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Effect of carrot leaf juice (*Daucus carota*) on the lung histology structure of white rats (*Rattus norvegicus*) induced by cigarette smoke

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Abstract. *Utami AS, Harini M, Handajani NS, Hidayat LN. 2020. Effect of carrot leaf juice (*Daucus carota*) on the lung histology structure of white rats (*Rattus norvegicus*) induced by cigarette smoke. Cell Biol Dev 4: 40-45.* Cigarette smoke is hazardous to both active and passive smokers. The impact results in oxidative stress, which results in cell damage across the lung's histological structure, including tissue dilation in the alveoli, epithelial abrasion, and other abnormalities. Carrot leaves contain beta carotene, an antioxidant that can help repair damaged cells caused by cigarette smoke's oxidative stress. The goal of this study was to assess the histological structure of the lungs following induction with cigarette smoke and the influence of carrot leaf juice (*Daucus carota* L.) on the lung structure following induction with cigarette smoke. A completely randomized design (CRD) with five replications was adopted in this investigation. The rats were divided into four groups: those induced by cigarette smoke and given 4 mL distilled water, those induced by cigarette smoke and given 4 mL carrot leaf juice (2 mL carrot leaf juice added to 2 mL distilled water), those induced by cigarette smoke and given 4 mL carrot leaf juice (3 mL carrot leaf juice added to 1 mL distilled water), and those induced by cigarette smoke and given 4 mL oat straw juice. For 14 days, white rats were provoked with three cigarettes every day. On the 31st day, rats were killed via cervical dislocation, their lungs were removed, and histological preparations using the paraffin method and Hematoxylin-Eosin staining were performed. Quantitative data were analyzed using non-parametric techniques Anova test, and qualitative data collection by describing the effect of cigarette smoke before and after carrot leaf juice administration.

Keywords: Beta carotene, carrot leaf, *Daucus carota*, histopathology, lung

INTRODUCTION

Cigarette smoke significantly negatively influences the health of active smokers, the environment, and passive smokers. Cigarettes and carcinogenic chemicals are inextricably linked. The consequences are severe, one of which is lung cancer. Several components can reduce the carcinogenic mechanism of the lungs due to smoking, such as anti-inflammatory drugs (budesonide, celecoxib, aspirin, naproxen, licofelone), antidiabetic drugs (Metformin, pioglitazone), antineoplastic agents (lapatinib, Bexaroterie, vorinostat), and other drugs and supplements (phenethyl isothiocyanate, Myo-inositol, N-acetylcysteine, ascorbic acid, berry extract). Pharmacological developments here are intended to prevent the impact of cigarette smoke on the body (Flora et al., 2016).

Oxidative stress that occurs in the lungs due to cigarette smoke has been shown to increase the levels of ceramides that accumulate in cells. The concentration of ceramide in the lungs will increase the release of superoxide (O^{2-}), which acts as a free radical. The accumulation of ceramide from cigarette smoke in the cells causes lung emphysema and cell death in the alveoli (Petrache et al. 2008).

The content of cigarettes, such as tar, nicotine, and carbon monoxide, has a harmful effect on the body. However, cigarettes also contain metal elements such as Al, As, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Po-210, Se, and Zn, which have harmful effects on the organs of active and passive smokers. The content of these metals, if inhaled by passive smokers, will impact lung tissue and be deposited in alveolar tissue. Besides that, it is also toxic to the fetus. Carbon monoxide, tar, nicotine, and other metal elements in cigarettes can be carcinogenic in the body (Chiba and Masoroni 1992).

One of the effects of smoking on lung health is causing emphysema in the terminal bronchioles. Emphysema is a clinical condition with abnormalities in the anatomical structure of the lungs in the form of widening and destruction of the walls of the terminal bronchioles. Emphysema can also occur in the alveolar walls (Alsagaff et al. 1989).

