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THE EFFECT OF GREEN TEA (*Camellia sinensis*) EXTRACT ON THE THICKNESS OF BRONCHIAL EPITHELIUM AND PULMONARY ARTERY WALL OF MICE (*Mus musculus*) EXPOSED TO CIGARETTE SMOKE

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Abstract

The study aims to investigate the impact of green tea extract on the thickness of bronchial epithelium and pulmonary artery walls in mice lungs exposed to cigarette smoke. Twenty-five 12-week-old mice (Mus musculus) weighing between 20-25 grams were used in the experiment. The control group (K(-)) received no cigarette smoke exposure and was administered 0.5 mL of 1% Na-CMC, while the positive control group (K(+)) was exposed to cigarette smoke without green tea leaf extract and received 0.5 ml of 1% Na-CMC. Groups exposed to cigarette smoke (P1, P2, and P3) were given green tea extract at doses of 20 mg/kgBW, 40 mg/kgBW, and 60 mg/kgBW respectively, each in a daily dose of 0.5 ml. Mice were exposed to one cigarette per group per day. The findings indicate that administering green tea extract has the potential to reduce the thickening of both the bronchial epithelium and pulmonary artery walls, with the optimal dose being 40 mg/kgBW. ANOVA analysis revealed significant differences in the thickness of the bronchial epithelium and pulmonary artery walls among the groups ($p < 0.05$). This study demonstrates that administering green tea extract suspension (Camellia sinensis) can effectively mitigate the thickening of the bronchial epithelium and pulmonary artery walls in mice lungs exposed to cigarette smoke..

Keywords: *Cigarette smoke, green tea extract, Mus musculus, thickening bronchial epithelial, thickening pulmonary artery wall.*

INTRODUCTION

Smoking poses significant health risks due to the presence of over 4000 harmful substances in cigarette smoke, including tar, nicotine, carbon monoxide, and polycyclic aromatic hydrocarbons (Mallah et al., 2023). The lungs, being regularly exposed to the free radicals in cigarette smoke, undergo structural and functional changes in the airways and lung tissue. The accumulation of mucus in bronchial tubes leads to inflammation and thickening of the bronchial epithelium (Suryadinata, 2018). This thickening involves the infiltration of macrophages or neutrophils and adaptive inflammatory immune cells, forming lymphoid follicles. Macrophage infiltration and goblet cell metaplasia contribute to bronchial epithelial thickening, causing airway resistance and obstruction (Thorley & Tetley, 2007). Nicotine in cigarette smoke also negatively impacts blood vessels, increasing blood pressure, heart rate, and vessel constriction due to thickened vessel walls (Leone & Landini, 2013).

Efforts to mitigate the adverse effects of cigarette smoke include exploring herbal remedies capable of combating smoke-induced oxidative stress. Green tea, known for its antioxidant properties, is one such herbal remedy. It contains catechin antioxidants with potent scavenging abilities, surpassing those of

vitamins C and E (Sajida et al., 2020). Regular consumption of green tea can enhance the body's defense system and organ function due to its high polyphenol content, which ranges from 30-40% in green tea leaves compared to 3-10% in black tea leaves (Zowail et al., 2009). This study aims to investigate the impact of green tea on the thickness of bronchial epithelium and pulmonary artery walls in the lungs of mice exposed to cigarette smoke.

MATERIALS AND METHODS

The research protocol was reviewed and approved by the Animal Care and Use Committee of Universitas Airlangga, Surabaya, Indonesia, under approval No. 324/HRECC.FORM/IV/2020. This study utilized 25 healthy male mice (*Mus musculus*), aged 12 weeks and weighing between 20-25 grams. These mice had not previously been subjected to any research activities. They were housed in cages with sawdust bedding and provided with ad libitum access to pellet food and drinking water.

