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Potential of shallot peels as a daily antioxidant supplement against cigarette smoke-induced lung damage

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ABSTRACT

Background: Cigarette smoking damages the alveoli through oxidative stress. Shallot peels containing flavonoids, especially quercetin, potentially serve as a daily antioxidant supplement to impede lung tissue damage induced by cigarette smoke. However, the maximum effective dose is yet to be determined.
Objective: This research was designed to establish the maximum effective dose of shallot peel infusion (SPI) to prevent oxidative stress and histopathological lung damage induced by cigarette smoke.
Methods: This experimental laboratory was a posttest-only control group design. A total of 24 male *Rattus norvegicus* Wistar strain, were allocated into 6 groups: a control group and 5 SPI-treated groups. All rats were exposed to 2 cigarettes/day and were treated for 28 days with aquabidest and different doses of SPI (0 mg/kgBW; 125 mg/kgBW; 250 mg/kgBW; 500 mg/kgBW; 1,000 mg/kgBW; and 2,000 mg/kgBW). The level of oxidative stress in serum was measured malondialdehyde (MDA) level with ELISA, and histopathological lung damage was estimated using the lung histopathological damage scoring method assessing inflammatory cells, alveolus lumen and inter-alveoli junction.
Results: The quadratic regression analysis revealed the maximum effective dose of SPI to prevent oxidative stress and lung damage was 1,435 mg/kgBW and 1,206 mg/kgBW, respectively. In the histopathological examination of the lungs, the administration of SPI up to a dose of 1,206 mg/kg BW prevents the inflammatory process caused by cigarette smoke, which is indicated by the number of inflammatory cells, the thickness of the alveolar septum, and the increasingly normal shape of the alveolar lumen.
Conclusion: The SPI doses of less than 1,206 mg/kgBW are safe and effective daily antioxidant supplements in rats exposed to cigarette smoke and have the potential to be further studied for application in humans.

INTRODUCTION

The incidence of smoking-related respiratory diseases remains high and potentially increases with the growing number of smokers. The World Health Organization (WHO) reports that smoking causes the deaths of 8,000,000 people per year, including 60,000 infants as passive smokers due to lower respiratory tract infections.[1] Passive smokers inhale toxic chemicals and oxidants into lung tissue, inhibiting the lung's repair mechanism. Passive smokers are exposed to chemicals 50 times higher than active smokers.[2]

Indonesia is the third country with the highest number of smokers worldwide (65 million smokers), following China and India.1 It has been reported that 35% of women are non-smokers, 33% of men are non-smokers, and 40% of children worldwide are passive smokers.[3] Both actively and passively, smoking has been proven to cause gradual lung damage, especially to alveolar

structures. Despite the absence of symptoms, lung tissue damage is inevitable.[2]

Cigarette smoke contains free radicals; continuous inhalation can disrupt the body's antioxidant defense, triggering oxidative stress. One parameter of oxidative stress is malondialdehyde (MDA). Malondialdehyde is a degradation product of lipid peroxidation that significantly increases in smokers. Oxidative stress conditions cause inflammation, including the accumulation of neutrophils, macrophages, and lymphocytes in the respiratory tract and lungs. This inflammation develops into a complex, involving lung tissue damage and remodeling, as well as airway narrowing, indicative of Chronic Obstructive Pulmonary Disease (COPD), the third leading cause of death worldwide according to the Global Burden of Disease Study.[4,5]

Efforts to neutralize oxidative stress immediately after exposure to cigarette smoke are critical steps in preventing lung damage. One approach is through a high intake of antioxidants that can be consumed daily, possibly in functional beverages with ingredients easily accessible to the public. The shallot peel, a household waste, is a natural source of high antioxidant flavonoids, especially quercetin, and can potentially be a daily antioxidant supplement in preventing lung tissue damage due to cigarette smoke.[6]

Red shallot peel extract for seven days could repair gastric damage caused by toxic doses of mefenamic acid.[7] Shallot peel extract neutralized hepatic oxidative stress in rats induced by diazinon.[8] However, research on shallot peels potential against the harmful effects of cigarette smoke on health, especially lung diseases, which remain a health concern to date, is yet to be conducted. Determining the maximum effective dose is necessary to ensure its safety for daily consumption.

Therefore, research on the potential of shallot peels to prevent oxidative stress and lung damage due to cigarette smoke is focused on the maximum effective dose that needs to be conducted. This study uses Wistar rats and chooses an infusion preparation to be applied as a daily functional beverage in the community. The oxidative stress parameter used here is MDA which is a lipid peroxidase degradation product that is proven to increase significantly in smoker.[9] Malondialdehyde can be easily detected in the blood plasma, making it commonly used as the oxidative stress marker, while lung damage is observed histopathologically.[10] The results of this study will determine the dose of SPI that has the potential to be a safe and effective daily antioxidant supplement in rats exposed to cigarette smoke, allowing for further investigation to apply it in humans. The findings are expected to provide an alternative natural antioxidant option for both active and passive smokers in Indonesia by utilizing everyday household waste.

