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Effect of Garlic Ethanol Extract Administration on Gluthatione Levels to Prevent Oxidative Stress in Smoker Rat Model

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ABSTRACT

Background: Sickle Garlic (Allium sativum L.) is known as a spice native to western Asia has a strong antioxidant effect and revealed it functions as an antioxidant by increasing ROS-capture activity, cellular antioxidants, SOD, CAT, and GSH levels in cells. Cigarette smoke is very dangerous because it can cause serious illness and death. Cigarette smoke is a major source of exogenous ROS because its particles are high in free radicals. Smoking is also related to a decrease in the body's natural antioxidant levels. Glutathione (GSH) synthesis and expression were found to increase initially and then decrease after being exposed to cigarette smoke. **Objective:** The aim of this study is; to analyze effect of garlic ethanol extract administration on gluthatione levels to prevent oxidative stress in smoker rat model. **Methods:** This was a case-control study with a control group design, with 15 healthy rats (Rattus norvegicus, sp.) divided into three groups, KN untreated animals (control), K1 animals exposed to cigarette smoke for 40 days (smoker), and K2 animals exposed to cigarette smoke for 40 days and treated with Allium sativum 0.1 g per day for 40 days (smoker and Allium sativum L.). After 40 days of treatment, all animals, including the control, were sacrificed with 30 mg/IP ketamine injections, and the blood plasma were taken for examination. **Results:** there were significant difference in glutathione levels between the treatment groups (K2) with the control group (KN) and the smokers group (K1) ($p < 0.05$). **Conclusion:** garlic ethanol extract administration can increase gluthatione levels and prevent oxidative stress in smoker rat model.

Keywords: Allium sativum, garlic, gluthatione, smoker rat model, inful crises.

1. BACKGROUND

Garlic (Allium sativum L.) is known as a spice native to western Asia and has been used for culinary purposes as well as a natural medicine for centuries. It appears to contain antioxidant properties that have the capacity to neutralize free radicals, reduce lipid peroxidation, and oxidize lipoproteins. This is because Allium sativum L., which contains allicin, diallyl sulfides, and other compounds, shows a wide range of physiological effects and activities in various metabolism pathways (1). Allium sativum L. has a strong antioxidant effect that is believed to prevent cancer, thrombus formation, heart disease, and other diseases. Allium sativum L. has been demonstrated to possess antioxidant properties both in vivo and in vitro. Allium sativum L. extract protects cells from ROS, according to biochemical studies. Its antioxidant capacity was also examined in animals using the trolox equivalent and antioxidant capacity assay to measure its activity in capturing free radicals (2). Allium sativum L. is thought to have an antioxidant effect by modulating ROS, increasing GSH, and increasing SOD activity (3). Through its antioxidant properties, Allium sativum L. protects tissue from injury and various diseases (4).

A previous study aimed to investigate the hepatoprotective mechanism of Allium sativum found a significant increase in antioxidant enzymes such as superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) in the Allium sativum extract-treated group compared to the CCL4-induced group (4). Study conducted by Xiaoshu Chen et al. in 2016 found that allicin could protect vascular endothelial cells from cell death caused by oxi-

dized low density lipoprotein (LDL) by inhibiting apoptosis and acting as an antioxidant (5). Allium sativum L. also contains enzymes including peroxidase, allinase, and myrosinase, and it also contains minerals and amino acids. These active compounds also help to protect tissues from various types of damage (2). A study on the effect of allicin on Pasteurella multocida infection found that rabbits infected with Pasteurella multocida had milder symptoms after receiving allicin. This might be due to allicin's anti-inflammatory, immunomodulatory, and antioxidant properties, as the antioxidant allicin was found to increase SOD expression (6). Several studies have claimed that Allium sativum L. could really improve spermatozoa quality because it contains allicin, selenium, zinc, vitamin C, and vitamin E, all of which are antioxidants that can protect cell membranes and organelles from peroxidative damage (7). The effect of Allium sativum L. extract on alcoholic patients was also revealed it functions as an antioxidant by increasing ROS-capture activity, cellular antioxidants, SOD, CAT, and GSH levels in cells. Allium sativum L. reduces ischemia and inhibits oxidative stress-induced LDL modification. Allium sativum L. also shields DNA from mutations and damage caused by reactive oxygen species (ROS) (2).

