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Bronchus Histology of Wistar Rats (Rattus norvegicus L) that are exposed to the cigarette smokes given with Angel's Tears leaves (Brugmansia suaveolens) smoke treatment

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Abstract— *Angel's tears (Brugmansia suaveolens Bercht. & Presl) contain tropane alkaloids, such as atropine, hyoscyamine and scopolamine which have anticholinergics potential. This study aims to analyze the effect of Angel's tears leaf smoke on the thickness of the mucous-submucosal membrane and the number of bronchial goblet cells of white rats induced by cigarette smoke exposure. Two months old male Wistar rats (Rattus norvegicus L.) were used (average weight 200 g). This study was an experimental study with a completely randomized block design consisting of 5 treatments and 4 replications, namely K0: negative control, K1: Positive control was given exposure to 1 cigarette / day, P3: K1 + Aminophylline 8.1 mg / 200 g BW, and two treatment groups (P1, P2) were given the smoke of Angel's tears with different doses. White rat bronchi were prepared for histological observation using the paraffin method and Hematoxylin-Eosin staining. Data analysis using ANOVA followed by Duncan's Multiple Range Tests with a confidence level of 95%. The results showed that the smoke of Angel's tears can reduce the thickness of the mucous-submucous membrane and the number of goblet cells in white rats. This study provides information to the public that Angel's tears can be used as an alternative treatment for respiratory disease.*

Keywords— ***Brugmansia suaveolens, cigarette smoke, goblet cells, mucosal-submucosal membrane thickness***

I. INTRODUCTION

Non-Communicable diseases (NCDs) have increased, especially in developing countries, one of which is chronic obstructive pulmonary disease (COPD). COPD has a prevalence of 3.7% occurring in the age group 30 years [1]. One of the main causes of COPD is smoking [2]. The components of cigarette smoke gas are CO, ammonia, hydrocyanic acid, nitrogen oxides, and formaldehyde. The particles consist of tar, indole, nicotine, carbazole and cresol. Particles that enter the airway can settle there and cause ineffective airway clearance, thereby increasing resistance or obstructive respiratory distress [3].

Each inhaled cigarette smoke contains 10^{17} Reactive Oxygen Species (ROS) molecules. ROS causes hyperplasia as a consequence of the inflammatory process. Hyperplasia and an increase in the number of epithelial cells due to oxidative stress are caused by the activity of the Epidermal Growth Factor Receptor (EGFR). These receptors are involved in the process of cell proliferation and deformation, so that EGFR activation will stimulate the differentiation of ciliated epithelial cells into goblet cells or mucus products [4].

Continuous exposure to allergens can stimulate inflammation in the respiratory tract which results in airway remodeling in the form of epithelial damage and smooth muscle hypertrophy. The epithelial damage is characterized by a change in the epithelial structure, which was originally cylindrical in the form of ciliated stratified epithelium to no flow and the release of the epithelium from the basement membrane. This is in line with previous studies that exposure to cigarette smoke can cause goblet cell hyperplasia [5].

Exposure to secondhand smoke has an adverse effect on breathing. Rats exposed to cigarette smoke for 20

days developed hyperplasia and an increased number of epithelial cells in the airway [6]. The irritant in the form of cigarettes contains free radicals that cause hyperplasia as a consequence of the inflammatory process. Giving exposure to cigarette smoke for 2 cigarettes per day for 28 days can increase the presentation of goblet cell hyperplasia in the bronchial airways. Harmful free radicals from inhaled cigarette smoke can irritate the bronchial airways. Irritation of the bronchial airways. Irritation of the bronchial airways is characterized by mucus hypersecretion caused by hyperplasia of goblet cells in the bronchi. [4].

