第 48 卷 第 12 期
2021 年 12 月

湖南大学学报（自然科学版）
Journal of Hunan University（Natural Sciences）

Vol. 48 No. 12
December 2021

Open Access Article

Tender Coconut Water Can Inhibit Inflammation Caused by Cigarette Smoke

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Abstract: Cigarette smoke contains numerous free radicals that can trigger an inflammatory response. The response stimulates the release of pro-inflammatory cytokines such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and IL-8. Tender coconut water containing L-arginine has been shown to reduce inflammatory cytokines, including TNF-α, and IL-6. This study aims to determine the potential of tender coconut water in inhibiting inflammation in mice exposed to cigarette smoke. In this posttest only control group study, twenty-four male Wistar rats were randomly divided into 4 groups: K1 (standard *ad libitum*+ aqua diet); K2 (standard diet *ad libitum* + aqua + exposure to cigarette smoke 3 sticks/day); K3 (standard diet *ad libitum* + aqua+ exposure to cigarette smoke 3 sticks/day + vitamin E at a dose of 1.8 mg/200 grBW/day); K4 (standard diet *ad libitum* + aqua + exposure to cigarette smoke 3 sticks/day + tender coconut water at a dose of 8mL/200 grBW/day). The treatment was given for 14 days. On day 15, the blood samples were collected to evaluate CRP, IL-6, and TNF-α using ELISA (enzyme-linked immunosorbent assay). The data were analyzed using one way ANOVA test. The results showed that the level of CRP, IL-6m, and TNF-α in the group administrated with tender coconut water was significantly lower than that of those exposed to cigarette smoke alone (p<0.05). This study demonstrates that tender coconut water can inhibit inflammation in mice caused by cigarette smoke.

Keywords: tender coconut water, cigarette smoke, C-reactive protein, interleukin-6, tumor necrosis factor-alpha.

嫩椰子水安抑製香煙煙霧引起的炎症

摘要：香煙煙霧中含有大量可引發炎症反應的自由基。該反應刺激促炎細胞因子的釋放，如 C 反應蛋白、腫瘤壞死因子-α、白細胞介素 6 和 8。含有 L-精氨酸的嫩椰子水已被證明可以減少炎症細胞因子，包括腫瘤壞死因子-α和 6。本研究旨在確定嫩椰子水在抑制暴露於香煙煙霧的小鼠炎症方面的潛力。在此僅後測對照組研究中，將 24 隻雄性威斯塔大鼠隨機分為 4 組：K1（標準隨意+水飲食）；K2（標準飲食隨意+水+接觸香煙煙霧 3 根/天）；K3（標準飲食隨意 + 水 + 暴露於香煙煙霧 3 支/天 + 維生素乙，劑量為 1.8 毫克/200 公克體重/天）；K4（標準飲食隨意 + 水 + 暴露於香煙煙霧 3 支/天 + 8 毫升/200 公克體重/天劑量的嫩椰子水）。治療持續 14 天。在第 15 天，收集血液樣本以使用酶聯免疫吸附測定評估 C 反應蛋白、6 和腫瘤壞死因子-α。使用單向方差分析測試分析數據。結果顯示，軟椰子水給藥組的 C 反應蛋白、白細胞介素 6 米和腫瘤壞死因子-α水平顯著低於僅暴露於香煙煙霧的組

Received: August 11, 2021 / Revised: October 7, 2021 / Accepted: November 17, 2021 / Published: December 30, 2021

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（p<0.05）。這項研究表明，嫩椰子水可以抑製香煙煙霧引起的小鼠炎症。

关键词：嫩椰子水、香煙煙霧、C 反应蛋白、白細胞介素 6、肿瘤坏死因子-α。

1. Introduction

Smoking is a risk factor for a wide range of diseases, disability mortality [1]. Half a million Americans died from smoking or exposure to secondhand smoke early, while another 16 million suffer from serious illnesses each year [2]. According to the World Health Organization (WHO), the latest statistical data shows the percentage of smokers in Indonesia accounts for 33.8%, with 21.37% deaths from smoking [3]. Cigarette smoke can cause chronic obstructive pulmonary disease (COPD), cardiovascular diseases, and other diseases. Smoking has been shown to increase the risks of chronic systemic diseases through inflammatory mechanisms [4].