METHODS

Research design

We performed an experimental study, employing a post-test-only control group design model.

Sample

This study used 24 Rattus norvegicus Wistar strain, males, aged 8-10 weeks, weighing 120-150 g, which were exposed to cigarette smoke and randomly divided into 6 groups using a computer based random order generator. The rats were obtained from Wistar Farm, Malang, Indonesia. The sample size was determined using the Federer formula, requiring a minimum of 4 rats per group.[11] All experimental animals used in this study underwent an acclimatization phase for 1 week in their respective cages with ad libitum feeds and water, following the day and night cycle. The animals selected for treatment were healthy, actively moving, and did not experience a weight loss of more than 10% during the acclimatization period.

Shallot peel preparation

The main material used was shallot peel Blue Lancor variety, *Allium cepa* species L. var. ascalonicum Back obtained from Tanjung Market, Jember, East Java, Indonesia. The species was determined by Botany Laboratory, Faculty of Math and Natural Science, University of Jember (Identification Certificate No.044/2022). Fresh (indicated by bright red) shallot peel waste was

cleaned from dirt and pesticide residues by soaking in a 10% saltwater solution for 15 minutes, then rinsed with running water and dried under sunlight (before 9 a.m. and after 3 p.m.). The dried red onion skin was then ground using a blender to produce powder-like simplicia. As much as 10 grams of simplicia was mixed with 50 mL of distilled water to obtain an infusion with the highest concentration, which was 20%. The mixture was heated using an infusion pot for 15 minutes while stirring, starting from when the temperature reached 90°C. The solution was filtered using flannel cloth, and the resulting filtrate was added to distilled water until the total volume reached 50 mL.[12]

To obtain a tiered dosage, the 20% concentration infusion was diluted using a serial dilution method to achieve doses of 1,000 mg/kgBW; 500 mg/kgBW; 250 mg/kgBW; and 125 mg/kgBW. The volume of aquabidest and SPI administration was determined based on the maximum stomach volume that can be tolerated by the rat, which is 10 ml/kgBW.[13] Therefore, for a rat weighing 200 grams, the maximum volume that can be given, whether aquabidest or SPI, is 2 mL. The determination of the tiered SPI dose refers to the same volume administered to each rat so that the difference in doses is indicated by the concentration difference. The highest concentration of SPI that can be made is 20%. This is because the determination of concentration is based on the weight of the simplicia (grams) and the volume of the solvent (mL). Shallot peel infusion has a light mass. Therefore, even at 1 gram, it has a relatively large volume. Additionally, the simplicia used is in powder form, making it easy to absorb water. Therefore, with a concentration of only 20%, the solution appears thick.

Experimental treatment

The Wistar rats were divided into 6 groups, i.e. the cigarette smoke group administered aquabidest (SPI dose of 0 mg/kgBW as control) and 5 SPI groups with doses of 125 mg/kgBW, 250 mg/kgBW; 500 mg/kgBW; 1,000 mg/kgBW and 2,000 mg/kgBW, respectively. Administration of SPI and aquabidest was performed orally using feeding tube, carried out 2 hours before exposure to cigarette smoke. All animals were subsequently exposed to cigarette smoke (2 cigarettes/day/group) using a smoking chamber (Figure 1).[14]