Hypersecretion of mucus will cause thickening of the airway membrane resulting in vasoconstriction. A pharmacological therapy to reduce clinical symptoms is one of them using aminophylline [7]. Aminophylline is a ß2 agonist drug which acts as a bronchodilator [8]. The mechanism of action of Aminophylline is to block acetylcholine receptors in the parasympathetic nerves of the bronchial muscles, causing stimulation of guanylate cyclase and suppressing the increase in bronchoconstriction mediator or cyclic-GMP (cGMP) and causing bronchodilation. In addition, aminophylline works to inhibit the phosphodiesterase enzyme, resulting in the cyclic-AMP (cAMP) buildup. The cAMP is a compound that causes relaxation of the bronchial catecholamine muscle as well as increases cyclic tissue cAMP levels by different mechanisms. This process is the basis of theophylline's action on bronchodilation. Long-term use of synthetic drugs can cause side effects such as nausea, itching, headaches, psychological disorders, hair loss, tachycardia, hyperuricemia, and even impaired liver function [9].

Efforts to reduce the negative effects of pharmacological drugs are to use natural medicines or traditional medicines from plants, one of which is Angel's tears. Previous research stated that the water extract of Mount Angel's tears at a dose of 47.55 mg kg BW had an effect equivalent to a comparison of methylprednisolone with the ability to suppress inflammation formed by 85% [10]. This is because Angel's tears contain bioactive compounds such as tropane alkaloids, flavonoids, terpenoids. The antioxidants contained in Angel's Tears have a function to stop or break the chain reaction of free radicals present in the body, thereby preventing damage to body cells [11]. This study aims to analyze the effect of Angel's tears leaf smoke on the histopathology of white rats exposed to cigarette smoke. This study can provide information to the public that Angel's tears with different doses will not cause toxicity effects on the bronchi and lungs so that they can be used as an alternative to prevent respiratory disease.

II. METHODS

Handling, facilities and management of animals during the experiment were carried out by the Guidelines for the Care and Use of the Biology Laboratory of the University of Semarang and approved by the Research Ethics Committee of the Diponegoro Faculty of Medicine University Number 12 / EC / H / FK-RSDK / III / 2020. from April to November 2020 at the Biology Laboratory of Semarang State University.

2.1 **Preparation of Angel's Tears extract**

Angel's tears were obtained from the Pondok Kopi area, Umbul Sidomukti. The leaves were oven-dried at 40-50º C for 10 days [13].

2.2 Laboratory Animal Care

Twenty healthy male Wistar rats (Rattus norvegicus L.) were obtained from the Laboratory of the Department of Biology, Semarang State University with an average age of 2 months (average weight 200 g. Acclimation was carried out for one week, commercial feed and water were given by ad libitum.

2.3 Experimental Design and Treatment Procedures

This research is an experimental study with a completely randomized block design consisting of 5 treatments and 4 replications, namely K0 = negative control given standard feed, K1 = positive control given exposure to 2 sticks of non-filter cigarette smoke per day, P1 = K1 given 25 mg / 200 g BW exposure to mountain Angel's tears, P2 = K1 given 50mg / 200 g BW exposure to mountain Angel's tears, P3 = K1 given 8.1 mg / 200 g BW aminophylline in 1 ml distilled water. Kretek cigarette smoke is given every morning for sixty days as much as 1 stick per day using a smoking pump, then given drinking water every day along with 75 ml rations. Angel's tears were given once a day at 8.00 a.m. using a nebulizer, while the administration of simvastatin extract and ethanol from Angel's tears was given every afternoon by ip using a disposable syringe. The treatment was carried out for 60 days.

2.4 Preparations and Coloring Procedures

Bronchial preparations were made by the paraffin method and fixed in 10% buffered formalin, then processed for paraffin sectioning, the bronchial organs were cut at ± 4 µm. The next stage is dehydration using alcohol graded 80%, 90%, 95% for 2 hours at each concentration. Then the tissue was cleaned using xylol I and II for 60 minutes then continued in the infiltration process using paraffin. The next process was deparaffinized using xylol I and II for 5 minutes each, alcohol with a grade of 95%, 90%, and 80% each for 2 minutes, and rinsed with distilled water. The staining process uses Hematoxylin-Eosin. The slides were put in Hematoxylin for 2 minutes and rinsed with

water. The next step was to put in the eosin for 5 minutes, then 80%, 90%, 95% alcohol was graded for 2 minutes, and xylol I and II for 2 minutes each. The final stage is the installation process, namely by applying adhesive to the preparation and covering it with a glass cover.

