ORIGINAL ARTICLE

Effects of E-Cigarette Vapor Smoke on Pulmonary Alveoli in *Rattus norvegicus* Lungs

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ABSTRACT

Introduction: Vapor is considered a safer alternative to tobacco cigarettes because the high nicotine content is less. However, vapor still contains substances that are classified as toxic to humans. Short-term exposure to vapors from liquids can induce an inflammatory response in the lungs and cause oxidative stress. This study aimed to determine the effects of e-cigarette vapor smoke on pulmonary alveoli in *Rattus norvegicus* lungs.
Methods: This was an experimental study on 32 adult male *Rattus norvegicus* rats. They were divided into two groups exposed to nicotine-containing vapor smoke two times a day for one and three months, respectively, and one group as control. After vapor exposure, the lung tissues of the rats were taken and then subjected to histopathological examination under a microscope.
Results: After exposure for one month, epithelial and endothelial cells degenerated, characterized by a decrease in collagen and elastin fibers in the extracellular matrix. For three months, there were changes, the alveolar membrane had no nucleus, the surrounding endothelial cells were not visible due to damage to the extracellular matrix, the alveolar lumen had widened, causing edema in the lumen of the alveoli, and the alveoli wall was destroyed. Therefore, the connection between the alveoli was stretched.
Conclusion: This study found that short-term exposure to nicotine vapor causes damage to the alveoli membrane.

INTRODUCTION

Electronic cigarettes, or e-cigarettes, have become increasingly popular as a safer alternative to traditional cigarettes.[1] E-cigarettes work by heating a liquid, which usually contains nicotine and other chemicals that are classified as toxic to humans, such as tobacco-specific nitrosamines (TSNA), carbon monoxide and propylene glycol, and other flavorings that are classified as toxic, to produce an aerosol that users inhale into their lungs.[2] While e-cigarettes are marketed as a less harmful alternative to traditional cigarettes, the long-term health effects of e-cigarette use are still unclear and require further study.[1] Some studies suggest that e-cigarettes may pose unique health risks, including respiratory and cardiovascular problems.[3] Since their inception 15 years ago, the prevalence of e-cigarette use has varied by continent and area, with Europe and Oceania having the highest prevalence. According to a systematic review, the incidence of current e-cigarette usage among middle and high schools in the United States (US) was 3.3% and 14.1%, respectively. At the same time, in Southeast Asia, it ranged from 3.3% to 11.8%.[4–6]

The impact of cigarette smoke is not only for active and passive smokers. People who do not smoke (passive smokers), if exposed to cigarette smoke, will inhale two times the toxins exhaled by

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active smokers. Short-term exposure to liquid from e-cigarettes can induce an inflammatory response in the lungs and cause oxidative stress. Therefore, vapor smoke can interfere with the activity of vibrational hairs in the respiratory tract and the macrophage function, causing hypertrophy of mucosal glands. The effects caused by vapor smoke depend on the duration of exposure, the concentration of exposure, and the immunity of the experimental subjects. The shorter the exposure, the lower the concentration and the milder the effect. Likewise, the longer the exposure to cigarette smoke, the more severe the effects.[7,8] The vapor examined has a higher abuse liability than nicotine inhalers but lower than combustible cigarettes.[8] This study aimed to determine the effects of e-cigarette vapor smoke on pulmonary alveoli in *Rattus norvegicus* lungs.

METHODS

The experimental method utilized in this study employed a research design known as the posttest-only control group design. The primary objective of this method was to elucidate the characteristics of rat lung histopathology subsequent to exposure to nicotine smoke from vapor. The research was conducted at the Research Laboratory of the Faculty of Medicine, Universitas Muslim Indonesia. Male *Rattus norvegicus* rats (n = 32), aged three weeks, were procured for the study. Inclusion criteria encompassed a body weight ranging from 200 to 300 grams, male, mobility, and the absence of macroscopic morphological abnormalities. Rats exhibiting signs of illness or defects, those that died following exposure before the designated surgery time, and those subjected to unsuitable vapor smoke were excluded from the study.

