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RESEARCH ARTICLE

Selenium Linked to Increased Antioxidant Levels and Decreased Free Radicals in Lung Tissue of Wistar Rats Exposed to E-Cigarette Smoke

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Abstract

Objective: This study aimed to find out whether selenium could increase levels of antioxidants and decrease free radicals in lung tissue of Wistar rats after exposure to e-cigarette smoke. Methods: This study used an experimental method with control group design. Male Wistar rats with criteria aged 2-3 months, weighing 200-250 grams were used as animal models in this study to assess cell damage through the expression of Superoxide Dismutase, Glutathione Peroxidase and Malondialdehyde in the lung tissue using immunohistochemical staining. E-cigarette smoke containing 6 mg of nicotine was given to each group of animal models with differences in the amount and duration of time. The adaptation process in experimental animals was carried out for approximately 5 days. Results and Conclusion: Selenium has been shown to increase the expression of antioxidant superoxide dismutase and glutathione peroxidase, and decrease the levels of malondialdehyde in cells following oral administration of selenium in Wistar rats ($p < 0.05$). In addition, selenium also reduced the amount of free radical in the cells; when it was given before exposed to e-cigarette smoke. It can be concluded that oral selenium can increase antioxidants levels and decrease free radicals in lung tissue cells due to exposure to e-cigarette.

Keywords: *Selenium, Glutathione peroxidase, Malondialdehyde, E-Cigarette, Immunohistochemistry.*

Introduction

Electric cigarette (E-cigarette) is first marketed as nicotine substitutes in tobacco cigarettes which had smaller health risks. E-cigarette is believed as an alternative way to reduce tobacco cigarette use throughout the world. While the debate over the long-term health implications of using e-cigarette is not yet known, e-cigarette users have exploded.

This kind of product is immediately accepted by the public and experienced an increase in the number of e-cigarette users worldwide [1, 2]. A good marketing system, various designs and choices of e-cigarette flavours, as well as public misperceptions that e-cigarette has smaller side effects compared to tobacco cigarette has resulted in an increase in the use of e-cigarette among young people [3,4]. Many teens are so curious to try e-cigarette and follow trends of young people's smoking behaviours. The negative influence of peer group is known as a potential source by which e-cigarette use affects their smoking behaviours. There is an increasing trend in e-cigarette users in many countries. It is estimated that around 5.5 million adult workers using e-cigarettes in US [5, 6].

There were approximately 1.5% adolescent e-cigarette users in 2011, and the number increased to 20.8% in 2018 [7]. In Malaysia, the users of e-cigarette in 2016 reached 3.2%, most of them were from urban areas, at young age, and had a high level of education. However, 74% of e-cigarette users were also tobacco cigarette users [8].

The number of e-cigarette users is still relatively small when compared to tobacco cigarettes. This can be seen from the sales of

e-cigarettes from 2010 to 2015 which was estimated to have reached $ 3.5 billion with the majority of users from beginners and former tobacco cigarette users in the adolescent and adult age groups. The dramatic rise in electronic smokers continues to grow every year and that causes further health issues in the society [5]. Some studies have shown various negative effects caused by e-cigarette. It is the number one risk factor for developing lung cancer as it delivers carcinogens though it is slightly lower than conventional cigarette.

Chemicals in e-cigarette smoke carry a much higher risk of developing heart and lung disease, including nicotine addiction and other psychological changes [9-11]. E-cigarette smoke contains various hazardous substances that generate free radicals. An excess of free radicals over antioxidant defences was associated with cell damage to a wide range of diseases [12]. Free radicals are one of the residuals of the body's metabolism and commonly play a role in affecting protein phosphorylation, apoptosis, and body immunity [13]. Excessive numbers of free radicals have a harmful effect on cellular structures of lipids, proteins, and nucleic acids [14].