2.5 Preparation for Observation

Bronchial thickness was measured from mucous membrane to submucous membrane, while the number of goblet cells was measured using a 100 x 10 magnification with light microscope. All slides were observed under an Olympus BX51 microscope equipped with a photomicrograph with a magnification of 400x, 1000x. Measurement of mucous-submucous membrane thickness and the number of goblet cells in the bronchi were carried out at 5 points which represented the thickness of the mucous-submucous membrane and the number of bronchial goblet cells in the whole and then averaged.

2.6 Statistic Analysis

The results of measurements of mucous-submucosal membrane thickness and the number of goblet cells in the bronchi were analyzed using the Shapiro Wilk normality test (sample size <50) and the homogeneity test. Data that were normally distributed and homogeneous were analyzed using one-way ANOVA at a 95% level of confidence using SPSS 22.0 software, then continued with Duncan's Multiple Range Test if there were significant differences.

III. RESULTS

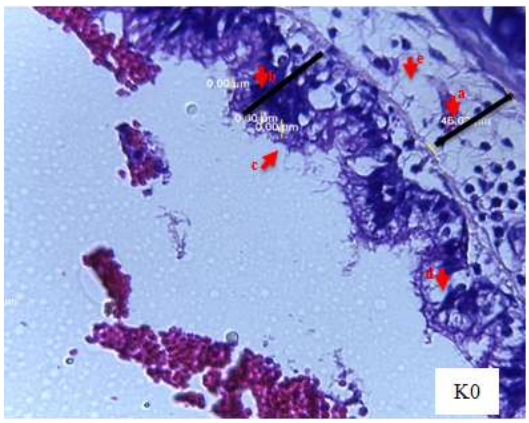

Fig.1. The bronchial histology of negative control rats fed standard feed with a magnification of 40x10 and staining using Hematoxylin, information: (a) thickness of bronchial mucosal-submucosal tissue, (b) thick layer of stratified cilia cells, (c) bronchial cilia, (d) goblet cells in the bronchial epithelium, (e) no thickening of the seromucous glands.

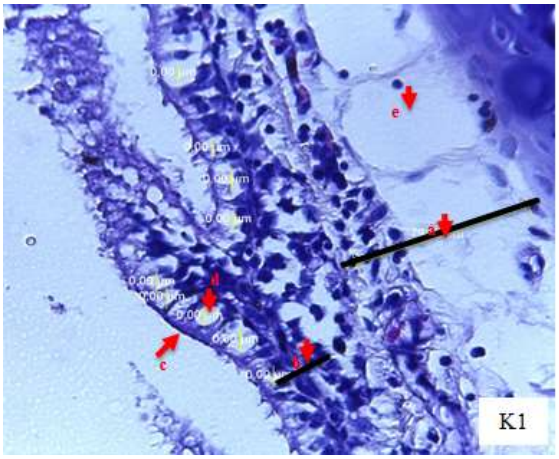

Fig.2. The bronchial histology of positive control rats exposed to 2 sticks / day of non-filter kretek cigarette smoke with a magnification of 40x10 and staining using Hematoxylin, information: (a) there is a thickening of the bronchial mucosal-submucosal tissue, (b) the thickness of the cilia cell layer is thinning. , (c) depletion of bronchial cilia, (d) an increase in the number of goblet cells in the bronchial epithelium, (e) thickening of the seromucous glands.

