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The Effect of Papaya Leaf Juice Jelly (*Carica Papaya*, Linn) on the Levels of SOD (Superoxide Sismutase) and MDA (Malondialdehyde)

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Abstract. Papaya leaves are commonly consumed by people as vegetables and also as traditional medicine. The use of papaya leaves as herbal medicine is due to chlorophyll's active substance content, which is included in the antioxidant. Papaya leaves able to be processed in jelly form so that it able to use as snacks. This study conducted to examine the effect of papaya leaf juice jelly was treated on levels of Malondialdehyde (MDA) and Superoxide dismutase (SOD). The design of this study was a randomized pre-posttest on 20 healthy people. Papaya leaf juice jelly was given 24.6 grams perday for 20 days. Data were collected by blood analysis using the TBARS method for MDA and Elisa for SOD; the pairedt-test analyzed data. The MDA and SOD levels before treatment were respectively 10.13 mmol/mL and 45.36 mmol/m. The MDA and SOD levels after treatment were respectively 9.03 mmol/mL and 75.09 mmol/mL. There was a significant effect of papaya leaf juice jelly on decreasing MDA (p= 0.002) and increasing SOD (p= 0.001). The treatment of papaya leaf juice jelly per day for 20 days had a significant effect on decreasing MDA levels and increasing levels of SOD.

INTRODUCTION

Indonesia has various kinds of natural wealth, including the natural wealth of plants which include medicinal plants. Papaya is a local plant that is easily found in various regions of the world, including Indonesia. The community has long carried out medicinal plants and has been passed down from generation to generation as traditional medicine [1]. Papaya leaves are commonly consumed by people as vegetables and also as traditional medicine. The use of papaya leaves as herbal medicine is due to the active substance chlorophyll, which is included in the antioxidant group.

Antioxidants effectively reduce disease complications, especially from biological sources, one of which is chlorophyll. The amount of chlorophyll content in plants (± 1% dry weight), very potential as a food supplement / functional food [2].

Chloroplasts contain the enzyme superoxide dismutase, a potent antioxidant in neutralizing free radicals [3]. In addition to enzymes, chloroplasts contain 30-40% of the total ascorbic acid in plant tissues [4]. The role of ascorbic acid as an antioxidant is to become a hydrogen ion donor for free radicals so that radicals free to become more stable molecules. This makes the superoxide dismutase enzyme lighter in catalyzing the superoxide radical dismutase reaction into H2O2 so that its levels in cells are more maintained [5].

Increased consumption of phytochemicals with antioxidant activity can reduce the incidence of degenerative diseases such as atherosclerosis, cancer, and diabetes mellitus. Another factor that can trigger degenerative diseases is free radicals. Free radicals play an essential role in several biological processes, some essential for life, such as killing intracellular bacteria by phagocytic cells such as granulocytes and macrophages [6]. Excessive amounts of free radicals can cause cell injury and death, leading to many diseases such as cancer, stroke, myocardial infarction, diabetes [7]. Oxidative stress causes damage to nucleic acids, lipids and proteins that affect cell health and viability or induces various cellular responses through the formation of secondary reactive compounds and ultimately cell death due to necrosis or apoptosis [8].

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Prevention of degenerative diseases caused by free radicals can be done by improving food consumption patterns so that they fulfil all the nutrients needed by the body, including antioxidants [9]. To improve the taste of papaya, leaves can be processed in the form of jelly to be used as snack food for the community. Jelly is a food that is readily accepted by the community because of its small portion and refreshing taste. Due to the refreshing nature of jelly, people usually consume jelly after consuming foods with a sharp taste. Papaya leaf jelly has gone through laboratory tests, and the results show that 8.1 grams of papaya leaf jelly contain 7.4% (60 mg) of chlorophyll and 50.3% of antioxidants.

