

4. SITI THOMAS_The Impact Of Tender Coconut Water On Preventing Lipid Peroxidation And Increasing Antioxidant Enzymes In Lead Induced Rats

by Siti Thomas

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The Impact Of Tender Coconut Water On Preventing Lipid Peroxidation And Increasing Antioxidant Enzymes In Lead Induced Rats

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ABSTRACT

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Plumbum is a heavy metal that can trigger the formation of *Reactive Oxygen Species* (ROS), increasing the generation of free radicals, lipid peroxidation and lowering The antioxidant enzymes such as GPx. Tender Coconut Water contains Antioxidants, L-arginine and other compounds that can reduce the effect of Pb exposure. The Purpose of this research is to know the effect of tender coconut water to prevent lipid peroxidation and increase antioxidant enzymes in Pb-induced rats. Experimental study using *post test only control group design*, with 18 white male wistar strain rats randomly divided into 3 groups. K1 (standard feed only), K2 (standard feed + Pb), K3 (standard feed + Pb + tender coconut water). The induction of Pb is performed by inhalation at a dose 10 mg/day/ratekor, while tender coconut water were administered by sonde dose 8 mL/200grBW/day together for 4 weeks. Rat blood from the opthalmicus vein was examined using Elisa to measure Malondialdehyde (MDA) levels and glutathione peroxidase (GPx). Data were analyzed using Anova test. Results showed that the average rate of MDA in Group 2 was increased compared to group 1, but in Group 3 decreased compared to group 2. The GPx average in group 2 decreased compared to group 1, but in group 3 is increased compared to group 2. The statistical analysis obtained p values of <0,05. Tender coconut water administration is proven to be able to prevent lipid peroxidation and increase the antioxidant enzymes characterized by increased GPx levels of Pb induced wistar strain rats.

Key words: Plumbum (Pb); Tender coconut water; MDA Levels; GPX levels

Introduction

Indonesia is the world's third-highest air pollution level. Of the many sources of air pollution, motor vehicles (transportation) are the largest source of air pollution (85%)¹. In the last decade, the NT exposure has expanded and became a health problem in society. The use of Pb in the workplace today must be controlled and restricted because it provides a detrimental effect to health² detection of lead can be seen in the blood because more than 90% of lead metals are absorbed by the blood with red blood cells. Continuous Pb exposure will trigger the formation of *Reactive Oxygen Species* (ROS), increase lipid peroxidation, cause oxidative stress in cells or tissues, inhibit enzymes, breakdown of PROTEIN structures, DNA damage, interfere with The body's antioxidant metabolism is particularly superoxide dismutase (SOD) and glutathione peroxidase (GPX), lowering glutathione (GSH) and vitamin C as well as inhibiting thiamin (B1) and Pyridoxin (B6).³ Malondialdehyde (MDA) is a highly reactive compound and is the end product of lipid peroxidation, usually used as a sign of the occurrence of lipid peroxidation to assess oxidative stress.⁴ Oxidative stress due to Pb exposure can occur through depletion of antioxidants and increased ROS. Pb forms a covalent bond with sulfhydryl groups in antioxidants such as GSH, glutathione reductase (GR), and glutathione S-transferase (GST). Pb also reduces the absorption of GST so that it can cause a decrease in the activity of antioxidant enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD) and KATALASE (CAT), so that there is depletion of glutathione levels Reduction (GSH) and buildup of H₂O₂ which ultimately leads to oxidative stress.⁵

In normal physiological conditions, the amount of endogenous antioxidants (antioxidant enzymes) is sufficient to ward off free radicals, but in conditions of increased free radicals caused by environmental pollution, there is an imbalance Between free radicals with antioxidants so that it can cause oxidative stress. This is where exogenous antioxidant roles of food, fruit, or vegetables are essential to prevent various effects such as heavy metal toxicity³. Foods containing natural antioxidants can be used as a strategy to reduce morbidity and mortality rates in particular due to oxidative stress. Natural antioxidants are able to protect the body against cell damage caused by ROS, able to inhibit the occurrence of degenerative diseases and able to inhibit lipid peroxidation⁴

