Anti Inflammatory Action of Allium Sativum Ethanol Extract to Prevent Lung Damage in Smoker Rat Model

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ABSTRACT

Background: Smoking is the leading cause of death in worldwide and is known as one of the risk factors in the development and pathogenesis of several diseases and most are respiratory and cardiovascular diseases. Secondhand smoke (SHS) exposure is associated with negative health consequences including respiratory tract infection, asthma, and cancer. One of the pathogenesis that has known to cause these diseases is inflammation. Garlic (Allium sativum) is a medicinal herb that contains Allicin and other active constituents that are known to have anti-inflammatory ability by suppressing the expression and production of proinflammatory cytokines that will cause inflammation. Objective: The aim of this study is; to analyze the anti-inflammatory action of Allium sativum ethanol extract to prevent lung damage in the smoker rat model. Methods: This is a case-control study with five groups of rats each group contains of three rats. The five groups were negative control (KN), 10 days (10d) smoker (K1), 20 days (20d) smoker (K2), 20d smoker treated with Allium sativum for 10 days (K3) and 20d smoker treated with Allium sativum for 20 days (K4). After 20 days all animals were sacrificed and histological preparation of lung organs was observed under a microscope with 100 dan 400 times magnification and then captured by photomicrograph for analyzed. Results: There were improvements in lung structure both in group K3 and K4. There was a decrease of leucocytes and inflammatory cells infiltration that covered almost all alveolar surface to 10-20% surface area and the dilated alveoli decrease from more than 50% to less than 30% area. The bronchus was clean in both two groups compared to the groups that were not treated with Allium sativum Conclusion: This study shows that Allium sativum ethanol extract has the ability to prevent lung damage in the smoker rat model.

Keywords: Allium sativum, anti inflammatory, lung damage, smoker rat model.

1. BACKGROUND

Cigarette smoking becomes a worldwide health problem that has known as a major cause of death. It causes various diseases but the most common diseases that increase mortalities are respiratory and cardiovascular diseases (1). Cigarette smoking alters lung function and affects other systemic functions causing smoking-related death. In the United States according to Surgeon General’s report in 2014, cigarette smoking cause >480,000 deaths per year. In Indonesia reported by World Health Organization (WHO) for South-East Asia in 2018 cigarette smoking caused >200,000 deaths per year (2,3). Exposure to secondhand smoke (SHS) is also very dangerous and can cause many health problems that lead to death as well, it is because the SHS contained toxins that are inhaled by secondhand smokers is not filtered so that the amount of hazardous substances are greater in secondhand smokers than active smokers (4,5). SHS can cause sudden death syndrome, respiratory and ear infection in infants and children (4). Despite all the carcinogenic materials contained in a cigarette, some studies have reported the relationship between cancer and SHS, it is recorded that one of the risk factor of cancer is SHS. Previous study conducted on animals reported SHS causes lung and nasal cancer (6).

It has been reported by many studies that cigarette smoking can cause chronic inflammation and autoimmunity on a systemic level by secreting pro-inflammatory and anti-inflammatory cytokines. Previous studies have shown that cigarette smoking has an impact on lymphocytes, especially T cell.
cells, and their function in secreting proinflammatory mediators (7). There is also evidence showing that cigarette smoking affects airflow, vascular, and immune functions. Hazardous materials contained in cigarette smoking have the potential to cause endothelial cell injury. It has long been known that the lung endothelium is not only a passive barrier used for gas exchange but is also metabolically active and plays a key role in mediating inflammation (2). Particulate matter inhaled during inspiration increases reactive oxygen species in lung tissues, resulting in oxidative stress and an increase in inflammatory cells in both lung tissues and bronchoalveolar lavage fluid (BALF), as well as an increase in the concentration of inflammatory mediators IL-1, IL-6, IL-8, and MMP-9 in BALF (8). Previous studies analyzing the microscopic changes of the lungs in rats have shown that there is increased activity of inflammatory cells after exposure to cigarette smoke, and there are also changes in lung histopathology in almost all parts of the lungs, which are the ductus alveolary, saccus alveolary, and the alveoli, caused by the inflammation reaction (9,10,11).

Garlic (Allium sativum), an Alliaceae family member, is the oldest cultivated herb and has been used for over 10,000 years. It is not only used for culinary purposes but also as therapy for several diseases; it contains allicin, which has a higher concentration of sulfur compounds, and other active compounds such as diallyl disulfide, S-allylcysteine, and diallyl trisulfide, which have anti-inflammatory properties (12-14).

