The effects of red guava (*Psidium guajava* L) fruits on lipid peroxidation in hypercholesterolemic rats

Sugeng Maryanto

Nutrition Science Study Program, School of Health, Ngudi Waluyo Jl. Gedongsongo-Candirejo, Ungaran-Semarang-Indonesia

*Corresponding author email: sugengmaryanto99@yahoo.co.id

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Background: Lipid peroxidation is a mechanism of cell injury, characterized by the formation of free radicals including malondialdehyde (MDA). To prevent or reduce the injury by free radicals, antioxidants are needed. Red Guava Fruit (*Psidium guajava* L) contains high antioxidants including vitamin C. This study aimed to determine the effects of red guava fruit on lipid peroxidation in hypercholesterolemic rats. Method: This study, a randomized Pre test-Post test control group design, was done on hypercholesterolemic Sprague Dawley rats. The rats were treated with 0.72 g red guava flour and vitamin C supplements equal to that in the red guava flour as a comparison for 14 days. SOD was measured by using the reaction of xanthine and xanthin oxidase (XOD). MDA was measured with tiobarbiturat acid (TBA) reaction. Data were analyzed by using a t-test and one way Anova with 5% level of significant. Result: There were an increase of SOD and decrease of MDA at both groups at almost the same percentage, 73% to 74% and 61% to 62% respectively. The mean of SOD and MDA post supplementation values of guava and vitamin C groups were almost at the same level with the group receiving standard diet, which were significantly higher and lower than the high cholesterol diet group respectively. Conclusion: Red guava fruit has the same antioxidant potency as vitamin C.

Keywords: red guava fruit, SOD, MDA, lipid peroxidation.

INTRODUCTION

Lipid peroxidation, a mechanism of cell injury, is characterized by unstable free radical formation and is decomposed to form some complex compounds including reactive carbonyl compounds, mainly malondialdehyde (MDA). MDA is a highly reactive compound that is the end product of lipid peroxidation and is usually used as a biomarker for assessing lipid peroxidation of biological oxidative stress (Guéraud, 2010).

To prevent or reduce chronic diseases by free radicals, antioxidants are needed. Antioxidants stabilize free radicals by completing the deficiency of electrons possessed by free radicals, inhibiting the chain reaction and the formation of free radicals that can cause oxidative stress. There are two types of antioxidants that are exogenous and endogenous antioxidants. Exogenous antioxidants are material from outside the body, entering through food or supplements. Food included in the exogenous antioxidants are: Alpha lipoic acid (ALA), Bilberry (Vaccinium myrtillus), Burdock (Artium lappa), Carotenoids, Coenzyme Q 10, Curcumin (Tumeric), Flavonoids, Garlic, Ginkgo biloba, Glutathione, Grape seed extract, Green tea, Melatonin, methionine, N-
Acetylcysteine (NAC), Nicotinamide Adenine dinucleotide (NADH), oligomeric Proanthocyanidins (OPCs), Pycnogenol, Selenium, Silymarin, Vitamin A, Vitamin C, Vitamin E and Zinc. While endogenous antioxidant enzymes in the human body which are superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (Bender, 2009).

Both supplement and antioxidant intake in the diet can increase the synthesis of endogenous antioxidant enzymes in the blood such as SOD (superoxide dismutase), GPx (glutathione peroxidase) and catalase (Castilla et al., 2008; Herrera et al., 2009). The presence of endogenous antioxidant enzymes in the blood plays a role in the inhibition of atherosclerosis by preventing the oxidation of LDL, in addition, to the endogenous antioxidants can reduce lipid peroxidation. There were clinical studies mentioning that the daily consumption of antioxidants in the diet can reduce the risk of atherosclerosis due to the oxidation of LDL and can prevent premature vascular diseases (Mukherjee and Das, 2011).

Fruits and vegetables contain high ascorbic acid, β-carotene and folic acid which are useful to the body as a bioactive that is directly related to the prevention of lipid peroxidation and vascular disease (Farinazzi-Machado et al., 2012). Guava (Psidium guajava L) which is rich with antioxidant is a tropical plant from America, spread to Southeast Asia, including Indonesia. Several types of guava are available, one of the most favored by Indonesia people is red guava (Ditjen BPPHP Departemen Pertanian, 2002).

