Research Article

Potency of Red Guava Fruitghurt as an Antioxidant

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Abstract.
Antioxidants are compounds needed by the body to protect body cells from damage caused by free radicals. Free radicals can damage macromolecules such as cell membrane lipids, DNA, and proteins, and cause cell oxidative stress. Red Guava Fruitghurt is a probiotic with the basic ingredients of fruit. The purpose of this study is to determine the effect of fruitghurt on the MDA levels in the blood of mice induced by CCl4. This was an experimental study conducted using mice (Mus musculus) which were divided into three treatment groups – the negative control group (that were not induced and were not given fruitghurt), the positive control group (that were induced with CCl4 but not given fruitghurt), and the treatment group (that were induced with CCl4 and given fruitghurt). The data obtained in the study were analyzed using the one-way ANOVA test, and the results showed significant differences between the treatment groups. Giving fruitghurt to the P1 group had a significant effect on reducing malondialdehyde levels compared to the positive control group, with a *P*-value of 0.03. Lactobacillus acidophilus bacteria found in this fruitghurt can reduce oxidative stress by inhibiting ascorbic acid oxidation, reducing activity and capturing radial superoxide anions, hydrogen peroxide, and free radicals, and inhibiting lipid oxidation. So this fruitghurt has antioxidant properties. This is also strengthened by the content of red guava which is rich in phenolics, flavonoids, and vitamin C that have good free radical scavenging activity, so guava fruit is a natural source of antioxidants.

Keywords: antioxidants, fruitghurt, malon dialdehyde, probiotics, red guava

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1. Introduction

A free radical is an atom or molecule that has one or more unpaired electrons. Due to these unpaired electrons, free radicals tend to be highly reactive and can interact with other molecules in an effort to acquire additional electron pairs [1,2]. Free radicals can form naturally in the human body as a result of normal chemical reactions, but they can also be generated by external factors such as air pollution, excessive sunlight exposure, smoking, or exposure to hazardous chemicals [3]. And the habit of consuming unbalanced fast food meals, lacking in proper proportions of protein, carbohydrates, and fats [4]. Free radicals can interact with various molecules within cells, including proteins, lipids, and DNA, thereby causing oxidative damage to cells and tissues, and contributing to the development of various diseases such as cardiovascular disease, cancer, and aging and other degenerative diseases [3]. This is because the antioxidants in the body are unable to balance the influx of oxidants entering the body [1].

Our bodies have a natural defense mechanism to combat free radicals, known as the antioxidant system. Antioxidants are compounds that can provide additional electrons to free radicals, thereby helping to neutralize them and protect cells from damage [5].

Our bodies can produce endogenous antioxidants, including glutathione peroxidase, superoxide dismutase (SOD), catalase, and glutathione S-transferase [6]. However, endogenous antioxidants within the body may not necessarily protect cells as a whole against external oxidants. Therefore, the body requires exogenous antioxidants, which come from outside the body [1]. External antioxidants can come in the form of foods or beverages that we consume, including Vitamin C, Vitamin E, beta-carotene, selenium, and various phytochemicals found in fruits, vegetables, grains, and spices [3,7].

Fruitghurt is a beverage produced from fruit juice that is fermented by lactic acid bacteria such as Lactobacillus acidophilus. Lactose from the fruit juice can be converted into lactic acid by lactic acid bacteria (LAB) [8].

Red guava Fruitghurt utilizes red guava as its main ingredient. Red guava is a fruit that has a sweet and sour taste, and it is rich in vitamin C, fiber, which is important for the immune system and antioxidants that protect cells from oxidative damage. Additionally, it also contains protein, calcium, and probiotics that are beneficial for health [8,9].

The LAB bacteria found in Fruitghurt, such as Lactobacillus acidophilus, also exhibit high antioxidant activity. Previous studies have found that lactobacillus bacteria in Fruitghurt have high antioxidant activity, thereby providing benefits by supplying safe and effective natural antioxidants to consumers [10,11]. In addition, Fruitghurt has an advantage over regular yogurt as it can be consumed by individuals with cow's milk allergy (lactose intolerance) [12].

Red guava (Psidium guajava L) is one of the tropical fruits characterized by its bright red color and sweet taste with a hint of acidity. Red guava can be utilized as a functional food component due to its beneficial effects on human health. It contains relatively high levels of vitamin C and is rich in carbohydrates, iron, phosphorus, calcium, vitamin A, and beta-carotene [13] These components can limit the development of free radicals in the body or act as antioxidants. These antioxidants can prevent and repair liver cell damage caused by free radicals from used cooking oil by inhibiting the formation of free radicals at the initiation stage or limiting the continuation of chain reactions at the propagation stage [14,15]. In addition, red guava is rich in phenolic compounds and flavonoids, which can reduce oxidative stress by binding to free radicals. Flavonoids, in particular, contribute to 51.28% of the radical scavenging activity [4]. Flavonoids function as antioxidants by interrupting or trapping the chain reactions of free radicals' oxidation. As a result, free radicals are unable to react with biological components [16].