Green Tea Leaf Extraction

Green tea leaves (*Camellia sinensis*) were sourced from the Wonosari tea garden located in Malang Regency, East Java, Indonesia. The extraction process utilized the maceration method employing ethanol solvent. One kilogram of green tea leaf powder was immersed in eight liters of 96% ethanol for a duration of three days. Subsequently, the macerated mixture was subjected to evaporation using a rotary evaporator set at 50˚C and 45 rpm for a duration of 5 hours, resulting in the production of a concentrated extract. This extract was then freeze-dried according to the procedure outlined by Khoirunnisa et al. (2019). The obtained extract powder was dissolved in 1% Na-CMC solution and adjusted accordingly to achieve the desired dosages.

Treatment of animals

The mice underwent a one-week acclimatization period in cages before commencing the 36-day treatment regimen. A total of twenty-five mice (*Mus musculus*) were randomly assigned to five groups. The K- group served as the negative control, where mice were neither exposed to cigarette smoke nor administered green tea extract (GTE), receiving only 0.5 mL of 1% Na-CMC. The K+, P1, P2, and P3 groups were exposed to cigarette smoke and received doses of 0, 20, 40, and 60 mg/kg body weight of GTE, respectively. GTE was orally administered to each mouse using a gastric probe at a dosage of 0.5 mL/mouse/day. Each group was put into specialized exposure chamber (31 x 19 x 22 cm) equipped with two openings for smoke input and output to be exposed to smoke from cigarette at nicotine dose of 2.2 mg for 20 minutes (Fadhilah et al., 2023). Cigarette smoke was generated from a single clove cigarette per day, as outlined in the study by Morales-Mantilla et al. (2020). On day 37, all mice were euthanized via cervical dislocation, and their lungs were harvested. The lung tissues were then fixed in a 10% formalin solution for subsequent histopathological examination using hematoxylin and eosin staining. Histopathological analysis of the lung tissues was performed utilizing a Nikon Eclipse microscope at 100x magnification.

Histopathological Evaluation

The thickness of the bronchial epithelium was assessed by examining histopathological preparations of mouse lung tissue stained with Hematoxylin and Eosin (HE) at a magnification of 100x. Measurement involved determining the distance from the tip of the cilia to the basement membrane. Five visual fields within one bronchial lumen were observed in each lung preparation to ensure comprehensive assessment. Similarly, the pulmonary artery wall thickness in mice was evaluated using HE-stained lung tissue preparations at a magnification of 100x. The measurement spanned from the tunica intima to the tunica adventitia to capture the entire arterial wall thickness (Zhao *et al*., 2014).

Data Analysis

Statistical analysis of the collected data was conducted using ANOVA, followed by Duncan's multiple range test for comparison between groups. The analysis was carried out using the Statistical Program and Service Solution (SPSS) version 23 software for Windows.

RESULT

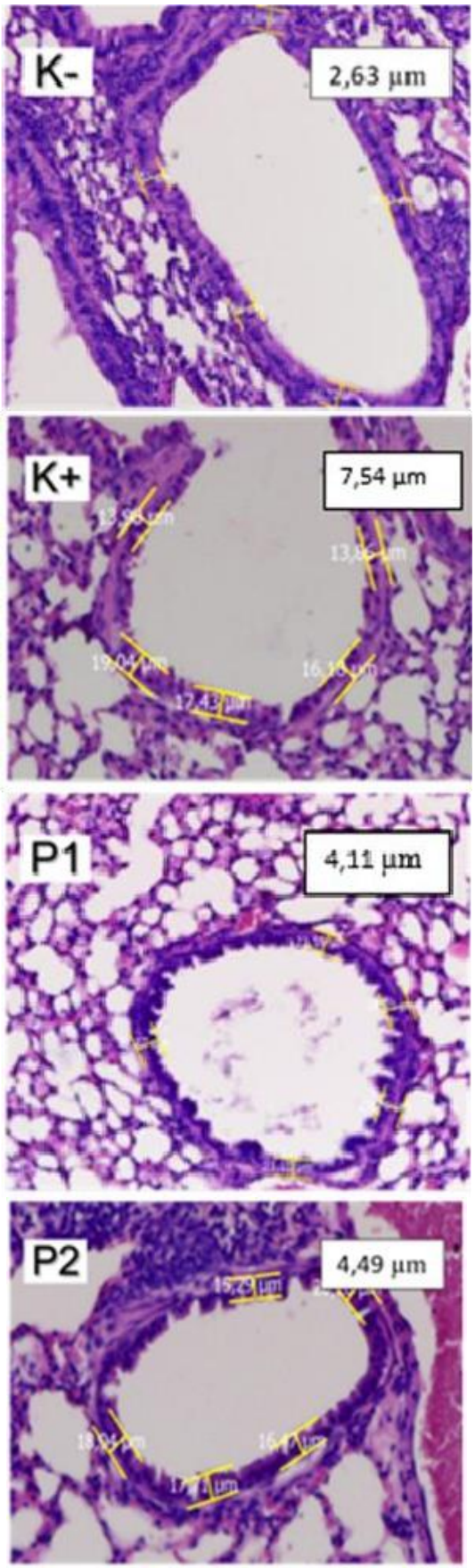
Table 1. Mean and Standard Deviation of Green Tea Extract (*Camellia sinensis*) Administration to Thickness of Bronchial Epithel and Pulmonary Artery Walls of Mice (*Mus Musculus*) Exposed to Cigarette Smoke