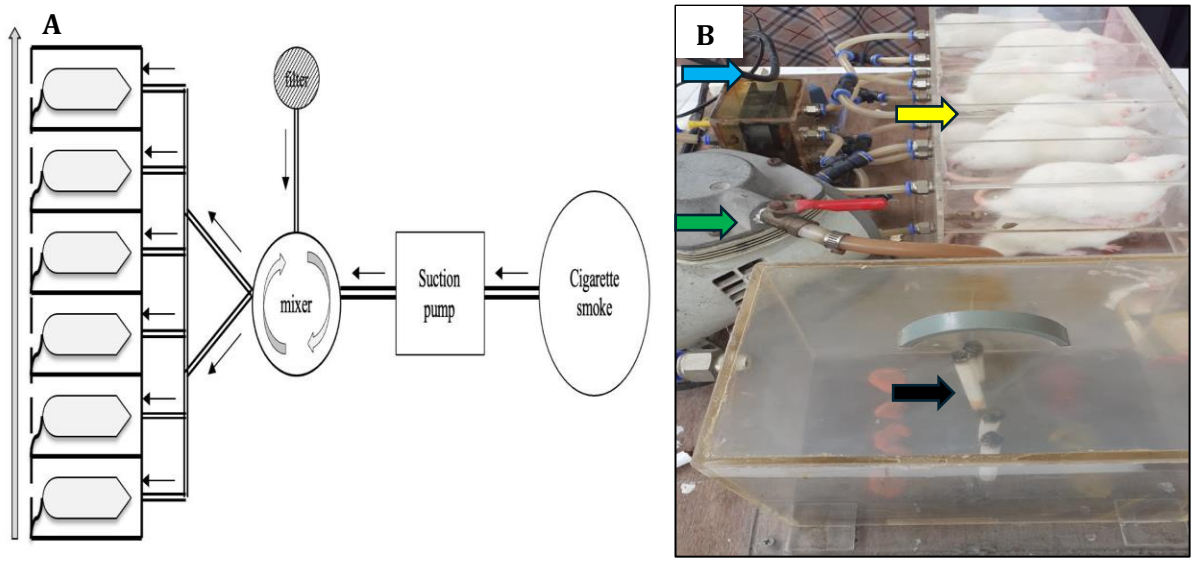

Figure 1. Smoking chamber model. A. Schematic figure, B. Smoking chamber used in this current research. The smoking chamber equipment consists of a cigarette holder (black arrow), vacuum (green arrow), aspirator (blue arrow), mixe (red arrow), and exposure chamber (yellow arrow). The number of cigarettes given is according to needs. There are 12 holes for cigarettes, holes that are not filled with cigarettes can be covered with cotton or playdough. The vacuum was used to exhaust cigarette smoke from the cigarette holder to the mixer room, while aspirator was used to aspirate and filter outside air into the mixer room. The exposure chamber had a small hole at the back for air circulation.

All experimental animals were marked and placed in their respective cages labeled with their group names. Treatment was administered according to the allocation of animals in each group. During the serum and lung organ preparation stage, the samples were coded to make sure the examiners of the slides were blinded to the group origins or treatment differences between the samples. The treatment was conducted for 28 days. On day 29, the Wistar rats were euthanized using ketamine and xylazine at 10:1 intraperitoneally. Blood was then collected through the heart to measure serum MDA levels using the ELISA method, and lung organs were harvested for histopathological preparation with HE staining.

Serum MDA level analysis

Serum MDA levels were measured using ELISA (Bioassay Technology Laboratory, Shanghai, Catalog Number: E0156Ra). Serum was obtained by extracting blood from the heart using a syringe, incubating it at room temperature for 30 minutes, followed by centrifugation for 15 minutes at a speed of 3,000 rpm. As many as 100 µL of standards and 40 µL of the samples were added into the appropriate wells, incubated with the detection antibody, washed, and given horseradish peroxidase (HRP) and colorimetric 3,3',5,5'-tetramethylbenzidine (TMB) reagent according to the protocols. The sample absorbance was read using a wavelength of 450 nm in a spectrophotometer, and each set of standards, and controls, as well as samples was calculated against the optical density with zero standard. The standards were used to construct the graph plot, and the sample absorbance reading was subsequently converted into concentration using the curve plotted.

Histopathological analysis

The preparation of the histopathological slide included fixation, dehydration, clearing, impregnation, making paraffin blocks, cutting, deparaffinization, rehydration, and staining using hematoxylin eosin (HE). The fixation stage was carried out by soaking the tissue using a 10% neutral-buffered formalin (NBF) with a minimum volume of 5x the specimen volume until the tissue consistency was hard and not reddish. The dehydration stage was carried out using graded alcohol, starting from 70% alcohol, 95%, and 100%, each for 30 minutes, continued using 100% alcohol for one hour, three times, and finally using 100% alcohol or xylol for 30 minutes. The clearing stage was performed using xylol for 1 hour and 2 hours. The impregnation stage was performed by soaking the tissue using liquid paraffin with a melting point of < 60°C for 2.5 hours and for 4 hours. Tissue incubation was carried out using an incubator or oven with a temperature of 55-57°C. At the stage of making paraffin blocks, the tissue was embedded in paraffin and stored at a temperature of 20-25°C. The cutting stage was conducted using a microtome, then the HE staining procedure was carried out (Leica Biosystems, United States) through the stages of deparaffinization, rehydration, staining, dehydration, clearing, and mounting. Histopathological observation was conducted at 4 randomly selected areas, avoiding regions containing bronchial structures. Histopathological observations were conducted using lung histopathological damage score (Table 1) using a Leica DM500 microscope at a magnification of 100x.[15,16]

Table 1. Histopathological lung damage score[15,16]

Histopathology appearance	Scoring		
	1	2	3
Inflammatory cells (polymorphonuclear and phagocytes)	Inflammatory cells < 25%	Inflammatory cells 25-75%	Inflammatory cells > 75%
Alveolus lumen	Proportional size >75%	Proportional size 25-75%	Proportional size <25%
Inter-alveoli junction (alveolus septum thickness)	Tight > 75%	Tight 25-75%	Tight <25%

Statistical analysis

The average MDA levels and lung scores across treatment groups were analyzed using regression analysis. The determination of the maximum effective dose was based on the curve

and quadratic equation, calculated using IBM SPSS Statistics version 26.[16,17]

Ethics

Ethical approval was received from the Ethics Commission of the Faculty of Medicine, University of Jember No. 1.623/H25.1.11/KE/2022.