Red guava Fruitghurt is a probiotic beverage made from red guava as the main ingredient. The combination of these two ingredients is expected to provide greater health benefits, including its antioxidant properties in combating free radicals in the body.

Malondialdehyde (MDA) is a biomarker or laboratory parameter commonly used as an indicator of oxidative stress or free radicals [12,14]. Malondialdehyde is a highly reactive organic compound produced during lipid peroxidation, which is the oxidative degradation process of lipids. It is a byproduct of lipid peroxidation. When cells are exposed to toxins or free radicals, the lipids in cell membranes can become damaged, leading to the production of reactive oxygen species (ROS), including MDA [2]. MDA can be found in lipid peroxidation plasma, serum, and urine [14].

Carbon tetrachloride (CCl4) is a xenobiotic commonly used to induce and poison lipid peroxidation. The enzyme cytochrome P450 converts the CCl4 molecule into the free radical CCl3, which is responsible for the toxicity of CCl4 [2].

Assessing the potential health effects of regular consumption of red guava fruit yogurt on the body's antioxidant levels is crucial as it's highly associated with various conditions and diseases related to oxidative stress, such as cardiovascular diseases, diabetes, or cancer. Regular consumption of red guava fruit yogurt is expected to increase antioxidant substances in the body, which can counteract the dangers caused by oxidative stress.

Therefore, this research was conducted to determine the effectiveness of red guava Fruitghurt as an antioxidant in reducing MDA levels in the blood of mice induced by CCl4.

2. Materials and Methods

This study was a laboratory experiment using an experimental approach to evaluate the changes that occurred after the intervention.

The study subjects used were mice (Mus musculus), which had to meet inclusion criteria including being active and mobile (to ensure the mice were in a healthy condition), aged 10-14 weeks, and weighing between 20-40 grams. The exclusion criterion was a weight loss of more than 10%. Significant weight loss could indicate health problems or stress that might affect the research outcomes.

The object of this research is red guava Fruitghurt, which is made from red guava fruit fermented by Lactobacillus acidophilus. The inclusion criteria include the use of Lactobacillus acidophilus ATCC 4356 bacteria, a minimum bacterial count of 107 CFU/ml for Fruitghurt production, and the exclusion criteria are Fruitghurt with a foul odor and Fruitghurt contaminated with other substances.

The research subjects consisted of 27 mice, divided into 3 treatment groups: negative control group (NC), in which mice were not induced with Carbon Tetrachloride (CCl4) and were not given Fruitghurt; positive control group (PC), in which mice were induced with Carbon Tetrachloride (CCl4) and were not given Fruitghurt; and treatment group (T1), in which mice were induced with Carbon Tetrachloride (CCl4) and were given Fruitghurt.

Ethical approval was obtained from the Ethics Committee of Universitas Jenderal Achmad Yani with Approval No. 26/UH1.11/2022.

The research procedure began with the preparation of materials, tools, and bacteria. Then, Fruitghurt was prepared by adding 10% sucrose. Subsequently, the mice were acclimatized for 7 days in the Animal Laboratory of the Faculty of Medicine, Universitas Jenderal Achmad Yani. Blood samples were collected from the mice to examine the MDA levels, and then the mice were induced with CCl4, followed by the measurement of MDA levels in the mice. After 24 hours, Red Guava Fruitghurt was administered to the mice for 7 days, and then MDA levels were measured in the mice again (see Table 1).

The data testing began with the normality test using the Shapiro-Wilk test since the data sample size is less than 50. Then, data analysis was conducted using one-way analysis of variance (ANOVA) to assess the differences in antioxidant effects among the groups. Post hoc Tukey test was performed to evaluate the observed differences between the groups.

TABLE 1: Research groups.

