

## **The Usefulness of Green Coconut Water Supplementation in Overcoming The Toxic Effect of Mercury Chloride in The Blood and Blood Profile (*Study In Sprague Dewly Rats*)**

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**Abstract :** When people are exposed to mercury chloride, it can produce a variety of health effects in the blood and liver. Coconut water contains Zn, Fe, Vit. C, Vit B<sub>11</sub>, Vit. B<sub>6</sub>, and Se to reduce mercury chloride level in the blood and improve blood profile. Aim of this study was to analysis the effect of green coconut water supplementation in overcoming the toxic effect of Hg chloride in the blood of Sprague daily rats exposed to Hg chloride. Samples were randomly about 36 rats exposed to HgCl<sub>2</sub> through forced feeding by 20 mg/kgBW sondage perday for 14 days, which divided into control group, and intervention groups were given fresh green coconut water in each by 6, 8, and 10 mL/kgBW for intervention 7 and 17 days. The result of this study showed that there is a significant effect and the decrease in mercury levels in the blood. There is no significant affect on the hemoglobin level, hematocrit level and platelet count with the treatment of green coconut water in the rats with exposure Hg. New findings/significance of contribution were the effectiveness of green coconut water to reduce Hg level in blood and improve blood profile.

**Keywords :** green coconut water, mercury chloride, blood profile, SGPT, liver cells.

### **Introduction**

Heavy metals such as arsenic, cadmium and mercury are very dangerous for human health. There are two main sources of heavy metals contamination, i.e., natural (e.g. volcanic eruption) and anthropogenic activities (e.g. mining). Gold mining is one of the world's top source of contamination by Hg, especially in developing countries<sup>1</sup>. Blood is being an important component in the body, an exposure to contaminants will matter to its constituents of hemoglobin, platelets, erythrocytes, and leukocytes. The pollutants will bind to the active groups of enzymes Aminolaevulinic acid dehydratase (ALAD) in the body. Bond is formed between the metal Hg with clusters of ALAD, which results in the formation of intermediates porphobilinogen. Poisoning due to air pollutant Hg can result in disruption of the blood components (blood profiles) that elevated levels of levulinic Amino acid (ALA) in the blood and urine, improving protoporphyrin levels in red blood cells, lowering the amount of red blood cells, lowering the number of erythrocytes causing hematopoietic and increase hematocrit levels in the blood. It can be seen from the value of mean corpuscular volume/volume of blood cells (MCV), Mean Corpuscular Hemoglobin/Weight Hemoglobin average in 1 erythrocytes (MCH), and Mean Corpuscular Hemoglobin Concentration/Hemoglobin concentration erythrocytes average (MCHC)<sup>2</sup>.

Coconut water (coconut liquid endosperm), with its many applications, is one of the world's most versatile natural product. This refreshing beverage is consumed worldwide as it is nutrition's and beneficial for health<sup>3</sup>. There is increasing scientific evidence that support the role of coconut water in health and medicinal application. Coconut water is traditionally used as a growth supplement in plant tissue culture or micropropagation. The wide applications of coconut water can be justified by its unique chemical composition of sugars, vitamins, minerals, amino acids and phytohormones<sup>4</sup>.

Most of the detoxifying methods of Hg are problematic and have to be adjusted to the patient individually. So, Hg can be absorbed through the intake of selenium, but then it is often deposited in the body and cannot be excreted. Folic acid may also absorb Hg; however, in this way organic Hg chloride can emerge which is even a hundredfold more toxic. So-called chelating agents like DMPS (dysmyelopoietic syndrome) absorb Hg very well, but at the same time absorb all other metals in the body as well, so that a long-term treatment is not possible: only single use for a one time "remove" of heavy metals. Single homeopathic detoxifying methods have to start with low potencies which, however, can lead to a re-contamination with Hg because the oxidation<sup>4</sup>. Coconut water contains fatty acids which can extract Hg stored in fatty tissue<sup>5</sup>. Based on this background, this research will examine the effects of green coconut water supplementation to overcome the toxic effect of Hg in the blood and liver of Sprague dawley rat exposed to Hg chloride.

Aim of this study is to analysis the effect of green coconut water supplementation in overcoming the toxic effect of Hg chlorid in the blood and liver of Sprague dawley rats exposed to Hg chloride. The benefit of this study is to contribute ideas for the implementation and development of the substance in the medical health faculty, especially about the effectiveness of green coconut water to reduce Hg level in blood and improve blood profile.

