

Tender Coconut Water (*Cocos nucifera* L.) Can Increase Antioxidant Enzymes and Decrease MDA Levels: Experimental Study on Cigarette Smoke-Exposed Rats

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ABSTRACT

Background: Cigarette smoke exposure increase free radicals in the body in the form of Reactive Oxygen Species (ROS). Excessive levels of ROS can cause an imbalance between the amount of oxidants and antioxidants that trigger oxidative stress. The state of oxidative stress can be seen from the decrease in antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and increased levels of malondialdehyde (MDA). Tender coconut water contains a variety of substances that are beneficial to the body, including L-arginine and Vitamin C which can increase antioxidant enzymes and reduce oxidative stress. **Aim and Objectives:** This study aims to determine the effect of giving tender coconut water on increasing antioxidant enzymes such as SOD and CAT and decreasing MDA in rats exposed to cigarette smoke. **Material and Methods:** Experimental research by Post-Test Only Control Group Design study used 24 rats divided into 4 groups, K1 was the control, K2 was the negative control exposed to cigarette smoke, K3 the positive control was given vitamin E and K4 the treatment group. Furthermore, sampling was continued by examining the levels of SOD and CAT using ELISA (Enzyme-Linked Immunosorbent Assay), while MDA using spectrophotometry. The SOD, CAT, and MDA data were processed using SPSS with the One Way Anova. **Results:** The administration of tender coconut water at a dose of 8mL/200grBW/day and vitamin E at a dose of 1.8 mg/200gBW/day for 14 days increasing SOD levels, CAT levels and reduce MDA levels. **Conclusion:** It can be concluded that tender coconut water can increase antioxidant enzymes and reduce MDA in rats exposed to cigarette smoke.

Key words: Tender coconut water, Superoxide dismutase, Catalase, Malondyaldehyde, Cigarette smoke.

INTRODUCTION

Smoking is one of risk factor for various degenerative diseases such as coronary heart disease, COPD, and other non-communicable diseases.¹ Smokers are 2-4 times more likely to develop coronary heart disease than non-smokers.² In the WHO study, every year in Indonesia there are about 225,700 people die because of smoking. The previous WHO study also stated that smoking is a major risk factor for premature death and disability worldwide. Indonesia ranks third in the number of smokers in the world, the first is filled by China and the second is filled by India (WHO, 2020). Based on data from the 2018 Basic Health Research (RISKESDAS) as many as 28.8% of the total population of Indonesia with the age of 10 years are active smokers.³

Exposure to cigarette smoke can trigger an increase in free radicals in the body. Free radicals in the body can be in the form of oxygen and its derivatives called Reactive Oxygen Species (ROS). Excessive levels of ROS can cause an imbalance between the number of oxidants and antioxidants that trigger oxidative stress.⁴ The state of oxidative stress can be seen from increasing levels of malondialdehyde (MDA) and decreasing antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx).^{5,6} Tender coconut water is a drink that contains a variety of beneficial

substances for the body, including various nutrients such as L-arginine, Vitamin C, amino acids and minerals such as magnesium, potassium, calcium, selenium, methionine, zinc, iodine, manganese, and cuprum.^{7,8} Research about tender coconut water on MDA, SOD, and CAT levels in cigarette smoke exposure have not been well studied.

Research conducted in previous studies also stated that cigarettes significantly affect antioxidant biomarkers, such as SOD, CAT, and GPx.⁹ The body has special compounds, namely endogenous antioxidants that function to ward off free radicals in the body. Antioxidants can be enzymatic and non-enzymatic, antioxidants that are enzymatic or also referred to as primary antioxidants can be in the form of SOD, CAT and GPx. Non-enzymatic antioxidants, also known as secondary antioxidants, from foods such as vitamins C, E, A, and -carotene, as well as flavonoids, uric acid.¹⁰ SOD is the first antioxidant enzyme in the defense mechanism against superoxide anion, and then followed by CAT and GPx enzymes to form H₂O.¹¹

The imbalance between free radicals and antioxidants can also cause free radicals to react by adding hydrogen ions from the methylene carbon chain to form lipid radicals. The lipid radicals then react with oxygen to form lipid peroxy radicals. Then, lipid peroxy radicals form new lipid radicals and cause changes in Polyunsaturated Fatty Acids (PUFA) into

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lipid hydroperoxides which are easily decomposed into MDA. This situation results in a change in the strength of the cell membrane.¹²

Tender coconut water contains complete and beneficial nutrients for the body. The nutritional content of tender coconut water includes sugar, inorganic ions, vitamins, amino acids, and phytohormones.¹³ L-arginine in tender coconut water is one type of amino acid, this compound is believed to significantly reduce free radicals by assisting the synthesis of enzymatic antioxidants so as to minimize oxidative stress.¹⁴ In previous studies shows that the presence of vitamin C in tender coconut water can significantly maintain kidney antioxidant levels and prevent kidney damage.¹⁵

This study aims to determine the effect of tender coconut water on increasing SOD and CAT levels, as well as decreasing MDA level in cigarette smoke-exposed rats.

