

The Effect Of Wild Guava (*Bellucia Pentamera Naudin*) Yogurt On Cholesterol Levels In Mice (*Mus Musculus*)

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Abstract: Based on 2018 Riskesdas data, the proportion of total cholesterol levels in Indonesia is 21.2%. Cholesterol poses a complex issue in the human body. Managing high cholesterol involves limiting fat intake and maintaining a balanced diet. Wild guava, a fruit native to West Kalimantan, has a high Vitamin C content. Vitamin C serves as a natural antioxidant that protects the body from various diseases. Wild guava can be processed into yogurt, a functional food containing probiotics like Lactic Acid Bacteria (LAB) which have potential anti-cholesterol properties. Method: This study employed an Experiment Pre-Post Control Group Design with treatment and control groups. The research design involved five randomly divided groups, with three as treatment groups and two as control groups for comparison. Data were analyzed using Sample Paired T-test and One Way ANOVA to assess average differences before and after treatment and between treatment groups. Results: The average cholesterol levels pre and post-intervention in all groups showed a significant reduction in cholesterol levels with a p-value < 0.05. Additionally, the comparison of cholesterol levels between intervention and control groups also exhibited a significant decrease in cholesterol levels with a p-value < 0.05, particularly with a 15% fruit concentrate treatment showing differing averages. Conclusion: Administering wild guava yogurt (*Bellucia pentamera naudin*) led to a decrease in cholesterol levels in mice (*Mus musculus*), especially with the addition of a 15% fruit concentrate.

Keywords: wild guava; cholesterol levels; mice

A. Introduction

Cholesterol is a complex problem in the human body. Apart from having good benefits in the human body, on the other hand, cholesterol is very dangerous in the body if the amount of bad cholesterol (*low density lipoprotein*) dominates the amount of good cholesterol (*high density lipoprotein*) can cause several diseases such as heart disease, diabetes, and atherosclerosis. Cholesterol is one of the causes of cardiovascular disease, which is a deadly disease and has become a serious problem in developed and developing countries. (Rucita & Rahayuningsih 2013; Widyaningrum 2015).

Cardiovascular disease in Indonesia is the number one killer along with changes

in diet which tends to be high in fat and low in fiber. High cholesterol levels will cause the formation of plaques starting with the infiltration of fat/LDL proteins (*Low Density Lipoprotein*) into the arterial wall causing atherosclerosis. (Umamiet *al.* 2016).

Based on data from Riskesdas in 2018, the proportion of total cholesterol levels among people in Indonesia is 21.2% (Ministry of Health of the Republic of Indonesia 2018). According to WHO (2014), 37% of deaths in Indonesia are caused by heart and blood vessel disease (Balitbangkes RI 2018).

According to the book *Healthy Solutions* by Eliana & Sitanggang (2009), consuming too much and often food with



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high fat content, rarely doing physical activity and smoking are some of the main causes of hypercholesterolemia. Apart from fat, consuming carbohydrates can also increase cholesterol in the body. Therefore, consuming foods that contain high calories such as rice, cakes, noodles, snacks and bread must also be limited.

The way to deal with high cholesterol is to limit fat intake, avoid smoking, exercise regularly and adjust your diet to live a healthy life. Consuming more fiber such as vegetables and fruit is very good because fiber in food is related to cholesterol levels because it functions to bind fat that occurs in the intestines and stomach. Fiber forms gelatin and in the digestive process binds bile acids and cholesterol which is then excreted through feces, so that cholesterol levels entering the blood decrease (Djameludin & Tabrani 2020).

Forest guava fruit or forest monkey guava fruit is a term used by the people in Kapuas Hulu, West Kalimantan or in accordance with the Regulation of the Minister of Environment and Forestry of the Republic Indonesia Number P.94/Menlhk/Setjen/Kum.1/12/2016 with the name *Bellucia pentamera naudin*. This fruit belongs to the Melastomataceae family with the genus *Bellucia* and the species name *Bellucia pentamera Naudin*. This fruit is rarely consumed but is loved by fruit-eating animals in the forest such as civets, monkeys and birds (Setiawan et al. 2022).