## Experimental

The populations in this study were *Sprague dawley* rat originated from Animal Care Laboratory Pharmacology and Therapeutics Faculty of Medicine Gadjah Mada Unversity total of 36 Rats. The sampling technique have been done by Simple random sampling is the selection of a group of *Sprague dawley* rat based on equality chances every individual of *Sprague dawley* rat to be a member of the experimental Group I, II, or III.

## Experimental Design

All animals were acclimate and put in place with good environment, food, and at libitum drinks for 7 days. The experimental rats will be exposed to HgCl<sub>2</sub> through forced feeding by 20 mg/kgBW sondage perday for 14 days. Aminals are randomly about 36 animal, it divides as a control group, and intervention. Intervention groups give fresh green coconut water in each by 6, 8, and 10 mL/kgBW for intervention for 7 and 17 days. The amount of fresh green coconut water will be served according to each rat's body weight by the doses of 6 mL/kgBW, 8 mL/kgBW and 10 mL/kgBW for group I, II, and III respectively for 7 and 17 days. Measurement of Hg level in that blood sample done at BPTLPP YOGYAKARTA using Mercury analyzer. Researcher has observed any changes of blood mercury level, and blood profile on the experimental animal.

Data recording, processing and analyzing will be done according to the latest methodology available and assisting by computer. The first data will be analized normality to find the suitable of statistics analyze. Secondly, statistical analysis of univariate data presented (mean, SD), bivariate (Wilcoxon, Pairs t-test), bivariate (Kruskal Wallis) unless the data is no normality, and bivariate (Pairs t-test, ANOVA / Post Hoc Duncan's Multiple Range Test) by significance level 0.05.

## Result and Discussion

The results (Table 1) of this study revealed that there the mean values of blood Hg, hemoglobin, hematocryt, WBC, and platelet with the mean values of the control group.

**Table 1. Result Analysis Before and After Treatment with Green Coconut Water**

	Dose I			Dose II			Dose III		
	Day 0	Day 7	Day 17	Day 0	Day 7	Day 17	Day 0	Day 7	Day 17
<b>Blood Hg</b>	9.80	8.10	8.13	8.74	2.21	3.48	5.96	3.00	1.85
<b>Hemoglobin</b>	17.32	17.07	16.65	16.95	16.60	16.75	15.52	15.62	16.90
<b>Hematocryt</b>	51.15	51.30	49.77	48.02	46.52	49.52	49.05	46.55	48.77
<b>WBC</b>	11550	9350	11866	11050	9825	9000	10775	9400	10125
<b>Platelet</b>	1050	1235.25	1272.75	929.25	1207.75	1118.75	1304.25	1289	1418.50

Note: dose I = 6 mL/kgBW; dose II = 8 mL/kgBW; dose III = 10 mL/kgBW

### Blood Hg level

The distribution of Hg levels after 14 days was exposed by 20 mg/KgBW before they were treated by fresh green coconut water in animal experiments can be seen in table 1. The highest Hg level was group A, and the lowest was group B. The distribution of mercury levels after 7 days treatment of fresh green coconut water in animal experiments, the highest mercury level at day 7 was group A, and the lowest was group B. The distribution of Hg levels after 17 days treatment of fresh green coconut water in animal experiments, the highest Hg level at day 17 group was group A, and the lowest was group C.

In second group day 0 and day 17 there are significant differences in blood Hg level before and after 17 days intervention with dose I of fresh green coconut water. There is significant difference in blood Hg level before and after 7 and 17 days of intervention with dose I of fresh green coconut water. There is significant difference in blood Hg level before and after 7 and 17 days of intervention with dose III of fresh green coconut water.

### Blood profile

#### 1) Hemoglobin level

The distribution of hemoglobin levels after 14 days was exposed by 20 mg/KgBW before they were treated by fresh green coconut in animal experiments can be seen in table 1. The highest hemoglobin level was group A, and the lowest was group C. The distribution of hemoglobin levels after 7 days treatment of fresh green coconut water in animal experiments, the highest hemoglobin level at day 17 was group A, and the lowest was group B. Data distribution of hemoglobin levels after 17 days treatment of fresh green coconut water in animal experiments, the highest hemoglobin level at day 7 was group C, and the lowest was group A.

#### 2) Hematocrit level

The distribution of hematocrit levels after 14 days was exposed by 20 mg/KgBW before they were treated by fresh green coconut in animal experiments can be seen in table 1. above. The highest hematocrit levels was group B, and the lowest was group C. The distribution of hematocrit levels after 7 days treatment of fresh green coconut water in animal experiments, the highest hematocrit levels at day 7 was group C, and the lowest was group B. Data distribution of hematocrit levels after 17 days treatment of fresh green coconut water in animal experiments, the highest hematocrit level at day 17 was group A, and the lowest was group B.

