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The Protective Effect of Aloe vera L. on Lipid Profile in Rat after being Chronic Exposed to Cigarette Smoke

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Abstract— Smoking epidemic remains to be one of the biggest public health issues the world has ever faced. Cigarette smoke can stimulate peroxidation of lipid and oxidation of proteins and become a risk factor for atherosclerosis and cardiovascular disease. *Aloe vera* has been used for medicinal purposes for centuries and reported to have potent antioxidant capacity. Considering its good potential as alternative medicine in the future, this study aimed to evaluate the effect of *Aloe vera* on serum lipid profile in the rat after being exposed to cigarette smoke. The experimental study was conducted on male rats which were divided into 3 groups. The first group was a control, the second group was exposed to the smoke of 8 cigarettes/ day for 30 minutes. The other group was given 1 mL/ day of *Aloe vera* gel an hour before the exposure of the smoke for 42 days. The blood sera were measured for lipid profile, consisting of total cholesterol, low density lipoprotein (LDL), and high density lipoprotein (HDL). The results were statistically analyzed using Kruskal-Wallis and post hoc test Mann-Whitney. There was a significant increase in serum LDL and a decrease in HDL between control and cigarette smoke group ($p<0.05$). A comparison of results between cigarette smoke and cigarette smoke plus *Aloe vera* groups showed a decreasing concentration of serum LDL ($p<0.05$). *Aloe vera* L. may prevent dyslipidemia in rats after being chronically exposed to cigarette smoke.

Keywords— *Aloe vera* L.*; Lipid Profile; Dyslipidemia, Cigarette Smoke.*

1. Introduction

The smoking epidemic remains to be one of the biggest public health issues the world has ever faced [1]. Currently, the number of smokers reaches 1.2 billion people globally and most of them live in countries with low- and middle-income. World Health Organization (WHO) reported Indonesia ranks the third for having the largest number of smokers in the world following China and India [2]. The prevalence of smoking in Indonesia is high, especially in male consisting of children, adolescents, and adults [3].

Cigarette consumption has a serious impact on the increasing burden of smoking-related diseases and deaths [4]. Cigarette smoking causes more than seven million deaths and economic losses of USD 1.4 trillion each year, calculated from medical care expenses and loss of productivity [5]. WHO estimates that direct tobacco use results in more than six million deaths while the non-smokers being exposed to second-hand smoke causes around 890.000 deaths [6]. Brief exposure to cigarette smoke can even cause considerable harm to the body since there is no safe level of exposure to second-hand smoke. Cancer, cardiovascular diseases, and chronic obstructive pulmonary disease are the major health problems affecting smokers [1,6-8].

Cigarette smoke contains more than 4000 toxic and mutagenic ingredients, including nicotine, ammonia, acrolein, phenols, acetaldehyde, polycyclic aromatic hydrocarbons, polyphenols, carbon monoxide, nitrogen oxides, hydrogen cyanide, and trace metals. It has a broad spectrum of free radicals and non-radical oxidants [9]. Free radicals have a high reactive power and are able to damage some vital macromolecules. Free radicals, such as peroxyl (ROO), superoxide radical (O2-), and particularly hydroxyl radical (OH) can induce oxidative damage in the form of lipid peroxidation and oxidation of proteins and DNA bases [8,9]. Studies have shown that smokers exhibited an elevation of LDL level and a decrease in HDL level, as compared with nonsmokers [10, 11].

The oxidative stress caused by reactive oxygen species (ROS) in cigarette smoking occurs as an imbalance between production and detoxification of these free radicals [12]. Antioxidants provide defense against this oxidative stress by preventing excess production of ROS and neutralizing free radicals. Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) play an important role to prevent cell damage, peroxidation of lipid, and oxidation of protein and DNA [9,13].

