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Tender Coconut Water To Prevent Oxidative Stress Due To Mercury Exposure

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Abstract: *Gold mining is the global largest source of mercury pollution. In Indonesia, Wonogiri Regency, Central Java, mining workers in one of the traditional mining area use mercury for amalgamation of gold. A preliminary survey showed that about 57,5% of the workers traditional gold miners had a high blood mercury levels. The aim of research was to confirm that coconut water can prevent oxidative stress resulting from mercury exposure. This was an experimental research with a randomized pre and post test control group design. The treatment group was given tender coconut water (450 ml/ day for 30 days) and the control was given water. The 38 mining workers recruited randomly were given a questionnaire and were subjected to MDA level using Elisa method. The Data were analyzed using Mann Whitney test for abnormal distribution. There was no difference between the two groups. The mean of MDA levels after treatment in the control group and treatment were 4.5 ± 0.5, 3.6 ± 0.4 respectively. This study resulted in p-value: 0.000. These results indicate that intake of tender coconut water can prevent oxidative stress due to mercury exposure the traditional gold miners.*

Keywords: *Mercury, traditional gold miners, tender coconut water, oxidative stress, MDA,*

I. Introduction

Gold mining is the largest source of mercury pollution.. In Indonesia, Wonogiri Regency, Central Java, there are traditional gold mining activities using amalgamation method to extract gold from ore using mercury. A preliminary survey showed that about 87 workers of the gold mine had a high blood mercury levels (6.07 g / L-257,87μg / L).[1] High levels of free radicals in the body can be indicated by high levels of plasma MDA, MDA is used as a marker of lipid peroxidation in the body and can increase the lipid peroxidation hydroxyl radicals (OH) triggering oxidative stress.[2]

A continuous exposure to mercury will trigger the formation of Reactive Oxygen Species (ROS), and cause oxidative stress in cells.[3] Oxidative stress can occur as a result of ROS amount exceeds the amount of antioxidants in that attack the components of the lipid, protein, and DNA.[2] The emergence of ROS action caused by low antioxidant system leading to less effective ROS scanvanging. Low antioxidant defense system is due to reduced thiol groups on the protein antioxidants and antioxidant bounding to mercury, causing depletion of antioxidants and decreased activity of antioxidant enzymes. A study by Al-azzawie et al,[4] showed duration of mercury exposure is significantly associated with levels of blood mercury and mercury levels in the blood affect MDA levels in workers exposed to mercury.

Curent studies have focused on strategies to protect cells and organs from oxidative stress caused by free radicals. Since the possibility of side effects of synthetic antioxidants has not been established, natural antioxidants have a potentive to be developed. In recent years, food sources with naturally occurng antioxidants can be used as a strategy to reduce morbidity and mortality, especially due to oxidative stress.[5].

Coconut water is a healty natural drink, , nutritious containing a variety of nutrients that lower levels of MDA. Bhagya et al,[6] the present study that tender coconut water effectvely reduced the oxidative stres in fruktosa rats. Loki and Rajamohan[7] have shown that the administration of tender coconut water significantly reduce lipid peroxidation on carbon tetrachlorida (CCl_4) induced in rats. Anurag and Rajamohan[8] state in their research that tender coconut water can provides protection against the free radical mediated damage, induced by isoproterenol in rats. The purpose of this study was to evaluate that coconut water can prevent oxidative stress in the traditional gold miners exposed to mercury.

This was a true experimental study with a randomized pre and post test control group design.[9] The population were all traditional gold miners in Selogiri Wonogiri listed in the mining worker as many as 400 people. Samples are calculated based on the formula of the samples and obtained a sample of at least 38 people, a technique done by *simple random* sampling.[10,11] Inclusion criteria included living in Wonogiri, male, 20-60 years of age with an employment duration ≥ 5 years, normal (BMI) and willing to become respondents, while the exclusion criteria were having immune system disorder, a history of heart brain infarction, pulmonary

tuberculosis, diabetes, sepsis and severe infections or other disorders brain infartion and disease, liver disease, lung disease, kidney disease, diabetes, and other severe diseases. The sample used for the examination of mercury levels and venous blood levels of MDA was the respondent. Sampling of venous blood was carried out at the site of the respondents, in Selogiri Wonogiri, while mercury levels checked at the Center for Environmental Health Engineering for Disease Control and Yogyakarta (BBTKL) method Mercury Analyser Cold Vapour, and examination of MDA levels as parameters of lipid peroxidation was done in the laboratory PAU UGM Yogyakarta using reagent kit BIOXYTECH MDA-586.

