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Rat Lung Histology After Exposure to Cigarette Smooke Made from *Brugmansia suaveolens* Plant

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Abstract**—***Brugmansia suaveolens contains tropane alkaloids such as atropine, hyoscyamine and scopolamine which have anticholinergic properties. This study aims to analyze the effects of exposure to cigarette smoke made from amethyst leaves and flowers on the lungs based on histopathological features. This study used 20 male Rattus norvegicus strains of Wistar, consisting of 4 treatments and 5 replications. This study used a completely randomized design (CRD) with treatment, namely P0: rats without exposure to cigarette smoke (control), P1: rats were exposed to cigarettes from amethyst leaves, P2: rats were exposed to cigarettes from amethyst flowers, P3: rats were exposed to cigarettes from amethyst mix of leaves and amethyst. The treatment was carried out for 21 days. The parameters observed in this study were alveolar septal destruction, increased black particles in lung tissue, alveolar diameter, and alveolar epithelial cell thickness. Data were analyzed using the normality Shapiro-Wilk test because the sample size was ≤50, followed by thetest method one way Anova. The results showed that there was a significant difference (P> 0.05) in the variable diameter of the alveolar epithelial cell thickness, namely treatment P1 to P0. Exposure to cigarette smoke from leaves, flowers, leaf mixtures and mountain amethyst (Brugmansia suaveolens) can cause disturbances in lung structure and function characterized by destruction of the alveolar septum, inflammatory cell infiltration, the emergence of black particles, and an increase in the diameter and thickness of the alveolar epithelial cells. Exposure to cigarette smoke from mountain amethyst leaves (Brugmansia suaveolens) has the most toxic effect on the lungs than exposure to cigarette smoke from flowers or a mixture of the two.*

Keywords**—Brugmansia suaveolens, cigarettes, lung histopathology.**

I. INTRODUCTION

Brugmansia suaveolens is a plant in the eggplant family (Solanaceae). This plant contains tropane alkaloids in the form of atropine, hyoscyamine, and scopolamine with varying concentrations depending on geographical distribution, climate, season, and plant parts. The highest alkaloid content is usually produced when the plants flower. Mountain amethyst when blooming contains about 0.65 mg scopolamine and 0.3 mg atropine, while for hyoscine content is 0.83%. Flowers on older plants contain more hyoscine, which is 3mg so that they are more toxic. Tropane alkaloids affect nerve activity and are known to have hallucinogenic effects. The effect that causes hallucinations is a high potential for abuse. Tropane alkaloids are very toxic, toxicity can occur through consumption, smoking, and topical absorption, especially through mucous membranes [1].

In Indonesia, especially in Javanese society, amethyst is used as a mixture of cigarettes with a weight of 1-9 g per cigarette. Cigarettes made from the mountain amethyst plant have antidepressant effects to hallucinogenic effects [2]. The antidepressant effect is misused by some people, due to several factors, including the way to obtain this plant is very easy, and relatively cheap. Amethyst misuse

in Indonesia reaches 0.3% for the hallucinogenic category of drugs [3].

Natural alkaloid compounds from amethyst leaves and flowers in the form of atropine, hyoscyamine, and scopolamine will enter the respiratory tract. When inhaled, the compound will enter through the nose and mouth while breathing, then into the trachea, bronchi, bronchioles, and into the alveoli. These compounds will diffuse into the blood, then pass to the left atrium through the pulmonary vein. When the left atrium relaxes the blood will be pumped into the heart. Toxicity causes confusion, hallucinations, tachycardia, urinary retention, midriation, respiratory failure, and death [4]. The results showed that smoking habits using mountain amethyst as raw material can cause disturbances in the organs and respiratory tract.