Apart from that, forest guava fruit (*Bellucia pentamera naudin*) is a high quality fruit (high sugar content and low in defensive compounds). The use of forest guava fruit is still largely unknown. Although there are claims that it can be used as food, medicine and animal feed. There are several studies that use it on leaves, fruit and stems, such as using stem bark extract to increase antibacterial activity in *Escherichia Coli* and *Salmonella Typhi*, use of leaves as an anti-lavarricide,

and potential antioxidant compounds from endophytic fungi.

According to research on the nutritional content of forest guava fruit by FKIP students at Tanjung Pura University in 2018, it can be seen that the Vitamin C content in forest guava fruit is 46.93 gr/100 gr of fruit (Tendry et al, 2018).

Vitamin C is a natural antioxidant that can protect the body from various diseases related to the presence of free radicals if consumed regularly. Clinical research also shows that vitamin C can lower cholesterol and triglycerides in people with high levels, but not in those with normal levels. This means that vitamin C plays a homeostatic role to achieve balance (Aufa et al. 2020; Sunarsih et al. 2007)

In research on the effect of giving vitamin C on total serum cholesterol in hyperlipidemic male Wistar rats conducted by the Faculty of Medicine, Diponegoro University, the results were that giving vitamin C was able to reduce total serum cholesterol levels due to an increase in bile acid production caused by vitamin C which helps the hydroxylation reaction in the formation of bile acids thereby increasing cholesterol excretion, and ultimately total cholesterol levels will decrease (Sunarsih et al. 2007).

Yoghurt is a functional food that uses probiotics. Yoghurt is generally made using lactic acid bacteria (LAB), *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. These two bacteria cannot withstand the acidity level in the digestive tract, so probiotics are added so that the benefits of LAB last until digestion. The probiotics that are widely used in yoghurt are *Lactobacillus acidophilus* and *Lactobacillus casei* (Zulaikhah & Fitria 2020).

Yoghurt contains Lactic Acid Bacteria (LAB), namely *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Lactic Acid Bacteria (LAB) have the potential to act as anticholesterol because the first factor is the presence of exopolysaccharides or EPS which can bind cholesterol in the blood.

The second is because lactic acid produced by Lactic Acid Bacteria (LAB) degrades cholesterol into coprostanol which is a substance that cannot be absorbed by the intestines. Coprostanol and remaining cholesterol will be excreted in the feces. Apart from that, lactic acid can increase intestinal peristalsis so that less cholesterol is absorbed (Fitriyana 2017).

This research, conducted by Siraz University of Medical Science and Armenian Agricultural Academy, gathered respondents who had cholesterol levels of 200-304 mg/dl. For three months, they consumed 300 g of yoghurt every day and the results of the respondents' LDL (Low Density Lipoprotein) or bad cholesterol levels decreased.

The vitamin C content in forest guava fruit combined with the provision of good bacteria in the form of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in making yoghurt is expected to effectively reduce blood cholesterol levels.

This research will test the effect of forest guava fruit yoghurt on cholesterol levels using mice. Mice are widely used as laboratory animals because they have advantages such as a relatively short life cycle, large number of offspring per birth, easy to handle, reproductive characteristics similar to other mammals, anatomical structure, physiology and genetics similar to humans.

Based on the background above which shows that the availability of forest guava fruit is underutilized and has a fairly high vitamin C content, researchers are interested in conducting research with the title "The Effect of Forest Guava Fruit Yoghurt (*Bellucia pentamera naudin*) on Cholesterol Levels in Mice (*Mus musculus*)".

B. Materials and Methods

This study employed an Experiment Pre-Post Control Group Design with treatment and control groups. The research design involved five randomly divided groups, with three as treatment groups and two as control groups for comparison. Data

were analyzed using Sample Paired T-test and One Way ANOVA to assess average differences before and after treatment and between treatment groups.

In this research, a normality test was first carried out to see whether the resulting data was normally distributed or not so that we could continue with the statistical test of differences in means. Normality test results use the normality test *Shapiro Wilk* which shows that each data has a sig value > 0.05, which means all data is normally distributed. Therefore, The next data analysis will use parametric data processing analysis, namely the Paired Sample T-test.

