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The Effect of Cigarette Smoke Exposure Time Difference on the Histopathological Images of Mice Lungs (*Mus musculus*)

El efecto de la diferencia de tiempo de exposición al humo del cigarrillo en las imágenes histopatológicas de los pulmones de ratones (*Mus musculus*)

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SUMMARY

An electric cigarette is a product that releases an aerosol containing nicotine to users by heating a liquid consisting of propylene glycol or glycerol (glycerine), nicotine, and flavoring agents. This study aims to determine the effect of the difference in the exposure time of electric cigarette smoke on histopathological images of the lungs of mice (Mus musculus).
Methodology: *This study was a laboratory experimental study. All data were tested statistically using ANOVA parametric test. If the data were not distributed normally then they were tested with a non-parametric alternative test which is Kruskal-Wallis. The subjects of this study used 25 mice divided into 5 treatment groups i.e., control, and four treatment groups exposed for different times ranging from 15 minutes up to 60 minutes per day. Changes were observed microscopically after 14 days of treatment and assessed using the Lung Damage Score by Marianti.*
Results: *In this study obtained results p= 0.0001.*
Conclusion: *There is an effect of the time difference of exposure to cigarette smoke on the histopathologic picture of mice.*

Keywords: *Electric Cigarette, (E-cigarettes), Vape, ENDS (Electronic Nicotine Delivery Systems), ENNDS (Electronic Non-Nicotine Delivery Systems).*

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RESUMEN

Un cigarrillo eléctrico es un producto que libera un aerosol que contiene nicotina a los usuarios al calentar un líquido que consiste en propilenglicol o glicerol (glicerina), nicotina y agentes saborizantes. Este estudio tiene como objetivo determinar el efecto de la diferencia en el tiempo de exposición del humo del cigarrillo eléctrico en imágenes histopatológicas de los pulmones de ratones (Mus musculus).
Metodología: *Este estudio fue un estudio experimental de laboratorio. Todos los datos se probaron estadísticamente usando la prueba paramétrica ANOVA. Si los datos no se distribuyeron normalmente, se probaron con una prueba alternativa no paramétrica*

que es Kruskal-Wallis. Los sujetos de este estudio utilizaron 25 ratones divididos en 5 grupos de tratamiento, es decir, control, y cuatro grupos de tratamiento expuestos durante diferentes tiempos que van desde 15 minutos hasta 60 minutos por día. Los cambios se observaron microscópicamente después de 14 días de tratamiento y se evaluaron mediante el Lung Damage Score de Marianti.
Resultados: *En este estudio se obtuvieron resultados p = 0,0001.*
Conclusión: *Existe un efecto de la diferencia de tiempo de exposición al humo del cigarrillo en el cuadro histopatológico de los ratones.*

Palabras clave: *Cigarrillo eléctrico, (cigarrillos electrónicos), Vape, ENDS (Sistemas electrónicos de suministro de nicotina), ENNDS (Sistemas electrónicos de suministro sin nicotina).*

INTRODUCTION

An electric cigarette is a product that releases a nicotine-containing aerosol (usually called a vapor) to the user by heating a liquid consisting of propylene glycol or glycerol (glycerin), nicotine, and a flavoring agent (1,2). Electric cigarettes are used as a substitute or transition from conventional cigarettes in an attempt to quit smoking (3,4).

The workings of electric cigarettes differ from conventional cigarettes which are inhaled by burning; electric cigarettes vaporize the liquid in the apparatus so that the outflow is smoke evaporated and not burned by fire (1,4); therefore, electric cigarettes are also often called 'vapor/ vape', while the electrical smoking activity is called 'vaping' (3,5). Many say that vaping is not smoking. However, previous research has shown that smoke from electric cigarettes can cause damage to mouse fibroblast cells, especially in e-liquids containing flavoring agents (6-8).