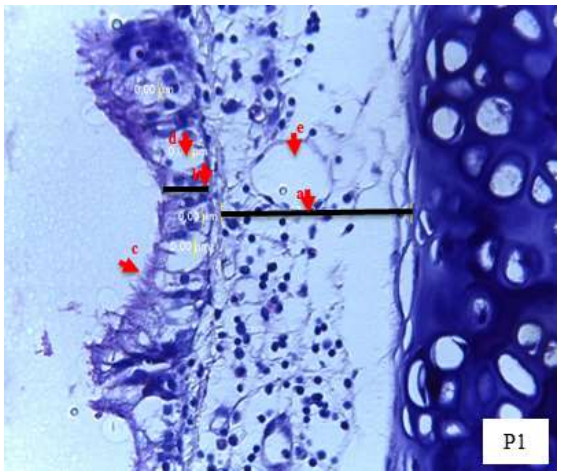

Fig.3. Histology of the bronchi of P1 = K1 rats given exposure to the smoke of Angel's tears at a dose of 25mg / 200gBB with a 40x10 magnification and staining using Hematoxylin, information: (a) there is still thickening of the bronchial mucosal-submucosal tissue, (b) thick layer of cilia cells is thinning, (c) bronchial cilia begin to regenerate, (d) there is still an increase in the number of goblet cells in the bronchial epithelium, (e) thickening of the seromucous glands.

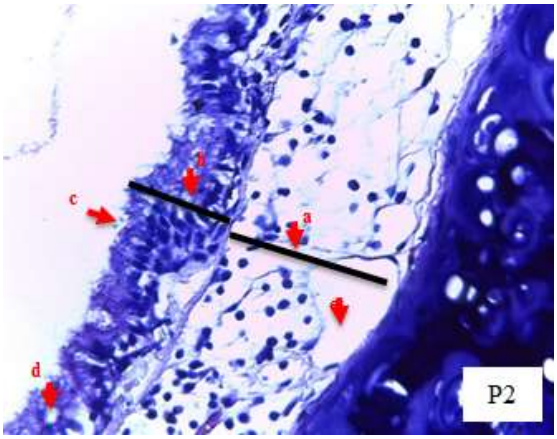

Fig.4. Histology of the bronchi of P2 = K1 rats treated with exposure to the smoke of Angel's tears at a dose of 25mg / 200gBB with a 40x10 magnification and staining using Hematoxylin, information: (a) there is a reduction in the thickness of the mucosal-submucosal tissue of the bronchi, (b) the thickening of the cilia cell layer stratified, (c) bronchial cilia begin to regenerate, (d) there is a decrease in the number of goblet cells in the bronchial epithelium, (e) there is still thickening of the seromucous glands.

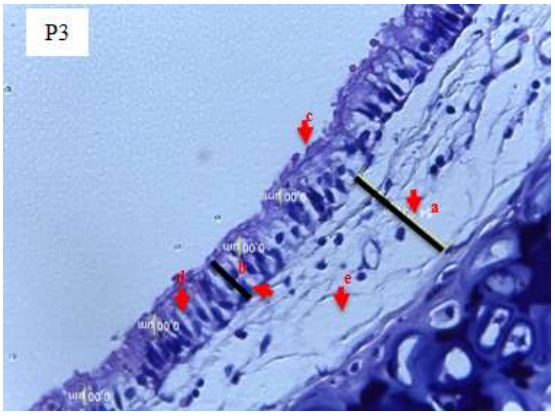

Fig.5. The bronchial histology of P3 = K1 rats treated with exposure to Angel's tears leaf smoke at a dose of 50mg / 200gBB with a 40x10 magnification and staining using Hematoxylin, information: (a) there was a reduction in the thickness of the bronchial mucosal-submucosal tissue, (b) the thickening of the cilia cell layer stratified, (c) the bronchial cilia regenerate a lot, (d) a decrease in the number of goblet cells in the bronchial epithelium, (e) no thickening of the seromucous glands.

Table 4.1. The average thickness of the mucosal-submucosal gland walls and the number of goblet epithelial cells in the bronchi of the Wistar White Rat

Treatment	Variabel	
	Wall Thickness (µm) ± SD	Number of goblet cells ± SD
K0	$55,31^{c}$ ± 1,22	$3,82^{c}$ ± 0,17
K1	$163,87^{a}$ ± 8,67	$9,97^{a}$ ± 0,33
P1	$155,29^{a}$ ± 13,18	$9,27^{a}$ ± 0,71
P2	$89,87^{b}$ ± 4,67	$6,32^{b}$ ± 0,62
P3	$56,48^{c}$ ± 3,26	$4,35^{c}$ ± 0,58
Annotation:	Figures with different superscripts in the same column showed a significant difference between treatments at the 95% level (P <0.05). K0 = negative control given standard feed, K1 = positive control given exposure to 2 sticks of non-filtered cigarette smoke / day, P1 = K1 given 25mg / 200 g BW exposure to mountain Angel's tears, P2 = K1 given 50mg / 200 g BB exposure to leaf Angel's mountain tears, P3 = K1 given 8.1 mg / 200 g BW of aminophylline in 1 ml of distilled water.	