Delivery of blood samples from Wonogiri to Yogyakarta is conditioned at a temperature of 4-10^{0}C, venous blood is inserted into a test tube which already contains EDTA, the tube is then inserted into the cold box by ice, then taken to the laboratory. A pre-test was done by measuring worker's blood levels of MDA, after the pre-test sample treatment group were given coconut water 450 mL / day for 30 days and the control group was given water. Thirty days after the pre-test and post-treated test is performed to measure levels of MDA in both sample groups, pretest and post test results were compared. Data were analyzed using SPSS, normality test was performmed on data because the data was a ratio scale, using *Shapiro Wilk* normality test because the sample size <50, the second data normality test resulted in variables p-value <0.05, meaning data distribution was not normal. To test the hypothesis , Mann Whitney test with α 5% was performed.[12] This study has obtained permission (ethical clearance) from the Health Research Ethics Committee (IEC) Diponegoro University School of Medicine and Hospital, Dr. Kariadi with (registration number 481 / EC / FK-RSDK / 2014).

The mean levels of MDA in treatment group after administration of coconut water 450 mL / day for 30 days in the traditional gold miners exposed to mercury decreased from 4.5 nmol / mL to 3.6 nmol / mL, while the control group given water has increased from 4.4 nmol / mL to 4.5 nmol / mL. The mean levels of MDA in the control and treatment groups after treated decreased by 0.9 nmol / mL (20%). The results of the analysis of the average levels of MDA after being treated in the control group and the treatment group resulted in p = 0.000 means at α = 5% Ha accepted that the provision of coconut water 450 ml / day for 30 days lowered MDA levels in traditional gold mining workers who are exposed mercury. The results can be seen in table 2.

Table 2. The mean MDA levels before and after the control group and the treatment group.

variable	Treatment (n: 38)		Controls (n: 38)		p-value
	pre	Post	pre	Post	
MDA Levels	4.5 ± 0.5	3.6 ± 0.4	4.4 ± 0.5	4.5 ± 0.5	0,000

Coconut water given to the treatment group was coconut water that comes from Pacitan, to ensure that the tender coconut water has not been contaminated with mercury because of the distance between the location of research with Pacitan ± 50 km, another reason for the region Selogiri and surrounding oil demand is supplied from Pacitan , Coconut water is taken from coconut aged 5-7 months, because at this time of coconut water has a taste of the most sweet and tasty, water and sugar content reaches a maximum at that age and will be reduced with age maturity of the coconut fruit.[13-15] Lipid peroxidation in this study was measured by the levels of malondialdehyde (MDA) in plasma. MDA is one of the products of lipid peroxidation oxidation of polyunsaturated fatty acids in cell membranes, increased levels of MDA is an important indicator of lipid peroxidation and lipid peroxidation can improve hydroxyl radicals (· OH) that would trigger oxidative stress. High levels of free radicals in the body can be demonstrated by the high levels of malondialdehyde (MDA) in plasma.[2]

Mercury is a free radicalhavinga strong affinity with thiol or sulfhydryl.[16,17] Mercury will bind the thiol group contained in the antioxidant protein that antioxidant activity. This condition causes the work activities of antioxidant enzymes inhibited so there was a decrease in glutathione and increased H_2O_2.[16] Hydrogen peroxide when reacted with a transition metal *(*transitional metals*)* such as Fe^{++} and Cu^{+} in the Fenton reaction will produce hydroxyl radicals(OH) which is the most dangerous because of very high reactivity, these compounds can lead to oxidative stress.[2,18] Hydroxyl radicals can damage the unsaturated fatty acids which are components of the phospholipid constituent of cell membranes rich source of *poly unsaturated fatty acid* (PUFA) which can cause lipid peroxidation. MDA is the most widely used marker for measuring lipid peroxidation in humans and animals.