No	Groups	Standard diet	Carbon Tetra-clorida (CCl4)	Fruitghurt	Duration of treatment
1	NC	+	-	-	16 days
2	PC	+	0,5ml/gr bw	-	16 days
3	P1	+	0,5ml/gr bw	1 ml/20gr bw	16 days

Description:
KN: Negative control group of mice not induced with Carbon Tetrachloride (CCl4) and not given Fruitghurt.
KP: Positive control group of mice induced with Carbon Tetrachloride (CCl4) and not given Fruitghurt.
P1: Treatment group of mice induced with Carbon Tetrachloride (CCl4) and then given Fruitghurt

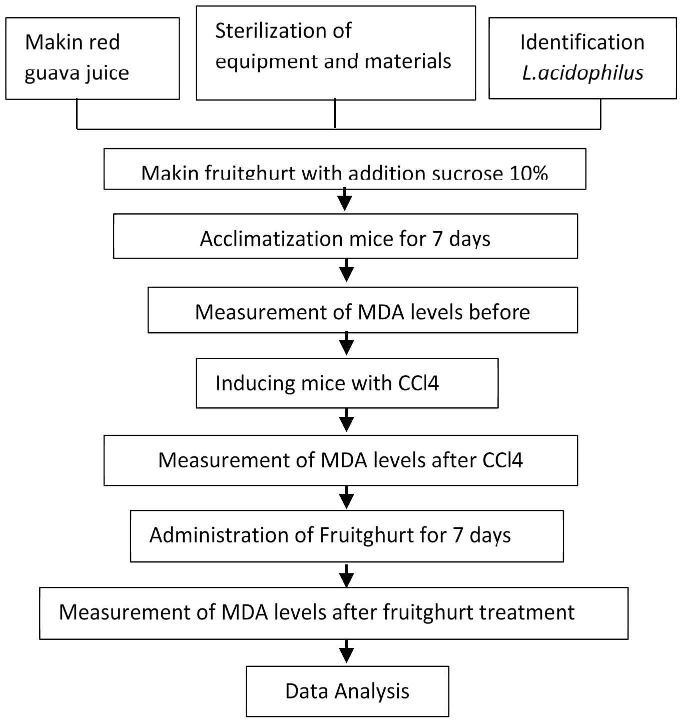

Figure 1: Research flow diagram.

3. Results and Discussion

The measurement of MDA levels was first conducted on the 8th day after the mice were given standard pellet feed and ad libitum drinking water, and before the induction of Carbon Tetrachloride (CCl4) at a dosage of 0.5ml/20g bw for the treatment group.

The effect of administering fermented red guava fruitghurt with Lactobacillus acidophilus as an antioxidant on the reduction of Malondialdehyde (MDA) levels is presented in Table 2.

TABLE 2: Presents the mean values of MDA levels in mice (Mus musculus) after the treatments.

Groups	Mean value of MDA (mg/dl)	Standar Deviasi (SD)	Min-Max
Negative control (NC)	1.096	0.275	0.81 - 1.36
Positive control (PC)	1.683	1.459	0.24 - 2.59
Treatment Group (P1)	0.996	0.267	0.69 - 1.18

Note:
KN: Negative control group of mice not induced with Carbon Tetrachloride (CCl4) and not given Fruitghurt.
KP: Positive control group of mice induced with Carbon Tetrachloride (CCl4) and not given Fruitghurt.
P1: Treatment group of mice induced with Carbon Tetrachloride (CCl4) and then given Fruitghurt

MDA levels are used as an indicator of oxidative stress or oxidative damage in experimental animals. The higher the MDA levels, the higher the level of oxidative stress. In this study, the mean MDA values were used to compare the levels of oxidative stress between the treatment groups.

In the negative control group, the mean MDA value was 1.096 mg/dL. The relatively low standard deviation indicates consistency in the MDA values within this group. The observed range of MDA values ranged from 0.81 mg/dL to 1.36 mg/dL.

In the positive control group, the mean MDA value was 1.683 mg/dL. The high standard deviation indicates a large variation within this group. The observed range of MDA values ranged from 0.24 mg/dL to 2.59 mg/dL. The high results in the positive control group compared to the negative control group indicate an increase in oxidative stress due to CCl4 induction.

In the treatment group, the mean value of MDA is 0.996 mg/dl. The low standard deviation indicates consistency in MDA values in this group. The observed range of MDA values is between 0.69 mg/dl and 1.18 mg/dl. The lower results compared to the positive control group indicate that the administration of fruitghurt to CCl4-induced mice can reduce the level of oxidative stress.

Table 3 presents the results of the normality test on the MDA levels in mice for each treatment group using the Shapiro-Wilk test. From the normality test results, it can be concluded that the MDA levels in each treatment group follow a normal distribution ($p > 0.05$). This indicates that the assumption of normal distribution is fulfilled, allowing for further analysis, in this case, using the one-way ANOVA test to compare significant differences among the treatment groups.