Carrot leaves contain vitamin C, -carotene, fiber, and minerals such as Na, P, K, Ca, Mg, Mn, Zn, and Fe. Besides that, they also contain omega-3 fatty acids. Alpha-linolenic acid (LNA) is an essential fatty acid in omega-3, which plays an important role in human health (Almeida et al., 2009). Carrot leaf paste with a concentration of 5% can increase levels of antioxidants and omega-3 fatty acids. The compounds responsible for the antioxidant function in carrot leaves are flavonoids. The function of the antioxidant itself is to prevent cell damage such as cancer, inflammation, atherosclerosis, or other cell damage caused by free radicals (Boroski et al., 2011).

Omega-3 fatty acids and antioxidants in carrot leaves serve as nutritional enhancers for the body. Carrot leaf paste is made by extracting dried carrot leaves. The results

showed that carrot leaf paste with a concentration of 5% increased levels of antioxidants and omega-3 fatty acids. Flavonoids are one of the compounds responsible for the antioxidant function in carrot leaves. The function of antioxidants is to prevent cell damage such as cancer, inflammation, atherosclerosis, or other cell damage caused by free radicals (Boroski et al., 2011).

Beta carotene in carrot leaves is a group of carotenoids that act as antioxidants. In addition, carotenoids play an important role as a secondary defense system, warding off free radicals such as peroxyl radicals. Besides that, carotenoids act as pro-oxidants (Stahl 2016; Abidin et al. 2019). Beta carotene is important for cardioprotection or other organs and binds free radicals that enter the body. And it has a significant effect on preventing damage due to increased radicals, one of which is cigarette smoke (Csepanyi et al., 2015).

MATERIALS AND METHODS

Research time and location

The research was carried out from April to August 2017. First, it was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences; and Histology Laboratory, Faculty of Medicine at the Universitas Sebelas Maret, Surakarta; then Laboratory of Animal Anatomy, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Methods and materials

Carrot leaves, kretek cigarettes, 2 of 3-month-old male white rats weighing 200 grams, pellets as white rat feed, aquadest as a white rat drink, and aquadest, a package of ingredients for making sliced preparations using the paraffin method, were utilized in this investigation (70 percent ethanol, xylol, paraffin, hematoxylin-eosin staining kit).

The experimental design employed total randomization (CRD). Twenty-eight male white rats were divided into four treatment groups: group p1 was exposed to only cigarette smoke, group p2 was exposed to cigarette smoke and given 2 mL carrot leaf juice per day, group p3 was exposed to cigarette smoke and given 3 mL carrot leaf juice per day, and group p4 was exposed to cigarette smoke and given 4 mL carrot leaf juice per day.

White rats were exposed to cigarette smoke from three cigarettes daily for 14 days (Tohomi et al. 2014), beginning on day eight, in a rat cage enclosed in a cardboard lid measuring 50 x 35 x 20 cm with a ventilation hole measuring 20 x 10 cm. Carrot leaf juice was administered once daily to white rats exposed to cigarette smoke for seven days, beginning on day 22. On day 29, before surgery, the white rats were killed via cervical dislocation. The lungs of white rats were removed and preserved. The results of observations of damage to the histological structure of the lungs will be scored, namely:

0 = no changes in histological structure
1 = damage to less than 25% of the total field of view
2 = damage to 26-50% of the entire field of view
3 = damage to 51-75% of the total field of view
4 = damage to 51-75% of the entire field of view

The scoring results will be used as quantitative data for the ANOVA statistical test.

RESULTS AND DISCUSSION

Alveoli cross section

For each of the two preserved preparations, the degree of tissue damage was assessed using a scoring system (see Table 1). So, for the two preserved preparations that were seen in 50 fields of vision at a magnification of 100x, there were 100 scoring data points.

Bronchi cross-section

The degree of tissue damage was evaluated using a scoring system (Table 2). Each preparation was observed in 50 fields of view, resulting in 100 score data for the two preserved preparations at a magnification of 100x.