MDA has been used as a clinical marker of lipid peroxidation. Increased content of MDA is an important indicator of lipid peroxidation. Lipid peroxidation is a chemical mechanism that is capable of disrupting the structure and function of biological membranes that occurs as a result of free radical attack on lipids.[2] The ability of mercury to produce ROS are generated in this study with an increase of lipid peroxidation. This result is supported by Alfanie's stating that mercury causes increased levels of MDA. The results of this study showed that tender coconut water is proven to reduce levels of MDA, there is a significant difference in the mean levels of MDA before and after administration of coconut water. The results are consistent with a research conducted by Bhagya, et al.[6] that the tender coconut water is proven to reduce lipid peroxidation characterized by decreased levels of MDA in mice with oxidative stress due by dietary fructose, of MDA in the

group given coconut water is lower when compared with in control group. Loki and Rajamohan[7] reinforce the results of this study, which states that coconut water can reduce levels of MDA in rats CCl_4, the mean levels of MDA in rats CCl4 without coconut water is 1.680 ± 0.034 mM / 100g, while the average level of MDA in mice given CCl4 and were given coconut water 1,147 ± 0.023 mM / 100 g, no significant difference in the mean levels of MDA in mice that were given CCl_4 and the coconut water with a group of mice that were given the CCl_4 without coconut water ($p < 0.05$).

Tender coconut water is rich source of free amino acids L-arginine and vitamin C, which can prevent heart disease and prevent lipid peroxidation.[6,8]. Vitamin C in coconut water 3,25 mg / 100mL, this vitamin has a role as an electron donor, is able to neutralize and reduce ROS that can prevent oxidative stress resulting from exposure to free radicals and can reduce lipid peroxidation. Vitamin C and vitamin E has a protective effect the toxicity of mercury.[4] The role of vitamin C works synergistically with vitamin E in inhibiting lipid peroxidation process. Vitamin E is an antioxidant chain breaker of the membrane that can prevent cell damage by inhibiting lipid peroxidation and free radical formation.[2,19] Vitamin E is oxidized by free radicals can react with vitamin C, after obtaining the hydrogen ion of vitamin C will be transformed into vitamin E.[20] Vitamin E in the membrane to react with lipid radicals ($LOO^{\bullet}$) radicals form of vitamin E (vit. $E^{\bullet}$). Radical vitam E reacts with vitamin C to form free radicals vitamin C (vit. $C^{\bullet}$). Radical vitamin C (vit. $C^{\bullet}$) will regenerate into vitamin C, involving glutathione (GSH). GSH is oxidised to oxidized glutathione (GSSH) by the enzyme glutathione peroxidase (GPx), GSSH will be reduced back to form GSH by the enzyme glutathione reductase (Gred) involving NADPH as an electron donor. Methionine contained in coconut water can be used as a source of thiol. The content of methionine in coconut water measured simultaneously with tryptophan as much as 23.5 mg / 100mL. Methionine acts as a precursor for the formation of cysteine is the main compound in the synthesis of glutathione (GSH). The role of vitamin C and E in inhibiting lipid peroxidation presented in Figure 1.

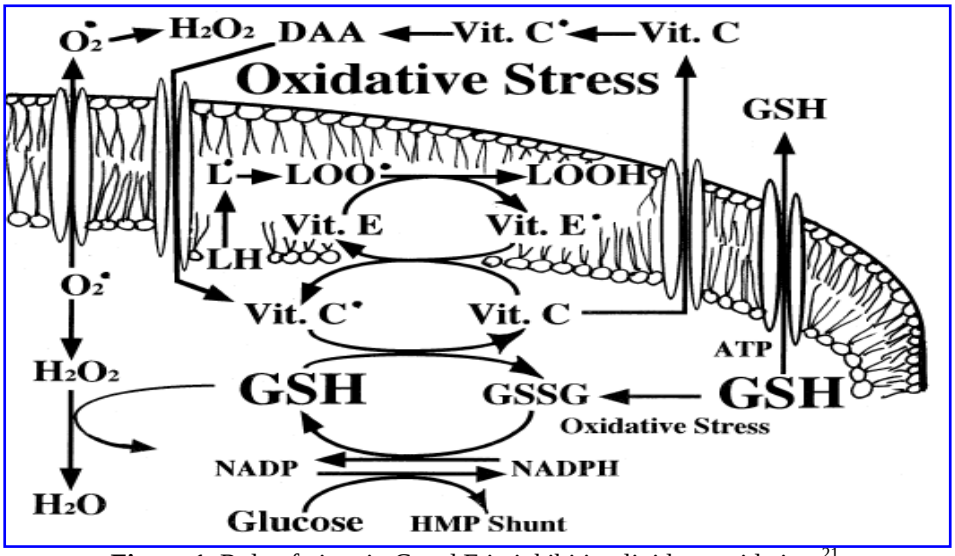

Figure 1. Role of vitamin C and E in inhibiting lipid peroxidation.[21]

The present study indicates that intake of tender coconut water can prevent oxidative stress due to mercury exposure the traditional gold miners.