#### 3) White Blood Cell (WBC) count

The distribution of WBC count after 14 days was exposed by 20 mg/KgBW before they were treated by fresh green coconut in animal experiments and after they were treated by fresh green coconut in animal experiments can be seen in table 1 above. The highest WBC count was group C, and the lowest was group B. The distribution of WBC count after 7 days treatment of fresh green coconut water in animal experiments, the highest WBC count at day 7 was group A, and the lowest was group A. The distribution of WBC count after 17 days treatment of fresh green coconut water in animal experiments, the highest WBC count at day 17 was group A, and the lowest was group B.

#### 4) Platelet count

Data distribution of platelet count after 14 days were exposed by 20 mg/KgBW before and after they were treated by fresh green coconut in animal experiments can be seen in table 1 above. The highest platelet count was group C, and the lowest was group A. Data distribution of platelet count after 7 days treatment of fresh green coconut water in animal experiments, the highest platelet count at day 7 was group B, and the lowest was group A. Data distribution of platelet count after 17 days treatment of fresh green coconut water in animal experiments, the highest platelet counts at day 17 was group C, and the lowest was group B.

### Discussion

#### Blood Hg Level

There is a significant effect and the decrease in mercury levels in the blood. This is because coconut water contains fatty acids which can extract mercury stored in fatty tissue. At the same time the sulfur-containing amino acids of coconut water bind the mercury and prevent redistribution in the body. Mercury levels in the blood reduce by treatment fresh green coconut water, because for a definite detoxifying, sulfuric amino acids in the green coconut were required to absorb mercury and to allow excretion through urine and purgation<sup>6</sup>.

Coconut water was rich in free amino acids, the source of L-arginine and vitamin C, which can prevent heart disease and peroxidation<sup>7</sup>. A high blood mercury level indicated that the level of free radicals in the body was high. The main target of free radicals is a protein, unsaturated fatty acids, as well as elements of DNA, including carbohydrates. The three molecular targets, unsaturated fatty acids were those most susceptible to free radical attack. A high level of free radicals in the body can be demonstrated by the low activity of antioxidant enzymes and high levels of malondialdehyde (MDA) in the blood plasma<sup>8</sup>. Coconut water contains antioxidants like vitamin C, vitamin B1, vitamin B6, amino acids such as methionine, L-Arginine, selenium, cytokines and minerals that were helpful to protect the body's cells against the risk of free radical attack and prevent the effects resulting from exposure to mercury<sup>7</sup>. A component such as the amino acid methionine found in tender coconut water is the source for cysteine sulfhydryl. Methionine acts as a precursor for the formation of cysteine was the main compound in the synthesis of glutathione (GSH). Methionine was being synthesized into S-adenosyl methionine (SAM) is assisted by the enzyme methionine catalyst adenosyltransferase. Then, S-adenosylmethionine (SAM) will be converted into the S-adenosylhomocysteine (SAH). SAH is converted into homocysteine by the enzyme adenosylhomocysteine and by methionine synthase (MS) homocysteine can back into methionine. In the synthesis of cysteine, homocysteine is converted into cystathionine with the help of the enzyme cystathionine  $\beta$ -synthase (CBS) and vitamin B6, and then converted to cysteine with the help of cystathionine lyase enzyme and vitamin B6, cysteine eventually be synthesized into glutathione (GSH)<sup>9</sup>.

GSH was a substrate for the antioxidant glutathione peroxidase (GPx) to decompose hydrogen peroxide. If glutathione synthesis would be disturbed, it will cause a decrease in GSH. There will eventually dump hydrogen peroxide and hydroxyl radicals, so as result it will be no increase is more dangerous to the human body. GSH also serves as a substrate in the reaction radical regeneration of vitamin C, so that if a drop of GSH occurs, it will interfere with the reaction and will cause lipid peroxidation. Methionine works in synergy with vitamin B6. Vitamin B6 acts as a cofactor of the enzyme of cystathionine  $\beta$ -synthase and the lyase cystathionine cysteine and GSH synthesis process.