Radical Superoxide (O_2^-) is a free radical type of Reactive Oxygen Species (ROS) produced by e-cigarettes [15]. At normal amounts, Superoxide (O_2^-) will naturally be neutralized by the body using the enzymatic antioxidant superoxide dismutase (SOD) producing hydrogen peroxide (H_2O_2). Then, hydrogen peroxidase (H_2O_2) is converted into water (H_2O) and oxygen (O_2) through the antioxidant Glutathione peroxidase (GSH-Px) [16]. An excessive increase in Superoxide (O_2^-) contributes to lipid peroxidation process, thus causing oxidative stress and cell damage [17].

Additional administration of antioxidants is one way to reduce the negative effects caused by free radicals in e-cigarette [18]. Increasing the production of enzymatic antioxidants could be achieved by providing micronutrient intake in the form of selenium. It will increase antioxidant levels to fight oxidative stress and reduce the risk of disease. Selenium micronutrients are expected to help neutralize free radicals in the cells and protect against the cell damage [19]. However, it is recommended that the administration of selenium should consider the dose of selenium given and the time period of exposure to e-cigarette smoke [20]. Previous studies related to the safety and health risks posed by e-cigarettes health risks compared to tobacco cigarettes are still limited and inconclusive. This is because e-cigarettes are relatively new so the long-term effects on health have still not yet been proven [21]. In this study, the impact of exposure to e-cigarette smoke through intracellular administration of selenium was analyzed by examining the expression of superoxide dismutase, glutathione peroxidase and malondialdehyde in the lung tissue.

Materials and Methods

Study Design

This study was an experimental method using post-test control group design. There were 6 groups, each of which contained 5 experimental animals which were given treatments in the form of oral selenium and different exposures to e-cigarette smoke in each group. Ethical approval was obtained from Health Research Ethics Committee, Faculty of Public Health, Universitas Airlangga (No. 103/EA/KEPK/2018) prior to commencement of a study.

Sample and Setting

Exposure to e-cigarette smoke was carried out for 2 minutes per day on each intervention, and the difference in the treatment in each group was the order of administration of selenium and exposure to cigarette smoke. The first group was the negative control group that was not given selenium nor exposed to e-cigarette smoke for 4 weeks. The second group was the positive control group of cigarettes which was exposed to e-cigarette smoke without the administration of selenium for 4 weeks. Next, the third group was the selenium control group who received selenium intake without exposure to e-cigarette smoke for 4 weeks.

Then, the fourth group was the first treatment group where selenium was given from the first week, then selenium and cigarette smoke was given simultaneously in the second to the fifth week. In addition, the fifth group was the second treatment group where selenium and cigarette smoke were given from the beginning simultaneously for 4 weeks.

Finally, the sixth group was the third treatment group where the exposure to e-cigarette smoke was given from the first week, then selenium and cigarette smoke were given simultaneously in the second to fifth week. Afterwards, the lung tissue was observed using Immunohistochemistry (IHC) staining to observe the tissue damage.

Mice

This research used Wistar male rats (Rattus novergicus) as experimental animals with criteria aged 2-3 months, weighing 200-250 grams and healthy. The adaptation process in experimental animals was carried out for approximately 5 days at the Laboratory of the Faculty of Medicine, Universitas Airlangga. The experimental animals in this study were treated according to the 3R principle (Replacement, Reduction and Refinement).

E-Cigarette

E-cigarette used in this study contains 6 mg of nicotine. Rats were exposed to e-cigarette smoke. In the space of 50 cm x 40 cm x 20 cm was given a hole that will be used to drain e-cigarette smoke as well as several small holes as a place for air exchange. Time duration and experimental groups given exposure to cigarette smoke were adjusted.

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) assessed the expression of each sample quantitatively. The value obtained was the average of positive cell observations in each preparation observed in 10 (ten) Field View (LP). Observations were made using a light microscope through 400x magnification.

Statistics

The results of the study were obtained data ratios in the form of a mean magnitude of Immuno Reactive Score (IRS) which was the expression value of superoxide dismutase, gluthatione peroxidase and malondialdehyde lung tissue in each group.

