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RESEARCH ARTICLE

EFFECTS OF TURMERIC (CURCUMA LONGA L.) RHIZOME EXTRACT ONLUNGHISTOLOGICAL STRUCTUREIN MICE(MUSMUSCULUSL) INDUCEDNICOTINE

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Abstract

Nicotine is an alkaloid compound found in tobacco leaves. The use of nicotine in a long period can cause lung damage.The aim of this study was to determine the effect of giving turmeric extract (*Curcuma longa* L.) on lung histological structure in nicotine-induced mice. In this study, 25 male mice were divided into 5 groups. Group 1 was a negative control group without nicotine-induced and turmeric extract. Group 2 was a positive control group induced by nicotine without turmeric rhizome extract, groups 3, 4 and 5 were groups exposed to nicotine followed by giving turmeric extract at doses of 0,98, 19,6 dan 29,4 mg/kg BW. The dose of nicotine given was 2.5 mg/kg BW, given intraperitoneally for 28 days. Turmeric extract was given as gavage for 14 days. The results showed that nicotine exposure resulted in an increase in mucosal thickness of the bronchioles and alveolar septa of mice. The administration of turmeric rhizome extract resulted in a decrease in the thickness of the bronchial mucosa and alveolar septa. Giving turmeric extract at a dose of 29,4 mg/kg BW resulted in a decrease in the thickness of the bronchial mucosa and alveolar septa. The conclusion of this study is that the 29,4 mg/kg BW dose of turmeric extract is effective in repairing histological damage to lung tissue in mice.

Introduction:-

Nicotine is the main alkaloid compound contained in tobacco leaves. The nicotine content in tobacco leaves ranges from 2-8%, depending on the tobacco species, the position of the leaves on the stem [1]. The content of nicotine compounds in cigarettes can have a detrimental effect on the heart, reproductive system, kidneys and lungs [2]. Nicotine that enters the body will be metabolized in the liver, into cotinine compounds (about 70-80%), which will be excreted in the urine around 5-10% [3]. Nicotine and its metabolites will form Reactive Oxygen Species (ROS) which are mostly formed in mitochondria [4]. An imbalance of ROS and antioxidants in the body will trigger oxidative stress [5]. Increased oxidative stress will trigger peroxidation in cells which results in damage and cell death [6].

One of the cell damage caused by nicotine is lung epithelial cells and also triggers an inflammatory reaction [7]. Damaged epithelial tissue will trigger the release of inflammatory cytokines, among others Interleukin-6 (IL6) [8]. Furthermore Interleukin-6 (IL6) will trigger the increasing Transforming Growth Factor β1 (TGF β1) which causes

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the deposition of extracellular matrix or collagen by myofibroblast cells [8];[7]. [9] stated that nicotine-induced mice subcutaneously at a dose of 2.5 mg/kg/day for 1 month showed thickening of the interalveolar septum (IAS). Another study stated that nicotine-induced mice subcutaneously at a dose of 2.5 mg/kg BW (5 days a week, for 22 weeks) showed an increasing in the percentage of DNA fragmentation [10].

Increased ROS which causes a decrease in antioxidant activity in the body can be overcome by adding external antioxidants from plants, one of which is turmeric rhizome (Curcuma longa L.) [11]. The research showed that turmeric rhizome has a high antioxidant content [12]. Another study stated that curcumin in turmeric rhizome was able to inhibit damage to the lung tissue of white rats exposed to nicotine by gavage [13]. The results of research [14] showed that administration of turmeric rhizome extract (Domestic turmeric) at a dose of 0.25% as much as 1 ml/day by gavage in male wistar rats that have been exposed to cigarette smoke containing nicotine 3 mg/day (9 cigarettes) can reduce BALT hyperlasia (bronchus associated lymphoid tissue) and macrophage infiltration of the alveolar septa of the lung.

The purpose of this study was to determine the effect of turmeric rhizome extract (Curcuma longa L.) on the histological structure of the lungs including the thickness of the bronchiolar mucous layer and the thickness of the alveolar septa of mice after exposure to nicotine.

Material and Method:-

Place and time of research

This research was conducted from October to December 2020 at the Zoology Laboratory and Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember.

Tools and materials

The tools used in this study included mice cages , mice drinking bottles, mice feeding containers, sonde, surgical board, analytical scales (Ohaus balance), beaker glass, schott bottle, small plastic funnel, knife, cutting board, spatula, porcelain cup, rotary evaporator, blackstainless steel, oven, rotary microtom, blender, flakonbottles,staining jar, OptiLab, scalpel, hot plate, Olympus microscope.

