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Research Article

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The effect of exposure to liquid air freshener on histological of the bronchial mice (*Mus musculus*)

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ABSTRACT

Air fresheners are products that contain chemicals aimed at reducing unpleasant odours in confined spaces. The use of synthetic air fresheners turns out to harm health because some of their leased Volatile Organic Compounds are classified as toxic compounds and are carcinogens. Air freshener enters the body through the inhalation process in the respiratory system. Modern air fresheners are available in liquid (aerosol) and gel forms. Air fresheners contain addictive substances and solvents such as 1,4-dichlorobenzene which can affect pulmonary function. The purpose of this study is to know the effect of exposure to liquid air freshener on the histology of bronchi of mice (*Mus musculus*). This study used 20 male mice (*Mus musculus*) consisting of 4 treatments with 5 replications. The research design is true experimental in the form of a post-test only control group with Completely Randomized Design (CRD). The post-test was done by observing the histological picture of the mice's bronchi after exposure to liquid air freshener given 3x/day for a period of P1 = 2 weeks, P2 = 4 weeks and P3 = 6 weeks. Quantitative data on bronchial histology was tested using the One Way ANOVA statistical test followed by the Post Hoc Tukey test. The results of the study found changes in the histology of the bronchi, thickening of the epithelial tissue of mice. Analysis of comparative data between the control and treatment groups statistically obtained p = 0.010 <0.05, meaning that there was a significant effect and change in the bronchial tubes exposed to liquid air freshener. Histologically there were differences in histology between the control and treatment groups. It was concluded that liquid air freshener had a significant effect on the histological picture of the mice's bronchi.

Keywords: Bronchial histology; liquid air freshener; *Mus musculus*

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INTRODUCTION

Air is a mixture of gases that exist in the layers of the earth. As a result of the times, the quality of clean air has decreased and has an impact on air pollution. The quality of a healthy environment is a key part of the health sector. Air as an important environmental component in life needs to be maintained and improved in quality so that it can provide support for living things to live optimally (Prabowo, 2018).

The quality of a healthy environment is a major part of the health sector. A person's health cannot be separated from the influence of his environment. Air is one of the most important environmental components in life. Air quality needs to be maintained and improved so that it can provide support for living things to live optimally. Nowadays air quality is increasingly showing a very concerning condition due to the increase in air pollution in the environment (Damayanti, Yuningtyaswari, & Susanti, 2016).

Clean air is a basic need for human health. A comfortable and healthy home is not only because it is clean, but the fragrance in every room of course also plays a role in creating a comfortable atmosphere in the house. A fragrant house in every room will make the residents feel at home. Unpleasant odours in the house can be caused by a lack of natural light entering, this causes the room to become humid. Air circulation is not smooth is also a contributing factor. The air that is in the house and does not change quickly, in addition to making the room humid, if there is an unpleasant odour, it will not quickly disappear. To make the space in the house fragrant, many use air fresheners (Aksari, 2018).

Air pollution is the pollution of the environment inside (indoor) and outdoors (outdoor pollution) by chemical, physical or biological agents that modify the natural characteristics of the atmosphere. According to WHO 2011 data, worldwide it is estimated that 2.7 million people died from air pollution, 2.2 million of them were due to indoor pollution. The EPA (Environmental Protection Agency of America) consistently ranks indoor pollution as the fifth most risky to the environment on public health (Damayanti, Yuningtyaswari, & Susanti, 2016).

Air pollutants found indoors are of particular concern as the average person spends 85-90% of their time indoors. Many household products are sources of volatile organic compounds. Air pollutants are usually found in concentrations several orders of magnitude higher indoors when compared to outdoors. Air fresheners cause sensory, lung irritation and a decrease in airflow velocity (Zuhair, 2012).

At this time, pollution is happening everywhere at such an unprecedented rate. The trend of pollution, especially since the Second World War, has led to two things, namely, the increasing disposal of certain chemical compounds mainly due to industrial and transportation activities. In addition, it is also due to the use of various bioxide products and hazardous materials in human activities (Irianto, 2015).