RESULTS

The results of serum MDA level and histopathological lung damage score of each group are shown in Table 2. The highest serum MDA level was obtained in the cigarette smoke group with an average value of 1.25 µM/mL, while the lowest serum MDA level was obtained in the 2,000 mg/kgBW SPI group with an average value of 0.71 µM/mL. These data demonstrated that exposure to cigarette smoke increases the serum MDA levels in Wistar rats. With the administration of SPI doses of 125 mg/kgBW, 250 mg/kgBW, 500 mg/kgBW, 1,000 mg/kgBW, and 2,000 mg/kgBW, the serum MDA levels decreased successively to 1.16 µM/mL, 0.94 µM/mL, 0.78 µM/mL, 0.77 µM/mL, and 0.71 µM/mL. The higher the dose of SPI given, the lower the serum MDA levels.

Table 2. Serum malondialdehyde level (µM/mL) and histopathological lung damage score

Groups	n	Serum malondialdehyde level (µM/mL)	Histopathological lung damage score
Cigarette smoke	4	1.25 ± 0.11	7.15 ± 0.17
P1 SPI 125 mg/kgBW	4	1.16 ± 0.19	5.80 ± 0.43
P2 SPI 250 mg/kgBW	4	0.94 ± 0.11	6.02 ± 0.79
P3 SPI 500 mg/kgBW	4	0.78 ± 0.09	5.42 ± 0.19
P4 SPI 1,000 mg/kgBW	4	0.77 ± 0.08	4.52 ± 0.95
P5 SPI 2,000 mg/kgBW	4	0.71 ± 0.03	5.47 ± 0.46

Values are in mean ± standard deviation, SPI: shallot peel infusion, kgBW: kilogram body weight

A regression test was used to determine the maximum effective dose of SPI in preventing oxidative stress due to exposure to cigarette smoke (Figure 2). We obtained a regression equation ($Y= 1.2 – 7.95E – 4x + 2.77E – 7x^2$). The maximum effective dose of SPI in preventing oxidative stress caused by cigarette smoke obtained from the calculation using the first derivative (y'=0) was 1,435 mg/kgBW. It indicates that the maximum effective dose of SPI in this study does not exceed 2,000 mg/kgBW even though the lowest serum MDA level is at a dose of 2,000 mg/kgBW.

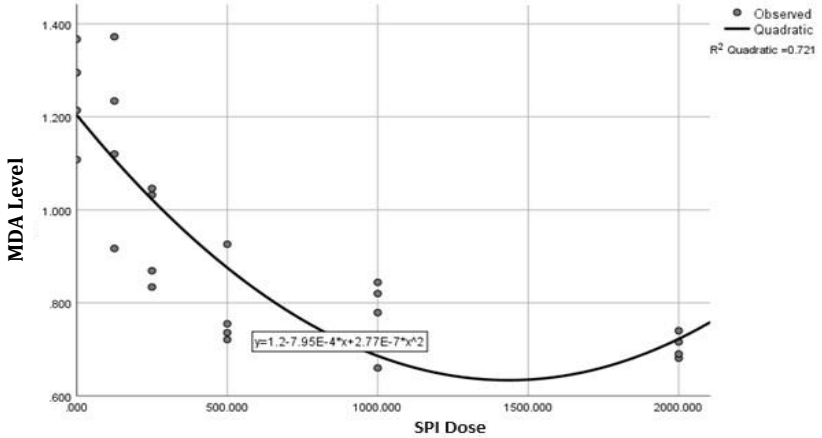

Figure 2. Regression curve of shallot peel infusion dose (SPI) and serum malondialdehyde (MDA) level

The histopathological depiction of the lungs in each treatment is presented in Figure 3. Based on histopathological lung damage score, the highest lung damage score was found in the cigarette smoke group with an average value of 7.20 whereas the lowest lung damage score was

obtained in the 1,000 mg/kg BW SPI group with an average value of 4.53. These data show that exposure to cigarette smoke causes lung damage in Wistar rats. With the administration of SPI doses of 125 mg/kgBW; 250 mg/kgBW; 500 mg/kgBW; 1,000 mg/kgBW; and 2,000 mg/kgBW, the average lung damage score showed a decreasing trend, namely 5.8; 6.03; 5.43; 4.53; and 5.48 Administration of SPI reduced the lung damage score, but at doses of 250 mg/kgBW and 2,000 mg/kgBW, the increase in the SPI dose did not correspond to a decrease in the lung damage score. The lung histopathology observation data above show that the administration of SPI up to a dose of 1,000 mg/kg BW prevents the inflammatory process, as indicated by the number of inflammatory cells, the thickness of the alveolar septum, and the increasingly normal shape of the alveolar lumen.