The materials used in this study consisted of mice (*Mus musculus* L.) DDY Strain males aged 6 to 8 weeks with a body weight of around 20-25 grams obtained from the Center of VeterinariaFarma, Surabaya (Pusvetma) as many as 25 mice, broiler pellet feed (BR1 Plus), distilled water, corn oil, turmeric rhizome from Madura, pure nicotine (Nicotine hydrogen tartreate salt) Sigma, filter paper, glass object, cover glass, Phosphate Buffer Saline (PBS) solution, formaline 10%, NaCl 0.9%, absolute alcohol, paraffin, glycerin, albumin, xylol, Haematoxylin Eosin (HE), chloroform, N-hexane and ethyl acetate.

Preparation of Turmeric Rhizome Extract

The turmeric rhizome was peeled and washed with water, then drained. The clean rhizomes were then sliced thin and dried in an oven at 50°C for 48 hours. The dried turmeric was ground and sieved using a 60 mesh sieve until it became turmeric rhizome powder. Turmeric rhizome powder was macerated using N-hexane at a ratio of 1:5 for 24 hours and filtered using filter paper. Furthermore, the resulting supernatant was macerated using ethyl acetate with a ratio of 1:5 for 24 hours, filtered using filter paper. The maceration results are filtered using filter cloth and filter paper, then put insiderotary evaporator with a temperature of 80°C to obtain turmeric rhizome extract.

Preparation of Test Animals

The animals used in this study were mice (*Mus musculus* L.) male DDY strain aged 6-8 weeks with a body weight of 30-35 grams. Mice were placed in a cage with broiler pellets (BR 1 plus) and given drinking water ad libitum.

Animal Treatment

Mice were divided into 5 groups, namely K- (without exposure to nicotine and without administration of turmeric rhizome extract), K+ (given nicotine only), D1, D2 and D3 were exposed to nicotine followed by administration of turmeric rhizome extract at doses of 0.98, 19.6 and 29.4 mg/kg BW. Administration of nicotine is carried out intraperitonial (IP) at a dose of 2.5 mg/kg BW for 4 consecutive weeks. Nicotine is dissolved in distilled water and the volume of administration is 1 ml. After the last nicotine administration, it is continued with the administration of turmeric rhizome extract which is carried out orally (gavage) for 2 weeks. Turmeric rhizome extract was dissolved in corn oil and the volume of administration was gavage 1 ml.

Preparation of Lung Histology

Lung organ harvesting was carried out one day after the last treatment. Mice were anesthetized with chloroform and then placed on a surgical board in a supine position. The abdomen and thorax of mice were dissected, then the left lung organ was taken. The taken lung was cut 1/3 of the middle and washed using 0.9% NaCl. Preparations were made using the paraffin method, with Hematoxylin-Eosin (HE) staining.

Data analysis

The data obtained in the form of thick bronchiolar mucosa and thick alveolar septa of mice were analyzed using one way ANOVA with a confidence level of 95% or $\alpha = 0.05$. If there is a significant difference, continue with the test Duncan Multiple Range Test (DMRT) to see significant differences between treatment groups [15].

Results and Discussion:-

The results of measuring and calculating the thickness of the bronchiolar mucosa and the thickness of the alveolar septa given turmeric rhizome extract after exposure to nicotine can be seen in Table 1. Based on the test results One Way Anova obtained a significance value of $p = 0.000 < 0.05$ either in the thickness of the mucosa or the thickness of the alveolar septa. These results indicate that administration of turmeric rhizome extract has a significant effect on the average bronchiolar mucosa thickness and alveolar septa thickness after exposure to nicotine. The results of the Duncan Multiple Range Test (Table 1) after nicotine administration, showed the average bronchiolar mucosa thickness and alveolar septa thickness in the positive control (K+) group were significantly different from the negative controls (K-). This shows that administration of nicotine at a dose of 2.5 mg/kg body weight caused thickening of the bronchiolar mucosa and alveolar septa, which was thought to be due to an increase in free radicals. [9] stated that nicotine-induced mice subcutaneously at a dose of 2.5 mg/kg/day for 1 month showed thickening of the interalveolar septum (IAS). Exposure to e-cigarette smoke containing 6 mg/ml of nicotine by administering a volume of 1 ml of e-cigarette fluid for 4 weeks in male Balb'C Strain mice causes an increase in the thickness of the bronchial mucosa [16].

Table 1:- Bronchial mucosal thickness and alveolar septa after administration of turmeric rhizome extract after exposure to nicotine.