MATERIALS AND METHODS

Reagents and equipment

Digital scale, experimental animal cage, feeding and drinking chamber, smoking chamber, drop pipette, scientific centrifuge, test tube, measuring cup, 96-well plate, cuvette, microhematocrit for taking rat blood samples, ELISA (Enzyme-Linked Immunosorbent Assay) kit, Automatic Spectrophotometer Unit, Wistar strain male rats, tender coconut water, cigarettes, BR-12 standard feed, aquadest, buffer diluent solution, enzyme reagent solution.

In vivo assay

The 24 male Wistar strain rats were divided into 4 groups and each group consisted of 6 rats. This study had been approved by ethical committee with the legal number 349/XI/2020/Komisi Bioetik. The experimental animals will be given 7 days of adaptation time to avoid stress on the experimental animals. Each experimental animal was treated for 14 days, according to the following group divisions:

Group 1 (K1): fed standard diet + distilled water ad libitum) for 14 day	
Group 2 (K2)	fed standard diet + distilled water ad libitum + cigarette smoke exposure (3 cigarettes/day) for 14 day
Group 3 (K3)	fed standard diet + distilled water ad libitum + cigarette smoke exposure (3 cigarettes/day) + vitamin E at the dose of 1.8 IU /200gBW/day for 14 day
Group 4 (K4)	fed standard diet + distilled water ad libitum + cigarette smoke exposure (3 cigarettes/day) + tender coconut water muda (<i>Cocos nucifera</i> L.) at the dose of 8 mL/200 grBW/day for 14 day

Cigarette smoke exposure

The rats were transferred to the smoking chamber according to their group when they were exposed to cigarette smoke. The chamber is a smoking box with a barrier to separate the experimental animals from the burning ends of the cigarettes. Cigarette smoke is exhaled repeatedly with the help of an injection tube until the cigarette burns out. The number of cigarettes used in this study was 3 cigarettes/day and administered for 14 days.

Blood sampling

Blood was taken by inserting a microhematocrit tube into the ophthalmic vein at the corner of the rat's eyeball periorbitally then rotated slowly until the blood came out. The blood is collected in 2cc endendorf. Remove the micro-hematocrit tube if the blood is sufficient, clean the blood in the corner of the rat's eyeball with sterile cotton.

Analysis of SOD and CAT in serum

SOD and CAT levels were measured using the ELISA method. Blood samples of male Wistar rats were put into a tube, then added a buffer

solution with a ratio of 1:500. Incubate for 30 minutes at room temperature, then add 500 μ L of diluent, and then homogenize. Place 100 μ L of sample into the microplate, then incubate for 30 minutes at room temperature. Put 100 μ L of conjugate solution into a microplate, both negative, positive and sample control, and incubate for 30 minutes at room temperature.

Add 100 μ L of tetramerhybenxidine (TMB) substrate to the microplate, and incubate for 30 minutes at room temperature. Add 100 μ L of the reaction-stopping solution to the microplate. Immediately measure the absorbance level using a microplate reader.

Analysis of Malondialdehyde (MDA) in serum

Wistar male rat blood 2 mL was taken into a tube containing EDTA. The level of malondialdehyde (MDA) was measured by the concentration of Thioarbituric Acid Reactive Substances using the TBARSC method. Then, as much as 750 μ L of phosphoric acid was put into a 13 mL polypropylene tube. 50 μ L standard TEP/aquadest was put into the tube. Then the mixture was homogenized and 250 μ L of 40 Mm TBA solution was added. Then 450 μ L of distilled water was added to the tube and closed tightly. The mixture was heated for 1 hour, then placed into an ice bath. The cooled sample was applied to an 18-column Set Pack C. The absorbance was measured by a spectrophotometer with a wavelength of 340 nm.