Coconut water as a source of methionine and vitamin B6<sup>10</sup>. The high content of L-arginine in tender coconut water can be used to reduce the generation of free radicals, increase antioxidant activity and inhibit the process of lipid peroxidation<sup>7</sup>. L-arginine plays an important role in the detoxification of mercury and could potentially serve as a preventive and therapeutic strategy against occupational or environmental exposure to mercury<sup>11</sup>. NO can inhibit xanthine oxidase (XO), increases levels of SOD, the number of thiol (T-SH), vitamin C, the number of antioxidants (TAC)<sup>12</sup>. If the XO activity decreases, the amount of superoxide also is decreased so that the rate of increase SOD, because of the need to decipher superoxide to hydrogen peroxide is also lacking. Superoxide can also be formed due to the reduction of O<sub>2</sub> by NO synthase, when deprived of arginine and tetrahydrobiopterin (BH<sub>4</sub>) occurs, so that L-arginine may be used as an alternative to reduce the production of superoxide.

Thiol levels after administration of L-arginine can improve ascorbic acid radical conversion into ascorbic acid<sup>11</sup>. Treatment with L-arginine can increase the activity of GPx in rats exposed to Pb<sup>12</sup>. The high content vitamin C in tender coconut water makes coconut water effective to raise the level of antioxidants such as vitamin C can reduce superoxide radicals, hydrogen peroxide and other reactive oxygen derived from activated neutrophils and monocytes. As an antioxidant, ascorbic acid was not directly give birth membrane-bonding antioxidants, such as  $\alpha$ -tocopherol, with increasing peroxy radicals and singlet oxygen. Vitamin C works synergistically with vitamin E. Vitamin E is oxidized by free radicals can react with vitamin C, after obtaining the hydrogen ion of vitamin C and then be converted into tocopherols<sup>8</sup>. Tender coconut water contains selenium, GPx activity was strongly influenced by the presence of selenium, selenium deficiency in the body can reduce the activity of GPx<sup>13</sup>. Mercury has a strong affinity for binding selenium<sup>14</sup>, and the two antagonists<sup>15</sup>. Provide gentle coconut water after exposure to mercury can be used as a source of selenium to increase the activity of GPx.

High nutrient content in tender coconut water can be used as a source of minerals that are important for the body, one of which is as natural cofactor antioxidant SOD contained in the body. Mineral deficiency Cu, Zn and Mn can lower the activity of Cu-Zn SOD and Mn-SOD. Mn-SOD function as a catalyst for the reaction of superoxide anion to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and oxygen (O<sub>2</sub>) in the mitochondria, while the Cu-Zn SOD function as a catalyst for the reaction of the anion superoxide dismutase into hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and oxygen (O<sub>2</sub>) in the cytosol. In the Cu-Zn SOD is, Cu minerals required for the proper functioning of the catalytic enzyme, whereas Zn is important for the structural function. Mineral Cu, Zn and Mn work synergistically<sup>8</sup>.

## Blood profile

### a) Hemoglobin level

After 7 days of treatment have no significant affect on hemoglobin levels and also on the increasing doses of green coconut water given after 17 days of treatment no significant affect on the hemoglobin level with the treatment of green coconut water in the mice with exposure Hg.

The process of formation of red blood cells (erythropoiesis) and of hemoglobin synthesis occurs in red bone marrow. Iron contained in coconut water is thought to have a role as a main constituent of hemoglobin chemical bonds that became one component of red blood cell formation. Heme iron by binding to globulin protein to form hemoglobin<sup>8</sup>. The absorption of iron from foods that will be used in the formation of hemoglobin allegedly influenced by other substances, namely vitamin C. Vitamin C is contained in coconut water has been implicated in increasing the absorption of iron in the body to speed up the synthesis of hemoglobin. Vitamin C serves to reduce the ferric iron (Fe 3+) to ferrous (Fe 2+) in the small intestine so easily absorbed<sup>16</sup>. Vitamin C and iron to form iron ascorbate complex compounds soluble and easily absorbed to the synthesis of hemoglobin in erythroblast<sup>17</sup>. Vitamin B6 is also contained in coconut water has been implicated as a coenzyme in the reaction of the formation of hemoglobin. Other substances that alleged role in the formation of red blood cells are also found in coconut water is folic acid has a role in the synthesis of DNA to control the maturation of the core erythroblast (cells found in red bone marrow, which will develop into erythrocytes)<sup>18</sup>. However, in this research the iron and the vitamin C contain in coconut water could not help to rise the hemoglobin level, probably due to the iron contain was not enough to repair hemoglobin level.