IV. DISCUSSION

The analysis showed that there was a significant difference (P <0.05) in the thickness of the mucosal membrane and the number of goblet cells in white rats exposed to cigarette smoke after being given smoke from Angel's tears. Table 1 shows that the mucosal-submucosal membrane thickness of the negative control white rats had a lower mean thickness and fewer goblet cells, namely 55.31 ± 1.22µm and 3.82 ± 0.17 compared to the control group. Positive and other treatment. The K0 bronchial histology presented in Figure 1 shows a normal histological picture, meaning that there is no mucus accumulation on the mucous-submucosal membrane and damage to the epithelial cell structure which causes thickening of the bronchial diameter. According to previous studies, the bronchial epithelium in control rats was seen in normal conditions where the structure of the epithelial cells was still compact and in the form of cylindrical stratified ciliated epithelium. This suggests that feeding and drinking standard water for 60 days does not affect the bronchial histopathology of white rats [14].

Based on Table 1, it was found that the positive control had a higher mean thickness and a higher number of goblet cells, namely 163.87 ± 8.67 and 9.97 ± 0.33. Microscopic observation of the bronchi in the positive control group showed thickening of the mucosa-submucosa layer and hyperplasia of goblet cells accompanied by stratified cilia cell depletion. Based on previous studies that there was an increase in black particles adhering to the alveolar walls of rats' lungs after exposure to cigarette smoke [12]. This suggests that exposure to cigarette smoke at a dose of 1 stick per day for 60 days is thought to cause thickening of mucosal-submucosal membrane thickening and an increase in the number of goblet cells in rat bronchi.

The thickening of the mucosal layer that occurred in this study indicated the presence of mucus hypersecretion which was indicated by an increase in the number of goblet cells. This is consistent with previous studies that showed that in bronchial epithelium, free radicals cause hyperplasia as a consequence of the inflammatory process. Cigarette smoke exposure contains free radicals that are thought to affect bronchial diameter. Continuous exposure to allergens can stimulate inflammation in the respiratory tract which results in airway remodeling in the form of epithelial damage to the airway [4]. The epithelial damage can be seen in Figure 2, characterized by a change in the epithelial structure, which was originally cylindrical in the form of ciliated stratified epithelium to no meaning and the release of the epithelium from the basement membrane.

Each inhaled cigarette smoke contains 10^{17} Reactive Oxygen Species (ROS) molecules. ROS from cigarette smoke is mediated by hydroxyl radical (OH-), peroxynitrite (ONOO-), superoxide anion (O2-), and hydrogen peroxide (H2O2). ROS will inactivate secretory leukoprotease inhibitors (SLPI) and α1-antitrypsin (α1-AT) and mediate the increase in proteolysis, causing decreased anti-protease defenses. Stimulating the incoming cigarette smoke allergens will stimulate the postganglionic fibers to release acetylcholine. Acetylcholine binds to muscarinic and nicotinic receptors in the parasympathetic ganglion located on the wall of the airway. One of the active substances contained in cigarettes, namely nicotine, will bind to nicotinic receptors. The nicotine compounds in cigarettes have cholinergic properties which act as agonists at nicotinic receptors, causing bronchoconstriction of the airways [15].