Data analysis was performed to see differences in expression of gluthatione peroxidase and malondialdehyde in lung tissue using ANOVA test analysis with SPSS version 22. The next stage of the data was analyzed using the Least Significant Differences (LSD) test to see differences between groups. The relationship between the value of expression of superoxide dismutase, gluthation peroxidase and malondialdehyde was analyzed using Pearson correlation test.

Result

Expression of Superoxide Dismutase

Table 1 shows mean values and standard deviations of superoxide dismutase in each group. The results show that administration of selenium increases the number of positive cells from superoxide dismutase in each group, while exposure to cigarette smoke decreases the number of positive cells. In group III, the mean value of superoxide dismutase reaches 4.06 ± 0.13, which is the lowest mean value in all groups. While the highest mean value is obtained in group II which reaches 13.36 ± 0.63.

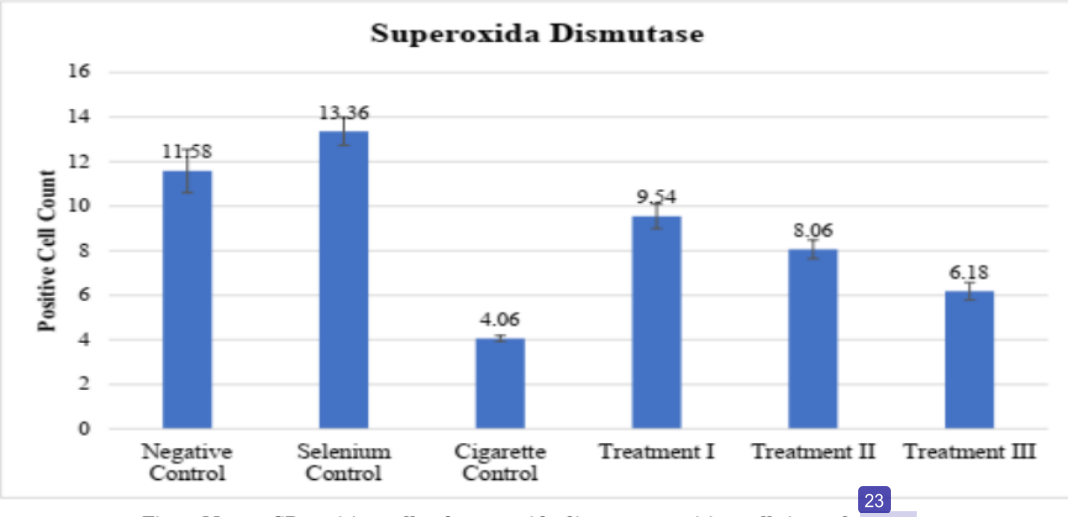

Fig. 1: Mean ± SD positive cells of superoxide dismutase positive cells in each group

The difference in the number of positive cells superoxide dismutase in various groups was analyzed using the ANOVA Test (p = 0.000). The LSD (Least Significant Differences) analysis of the number of positive superoxide dismutase cells illustrate a significant difference between groups (p = 0.000) (Table 1).

Table 1: ANOVA test results for malondialdehyde in each group

Groups	Mean ± SD	Maximum	Minimum	ANOVA Test
Negative Control	11.58 ± 0.97	13.10	10.40	0.000
Selenium control	13.36 ± 0.63	14.30	12.60	
Cigarette Control	4.06 ± 0.13	4.20	3.90	
Treatment I	9.54 ± 0.55	10.10	8.80	
Treatment II	8.06 ± 0.42	8.70	7.60	
Treatment III	6.18 ± 0.39	6.60	5.60	

Figure 2 proves the number of positive cells in each visual field in the lung tissue using immunohistochemical staining (IHC).