Statistical analysis

Analysis of research data was processed using SPSS (Statistical Product and Service Solution). The data on SOD, CAT, and MDA levels were presented descriptively in the form of the average value and standard deviation, then the normality of the data was analyzed using the Shapiro Wilk Test and continued with the analysis of the homogeneity of variance with the Leuvene test. SOD, CAT, and MDA data were tested using One Way Anova to prove the effect of giving tender coconut water. The levels of SOD and MDA in rats exposed to cigarette smoke were normal and homogeneous, then followed by a different test of SOD levels between the two groups using a post hoc LSD test, while the CAT data were found to be normally distributed but not homogeneous, so a different test was used with the post hoc Tamhane test. Significance was assessed at $P < 0.05$ or more ($*P < 0.05$, $**P < 0.01$, and $***P < 0.001$).

RESULTS

Mean Level of SOD in serum

The results of SOD levels between groups can be seen in Figure 1. Figure 1 shows the highest mean SOD levels were found in group K1 (83.1 ± 3.57 U/ml), followed by group K3 (72.5 ± 3.80 U/ml), then group K4 (69.9 ± 6.22 U/ml), and the lowest was the K2 group (25.2 ± 3.88 U/ml). SOD levels appeared to be lower in K2, K3 and K4 compared to K1 because of the impact of exposure to cigarette smoke, and SOD levels seemed higher in K3 and K4 compared to K2 because in the K3 group there was Vitamin E administration, while in the K4 group there was tender coconut water administration.

The results of statistical analysis of the mean SOD levels on the administration of tender coconut water between groups can be seen in Table 1.

Table 1 shows the data normality test with the Shapiro Wilk obtained $p > 0.05$ in the four groups, it means that the all groups in this study had normal distribution of SOD levels.

Based on the data from the Leuvene's Test, p value = 0.352 ($p > 0.05$), it is stated that the variance of the SOD level data between all groups is homogeneous. The assumption is that the data are normally distributed and homogeneous, so that the differences in SOD levels between the

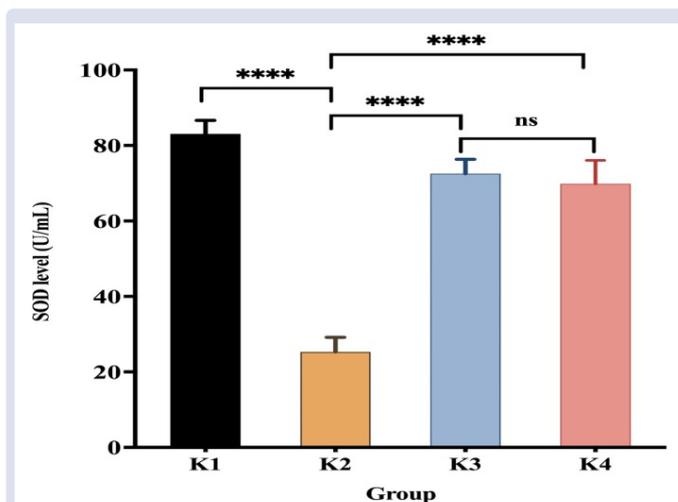


Figure 1: Mean Levels of SOD each group in Cigarette smoke – exposed rats.
 **** Significant $p < 0,0001$
 ns - Non –significant

Table 1: Statistical Analysis Results on Mean Level of SOD.

Group	Mean \pm SD (U/mL)	Shapiro Wilk	Leuvene's test	One Way Anova (p-value)
K1	83.1 \pm 3.57	1.000***		
K2	25.2 \pm 3.88	0.863***		
K3	72.5 \pm 3.80	0.945***	0.352 **	0.000*
K4	69.9 \pm 6.22	0.584***		

*** = normal data distribution ($p > 0.05$)
 ** = homogeneous data variance ($p > 0.05$)
 * = significant ($p < 0.05$)

Table 2: Results of post hoc LSD on SOD levels.

Group (I)	Group (J)	p-value
K1	K2	0.000*
	K3	0.001*
	K4	0.000*
K2	K3	0.000*
	K4	0.000*
K3	K4	0.312

* $p < 0.05$ significant difference

four groups can be analyzed using the One Way Anova test. The One Way Anova test resulted in a p-value of 0.000 ($p < 0.05$), meaning that the mean (mean) levels of SOD between the four groups were significantly different. Next, a post hoc LSD test was conducted to determine the difference in mean SOD levels between groups. The results of the post hoc LSD analysis are shown in (Table 2).