In recent years the use of electric cigarettes has been increasing in all circles around the world, especially in adolescence as evidenced by data from the WHO that the use of electric cigarettes increased in 2014 and is predicted to increase (9).

The role of electric cigarettes as an alternative to or substitute for conventional cigarettes is a problem in itself. Research on histopathological changes in the lungs of mice after exposure to electric cigarette smoke was never implemented but no significant changes were suspected due to several unmet factors (10-12).

Based on the description above, the researcher is interested in researching the effect of the time difference of exposure to electric cigarette smoke on the histopathological picture of the lungs of mice (Mus musculus).

The purpose of this research is to know the effect of different lengths of exposure to electric cigarette smoke on the histopathological picture of the lungs of mice.

METHODS

We strongly the research is laboratory experimental research, conducted in July-September 2017 at the Animal Laboratory of Universitas Nusa Cendana Kupang, Faculty of Medicine.

The subjects of this study were 25 mice (*Mus musculus*). Animals are adapted for seven days to observe the general condition of rats and keep the weight of mice to remain stable.

Mice were divided into 5 treatment groups. Each group consisted of 5 mice. The first group was the control group (K) that was not exposed to smoke, the second group was the treatment group (P1) with exposure to second-hand smoke for 15 minutes per day, the third group was the treatment group (P2) with exposure to second-hand smoke for 30 minutes per day, the fourth group was a treatment group (P3) with exposure to second-hand smoke for 45 minutes per day, and the fifth group was a treatment group (P4) with exposure to cigarette smoke for 60 minutes per day. All treatments were given cigarette smoke at a dose of 100 mL / min channelled into the isolation box via a disposable syringe.

The mice were then terminated and lung procedures were taken and histopathologic preparations were made with Hematoxylin Eosin (HE) and interpreted using Marianti's lung damage scores as the gold standard for this experiment (13); Table 1. Observations were made on 5 randomly selected fields of view which would then be averaged. After the data

were collected, the data were tested statistically using the ANOVA parametric test. If the data were not normally distributed then the nonparametric alternative test was the Kruskal-Wallis, continued by Post-Hoc LSD analysis.

Table 1

Lung Damage Score

Histopathology image	Score 1	2	3
Alveoly Membrane	Alveolar membranes are intact, core, and complete with endothelial cells> 75 %	Alveolar membranes are intact, core, and complete with endothelial cells 25 %-75 %	Alveolar membranes are intact, core, and complete with endothelial cells <25 %
Alveoli Membrane	Alveolar membranes are intact, core, and complete with endothelial cells> 75 %	Alveolar membranes are intact, core, and complete with endothelial cells 25 %-75 %	Alveolar membranes are intact, core, and complete with endothelial cells <25 %
Alveoly Lumen	Rounded proportion > 75 %	Rounded proportion 25 %-75 %	Rounded proportion <25 %
Between alveoli	Density >75 %	Density 25 %-75%	Density < (25 %)

RESULTS

Measurement weight results during adaptation period

The results of the bodyweight measurements of mice during the adaptation period were carried out for 7 days. In this adaptation process, researchers conducted observations of the general condition of the mice and measured the bodyweight of mice regularly every two days. Based on the observation of the general condition of mice for 7 days there was no appearance of dull hair, hair loss, less activity, and abnormal exudate from the eyes, mouth, anus, and genitals. This confirms that the sample had good general conditions during the adaptation process.

Macroscopic observation results

Microscopic lung observation began with lung removal by thoracic surgical procedure. The preparations were undertaken by the researchers, then the histopathologic preparations were made in the Anatomy Pathology Laboratory Siloam Kupang Hospital.

The assessment of the rate of lung damage microscopically was done using the lung damage score by Marianti (13). The lowest score was three and the largest was nine, the greater the score indicating the greater the damage that has occurred. The results of the assessment of each group observation can be seen in Table 2 and the average score of the group can be seen in Table 3, and the results of the statistical processing can be seen in Table 4.