C. Result

Product Description Guava Fruit Yoghurt (*I enjoy Bellucia Pentamera*)

Forest guava fruit (*Bellucia pentamera I enjoy*) is one of the typical fruits of the Kalimantan forest. This fruit is often found in the Kapuas Hulu area and is known as forest monkey guava fruit. In the Pontianak City area, especially North Pontianak District on Jalan Selat Panjang, there are many forests that still grow this type of fruit which is not used as a food product or for personal consumption. Therefore, this research uses forest guava fruit in the Jalan Selat Panjang forest to make Forest Guava Fruit Yoghurt which is divided into 3 different concentrates, namely with concentrate doses of 5%, 10% and 15%.

Based on the results of making forest guava fruit yoghurt with 3 concentrates, it produces 3 types of forest guava fruit yoghurt. In forest guava fruit yoghurt with a concentrate dose of 5% using 50 mL of forest guava fruit juice, it produces yoghurt with a white, slightly brownish color with seed spots with a thick texture like yoghurt, with a dominant sour and slightly sweet taste. For forest guava fruit yoghurt with a concentrate dose of 10%, using 100 mL of forest guava fruit juice produces a slightly brownish white color which is more visible than yoghurt with a concentrate dose of 5%, there are also seed spots with a thick texture like yoghurt in general with a

dominant taste. sour and slightly sweet. In forest guava fruit yoghurt with a concentrate dose of 15% using 150 mL of forest guava fruit juice, it produces yoghurt with a dominant brown color with seed spots with a thick texture like yoghurt in general, but has a stronger sour taste than the two previous concentrate doses. Apart from these 3 yoghurts, this study also had a control group as a comparison using pure yoghurt without the addition of guava juice and a control group without giving yoghurt.

After the guava fruit yoghurt and pure yoghurt products were finished, the Vitamin C levels were checked to see the differences in Vitamin C levels for each dose of fruit juice concentrate used by means of the Vitamin C Titration Test. The results of the vitamin C titration test showed that the average vitamin levels were C in pure yoghurt is 88 mg, 5% forest guava fruit yoghurt is 90.2 mg, 10% forest guava fruit yoghurt is 92.4 mg, and 15% forest guava fruit yoghurt is 101.2 mg. These results are in line with research by FKIP UNTAN students regarding the fairly high vitamin C content in forest guava fruit, where the more forest guava fruit juice is added, the higher the vitamin C level will be.

Induction of Hypercholesterolemia in Animal Experiments in Mice

In this study, experimental animals were used in the form of mice (*Mouse muscle*) with the characteristics of healthy white mice, males aged 2-3 months with a body weight ranging from 20-30 g. The mice were obtained from mouse and Wistar rat breeders from Pontianak.

Before the intervention was carried out, the mice were first acclimated to adapt to the new drum conditions for 7 days. After carrying out the acclimation process, the mice were induced with hypercholesterolemia to become mice with total cholesterol levels >130 mg/dl. The process of inducing hypercholesterolemia in mice is divided into 3 induction phases with different inducers. Induction in the first phase is carried out by providing a

mixture of 0.1% Profiltiuracil (PTU) in 1 L of water, 30 g of quail egg yolk and 120 mL of vegetable oil which is a modification of hypercholesterolemic feed (Umamiet *al.*, 2016). This preparation is given as much as 0.5 ml per day for 10 days.

Based on the results of the induction carried out in the first phase, the cholesterol level results obtained from several randomly taken samples showed that the cholesterol levels of the mice were in the Lo state (cholesterol levels < 100 mg/dl) where the mice were not yet considered hypercholesterolemic. Therefore, induction was carried out in the second and third phases by giving 0.5 mL of egg yolk per day for 5 days and giving 7 g of boiled eggs per day for 5 days and giving unlimited drinks mixed with 0.1% Profiltiuracil (PTU). in 1 L of mice drinking water. So, the total length of induction time is 20 days. After the induction process was carried out, the mice were then fasted for 12 hours before checking their total cholesterol levels. After undergoing the induction process, the mice's cholesterol levels reached hypercholesterolemia with cholesterol levels > 130 mg/dl. The following is the induction flow that is carried out to make mice hypercholesterolemic.