A sample of 32 rats was divided into control and experimental groups, each comprising eight rats. In this study, three experimental groups were established. Group 1 was not exposed to vapor smoke, Group 2 was subjected to vapor smoke for a duration of one month, and Group 3 for a duration of three months. The vapor employed in this study was derived from low-nicotine liquid with a nicotine composition of 3 mg, obtained from Vape Inc. Exposure to vapor smoke was administered twice daily to each group, with varying administration durations within the experimental groups. Following the completion of the predetermined exposure period, this study assessed any potential changes in the samples' body weight resulting from vapor smoke exposure. Subsequently, surgical procedures were conducted to extract lung tissue from the rats, which was examined under a microscope using Hematoxylin and Eosin (H&E) staining. The degree of severity of lung histopathological effects, determined by the extent of lung alveolar damage, was evaluated by an anatomical pathologist.[9]

The data collected from microscopic observations were analyzed using the Statistical Package for the Social Sciences (SPSS) version 23. The data underwent testing via non-parametric methods, including the Kruskal-Wallis and Mann-Whitney tests, to identify distinctions among the groups. Ethical clearance was approved by the Ethics Committee of the Faculty of Medicine, Universitas Muslim Indonesia (No. 169/A.1/KEPK-UMI/VII/2019).

RESULTS

This study was conducted in January 2022 with a total sample of 32 rats. The body weight of each experimental group was examined. Based on Table 1, Group 1, as the control and also the weight data before exposure to vapor smoke, showed an average weight of 224.50 ± 0.04 compared to Group 2, which had been exposed to vapor and had a body weight loss with an average of 196.97 ± 0.11, and Group 3 had 157.88 ± 12.09 of body weight loss data.

Table 1. Analysis of differences between body weight before and after exposure to e-cigarette vapor smoke using the Kruskal-Wallis test

Characteristic	Group	Mean ± SD	p-value
Body weight (gram)	1	224.50 ± 0.04	0.000
	2	196.97 ± 0.11	
	3	157.88 ± 12.09	

Furthermore, this study tested the collected body weight data for the Kruskall-Wallis statistical difference test, which showed a significant difference in body weight before (Group 1) and after (Groups 2 and 3) exposure to cigarette smoke (p = 0.000) (Table 1).

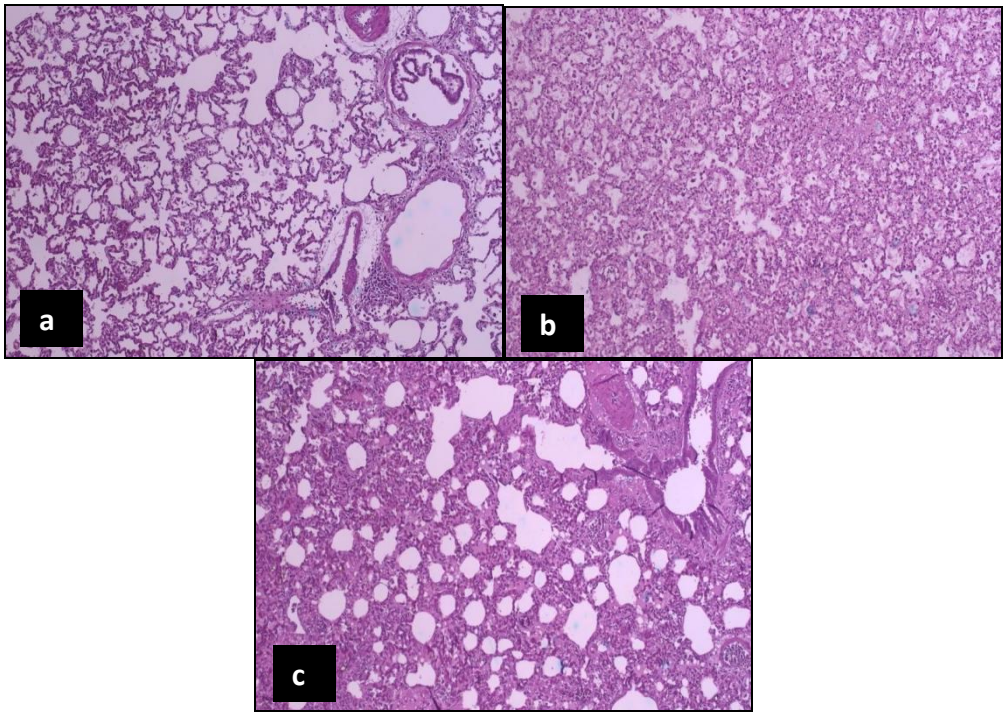

Figure 1. Histopathology of the pulmonary alveolar membrane of *Rattus norvegicus*. (a) Group 1, as control, was not exposed to vapor smoke; (b) Group 2 was exposed to vapor smoke for one month; (c) Group 3 was exposed to vapor smoke for three months.