### b) Hematocrit level

Based on the results based on the increasing doses of green coconut water given on control group, after 7 days of treatment, increasing doses of green coconut water given after 17 days of treatment have no significant affect on hematocrit level with the treatment of green coconut water in the mice with exposure Hg. Based on the iron content and Vitamin C contained in the green coconut water, green coconut water should be able to increase or improve blood profile. However, in this study there is a decrease in blood profile in mice with exposure by Hg. This is because the Hg content in the drinking water of mice had damaged tissue cells in mice that treatment using green coconut water does not have a significant effect on the blood profile of rats. Hg directly damage the cells of tissues of living beings with their deployment to air, water and food contamination<sup>19</sup>.

Hg inhibits the synthesis of heme and shorten the life of erythrocytes. This is due to the activity of various enzymes necessary for heme synthesis is inhibited. One example of the enzyme glutathione peroxidase, this enzyme has sulfhydryl groups where the presence of Hg in the body of the sulfhydryl groups are bound with Hg so that the enzyme becomes inactive and enzyme concentration decreases. The decline in the activity of glutathione peroxidase will also interfere with the process of glycolysis resulting in energy reduction of erythrocytes is reduced so as to cause a short erythrocyte lifespan<sup>19</sup>.

Hg has a high affinity against erythrocytes, approximately 95% bound in blood erythrocytes. Hg has a half-life in the blood very slowly about 25 days, at 40 days and the soft tissue on the bones of 25 years. Given the nature of this very slow excretion of Hg easily accumulate in the body. Hg deposited in the red blood cells and cause damage to red blood cells into the blood Hg of blood, 95% attaches to red blood cells, 5% in the blood plasma. Damage to red blood cells that contain Hg i.e. before the rupture of red blood cells mature red blood cells, resulting in a decrease in the number of red blood cells in the blood. Eritrosit regenerated cell, so Hg accumulated momentarily in erythrocytes, will be lessened follow released of red blood cells, but if exposure to Hg continuously at levels above the normal threshold will together of red blood cells to the heart and other organs, namely bone marrow and accumulate settles<sup>10</sup>.

### c) White blood cell count

The increasing doses of green coconut water given to control group have a significant effect on the WBC level. After 7 days of treatment and also on the increasing doses of green coconut water given after 17 days of treatment no significant affect on the WBC level with the treatment of green coconut water in the mice with exposure Hg. Coconut water also contains folate which is also known as vitamin B9. These were identified in the late 1930s as nutrients needed in reducing anemia in pregnancy which also helped prevent mitochondrial toxicity caused by methanol metabolism<sup>13</sup>. This contrasts with the results of research that states the number of leukocytes decreases with increasing doses of green coconut water. High mercury content can inhibit heme red blood cell synthesis after poisoning<sup>8</sup>. Anemia is caused not only by increased erythrocyte impairment<sup>16</sup>, but also by decreasing the synthesis and release of erythrocytes into the blood circulation<sup>17</sup>. Connects anemia with erythropoiesis disorders caused by the direct effects of metals on hematopoietic (kidney / spleen) centers, accelerated erythematic due to increased membrane permeability and / or increased mechanical fragility<sup>18</sup>.

### d) Platelet count

No significant affect on the platelet count with the treatment of green coconut water in the mice with exposure Hg. A disturbance in the bone marrow lead to failure or suppression of stem cells that result in disruption of the process of formation of blood cells (hematopoiesis). Hematopoiesis disorders can occur in the process of formation of red blood cells, which cause a reduction in the number of red blood cells. All blood cells originate from stem cells pluripotential which then differentiate into: stem cell lymphoid forming lymphocytes and plasma cells, stem cells are multi potential myeloid (non-lymphoid) which then develop into different types of hematopoietic cell to another, such as platelets, erythrocytes, granulocytes and monocytes<sup>19</sup>.

## Conclusion

Green coconut water can decrease Hg concentrations in the blood after supplementation with coconut water given to Sprague dewly rats exposed to mercuric chloride. There were no significant differences in blood profile (hemoglobin, hematocrit, WBC and platelet count) between before and after supplementation green coconut water. Decreased levels of Hg in the blood that is effective in treatment after a day 7 at a dose of 8 ml of green coconut water. Increased hematocrit effective in treatment after day 17 with a dose of 6 ml and 8 ml of green coconut water, increasing WBC effective in the treatment after 7 days with a dose of 8 ml and treatment after day 17 with a dose of 10 ml of green coconut water, whereas the increase in platelet count that is effective on the 17th day of treatment using green coconut water to Sprague dewly rats exposed to mercuric chloride.

For the development of science, suggestion to the further research is increasing the number of rats for the better result of the research. Recommendation to decrease mercury in the blood for human can be used green coconut water supplementation as showed in this research.

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