In some countries, *Aloe vera* has been used for medicinal purposes for centuries. *Aloe vera* has more than 200 active components and has anti-inflammatory, antimicrobial, antioxidant and anticancer effects [13-15]. It has been reported that potent antioxidant capacity of *Aloe vera* could scavenge oxygen radicals. The activity of enzyme GPX, enzyme SOD, and a phenolic antioxidant in *Aloe vera* gel might be responsible for these antioxidant effects [15]. Health benefits of *Aloe vera* also included its effect on wound healing, increasing HDL, reducing LDL, and reducing blood sugar in diabetics [13,15]. Considering its good potential as alternative medicine in the future, this study aimed to evaluate the effects of *Aloe vera* on serum lipid profiles in the rats after being exposed to cigarette smoke.

2. Materials and Methods

2.1. Animal, Treatments, and Samples Collection

This experimental study was conducted on 24 male rats (*Rattus norvegicus*) Wistar albino strain aged 9–11 weeks. The inclusion criteria were rats weighing 200-250 grams and in good health, while the exclusion criteria were sick conditions of rats such as lacking agility or appetite loss. The rats were divided into three groups. The first group was a control group and there was no intervention given to this group. The second group was cigarette smoke group and exposed to the smoke of eight cigarettes per day for 30 minutes. The last group was given 1 mL/ day of *Aloe vera* gel an hour orally before the exposure of the smoke with the same amount of cigarettes for 42 days [7]. On the 42nd day, the blood samples were collected from the rats followed by lipid profiles analysis. During the study, all groups were exposed to the equal environmental condition, given ad libitum feedings as well as unlimited access to water.

2.2. Passive Cigarette Smoke Inhalation

The animals were exposed at a time to cigarette smoke from commercially available cigarettes in an inhalation apparatus designed in our laboratory. The inhalation apparatus was designed in a manner that it included a nonpermanent cover attached to a Perspex chamber (Figure 1). The chamber consisted of separate inlets for smoke and fresh air and to control the air flow. Animals were exposed to the smoke of 8 cigarettes for 30 minutes daily. Each cigarette took approximately 4 to 6 min to burn completely. This procedure had been standardized in such a way that animals inhaled cigarette smoke without any respiratory stress as is evident from carboxy-hemoglobin (CO-Hb) levels [16].

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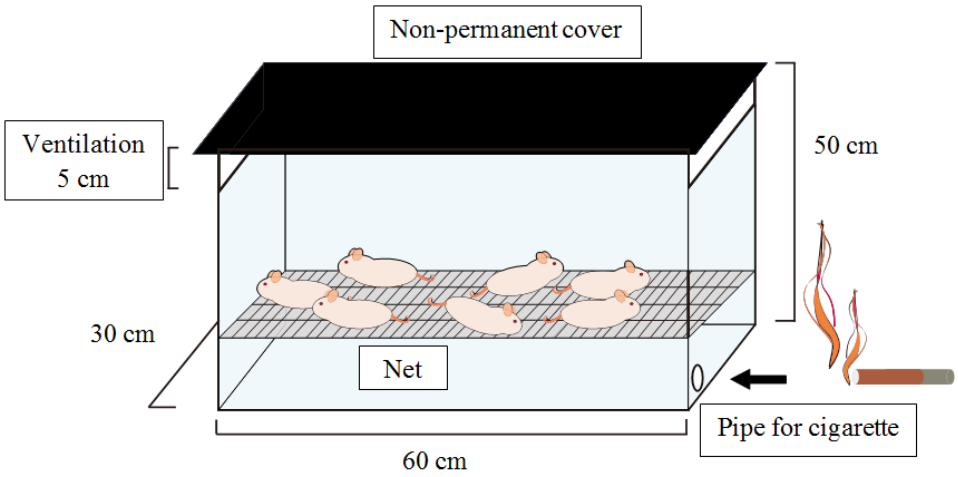

Figure 1. Apparatus model for Inhalation. Apparatus model was made by transparent glass. There was ventilation to guarantee the rats still got fresh air during cigarette smoke inhalation procedure.

2.3. Aloe vera gel

Fresh leaves of *Aloe vera* were obtained from Cimande Village, Bogor, and West Java, Indonesia. Wash the *Aloe vera* then proceed to gel collection. The colorless gel was separated from the thick outer green cuticle and stored at 4^{o}C refrigerator. The fresh gel should be obtained every 2 days.