Guava plants have many benefits. The leaves are used traditionally by many people to overcome a wide range of diseases, while the guava fruits are believed to overcome various of health problems including and a source of antioxidants (Sandra et al., 2012; Gutiérrez et al., 2008). Red guava fruit contains vitamin C, two times higher than other fruits such as orange (Mahattanatawee et al., 2006; Lim et al., 2006). Vitamin C is an important compounds that has an antioxidant activity (Thaipong et al., 2005; Monárrez-Espino et al., 2011; Thuytong and Anprung, 2011). Other compounds in guava fruit are carotenoids such as beta-carotene, lycopene, and beta-cryptoxanthin, and polyphenols (Nascimento et al., 2010; Oliveira et al., 2010; Ordóñez-Santos and Vázquez-Riascos, 2010). Lycopene is associated with the prevention of cardiovascular damage due the LDL oxidation, as the impact of dyslipidemia (Lorenz et al., 2012; Sesso, 2012).

Several studies about the benefits of guava have been done, either using guava leaves or the fruit. The research using guava leaves are proven to be beneficial for health, such as for cough medicine, antidiabetic, antimicrobes, antihypertensives, anti-inflammatory, analgesic, anti-diarrhea and spermatoprotective (Jaiarj et al., 1999; Oh et al., 2005; Deguchi and Miyazaki, 2010; Qadan et al., 2005; Ojewole, 2005; Ojewole, 2006; Akinola, 2007; Gonçalves et al., 2008). Several studies have proven the benefits of guava fruit for health. Antioxidant content in guava fruit can be used as a source of natural antioxidants (Jiménez-Escrig, 2001). Lycopene content in guava fruit is proven to prevent or delay the oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species (ROS) (Lim et al., 2006).

This study aimed to determine the effects of red guava fruit on lipid peroxidation in hypercholesterolemic rats. The expected benefit is the find of scientific explanation on the potency of red guava fruit in lowering lipid peroxidation as a contributing factor to atherosclerosis.

METHODS

Experimental design

The study was an experimental design with randomized Pre test-Post test control group design (Campbell and Stanley, 1966). The minimum sample size of each group was calculated using Federer formula, and was set out a number of 7 animals in each group (Federer, 1991).

MATERIALS

Red guava fruit in this study was the ripe fruit from the tree plantation in Sukorejo district Kendal regency, Central Java, Indonesia. Guava pulp was dried in an oven at a temperature of 60 °C to be used as an animal experiment food. Vitamin C content in the flour was analyzed by using AOAC titrimetric method (Ball, 2006). The content of vitamin C in red guava fruit flour was 0.398 mg/g.

Animal care and maintenance

Twenty-eight male Sprague Dawley rats aged 2 months weighing range of 150 -160 g from integrated research and testing laboratories (LPPT) Gadjah Mada University, were divided into 4 groups, fed with the American Institute of Nutrition Rodent Diets (AIN 93) standard for 3 days, followed by a high-cholesterol food of AIN 93.
standard (by adding 1% cholesterol crystals and sodium cholate) for 14 days (Reeves, 1997). After doing randomization, they were clustered in individual cages and continued the treatment for 28 days. First group received normal food (N) and the control group (C) was given high-cholesterol only; treatment group I (E₁) received high-cholesterol food plus red guava fruit; and treatment group II (E₂) received vitamin C equal to the content in red guava flour. Red guava fruit flour and vitamin C treatment were calculated as an adult dose converted based on Laurence and Bacharach to 200g rats (Ngatijan, 2006).

Collection of blood samples and determination of biochemical profiles

Blood samples were taken using a hematocrit pipette through retro-orbital sinus in 1 ml to measure the levels of Superoxide dismutase (SOD) and Malondialdehyde (MDA) were performed before and after supplementation. (Suckow et al., 2000). SOD was measured based on the reaction of xanthine and xanthin oxidase (XOD) to generate superoxid radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) superoxid formazan dye which produces one unit of SOD inhibits 50% reduction of INT (Matsunami et al., 2010). MDA was measured with the acid-base reaction tiobarbiturat MDA (TBA) which form colored compounds MDA-TBA2 and absorb light at a wavelength of 532-534 nm, using spectrophotometer (Tüközkan et al., 2006).

Statistical analysis

The variables are presented as means and standard deviations. The data were analyzed by T-test and LSD-test with 5% level of significance. The study was conducted after obtaining the Ethical Clearance from the Health Research Ethics Committee of Diponegoro University and Dr. Kariadi Hospital.

RESULT

SOD and MDA profiles

Table 1 and 2 depict the level of SOD and MDA before and after the supplementation. There were significant increase in SOD level in groups receiving supplementation up to 73%-74%. While in MDA, the supplementation resulted the decrease value in both groups by 61%-62%.

The Pre test mean value of the groups given high cholesterol food had lower SOD values, half of the group receiving normal food. However, with vitamin C and guava supplementation both groups had higher SOD level than the group receiving high cholesterol food with no supplementation.