The respiratory tract is the organ most easily exposed to volatile compounds that enter through inhalation [5]. The use of Brugmansia leaves and flowers as raw material for cigarettes can cause pathological effects on the lungs. Lung cells exposed to cigarettes can undergo histological changes that vary, including thickening of the alveolar septum to fibrosis, thickening of the alveolar wall, macrophages whose cytoplasm contains brownish dust, and lymphocyte infiltration [6]. Individuals who often inhale amethyst cigarette smoke have the potential to experience lung problems. Pressure on the respiratory tract caused by cigarette smoke continuously can trigger leakage of blood vessels in the lungs, plasma protein levels come out with fluids and accumulate in the alveoli. This condition is characterized by a microscopic image of the alveoli, which shows that the air spaces are widened, the walls are thinning, and damage to the interalveolar septum [7].

Several studies have shown case reports of the toxic effects of the amethyst plant that cause many health problems. Previous research results showed that giving doses exceeding 5,000 mg / kgBW by inhalation can cause toxic effects [8]. Research on the effect of using Brugmansia plants as raw material for cigarettes on the lungs is still very limited. This study was conducted to determine the histopathological changes that may occur in the lungs of male white rats Wistar strain after exposure to cigarette smoke from Brugmansia plants.

II. METHODS

2.1 Research Sample

The method used in this study was a completely randomized design with four treatment groups and five replications in each group. The sample used in this study was 20 male rats (*Rattus norvegicus*) aged about 2 months with an average weight of 100-180 g. Permission to use these animals has been granted by the Health Research Ethics Committee Dr. Kariadi, Faculty of Medicine, Diponegoro University Semarang number 12 / EC / H / FK-UNDIP / III / 2020. The brugmansia used in this study were obtained from the Bandungan area, Semarang Regency, Indonesia.

2.2 Preparation ofBrugmansia Cigarette Stocks.

Mountain amethyst (B. suaveolens) leaves and flowers were obtained from the Bandungan area, Semarang Regency. Leaves are taken from 4-6 branches from the shoots. Making Brugmansia cigarettes begins with washing the fresh leaves and flowers thoroughly using running water, then slicing them into small pieces and then drying them in the oven at 40^{o} C for 24 hours until the water content is <10%. The dried samples were then rolled using a cigarette rolling device. The weight for each cigarette used is 1g.

2.3 Grouping of Samples

After one week of acclimatization, the rats were randomly divided into four groups. Each group contains five rats. Group P0: rats were not exposed to cigarette smoke. Group P1: rats were exposed to cigarette smoke made from leaves *Brugmansia suaveolens*. Group P2: rats were exposed to cigarette smoke made from flowers *Brugmansia suaveolens*. Group P3: rats were exposed to cigarette smoke made from a combination of leaves and flowers *Brugmansia suaveolens.*

2.4 Treatment of Test Animals

Exposure to cigarette smoke is carried out after the acclimation process for 1 week. Mountain amethyst cigarette smoke is used as a form of exposure to free radicals. The smoking procedure uses a modified used mineral bottle that can be used to collect cigarette smoke so that it can then be exhaled into a cage filled with mice. 1 cigarette per day was used for each treatment group. The plastic tub used for smoking is given a hole on one side as an air vent and a hole on the opposite side as a place for giving cigarette smoke. The rat smoking tub is closed using clear glass. Amethyst cigarettes are burned and put into a hole in the smoking tub while being pumped by hand. After the last cigarette is used up, the sample is removed from the treatment bath. Cigarette smoke exposure is carried out every consecutive day starting at 16.00 for 21 days.

2.5 Surgical Procedures and Making Histological Preparations

At the end of the treatment, the test animals were sedated until they fainted using cotton with 2-3 cc of chloroform for 3-5 minutes. After the test animal has passed out, the surgery is carried out. The lung was taken and then washed with a 0.9% physiological NaCl solution so as not to damage the tissue. The organs were inserted into BNF 10% as fixation material, then carried out by paraffin block. The initial stage is dehydration, after the organs are fixed, dehydration of the organs in acetone (I, II), each for 1 hour at room temperature. Furthermore, the clearing process is carried out with xylol (I, II) for 0.5-1 hour each. Then the infiltration process with xylol paraffin (I, II) each for 30 minutes at a temperature of 54-56° C. The next process is embedded in paraffin and cooled at room temperature. The blocks were collected and cut with a thickness of 5 µ and placed on a glass slide. These preparations are ready for routine hematoxylin and eosin staining. The object glass containing the sliced preparations is closed with a cover glass that has been previously dripped with canada balm [9].