Treatment Group	Bronchial Epithel Thickness (µm) (Mean ± SD)	Pulmonary Artery Wall Thickness (µm) (Mean ± SD)
K-	18,18^{a} ± 1,28	11,83^{a} ± 1,68
K+	35,65^{d} ± 1,51	23,84^{d} ± 1,61
P1	27,91^{c} ± 2,27	18,81^{c} ± 1,48
P2	20,77^{b} ± 1,52	13,86^{b} ± 1,58
P3	22,45^{b} ± 1,49	15,42^{b} ± 1,23

Note: Differences in superscripts a, b, c, d in the same column indicate significant differences ($p<0.05$).

From Table 1, it can be observed that the K- group, which represents the normal mice group, has the lowest thickness of bronchial epithelium and pulmonary artery wall, with significant differences compared to the K+ group exposed to cigarette smoke. The results indicate that in the mice of the K+ group exposed to cigarette smoke, there is the highest thickness of bronchial epithelium and pulmonary artery wall, significantly different from all treatment groups P1, P2, and P3.

Comparison of the average thickness of bronchial epithelium and pulmonary artery wall between groups P1, P2, and P3 exposed to cigarette smoke and green tea extract at different doses shows significant differences. P2, with the administration of green tea extract at a dose of 40mg/kg BW, shows measurement results similar to the normal group (K-). The histopathological depiction of bronchial epithelium thickness among treatments can be seen in Figure 1, and the histopathological depiction of pulmonary artery wall thickness can be seen in Figure 2.



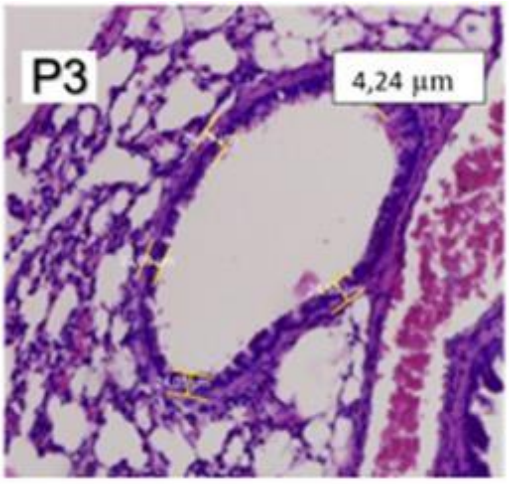
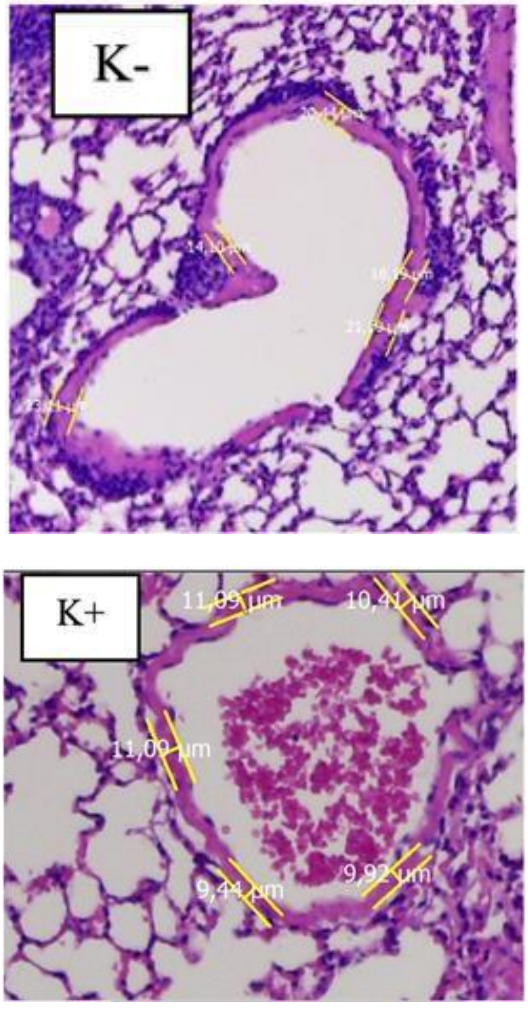