Treatment	Bronchiolar mucosa thickness (µm) (X ± SD)	Alveolar septa thickness (µm) (X± SD)
K- (without treatment)	22.44 ±1.23 [a]	16.95±0.44 [a]
K+ (Nicotine 2.5 mg/kg bw)	26.10 ±1.05 [c]	22.07±0.48 [c]
D1 (Nicotine + Turmeric Rhizome Extract 0.96 mg/kg bb)	25.04±1.49 [b]	22.69±1.32 [c]
D2 (Nicotine + Turmeric Rhizome Extract 19.6 mg/kg bb)	22.97±1.15 [ab]	22.01±0.88 [c]
D3 (Nicotine + Turmeric Rhizome Extract 29.4 mg/kg bb)	22.20±1.67 [a]	18.65±1.53 [b]

Nicotine in this study was administered intraperitoneally. Exposure to nicotine carried out intraperitoneally will enter the abdominal cavity which is carried by blood capillaries to the portahepatic vein and is carried into the liver. Nicotine will experience metabolism in the liver. In the liver nicotine will be metabolized into kotinine, nicotine glucoronid, nornikotine. Most of the nicotine will be converted into cotinine (about 80%) which has a longer half-life of around 17 hours compared to nicotine's half-life of 2-3 hours [3]. Nicotine and its metabolites are free radicals. Cotinine metabolites can form ROS in the form of peroxide anion compounds and hydrogen peroxide. An increase in free radicals will cause oxidative stress which can damage cell membranes through a series of lipid peroxidation reactions [4]. Tissue damage will stimulate the release of inflammatory cytokines such as IL6 [8]. Furthermore, IL6 will induce the expression of TGF β. The role of TGF β is to activate fibroblasts to become myofibroblasts which cause the synthesis of extracellular matrix including collagen, laminin and fibronectin [17], which plays an important role in the process of pulmonary fibrosis. Nicotine can increase the production of fibroblast proliferation and collagen synthesis by fibroblasts, causing an increase in the thickness of the bronchiolar mucosa and alveolar septa [7]. The thickness of the bronchiolar mucosa and alveolar septa can be seen in Figures 1 and 2.

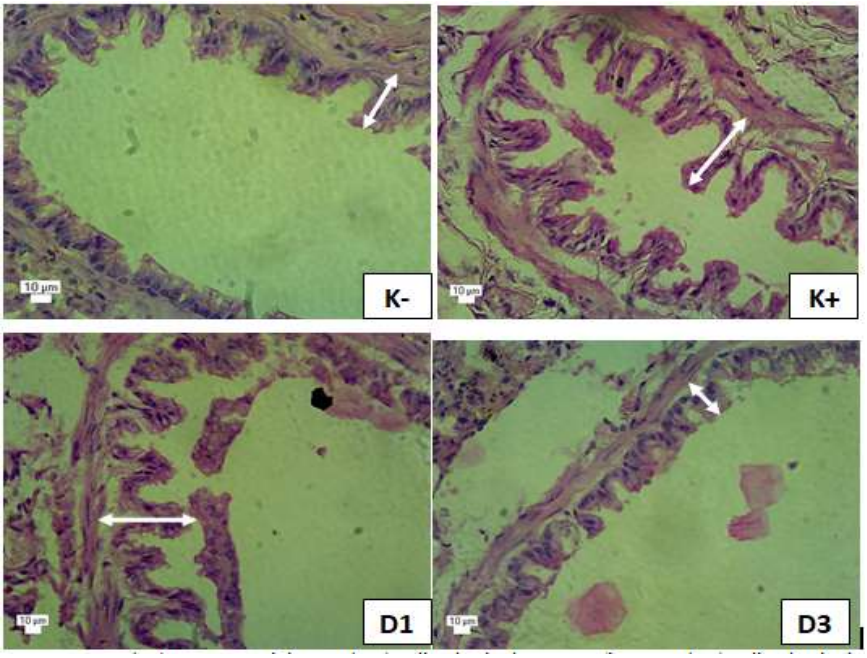

Figure 1:-The thickness of the bronchial mucosa of mice (shown by white arrows) after administration of turmeric rhizome extract. Microscope magnification 400 x. K- (without treatment); K+ (giving nicotine at a dose of 2.5 mg/kg BW); D1 (giving nicotine at a dose of 2.5 mg/kg BW + turmeric rhizome extract at a dose of 0.96 mg/kg BW); D3 (giving nicotine at a dose of 2.5 mg/kg BW + turmeric rhizome extract at a dose of 29.4 mg/kg BW).