2.6 Observation of Histological Preparations

Observation of the lungs using an Olympus BX51 microscope to observe histopathological changes that may occur during exposure to cigarette smoke from brugmansia plants, is equipped with DP2-BSW computer software linked to a photomicrograph. Each rat was made 1 preparation so that 1 group had 5 preparations. Each preparation consists of 2 to 4 incisions. From each preparation 3 fields of view were taken in a certain area. The histopathological features of the rats' lungs were observed using a photomicrograph with a 400x magnification.

2.7 Data Analysis

The observational data were analyzed by Analysis of Varian (ANOVA) on the 95% ($P<0.05$) of confidence level. Analysis was performed using SPSS 20 software for windows.

III. RESULT AND DISCUSSION

The pulmonary histology of the test animals in treatment P0, P1, P2, and P3 with hemactosillin and eosin staining, is shown in figure.

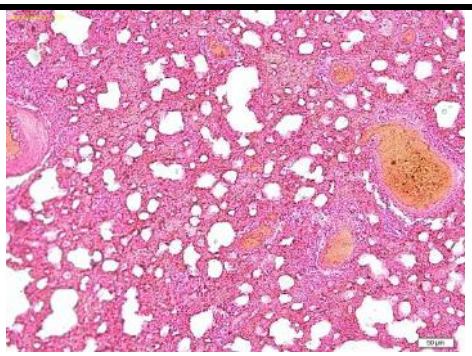

Fig. 1:Histologic structure of control rats' lungs (P0), HE staining with 400x magnification

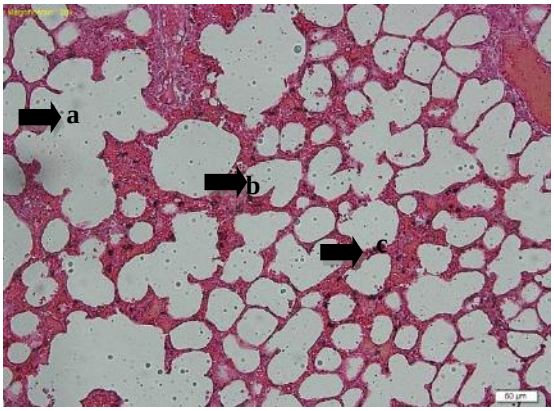

Fig. 2:Histological structure of the lungs of group P1 rats, HE stained with 400x magnification

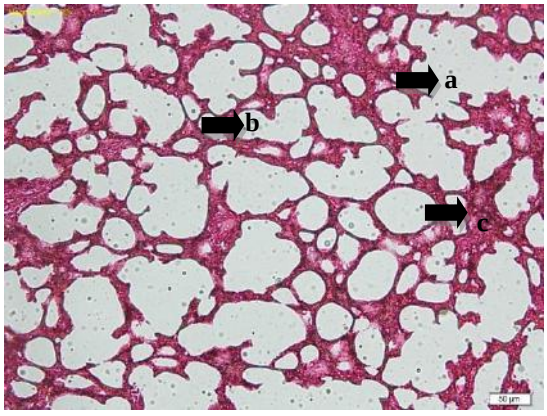

Fig. 3:Histological structure of the lungs of group P2 rats, HE stained with 400x magnification