2.4. Lipid profile analysis

Blood was collected and plasma was separated by centrifugation for 10 min at 5900 ×g at 4 °C. The sera were measured using diagnostic kits and by the spectrophotometric method for serum lipid profiles, consisting of total cholesterol, LDL and HDL [17].

2.5. Statistical Analysis

Data were analyzed with Kruskal–Wallis test followed by Mann–Whitney U-test for secondary comparisons using SPSS V.13. Statistical significance was designated at $p<0.05$.

3. Results

According to Table 1, the mean total cholesterol in the control group, cigarette smoke group, and cigarette smoke plus *Aloe vera* group was 63.57 ± 2.349 mg/dL, 62.00 ± 5.807mg/ dL and 55.29 ± 4.789 mg/ dL, respectively. The results were measured using Kruskal-Wallis and showed p-value of 0.203. Hence, the observed difference in mean total cholesterol of the three groups was not significant ($p>0.05$).

Table 1. Mean Serum Total Cholesterol

Group	Mean ± SEM (mg/dL)	p-Value
Control	63.57 ± 2.349	
Cigarette smoke	62.00 ± 5.807	0.203
Cigarette smoke + *Aloe vera*	55.29 ± 4.789	

The mean HDL in the control group, cigarette smoke group, and cigarette smoke plus *Aloe vera* group was 31.29 ± 2.456 mg/ dL, 19.89 ± 1.954 mg/ dL and 17.14 ± 1.056 mg/ dL, respectively. The observed difference in mean serum HDL of the three groups showed significant result ($p = 0.001$) as shown in Table 2.

Table 2. Mean Serum HDL

Group	Mean ± SEM	p-Value

	(mg/ dL)	
Control	31.29 ± 2.456	
Cigarette smoke	19.89 ± 1.954	0.001
Cigarette smoke + *Aloe vera*	17.14 ± 1.056	

The mean serum LDL measured was 10.14 ± 0.800 mg/ dL, 14.78 ± 1.470 mg/ dL, 10.71 ± 0.994 mg/ dL in the control group, cigarette smoke group, and cigarette smoke plus *Aloe vera* group respectively (Table 3). The result of p value observed in the three groups was 0.041. Hence, the LDL mean of the three groups was significant (Table 4).

Table 3. Mean Serum LDL

Group	Mean ± SEM (mg/ dL)	p-Value
Control	10.14 ± 0.800	
Cigarette smoke	14.78 ± 1.470	0.041
Cigarette smoke + *Aloe vera*	10.71 ± 0.994	

The result showed a significant difference ($p < 0.05$) in the serum HDL and LDL with p-value 0.001 and 0.041 respectively. However, this test could not tell which specific groups of independent variable differed statistically significant from each other. We had to determine which of these groups differed from each other and did post hoc analysis using Mann-Whitney test.

The result showed serum HDL was significantly decreasing between the control group and cigarette smoke group with p-value 0.004 (Figure 2). The mean serum HDL between cigarette smoke and cigarette smoke plus *Aloe vera* was decreasing in number. However, the differences were not significant statistically ($p > 0.05$).

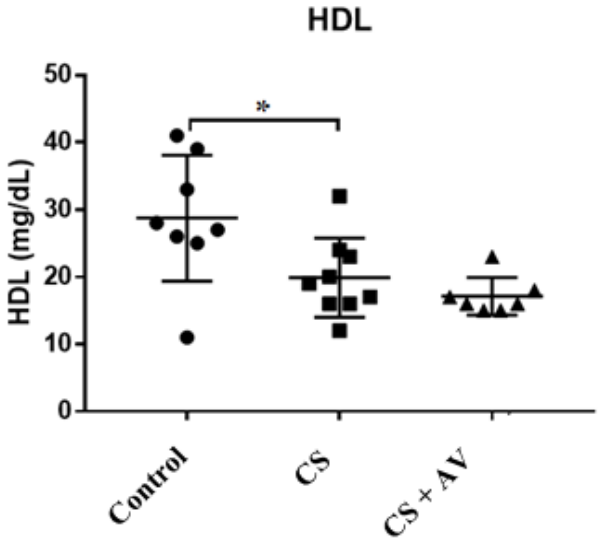

Figure 2. Comparison of serum HDL between groups. The rats were exposed with cigarette smoke for 6 weeks and HDL serum was analyzed. HDL serum concentration of cigarette smoke exposed group was lesser than the control group (P*<0.05). CS: Cigarette Smoking; CS + AV: Cigarette Smoking plus *Aloe vera*.