Air pollution is the presence of foreign materials or substances in the air that cause changes in the composition (composition) of the air from its normal state. The presence of foreign materials or substances in the air in a certain amount and for a long time can interfere with human life. If such a situation occurs, it can be said that the air has been polluted (Prabowo, 2018).

Air fresheners are products that contain chemicals aimed at reducing unpleasant odours in enclosed spaces. The dangers of air fresheners generally depend on the type, form, fragrance and active chemical components contained in them and are influenced by the length of exposure. Modern air fresheners are available in liquid (aerosol) and gel forms. Most of the chemical content of air freshener products is not disclosed, making it difficult to judge whether it is safe or not (Yuningtyaswari, 2015).

Air fresheners can produce various volatile organic compounds (VOCs), such as terpenes such as limonene, which often dominate the pollutants found in the room, and produce secondary pollutants such

as formaldehyde. Air fresheners have been linked to a variety of adverse health effects, such as migraine headaches, asthma attacks, breathing difficulties, and neurological problems. In two national surveys of the US population, 19% reported difficulty breathing, headaches, or other health problems when exposed to air fresheners (Steinemann, 2016).

Household chemicals are the most common source of indoor air pollution. One of the widely used household chemicals is an air freshener. Air freshener is a household chemical that is considered as one of the air pollutants from inside the room. The use of air fresheners is now increasingly questionable, especially about the content in it. In most areas of the world, manufacturers of consumer products are not required by law to disclose their ingredients. Air fresheners enter the body through the inhalation process in the respiratory system. Air fresheners are expected to give negative responses both psychologically and physiologically, such as respiratory disorders, allergic responses and various non-specific symptoms such as headaches, irritation of the nose, eyes and others (Yuningtyaswari, 2012).

Regarding the use of air freshener on the histology of the bronchial tubes of mice, several studies have been carried out previously, namely by Yuningtyaswari and Asti Haryani in 2015, explaining that there is an effect of exposure to liquid and gel air fresheners on the pulmonary histology picture in white rats (*Rattus norvegicus*) exposed during 8 hours/day for a period of 15 days, there was a picture of pulmonary histology damage, especially the alveolus of white rats in the form of thickening of the interalveolar septum. In addition, research conducted by Novia Chamala Sari Dewi, Sudiastuti and Agung Nugroho in 2015, explained that the active ingredient transfluthrin contained in paper mosquito repellents can cause damage to lung structure in the alveoli of mice (*Mus musculus* L.). Parameters observed were bronchial histology in mice. Therefore, in this study, precise accuracy is needed because the bronchial size of mice is very small. Based on this background, it is important to research to see the effect of exposure to liquid air freshener on the histological picture of the bronchi of mice (*Mus musculus*)".

RESEARCH METHODS

This research was conducted in March 2020 − May 2020 at the Laboratory of Animal House Abduh Tikus Center Palembang and Dyatnitalis Laboratory of Morphology and Anatomy Palembang. This type of research is quantitative research. The design of this research is true experimental in the form of a post-test only control group with a completely randomized design. Table 1 shows the observational data used for RAL research.

Table 1. Observation Data for RAL

Treatment	Repeat (Coating Epithelial Thickness) I	II	III	Average
Control (C)				
Treatment 1 (P1)				
Treatment 2 (P2)				
Treatment 3 (P3)				

The experiment used male mice (*Mus musculus*) with a swiss webster strain that was 3 months old and weighed 20 grams. The number of mice used was 20 in 5 mice in each group. Experimental animals were divided into 4 groups consisting of 1 control group and 3 treatment groups with 5 repetitions. Determination of sample size is based on WHO provisions with a minimum sample size of 5 individuals per group (Arumingtyas, 2010). Meanwhile, the number of repetitions is determined based on the Federer formula (Nugroho, 2018).