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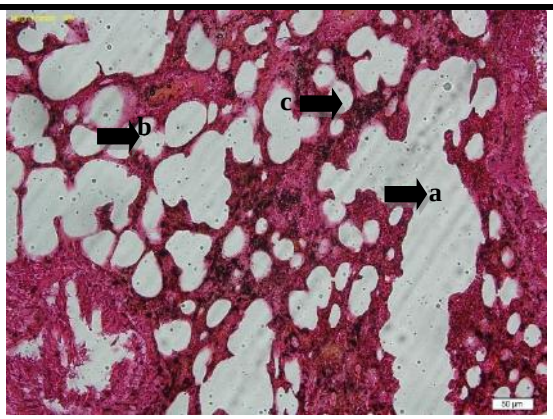

Fig. 4:Histological structure of the lungs of group P3 rats, HE stained with 400x magnification

Table 1. Themean diameter and thickness of the alveolar epithelial layer of the test animals in various treatments

Mean±standard deviation				
Group	**P0**	**P1**	**P2**	**P3**
Diameter(µm)	26.85±2.25	43.87±2.74	35.00±6.93	33.63±7.03
Thick (µm)	2.59±0.30	5.26±1.52	4.21±1.75	3.22±0.65

The results of the analysis of the effect of exposure to cigarette smoke from the leaves and amethyst flowers on the diameter and thickness of the alveolar epithelial layer showed significant results (P> 0.05).

IV. DISCUSSION

The results of the photomicrograph as shown in Fig. 1 for control (P0), show that the pulmonary histology in the test animals appears in normal conditions. Another indication is the absence of inflammatory cell infiltration or thickening of the alveoli and the alveolar diameter is relatively normal. Normal lung histology is characterized by specific characteristics, which include the outer layer of the thin alveoli, and is coated with two types of epithelial cells (pneumocytes), namely squamous epithelial cells (type I pneumocytes) and large cuboidal cells. (Type II pneumocytes) [6]. The pulmonary blood vessels appear to be normally distributed in the lung parenchyma. The blood capillaries that vascularize the alveolar septum are normal in size, and the alveolar septum thickness also indicates normal size.

The results of histological observations on treatment (P1, P2, P3) showed significant damage to lung tissue. The damage is characterized by the appearance of brown or black particles on the alveoli. Another indication of damage is the presence of inflammatory cell infiltration and thickening that occurs in the outer layer of the alveoli. The brown or black particles are thought to be caused by the effect of iron (Fe oxidation$^2O^2$). The emergence of brown or black particles on the outer alveolar layer is a deposit of toxic compounds that accompany cigarette smoke. Cigarette smoke is a heterogeneous aerosol particle. These particles consist of gas components, volatile compounds, oxidants, pharmacologically active compounds such as alkaloids, mutagens and carcinogens, as well as antigenic components and cytotoxic compounds. Exposure to these various compounds along with cigarette smoke is thought to be a factor in the appearance of black or brown particles in lung tissue [10].

Infiltration of inflammatory cells in lung tissue occurs as a result of the effects of free radicals due to the influence of cigarette smoke brugmansia. The inflammatory process will activate the macrophage cells in the tissue as a nonspecific form of defense. Inflammatory cells will release neutrophil chemotactic factors, such as interleukin 8 and leukotriene B4. These two kinds of chemotactic factors will stimulate neutrophils to synthesize and release proteases which have the potential to damage connective tissue and lung parenchyma in the form of damage to the outer alveolar layer. The occurrence of damage to lung tissue is indicated by the infiltration of inflammatory cells in response to internal or external stimulants accompanied by the formation of free radicals and the occurrence of free radical chains in the tissue [13].

The observations also showed a thickening of the outer alveolar layer. This thickening is caused by a buildup of inflammatory cells in that section. Cigarette smoke can cause a buildup of inflammatory cells in lung tissue. These inflammatory cells can trigger damage to the alveolar structures of the lung as a result of increased secretion of proteolytic enzymes and free radicals in the extracellular space [11].

