Larsen BS, McEwen CN 1996 Gas Chromatography and Mass Spectrometry London. Academic Press
[15] Burke RW, Diamondstone BI, Velapoldi RA, and Menis O 1974 Clin. Chem. 20/7, 794-801
[16] Quan Z, Jiang W, Jie T, Jia-Ji W , Chu-Hong L, and Pei-Xi W 2015 Int. J. Environ. Res. Public Health, 12: 4726-4738; doi:10.3390
[17] Marsono Y 2004 Serat Pangan Dalam Perspektif Ilmu Gizi. Pidato pengukuhan Guru Besar pada Fakultas Teknologi Pertanian UGM
[18] Brownlee IA, Dettmar PW, Strugala V and Pearson JP 2006 Curr Nutr Food Sci 2006; 2:243–264
[19] Demigne C, Morand C, Levrat MA, Besson C, Moundras C and Remesy C 1995 British Journal of Nutrition; 74: 209-219
[20] Kristensen M, Jensen MG, Aarestrup J, Petersen KEN, Søndergaard L, Mikkelsen MS and Astrup A 2012 Nutr Metab ; 9(8)