This study conducted histopathological examinations of *Rattus norvegicus* lungs, focusing on the appearance of the alveolar membrane. A comparison of the pulmonary alveolar tissues is presented in Figure 1. The tissues were stained using H&E. Based on the pre-exposure appearance of the alveoli in healthy rats and those not exposed to vapor smoke, the following was observed:

In Group 1 (a), the alveolar membrane remained intact, nucleated, and complete, with endothelial cells comprising over 75% of the structure. The lumens of the alveoli were rounded, with sizes exceeding 75%, and the connections between adjacent alveoli were tightly integrated, also exceeding 75%. The extracellular matrix, consisting of collagen and elastin fibers, appeared intact.

In Group 2 (b), exposed to vapor smoke for one month, the alveolar membranes showed level 2 damage, characterized by epithelial and endothelial cell degeneration. This was manifested by reduced collagen and elastin fibers within the extracellular matrix. While the lumens of the alveoli remained relatively rounded, and the connections between adjacent alveoli were somewhat tight, these aspects were not as pronounced as in Group 1.

In Group 3 (c), exposed to vapor smoke for three months, the alveolar membranes displayed level 3 damage, indicating a lack of nuclei in the alveolar membrane and the absence of visible surrounding endothelial cells due to extracellular matrix damage. The lumens of the alveoli were widened, and the connections between adjacent alveoli were stretched. Overall, this study provided insights into the progressive effects of vapor smoke exposure on the structural integrity of alveolar membranes and their components.

DISCUSSION

Based on the results of this study, Group 1 provided a histological appearance that was still included in the normal level, meaning a score of 1 with the alveolus membrane intact, nucleated, and complete with endothelial cells >75%. This was because the control group was not exposed to irritants contained in smoke vapor. Hence, the cells were not damaged. In Group 2, the degree of damage obtained a score of 2, meaning that there was degeneration of the alveolar membrane of epithelial and endothelial cells marked by reduced collagen and elastin fibers in the extracellular matrix, the lumen of the alveolus was still relatively rounded, and the relationship between the alveolus was still relatively tight. This is similar to previous studies stating that the number of alveolus macrophages in rats exposed to e-cigarette smoke is significantly more than those not exposed to e-cigarette smoke.[10–12] Exposure to e-cigarette smoke in experimental mice causes lung damage in the form of alveolar membrane damage.

Whereas in Group 3, the results of histopathological damage to the lung alveoli had a score of 3, meaning that the alveolar membrane did not have a nucleus and the surrounding endothelial cells were not visible due to damage to the extracellular matrix. The

alveolar lumen widened due to an increase in cytoplasmic fluid in the interstitial space, causing edema in the lumen of the alveoli, eventually damaging the walls of the alveoli, and the connection between the interalveolar was stretched due to infiltration of lymphocytic inflammatory cells into the interstitial septa of the alveoli. The lungs have proteinase inhibitors which function to provide a protective effect on the lungs from proteinases produced by phagocytosis and inflammatory responses to fight foreign particles that enter the lungs. Lung proteinases as a defense system include 1-antitrypsin, 2-macroglobulin, 1-antichymotrypsin, inter-α-trypsin inhibitors, and secretory leukocyte protease inhibitors. When the biological or chemical substances that act as free radicals are inhaled into the alveolus, it will cause an inflammatory response. Complement components will increase vascular permeability and increase the involvement of inflammatory cells. Macrophages are activated and secrete proinflammatory cytokines. Thus, damage to the extracellular matrix and elastin fibers will occur, resulting in histopathological changes. Some previous studies have shown that exposure to e-cigarette vapor can induce inflammatory responses in the lungs.[13–15] Inflammation in the pulmonary alveoli could damage the alveolar walls, potentially compromising their ability to exchange gases efficiently. Chronic inflammation might contribute to conditions like chronic obstructive pulmonary disease (COPD) and other respiratory ailments.

