Maximum Effective Dose of Shallot Peel in Preventing Cigarette Smoke-Induced Lung Oxidative Stress

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Abstract. Cigarette smoke is a source of free radicals and a trigger for the production of free radicals in the body and has been proven to cause oxidative stress, especially in the lungs. Continuous oxidative stress, causes an inflammatory process and lung tissue damage, the pathophysiology of chronic obstructive pulmonary disease (COPD), lung fibrosis and pulmonary cancer. Shallot peel, with a high flavonoid antioxidant content, can potentially prevent cigarette smoke-induced lung oxidative stress. This study was designed to determine the maximum effective dose of shallot peel infusion form (SPI) in preventing lung oxidative stress induced by cigarette smoke. The type of experimental laboratory was a posttest-only control group design. Rats were exposed to 2 cigarettes/day and divided into a cigarette smoke group administered aquabidest (SPI 0 mg/kgBW) and 5 SPI groups administered SPI doses of 125 mg/kgBW, 250 mg/kgBW, 500 mg/kgBW, 1,000 mg/kgBW and 2,000 mg/kgBW in 28 days treatment. Oxidative stress was measured in lung tissue using the ELISA method. Based on the regression analysis quadratic model, it was found that the maximum effective dose of SPI to prevent lung oxidative stress was 1046 mg/kgBW. It can be concluded that SPI with amounts more than 1,046 mg/kgBW is no longer effective in preventing pulmonary oxidative stress induced by cigarette smoke.

Keywords: shallot peel, cigarette smoke, oxidative stress, lung malondialdehyde

1 Introduction

Indonesia ranks as the third country in the world with the highest number of smokers (65 million smokers or 27.6% of the population) after China and India [1]. Cigarette smoke has been proven to cause many adverse effects, both in active and passive smokers. Passive smokers inhale toxic chemicals and oxidants into lung tissues, preventing lung repair mechanisms. Passive smokers are exposed to chemicals 50 times higher than active smokers [2]. It has been reported that 35% of women, 33% of men, and 40% of children worldwide are passive smokers [3]. The World Health Organization (WHO) reports that smoking causes 8 million deaths annually, including 60,000 infants as passive smokers due to lower respiratory tract infections [1].

Cigarette smoke contains nicotine, CO, tar, and various chemicals and oxidants that can trigger multiple pathological effects [4]. Cigarette smoke is a source of free radicals, and continuous inhalation leads to the accumulation of free radicals, causing

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Cigarette smoke contains nicotine, CO, tar, and various chemicals and oxidants that can trigger multiple pathological effects [4]. Cigarette smoke is a source of free radicals, and continuous inhalation leads to the accumulation of free radicals, causing their production in the body and disrupting the body's antioxidant defense, resulting in oxidative stress. This oxidative stress directly causes cell damage by the oxidizing cell membrane lipids, proteins, and DNA. Indirectly triggers inflammation, including activating inflammatory cells and releasing proinflammatory cytokines and chemokines. This condition exacerbates oxidative stress and leads to tissue damage. If this condition persists chronically, it can lead to persistent oxidative stress and diseases such as chronic obstructive pulmonary disease, pulmonary fibrosis, or lung cancer.

Based on the above explanations, oxidative stress due to cigarette smoke is a trigger for lung tissue damage. Efforts to neutralize free radicals immediately after continuous exposure to cigarette smoke are a crucial step in preventing oxidative stress and lung damage. One way to achieve this is through daily consumption of antioxidant supplements, possibly functional drinks made from easily accessible ingredients. Shallot peel, with its high flavonoid antioxidant content, has the potential to prevent oxidative stress in the lungs caused by cigarette smoke. Research by Rahima et al., 2022, showed that the flavonoid content of shallot peel in extract form was 228.1 mg QE/g, while Helianti et al., 2023, showed a flavonoid content of 96.8 mg QE/L in the form of an infusion. The flavonoid content in the extract form was 2.5 times higher than in the infusion form, but the infusion form is more readily applicable in daily life.

This study is designed to determine the maximum effective dose of shallot peel in infusion form (SPI) in preventing oxidative stress in the lungs due to cigarette smoke [5]. Research on the effectiveness of shallot peel antioxidants has begun in recent years. Putri et al., 2020, reported that shallot peel extract for seven days could repair gastric damage caused by toxic doses of mefenamic acid. In addition, the administration of shallot peel extract has been reported to neutralize oxidative stress in the liver and kidneys of rats induced by diazinon [6,7]. However, the protective potential of shallot peel against oxidative stress in the lungs due to cigarette smoke has not been investigated. Determining the maximum effective dose is necessary to assess that shallot peel is a safe substance and can be used daily with adequate dosage.

Therefore, research on the potential of shallot peel in preventing oxidative stress in the lungs due to cigarette smoke, focusing on determining the maximum effective dose, must be conducted. This study uses Wistar rats and opts for an infusion form for applicability in the community. The oxidative stress indicator used is malondialdehyde (MDA) levels, which is the end product of cell membrane lipid oxidation, easily detected, and often used as a marker of oxidative stress [8,9]. Lung tissue is used in measuring MDA levels as it is exposed to cigarette smoke directly and indirectly.