Forest Guava Fruit Yogurt Intervention

In this study, an intervention was carried out giving forest guava fruit yoghurt which was divided into several groups, namely the 5% dose yoghurt intervention group, the 10% yoghurt intervention group, the 15% yoghurt intervention group, the pure yoghurt control group and the no treatment control group. The intervention was carried out for 15 days by giving 0.5 mL of forest guava fruit yoghurt per mouse per day. This administration is based on the Food and Drug Administration Regulations regarding Pharmacodynamic Guidelines that the maximum volume of oral test preparations in mice is 1 mL. This intervention is accompanied by the provision of food and water as usual. The

control group without treatment was only kept and given food and water only. From the results of the 15 day intervention, the mice's total cholesterol levels experienced changes in decreasing cholesterol levels.

In this research, a test was used *paired sample t-test* which is a different (comparative) test analysis method. The aim is to find out whether there is a difference in the average between 2 paired samples. Therefore, this paired sample t-test was carried out to determine the average

Group	Min	Max	Mean	SD	Sig.
P1	-64	-20	-34.71	15.924	
P2	-50	-25	-36.14	10.915	
P3	-76	-27	-50.86	17.827	0.002
K (+)	-41	-20	-33.29	7.2014	
K (-)	-33	-10	-18.86	9.082	

differences between the groups before and after the intervention as follows (table 1).

Tabel 1. Average Cholesterol Levels Before and After Intervention

Paired Sample T-Test

Based on the results in Table 1, it can be seen that there was a change in the average reduction in cholesterol levels before and after the intervention in all groups, namely in the P3 intervention group with the highest average decrease before the intervention of 163.86 mg/dl, experiencing a decrease of 163.86 mg/dl. 50.86 mg/dl to 113 mg/dl with a significance of 0.000 < 0.05, so this change has a significant effect on reducing cholesterol levels. However, the control group experienced the smallest decrease in average cholesterol levels in the K (-) group or the group that was not given yoghurt treatment, with the average cholesterol level before being 178 mg/dl experiencing a decrease of 18.86 mg/dl to 136.43 mg/dl with a significance of 0.002 < 0.05, then this change still had a significant effect on reducing cholesterol levels, although not as much as the reduction in cholesterol levels in the group given the forest guava fruit yoghurt intervention. The decrease in cholesterol levels that occurs is directly proportional to the addition of forest guava fruit juice, where the more addition of forest

guava fruit juice can reduce cholesterol levels more quickly than not being given forest guava fruit yoghurt or pure yoghurt at all.

After knowing the average difference before and after the intervention, this research continued with testing *One Way ANOVA* which is a multivariable comparative analysis method where the data used must be normally distributed and homogeneous with the aim of determining the average differences between treatment groups as follows.

Tabel 2. Results of One Way ANOVA Test Between Treatment Groups

Group	Min	Max	Mean	Mean'	Δ	SD	Sig.
P1	103	199	157.2	122.5	-34.72	15.9	0.001
P2	110	186	159	122.8	-36.14	10.9	0.000
P3	103	199	163.8	113	-50.86	17.8	0.000
K (+)	103	186	157.7	124.4	-33.28	7.2	0.000
K (-)	119	178	155.2	136.4	-18.86	9.0	0.002

One Way ANOVA

Based on Table 2, it can be seen that the average cholesterol levels of the intervention group and the control group experienced a change towards decreasing cholesterol levels with significant results for all groups of 0.002 < 0.05. So, it can be concluded that the cholesterol levels of the intervention group and the control group have a significant average difference in reducing cholesterol levels in mice.

D. Discussion

Effect of Differences in Yogurt Vitamin C Levels

In this study, forest guava fruit yoghurt was made from 3 different concentrates with the results of differences in vitamin C content. The more forest guava juice added, the greater the vitamin C content contained in the yoghurt. Vitamin C levels in yoghurt produced by each treatment with the addition of 50 ml of forest guava juice resulted in an increase in vitamin C levels of 2.2-8.8 mg/dl. This is because the vitamin C content in forest guava fruit is quite high and when it is processed into yoghurt which basically also contains vitamin C, the vitamin C level in the fruit does not decrease too much because the nature of vitamin C is stable in acidic conditions (Chairunnisaa et al. 2021). This

is also directly proportional to the test on reducing cholesterol levels, that in the yoghurt group with a concentrate dose of 15% or the addition of 150 ml of forest guava fruit juice with a vitamin C content of 101.2 mg/dl had greater success in reducing cholesterol levels. This is supported by the results of scientific research conducted by Putri et al in 2017 which showed that increasing the dose of pineapple extract was able to reduce cholesterol levels caused by Vitamin C which could improve the lipid profile by forming bile fluid through extrahepatic cholesterol excretion (Octadiani Putri et al al. 2017). Apart from that, research was conducted on the effect of giving methanol extract of okra seeds, where okra seeds also contain vitamin C compounds which have the potential to reduce cholesterol levels. Giving high doses of okra seed methanol extract was able to reduce total cholesterol levels in hypercholesterolemic mice (Tandraini et al. 2020).

