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Pengaruh Jus Apel Hijau terhadap Kadar SOD dan IL-6 pada Tikus yang Terpapar Asap Rokok

Effect of Green Apple Juice on SOD And IL-6 in Cigarette Smoked Rats

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Abstract

*Cigarette smoke contains oxidants or free radicals and approximately 4,700 hazardous chemicals. Elevated levels of free radicals in the body can trigger the generation of Reactive Oxygen Species (ROS), leading to oxidative stress. This condition arises when there is an imbalance between oxidants and antioxidants. During this process, oxygen (O2) leakage occurs, resulting in the formation of superoxide radicals (*O2), which can subsequently induce the production of pro-inflammatory cytokines such as IL-6. The objective of this study was to evaluate the effect of green apple juice supplementation on superoxide dismutase (SOD) and IL-6 levels in male Wistar rats exposed to cigarette smoke. The study employed a Post-Test Only Control Group Design. A total of 20 male rats that met the inclusion criteria were randomly assigned to four groups: K1, K2, K3, and K4. Group K3 received standard feed supplemented with green apple juice at a dose of 13.5 grams per day, while group K4 received standard feed supplemented with green apple juice at a dose of 27 grams per day. Both groups were exposed to cigarette smoke for 14 consecutive days. On the 15th day, blood samples were collected from the male Wistar rats to measure SOD and IL-6 levels using the ELISA method. Data were analyzed using a One-Way ANOVA test, followed by Tukey's Post Hoc test. The results showed that the highest average SOD level was observed in group K2 (1.76 ng/L), while the highest IL-6 level was recorded in group K1 (4.08 ng/L). The One-Way ANOVA test indicated significant differences in SOD and IL-6 levels among the groups, with a p-value of <0.05.*

Keywords**:** *Green Apple Juice; Rats; Smoke exposure; SOD IL-6*

1. INTRODUCTION

Smoking is a lifestyle habit that significantly impacts human health. Cigarette smoke contains approximately 10^{15}-10^{17} oxidants or free radicals and around 4,700 hazardous chemicals, including aldehydes/carbonyls, nitrogen dioxide (NO_2), and sulfur dioxide (SO_2) [1]. High levels of free radicals in the body can trigger the formation of Reactive Oxygen Species (ROS), which lead to oxidative stress. This condition arises when there is an imbalance between the number of oxidants and antioxidants. The primary antioxidant that serves as the first line of defense against free radical compounds is superoxide dismutase (SOD). SOD is a key enzymatic antioxidant in the defense mechanism against superoxide anions[2,3].

Free radicals in the human body are generated through various processes, including oxidation, cellular respiration, metabolism, inflammation, and exposure to environmental pollutants such as vehicle emissions and cigarette smoke[4,5]. These free radicals react with cellular molecules to gain electron pairs, thereby stabilizing themselves; however, the affected cellular molecules become free radicals in the process. This chain reaction continues unless interrupted, ultimately leading to oxidative stress, which can result in inflammation, DNA and cellular damage, and the onset of degenerative diseases. Free radicals can also be generated during inflammatory processes, such as the conversion of NADPH to NADP catalyzed by NADPH oxidase [6].

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During this process, oxygen (O_2) leakage can produce superoxide radicals ($*O_2$), which stimulate the formation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) [7]. IL-6 is a pro-inflammatory cytokine widely used as a biomarker for assessing inflammation levels. Cigarette smoke contains significant amounts of free radicals, which can cause structural tissue abnormalities related to the inflammatory response. This occurs because cigarette smoke generates ROS, which stimulates the production of pro-inflammatory cytokines such as IL-6 [8].

A study conducted by Jennifer *et al.,* (2015) in Taiwan reported that SOD levels in smokers were significantly lower than in non-smokers [9]. The reduction in SOD concentration is attributed to the ability of ROS to oxidize proteins, including enzymatic proteins, leading to the loss of SOD activity and function. Several studies have reported that smoking behavior increases serum IL-6 levels [10]. Another research by *Mokkem et al.,* (2018) have shown a relationship between elevated serum IL-6 levels in smokers and non-smokers, wherein the oxidative stress induced by cigarette smoke stimulates the release of pro-inflammatory cytokines such as IL-6 [11].

However, conflicting findings exist, with some studies reporting no significant differences in mean serum IL-6 levels between smokers and non-smokers. Green apples are nutrient-dense fruits rich in antioxidants [12]. The flavonoid content in green apples exhibits anti-inflammatory properties, as flavonoids can inhibit the production of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β, and interferon-γ [13].