Cross-section of bronchioles

Scoring (Table 3) was used to determine the degree of tissue damage in each of the two conserved preparations. Using 50 fields of view for each preserved preparation, the total scoring data for the two preserved preparations was 100 with a magnification of 100x (Figure 4-11).

Table 1. Description of alveolar damage scoring from the One Way ANOVA Test

Treatments	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	2	3.0000	.00000	.00000	3.0000	3.0000	3.00	3.00
P1	2	3.0000	.00000	.00000	3.0000	3.0000	3.00	3.00
P2	2	3.0000	.00000	.00000	3.0000	3.0000	3.00	3.00
P3	2	3.0000	.00000	.00000	3.0000	3.0000	3.00	3.00
Total	8	3.0000	.00000	.00000	3.0000	3.0000	3.00	3.00

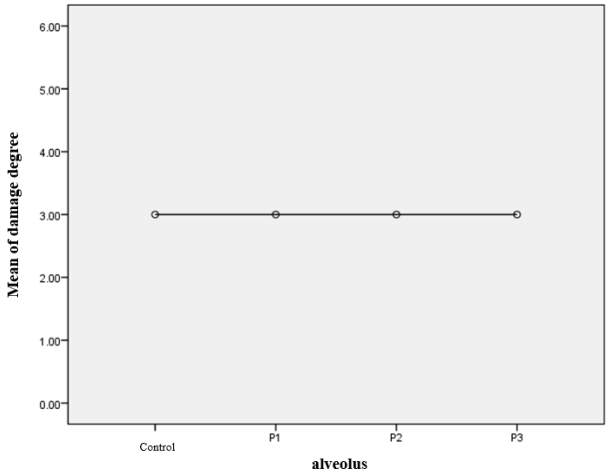

Figure 1. Means plots One Way Anova Test

Table 2. Description of bronchial damage scoring from One Way Anova Test

Treatments	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control					4.0000	4.0000	4.00	4.00
P1	2	4.0000	.00000	.00000	4.0000	4.0000	4.00	4.00
P2	2	4.0000	.00000	.00000	4.0000	4.0000	4.00	4.00
P3	2	4.0000	.00000	.00000	4.0000	4.0000	4.00	4.00
Total	8	4.0000	.00000	.00000	4.0000	4.0000	4.00	4.00

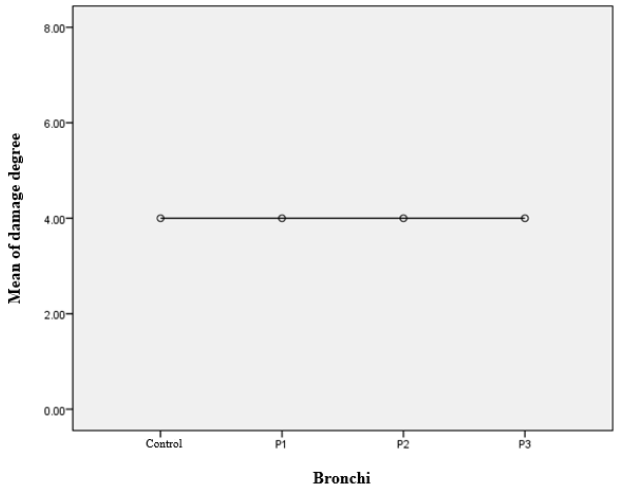

Figure 2. Means plots of One Way ANOVA Test

Table 3. Description of bronchiolar damage scoring from One Way ANOVA test

Treatments	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	2	3.5000	.70711	.50000	-2.8531	9.8531	3.00	4.00
P1	2	3.5000	.70711	.50000	-2.8531	9.8531	3.00	4.00
P2	2	3.0000	.00000	.00000	3.0000	3.0000	3.00	3.00
P3	1	3.0000	.	.	.	.	3.00	3.00
Total	7	3.2857	.48795	.18443	2.8344	3.7370	3.00	4.00