$(5-1)(n-1) \geq 15$
$4(n-1) \geq 15$
$4n - 4 \geq 15$
$4n \geq 19$
$n \geq 4,75$
$n \geq 5$
t = treatment group
n = number of repetitions or samples of each group

The control group without treatment, treatment group 1 (P1) was given 1 ml of liquid air freshener 3 times a day for 2 weeks, treatment group 2 (P2) was given 1 ml of liquid air freshener 3 times a day for 4 weeks, and treatment group 3 (P3) was given 1 ml of liquid air freshener 3 times a day for 6 weeks. After finishing the last treatment, all experimental animals were surgically treated (according to the code of ethics for veterinary surgery) and the bronchial organs were taken and made histological preparations. In this study, there are two variables, namely variable X (influence) and variable Y (influenced). The variable X was exposure to liquid air freshener and variable Y was bronchial histology of mice (*Mus musculus*). Quantitative data were collected by measuring the thickness of the lining epithelium in the bronchi of mice using raster images and micrometers. The research data in the form of the thickness of the lining epithelium in the bronchi of mice were tested using the One Way ANOVA statistical test followed by the Post Hoc Tukey test. The analysis used in this study used normality and homogeneity tests. After the data is proven to be normally distributed and homogeneous, it will be continued with the One Way Anova test. If it is proven that there is an effect, it will be carried out with a Post Hoc Tukey further test.

FINDING AND DISCUSSION

The study was conducted on experimental animals starting from the acclimatization of experimental animals for 7 days and then given treatment from each group. The results of the study showed changes in the histological picture of the bronchi in the form of thickening of the lining epithelium. In Figure 1 observations are using a microscope with a magnification of 100x and 400x. Observations and measurements of the thickness of the epithelial lining quantitatively for each preparation were carried out in 3 fields of view and using a raster image application.

The results of histological observations of the bronchi of male Swiss webster mice exposed to liquid air freshener showed a thickening of the bronchial epithelium which was indicated by the thickness of the lining epithelium in the bronchi. Epithelial tissue (epithelium) is composed of similar cells that cover the outer and inner surfaces of organs in the form of channels (tubules) and cavities (cavum). Epithelial tissue is a very dense layer of cell arrangement. The lining epithelium is the superficial epithelium which is a membrane or sheet/layer. The type of epithelium found in this study is the ciliated stratified columnar epithelium. Table 2 shows the thickness of the bronchial epithelium in each treatment.

Based on the analysis of the data in Table 3, it is known that liquid air freshener affects the thickening of the epithelium in the bronchi ($P = 0.010 < 0.05$), then Ha is accepted and Ho is rejected. This means that liquid air freshener can affect the bronchi. Therefore, further tests were carried out to see the effect between treatments, namely by conducting the Tukey Post Hoc test.