Allium sativum plays a key role in protecting tissue from tissue injury and various diseases through its antioxidant and anti-inflammatory abilities. A previous study aimed to analyze the hepatoprotective mechanism of Allium sativum and recorded a significant increase in antioxidant enzymes including superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) in the group treated with Allium sativum extract compared to the CCL4-induced group (14). Many previous studies have also documented Allium sativum’s anti-inflammatory ability by observing a significant decrease in inflammatory markers such as TNF-, IL-6, IL-1, CRP, and ICAM1 in any groups that were treated with Allium sativum (14,15,16,17,18). It has also been reported that nuclear factor κB (NF-κB), which is known to be associated with inflammatory reactions, was increased in the kidney cortex of diabetic-induced animals, followed by its inhibitor (I-κB); additionally, NF-κB was decreased and I-κB was prevented from decreasing in groups treated with Allicin (16).

In other research, garlic has shown the ability to reduce nitric oxide (NO) and prostaglandin E2 (PGE2) production by decreasing the expression of inducible NO synthase [iNOS] and cyclooxygenase-2 [COX2] (19). Various diseases will cause tissue injury; they are related to inflammation reactions. Many types of therapeutics, including various drugs and herbal medicines, have been used to prevent this reaction. For example, garlic has shown its anti-inflammatory ability in many studies by suppressing the proinflammatory cytokines.

2. OBJECTIVE
This study aimed to analyze the anti-inflammatory effect of Allium sativum to prevent the lung damage caused by inflammation reaction on smoker rat model.

3. MATERIAL AND METHODS

Subjects
The research conducted was a case-control study, the subjects were 15 healthy rats (Rattus norvegicus, sp.) aged 8 weeks and weighing 180–200 g, divided into 5 groups, and kept under standard conditions. The materials used were unfiltered Marlboro cigarettes with 2.4 mg of nicotine per cigarette, Allium sativum, and a smoking chamber. The identification of Allium sativum was performed at the Badan Riset dan Inovasi Nasional (BRIN) laboratory in Jakarta, Indonesia and the extraction was done using the maceration technique with 96% ethanol (20).

Rats were placed in cages, and water and standard pellet diets were available throughout the experiment period. 15 rats were divided into 5 groups: the first group was KN untreated animals (negative control), the second was K1 animals exposed with cigarette smoke for 10 days (10-day smoker), the third was K2 animals exposed with cigarette smoke for 20 days (20-day smoker), the fourth was K3 animals exposed with cigarette smoke for 20 days and treated with Allium sativum 0.1 g per day for 10 days, and the fifth was K4 animals exposed with cigarette smoke for 20 days and treated with Allium sativum 0.1 g per day for 20 days. Cigarette smoke was exposed twice per day, every morning and afternoon, for 30 minutes each (10), and Allium sativum was given 0.1 g per day (21). After being treated for 20 days, all animals, including the control, were sacrificed with ketamine injections of 30 mg/IP, and the lung organs were taken to be examined.

Procedure and ethical considerations
This research received ethical approval from the Health Research Ethical Committee of Universitas Sumatera Utara Medan Indonesia (No. 59/KEPK/USU/2022).

Histopathology
After being treated for 20 days, all animals, including the control, were sacrificed with ketamine injections of 30 mg/IP, and the lung organs were taken, and histological preparation was done with histochemical staining, haematoxylin eosin (HE) (22).

Image Acquisition
Histopathological preparations from each group were examined using microscopic images captured with an Olympus BX43 microscope linked to a computer system. Microscopic images were taken between 100 and 400 times magnification on 10 different areas; all images were saved in TIF format.

Image Analysis
Image analysis was performed using ImageJ Analysis software downloaded from the internet with version ImageJ1.48 (NIH, Bethesda, Maryland) (Java 1.8.9 66). Image analysis is done by segmenting the part to be analyzed for intensity.
4. RESULTS

The analysis of photomicrographs from group KN revealed that the lung structure was not damaged. The bronchus was clean, the size of the alveoli was normal, and no inflammatory cells had infiltrated the bronchus or the alveoli (Figure 1A). In group K1, there were changes in lung structure. The bronchus was clean, but there was inflammatory cell infiltration in the alveoli. Alveoli are dilated in more than 50% of areas, and lymphocytes and polymorphonuclear (PMN) cells were increased (Figure 1B). K2, as there was inflammatory cell infiltration in both the bronchi and the alveoli in group well as an increase in lymphocytes and a decrease in PMN. Alveoli were not clearly visible because they were covered with diffuse inflammatory cells (Figure 1C).