The epithelial cells lining the alveoli are crucial in maintaining lung health. Studies on *Rattus norvegicus* have indicated that exposure to e-cigarette vapor can cause damage to these cells, affecting their integrity and function. This damage could disrupt the barrier between the alveoli and blood vessels, potentially allowing harmful substances to enter the bloodstream, leading to vaping-associated pulmonary injury (EVALI) or vaping-associated respiratory distress syndrome (VARDS). Christiani (2019) and Bhatt, *et al*. (2020) describe EVALI and VARDS as a sign of vapor exposure-induced hypoxemia with abnormal X-ray features and respiratory failure due to severe inflammatory response.[16,17] Histopathological features include pneumonia, diffuse alveolar damage, eosinophilic pneumonia, diffuse alveolar hemorrhage, acute fibrous pneumonitis, foamy and vacuolated macrophages, foamy and vacuolated pneumocytes, intra-alveolar fibrin, bronchiolitis, bronchial mucosal ulceration, intestinal edema, inflammation, neutrophils, chronic intestinal inflammation, and pigmented macrophages.

In addition, e-cigarette vapor contains various chemicals, including nicotine and flavoring agents. When inhaled, these chemicals can generate oxidative stress within the lung tissues. Oxidative stress occurs when there is an imbalance between the production of harmful reactive oxygen species (ROS) and the body's ability to detoxify them. The ROS can damage cells, including those in the alveoli, contributing to lung dysfunction and promoting the development of respiratory diseases. The primary function of pulmonary alveoli is to facilitate efficient gas exchange. Any disruption to the structure or function of these alveoli can impair oxygen uptake and carbon dioxide removal. Studies on *Rattus norvegicus* lungs have suggested that exposure to e-cigarette vapor might compromise the thinness of the alveolar walls, leading to reduced gas exchange capacity.[18]

From the observation of the analyzed body weight of rats, it was found that there was a significant difference in the weight loss of rats with a p-value = 0.000 ($p < 0.05$). In Group 1, the average body weight was 224.50. In Group 2, exposed to cigarette smoke for one month, the average value was 196.97. For Group 3, exposed to cigarette smoke for three months, the average body weight was 157.88. Hence, it can be concluded that a relationship between active and passive smokers with doses given for one month and three months can cause a decrease in immune response, resulting in anorexia, whereby anorexia can affect weight loss. The effect of significant weight loss can cause changes in the histopathological picture of the lung alveoli. Chen (2021) stated that rats exposed to e-cigarette smoke can cause weight loss and more significant damage to the pulmonary alveoli, affecting each group's average score.[19] The greater damage is due to the immune response in rats with low body weight, which is not as good as in rats with normal body weight. Therefore, the protective function against damage decreases, and more severe damage occurs. Changes in the histopathological picture in this study occurred due to exposure to vapor smoke containing harmful substances such as formaldehyde, TSNA, and propylene glycol, which is a carcinogen, then glycidol acetaldehyde, acetol, and acrolein, which are strong irritants, while nicotine and nicotirin (the result of nicotine combustion) are psychoactive substances with high addictive properties. In addition to the harmful ingredients, e-cigarettes also contain carbon monoxide (CO), which causes lung damage, tissue cell death, and structural changes in the lung alveolar tissue.[19,20]

The limitations of this study were that there was no examination of the content of harmful substances in the e-cigarette smoke used, the dose of liquid that can cause lung alveolus damage in rats was not determined, and the amount of smoke inhaled by each experimental animal was not necessarily the same because it was in an isolation box, some experimental animals were included

at once and were not separated in their respective isolation boxes. For suggestion, it is better to separate each experimental animal in a separate isolation box to control the amount of smoke inhaled by each sample.

CONCLUSION

Based on the histopathological findings, this study found that short-term exposure to nicotine vapor can cause damage to the alveoli membrane. Further research needs to be performed on the levels of harmful substances in e-cigarette smoke used in the study and review the dose of liquid given to rats that can cause damage to the pulmonary alveoli.

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Conflict of Interest
The authors declared there is no conflict of interest.

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Authors' Contributions
Planning and supervising the manuscript: EPW, DR. Performing the measurements, processing the experimental data, performing the analysis, drafting the manuscript, and designing the figures: MNZ. Interpreting the histopathological results: SR, DI. Giving feedback and reviewing the manuscript: UAP. All authors contributed and approved the final version of the manuscript.

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