METHOD

This research aims to examine the effect of papaya leaf extract jelly against MDA and SOD. The design of this study was an experimental design with a randomized pre-posttest design. The study population was Ngudi Waluyo University Ungaran, Central Java, with healthy categories seen from weight, height, Body Mass Index (BMI), blood sugar levels, and blood pressure.

Respondents were selected by randomized sampling with a total of 20 people from various study programs. Before and after the intervention, SOD and MDA levels were measured. The jelly intervention was given as much as 24.6 grams for 20 days. Controlled food intake using a food recall 3 x 24 hours.

Data collection by blood analysis using the TBARS method for MDA was carried out at the Diponegoro University Laboratory. The Elisa method for SOD was carried out at PAU Gajah Mada University. Data analysis in this study used a normality test and paired t-test.

RESULT AND DISCUSSION

Table 1 indicates that the respondents who were included into early adulthood are in the age of 19-35 years. BMI of all respondents was classified as usual (BMI > 18.5 – 25.0) with an average BMI of 20.24 kg/m2. Blood sugar and blood pressure within normal limits respondents classified. It can be concluded that the 20 respondents were in a healthy state.

TABLE 1. Characteristics of Respondents.

Characteristics	Average ± SD
Age	20,50 ± 1,54
BMI	20,24 ± 4,20
Blood sugar levels	125,95 ± 16,31
Blood pressure	126,5 ± 4,89

Table 2 shows that the level of fibre intake is shallow because the respondents' consumption of vegetables and fruit is still significantly less than what is recommended by WHO, which is about five servings/day.

TABLE 2. Description of Respondents Intake Level.

Variable	Average ± SD	P
Energy intake level	48,43±7,75	0,008
Protein intake level	68,04±15,49	0,035
Fat intake level	52,86±15,61	0,002
Carbohydrate intake level	50,06±9,20	0,005
Fiber intake level	34,46±13,08	0,011

The results of the paired sample t-test in Table 3, p-value = 0,001 was obtained. Statistical test result $p < 0.05$ indicates different levels of SOD scores before and after intervention jelly papaya leaf chlorophyll. Chlorophyll contained in papaya leaves has a positive effect on the body because the bioactive compound chlorophyll is a chain-breaking antioxidant that works by donating its electrons to free radicals, especially at initiation before hydroperoxide is formed. The critical structure of chlorophyll, which has antioxidant activity, is in the structure of the porphyrin.

TABLE 3. Effect of Papaya Leaf Chlorophyll Jelly *(Caricapapaya* Linn) on Superoxide Dismutase (SOD) Levels.

Variable	Min	Max	Average ± SD	p
Before treatment	16	75	45.36±14.97	0,001
After treatment	47	87	75.09±10.21	

The content other than chlorophyll in chloroplasts has the antioxidant capacity, namely exogenous superoxide dismutase, especially Fe-SOD, Cu, Zn-SOD, and Mn-SOD and ascorbic acid. Ascorbic acid makes superoxide dismutase lighter in catalyzing the reaction of superoxide radical dismutase to become more stable H2O2, so cell levels are maintained [10].

The role of other components left in papaya leaf extract, which is rich in chlorophyll and has antioxidant activity, is thought to be saponins. Saponins increased the antioxidant defence system by activating catalase and superoxide dismutase to provide ion hydrogen [11].

The combination of antioxidant components in papaya leaf extract rich in chlorophyll, including chlorophyll, magnesium, exogenous superoxide dismutase, vitamin C, and saponins, may not have a pro-oxidant effect in experimental animals. Physiologically presence of chlorophyll in the leaves of papaya also is a consideration because the body will form its homeostasis.

Giving oral treatment also gives effect to be safer than other means. Saponins have a toxic effect when given intravenously, but the toxic effect will be reduced if given orally. Interaction between antioxidants in papaya leaf extract rich in chlorophyll possible synergy so that the antioxidant activity and potential hypoglycemic more optimal. The presence of vitamin C increases protective proteins (superoxide dismutase and catalase) and reduces oxidative stress [12]. This is supported by previous studies, which suggested vitamin C can increase the activity of Cu, ZnSOD in the liver. Previous research also explained that magnesium in the form of chelates, when combined with protein and porphyrins to form chlorophyll, also synergizes in antioxidant function.