Based on the post hoc LSD analysis, almost all pairs of groups showed a significant difference in the mean of SOD levels ($p < 0.05$), except between the K3 and K4 groups ($p > 0.05$). Based on the results of this analysis, it can be concluded that exposure to cigarette smoke causes a decrease in SOD levels, and the administration of vitamin E and tender coconut water has an effect on increasing SOD levels in male wistar rats cigarette smoke-exposed. The effect of giving tender coconut water in increasing SOD levels is equivalent to the effect of giving vitamin E, there is no significant difference between K3 and K4 (p-value: 0.312 > 0.05).

Mean Level of CAT in serum

The results of the examination of CAT levels between groups can be seen in Figure 2. Figure 2 shows the highest mean CAT levels were in

group K1 (6.68 \pm 0.07 U/ml), followed by group K3 (4.95 \pm 0.37 U/ml), then group K4 (4.26 \pm 0.26 U/ml), and the lowest was the K2 group (1.71 \pm 0.07 U/ml). CAT levels appeared to be lower in K2, K3 and K4 compared to K1 because of the impact of exposure to cigarette smoke, and CAT levels were higher in K3 and K4 compared to K2 because in the K3 group there was Vitamin E administration, while in the K4 group there was tender coconut water administration.

The CAT level data was then analyzed statistically using the Shapiro Wilk test to determine the normality of the data distribution and the Leuvene's Test to determine the homogeneity of the variance, and continued with the One Way Anova test which can be seen in Table 3.

Table 3 shows the results of the Shapiro Wilk analysis show that all groups have normal data distribution. The results of the homogeneity test using Leuvene's obtained a value of $p = 0.010$ ($p < 0.05$) so that the data is not homogeneous. Furthermore, the data were analyzed using the One Way Anova, the result was a p-value of 0.000 ($p < 0.05$) so that the working hypothesis of this study was accepted, meaning that there was a significant difference in the mean CAT levels in the four groups. Because the mean CAT data obtained were normally distributed but not homogeneous, a post hoc Tamhane test was conducted to determine the difference in mean CAT levels between groups. Tamhane's post hoc results are shown in Table 4.

Table 3: Statistical Analysis Results on Mean Level of CAT.

Group	Mean \pm SD (U/mL)	Shapiro Wilk	Leuvene's test	One Way Anova (p-value)
K1	6.68 \pm 0.07	0.842***		
K2	1.71 \pm 0.07	0.408***		
K3	4.95 \pm 0.37	0.343***	0.010	0.000*
K4	4.26 \pm 0.26	0.073***		

*** = normal data distribution ($p > 0.05$)
 * = significant difference

Table 4: Results of post hoc Tamhane on CAT levels.

Group (I)	Group (J)	p-value
K1	K2	0.000*
	K3	0.000*
	K4	0.000*
K2	K3	0.000*
	K4	0.000*
K3	K4	0.029*

* $p < 0.05$ significant difference

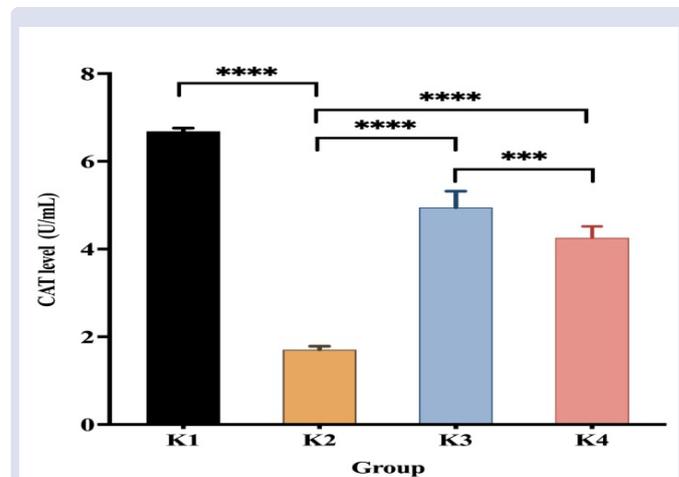


Figure 2: Mean Levels of CAT each group in Cigarette smoke – exposed rats.
 **** Significant $p < 0,0001$

Based on Tamhane's post hoc analysis, it was found ($p < 0.05$) significant difference between groups in the mean of CAT levels. Based on the results in this analysis, it can be concluded that exposure to cigarette smoke causes a decrease in CAT levels, and the administration of vitamin E and tender coconut water has an effect on increasing CAT levels in male Wistar rats with cigarette smoke-exposed. The results showed that giving tender coconut water at a dose of 8mL/200grBW/day and vitamin E at a dose of 1.8 mg/200gBW/day for 14 days could increase CAT levels in male Wistar strain rats with cigarette smoke-exposed, but the CAT levels in the administration of vitamin E (K3) was higher than the administration of tender coconut water (K4).