Cigarette smoke is a source of exogenous free radicals with more than 7000 chemicals. One cigarette contains 107 oxidant molecules [5]. There are two main phases in cigarette smoke that play a role in pathogenesis: the tar and gas phase. Both phases are rich in free radicals and non-free radicals. One puff of cigarette smoke contains 1017 free radicals in the tar phase and 1015 in the gas phase. Nicotine found in cigarettes can increase ROS reactive oxygen species (ROS), decrease the antioxidant capacity of enzymes, and induce the formation of active compounds that interfere with blood flow by inducing inflammation in blood vessels [1].

Cigarette smoke exposure can cause airway inflammation characterized by increased macrophages, neutrophils, and T lymphocytes that trigger the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and IL-8 [6]. Several studies have shown increased levels of C-reactive protein (CR), TNF-α, IL-6, and IL-8 in COPD patients with inflammation due to exposure to cigarette smoke. Levels of C-reactive protein (CRP), TNF-α, IL-6, and IL-8 in smoking COPD patients are higher than that of non-smoking; exposure to cigarette smoke increases levels of pro-inflammatory cytokines [5].

Tender coconut water contains L-arginine, polyphenols, vitamin C, selenium, and minerals (Cu, Mg, Mn, K, Na, Zn). L-arginine in tender coconut water can significantly reduce free radicals. Oxidative stress decreases along with the decrease in free radicals in the body. L-arginine can inhibit inflammation indicated by the decrease in levels of pro-inflammatory cytokines such as CRP, TNF-α, IL-6, and IL-8 [7]. This study was aimed to determine the potential of tender coconut water in inhibiting inflammation due to cigarette smoke exposure.

2. Method

In this posttest experimental study of control group only, twenty male Wistar rats, kept in Inter-University Research Nutrition Laboratory of PSPG Faculty of Medicine, Gadjah Mada University, Yogyakarta, were randomly divided into four groups (n=6 rats). Group division and treatment can be seen in Table 1.

Table 1 Group division and treatment

Group	Treatment
Negative control (K1)	Standard diet *ad libitum* + aqua (morning-afternoon) for 14 days
Positive control (K2)	Standard diet *ad libitum* + aqua + cigarette smoke exposure (3 cigarettes/day (morning-afternoon) for 14 days
Treated group 1 (K3)	Standard diet *ad libitum* + aqua + cigarette smoke exposure (3 s cigarettes/day + vitamin E at the dose of 1.8 mg/200gBW/day (morning-afternoon) for 14 days
Treated group 2 (K4)	Cigarette smoke3 (3 cigarettes/day + tender coconut water at the dose of 8mL/200gBW/day (morning-afternoon) for 14 days.

On day 15, blood samples were collected to evaluate levels of CRP, IL-6, and TNF-α using the ELISA (enzyme-linked immunosorbent assay) method.

2.1. Cigarette Smoke Exposure

According to their group, the rats were transferred to the smoking chamber (special cage) to expose cigarette smoke. The cage was a smoking box with a barrier to separate the experimental animals from the burning ends of the cigarettes. Cigarette smoke was exhaled repeatedly with the help of an injection tube until the cigarette burned out. The number of cigarettes used in this study was 3 cigarettes/day and administered for 14 days.

2.2. Blood Sampling

The equipment used was sterile micro-hematocrit tubes, blood collection bottles, and sterile cotton. A blood sample was taken by inserting a micro-hematocrit tube into the ophthalmic vein in the rat's eyeball periorbital corner and then slowly rotated until the blood came out. The blood was accumulated in 2cc Eppendorf. The micro-hematocrit tube was removed after the required blood obtained. The blood in the rat's

eyeball corner was cleaned with sterile cotton.

2.3. Measurement of CRP, IL-6, and TNF-α

CRP, IL-6, and TNF-α were assessed using the ELISA (*Enzyme-Linked Immunosorbent Assay*) kit.

2.4. Determination of Tender Coconut Water Dosage

The Dosage of tender coconut water was determined based on that proposed by Zulaikhah et al., i.e., 4 mL/100gBW.

2.5. Research Place

Treatment of rats and measurement of CRP, IL-6, and TNF-α took place in Inter-University Research Nutrition Laboratory of PSPG Faculty of Medicine, Gadjah Mada University, Yogyakarta.