Table 3: Results of Normality Test for MDA Levels in Mice (Mus musculus) After Treatment.

Groups	Normality testfor MDA levels		
	P value	Distribution of data	Significant value
Negative control (NC)	0.860	Normal	0.033
Positive control (PC)	0.085	Normal	
Treatment Group (P1)	0.215	Normal	

Note: Shapiro-Wilk Test: p-value > 0.05 indicates normal distribution of data.
One-Way ANOVA Test: p-value < 0.05 indicates a significant difference

The one-way ANOVA test in Table 2 shows a p-value of 0.033. The obtained p-value is smaller than the predetermined significance level (0.05), indicating that there is a significant difference among the treatment groups in this study. Thus, based on the one-way ANOVA test, it can be concluded that the administration of fruitghurt derived from fermented red guava with Lactobacillus acidophilus as an antioxidant has a significant effect on reducing MDA levels in the experimental animals.

Table 4: Comparison of mean MDA levels among groups of mice.

	Groups	comparator	p-value
MDA	NC	PC	.058
		P1	.962
	PC	NC	.058
		P1	.042*
	P1	NC	.962
		PC	.042*

Note : The Tukey post hoc test results showed that the p-value was <0.05.
The asterisk (*) indicates statistical significance

In Table 4, a post hoc Tukey test was conducted to compare the differences in mean MDA levels between treatment groups. The comparison between the negative control group and the positive control group yielded a p-value of 0.058, which is greater than 0.05. Therefore, it can be concluded that there is no significant difference in MDA levels between these two groups..

The comparison between the negative control group and the treatment group resulted in a p-value of 0.962. This value is greater than 0.05, indicating that there is no significant difference in MDA levels between these two groups. Similarly, when comparing the positive control group (PC) with the treatment group (P1), the obtained p-value is 0.058, which is greater than 0.05, indicating no significant difference between the treatment groups.

However, there is a significant difference in MDA levels between the treatment group and the positive control, as well as between the treatment group and the negative control, with a p-value of 0.042, which is smaller than 0.05. Therefore, based on the post hoc Tukey test, it can be concluded that there is a significant difference in MDA levels between the treatment group and both the positive and negative controls.

In a previous study conducted by Trisnowati, it was found that red guava fruit contains beneficial antioxidants for the body. Antioxidants can inhibit and repair liver cell damage caused by free radicals by either inhibiting the formation of free radicals in the initiation stage or inhibiting the continuation of chain reactions in the propagation stage [15].

Red guava fruit possesses functional properties, particularly its high content of vitamin C, which can act as an antioxidant. Vitamin C in red guava fruit plays a crucial role as an electron donor (reducing agent) by transferring one electron to metal compounds such as Cu, thus stabilizing reactive oxygen compounds. Vitamin C donates electrons as part of hydrogen atoms and captures free radicals. Research conducted by Norazmir indicates that administering red guava fruit at doses ranging from 0.5 to 2.0 g/kg body weight of the test animals can reduce free radicals. This is because red guava fruit can enhance the activity of antioxidant enzymes in the blood of the test animals [17]. In addition, red guava fruit contains high levels of flavonoids. Flavonoids bind to peroxyl radicals formed during the chain reaction of lipid peroxidation and donate an unpaired electron to the free radicals, thereby breaking the chain. Flavonoids contribute approximately 51.28% of the radical scavenging activity [15].

In this study, the preparation of fruitghurt involved the addition of 10% sucrose. Lactic acid bacteria, including Lactobacillus acidophilus, are capable of breaking down glucose into lactic acid and other sugars such as lactose, galactose, fructose, sucrose, and maltose during the fermentation process. Lactic acid bacteria utilize fructose and glucose in the fruit extract present in the probiotic beverage as a carbon and nitrogen source for their growth during fermentation. As a result, the growth of lactic acid bacteria in this probiotic beverage will increase [18]. During fermentation, lactic acid bacteria (LAB) convert sugars into lactic acid through a series of chemical reactions. These bacteria utilize sugars as substrates to produce energy through their metabolic pathways. The process involves the breakdown of sugars into simpler components and their utilization within the bacterial metabolic pathways, ultimately resulting in the production of lactic acid as the end product [19,20].

4. Conclusion

Based on the conducted research, it can be concluded that the highest mean MDA level was observed in the Positive Control (KP) group, which was induced with Carbon Tetrachloride (CCl4) and not given fruitghurt. Furthermore, there was a significant difference between the positive control group and the treatment group, indicating that fruitghurt has a significant effect in reducing MDA levels.

Declaration of Interests

The author hereby declares that there is no conflict of interest in the scientific articles that we write

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