The mechanism of chlorophyll in hepatoprotection is not yet known with certainty. So far, chlorophyll is believed to have antioxidant activity and protect body cells from the harmful effects of ROS. This is supported by the understanding that tetrapyrrole (including chlorophyll) can form a group of prosthetic metalloenzymes such as sulphite reductase, catalase, peroxidase, and nitrite reductase, which are capable of carrying out various oxidation and reduction reactions in the body [16].

TABLE 4. Effect of Papaya Leaf Chlorophyll Jelly (*Caricapapaya* Linn) on Malondialdehyde (MDA) Levels.

Variable	Min	Max	Average ± SD	p
Before treatment	10,3	13,29	11,74±0,87	0,002
After treatment	9,03	12,98	11,74±0,87	

MDA in the body is formed due to oxidative stress conditions, namely an imbalance between the formation of reactive oxygen species (ROS) and the presence of antioxidants so that free radical levels are higher than antioxidants. Excess hydroxyl radicals and peroxynitrite can attack cell membranes and lipoproteins to form lipid peroxides. The reaction produces MDA and some conjugated diene components. Thus, the higher the MDA level, the greater the potential for damage to cell membranes and lipoproteins. Damage to cell membranes and lipoproteinsdue to MDA can increase cancer incidence and degenerative and atherogenic diseases [17].

Until now, no information states normal MDA levels in the human body, both men and women; however, the results of research in Iran showed that plasma MDA levels in healthy women aged 20-45 years with normal BMI (19-25 kg/m2), the value is less than 1.4±0.3 mol/L.117 Thus, the average MDA level of the subjects before andafter the intervention is above the average value of the study results in Iran. The higher MDA levels are thought tobe due to more air pollution in Indonesia compared to Iran.

Snacks that are usually sold in the market generally use oil that is used repeatedly. The research results on repeatedly breathed palm oil showed that the formation of free radicals was relatively high [18]. Statistical analysis showed a difference before and after treatment for 20 days by giving chlorophyll jelly with p-value ($p = 0.002$) through different test paired t-test. Based on the mean in Table 4, it is known that before and after treatment, there was a decrease in MDA levels in respondents.

Papaya leaf chlorophyll jelly given for 20 days was able to reduce malondialdehyde (MDA) levels with $p<0,05$, this study was able to prove previous research on experimental animals, which showed that chlorophyll was able to reduce MDA levels [10] significantly. The average level of malondialdehyde (MDA) before treatment was 11.74±0.87 nmol/ml. After administration of papaya leaf, chlorophyll jelly decreased to 10.82±1.06 nmol/ml, which illustrates a significant effect of giving chlorophyll jelly on subjects with healthy people.

This is in line with research with IPB student subjects that the intervention group of green grass jelly chlorophyll experienced a significant decrease in MDA levels between before and after the intervention for 21 days [13]. The statistical test results of the effect of giving papaya leaf chlorophyll jelly on malondialdehyde (MDA) levels can be seen in Table 4.

Consuming papaya leaf chlorophyll jelly has been shown to reduce MDA levels. The results of this study are in line with previous studies which were able to inhibit the occurrence of lipid peroxidation. Another study showed that rats that were intervened with a chlorophyllin diet reduced MDA levels and could prevent mutations from being

induced by fighting (reversed) oxidative stress [14]. Furthermore, it was also reported that chlorophyll could protect mice from oxidative damage caused by oxidants [15].

CONCLUSION

The provision of chlorophyll jelly is high in antioxidants from leaves of papaya (*Carica papaya* Linn), which amounted to 24,6 grams for 20 days, provides an effective reduction of the MDA and provide an effective increase in SOD levels in healthy people.