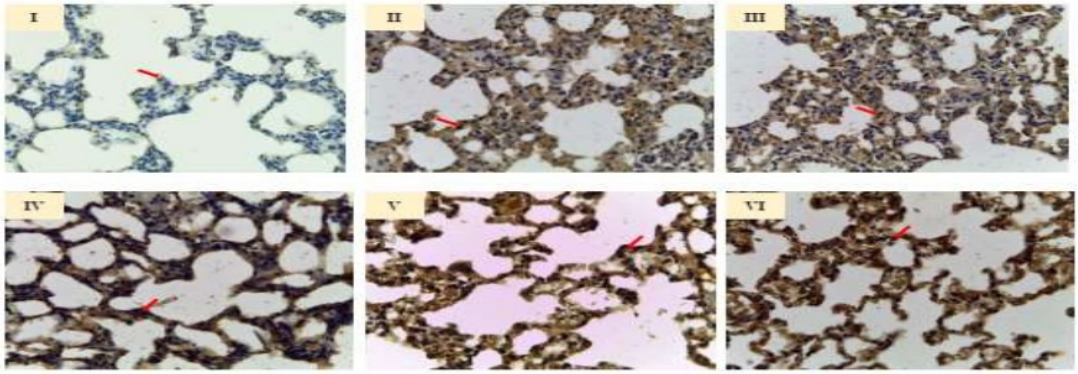

Fig. 2: Positive cells of superoxide dismutase in lung tissue

Expression of Glutathione Peroxidase

Table 2 signifies mean value and standard deviation of the number of glutathione peroxidase positive cells in each group. It is investigated that administration of selenium increases the number of glutathione peroxidase positive cells in each group, while exposure to cigarette smoke reduces the number of glutathione peroxidase positive cells. In group III the mean value of glutathione peroxidase positive cells reaches 6.14 ± 0.38 which is the lowest mean value in all groups. While the highest mean value is obtained in group II which reached 23.26 ± 0.42.

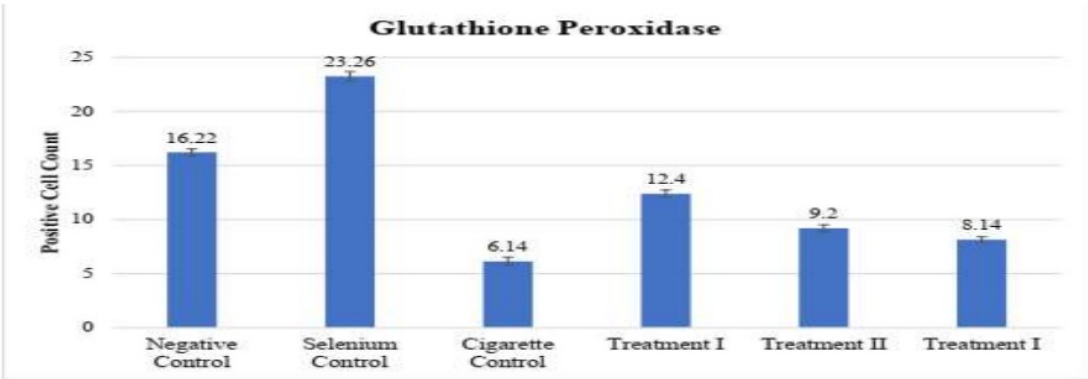

Fig. 3: Mean ± SD positive cells of glutathione peroxidase in each group

The results of LSD (Least Significant Differences) analysis on the number of glutathione peroxidase positive cells represent a major differences between groups ($p = 0.000$) (Table 2).

Table 2: ANOVA test results for malondialdehyde in each group

Groups	Mean ± SD	Maximum	Minimum	ANOVA Test
Negative Control	16.22 ± 0.32	16.60	15.80	0.000
Selenium control	23.26 ± 0.42	23.80	22.80	
Cigarette Control	6.14 ± 0.38	6.50	5.60	
Treatment I	12.40 ± 0.34	12.90	12.00	
Treatment II	9.20 ± 0.33	9.60	8.90	
Treatment III	8.14 ± 0.27	8.50	7.80	

Figure 4 shows the number of positive cells in each visual field in the lung tissue using immunohistochemical staining (IHC).