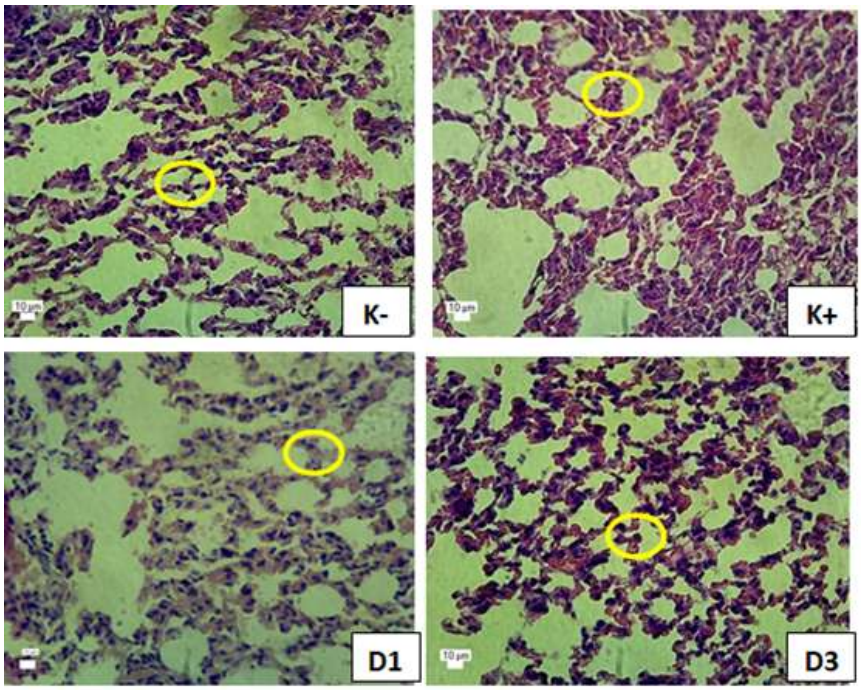

Figure 2:- Thickness of the alveolar septa of mice (shown by yellow circles) after administration of turmeric rhizome extract. Microscope magnification 400x. Description: K- (without treatment); K+ (giving nicotine at a dose of 2.5 mg/kg BW); D1 (giving nicotine at a dose of 2.5 mg/kg BW + turmeric rhizome extract at a dose of 0.96 mg/kg BW); D3 (giving nicotine at a dose of 2.5 mg/kg BW + turmeric rhizome extract at a dose of 29.4 mg/kg BW).

The thickness of the bronchiolar mucosa and the thickness of the alveolar septa after administration of turmeric rhizome extract after exposure to nicotine decreased with increasing doses of turmeric rhizome given. The thickness of the bronchiolar mucosa began to decrease when given turmeric rhizome extract at a dose of 0.96 mg/kg body weight, while the thickness of the alveolar septa decreased significantly compared to group K.+. The decrease in the thickness of the bronchiolar mucosa and the thickness of the alveolar septa is thought to be due to the extract of turmeric rhizome being able to inhibit the increase ROS (Reactive Oxygen Species) caused by nicotine.

Curcumin (diferuloylmethane) is an active component of turmeric which functions as a natural antioxidant to neutralize free radicals [18]. [19] stated that giving curcumin at a dose of 50 mg/kg BW gavage in wistar rats that were injected subcutaneously with nicotine 0.6 mg/kg BW for 2 months, it caused a decrease in MDA levels and increased levels of gluthation-S-Transferase Turmeric rhizome contains the main substance, namely curcumin, which can act as an anti-inflammatory [20]. Curcumin modulates the inflammatory response by reducing the production of tumor necrosis factor-alpha (TNF-a) cytokines, interleukins (IL-8) [21]. [13] stated that curcumininhibits cytokines and free radicals in epithelial cells, inhibits matrix production by fibroblasts, inhibits the production of ROS and TGF β by macrophages. More [22] stated that curcumin can inhibit fibroblast proliferation. Fibroblast proliferation is the cause of fibrosis. So the administration of turmeric rhizome extract in this study besides being able to ward off free radicals, is also thought to reduce inflammatory cytokines thereby reducing the thickness of the bronchial mucosa and alveolar septa.

Conclusion:-

The conclusion of this study was administration of turmeric rhizome extract at a dose of 29.4 mg/kg BW was able to reduce the thickness of the bronchiolar mucosa and the thickness of the alveolar septa of mice after exposure to nicotine.