2. Method

This study was a laboratory experimental research with a posttest-only control group design. Ethical approval was obtained from The Ethics Commission, Faculty of Medicine, University of Jember, with number 1.623/H25.1.11/KE/2022.

A total of 24 experimental animals, male Rattus norvegicus Wistar strain, aged 8-10 weeks, weighing 120-150 g were exposed to cigarette smoke, 2 cigarettes/day/group, using a smoking chamber consisting of a cigarette holder, vacuum, aspirator, mixer, and exposure chamber for each mouse. The experimental animals were divided into 6 groups, i.e. the cigarette smoke group administered aquabidest (SPI dose of 0 mg/kgBW) 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. The highest SPI dose (2000 mg/kgBW) obtained from 20% SPI was diluted using serial dilution method to obtain doses of 1000 mg/kgBW, 500 mg/kgBW, 250 mg/kgBW, and 125 mg/kgBW (BPOM RI, 2010). Treatment was carried out for 28 days. On the day 29, the experimental animals were terminated using ketamine and xylazine in a ratio of 10:1 intraperitoneally and lung MDA levels were measured using ELISA method.

Lung MDA levels of the treatment groups were analyzed using regression test and the maximum effective dose was determined based on quadratic curves and equations [10].

3. Result

The result of lung MDA levels are presented in Table 1.

Table 1. Lung MDA level (μM/mL)

Group	n	Mean ± SD
P0 cigarette smoke	4	0.964±0.134
P1 SPI 125 mg/kgBW	4	0.824±0.119
P2 SPI 250 mg/kgBW	4	0.771±0.171
P3 SPI 500 mg/kgBW	4	0.751±0.087
P4 SPI 1,000 mg/kgBW	4	0.738±0.023
P5 SPI 2,000 mg/kgBW	4	0.863±0.146

Based on lung MDA level data, the highest lung MDA level was found in cigarette smoke group with a mean value of 0.964 ſM/mL while the lowest lung MDA level was found in SPI group at a dose of 1,000 mg/kgBW dose with a mean value of 0.738 ſM/mL. These data show that SPI administration at doses of 125 mg/kg BW, 250 mg/kg BW, 500 mg/kg BW, and 1,000 mg/kg BW to mice exposed to cigarette smoke can reduce lung MDA levels (0.824 ſM /mL, 0.771 ſM/mL, 0.751 ſM/mL and 0.738 ſM/mL, respectively). The higher the SPI dose, the lower the lung MDA level.

However, SPI group at a dose of 2,000 mg/kgBW showed higher lung MDA level compared to a dose of 1,000 mg/kgBW.

The regression test was carried out to determine the maximum effective dose of SPI in preventing oxidative stress conditions due to cigarette smoke exposure. The regression test resulted a quadratic curve (see Fig. 1).

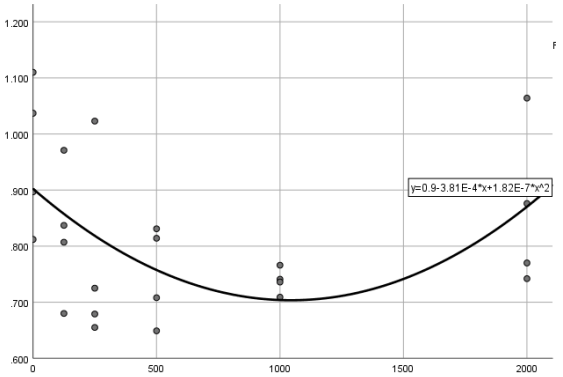

Fig. 1. Regression curve of SPI dose with lung MDA levels

Based on this curve, the regression equation was $y = 0.9 – 3.81E – 4x + 1.82E – 7x^2$. The maximum effective dose of SPI determined by calculating x value using the first derivate (y'=0) is 1,046 mg/kgBW.

4. Discussion

This research proved that exposure to two cigarettes of cigarette smoke per day for 28 days in Wistar rats significantly increases lung MDA levels. Similar research by Suryadinata et al. (2017) proved that exposure to cigarette smoke in Wistar rats for 28 days caused a significant increase in serum MDA [11]. In mice, research by Hu et al. (2014) showed that exposure to two cigarettes of cigarette smoke per day for 14 days increased serum MDA levels and decreased serum superoxide dismutase (SOD) levels [8]. In humans, Shah et al. (2015) research showed that MDA and xanthine oxidase levels in healthy adult smokers were significantly higher than in non-smokers [12]. Lymperaki et al. (2015) proved that exposure to cigarette smoke for 30 minutes significantly increased MDA levels in both active and passive smokers [13]. In a cross-sectional study, Ahmed NJ et al. (2020) proved a significant increase in serum MDA levels in active and passive smokers. It has been proven that acute and subchronic exposure to cigarette smoke causes an increase in serum MDA and tissue MDA levels in both active and passive smokers [14]. Different results were obtained in the research of Taito et al., 2017 which showed that a significant increase in hydrogen peroxide concentration as an indicator of oxidative stress was only found after smokers received exercise testing (single-sprint anaerobic exercise). Under

normal conditions, there is no significant difference in hydrogen peroxide concentrations between smokers and non-smokers [15] .