Figure 1. Histopathological image of lung showing bronchial epithelium thickness; K-: normal mice; K+: mice exposed to cigarette smoke; P1, P2, P3: mice exposed to cigarette smoke + green tea extract suspension doses of 20, 40, and 60 mg/kg BW; Hematoxylin Eosin (HE) staining, magnification 100x.



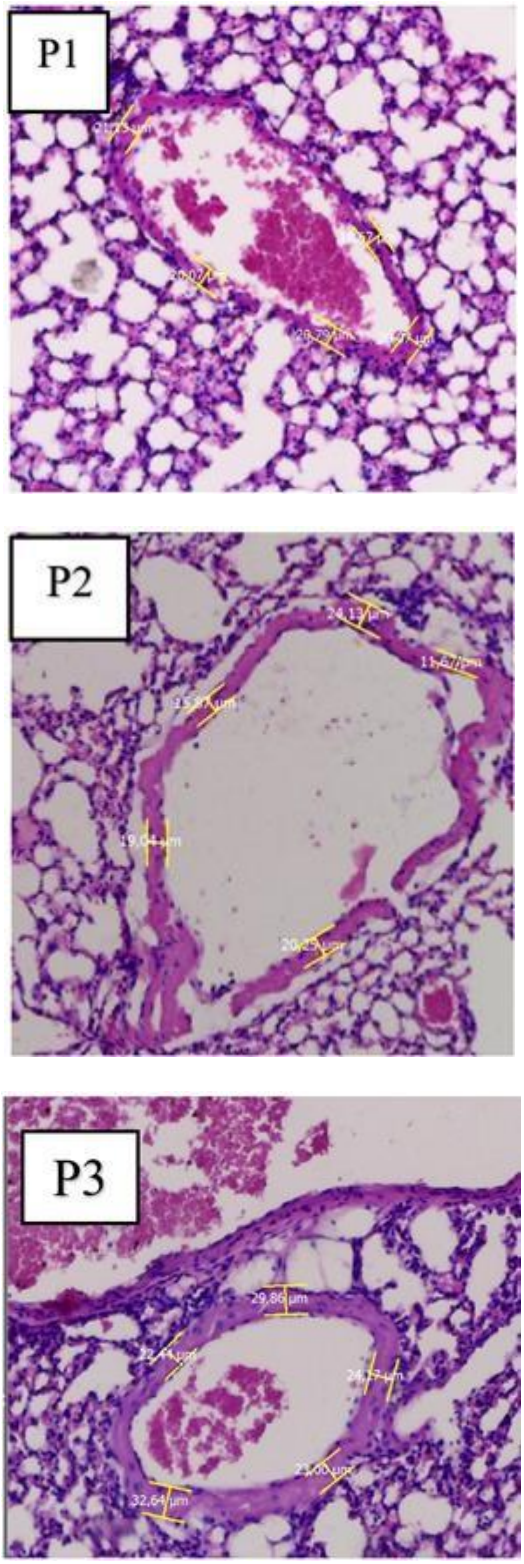


Figure 2. Histopathological image of lung showing pulmonary artery wall thickness; K-: normal mice; K+: mice exposed to cigarette smoke; P1, P2, P3: mice exposed to cigarette smoke + green tea extract suspension doses of 20, 40, and 60 mg/kg BW; Hematoxylin Eosin (HE) staining, magnification 100x.