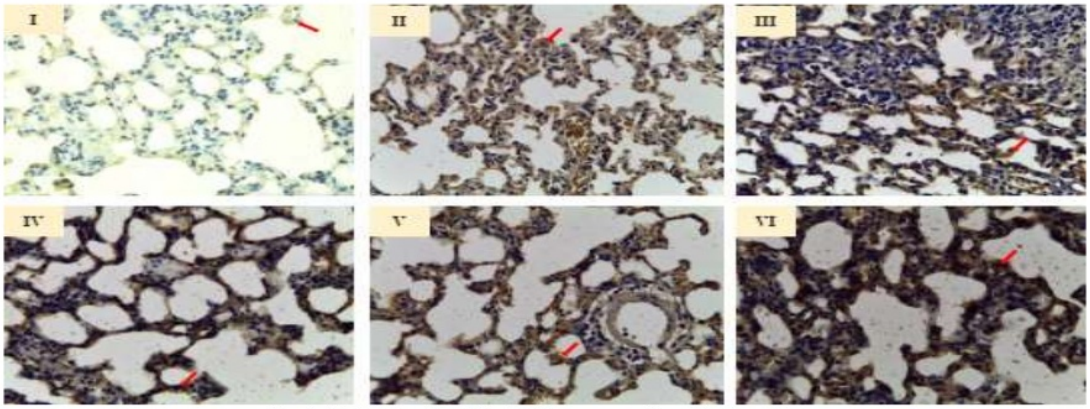

Fig. 4: Positive cells of glutathione peroxidase in lung tissue

Expression of Malondialdehyde

From table 3, it can be seen the mean value and standard deviation of the number of positive malondialdehyde cells in each group. It reveals that administration of selenium reduces the number of malondialdehyde positive cells in each group, while exposure to e- cigarette smoke increases the number of malondialdehyde positive cells. In group II the mean value of malondialdehyde positive cells reaches 5.70 ± 0.29 which is the lowest mean value in all groups. While the highest mean value is obtained in group III which reaches 24.22 ± 0.68.

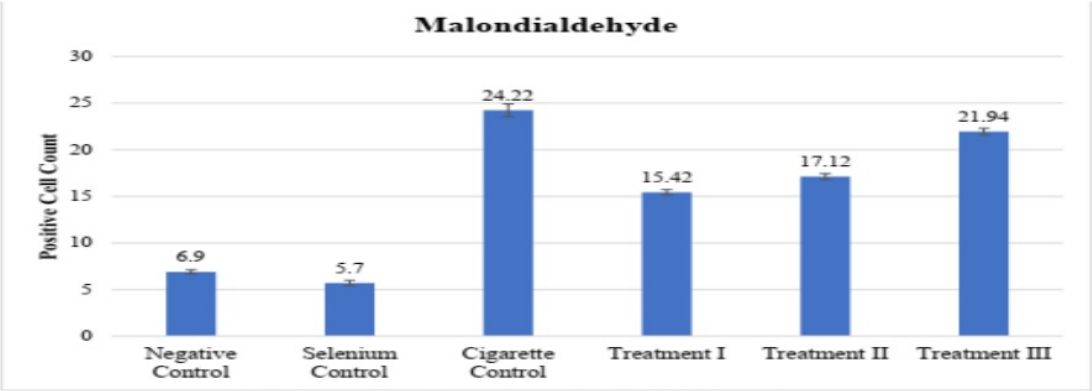

Fig. 5: Mean ± SD positive cells of malondialdehidyde in each group

The results of LSD (Least Significant Differences) analysis on the number of positive malondialdehyde cells show differences between each group (p = 0.000) (Table 3).