The mean serum LDL between control and cigarette smoke group increased significantly with p-value 0.029. Comparison of results between cigarette smoke and cigarette smoke plus *Aloe vera* group showed the decreasing number in serum LDL with p-value 0.048. Hence, the result was significant (Figure 3).

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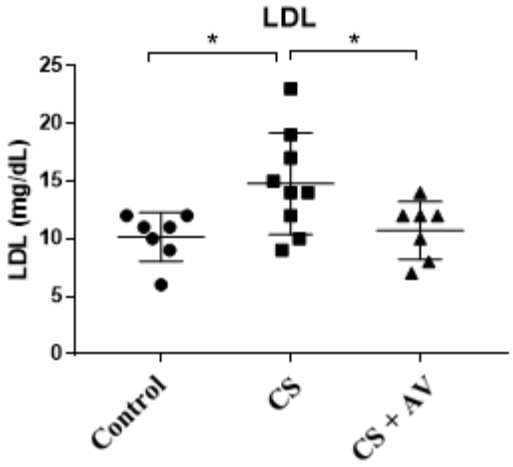

Figure 3. Comparison of serum LDL between groups. The rats were exposed with cigarette smoke for 6 weeks and LDL serum was analyzed. LDL serum concentration of cigarette smoke exposed group was higher than control and cigarette smoke exposed plus *Aloe vera* group (P*<0.05). CS: Cigarette Smoking; CS + AV: Cigarette Smoking plus *Aloe vera*.

The mean total cholesterol between control and cigarette smoke group showed no significant difference with p-value 0.314. The result of total cholesterol between cigarette smoke and cigarette smoke plus *Aloe vera* group also showed no significant difference (Figure 4).

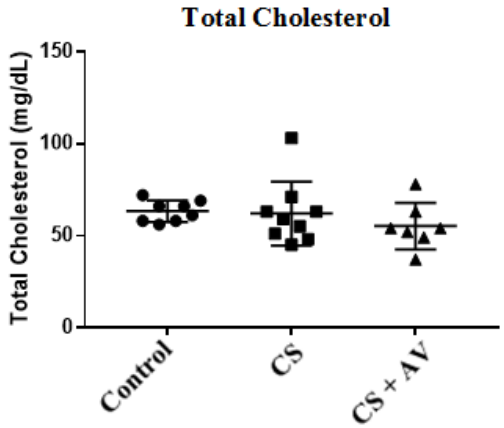

Figure 4. Comparison of serum total cholesterol between groups. The rats were exposed with cigarette smoke for 6 weeks and LDL serum was analyzed. The total cholesterol serum concentration from all groups was relatively similar. CS: Cigarette Smoking; CS + AV: Cigarette Smoking plus *Aloe vera*.

4. Discussion

This study aimed to analyze the effect of *Aloe vera* on serum lipid profiles in the rat after being exposed to cigarette smoke. The result showed there was a significant increase between the control group and cigarette smoke group for the mean serum LDL. The previous study stated that smokers had shown a tendency for LDL concentration to be higher than nonsmokers [18].

Cigarette smoke contains many oxidants and free radicals which can induce oxidative stress. Oxidative stress is the imbalance associated with the increase in the formation of oxidative products or decrease in endogenous antioxidant defense mechanisms [12]. Increased oxidative stress shows its harmful effects mostly on the cell parts, including the DNA bases, membrane lipids and proteins [8, 9, 19]. Therefore, smokers exhibit an elevation of LDL as compared with nonsmokers [10, 11].