This study aims to investigate the potential effects of green apple (Malus domestica) juice on the levels of superoxide dismutase (SOD) and interleukin-6 (IL-6) in male Wistar rats exposed to cigarette smoke. Specifically, the research seeks to determine whether the antioxidant compounds present in green apple juice can enhance enzymatic antioxidant activity and reduce inflammatory responses caused by cigarette smoke exposure, as indicated by changes in SOD and IL-6 levels.

Previous studies have demonstrated that green apples can prevent the increase in nitric oxide (NO) levels, thereby mitigating endothelial damage in blood vessels and the heart. However, research examining the effects of green apple juice on enzymatic antioxidant markers and inflammation in the context of cigarette smoke exposure remains limited. This study aims to evaluate the effects of green apple juice on SOD levels and IL-6 levels as markers of enzymatic antioxidants and inflammation in male Wistar rats exposed to cigarette smoke.

2. METHODS

Materials

20 male Wistar rats weighing between 120 and 180 grams were acclimatized in the laboratory for one week prior to the experiment. During this period, the rats were provided adaptive feed and placed in four separate cages under controlled conditions of temperature, humidity, and light. They were initially fed pellet feed and given access to drinking water via a sipper tube. The study was conducted at the Inkes Medistra Lubuk Pakam pharmaceutical laboratory, while experimental testing was performed at the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra. The research protocol was reviewed and approved by the Ethics Committee of the University of North Sumatra (No. 0735/KEPH-FMIPA/2024). Fresh green apples were thoroughly washed and then blended into a puree. The recommended human dose of green apples is 300 grams per day. The human dose (based on an average body weight of 70 kg) was converted for rats (average body weight = 200 grams) using a dose conversion factor of 0.018. As a result, the first dose for the rats was determined to be 13.5 grams per day, and the second dose was set at 27 grams per day.

Methods

Juice Preparation Process

The green apples were thoroughly washed before being blended into a puree. The standard human consumption dose of green apples is 300 grams per day. To convert this human dose (based on an average body weight of 70 kg) into the equivalent dose for rats (average body weight = 200 grams), a conversion factor of 0.018 was applied. Consequently, the first dose was calculated as 13.5 grams per day, and the second dose as 27 grams per day.

Blood Sampling Procedure

Blood samples were collected from the orbital sinus of the test animals. The procedure began by restraining the animal, holding the skin at the nape and back firmly with the left thumb and index finger. A glass pipette was used for the sampling, held in the right hand. The pipette was positioned at a 45-degree angle toward the orbital sinus area (medial canthus). It was then carefully inserted through the outer skin until a slight "click" sound was heard, indicating penetration. The mouse was gently tilted, allowing blood to drip into the pipette, which was then transferred into a collection tube for further analysis [14].

Preparation of Cigarette Smoke Exposure

The cigarettes used in this study were kretek cigarettes. Cigarette smoke exposure was conducted daily using four cigarettes per session. The exposure process was carried out over 14 consecutive days in a designated cigarette smoking chamber. During each session, the cigarettes were kept continuously lit to ensure maximum smoke exposure to the rats throughout the treatment period.

SOD Enzyme Assay

The SOD enzyme assay was performed as follows: 50 µL of sample diluent was added to the sample diluent wells, prepared in duplicate. Subsequently, 50 µL of pre-diluted standards (S1–S6) was added to the respective standard wells, and 50 µL of the serum sample was added to the sample wells. Next, 100 µL of HRP-Conjugate reagent was added to each well. The plate was mixed thoroughly and incubated at 37°C for 1 hour. After incubation, the wells were washed five times with a wash solution. Following the washing step, 50 µL of Chromagen A and 50 µL of Chromagen B were sequentially added to each well. The plate was then incubated at room temperature (20–25°C). Finally, the absorbance was measured at 450 nm using a microplate reader [15].

IL-6 Enzyme Assay

On the 15th day, blood samples were collected from the white mice via intracardiac puncture. The blood was then transferred into tubes without anticoagulant. The tubes were placed in a centrifuge and spun at 3000 rpm for 10 minutes to separate the serum. The resulting serum was carefully collected and analyzed for IL-6 levels using an IL-6 ELISA kit, with the readings obtained using an ELISA Reader [16].