Table 3: ANOVA test results for malondialdehyde in each group

Groups	Mean ± SD	Maksimum	Minimum	Anova Test
Negative Control	6.90 ± 0.22	7.20	6.60	0.000
Selenium control	5.70 ± 0.29	6.10	5.40	
Cigarette Control	24.22 ± 0.68	24.90	23.20	
Treatment I	15.42 ± 0.31	15.80	15.10	
Treatment II	17.12 ± 0.32	17.50	16.70	
Treatment III	21.94 ± 0.35	22.40	21.50	

Figure 6 shows the number of positive cells in each visual field in the lung tissue using immunohistochemical staining (IHC).

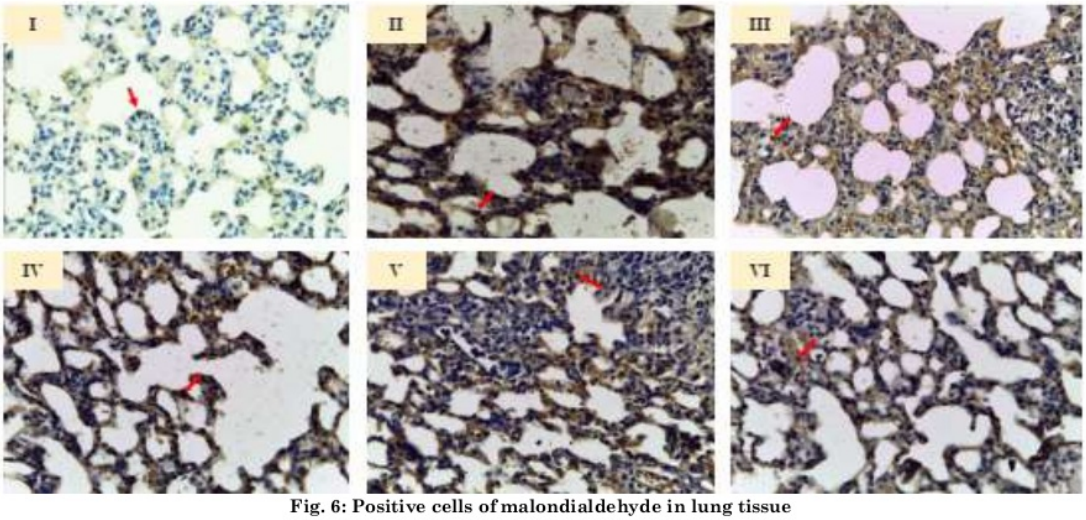

Fig. 6: Positive cells of malondialdehyde in lung tissue

Discussion

Various harmful chemicals contained in e-cigarettes have negative impacts on health. As e-cigarette smoke moves into the respiratory tract, it induces free radical levels in the airways [22]. This condition may decrease the expression of superoxide dismutase, glutathione peroxidase and increase the expression of malondialdehyde. The positive cigarette control group shows the effects of free radicals caused by exposure to e-cigarette smoke.

Decreased expression of superoxide dismutase and glutathione peroxidase is considered as a decline in body's antioxidant activity [23]. Both enzymatic antioxidants are expected to be able to neutralize free radicals produced by e-cigarettes.

Increased expression of malondialdehyde shows that excessive free radicals limit the ability of antioxidants to inhibit oxidation [24]. In addition, elevated malondialdehyde level in the lung tissue indicates the occurrence of oxidative stress which has a positive correlation with lipid peroxidation as a mechanism of tissue damage [25]. The administration of selenium could increase the expression of superoxide dismutase and glutathione peroxidase and decrease the expression of malondialdehyde [26].

Increasing the expression of antioxidants could neutralize free radicals that move into the airways. The results of this study strongly suggest that one of the main reasons

for high body's antioxidant levels could be managed by the duration of selenium administration. From this study, it could be interpreted that the earlier the administration of selenium, the far greater the quantity of antioxidant levels.

Conclusion

Free radicals in e-cigarette smoke have been considered to have negative consequences on health. However, selenium was found to be effective in increasing antioxidants levels and reducing free radicals in the cells when it was given earlier before exposed to e-cigarette smoke.

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