Mean Level of MDA in serum

The results of the examination of MDA levels between groups can be seen in Figure 3. Figure 3 shows the highest mean MDA levels were found in the K2 group (9.39 ± 0.38 mmol/mL), followed by the K4 group (3.09 ± 0.56 mmol/mL), then the K3 group (2.49 ± 0.53 mmol/mL), and the lowest was the K1 group (1.62 ± 0.24 mmol/mL). MDA levels appeared to be higher in K1, K3 and K4 compared to K2 because of the impact of exposure to cigarette smoke, and MDA levels were lower in K3 and K4 compared to K2 because the K3 group was given Vitamin E while in the K4 group there was given tender coconut water.

The MDA level data were analyzed statistically using the Shapiro Wilk test to determine the normality of the data distribution and the Leuvene's Test to determine the homogeneity of the variance, and continued with the One Way Anova test which can be seen in Table 5.

Based on Table 5, the results of the Shapiro Wilk show that all groups data have normal distribution. The results of the homogeneity test using Leuvene's obtained a value of $p = 0.130$ ($p > 0.05$) so that the data is homogeneous. Furthermore, the data were analyzed using the One Way Anova analysis, the results obtained p value of 0.000 ($p < 0.05$) so that the working hypothesis of this study was accepted, there was a significant difference in the mean of MDA levels in the four groups. Because the average MDA data obtained were normally distributed and homogeneous, a post hoc LSD was carried out to determine the difference in the mean of MDA levels between groups. The results of the post hoc LSD test are shown in Table 6.

Based on the post hoc LSD analysis, it was obtained ($p < 0.05$) so that there were significant differences between groups in the mean of MDA

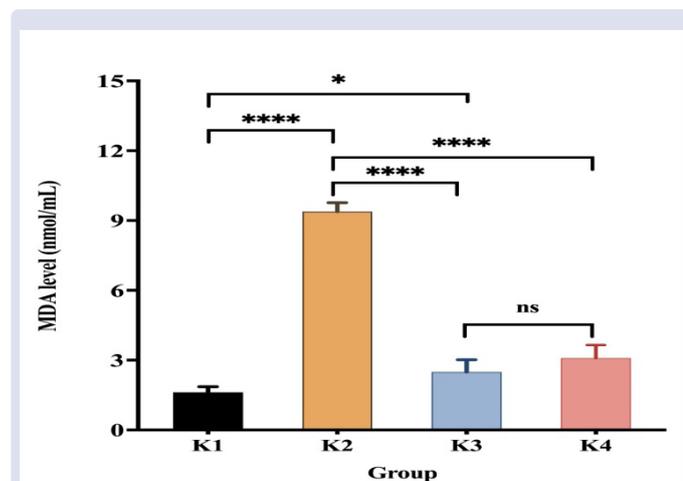


Figure 3: Mean Levels of MDA each group in Cigarette smoke – exposed rats.

**** Significant $p < 0.0001$
 * Significant $p < 0.005$
 ns Non-significant

Table 5: Statistical analysis results on mean level of MDA.

Group	Mean ± SD (mmol/mL)	Shapiro Wilk	Leuvene's test	One Way Anova (p-value)
K1	1.62 ± 0.24	0.429***		
K2	9.39 ± 0.38	0.995***	0.130**	0.000*
K3	2.49 ± 0.53	0.173***		
K4	3.09 ± 0.56	0.163***		

*** = normal data distribution ($p > 0.05$).

** = homogeneous data variance ($p > 0.05$).

* = significant ($p < 0.05$)

Table 6: Results of post hoc LSD on MDA levels.

Group (I)	Group (J)	p-value
K1	K2	0.000*
	K3	0.003*
	K4	0.000*
K2	K3	0.000*
	K4	0.000*
K3	K4	0.031*

* $p < 0.05$ significant difference

levels. Based on the results of this analysis, it can be concluded that exposure to cigarette smoke causes an increase in MDA levels, and the administration of vitamin E and tender coconut water has an effect on reducing MDA levels in male Wistar rats with cigarette smoke-exposed.