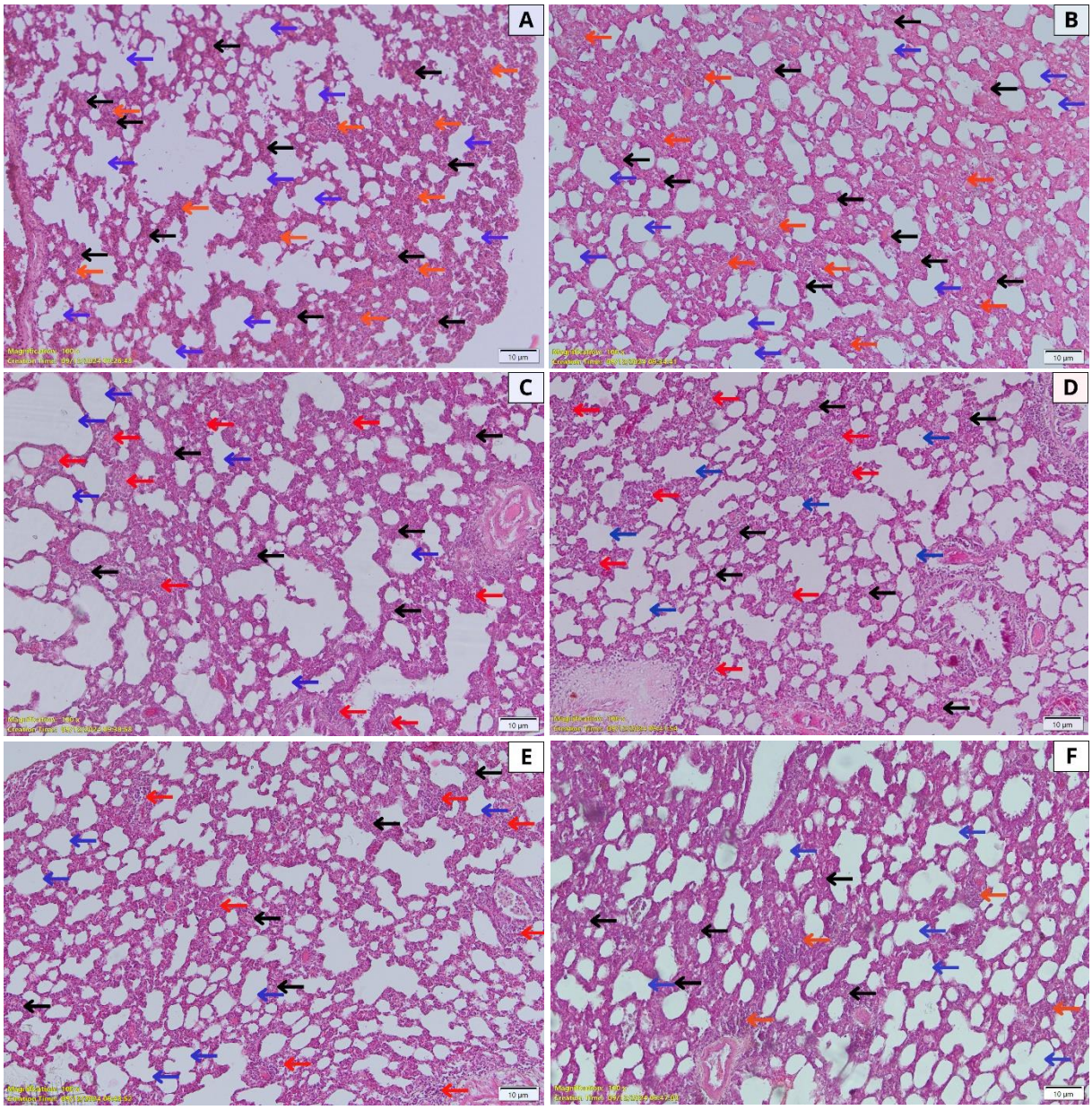

Figure 3. Lung histophatology (A) cigarette group, (B) SPI 125 mg/kg BW; (C) SPI 250 mg/kgBW; (D) SPI 500 mg/kgBW; (E) SPI 1,000 mg/kgBW; (F) SPI 2,000 mg/kgBW. Black arrow: thickened alveolar septa due to many inflammatory cells, red arrow: inflammatory cells, blue arrow: disproportionate airspaces due to thickening of the septa. SPI: shallot peel infusion. Hematoxycillin Eosin staining, 100x.

A regression test was used to determine the maximum effective dose of SPI in preventing lung damage due to exposure to cigarette smoke. The results of the regression test yielded a quadratic curve (Figure 4). The regression test revealed an equation (Y= 6.81 – 3.86E – 3x + 1.6E

– $6x^2$). The maximum effective dose of SPI in preventing lung damage caused by cigarette smoke obtained from the calculation using the first derivative (y'=0) was 1,206 mg/kgBW.