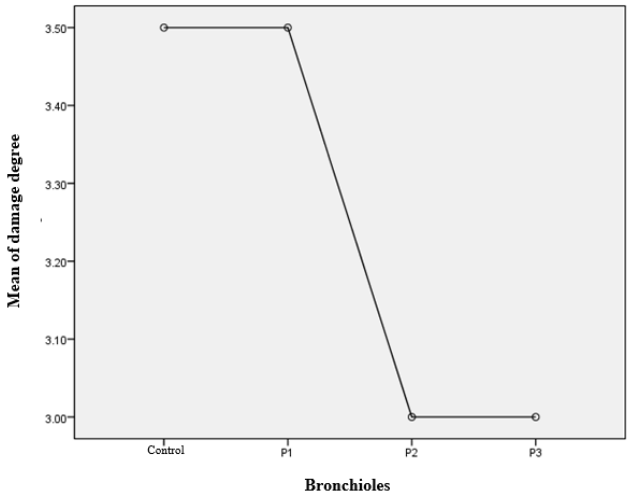

Figure 3. Means Plots of One Way ANOVA Test

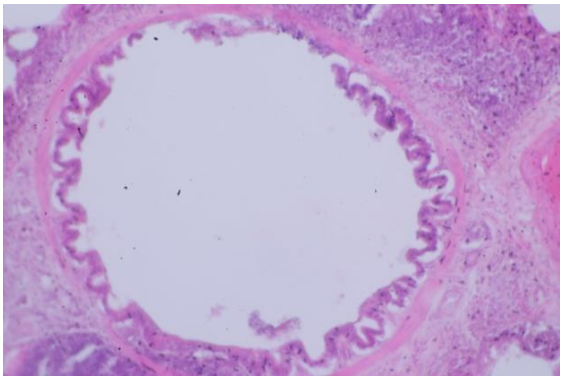

Figure 4. Cross-section of the bronchus control

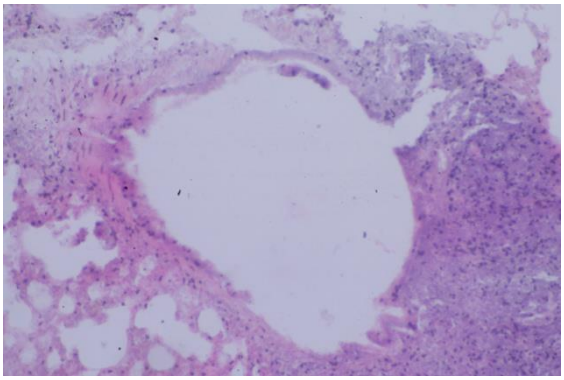

Figure 6. Cross-section of the bronchus P1

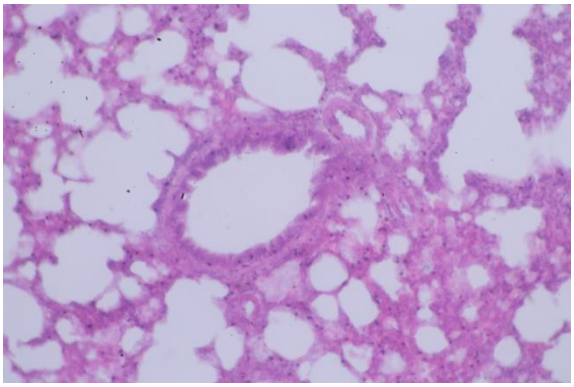

Figure 5. Cross-section of bronchioles control

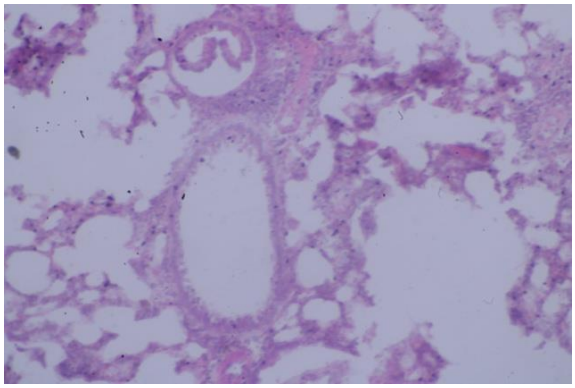

Figure 7. Cross section of bronchioles P1

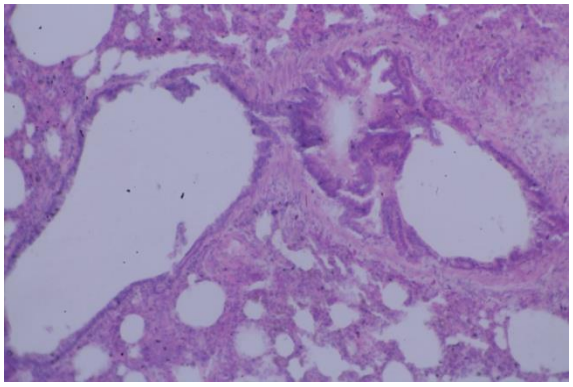

Figure 8. Cross-section of bronchus P2

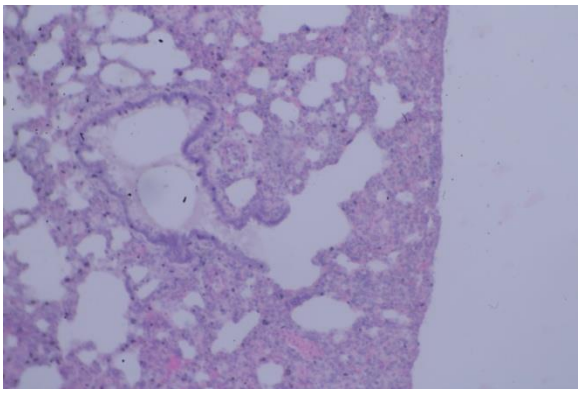

Figure 9. Cross section of bronchioles P2

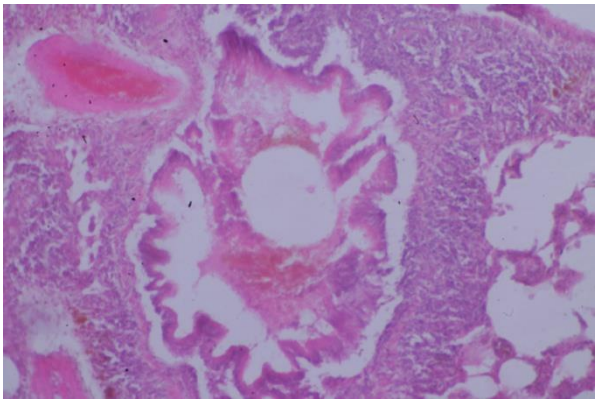

Figure 10. Cross-section of bronchus P3

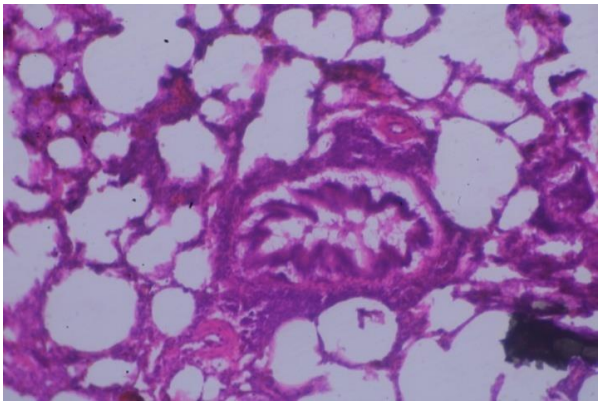

Figure 11. Cross-section of bronchioles P3

Discussion

Allevoluus histopathology

Observation of the alveolus on preserved preparations showed that the diameter of the alveolus experienced widening and lost its elasticity. Cigarette smoke causes fatty acid chains to break and produces various compounds, one of which is MDA (Malondialdehyde). These compounds are formed due to the reaction of lipid peroxides that react continuously. In addition to the peroxide reaction, cigarette smoke causes protein oxidation reactions, increasing the proteolytic enzyme reaction and inactivating the antiproteolytic enzyme. This condition causes the levels of the two enzymes to experience an imbalance. Antioxidants are one way to prevent a decrease in the percentage density per volume in the alveoli caused by the effects of free radicals from cigarette smoke. The content of phenol and ethanol from plants or fungi can prove this. Consumption of antioxidants can balance the levels of proteolytic and antiproteolytic enzymes (Rahimah et al., 2010).