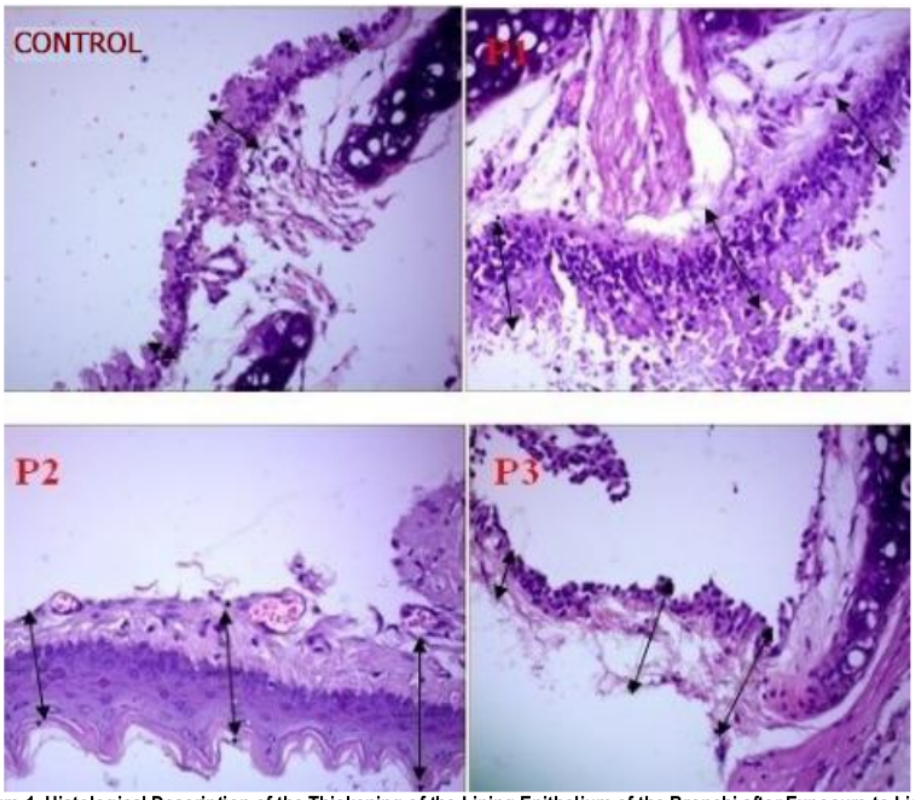

Figure 1. Histological Description of the Thickening of the Lining Epithelium of the Bronchi after Exposure to Liquid Air Freshener with HE Staining at 100x and 400x Magnifications

Table 2. Thickness of the Lining Epithelium in the Bronchi of Mice

Treatment	Coating Epithelium Thickness (mm)			Average
	I	II	III	
control (K)	0,20	0,24	0,16	0,20
Treatment 1 (P1)	0,56	0,65	0,43	0,54
Treatment 1 (P2)	0,57	0,73	0,78	0,69
Treatment 1 (P3)	0,26	0,61	0,62	0,49

Table 3. ANOVA Test

Variant Source	Sum of Squares	Free Degree	Mean Square	F_{count}	Level of Significance
Between Group	0,386	3	0,129	7.574	0,010
In Group	0,136	8	0,017		
Sum	0,521	11			

The results of the Tukey test can be seen in Table 4. From the results of the Anova statistical test regarding the thickness of the lining epithelium in the bronchi, there is a significant difference between the control group and treatment groups 1, 2, and 3.

Table 4. Tukey Post Hoc Follow-up Test

Group	N	α = 0.05	
		1	2
P0	3	0,2000	
P3	3	0,4967	0,4967
P1	3		0,5467
P2	3		0,6933
Value Sig.		0,090	0,320

Based on the data obtained from the results of research that has been calculated with the One Way ANOVA test and the post hoc Tukey follow-up test, it is proven that there is an effect of exposure to liquid air freshener on the histological picture of the mice's bronchi. The type of epithelium in the bronchi is the ciliated stratified columnar epithelium. Histologically, in cross-section, the cells are cylindrical in shape, stand on the basement membrane, and have a height that exceeds their width. The stratified columnar epithelium has a structure consisting of several layers of cells, the columnar cells are only on the surface layer.

According to Adi (2014), the ciliated epithelium in this system is very sensitive and is easily injured if exposed to toxic gas inhalation, viral infections and trauma. If exposed to foreign objects, these epithelial cells will swell, detach from the basement membrane or lose their cilia (deciliation). This process will heal quickly if the cause is removed. However, if the process is chronic, there will be hyperplasia and metaplasia of the epithelium. The deposition is the process of trapping particles of a certain size and shape in the respiratory system. Clearance is the process of destroying, neutralizing, and removing deposited particles from the mucosal surface. Several mechanisms play a role in the clearance process, namely sneezing, coughing, adsorption, mucociliary transport and phagocytosis.

The use of synthetic air fresheners turns out to harm health because some of the released Volatile Organic Compounds are classified as toxic compounds and are carcinogens. Air fresheners enter the body through the inhalation process in the respiratory system. Modern air fresheners are available in liquid (aerosol) and gel forms. Air fresheners contain addictive substances and solvents such as 1,4-dichlorobenzene which can affect pulmonary function (lungs).

Liquid air fresheners generally contain chemicals. Chemical substances can be inhaled through the nose, trachea and then into the bronchi. The bronchi are located in the middle of the airways so that absorption is not perfect like the lungs, but the absorption is better than the trachea so that the reaction to foreign bodies is more easily absorbed than the trachea so that the particles are more easily deposited in the bronchi. Any particles that are inhaled will collect in the respiratory tract and can reach the bloodstream. Based on research conducted (Damayanti, Yuningtyaswari, & Susanti, 2016), one of the chemical constituents contained in liquid air freshener is formaldehyde.