Tender coconut water is a natural drink that contains beneficial compounds such as iron, vitamin C, vitamin B6, folic acid, L-arginine and fatty acids (Zulaikhah, 2019). L-Arginine is known to act as an antioxidant and can reduce the formation of free radicals, vitamin C also acts as an antioxidant that prevents the occurrence of lipid peroxidase. Vitamin B6 plays a role in enhancing the production of GSH and Vitamin B1 (thiamine) can prevent the occurrence of lipid peroxidation on the liver and kidneys of a rat induction Pb⁵. Earlier research results stated that tender coconut water administration proved to lower MDA levels and increase GPx levels in people exposed to mercury.⁷(Zulaikhah, 2015) This research is to prove the effect of tender coconut water to prevent lipid peroxidation And increase GPX levels in Pb-induced wistar strain rats

¹ MATERIAL AND METHODS

This study was designed as experiment research with *Post-Test Control Group Design*

Ethical Clearance:

This study received ethical clearance from the Bioethics Committee of Medical Research / Medical Faculty of Sultan Agung Islamic University, Semarang (42/II/2019/Bioethics Commission).

Tender Coconut Water:

TCW used was coconut viridis variety (green coconut) aged 5-7 months and obtained from research surroundings. During the age 5-7 months, coconut has tender, thin, jelly like endosperm and it is edible using spoon. The dosage administered is 8ml/200grBW/day for 4 weeks¹¹.

Experimental Animal:

Lead induction (Pb):

The dosage of lead given is 10 mg/day by inhalation. 10 mg of lead powder is diluted with 1 mL aquadest , poured to open container, then the container is placed in the cage. This research one group consists of 6 rats, thus one cage has one 60mg lead container diluted with 6 mL aquadest¹². Male Wistar strain white rats that met the criteria which is 2 months old, weighed 180-220g, healthy looking, active motion, normal feeding and drinking, no injuries and no disabilities. The total of 18 rats were adapted for 1 day, and then randomly werw divided into 3 groups of 6 rats each and fed the following died:

Group 1 (K1):	fed standard diet + distilled water ad libitum) for 4 weeks
Group 2 (K2)	fed standard diet + distilled water ad libitum + Pb inhalation (10 mg Pb + 1 mL aquadest/day) for 4 weeks
Group 3 (K3)	fed standard diet + distilled water ad libitum + Pb inhalation (10 mg Pb + 1 mL aquadest/day) + 8 mL/200g body weight/day tender coconut water for 4 weeks.

After 4 weeks, blood was drawn to measure the MDA and GPx levels.

Blood drawing procedure:

The equipment used is sterile microhematocrit tubes, blood vials, and sterile cottons. Blood is taken by inserting a microhematocrit tube in the ophthalmic vein in the corner of the rats' eyes' periorbita then slowly rotated until the blood comes out. Blood coming out is contained in ependrof as much as 2cc. Plug out the micro hematocrit tube if the required blood is sufficient, clean the remaining blood in the corner of the rat's eye using sterile cotton. The examination of MDA and GPx levels using ELISA (*Enzyme-Linked Immunosorbent Assay*).

Research Location:

The treatment of experimental animal and examination of MDA and GPX levels was carried out in PAU Gadjah Mada University Yogyakarta.

Statistical Analysis

All statistical analyses were performed using SPSS software version 22.0. Data were tested for normality by *Shapiro-Wilk* and homogeneity testing with *Leuvene's Test*. Data on MDA and GPx levels were normally distributed and homogenous so that they were analysed by parametric statistical test which is *One Way Anova* followed by *Post Hoc LSD* to determine the difference between groups. P-values < 0,05 were considered to be statistically significant.

RESULTS

The effect of tender coconut water can lower MDA levels as a parameter to lipid peroxidation and increase the antioxidant GPx enzyme can be seen in table 1.