The results showed that the administration of tender coconut water at a dose of 8mL/200grBW/day and vitamin E at a dose of 1.8 mg/200gBW/day for 14 days could reduce MDA levels in male Wistar strain rats with cigarette smoke-exposed, but a decrease in the mean levels of The MDA in the administration of vitamin E (K3) was higher when compared to tender coconut water (K4).

DISCUSSION

The results of this study indicate that giving tender coconut water can increase levels of antioxidant enzymes, namely Superoxide Dismutase (SOD) and catalase (CAT), and reduce levels of Malondialdehyde (MDA) in male Wistar strain rats exposed to cigarette smoke. In the group exposed to cigarette smoke, the levels of antioxidant enzymes SOD and CAT were lower, but MDA levels were higher when compared to the group not exposed to cigarette smoke. These results are consistent with previous studies which state that cigarette smoke can reduce levels of antioxidant enzymes.

Cigarette smoke contains high concentrations of oxidant components and Reactive Oxygen Species (ROS), this causes smoking to cause a decrease in antioxidant activity.¹⁶ In cigarette smoke, there are two main phases that have been identified, namely the tar phase and the gas phase. Both of these phases are high in free radical components, and non-radical oxidants. Superoxide radicals (O_2^-), hydroxyl radicals (OH^-), and peroxy (ROO^-) can trigger oxidative damage in the form of lipid peroxidation.⁹ Excessive levels of ROS in the body, triggering an imbalance between the number of oxidants and antioxidants, this can cause oxidative stress.⁴ Exposure to cigarette smoke causes a decrease in GSH concentrations and the activity of several antioxidant enzymes such as GPx, SOD, and CAT.¹⁷ High levels of free radicals in the body will attack lipid, protein or DNA components that cause oxidative stress. Antioxidant status in the body can be observed in various parameters, namely through levels of SOD, GPx and CAT.⁸

The dangerous thing about smoking is the smoke produced by these cigarettes, because the content of cigarette smoke inhaled by smokers contains about 4000 chemicals including nicotine, hydrocarbons, carbon monoxide, and hydrogen cyanide.⁹ One of the dangerous

pathways from cigarette smoke to the body is because cigarette smoke is a free radical substance that can make the body experience oxidative stress which can damage protein, DNA, and RNA molecules in cells which in the long run will lead to chronic kidney disease, Alzheimer's in the brain, hypertension and coronary heart disease in the heart if left in the long term.¹⁸ Research conducted in previous studies also stated that cigarettes significantly affect antioxidant biomarkers, namely CAT, GPx, and SOD.⁹ Yadav *et al's* research also reported that betel nut and smoking habits led to a decrease in SOD activity.¹⁹ In previous studies it was found that exposure to cigarette smoke can cause liver damage in the form of liver necrosis and fatty liver.²⁰

Physiologically, the body has a balance between ROS or free radicals with antioxidant enzymes. Under certain conditions, the levels of free radicals and ROS produced are greater and cannot be suppressed by antioxidants, thus triggering oxidation and cellular damage occurs, this is called oxidative stress.¹² There are two ROS compounds that can initiate oxidative damage in the form of lipid peroxidation, namely hydroxyl radicals (HO•) and hydroperoxyl (HO₂•).⁹ Free radicals and antioxidants that are not balanced can cause free radicals to react by adding hydrogen ions from the methylene carbon chain to form lipid radicals. The lipid radicals then react with oxygen to form lipid peroxy radicals. Then, lipid peroxy radicals form new lipid radicals and cause changes in polyunsaturated fatty acids (PUFA) into lipid hydroperoxides which are easily decomposed into malondialdehyde (MDA). This situation results in changes in the strength of the cell membrane.¹²

Giving tender coconut water can increase the average levels of SOD and CAT, increase SOD levels up to 44.70 U/mL, while CAT levels are 2.55 U/mL, this indicates that tender coconut water can increase antioxidant enzymes due to exposure to cigarette smoke. On the other hand, the provision of tender coconut water can reduce the average level of MDA by 6.30 mmol/mL, this indicates that tender coconut water is able to reduce lipid oxidation due to exposure to cigarette smoke. These results are linear with previous studies which state that tender coconut water can increase levels of antioxidant enzymes and reduce lipid peroxidation.⁸

Coconut water has many health benefits. The content in coconut water includes vitamins, minerals, and amino acids.²¹ In previous studies, tender coconut water has been shown to improve lipid profiles, improve blood pressure status, and increase antioxidant levels in the body.^{7,8,15}