2.6. Data Analysis

The data were tested for normality with Shapiro-Wilk, resulting in a normal and non-homogeneous distribution. Then the data were analyzed using one way ANOVA test followed by the post hoc Tamhane test to compare treatment groups. The TNF-α level data had a normal and homogeneous distribution, and then the data were analyzed by one-way ANOVA test followed by Post Hoc LSD test to compare treatment groups. The decision to accept or reject the hypothesis was based on α 5%.

3. Findings

The effect of tender coconut water on CRP, IL-6, and TNF-α is shown in Table 2.

Table 2 Effect of tender coconut water on the level of CRP, IL-6, and TNF-α in the four groups

Variable	Group				p-Value
	K1	K2	K3	K4	
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
CRP level (ng/mL)	3.2±0.28	17.2±0.86	5.4±0.39	6.7±0.33	>0.05*
Shapiro wilk	0.161	0.091	0.988	0.1222	<0.05**
Levene test					0.0001***
One way ANOVA					
IL-6 level (pg/mL)	43.0±6.09	87.8±3.87	53.9±1.87	62.8±3.82	>0.05*
Shapiro wilk	0.111	0.996	0.616	0.961	<0.05**
Levene test					0.0001***
One way ANOVA					
TNF-α level	6.06±0.30	17.9±0.26	7.3±0.39	8.8±0.43	>0.05*
(pg/mL)	0.737	0.858	0.933	0.825	>0.05**
Shapiro wilk					0.0001***
Levene test					
One way ANOVA					

Significant *>0.05, **>0.05, ***<0.05

Mean CRP, IL-6, and TNF-α, and Post hoc Tamhane and LSD are presented in Fig. 1.

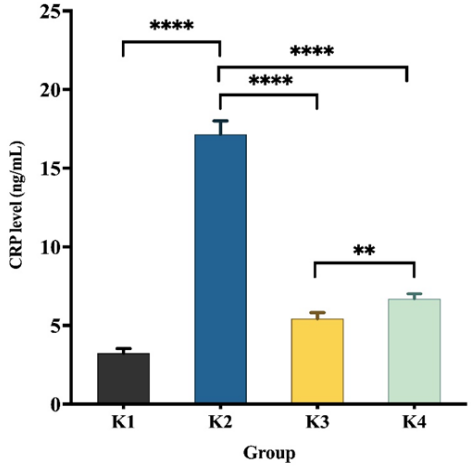

Fig. 1 Differences in mean CRP levels between groups
**** Significant p<0.0001
** Significant p<0.05

Table 1 and Fig. 1 show that the highest and the lowest mean levels of CRP were found in the K2 (7.2±0.86 ng/mL) and K1 (3.2±0.28 ng/mL) group, respectively. K3 and K4 (53.9±1.87 pg/mL and 62.8±3.82 pg/mL) had lower mean levels of IL-6 than K2 but higher than K1. The difference in the mean level of IL-6 between K2 and K3 was 11.8 ng/m; between K2 and K4 was 10.5 ng/mL. The results of the ANOVA showed that the administration of tender coconut water at the dose of 8mL/200gBW/day for 14 days reduced IL-6 levels (p<0.05). The mean IL-6 levels in K3 were lower than that of tender coconut water (K4). The analysis results using the Post hoc Tamhane test showed a statistically significant difference in mean IL-6 level between the two groups.

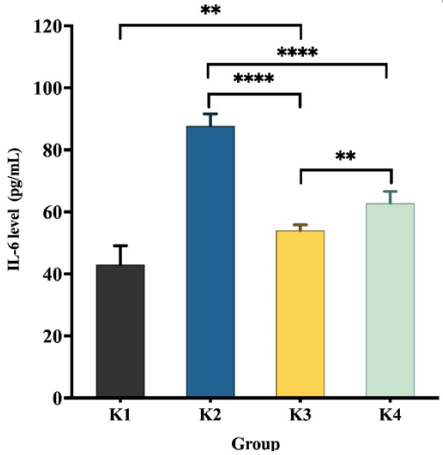

Fig. 2 Differences in mean IL-6 levels between groups
**** Significant (p<0.0001)
** Significant (p<0.05)

Table 1 and Fig. 2 show that K2 and K1 groups had the highest (87.8±3.8 pg/mL) and the lowest (43.0±6.09 pg/mL) mean IL-6 level, respectively. K3 and K4 had lower mean IL-6 levels (53.9±1.87 pg/mL and 62.8±3.82 pg/mL) than K2 but higher than K1. The difference in mean level IL-6 levels between K2 and K3 was 33.9 pg/mL, K2 and K4 was 25.0 pg/mL.