In the control preparation (P1) with exposure to cigarette smoke for 14 days and seven days in oral distilled water, observations were made with a magnification of 100x. The tissue damage was scored, one of which was alveolar dilation. The average number of observations is 3, with damage between 51% and 75%. Likewise, for preparations, P2 with treatment exposed to cigarette smoke for 14 days and seven days in oral 2 mL carrot leaf juice, P3 with treatment exposed to cigarette smoke for 14 days and seven days in oral 3 mL carrot leaf juice, and P4 with treatment in exposure to cigarette smoke for 14 days and seven days in oral 4 mL of carrot leaf juice, all of them gave observations with an average score of 3. The scores obtained were then carried out with a one-way ANOVA test. The results showed that carrot leaf juice did not affect repairing damage to the lung alveoli.

Histopathology of the bronchi and bronchioles

Observations of the bronchi and bronchioles of the lungs revealed that the epithelium had been rubbed, leaving only smooth muscle tissue. ROS are molecular byproducts of oxygen metabolism. Superoxide anion radicals (O_2-), singlet oxygen (O_2), hydrogen peroxide (H_2O_2), and highly reactive hydroxyl radicals are all ROS derivatives (OH). Although reactive oxygen species (ROS) are found in all aerobic cells, an excess of ROS in a cell results in oxidative stress, which is toxic. Additionally, these molecules are produced as a byproduct of cellular metabolisms, such as mitochondrial respiration. ROS that accumulates in tissues will bind to lipids in cellular membranes, causing damage to nucleotides in DNA (Waris and Ahsan. 2006).