The antioxidant content in coconut water including vitamin C and L-arginine can function as an antidote to the bad effects of free radicals because it can increase antioxidant activity in the body.¹⁵ Coconut water has also been shown to increase levels of CAT, SOD, and GPx in traditional mining workers exposed to mercury. The presence of the free amino acid L-arginine which is abundant in coconut water can significantly reduce the level of ROS in the body and can increase levels of antioxidant enzymes such as SOD and CAT.⁸

Through the provision of tender coconut water, the decrease in SOD levels due to exposure to cigarette smoke can be prevented. This result is related to the L-arginine content in it, a free amino acid that can reduce ROS levels in the body so that SOD levels increase. L-arginine can significantly increase the catalase enzyme and reduce lipid peroxidation which is characterized by a decrease in MDA levels.¹⁵ Minerals such as Cu, Zn and Fe as well as vitamin C are also contained in tender coconut water. These minerals act as antioxidants that work in the following ways: Zn accelerates the formation of proteins that can neutralize ROS levels and replace transition metals such as Fe²⁺ and Cu²⁺.²² The minerals in coconut water can also add to the mineral deficiency that occurs due to exposure to cigarette smoke. Deficiency of Cu, Zn and Mn minerals can reduce the activity of Cu-Zn SOD and Mn-Mn-SOD. Mn-SOD functions as a catalyst for the dismutase reaction from

superoxide anion to H₂O₂ and O₂ in the mitochondria, while Cu-Zn SOD functions as a catalyst for the dismutase reaction from superoxide anion to H₂O₂ and O₂ in the cytosol. Cu minerals in Cu-Zn SOD are required for the catalytic function of enzymes, while Zn is important for structural functions. These minerals work synergistically.⁸

The effect of tender coconut water in increasing levels of SOD and CAT enzymes was also shown by Zulaikhah *et al* in workers exposed to mercury. Similar to exposure to cigarette smoke, prolonged exposure to mercury can also cause ROS formation, increase lipid peroxidation, and cause oxidative stress dismutase and glutathione peroxidation. Tender coconut water contains various antioxidants such as vitamin C, vitamin B1, vitamin B6, amino acids such as methionine, L-arginine, selenium, cytokinins and minerals that are useful in protecting body cells from free radical attacks and preventing the effects resulting from exposure. mercury.⁸ L-arginine is one type of amino acid in tender coconut water, this compound is believed to be able to significantly reduce free radicals in the body, this is because amino acids which are protein-forming monomers are known to help synthesize enzymatic antioxidants so as to minimize oxidative stress.²³

Vitamin E or α-tocopherol is one of the important components in the human body that functions as an antioxidant. Vitamin E can significantly reduce free radicals and prevent cell damage due to oxidative stress conditions, this is because vitamin E is a lipophilic antioxidant that can protect lipoproteins, PUFA, cellular and intracellular membranes from free radical chain reactions. Vitamin E has many benefits for human health, including it can be used for the prevention of premature aging, prevention of heart disease, as an anticancer compound, and other uses.²⁴ In previous studies, it has been proven that the administration of vitamin E can reduce levels of malondialdehyde (MDA) in white rats (*Rattus novgericus*) with diabetes mellitus.²⁵ In order to fulfil the needs of vitamin E, it is necessary to consume vegetables, wheat, and whole grains.²⁶

Vitamin E is able to fight the damage caused by exposure to cigarette smoke by interfering with the aqueous-ROS phase from cigarette smoke and can also inhibit lipid peroxidation due to ROS. Vitamin E supplementation can also increase the levels of endogenous vitamin E which are deficient due to ROS exposure,²⁷ so that the antioxidant activity produced by vitamin E is not reduced and is able to increase the activity of SOD.²⁸

In this study, the ability of tender coconut water to increase SOD levels in rats exposed to cigarette smoke was relatively similar to the ability of vitamin E. These results were seen from the average SOD levels in the K3 group (72.5 ± 3.80%) which were relatively similar to SOD levels in group K4 (69.9 ± 6.22U/ml) (p>0.05). Thus, tender coconut water can be used to replace vitamin E supplementation as an antioxidant. Further research on the benefits of tender coconut water in increasing SOD enzyme levels in smokers can be carried out.