The analysis results with ANOVA showed that the administration of tender coconut water at the dose of 8mL/200gBW/day for 14 days reduced IL-6 levels (p<0.05). The mean IL-6 levels in K3 were lower compared to K4. The analysis results using the Post hoc Tamhane test showed that there was a statistically significant difference in mean IL-6 levels between the two groups.

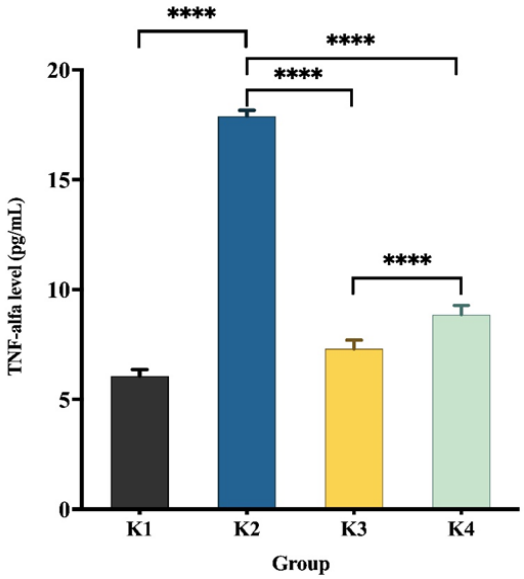

Fig. 3 Differences in mean TNF-α levels between groups
**** Sifgnificant (p<0.0001)
** Significant (p<0.05)

Table 1 and Fig. 3 show that the highest and the lowest mean levels of TNF-α were found in the K2 group (17.9±0.26 pg/mL) and K1 (6.06±0.30 pg/mL), group respectively. K3 (7.3±0.39 pg/mL) and K4 (8.8±0.43 pg/mL) had lower mean levels than K2 but higher than K1. The difference in the mean level of TNF α between K2 and K3 was10.6 pg/mL; K2 and K4 was 9.1 pg/mL.

The analysis results with ANOVA showed that the administration of tender coconut water at the dose of 8mL/200gBW/day for 14 days reduced IL-6 levels (p<0.05). The mean level of IL-6 in the K3 group was lower than in K4. The analysis using the Post hoc Tamhane test showed a statistically significant difference in mean levels of IL-6 between the two groups.

4. Discussion

This study showed that the mean levels of CRP, IL-6, and TNF-α in the group exposed to cigarette smoke were higher than those without exposure to cigarette smoke, indicating that tender coconut water has the potential to inhibit inflammation. These findings are consistent with that of the previous studies showing that smoking increased levels of CRP and IL-6, the presence of an inflammatory reaction increases levels of CRP, white blood cells, albumin, interleukin 6 (IL-6), and tumor necrosis factor (TNF-α) in the blood [8]. Long-term exposure to cigarette smoke can cause airway inflammation characterized by increased neutrophils, macrophages, T lymphocyte counts, and cytokines such as TNF-α, IL-6, and IL-8 [9]. The cytokine production is mediated, especially by free radicals derived from oxygen. These free radical mediators help the formation of cytokines by modulating nuclear factors (NFκB). Cigarette smoke contains free radical that causes oxidative stress. Cigarette smoke-induced oxidative stress causes a series of cellular and molecular reactions, activates cascades and transcription factors, releases inflammatory mediators, initiates inflammation, cell injury, and apoptosis [10].

One puff of cigarette smoke contains numerous free radicals, which play a role in pathogenesis. The major free radicals through cigarette smoke are superoxide (O_2), nitric oxide (NO), which combine to form peroxynitrite [11]. Due to the accumulation of free radicals, tissues are indented to deplete antioxidants such as ascorbic acid and protein sulfhydryl groups leading to the oxidation of DNA, lipids, and proteins. This ROS accumulation in tissues might be due to an increase in oxidant generation, decreased antioxidant defense agents, or failure to repair oxidative damage. The imbalance between oxidants and antioxidants causes oxidative stress initiating the inflammatory response [4]. The response begins with increased macrophages, neutrophils, and T lymphocytes that trigger the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and IL-8 [7]. Several studies have shown increased CRP, TNF-α, IL-6, and IL-8 in COPD patients with inflammation due to exposure to cigarette smoke. Levels of C-reactive protein (CRP), TNF-α, IL-6, and IL-8 in smoking COPD patients were shown to be higher than that of non-smoking. Exposure to cigarette smoke increases levels of pro-inflammatory cytokines [5, 12]. CRP, IL-6, and TNF-α levels are pro-inflammatory cytokines. Increased CRP, IL6, and TNF-α level indicate high levels of ROS and decreased nitrate oxidation. High levels of IL-6 stimulate the liver to synthesize and secrete markers of systemic inflammation, including CRP [13].