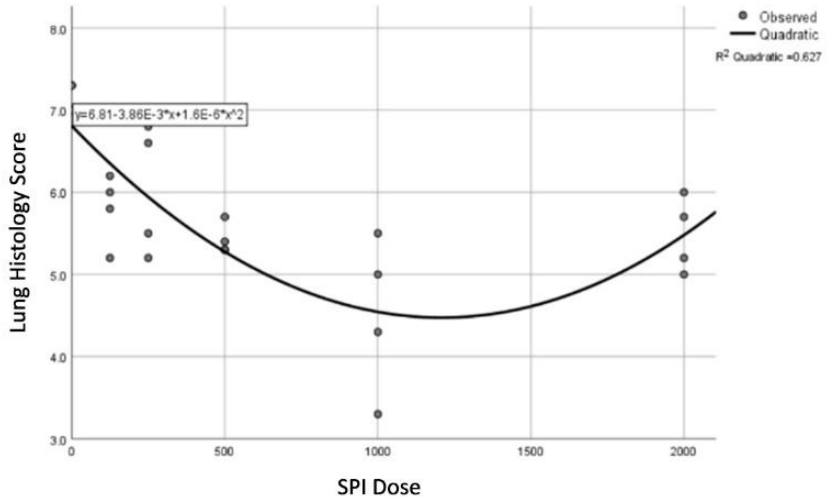

Figure 4. Regression curve of shallot peel infusion (SPI) dose and lung damage

DISCUSSION

This study used a 28-day treatment duration because it has been shown to significantly increase MDA levels. Previous research demonstrated increased MDA and decreased superoxide dismutase (SOD) levels following mice exposure to two cigarettes/day for 14 days.[18] A research performed 28 days of cigarette smoke exposure in Wistar rats, reported a similar finding for the MDA level.[9] A study in humans revealed that MDA and xanthine oxidase levels in healthy adult smokers were significantly higher than to non-smokers.[19] Serum MDA levels in both active and passive smokers highly increased after exposure to cigarette smoke.[20] A cross-sectional study resulted in a significant increase in serum MDA levels in active and passive smokers.[21] Hence, exposure to cigarette smoke, whether acute, subchronic, or chronic, causes an increase in MDA levels in both active and passive smokers.

Cigarette smoke is an aerosol consisting of solid and liquid droplets in the gas phase and contains more than 4,500 chemical compounds including oxidants and free radicals.[22] Each cigarette contains 1 x 1017 oxidant molecules and chemical components. These hazardous substances enter the body and produce free radicals or reactive oxygen species (ROS).[23] These chemical components include tar which produces super peroxide hydrogen peroxide radicals (H2O2), radicals (O2•–), and hydroxyl radicals (•OH), cadmium metal which triggers the Fenton reaction which produces hydroxyl radicals, oxidation of polyaromatic hydrocarbons (PAH) compounds which produces superoxide radicals, and NO oxidation which produces reactive nitrogen compound radicals (SNR).[24] In addition, cigarette smoke causes an increase in the release of iron and ferritin from dust cells, producing hydroxyl radicals (•OH).[22,25] The sustainable increase of free radical production in the body of smokers causes an imbalance between oxidants and antioxidants, thereby triggering oxidative stress. Under these conditions, ROS interact with cells and cause oxidative damage to genes, proteins, and cell membranes.[26] Oxidative damage to cell membranes occurs due to lipid peroxidation through binding hydrogen ions with the polyunsaturated fatty acids (PUFA) membrane. Super peroxide radicals and hydroxyl radicals from cigarette smoke are potent initiators of lipid peroxidation, which subsequently provokes a chain reaction to produce final products i.e. F2-isoprostanes, 4-hydroxy-2-nonenol (4-HNE), and MDA accumulating in biological systems. Malondialdehyde is a three-carbon compound from oxidized polyunsaturated fatty acids, especially arachidonic acid.[10] It reduces the activity of intracellular antioxidants such as glutathione, catalase, glutathione S-transferase, glucose-6-phosphate dehydrogenase, and superoxide dismutase so that antioxidant defenses in the body cannot neutralize the accumulation of free radicals.[27] Continuous collection of free radicals causes increased oxidative stress conditions, leading to loss of ability to regenerate and maintain

homeostasis.[28,29] In smokers, this condition is imperative in the lung disease pathogenesis, especially chronic obstructive lung disease.