Table 1: The average of MDA (nmol/mL) and GPx (u/mg) in 3 (three groups) (K1, K2, K3)

VARIABLE		Group			
		1	2	3	p-value
		Means \pm SD	Means \pm SD	Means \pm SD	
MDA	Levels	3,30 \pm 0,50	9,38 \pm 0,37	4,48 \pm 0,86	
(g/dL)					
Shapiro wilk		0,981	0,861	0,918	>0,05*
Levene test					0,339**
One way Anova					0,000***
GPx Levels()		66,75 \pm 3,26	23,02 \pm 2,03	59,93 \pm 2,56	
Shapiro wilk					
Levene test		0,871	0,867	0,956	>0,05*
One way Anova					0,341**
					0,000***
Keterangan: Signifikan		*>0,05			
		**>0,05			
		***<0,05			

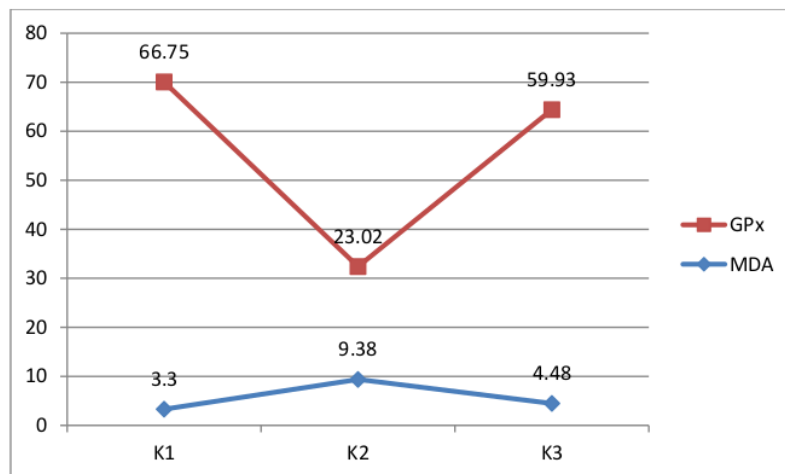


Chart 1 : The average of MDA (nmol/mL) and GPx (u/mg) in 3 (three groups) (K1, K2, K3)

Table 1. Indicates that the average rate of MDA in group 2 rating is compared to group 1, but on group 3 decreases when compared With group 2. Average of GPx on Grop 2 decreases compared to 1 grop, but on Grop 3 increases when compared to grop 2. Statistical analysis results with Anova test showed that tender coconut water administration at a dose of 8ml/200KGBW/day for 4 weeks can lower MDA levels and increase antioxidant levels of GPx enzymes (value $P < 0.05$).

Discussion

Continuous Pb exposure will trigger the formation of *Reactive Oxygen Species* (ROS), increase lipid peroxidation, cause oxidative stress in cells or tissues, inhibit enzymes, breakdown of protein structures, DNA damage, interfere with the body's antioxidant metabolism is particularly superoxide dismutase (SOD) and glutathione peroxidase (GPX), lowering glutathione (GSH) and vitamin C as well as inhibiting thiamin (B1) and Pyridoxin (B6).³

Pb exposure resulted in increased ROS production through inhibition of heme biosynthesis and activation of NAD (P) H oxidase; Increase lipid peroxidation; and inactivation and/or the depletion of antioxidant enzymes, can reduce the absorption of glutathione S-transferase (GST) so that occurs thinning gsh and thiol proteins so that GPX activity decreases.⁵ There is a positive correlation between the blood Pb levels and the decrease in glutathione (GSH). Pb exposure can also potentially reduce the absorption of selenium (the compounds needed by GPx) and/or thiol binding in antioxidant proteins. The higher the level of Pb in the blood, the higher the level of free radicals in the body. Pb will bind to the thiol cluster found in antioxidant proteins so that antioxidant activities and antioxidant enzymes such as glutathione peroxidase (GPX) will decreased. Deficiency of one of the antioxidant components causes a thorough decline of antioxidant

status⁶, This condition causes the depletion of GSH and increase H₂O₂. Hydrogen peroxide when it reacts with *transitional* Metals such as Fe⁺⁺ and Cu⁺ In Fenton reaction will produce radical hydroxyl (•OH) which is most dangerous because of its very high reactivation.^{7,8} Hydroxyl radicals can damage cell membranes that are rich in source *poly unsaturated fatty acids* (PUFA) so that it can cause lipid peroxidation, one of its parameters is increased MDA levels. The mechanism of oxidative stress due to Pb-induced can be seen in Figure 1.