In the control preparation (P1), after 14 days of exposure to cigarette smoke and seven days of oral distilled water, observations were made at a magnification of 100x. Then the bronchi and bronchioles were scored for tissue damage. The average number of bronchial observations was three, with damage ranging from 76% to 100%. Similarly, preparations P2, P3, and P4 all produced observations with an average score of 4. The obtained scores were then analyzed using a one-way ANOVA test.

The findings indicated that carrot leaf juice did not affect repairing bronchial lung damage. In addition, while all treatments had an average of 3.2 on the bronchioles, the one-way ANOVA test revealed that carrot leaf juice had little effect on repairing tissue even when the damage was severe.

Beta carotene plays a critical role in the body, particularly in the scavenging of free radicals. Beta carotene deficiency can result in the formation of cleavage products (CP) and oxidative stress (Alija et al. 2006). Antioxidants play a critical role in preventing damage caused by oxidative stress caused by cigarette smoke because the oxidative effects of cigarette smoke can cause damage to microsomal proteins and increase proteolysis so that it can cause DNA damage. Therefore, beta carotene is an antioxidant that should be consumed in the recommended dose by individuals who smoke or are exposed to cigarette smoke. Due to its critical role in reducing oxidative stress caused by free radicals, consuming beta-carotene-containing fruits and vegetables is essential in binding free radicals that enter the body (Omenn 1998).

This study indicates that carrot leaf juice has no significant effect on the histopathology of white rat lung's alveolus, bronchi, and bronchioles, including loss of alveolar tissue elasticity and abrasion of epithelial tissue in the bronchi and bronchioles.

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