The outcome of the MDA level show that at the pre supplementation, the normal food group had the lowest level value, and the all three groups receiving high cholesterol food had MDA level more than three times higher. At the end of the study of MDA level, the groups receiving guava and vitamin C had the same value, almost half of the group receiving high cholesterol food...
Table 3. SOD and MDA in different test groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>(Δ) means</th>
<th>± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. SOD</td>
<td>N</td>
<td>-1.21</td>
<td>± 66.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>30.3</td>
<td>± 34.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>E&lt;sub&gt;1&lt;/sub&gt;</td>
<td>266.9</td>
<td>± 26.54&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>271.2</td>
<td>± 35.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2. MDA</td>
<td>N</td>
<td>0.013</td>
<td>± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.3</td>
<td>± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>E&lt;sub&gt;1&lt;/sub&gt;</td>
<td>-4.1</td>
<td>± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-4.5</td>
<td>± 0.39&lt;sup&gt;c&lt;/sup&gt;</td>
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</tbody>
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Note: The number that followed by the same superscript letters indicate no difference

only. Table 3 shows that guava fruit and vitamin C supplementation give the same result in term of increasing the MDA level but gives different result in lowering the MDA level.

**DISCUSSION**

The study proves that red guava fruit increases levels of SOD significantly at p <0.001 (Table 1). Different test results between the groups shown that there were no differences between groups E<sub>1</sub> (guava) and E<sub>2</sub> (vitamin C) (Table 2). Based on these results it is said that red guava fruit has the similar to vitamin C which increases the levels of SOD in hypercholesterolemic rats. Guava fruit is a natural source of exogenous antioxidants including vitamin A, Phenols and Ascorbic Acid (Vitamin C) (Lim et al., 2006). The results of this study indicate that the consumption of guava fruit can increase endogenous antioxidant status, which is characterized by increased levels of SOD. The results are consistent with the research conducted by Hermsdorff of dietary antioxidants. It was reported that eating foods that contain high antioxidants (exogenous antioxidants) may increase the endogenous antioxidant status (enzymes SOD, GPx and catalase) (Hermsdorff, 2011).

SOD is produced naturally by the body, but the amount depends on the intake of exogenous antioxidants as well as the state of one’s body as well as the amount of free radicals in the body. The normal or balance amount of free radicals is important for the health and normal function of the normal to fight inflammation, kill bacteria, and control smooth muscle tone of blood vessels and organs in the body. In an excessive / unbalanced condition, free radical molecules which are not stable in the body are transformed into prey molecules, they will begin to move wildly and attack healthy or unhealthy body, causing disease. SOD is an endogenous antioxidant enzyme of glutathione peroxidase (GPx) and catalase, playing a role in counteracting free radicals in the body (Bender, 2009).

The presence of antioxidants is associated with a reduced risk of cardiovascular diseases, this is because the principle mechanism of antioxidants inhibits LDL oxidation and protects against nitric oxide. To repair this situation, it needs an exterminator of free radicals known as antioxidants. Free radicals in the blood vessels are associated with the oxidation of LDL, as the beginning of atherosclerosis. Knekt et al in their study explained that consumption of antioxidants can suppress the development of atherosclerosis by reducing oxidation of LDL to LDL-ox (Knekt et al., 2004). It was also reported by Plotnick et al in Katz that supplementation of vitamin C and E can prevent endothelial dysfunction, thereby potential as cardioprotective (Katz and Friedman, 2008).

Besides being able to enhance endogenous antioxidant SOD, red guava fruit consumption may reduce lipid peroxidation, which is characterized by decreased levels of MDA. This can be seen in Table 2, the red guava fruit (E<sub>1</sub>) showed to decrease the MDA hypercholesterolemic rats significantly (p <0.001). MDA is one of several lipid molecules as the result of decomposing endoperoksid form during the process of lipid peroxidation. MDA, therefore, is very appropriate to be used as an indicator of lipid peroxidation (Guéraud, 2010). Lipid peroxidation depends on the lipid intake and exogenous antioxidants from food and iodid level in blood. Knekt et al in their study of antioxidant vitamins and risk of CHD reported that antioxidants play a role in lipid peroxidation in the form LDL-ox. The resistance of antioxidant against lipid peroxidation may inhibit or prevent the formation of MDA which is characterized by reduced MDA levels (Knekt et al., 2004). The results also showed that red guava fruit was proven to lower LDL cholesterol significantly. Thus, LDL peroxidation in the form of formed LDL-ox is also reduced, so the MDA formed is reduced.

Based on the discussion, it was confirmed that the red guava fruit as a source of exogenous antioxidants is able
to reduce lipid peroxidation shown by the increase of endogenous SOD and the decrease of MDA level. Based on the results of this study, a further study of red guava fruit supplementation/consumption in human can be considered.

CONCLUSION

Red guava fruit is able to reduce lipid peroxidation showed by the increase of SOD and the decrease of MDA levels in animal experiment.

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