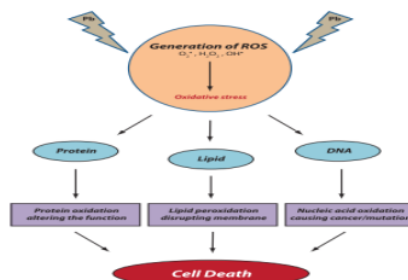


Figure 1. Possible mechanism and targets for lead-induced oxidative stress.³

Coconut water used in this research is the tender coconut water from the group of coconut cultivars, viridis variety (green coconut), aged 5-7 months, moderate antioxidant status measured is the antioxidant enzyme that is glutathione Peroxidase (GPx). The total content of sugar and water in coconut increases maximum when it is 5-7 months, so at this time coconut water has a very sweet taste and most delicious when compared to the older age, because the total content of sugar and water will be Decrease with the age of coconut until the coconut grows old which is aged \pm 12 months.⁹ In this study proved that administration of tender coconut water at a dose of 8ml/200KGBW/day in Pb-induced Wistar rats for 4 weeks could prevent the lipid peroxidation being marked With decreased MDA levels and increased antioxidant levels of GPx enzymes ($p < 0,05$). This research proves that the average MDA levels in group 2 when compared to group 1, but on group 3 has decreased when compared to group 2. Average GPx rate at group 2 decreased compared to group 1, but in group 3 increased compared to group 2. These results are linear with the research of Zulaikhah *et al* which proves that tender coconut water is able to lower MDA levels and increase the antioxidant levels of GPx enzymes on the mercury-exposed gold miners.¹⁰ Loki dan Rajamohan reinforcing this research stating that tender coconut water can increase GPx levels in mice that are induction with CCl₄.¹¹ Tender coconut water contains compounds that are very beneficial for health such as the amino acids L-arginine, methionine, vitamin C, B vitamins, selenium and others.⁹

Components of amino acids such as methionine contained in the tender coconut water is a sulfide source for the sestein. Methionine is an essential amino acid that cannot be synthesised alone by the body, its existence must be supplied from outside. Methionine serves as a precursor to the formation of cysteine which is the main compound in the glutathione (GSH) synthesis. Methionine will be synthesized into *S-adenosilmethionine* (SAM) with the help of the catalysts. Furthermore *S-adenosilmethionine* (SAM) will be changed to *S-adenosilhomocysteine* (SAH), SAH will be converted into homocysteine with the enzyme *adenosilhomosisteinase* and *methionine synthase* (MS) homocysteine can be changed back into Methionin. In cysteine synthesis, homocysteine will be converted into a cystathionine with the help of the enzyme *cystathionine β synthase* (CBS) and vitamin B6 and subsequently converted into cysteine with the help of the enzyme cystathionine liase and vitamin B6, finally Cysteine will be injected into glutathione (GSH).¹² GSH is a kind substrates for the antioxidant glutathione peroxidase (GPx) in describing hydrogen peroxide, when the glutathione synthesis is interrupted it will cause a decrease in GSH and there will be a buildup of hydrogen peroxide and will eventually occur the increase of hydroxyl radicals that are more harmful to the human body. GSH also serves as a kind substrates in the radical regeneration reaction of vitamin C to vitamin C, so that when a decrease in GSH will interfere with the reaction and will lead to lipid peroxidation. Methionine will work synergized with vitamin B6. Vitamin B6 serves as a cofactor of *cystathionine enzyme β synthesis* and cystathionine liase in the process of cysteine and GSH synthesis. Tender coconut water can be used as a source of methionine and vitamin B6.¹³

The content of other amino acids contained in the tender coconut water is L-arginine, high L-arginine content in tender coconut water can be utilized to reduce radical generation NO, increase antioxidant activity, lower MDA levels and Inhibits lipid peroxidation process.^{10,14} The role of L-arginine against decreased MDA levels and increased vitamin C through nitric oxide (NO) can be seen in Figure 2.