Effect of Cholesterol Levels Before and After Giving Guava Fruit Yoghurt and Pure Yoghurt

In this study, the experimental mice used were first induced to become hypercholesterolemic mice so that when the intervention was carried out, changes in cholesterol levels could be seen. Cholesterol levels before and after intervention in all treatment groups experienced changes towards decreasing cholesterol levels. However, the success of the intervention group given forest guava fruit yoghurt was greater compared to the control group given pure yoghurt and the control group without treatment.

The success rate in reducing cholesterol levels with an intervention period of 14 days in the control group without treatment was able to reduce cholesterol levels by 18.86 mg/dl, in the control group with pure yoghurt administration it was 33.28 mg/dl, while in the intervention group giving fruit yoghurt Forest guava at a dose of 5%, 10% & 15% can reduce cholesterol levels by 34.72 mg/dl, 36.14 mg/dl & 50.86 mg/dl. Where reducing cholesterol levels with the best success was in the P3 treatment by

administering forest guava fruit yoghurt at a dose of 15% or 150 ml of forest guava fruit juice.

The test results for the difference in average cholesterol levels in all groups before and after the intervention produced a p value <0.05, so it can be interpreted that there is a significant difference in the effect of reducing cholesterol levels in each group before and after the intervention. This is due to the effect of giving wild guava fruit yoghurt and pure yoghurt which was carried out for 14 days by not giving the mice any more hypercholesterolemia.

This research is directly proportional to the results of research conducted on the effect of giving tempeh flour on mice cholesterol levels, with the same length of intervention time it can have an effect on reducing cholesterol levels in the group without treatment but more cholesterol levels decreased rapidly in the treatment group with higher doses (Rumtal et al., 2021).

Effect of Cholesterol Levels in Mice between the Intervention Group and the Control Group

The test results show that the average difference between the intervention and control groups in all groups produces a p value of 0.02, where the p value is <0.05, so it can be interpreted that there is an average difference between the intervention and control groups in reducing cholesterol levels. in mice. In the intervention group, the average or change in cholesterol levels decreased more quickly than in the control group. Therefore, the addition of forest guava juice was proven to have an influence on changes in cholesterol levels with the result that cholesterol decreased when the intervention was carried out.

However, tests were carried out again to determine which groups had the same average or more significant reduction in cholesterol levels and it was found that the group treated with forest guava fruit yoghurt at a dose of 15% or 150 ml of forest guava fruit juice was much more significant in reducing cholesterol levels with a significance value of 0.001.

So, it can be concluded that the hypothesis which states that there is an

effect of forest guava fruit yoghurt on reducing cholesterol levels in mice can be accepted with a significant result of p value <0.05. Changes in cholesterol levels when giving forest guava fruit yoghurt at a dose of 15% had a greater effect on reducing cholesterol levels compared to other doses. This is caused by the fairly high vitamin C content due to the addition of 150 ml of forest guava juice.

E. Conclusion

The conclusion of this research is that there is an influence of vitamin C levels in yoghurt on the addition of forest guava juice, the more forest guava juice is added, the higher the vitamin C levels in yoghurt. The highest vitamin C levels were in the P3 group with the addition of 150 ml of forest guava fruit juice with the result of vitamin C levels of 101.2 mg/dl.

In addition, there was a difference in the average reduction in cholesterol levels before and after the intervention in all groups which was indicated by a significance result of <0.05. However, the group that was given a high dose of forest guava juice in the P3 group was faster in reducing cholesterol levels in mice, namely 50.86 mg/dl. The difference in the average reduction in cholesterol levels in the intervention group and the control group was also marked by a significance result of 0.002 <0.05. Apart from that, there was a treatment group that had a different or more significant average for reducing cholesterol levels, namely in the P3 treatment with a significant p value of 0.001.

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