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References

[1]. Rianto S. (2010) Analysis of the factors Factors Associated with Mercury Poisoning in the Traditional Village Gold Miners Jendi Selogiri District of Wonogiri. *Thesis.* Master of Environmental Health. Diponegoro University. Semarang.

[2]. Winarsi H. (2007) *Natural Antioxidants and free radicals, Potential and Its Application in Health*, Canisius, Yogyakarta.

[3]. Durak D, Calendar S, Uzun F G, Demir F, Calendar Y. (2010) Mercury Chloride-Induced Oxidative Stress in Human Erytrocytes and the Effect of Vitamin C and E in Vitro. *African Journal of Biotechnology* 9: 488-95.

[4]. Al-azzawie H F, Umran A, Hyader N H (2013) Oxidative Stress, Antioxidant and DNA Damage in a Mercury Exposure Workers. *British Journal of Pharmacology and Toxicology*. 4(3): 80-88.

[5]. Devasagayam T, Tilak J, Boloor K, Sane KS, Ghaskadbi SS, Lele R. (2004). Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. JAPI. Pp:52.

[6]. Bhagya D, Prema L, Rajamohan T. (2012). Therapeutic effect of tender coconut water on oxidative stress in fructose fed insulin resistant hypertensive rats, *Asian Pasific Journal of tropical Medicine*, vol 12, pp 270-276.

[7]. Loki AL, Rajamohan T.(2003). Hepatoprotective and antioxidant effect of tender coconut water on carbon tetrachloride induced liver injury in rats, *Indian Journal of Biochemistry and Biophysics*, vol. 40. pp 354-357
[8]. Anurag P., Rajamohan T.(2003). Beneficial effect of tender coconut water against isoproterenol induced toxicity on heart mitochondrial activities in rats, *Indian Journal of Biochemistry and Biophysics*, vol. 40:278-280.
[9]. Pratiknya A W (2003). *Basics of Research Methodology of Medicine and Health.* 5 th ed. PT. King Grafindo Persada. Jakarta. Pp: 117-32.
[10]. Dahlan M S (2010). Large *Sample and Sampling Method in Medical and Health Research.* Salemba Medika. Jakarta. pp.68-72
[11]. Sastroasmoro S. (2014). *Fundamentals of Clinical Research Methodology*, issue 5, Child Health Department of Medicine, University of Indonesia. Binarupa Script. Jakarta.
[12]. Dahlan M S (2014). *Gate understanding the statistics, Methodology and Epidemiology.* Sagung Seto. Jakarta.pp: 219-20.
[13]. Fife B. (2008). Coconut Water for Health and Healing. Piccadily Books, Ltd. USA.
[14]. Priya S.R. (2014). Ramaswamy L. Tender Coconut Water-Natures Elixir to Mankind. *International Journal of Recent Scientific Research*. 5(8):1485-90.
[15]. Alexia P, Dornier M, Diop N, Pain J-P. (2011). Coconut Water Uses, Composition and properties : a review. *Fruits*. 67:87-107.
[16]. SamZiff. (2002).*The Toxic Time Bomb Can The Mercury in Your Dental Fillings Poison You*. 4th ed. Aurora Press. USA.
[17]. Rooney JPK. (2007). The Role of Thiol, Dithiol, Nutrional Factors and Interacting Ligans in the Toxicology of Mercury. *Toxicology*. 234:145-56.
[18]. Suryohudoyo P. (2009). *Oxidants, Antioxidants and Free Radicals.* In: Molecular Medical Sciences. Capita Selecta. Sagung Seto. Jakarta
[19]. Maslachah L, Sugihartuti R, Kurniasanti. R. (2008). Hambatan produksi Reactive Oxygen Spesies Radikal Superoksida (O2•-) oleh antiokidan Vitamin E (α-tocoferol) pada tikus Putih (Rattus norvegicus) yang menerima Stressor Renjatan Listrik. *Media Kedokteran Hewan*. 24(1):21-6.
[20]. Hyman M. (2004). *The* Impact of Mercury on Human health and The Environment. Alternative Therapies. 10:70-5.
[21]. Inoue M. (2001). Protective Mechanism against Reactive Oxygen Species. In: Arias IM, Boyer JL, Chisari FV, Faustro N, Schachter D, Shafritz DA, editors. The Liver : Biology and Pathobiology. 4 th ed. Osaka City University Medical School. Japan. p. 281-90.