The high level of lung tissue damage in the test animals in the P1 treatment showed that the leaves of the mountain amethyst had a higher toxicity effect than the flower parts. This is supported by research evidence which shows that the use of amethyst plant parts in the form of leaves, roots and seeds can cause toxic effects on human lung tissue [4]. Another factor that is thought to be the cause of the high level of lung tissue damage in the test animals is the low antioxidant content in the tissue. This condition can be

caused by the low synthesis of endogenous antioxidants or exogenous antioxidants sourced from amethyst leaves. The low level of antioxidants in the lung tissue of the test animals did not result in an increased damage effect caused by the presence of free radicals and free radical chain reactions.

The results of the analysis of the effect of exposure to cigarette smoke from amethyst leaves and flowers on the diameter and thickness of the alveolar epithelial layer showed significant results (P> 0.05). This means that exposure to cigarette smoke from amethyst leaves and flowers can increase the diameter and thickness of alveolar epithelial cells. This condition is thought to be related to the occurrence of dilatation of the lumen and accumulation of inflammatory cells in lung tissue as a result of the toxic effects of various compounds from exposure to cigarette smoke. The data in table. 1 shows that the average alveolar diameter in the test animals (control / P0) is significantly different from the test animals in treatment P1, and is not significantly different in treatment P2 and P3.

The diameter and thickness of the alveolar epithelial layer in the control test animals that were not exposed to cigarette smoke (P0) were 26.85 µm and 2.59 µm. This value was statistically significantly different from the test animals in treatment P1, which were respectively 43.87 µm and 5.26 µm. The difference in the mean variable values at P0 and P1 is thought to be due to the effect of cigarette smoke made from amethyst leaves. Cigarette smoke from this material contains oxidants, volatile compounds, alkaloids, and irritant compounds in the form of aromatic hydrocarbons that trigger oxidative stress.

The diameter and thickness of the alveolar epithelial layer in the group of test animals that were not exposed to cigarette smoke (P0) were 26.85 µm and 2.59 µm, statistically not significantly different from the diameter and thickness of the alveolar epithelial layer in the test animal group exposed to smoke. cigarettes made of amethyst flower (P2) with respective sizes of 35.00 µm and 4.21 µm. This is presumably because the flowers resulting from the drying process using an oven at a temperature of 40˚ C for 24 hours have not been able to make the amethyst flowers dry completely, so that the resulting secondary metabolite content is less. T]emperature and drying time have a significant effect on the content of active substances contained in a material [12].

The size of the diameter and thickness of the alveolar epithelial layer in the test animal group that was not exposed to cigarette smoke (P0) was 26.85 µm and 2.59 µm, statistically not significantly different from the diameter and thickness of the alveolar epithelial layer in the test animal group that was exposed to it. cigarette smoke from a mixture of leaves and amethyst flower (P3) with a size of 33.63 µm and 3.22 µm, respectively. It is suspected that the flowers resulting from the drying process have not been able to make the amethyst flowers dry completely, so that the resulting secondary metabolite content is less, besides that another factor that affects is the leaf mixture. Although amethyst leaves had higher toxicity, for the P3 treatment group the dose of leaves used was less than in the P1 treatment group, which was 0.5 g per cigarette, so that oxidants, alkaloids, and other irritants tended to be less.

V. CONCLUSION

This study concluded that exposure to smoke from cigarettes made from leaves and flowers Brugmansia suaveolens for 21 days can cause disturbances in lung histology in the form of destruction of the alveolar septum, inflammatory cell infiltration, the emergence of black particles,and an increase in the diameter and thickness of the alveolar epithelial cells. Exposure to cigarette smoke from mountain amethyst leaves (*Brugmansia suaveolens*) has the most toxic effect on the lungs than exposure to cigarette smoke from flowers or a mixture of the two.Given the active metabolite content of of the active metabolite of Brugmansia plants that can suppress the function of the parasympathetic nervous system, it is expected that further research on the effects of cigarette smoke exposure on other organs Brugmansia to analyze the effects.

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