The most dangerous content of cigarette smoke causing oxidative stress and inflammation is nicotine, carbon monoxide, metals (arsenic, nickel, chromium, and lead), particulate matter, and caroline. Nicotine in cigarettes can cause an increase in ROS and a decrease

in the antioxidant capacity of enzymes and induce the formation of active compounds disrupting blood flow by inducing inflammation in blood vessels. Carbon monoxide in cigarettes can cause hypoxia and increase xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidases). Acrolein in cigarettes can cause a decrease in antioxidant enzymes, a decrease in the immune response, and an increase in inflammation markers [14].

Without excessive antioxidant reserves, the body requires higher exogenous antioxidants to eliminate the effects of free radicals exposure [15]. Different enzymatic mechanisms minimize radical damage and protect against excessive free radical production. Antioxidants play an important role in this defense mechanism [16]. In normal and healthy subjects, there is a balance between the formation of reactive oxygen species free radicals and endogenous antioxidant defense mechanisms [17]. If ROS amount is more than antioxidants, then the excessive amount of ROS will disturb lipid, protein, and DNA components called oxidative stress [15]. At present, the impact of oxidative stress and its related factors has become an important issue for human health. The World Health Organization (WHO) estimates that about 80% of the world's population uses traditional medicine for primary health care. In recent years, more interest has been focused on experimental research to verify natural antioxidants and anti-inflammatory drugs from natural resource products [18]. Foods containing natural antioxidants can be used as a strategy to reduce morbidity and mortality, especially due to oxidative stress [15]. Natural sources of antioxidants can be used as preventive medicine. Recent studies have shown an inverse relationship between the consumption of antioxidant-rich food and the prevalence of the human disease. Consuming antioxidants from natural plant sources as a daily diet can offer a solution to overcome human health problems [17, 19].

Tender coconut water contains various nutrients such as L-arginine, Vitamin C, amino acids, and minerals such as magnesium, potassium, calcium, selenium, methionine, zinc, iodine, manganese, and cuprum [15, 20]. This study shows that the administration of tender coconut can inhibit inflammation caused by cigarette smoke indicated by lower levels of CRP, IL-6, and TNF in the K4 group compared to the group exposed to cigarette smoke. The results of this study support that of a study conducted by Zulaikhah et al. showing that administration of tender coconut water at the dose of 8mL/200gr BW for 4 weeks prevents inflammation characterized by a decrease in TNF α, IL-1, and IL-6 in induced by streptozotocin (STZ) and nicotinamid (NA) [8].

Vitamin C in tender coconut water serving as an antioxidant is a micronutrient that plays an important role in the immune system with pleiotropic functions due to its ability to donate electrons, biosynthesis of enzymes, and function of gene regulation. Vitamin C also plays a role in cellular immunity, increasing epithelial barrier function, is needed in apoptosis, and can cleanse necrotic tissue (NETosis) to reduce the inflammatory process [21]. A study conducted by Dewi et al. proved that vitamin C reduces pro-inflammatory cytokines characterized by differences in IL-6 and CRP levels between the group a with vitamin C at a dose of 12 g/50 mL water every 12 hours for 7 days and the placebo group showing that mean levels of IL-6 and CRP in the group given vitamin C was lower compared to the placebo group [22].

Vitamin C has been shown to decrease nicotine toxicity by donating a hydrogen atom to nicotine metabolite indicated by the difference in CE expression between-group supplemented with vitamin C at the doses of 200 mg/day and 1000 mg/day compared with placebo. The decrease in CE excretion positively correlated with the increased dose of vitamin C supplementation [23].