In group K3, which was a 20-day smoker treated with Allium sativum for 10 days, the analysis of the photomicrograph showed that there were improvements in lung structure. The bronchus was clean; lymphocytes and inflammatory cells were reduced, followed by a reduction in inflammatory cell infiltration in the alveoli, which were reduced to 20% of their original size. Some alveoli remain dilated, but in less than 50% of cases (Figure 1D).

In group K4, which was a 20-day smoker treated with Allium sativum for 20 days, the analysis of the photomicrograph showed there were better improvements in lung structure. The bronchus was clean; there was a decrease in lymphocytes and inflammatory cells, which was followed by a decrease in inflammatory cell infiltration in the alveoli, which was decreased to 10% of the area and the dilated alveoli to 30% of the area (Figure 1E).

5. DISCUSSION

The analysis of the photomicrographs of group KN, K1 and K2 show that cigarette smoke exposure causes structural damage to the lung, and the longer the exposure, the greater the damage. The severity is directly proportional to the duration of exposure. It is well known that cigarette smoke exposure will cause changes in the production of many inflammatory mediators, both pro-inflammatory cytokines and anti-inflammatory mediators (Qiu, 2017). Research conducted on rats exposed to cigarette smoke at 10 cigarettes per head per day for 28 days showed lung damage in almost all parts of the alveoli, both in the alveolar ducts and in the alveolar sacs (11). Previous research has found that prolonged exposure to cigarette smoke causes inflammation. Innate and adaptive immune responses will both be activated and cause the entry of inflammatory cells (macrophages, neutrophils, T and B lymphocytes), the secretion of inflammatory mediators, and the production of growth factors (23). Exposure to cigarette smoke of 10 cigarettes per head per day on rats for 20, 40, and 60 days has been shown to cause lung damage, with a decrease in wet weight of the lungs at 20 days, followed by accumulation of black particles at 40 days. an increase in the number of inflammatory cells and enlargement of the alveoli on exposure for 60 days (9). In a study conducted on rats exposed to cigarette smoke containing 2.4 mg of nicotine in a smoking chamber for 30 minutes, twice a day for 35 days, an increase in the number of alveolar macrophages, widening of the alveolar lumen, thickening of the alveolar walls, and lymphocyte infiltration were found (10). The situation arises due to a recurring inflammation reaction. Exposure to cigarette smoke causes a repeated inflammation reaction, thus activating the immune system continuously. Cigarette smoke pollutants, which are antigens, will pass through the respiratory membrane between the alveoli and pulmonary
capillaries (23). These antigens will be recognized by innate immune system receptors involving neutrophils and alveolar macrophages, which then initiate inflammatory signaling cascades via activation of nuclear factor kappa B (NF-κB). TNF-α and its receptors, interleukin (IL)-1, IL-6, and IL-8, and granulosa cell-stimulating factor (G-CSF) colonies will all increase in response to such activation. This initiates the formation of reactive oxygen species (ROS) to mediate subsequent innate and adaptive immune responses (24).

The analysis of the photomicrographs of group K3 and K4 show improvements in lung structure and less damage to the alveoli compared to the groups that were not treated with Allium sativum, and the longer the treatment, the better the lung improvements and the less damage. It demonstrates that Allium sativum has anti-inflammatory properties. A previous study has proven the anti-inflammatory effect of Allium sativum by oral administration of Allium sativum, which caused an increase in Th2 response induced by increased production of IL-4 in spleen lymphocytes in treated rats (25). The effect of Allium sativum extract on Peyer’s patches increase in IFN-α and IL-4 while decreasing IL-2. Another study found an increase in IL-10, which became a negative feedback on IL-6 and IL-4 while decreasing IL-2. Another study found an increase in IL-10, which became a negative feedback on IL-6 and TNF (26). Many previous studies documented Allium sativum’s anti-inflammatory ability by observing a significant decrease in inflammatory markers such as TNF-α, IL-6, IL-1, CRP, and ICAM1 in any groups treated with Allium sativum (14-18). Allicin, the active substance in Allium sativum’s anti-inflammatory ability. Allicin has also been shown to inhibit the signaling pathways of transforming growth factor (TGF)-1 and PERK ½ (16).

6. CONCLUSION
It is clear that prolonged exposure to cigarette smoke produces structural damage to the lungs, with the severity of the damage being correlated with the exposure time. In a rat smoker model, Allium sativum ethanol extract can prevent lung damage, and the benefits to the lungs increase in direct proportion to the length of the treatment.

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