In the physiological process in the body when the initial exposure to irritant substances on the surface of the bronchi, the mucus will function to catch small and large particles so as not to reach the alveolus. The mucus is then removed from the respiratory tract by the cilia. Cilia move mucus out of the lungs slowly. The particles caught in it will then be swallowed or coughed up. However, due to a decrease in the function of ciliary cells, goblet cells have an increased function in producing mucus and an increase in the number of goblet cells in protecting the epithelial surface (Muhartiningsih, 2019).

Measurement of epithelial thickness was observed with 3 fields of view using raster image application and magnification of 100x and 400x. In Figure 2 group K, the histological picture of bronchial

epithelial thickness is 0.20 mm. In group P1, the histological picture of bronchial epithelial thickness was 0.54 mm. In group P2, the histological picture of bronchial epithelial thickness was 0.69 mm. In group P3, the histological picture of bronchial epithelial thickness was 0.49 mm. The significant difference in value may be influenced by chemical compounds contained in liquid air fresheners that can affect the respiratory system. The substance in liquid air freshener that can affect the thickness of the bronchial epithelium in experimental animals in the treatment group is thicker than in experimental animals in group K (without treatment).

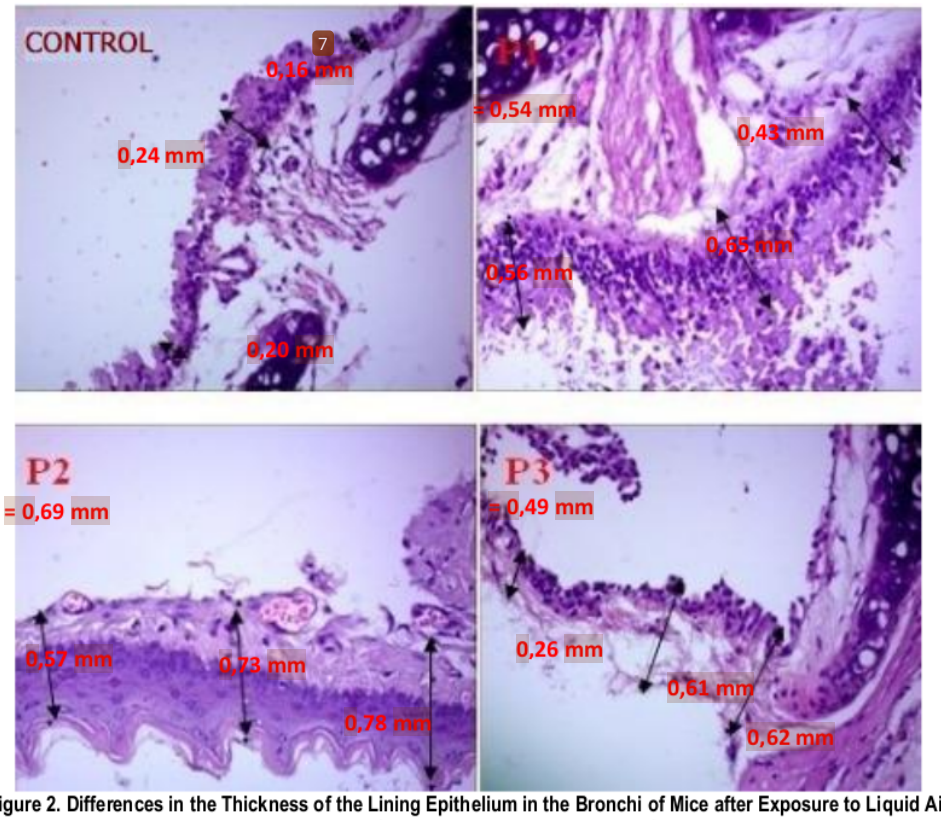

Figure 2. Differences in the Thickness of the Lining Epithelium in the Bronchi of Mice after Exposure to Liquid Air Freshener with HE Staining at 100x and 400x Magnifications

The thickness of the lining epithelium in the bronchi was the thinnest in the control group (K), while treatment group 2 (P2) had the thickest epithelial thickness. This proves that exposure to liquid air freshener at a dose of 3 ml for 4 weeks affect the bronchial epithelium. In treatment group 1 (P1) and treatment group 2 (P2) experienced hypertrophy. Hypertrophy is the enlargement of organs/tissues from normal size without cell division due to enlarged cells. Hypertrophy is characterized by enlarged cell nucleid.