Table 2

Observation of Histopathologic Preparations of Mice Lungs

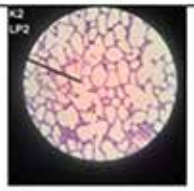
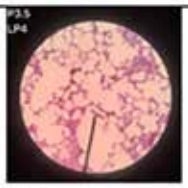
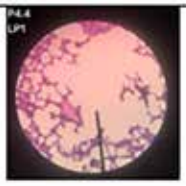
No	Group	Microscopic picture
1	Control	
2	P 1	
3	P 2	
4	P 3	
5	P 4	

Table 3

Histopathological Interpretation of the Lung

Mean Group Score	
Control	3.52
Treatment 1	5.28
Treatment 2	6.56
Treatment 3	5.16
Treatment 4	8.08

Table 4

The output of statistical processing results ANOVA

	df	Mean Square	Sig.
Between Groups	4	14,528	0,0001
Within Groups	20	0,562	
Total	24		

DISCUSSION

Interpretation of the results was conducted at the Wet Laboratory Faculty of Medicine, University of Nusa Cendana. Interpretation of the results was undertaken using the lung damage score by Marianti (2009) (13). Matters assessed were alveolar membrane state, lumen form, and interalveolar relationship at 400 μs microscopic magnification in 5 randomly selected fields of view. Scores for each item were rated then averaged and summed with the lowest score being three and the highest score being nine. The higher the score the greater the amount of damage that has occurred. From the interpretation of the results obtained, the greatest damage occurred in treatment four with an exposure to secondhand smoke for 60 minutes per day for 2 weeks with an average score of 8.08 and minimal damage occurred in the control group with an average score of 3.52.

Research on exposure to electric cigarette smoke and histopathological changes in mice lungs (*Mus musculus*) have been studied by Triana (2013) (14). The treatment given in the study was 20 times daily suction using a 60mL injection syringe. Results obtained showed no statistically significant changes were suspected due to short exposure times.

In this study, the dose was increased to 100mL of cigarette smoke per minute with exposure times according to the group ranging from 15 minutes to 60 minutes. In the research conducted there were statistically significant results though the experiment lasted for as long as the previous research, namely 2 weeks. From this, it can be concluded that certain doses may result in changes in histopathologic features of the lungs of mice.

The lungs have a proteinase inhibitor that serves to provide a protective effect on the lung from the proteinase produced by phagocytosis and the inflammatory response to fight foreign particles entering the lungs. Proteinases belonging to the lungs as defense systems include α1-Antitrypsin, α2-Makroglobulin, α1-Antichhemotripsin, inter-α-trypsin inhibitor, and secretory leukocyte protease inhibitor. If biological or chemical ingredients that act as free radicals are inhaled into the alveoli it will produce an inflammatory response. Complementary components will increase vascular permeability and increase the involvement of inflammatory cells. Macrophages become activated and secrete proinflammatory cytokines so that there will be damage to the extracellular matrix and elastin fibers so that histopathologic changes will occur (15).

Changes in histopathologic features in this study are suspected to occur due to toxic substances in electrical cigarette smoke, in this case, propylene glycol and other substances contained in electric cigarette smoke. The imbalance between the enzyme Proteinase inhibitor and the content of toxic substances affect the occurrence of damage to the continuity of the lung epithelial tissue resulting in microscopic observable histopathological changes.

The recommendations for further research are: it is necessary to check the levels of harmful substances in electric cigarette smoke used in the study; chronic exposure to electric cigarette smoke should be performed; research into the comparison of different levels of propylene glycol contained in e-liquid used and the dose of electric cigarette smoke that can lead to histopathological damage needs to be done.

CONCLUSION

The results of the influence of different time exposures to electric cigarette smoke on the histopathological images of the lungs of mice (*Mus musculus*) show that it can be concluded that there is an influence of different time exposures to electric cigarette smoke on the histopathological images of the lungs of mice (*Mus musculus*).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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