Shallot peel infusion administration in this study was aimed to determine the maximum effective dose of SPI in preventing oxidative stress due to cigarette smoke exposure. In this study, administration of shallot peel infusion improved the balance between free radicals and antioxidants, as indicated by differences in the MDA levels. The mechanism of action of flavonoids especially quercetin in preventing oxidative stress is increasing antioxidant defense by increasing intracellular antioxidant capacity through activation of the nuclear transcription factor erythroid 2-related factor (Nrf2).[30] In addition, flavonoids will donate hydrogen ions to excess reactive oxygen species, forming more stable compounds. That mechanism will impede oxidative damage to the lungs.[31,32]

The lowest serum MDA level in this study was found in the SPI group at a dose of 2,000 mg/kgBW, i.e. 0.71 μM/mL, while the maximum effective dose of SPI was 1,435 mg/kgBW. Therefore, although the lowest serum MDA level corresponds to the SPI dose of 2,000 mg/kgBW, it is not the most effective. Serum MDA levels up to 1,435 mg/kgBW showed that the higher SPI dose correlates to a better ability to prevent oxidative stress due to cigarette smoke exposure. Administration of SPI exceeding 1,435 mg/kgBW was no longer effective in neutralizing oxidative stress conditions induced by cigarette smoke, as excessive antioxidant intake might change its properties into pro-oxidants. The more •OH substitutions in the flavonoid structure, the stronger the pro-oxidant activity due to transition metals.[10]

It is necessary to calculate the amount of SPI consumption in daily life using the Laurence and Bacharach conversion table. The SPI dose in rats of 1,435 mg/kg BW was converted to 16,072 mg/day or 16.07 g/day in humans. If applied as a daily supplement in a high-concentration infusion (20%), it equals 80.36 mL, about half a glass. Hence, 20% SPI can be used as a natural drink to consume daily, which is easy to apply by the community.

Cigarette smoke causes histopathological damage to the lungs through direct contact between cigarette smoke and the pulmonary alveoli, resulting in irritation and inflammation of the alveoli. In addition, the free radicals of cigarette smoke enter the body, causing oxidative damage and systemic adverse effects, especially on the lungs, which are already irritated. The irritation process of the respiratory tract epithelium begins when cigarette smoke particles entering the lung are phagocytosed by alveolar macrophages and form dust cells, which will activate the inflammatory mediators release such as Tumor Necrosis Factor-α (TNF-α) and Interleukin-1β (IL-1β), followed by polymorphonuclear migration (monocytes and neutrophils) to the alveolar septum and alveolar. Neutrophils and macrophages will release free radicals and protease enzymes. In smokers, this process sustainably continues so that the alpha-antiprotease enzyme in the body cannot inhibit the activity of the protease enzyme, leading to the damage of the extracellular matrix especially elastin, and destruction of the alveolar walls so that the shape of the alveolar lumen is disproportionate. In addition, the continuing inflammatory process will disrupt the alveolar junction. The inflammatory process continues causing the junctions between the alveoli to become loose and subsequently decrease the air flow. These processes underlie the emergence of chronic obstructive pulmonary disease clinical manifestations.[33]

In this study, SPI was administered to determine the maximum effective dose of SPI in preventing lung damage due to exposure to cigarette smoke. We have previously explained the content and mechanism of action of antioxidants contained in SPI. The potential of SPI to reduce or prevent conditions of oxidative stress and inflammation caused by cigarette smoke will reduce or even prevent ongoing histopathological damage to the lungs. The maximum effective dose of SPI in preventing lung damage was 1,206 mg/kg BW. Administration of SPI exceeding 1,206 mg/kgBW is no longer effective in preventing lung damage due to cigarette smoke. The antioxidant properties of flavonoids, especially quercetin as pro-oxidants, have been previously described.

Based on the detail observation, the data on the decrease in lung damage scores are not fully correlated with the increase in the dose of SPI. The lung damage score data for the SPI group with a dose of 125 mg/kgBW is lower than the SPI group with a dose of 250 mg/kgBW so that it

is not in line with the decrease in lung damage scores for other groups. However, in this condition, the maximum effective dose of SPI in preventing lung damage due to cigarette smoke can still be determined in accordance with the objectives of this study. We verified this using a correlation test between the SPI dose and the lung damage score. Based on the results of the regression test (maximum effective dose of 1,206 mg/kgBW), we conducted a correlation test 2 times using data from 6 treatment groups (cigarette smoke and SPI groups with doses of 125 to 2,000 mg/kgBW) and data from 5 treatment groups (cigarette smoke and SPI groups with doses of 125 to 1,000 mg/kgBW). The results of the correlation test using 6 groups did not obtain linearity and correlation between the SPI dose and lung damage score, while the correlation test using 5 groups obtained linearity and a strong negative correlation between the SPI dose and lung damage score with a correlation coefficient value of -0.781. The results of the correlation test are in accordance with the regression test that the maximum effective dose of SPI is 1,206 mg/kgBW so that the correlation between the increase in the SPI dose and the decrease in lung damage score occurs up to a dose of 1,000 mg/kgBW. Meanwhile the addition of 2,000 mg/kgBW dose data becomes uncorrelated because the dose is no longer effective with the lung damage score which begins to increase.