Around 7000 chemicals are contained in cigarette, including oxidants and free radicals [16]. One cigarette has 1 x 10 oxidant molecules. In contrast, the main dangerous chemicals in cigarette smoke are monoxide, which interferes with the release of oxygen to body tissues; nicotine, which disrupts the sympathetic nervous system; and several compounds that produce reactive oxygen species (ROS), including tar has super peroxide radicals (O^{2-}), hydrogen peroxide radicals (H_2O_2) and hydroxyl radicals (OH); Cadmium metal triggers the Fenton reaction which produces hydroxyl radicals; Oxidized Polycyclic Aromatic Hydrocarbons (PAH) compounds will produce superoxide radicals; and oxidized NO produces reactive nitrogen compound radicals (SNR) [17]. During smoking activities, there is an increase in the production of free radicals in the smoker's body. If this condition occurs continuously, it will damage the body's antioxidant defenses so that the amount of antioxidants is insufficient to neutralize free radicals and repair the damage caused by free radicals; an imbalance occurs between oxidants and antioxidants and causes oxidative stress conditions.

This oxidative stress directly causes cell damage by oxidizing cell membrane lipids, proteins, and genes. In conditions of oxidative stress, reactive oxygen species that are not neutralized by antioxidants will interact with cells, and oxidative damage occurs to cell membranes, proteins, and genes [18]. Oxidative damage to cell membranes occurs through the process of lipid peroxidation, where ROS binds to polyunsaturated fatty acids (PUFA) in cell membranes and produces final metabolites in the form of 4-hydroxy-2-nonenol (4-HNE), F2-isoprostanes and malondialdehyde (MDA). ROS from cigarette smoke is a potent initiator of lipid peroxidation, namely superoxide radicals and hydroxyl radicals. Malondialdehyde (MDA) is a three-carbon compound formed from oxidized polyunsaturated fatty acids, especially arachidonic acid [9]. Malondialdehyde (MDA) reduces the activity of endogenous antioxidants such as glutathione, superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, glutathione S-transferase so that the body's antioxidant defenses are increasingly unable to neutralize the accumulation of free radicals [19].

In addition, oxidative stress indirectly triggers inflammation, including activating inflammatory cells and releasing proinflammatory cytokines and chemokines. This condition exacerbates oxidative stress and leads to tissue damage. Continuous accumulation of free radicals causes increased oxidative stress conditions, which result in the body losing its ability to regenerate and maintain homeostasis [20]. If this condition persists chronically, it can lead to persistent oxidative stress and diseases such as chronic obstructive pulmonary disease, pulmonary fibrosis, or lung cancer.

The aim of administering IKBM in this study was to determine the maximum effective dose of shallot skin infusion in preventing oxidative stress in the lungs due to exposure to cigarette smoke. The high flavonoid content in IKBM can improve the body's antioxidant defense system and maintain a balance between the number of free radicals and antioxidants in smokers' bodies by donating hydrogen ions to excess reactive oxygen species, thereby forming more stable compounds.

This study's lowest lung MDA level was at a dose of 1000 mg/kgBW, 0.738 ⌈M/mL, and the maximum effective dose measurement result was 1046 mg/kgBW. The amounts show that the IKBM dose with the lowest lung MDA levels and the maximum effective dose are not much different, with a difference of only 46 mg/kgBW.

The results of measuring lung MDA levels show that up to a dose of 1046 mg/kgBW, the higher the IKBM dose given, the better its ability to prevent oxidative stress conditions due to exposure to cigarette smoke. Giving IKBM exceeding 1046 mg/kgBB is no longer effective in neutralizing oxidative stress conditions caused by cigarette smoke. The dose can be explained by the fact that too high an intake of antioxidants will change the properties of antioxidants into pro-oxidants, namely electron-accepting compounds. In addition, transition metals originating from mineral intake will change the antioxidant properties of flavonoids into pro-oxidants [9,21].

The results of calculating the maximum effective dose of IKBM in this study were converted using the Laurence and Bacharach conversion table by changing the amount of IKBM in rat from 1406 mg/kg BW to 209 mg/head/day, then converted in humans to 11,704 mg/day or 11,704 g/day. If applied as a daily supplement in a high-concentration infusion preparation (20%), it takes 58.52 mL or about a third of a glass. The fact shows that IKBM, with a concentration of 20%, has the potential to become a natural drink high in antioxidants with a daily dose that is easy to implement in the community. This dose will be much reduced in red onion skin extract preparations with flavonoid levels 8-13x higher than in infusion preparations.

The results of this study cannot be compared with similar studies because there has been no research relating the administration of shallot skins to pulmonary oxidative stress due to cigarette smoke or other toxic substances.

5. Conclusion

Based on the research results, it can be concluded that IKBM, with a dose of more than 1,046 mg/kgBW, is no longer effective in preventing pulmonary oxidative stress due to cigarette smoke.

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Disclosure of Interests
Authors declare no conflict of interests.

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