The effect of giving tender coconut water and vitamin E to rats exposed to cigarette smoke for 14 days in this study has not been able to increase the levels of SOD and CAT enzymes to equal the normal conditions. These results can be seen from the levels of SOD and CAT respectively at K3 (72.5 ± 3.80 U/ml); (4.95 ± 0.37 U/ml) and K4 (69.9 ± 6.22 U/ml); (4.26 ± 0.26 U/ml) which was significantly lower than the levels of SOD and CAT in group K1 (83.1 ± 3.57 U/ml); (6.68 ± 0.07 U/ml) (p<0.05). The same thing with the effect of giving tender coconut water has not been able to reduce MDA levels to equal normal conditions, with a difference of 1.47 mmol/mL The dose of coconut water used in this study (8.0 ml/200 gbb) is almost equivalent to the therapeutic dose used in previous research, 450 ml for humans or 8.1 for rats weighing 200 g, only the duration of administration is relatively shorter, namely 14 days, whereas previous clinical trials were administered for 30 days.⁸

CONCLUSION

From this study, it can be concluded that tender coconut water has been shown to increase levels of antioxidant enzymes such as SOD and CAT and decrease lipid peroxidation through MDA levels in male Wistar strain rats exposed to cigarette smoke.

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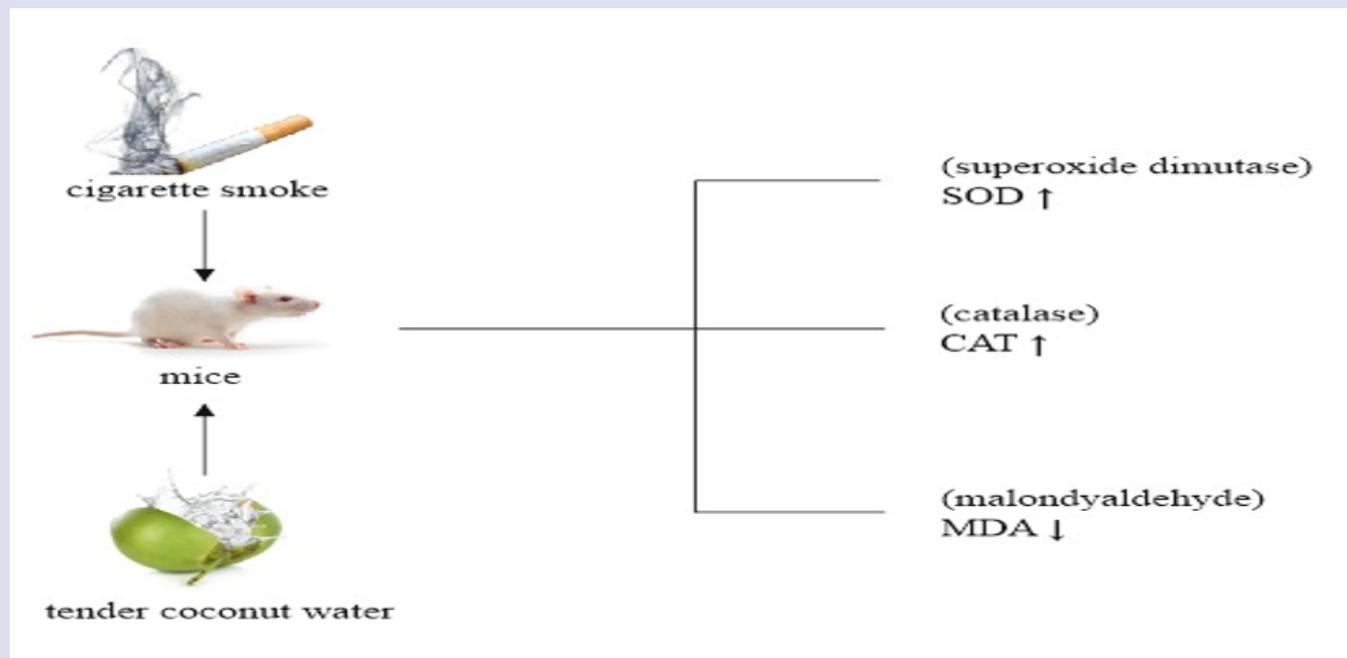
CONFLICTS OF INTEREST

There is no conflicts of interest.

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GRAPHICAL ABSTRACT



SUMMARY

Tender coconut water has been shown significantly increase levels of antioxidant enzymes such as SOD and CAT in male Wistar strain rats exposed to cigarette smoke.

Tender coconut water has been shown significantly decrease lipid peroxidation through MDA levels in male Wistar strain rats exposed to cigarette smoke.

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