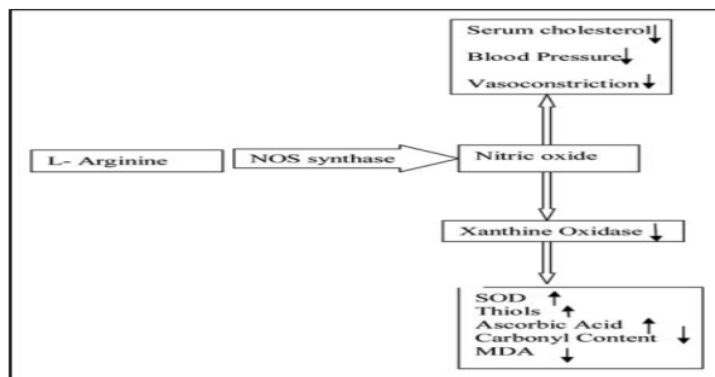


Figure 2. The role of L-arginine through nitric oxide(NO).¹⁴

Increased levels of group after L-arginine administration can increase the radical conversion of ascorbic acid into ascorbic acid.¹⁵ Treatment with L-arginine is able to increase GPx activity in rats induced with PB.¹⁶ The high content L-arginine in tender coconut water can be used to reduce the generation of free radicals, increase antioxidant activity and inhibit lipid peroxidation processes.¹⁷ Tender coconut water contains selenium, selenium is an essential mineral that is very important for human health, plays an important role in protein synthesis and enzyme activity of glutathione peroxidase (GPx). GPx activity is strongly influenced by the presence of selenium, selenium deficiency in the body can decrease GPx activity by up to 90%.¹⁰ Under normal conditions selenium needs in the body may have been fulfilled from the food, but for people exposed to Pb it is necessary to supply more selenium from outside. Pb has strong affinity for selenium binding, and both are antagonistic.¹⁸ Lipid peroxidation In this study is measured based on malondialdehyde (MDA) levels in plasma. MDA is one of the oxidation oxidizing products of polyunsaturated fatty acids on cell membranes, increased MDA levels are important indicator of lipid peroxidation occurrence.⁷

In this study tender coconut water proved to lower MDA levels as a sign of the occurrence of lipid peroxidation reduction. The lipid peroxidation process can be inhibited by compounds contained in tender coconut water. For example, the content of L-arginine contained in coconut water can be used as source no and no proved to decrease Fe and Cu, so as to inhibit Fenton reaction and inhibit the formation of hydroxyl radicals (OH*) will eventually inhibit lipid peroxidation.^{19,20} The vitamin C content contained in tender coconut water is also associated with decreasing lipid

peroxidation. Vitamin C and vitamin E have a protection effect against heavy metal toxicity.²¹ The role of vitamin C works synergized with vitamin E in inhibiting lipid peroxidation process. Vitamin E is an antioxidant chain breaker on membranes that can prevent cellular damage by lipid peroxidation and inhibit the formation of free radicals.⁶ Vitamin E which is oxidized by free radicals can react with vitamin C, after getting a hydrogen ion from vitamin C will turn into vitamin E.¹⁰ Vitamin E in membrane reacts with radical lipid (LOO[•]) Forms radical Vitamin E(vit. E[•]). The radical Vitamin E reacts with vitamin C to form free radicals vitamin C (Vit. C[•]). Radical vitamin C (Vit. C[•]) will experience regeneration into vitamin C by involving glutathione(GSH). GSH will be oxidized to oxidized glutathione (GSSG) by the enzyme glutathione peroxidase (GPx), GSSG will be reduced to a form of GSH by glutathione enzyme reductase (GRed).³ GSH metabolism due to Pb exposure can be seen in Figure 3.

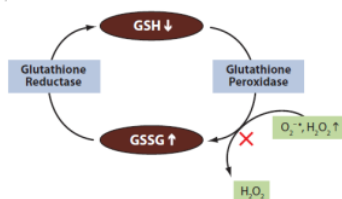


Figure 3. Effect of lead on GSH metabolism.³

CONCLUSION

The administration of tender coconut water at dose 8mL/200kgBW/day in Pb induced wistar strain rats for 4 weeks can prevent lipid peroxidation which marked by the decrease of MDA levels and increase the GPx antioxidant enzyme levels

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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