References:-

1. Aji, A., Maulinda. L, Amin, S. 2017. Isolation of nicotine from cigarette butts as an insecticide.Unimal Chemical Technology Journal. 4(1): 100-120.
2. Mishra, A., Chaturvedi, P., Datta, S., Sinukumar, S., Joshi, P, dan Garg, A. 2015. Harmful effects of nicotine. Indian Journal of Medical and Paediatric Oncologu. 36(1): 24–31.
3. Benowitz, N. L. 1996. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiologic Reviews. 18(2): 190-191.
4. Berawi, K.N, andTheodora, A. 2017. The effect of physical activity on the process of forming free radicals as a risk factor for atherosclerosis.Majority. 6(2): 85-90.
5. Ardhie, A. M. 2011. Free radicals and the role of antioxidants in prevent aging. Medicinus Scientific Journal of Pharmaceutical Development and Medical Application. 24(1): 4-9.
6. Suryadinata, R. V. 2018. The effect of free radicals on the inflammatory process in chronic obstructive pulmonary disease (COPD).Amerta Nutr. 317–324.
7. Jensen , K. , Damir , N. , Micheleine , G. , Syeda , A. , David , D. , Shannon , G. , 2012 .General mechanisms of nicotine-induced fibrogenesis. The FASEB Journal. Vol: 26.
8. Shochet, G.E., Brook, E., Bardenstein, W. B., Shitrit, D. 2020. TGF-β pathway activation by idiopathic pulmonary fibrosis (IPF) fibroblast derived soluble factors is mediated by IL-6 trans-signaling. Respiratory Research. 21:26
9. Zaghloul, D., Esam, K., Hekmat, O. Abdul, A., dan Mohammed, M. 2012. The possible protective role of curcumin on the toxic effect of nicotine in the lung of adult male mice: histological study. The Egyptian Journal of Histology. Vol. 35: 805-811.
10. Anany, M. G. E. A., Azza, M. K., and Kamla, E. S. 2015. Effects of curcumin and/or quercetin on nicotine-induced lung and liver toxicity in adult Male albino rat. Al-Azhar assiut Medical Journal. 13(2): 93-102.
11. Suprihatin, T., Rahayu, S., Rifa'i,M & Widyarti, S. 2020. Compounds in Turmeric Rhizome Powder (Curcuma longa L.) which have Potential as Antioxidants. Buletin Anatomi dan Fisiologi. Volume 5 Nomor 1 Februari 2020:35-42
12. Tsai, S., Huang, S., Chyau, C., Tsai, C., Weng, C., and Mau, J. 2011. Composition and antioxidant properties of essential oils from Curcuma rhizome. Asian J. Arts Scie. 2: 57-66.
13. Venkatesan, N., Durairaj, P., dan Mary, B. 2007. Protection from acute and chronic lung disease by curcumin in: the molecular targets and therapeutic uses of curcumin in health and disease. Journal of advance Experimental Medicine Biology. Vol. 595: 379-405.

14. Rahmad, L., Nida, U. M., Aagapietta, K., Novi, H., Kurniati, S., Bambang, Sutrisno., Hastari, W., and R. Wasito. 2015. The effect of giving turmeric rhizome extract (Curcuma domestica Val.) on histopathological features of the lungs induced by cigarette smoke in white Wistar rats. Journal of Veterinary Science. 33(1): 75-84.
15. Sugiyono. 2007. Statistika untuk Penelitian. Penerbit ALFABETA: Bandung.
16. Utami. E, T., Bella, D. A., Mahriani., Susantin. F. 2020. Effect of exposure to e-cigarette smoke on lung histology of male balb/c mice (Mus musculus L.). Bioeduscience: Journal of Biology & Science Education. 4(2): 129-135.
17. Saito, A., Masafumi, H., Takahide, N. 2018.TGF-β signaling in lung health and deasease. International Journal of Molecular Sciences. 19: 2460.
18. Shan, C.Y. dan Iskandar Y. 2018. Studi kandungan kimia dan aktivitas farmakologi tanaman kunyit (Curcuma longa L.). Farmaka. 16(2): 547- 555.
19. Thejomoorthy, M. 2017 Defensive role of indian space curcumin against nicotine induced damage on lung tissue in male albino rat. Asian Journal of Science and Technology. Vol. 08 (11): 6615-6623.
20. Labban, L. 2014. Medicinal and pharmacological properties of Turmeri (Turmeric is long): A review. International Journal Pharm Biomed Science.5(1):17-23.
21. Nasser, G. A. 2020. Turmeric as an anti-inflammatory agent. Wellness And Healthy Magazine, 2(1), 147-158.
22. Smith, M. R., Gangireddy, S.R., Narala, V.R., Hogaboam, C.M., Standiford, T.J., Christensen, P.J., Kondapi, A.K., dan Reddy, R.C., 2010. Curcumin inhibits fibrosis-related effects in IPF fibroblasts and in mice following bleomycin-induced lung injury. American journal of physiology-lung cellular and molecular physiology. 298(5): L616-L625.