Hypertrophy is an increase in the volume of cells in a tissue or organ, but the cells cannot reproduce but the cells that make up the tissue or organ of the body become larger than their normal size. In the bronchi there is hypertrophy of the bronchial mucus glands resulting in excessive and thicker mucus secretion. Histologically it can be proven by comparing the thickness of the mucous glands and bronchial

walls. Changes also occur in the bronchioli, namely in goblet cells, inflammatory cells in the mucosa and submucosa (Suyanto, 2016).

Respiratory disorders or changes in the airway epithelium due to chemicals can be in the form of hypertrophy Unitly (2018), in normal lungs, goblet cells are located mostly in the upper respiratory tract such as the bronchi, moving downwards in the bronchioles, the number of goblet cells decreases, while submucosal glands are only in the upper respiratory tract and are not found in the bronchioles. In respiratory tract disease, there is an enlargement of the submucosal glands (hypertrophy), resulting in thickening of the airways (Putra, Hanriko, & Kurniawaty, 2019).

Epithelial thickening is caused by hypertrophied and atrophic cells/tissues. According to (Romdhoni, 2001), the emergence of hypertrophy and atrophy is a mechanism of cell adaptation to the environment, which in this case is a response to chemical injury caused by formaldehyde contained in air fresheners. The pharmacodynamics of formaldehyde is by suppressing the function of cells and resulting in tissue necrosis. Pharmacokinetics of formaldehyde is absorbed through all routes of the stomach, intestine and lungs and is oxidized to formic acid and a small portion of methyl formate (formed methyl). Formic acid in the bronchi will bind stably to cellular macromolecules of DNA proteins that can be cross-linked, resulting in abnormalities in the gastric bronchial epithelium such as hypertrophy and atrophy.

In Figure 3 there is a comparison between treatment group two (P2) and treatment group three (P3). Treatment group two (P2) had thicker bronchial epithelium than treatment three (P3). This is presumably because the mice undergo a process of resistance or cell recovery to the chemicals contained in the liquid air freshener so that the cells can modify. When subjected to physiological stress or pathological stimuli, cells can adapt, reach new conditions, and maintain their survival.

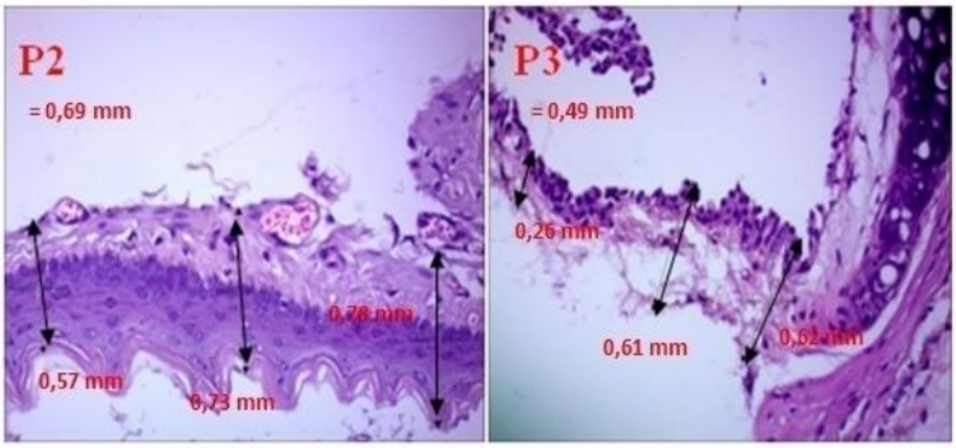

Figure 3. Differences in the Thickness of the Lining Epithelium in the Bronchi of Mice at P2 and P3 after Exposure to Liquid Air Freshener with HE Staining at 100x and 400x Magnifications

According to (Suyanto, 2016), chemicals can cause changes to cell function and cells to become damaged and die, so cells will adapt. The cell response can be reversible, i.e. the cell undergoes morphological changes but the cell does not die. One form of cell adaptation response is atrophy. whereas the process of cell adaptation in which the organ or tissue that is formed grows to reach normal limits but then undergoes shrinkage.