Aminophylline is a methylxanthine class that has lower efficacy than inhaled corticosteroids and long-acting β-2 agonists and is a potent bronchodilator with mild anti-inflammatory action [7]. The results in Table 1 show that the P3 bronchi have the second-lowest mean mucosal-submucosal membrane thickness and the second-lowest goblet cell count after negative control (K0), namely 56.48 ± 3.26 µm and 4.35 ± 0.58 compared with the K1 group. The histological picture of bronchus group P3 which is presented in Figure 5 shows a normal histological picture, there is thinning of the mucosal-submucosal tissue due to a reduction in mucus accumulation in the seromucosal tissue. In addition, there is also a decrease in the number of goblet cells, characterized by goblet cell hypoplasia which results in a decrease in the size of the tissue or organ due to a decrease in the number of constituent cells. This suggests that exposure to cigarette smoke together with aminophylline for 60 days in rats can reduce the risk of bronchoconstriction.

Treatments of P1 and P2 with the administration of 25 and 50 mg / 200 g BW of Angel's tears for 60 days in white rats exposed to cigarette smoke decreased mucosal-submucosal membrane thickness and the number of goblet cells compared to the K1 group. The decrease in mucosal-submucosal membrane thickening and the number of goblet cells occurred with an increase in the smoke dose of Angel's tears. The results of the mean mucosal-submucosal membrane thickness and the number of goblet bronchial cells of rats in the P1 and P2 groups were 155.29 ± 13.18 and 9.27 ± 0.71, respectively; 89.87 ± 4.67 and 6.32 ± 0.62.

The histological features of rat bronchi in P1 and P2 treatment respectively experienced better development. The bronchi of rats in P1 treatment still showed thickening of the mucosal-submucosal tissue due to mucus buildup in the seromucosal tissue but it was not worse than the K1 group. The mucosal-submucosal membrane in treatment P1 which was still experiencing thickening was thought to be due to the administration of Angel's tears leaf smoke at a dose of 25 mg / 200 g BW for 60 days and was unable to prevent mucus buildup on the mucous-submucosal membrane and goblet cell hyperplasia, but rat bronchi in treatment P2 showed there is a change, namely a decrease in the thickness of the mucous-submucosal membrane and the number of goblet cells. This means that the smoke of Angel's tears at a dose of 50 mg / 200 g BW has almost the same effectiveness as aminophylline, which can reduce the thickening of the mucous-submucosal membrane and decrease the number of goblet cells in the bronchi of rats.

The antioxidants contained in the leaves of Angel's tears, one of which is a flavonoid, can also function as a hepatoprotection, anti-inflammatory, or antihistamine. Flavonoids can also inhibit prostaglandin synthesis by inhibiting the action of cyclooxygenase (COX) so that arachidonic acid changes to prostaglandins as the main mediator of inflammation. The antioxidants in Angel's Tears also have a function to stop or break the

chain reaction of free radicals present in the body, thereby preventing damage to body cells. In addition, the plant Angel's Tears also contain tropane alkaloids which have anticholinergic properties acting as a competitive antagonist against acetylcholine at airway receptors [11]. This is in accordance with previous research which states that the water extract of Mount Angel's tears at a dose of 47.55 mg / kg BW affects equivalent to the comparison of methylprednisolone with the ability to suppress inflammation that is formed by 85% [10].

V. CONCLUSION

Exposure to the smoke of Angel's tears (Brugmansia suaveolens Bercht. & Presl) has a significantly different effect on the histopathology of white rats (Rattus norvegicus L.) exposed to cigarette smoke in terms of thickness of the mucous-submucous membrane and the number of goblet cells in the bronchial organs of rats, so it can be concluded that giving Angel's tears (Brugmansia suaveolens Bercht. & Presl) leaf smoke can reduce the mucosal-submucosal membrane thickening and the number of goblet cells in the bronchial organs of white rats (Rattus norvegicus L.).

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REFERENCES

[1] S. M. S. Islam, T. D. Purnat, N.T. Anh Phuong, U. Mwingira, K. Schacht, G. Fröschl, “Non Communicable Diseases (NCDs) in developing countries: A symposium report,” Globalization and Health., vol. 10, 2014, pp. 1–7.