Data Analysis

Data analysis of the research results was performed using the SPSS (Statistical Package for the Social Sciences) software, employing a one-way analysis of variance (One-Way ANOVA). If a significant difference was found, the analysis was continued with the Tukey HSD test to identify which variables showed differences. A significance level of $p < 0.05$ was considered statistically significant

3. RESULTS

This study utilized a sample of 20 male Wistar rats, each weighing between 150-200 grams, which were divided into four groups of five rats each: one control group and three treatment groups. Group 1 served as the control group, receiving standard feed without exposure to cigarette smoke. Group 2, the first treatment group, was given standard feed and exposed to cigarette smoke. Group 3 received standard feed and green apple juice at a dose of 13.5 g/KgBW/day, along with exposure to cigarette smoke. Group 4 was given standard feed and apple juice at a dose of 27 g/KgBW/day, also exposed to cigarette smoke. On the 15th day, levels of Superoxide Dismutase (SOD) and Interleukin-6 (IL-6) were measured using ELISA. This research investigated the effect of green apple juice (Malus domestica) on SOD and IL-6 levels in male Wistar rats exposed to cigarette smoke for 14 days. The results are presented in Table 1.

Table 1. Increased SOD and IL-6 activity was observed in the group treated with green apple juice.

Groups	n	Aktivitas SOD dan IL-6 dari rata-rata ± SD

		SOD (ng/mL)	IL-6 (ng/mL)
Normal	5	2,2203 ± 0,29[b]	5,2637 ± 1,04[b]
Negative	5	1,3050 ± 0,09[a]	4,3729 ± 0,24[a]
13,5 gr/ Kg BB	5	1,4992 ± 0,74[a]	4,0869 ± 0,22
27 gr/Kg BB	5	1,7605 ± 0,18[ab]	3,6031 ± 0,24[b]

Cigarette smoke contains free radicals, which, when present in high concentrations in the body, can trigger the formation of Reactive Oxygen Species (ROS), leading to oxidative stress. This imbalance between oxidants and antioxidants can result in cellular damage. In this study, the treatment groups exposed to cigarette smoke daily (four cigarettes per day) included K2, K3, and K4. The results of the SOD (superoxide dismutase) level examination showed that the group exposed to cigarette smoke without green apple juice had lower SOD levels than the control group, as shown in Table 1.

This suggests that cigarette smoke exposure can decrease SOD levels. The reduction is due to the oxidative stress induced by 14 days of cigarette smoke exposure. The prolonged high concentration of ROS during this exposure overwhelms the SOD antioxidant defense, impairing its ability to neutralize ROS and resulting in cell and tissue damage. SOD levels were higher in the group given green apple juice at a dose of 27 g/KgBW/day in addition to cigarette smoke exposure, compared to the group that received 13.5 g/KgBW/day of green apple juice. Although the 27 g/KgBW/day dose group had the highest SOD levels, they remained lower than the normal control group, as indicated in Table 1.

4. DISCUSSIONS

This can be attributed to the normal group not being exposed to cigarette smoke but receiving standard feed, which contributed to the highest SOD levels. Additionally, the group receiving 27 g/KgBW/day of green apple juice showed higher SOD levels than the group receiving 13.5 g/KgBW/day, indicating that a higher dose of green apple juice led to a higher SOD response. Green apple juice, rich in flavonoids, vitamins A, C, and E, has been shown to enhance antioxidant enzymes and reduce peroxide levels, as flavonoids help reduce the formation of free radicals in the body [17].

The study also revealed that IL-6 levels in the cigarette smoke-exposed group without green apple juice (K2) were significantly higher compared to the normal control group (K1), the group receiving 13.5 g/KgBW/day of green apple juice (K3), and the group receiving 27 g/KgBW/day of green apple juice (K4), as shown in Table 1. This suggests that cigarette smoke exposure increases free radical levels, which can disrupt tissue structure and trigger inflammatory responses, leading to elevated IL-6 levels. However, IL-6 levels were significantly lower in the group receiving 27 g/KgBW/day of green apple juice compared to the 13.5 g/KgBW/day group. This indicates that green apple juice can suppress IL-6, a pro-inflammatory cytokine, which is typically elevated under oxidative stress conditions induced by cigarette smoke [18].

The flavonoids in green apples inhibit the enzymes responsible for ROS production, thereby preventing cell and tissue damage and reducing oxidative stress. Furthermore, the vitamins A, B, C, and E in green apples can protect cells from oxidative damage caused by ROS [19]. A limitation of this study is that it did not measure ROS levels, which are a critical factor in causing oxidative stress.

5. CONCLUSION

The administration of green apple juice (Malus domestica) at a dose of 27 g/KgBW demonstrated the most significant effect in increasing SOD levels and decreasing IL-6 in male white mice induced by cigarette smoke

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