Exposure to liquid air freshener with an exposure duration of 6 weeks (P3) can experience cell atrophy, so that the cell structure in the bronchial epithelium may return to normal compared to P1 and P2. Based on research

conducted (Yunianto, Yanti, & Wulaningrum, 2014), atrophy of mucosal epithelial cells and loss of cilia from epithelial cells that occurred in the treatment group was an adaptive response to exposure to chemicals in liquid air fresheners. Atrophied cells are characterized by a reduction in the size of an organ or cell due to a decrease in cell size and or a decrease in the number of cells.

Meanwhile, according to (Utama, 2018), the cells that make up the lung organs undergo a transition becoming simpler as the airways become smaller. This can be observed from the epithelial changes in the bronchi. The epithelial cells types in the bronchi are ciliated columnar epithelial cells accompanied by goblet cells. Sections containing cartilage are found only in primary bronchi with ciliated columnar epithelial cell types accompanied by goblet cells. In addition, the amount of connective tissue and smooth muscle is decreasing.

Under normal circumstances, the walls of the bronchi are made of several layers of varying thickness and composition. The mucosal and submucosal layers contain cells that protect the respiratory tract and lungs from harmful substances. Normal cells can change if given continuous stimulation which can cause changes in cell structure. According to (Suyanto, 2016), the respiratory epithelium can make repairs that have an impact on changes in the anatomy and function of the airway. The process of tissue repair causes fibrosis of the extracellular matrix of connective tissue resulting in airway narrowing.

Histology of the bronchial wall is the same as that of the trachea, which consists of tunica mucosa, tunica muscularis, tunica adventitia. Branches that are already in the lung tissue have much-changed histology of the walls. Cartilage rings are lost, replaced by cartilage plates, which are irregularly arranged and support the entire circumference of the canal. Tunicamucosa in the branches and twigs of large bronchi has ciliated rod-shaped epithelium, while in small twigs the epithelium turns into cubes and is not ciliated. There is a thick basal lamina, separating the epithelial tissue from the lamina propria. The lamina propria contains many elastic fibers, and few collagen and reticulose fibers. Below the lamina propia is the tunica musculasris-mucosa (Yatim, 2006).

Histology is the study of tissue structure using a microscope on thinly cut tissue preparations. Histology studies the tissues that make up the body, the chemistry of tissues and cells is studied by microscopic and chemical analytical methods. Chemical substances in tissues and cells can be recognized by chemical reactions that produce insoluble coloured compounds, observed with a light microscope or by the scattering of electrons by precipitates that can be observed using an electron microscope. In addition to chemical reactions that occur in tissues, other methods, such as physical methods, are often used, such as interference microscopy which allows the determination of the mass of cells or tissues and spectrophotometric microscopy which allows determination of the amount of DNA and RNA in cells (Harjana, 2011).

Under the microscope, the bronchi usually have a grooved inner surface. This is because in the durable preparations the tunica musculasris-mucosa smooth muscle layer shrinks. The mucous glands are contained in the tunicamucosa and tunicasubmucosa. The tunicaadventitia contains connective tissue fibers, a little fatty tissue, and beneath it, there are pieces of cartilage that are irregularly arranged. The outermost layer consists of the mesothelium, as a continuation of the inner membrane of the pleura (Yatim, 2006).

Air freshener is a household chemical that is included as one of the air pollutants from inside the room. The use of air fresheners is now increasingly questionable, especially about the content in it. Air fresheners enter the body through the inhalation process in the respiratory system. Air freshener ingredients are expected to give negative responses both psychologically and physiologically, such as respiratory disorders, allergic responses and various non-specific symptoms such as headaches, irritation of the nose, eyes and others (Yuningtyaswari, 2012).

Air fresheners generally contain chemicals. Based on research conducted (Damayanti, Yuningtyaswari, & Susanti, 2016), one of the chemical constituents contained in liquid air freshener is formaldehyde. Chemical substances can be inhaled through the nose, trachea and then into the bronchi. The bronchi are located in the middle of the airways so that absorption is not perfect like the lungs, but the absorption is better than the trachea so that the reaction to foreign bodies is more easily absorbed than the trachea so that the particles are more easily deposited in the bronchi. Any particles that are inhaled will collect in the respiratory tract and can reach the bloodstream. The size of the incoming particles determines where the inhaled particles collect. The smaller the particle size, the farther it reaches in the respiratory tract.