The SPI dose calculation for humans uses the Laurence and Bacharach conversion table and was performed by changing the dose from 1,206 mg/kgBW to 241.2 mg/rat/day and converting to 13,507.2 mg/day or 13,507 g/day in humans. If applied as a daily supplement in a high-concentration infusion (20%), it equals 67.54 mL or about a third of a glass. This shows that 20% SPI has the potential to become a natural drink containing high antioxidants, which can be easily practiced in society. This amount is different from shallot peel ethanol extract, which has flavonoid levels of 8-13 times higher compared to infusion. Therefore, the shallot peel extract needed to achieve the maximum effective dose will be less than the infusion.

In this study, the flavonoid content in SPI prevents oxidative stress in lung tissue, so cell damage or inflammatory processes can be impeded. Irritation of the respiratory tract epithelium and epithelial injury due to direct contact with cigarette smoke will be minimized by the antioxidant activity by reducing free radicals and repairing injured cells. The other negative influence of cigarette smoke on the body is causing oxidative stress systemically and increasing toxic substances derived from cigarette smoke. The antioxidant activity of SPI prevents oxidative stress and neutralizes poisonous substances that enter the body by increasing liver activity.[32]

The maximum effective dose refer to the highest dosage of a substance containing a certain compound that can effectively repair pathological changes in the body.[34] In this study, there was a difference in the maximum effective dose of SPI between MDA levels and lung damage. This can occur because it uses different variables. Levels of MDA are indicators of oxidative stress, which is systemic, whereas lung damage is more specific to damage to the histological structure of the lung organ directly exposed to cigarette smoke. Measurement of these two variables is needed to analyze the pathologic mechanism of cigarette smoke on lung damage both directly and systemically through the oxidative stress process. The selection of the maximum effective dose in this study refers to a lower dose because if a higher dose is used, there is a risk of excessive doses becoming ineffective or even changing properties from antioxidants to oxidants.

The results of this study cannot be compared to similar studies because there has been no research related to the administration of shallot peels to lung histopathological damage induced by cigarette smoke or other toxic substances. If it is on the effectiveness of flavonoids contained in other natural ingredients, the antioxidant activity of flavonoids prevents damage to lung tissue induced by cigarette smoke. Berries contain flavonoids that can reduce pro-inflammatory mediators by macrophages by inhibiting Nuclear Factor-KappaB (NFκB).[35] Black cumin prevents the increase of MDA level and the decrease of glutathione level in lung tissue in white rats exposed to chronic cigarette smoke and reduces the number and function of alveolar macrophages in bronchoalveolar lavage fluid.[36] Other research showed red dragon fruit extract can prevent the increase of alveolar macrophages.[37] Previously, research on the maximum effective dose of SPI focused on the histopathological structure of the kidneys due to exposure to diazinon-type pesticides. The study found a maximum effective dose of 1,359 mg/kg BW.[38] This shows the

difference in the maximum effective dose of red onion peel in neutralizing the negative effects of cigarette smoke exposure and pesticides, but only by about 11.26%. The difference in treatments and variables measured will affect the determination of the maximum effective dose of a substance. The limitation of this study is that the maximum effective dose of SPI cannot be used to determine the level of toxicity of shallot peel. Further research regarding the toxicity of shallot peel is needed to ensure its safety in daily consumption.

CONCLUSION

Shallot peel infusion at less than 1,206 mg/kgBW potentially serves as a daily antioxidant supplement, which is safe for smokers. However, a toxicity study is needed to use shallot peel as a daily antioxidant supplement. The maximum effective dose of shallot peel in smokers has been determined, further research can be conducted by comparing the potential of shallot peel with other natural ingredients, such as vitamin C and curcumin as positive controls having been studied to have antioxidant potential in smokers. Shallot peel can be used as an alternative daily antioxidant for smokers. Molecularly, our further study is analyzing the antioxidants of shallot peel to prevent the inflammatory process in smokers by measuring pro-inflammatory indicators such as NFκB, TNF-α using through ELISA or immunohistochemistry methods.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the research and manuscript with any party.

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AUTHORS' CONTRIBUTION

DH & RD conceived the planned experiments, AM, SR & S conducted the experiments, DH, RD & CA contributed to the data interpretation and analysis, ASM provided the critical feedback, all author contributed to the manuscript writing with DH as the lead.

LIST OF ABBREVIATIONS

BNF: Buffered Neutral Formalin, COPD: Chronic Obstructive Pulmonary Disease, HE: Hematoxylin-Eosin, HRP: Horseradish Peroxidase, Interleukin-1β (IL-1β), MDA: malondialdehyde; NCR: Nitrogen Compound Radicals, NFκB: Nuclear Factor-KappaB, Nrf2: Nuclear Transcription Factor Erythroid 2-related Factor, PAH: Polyaromatic Hydrocarbons, PUFA: Polyunsaturated Fatty Acids, SPI: Shallot Peel Infusion, TMB: 3,3',5,5'-Tetramethylbenzidine, TNF-α : Tumor Necrosis Factor-α

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