DISCUSSION

Thickening of the bronchial epithelium by exposure to cigarette smoke occurs through increase the oxidative stress and reduced levels of glutathione with decreased glutathione peroxidase and glucose-6 phosphate dehydrogenase activity in alveolar cell type II, erythrocytes, and lung epithelial lining fluid. Endogenous antioxidant systems can no longer maintain cellular homeostasis due to exposure Very strong and long-lasting oxidants can produce conditions of oxidative stress, a number of pro-inflammatory pathways become active and result in chronic inflammation and tissue injury (Cipollina et al, 2022). The results in mice in the K+ group exposed to cigarette smoke showed the highest bronchial epithelial thickness. The K+ group showed a difference from the treatment groups P1, P2, and P3 which exposed to cigarette smoke and green tea extract doses of 20, 40 and 60 mg/kg BW. This shows that giving green tea extract at different doses can reduce the thickening of the bronchial epithelium due to exposure to cigarette smoke and giving a dose of 40 mg/kgBB shows the best results and approaches the group of normal mice.

Thickening of the pulmonary artery walls by exposure to cigarette smoke occurs through blockage, clotting of blood vessels and arteries and reduced capacity of extracellular and intracellular antioxidants which can increase phagocytes in the capillary walls due to chemicals in cigarette smoke (Alqarni et al., 2022). From the results of the study, showed differences between treatment groups. The group that was not exposed to cigarette smoke and green tea extract which was the group of normal mice had the lowest pulmonary artery wall thickness and was different from the group exposed to cigarette smoke but not given green tea extract. These results prove that exposure to cigarette smoke can cause an increase in the thickness of the pulmonary artery wall.

The results in the K+ group mice exposed to cigarette smoke showed the highest pulmonary artery wall thickness. The K+ group showed a difference from the treatment group P1, P2, and P3 which exposed to cigarette smoke and green tea extract doses of 20, 40 and 60 mg/kg BW. This shows that giving green tea extract at different doses can reduce pulmonary artery wall thickening due to exposure to cigarette smoke and giving a dose of 40 mg/kg BB in the P2 group showed the best results and approached the K- group which was a group of normal mice.

Green tea extract contains various bioactive compounds, including catechins and pholyphenol which are potent antioxidants. These antioxidants can effectively neutralize free radicals generated by cigarette smoke due to their ability to donate electrons and stabilize these reactive molecules (Sajida et al., 2020).

Cigarette smoke contains a plethora of harmful substances, including free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS). These radicals can cause oxidative damage to cells, DNA, proteins, and lipids, leading to inflammation, tissue injury, and various diseases of lungs (Wawryk-Gawda et al., 2020).

The catechins present in green tea extract, particularly epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), and epicatechin (EC), have been shown to scavenge free radicals effectively. They do so by donating hydrogen atoms or electrons to neutralize these radicals, thereby preventing them from causing oxidative damage to cellular components. Furthermore, green tea catechins can also upregulate endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which further enhances the antioxidant defense system of cells (Al-Anshori et al, 2022).

Moreover, the polyphenols in green tea extract have anti-inflammatory properties, which further contribute to protecting lung tissue from damage caused by cigarette smoke. These anti-inflammatory effects help to mitigate the inflammatory response triggered by oxidative stress, thereby reducing tissue inflammation and injury (Fu et al., 2022).

COCLUSION

The findings of this research indicate that administering green tea extract *(Camellia sinensis*) can effectively decrease the thickening of both bronchial epithelium and pulmonary artery walls in mice (*Mus musculus*) exposed to cigarette smoke, with the optimal dosage observed at 40 mg/kg

BW.

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