There are two types of chemical irritation in the respiratory tract, especially the nasal mucosa, namely primary irritation and secondary irritation. Primary irritants Exposure to irritants affects tissues by direct contact. Irritants will react directly with the epithelium triggering an inflammatory response. Secondary irritation will increase systemic responses, such as the emergence of dependence, nausea, and dizziness (Luttrel, William, Jederberg, Warren, & Still, 2007).

The bronchus is one of the organs in the respiratory system which is located at the bottom of the trachea. The bronchi are the branches of the trachea leading to the right and left lungs. Bronchi are part of the tracheobronchial tract, which is a structure that starts from the trachea and then continues into the bronchi and bronchioles. The trachea branches into the right and left main bronchi with the right bronchus being wider, shorter, and more vertical than the left bronchus. This causes foreign particles to be deposited more frequently in the right bronchus. The right main bronchus will branch into three lobes, namely the right upper lobe, right middle lobe, and right lower lobe. The left main bronchus is divided into two lobes, namely the left upper lobe and the left lower lobe. Each lobe of the bronchus will deliver air to a specific lobe of the lung.

The size of the bronchi as they go down will get smaller and their structure will change. Cartilage rings that support each branch will turn into irregular cartilage and eventually disappear when it reaches the bronchioles. Epithelial cells change from pseudostratified columnar cells to columnar cells and in the terminal part of the bronchioles will become cuboidal cells. Smooth muscle will increase in number. Cilia cells or cells that produce mucus are no longer present in the bronchioles so that foreign particles cannot be expelled through the mucociliary system but will be phagocytized by macrophages in the alveoli. According to (Paramita & Juniati, 2016), epithelial changes in the bronchi describe the function of the respiratory tract. The ciliated columnar epithelium in the initial branching functions to warm and circulate air and filter through the mucociliary role that pushes mucus upward towards the esophagus. The epithelium turns into cubes at the distal branching to allow gas exchange to occur.

In the bronchial epithelium, the tracheobronchial tract is histologically composed of three layers, namely the epithelial layer, the lamina propria, and the cartilaginous layer. The epithelial layer is composed mainly of pseudostratified columnar ciliated epithelium interspersed with submucosal glands. The basement membrane separates the epithelial layer from the lamina propria. The entire pulmonary tract is lined with epithelial cells that have an important function in maintaining the normal function of the respiratory system. These cells can function as a defence against foreign particles, carry out mucociliary transport, produce substances such as mucus, surfactant proteins, or antimicrobial peptides, and stimulate the response of other respiratory tract components such as smooth muscle cells and inflammatory cells (Paramita & Juniati, 2016).

Epithelial tissue consists of an arrangement of cells that are located close together and are held together by intercellular substances. Epithelial tissue is a very dense layer of cell arrangement and usually

limits a very dense layer of cell composition and usually limits the body to its external and internal environment such as intestinal walls, blood vessels, and others. The characteristics of epithelial tissue are that the cells are located adjacent to a certain arrangement, have a clear and strong linkage area, have a free surface and cells can form cytoplasmic projections with a specific purpose. Usually stands on the basement membrane (lamina basalis, membranaproporia). Rarely there are blood vessels in it (Hernawati, 2008).

CONCLUSION

Based on the results, it can be concluded that exposure to liquid air freshener has an effect on the histology of the bronchi of mice (*Mus musculus*). From the results of the Anova test, it was proven that the liquid air fresheners had an effect on the thickening of the epithelium in the bronchi (P = 0.010 <0.05), then Ha was accepted and Ho was rejected. This means that liquid air fresheners can affect the bronchi in mice. The bronchi experienced thickening of the lining epithelium with the type of epithelium, namely columnar ciliated epithelium. From the statistical test results regarding the thickness of the lining epithelium in the bronchi, there were significant differences between the control treatment and the 1, 2, and 3 treatments. So it is hoped that the results of this study in the form of bronchial histology preparations can be used in the learning process.

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