Vitamin C decreases inflammation and oxidative stress in patients with metabolic syndrome, indicated by decreased CRP and IL-6 levels [24]. Animal studies have shown that vitamin C deficiency exposed to cigarette smoke indicates the release of Troponin T and I in serum, oxidative stress, apoptosis, thrombosis, and myocardial deposition. There was a significant difference in plasma vitamin C levels between smokers and the non-smoker group [25].

Vitamin C is a micronutrient that plays an important role in the immune system with pleiotropic functions due to its ability to donate electrons, biosynthesis of enzymes, and function of gene regulation. Vitamin C also functions in cellular immunity, increases epithelial barrier function, is needed in the process of apoptosis, and can cleanse necrotic tissue (NETosis) to reduce the inflammatory process [23]. Research on experimental showed that vitamin C deficiency exposed to cigarette smoke showed the release of Troponin T and I in serum, oxidative stress, apoptosis, thrombosis, and myocardial deposition. There was a significant difference in plasma vitamin C levels in smokers compared to the non-smoker group [25].

CRP levels are inversely correlated with vitamin C levels in the body, meaning that the higher the intake of vitamin C, the lower the levels of CRP will be, and the lower the intake of vitamin C, the higher the levels of CRP will be [26]. Vitamin C acts as an antioxidant and immunomodulator in reducing the severity of COVID-19 infection [22]. The reduction of free radicals can prevent oxidative stress to inhibit the occurrence of inflammatory responses in the body. Vitamin C can also reduce the inflammatory response directly by suppressing the production of IL-6, CRP, and TNF-α [13, 22].

L-arginine in tender coconut water is a non-essential amino acid in nitric oxide synthase (NO Synthase). This compound is a substrate to produce citrulline and NO. NO can inhibit xanthine oxidase (XO), leading to increased SOD levels, total thiol levels (T-SH), vitamin C, total antioxidants (TAC) [20]. The results of previous studies showed that L-arginine inhibited the secretion of IL-1β, IL-6, and TNF-α by macrophages and other inflammatory infiltrating cells in the male dystrophin-deficient (MDX) diaphragm muscle. L-arginine inhibits the inflammatory cascade to reduce the number and activity of nuclear factor-kB (NF-kB) and metalloproteinase (MMP)-2 and MMP-9 activities in males dystrophin-deficient (MDX) mice [27].

L-arginine in tender coconut water is an antioxidant and can reduce free radicals characterized by increased SOD, catalase, and GPx [20]. L-arginine will bind to free radicals directly or damage free radical bonds [28]. L-arginine compounds can inhibit inflammation as seen from the decrease in levels of pro-inflammatory cytokines such as CRP, TNF-α, IL-6, and IL-8 [8].

5. Conclusion

Cigarette smoke can cause oxidative stress that can induce inflammatory responses [12, 14]. Tender coconut water consists of L-arginine, Vitamin C, and amino acids that can inhibit inflammation by decreasing levels of pro-inflammatory cytokines such as CRP, IL-6, IL-8, and TNF-α [8, 19].

Several studies consider the effect of tender coconut water. The oral administration of tender coconut water can lower TC, LDL, and TG levels and increase HDL serum levels in male rats fed a high-fat diet [15]. Other research has shown that consuming tender coconut water for 30 days increases antioxidant enzymatic SOD, CAT, GPx, and decreases lipid peroxidation in mercury exposure workers [20]. The administration of tender coconut water can decrease the levels of TNF-α, IL-1, and IL-6 in Streptozotocin (STZ) and Nicotinamide (NA) induced diabetic rats [8]. Coconut water can effectively reduce the level of heavy metal, especially Cd and Hg, in the blood of *Rattus norvegicus* [28].

This present study showed that the administration of tender coconut water has the potential effect of inhibiting inflammation due to cigarette smoke. This study demonstrates that the administration of tender coconut water can inhibit inflammation caused by cigarette smoke, characterized by decreased CRP, IL-6, and TNF-α in the K4 group compared to K2 in mice exposed to cigarette smoke.

6. Limitations and Further Study

The limitation in this study is that the study only uses tender coconut water with one dose and one type of tender coconut water. Despite its limitation, this study has strengths, including its study aim that find out the effect of tender coconut water in inhibits inflammation caused specifically by cigarette smoke, which is rarely studied. We suggest that researchers need to investigate the effect of tender coconut water in inhibiting inflammation caused by cigarette smoke using various dosages and different types of tender coconut water for further research.

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