The antioxidants provide defense against this oxidative stress by preventing excess production of ROS and neutralizing free radicals [20]. Antioxidants, such as SOD, CAT and GPX, are important to prevent oxidation of protein and DNA, peroxidation of lipid, and protect cells from damage [9, 13]. *Aloe vera* gel has been reported to have potent antioxidant effects. It was able to scavenge oxygen radicals due to its content of antioxidant enzymes [15, 20]. In research conducted on rats, *Aloe vera* gel could reduce blood LDL [22]. GPX activity, SOD enzymes and a phenolic antioxidant present in *Aloe vera* gel might be responsible for these antioxidant effects and preventing oxidative damage [15, 20]. This was also supported by the present study that showed administration of 1 mL/ day *Aloe vera* gel an hour before the exposure of the smoke was able to decrease the level of serum LDL in rats.

The mean HDL level decreased significantly between the control group and cigarette smoke group. Cigarette smoking exerts a negative effect on HDL, causing a reduced level of HDL [10,19,21]. Smoking can increase the release of catecholamine, causing a surge in circulating free fatty acids. This condition may lead to an elevation of VLDL and LDL concentrations and a reduced in HDL concentrations [11]. Smoking can affect HDL metabolism and function, including HDL biosynthesis and maturation, intravascular remodeling of HDL, HDL subfractions and catabolism which reduces the concentration of HDL. Oxidized HDL can block reverse cholesterol transport, enhance oxidation of LDL and increase vascular inflammation, which consequently makes HDL lose its protective function or even become atherogenic [10].

The protective function of HDL against atherosclerosis is done through reverse cholesterol transport. HDLs are important particles to transport excess cholesterol stored in peripheral cells, such as foam cells, to the liver for excretion into the bile. HDL also has an additional anti-atherogenic function which may be independent of its involvement in cholesterol homeostasis. HDL has been reported to reduce oxidation, improve endothelial function and promote its repair, reduce vascular inflammation and increase insulin sensitivity. Therefore, increasing HDL concentration may have the potential to reduce atherogenic processes which occur in people with dyslipidemia [19, 22].

In the randomized controlled-trial study conducted by Mofrad, *Aloe vera* has shown to improve HDL in pre-diabetic subjects [23]. Approved clinical trial data supported the effectiveness of *Aloe vera* for lowering LDL, increasing HDL and decreasing blood glucose level [11,15,24]. However, the comparison between cigarette smoke and cigarette smoke plus *Aloe vera* group of rats in this study showed no increase in serum HDL level. In a study, HDL concentration could be elevated by consuming certain polyunsaturated fats, including n-3-polyunsaturated fatty acids (PUFA), which was largely found in fish oil [22]. N-3-PUFAs were found to increase hepatic protein expression of apoA-I, the principal protein component of HDL particle, resulting in increased of HDL maturation [25].

The mean total cholesterol in this study showed no significant difference between control and cigarette smoke group. The result was consistent with the study by Kishizadeh which was conducted on rats [8]. Cholesterol enters the bloodstream in the form of lipoproteins. The lipoproteins can make cholesterol become soluble and transfer the lipids in the body. The total cholesterol level is defined as the sum of HDL, LDL and VLDL [26]. The present study showed HDL concentration decreased significantly in the serum while LDL concentration increase after being exposed to cigarette smoke. As for the mean total cholesterol after *Aloe vera* gel administration, HDL did not increase even though LDL decreased significantly. *Aloe vera* has anti-oxidative function which can scavenge free radicals and neutralize them. However, it was not able to increase HDL concentration in rats previously induced by cigarette. Therefore, the difference in total cholesterol between cigarette smoke and cigarette smoke plus *Aloe vera* group was not significant.

5. Conclusion

Administration of *Aloe vera* gel was able to decrease serum LDL level in rats exposed to cigarette smoke. It proved that *Aloe vera* L. may prevent dyslipidemia in rats after being chronically exposed to cigarette smoke. The existence of confounding factors in this research that might distort the interpretation also needs to be considered carefully. More study needs to be done in order to identify the compounds isolated in the gel.

6. Author's Contributions

NA, EA, DK, and RAK designed the experimental study and carried out the analysis. NA and DK contributed in preparing the manuscript and revision. All authors have read and approved the final manuscript.

7. Conflict of Interests

The authors have none declare.

8. Funding/Support

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9. Ethical considerations

All animal experiments conformed to the institutional guidelines and were approved by the ethical committee of Faculty of Medicine, Padjadjaran University.

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