REFERENCES

1. U. Rahmawati, E. Suryani, A. Mukhlason. *Pengembangan repository pengetahuan berbasis ontologi (ontology-driven knowledge repository) untuk tanaman obat indonesia*. Jurnal Teknik Pomits. 1-6 (2010)
2. S. Winarti. *Makanan Fungsional*. Yogyakarta: Graha Ilmu. 70-108 (2010)
3. H. Winarsi. *Antioksidan Alami Dan Radikal Bebas*. Yogyakarta: Kanisius (2007)
4. H. B. Shao, L. Y. Chu, Z. H. Lu, dan C. M. Kang. *Primary antioxidant free radical scavenging and redox signaling pathways in higher palnts cell.* Int J. Biology Science.**4**(1):8-14 (2008)
5. T. Wresdiyati, M. Astawan, D. Fithriani, I. K. M. Adnyane, S. Novelina, and S. Aryani. *Pengaruh α-tokoferol terhadap profilsuperoksida dismutase dan melondialdehida pada jaringan hati tikus di bawahkondisi stress*. Jurnalveteriner. (2011)
6. P. Pacher, J. S. Beckman, L. Liaudet. *Nitric oxide and peroxynitrite in helath and desease. Physiological reviews.***87**(1):315-424 (2007)
7. M. Karthikeyan, A. O. Dileep, B. A. Shamasundar. *Effect of water washing on the functional and rheological properties of proteins from threadfin bream (Nemipterus japonicus) meat.* International Journal of Food Science and Technology. **41**:1002–1010 (2006)
8. I. Dalle-Donne, R. Rossi, R. Colombo, D. Giustarini, A. Milzani. *Biomarkers of oxidative damage in human disease*. (2006)
9. Acha. *National college health assessment: reference group executive summary spring 2013.* Hanover (2013)
10. G. S. Pontang, *Efek pemberian chlorophyllin terhadap kadar nitric oxide dan malondialdehida tikus hiperkolesterolemia*. Jurnal Gizi Indonesia. (2014)
11. O.O. Elekofehinti. *Photosynthesis-making sugar from sunlight.* IUPUI Departement of Biology. (2004)
12. M. Khassaf, A. McArdle, C. Esanu, A. Vasilaki, F. McArdle, R. D. Griffith, D. A. Brodie, dan M. J. Jackson. *Effect of vitamin C supplement on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle.* J. Physiol. **549**(2):645-652. (2003)
13. I. Makaryani, L. Amalia *et al. Pengaruh Pemberian Pangan Antioksidan terhadap Malondialdehyde Plasma Mahasiswi Penyuka Gorengan.* Jurnal Gizi Klinik Indonesia. **10**:169-179 (2014)
14. G. S. Pontang. *Efe kpemberian chlorophyllin terhadap kadar nitric oxide dan malondialdehida tikus hiperkolesterolemia.* Jurnal Gizi Indonesia. (2014)
15. P. A. Egner, J. B. Wang, Y. R. Zhu, B. C. Zhang, Y. Wu, Q. N. Zhang, G. S. Qians, S. Y. Kuang, S. J. Gange, L. P. Jacobson *et al*. *Chlorophyllin intervention reduces aflatoxin – DNA adducts in individuals at high risk for liver cancer*. PNAS **98** (25) 14601-14606 (2001)
16. A. G. Smith, M. Witty. *Laboratory methods for the study of tetrapyrrol*. New Jersey: Humana Press (2002)
17. E. A. Shalaby, S. M. M. Shanab. *Antioxidant compounds, assays of determination and health*, Int J Biomed Sci. **4**(2):89-96. 2008
18. X. F. Leong, J. Salimon, M. R. Mustafa, K. Jaarin. *Effect of repeatedly heated olein on blood pressure-regulating enzyme activity and lipid peroxidation in rats*. Malays J Med Sci. **19**(1),9-20 (2012)