[2] M. D. Eisner, N. Anthonisen, D. Coultas, N. Kuenzli, R. Perez-Padilla, D. Postma, I. Romieu, E. K. Silverman, J. R. Balmes, “An official American Thoracic Society public policy statement: Novel risk factors and the global burden of chronic obstructive pulmonary disease”, American Journal of Respiratory and Critical Care Medicine,. vol 182, 2010, pp. 693–718.

[3] S. Wenny, “Bahaya Merokok Bagi Kesehatan,” UMM., vol. 1, 2010, pp. 1-8.

[4] R.V. Suryadinata., A. Lorensia, R. K. Sari, “ Differences in Nutrition Food Intake and Body Mass Index between Smoker and Non-smoker in Adult Indones,” J. Clin. Pharm., vol. 6, 2017, pp. 171–180

[5] M. Silva, and P. Bercik, “ Blood, Lymphatics, Immune System and Stem Cells. Macrophages are related to goblet cell hyperplasia and induce MUC5B but not MUC5AC in human bronchus epithelial cells,” Laboratory Investigation., vol. 92, 2012, pp. 937 – 948.

[6] A. Rohmani, “The Effects of Kersen Fruit Extract (Muntingia Calabura) on the Number of Bronchial Ciliated Epithellium Cells on Cigarette Smoke Exposed Wistar Rats”J. Faculty Medicine Muhammadiyah University of Semarang., vol. 1, 2018, pp.1-8.

[7] A. Lorensia, “Efektivitas dan Risiko Toksisitas Aminofilin Intravena pada Pengobatan Awal Serangan Asma,” Jurnal Farmasi Klinik Indonesia., vol. 7, 2012, pp. 8-18.

[8] Triyani, “Evaluasi Penggunaan Obat Asma Pada Pasien Asma Di Instalasi Rawat Inap Rsup Dr. Soeradji Tirtonegoro Klaten Tahun 2009,” Faculty Medicine Muhammadiyah University of Semarang., vol. 1, 2020, pp.21.

[9] Na’i, A, Y. Rakanita, and S. Mulyani, “Uji Efek Kombinasi EEDS dan DPW terhadap Penurunan Kadar Kolesterol Total Tikus Putih Jantan Hiperkolesterolemia-Diabetes,” Farmakologika Jurnal Farmasi., vol. 16, 2019, pp. 1–12.

[10] N.I. Anisa, S. A. Mutiara, A. S. Andreanus, “ Uji efek antiinflamasi ekstrak air daun Angel’s tears gunung [brugmansia suaveolens [humb. & bonpl. Ex willd] bercht. & presl] pada tikus wistar betina,” Jurnal Ilmiah Farmasi., vol. 2, 2015, pp.21-27.

[11] M. Aria, Verawati, A. Arel, and Monika, “Uji efek antiinflamasi fraksi daun piladang (solenostemonscutellarioides (l.) Codd) terhadap mencit putih betina,” Scientia., vol. 5, 2015, pp. 84-91.

[12] Unitly, Adrien, Jems, Akiles, N. Kusumorini, S.Agungpriyono, A. S. Satyaningtijas, A. Boediono, “Analisis mikroskopis paru-paru tikus jantan (rattus norvegicus)”., Journal Unsyiah., vol. 1, 2018, pp. 9.

[13] J. Sitasiwi, S. Isdadiyanto, and S. M. Mardiati, “The Estradiol 17- β Concentration in Mice After Treated with Ethanolic Leaf Extract of Azadirachta indica (Neem),” The 18th International Conference on Positron Annihilation., vol. 020014, 2017, pp. 1–8.

[14] Muhartiningsih, “Perbandingan Pengaruh Pendedahan Uap Bensin Jenis Pertamax Dan Premium Terhadap Gambaran Histologi Bronkus Tikus Putih (Rattus Norvegicus) Jantan,” Magna Medika., vol 6, 2019, pp. 1-5.

[15] L. Rahmad, “Pengaruh Pemberian Ekstrak Rimpang Kunyit (Curcuma Domestica Val.) terhadap Gambaran Histopatologis Paru-Paru yang Diinduksi Asap Rokok pada Tikus Putih Wistar,” Kedokteran Hewan., vol. 1, 2018, pp. 9.