Cigarette smoke is one of the pollutants that has a negative effect on health in the world, considering that the bad effects are very dangerous not only for people who actively smoke cigarettes but also for those around them. Cigarette smoke is very dangerous for health because it can cause serious illness and death. In America, it is estimated that one in four passive smokers, or approximately 58 million people, are exposed to cigarette smoke from active smokers during 2013–2014 (8). Tobacco use is estimated to kill 6 million people every year, either directly or indirectly through the smoke inhaled by active smokers or indirectly through passive smokers. Secondhand smoke has a negative impact on health, causing respiratory tract infections, COPD, ischemic heart disease, asthma, and lung cancer (9). Secondhand smoke exposure has been linked to an increased risk of several types of cancer and cardiovascular disease. Furthermore, data shows that secondary cigarette smoke contains more toxic substances, implying that exposure to secondary cigarette smoke causes serious harm (10). It is important to note that 10-15% of lung cancer patients have no smoking history, and lung cancer is the leading cause of death in both people who are exposed to secondhand smoke and those who do not smoke. Passive cigarette smoke exposure is related to cancer via both genotoxic and carcinogenic mechanisms (11).

Cigarette smoke is a major source of exogenous ROS because its particles are high in free radicals. Smoking is also related to a decrease in the body's natural antioxidant levels (12). Cigarette smoke contains a high concentration of free radicals; it is estimated that one puff of a cigarette contains 1,014 free radical molecules. The most dangerous type of CO is free radicals, which can cause cell membrane damage, structural changes, respiratory tract disease, and decreased immune response (13). According to BAL analysis, even acute cigarette smoke exposure can result in increased lipid peroxidation and degradation of extracellular matrix proteins, resulting in oxidative stress and tissue damage (14). The expression concentration or activity of several antioxidant enzymes, including glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), has been reported to decrease in response to oxidative stress. Glutathione (GSH) synthesis and expression were found to increase initially and then decrease after being exposed to cigarette smoke. Changes in alveolar and pulmonary GSH metabolism play a significant role in the inflammatory disease process (15,16).

2. BJECTIVE

This study aimed to analyze effect of garlic ethanol extract administration on gluthatione levels to prevent oxidative stress in smoker rat model.

3. MATERIAL AND METHODS

Subjects

The study was a case-control study with a control group design, with 15 healthy rats (Rattus norvegicus, sp.) aged 8 weeks and weighing 180-200 g divided into three groups and kept under standard conditions. Unfiltered Marlboro cigarettes with 2.4 mg of nicotine per cigarette, Allium sativum, and a smoking chamber were used. Allium sativum was identified at the Badan Riset dan Inovasi Nasional (BRIN) laboratory in Jakarta, Indonesia, and extracted using the maceration technique with 96% ethanol. (17).

Rats were housed in cages with access to water and standard pellet diets throughout the experiment. 15 rats were divided into three groups: KN untreated animals (control), K1 animals exposed to cigarette smoke for 40 days (smoker), and K2 animals exposed to cigarette smoke for 40 days and treated with Allium sativum 0.1 g per day for 40 days (smoker and Allium sativum L.). Cigarette smoke was exposed twice per day, for 30 minutes each, in the morning and afternoon (18), and Allium sativum was given 0.1 g per day (19). After 40 days of treatment, all animals, including the control, were sacrificed with 30 mg/IP ketamine injections, and the blood plasma were taken for examination.

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).

Statistical analysis

All the data of the study were tested by Shapiro–Wilk, the normal distribution variables ($p > 0.05$) because the data were normally distributed ($p > 0.05$), the data then were analyzed using the One-Way Anova test ($p > 0.05$), Statistical analysis was carried out using the SPSS program via the SPSS software version 24.0 (SPSS Inc., Chicago, Illinois).

4. RESULTS

In this study, it was found that the results of the analysis using the one-way ANOVA test showed that there

Group	Gluthatione levels ((x ± SD)	p
K1 (5)	188,5 ±16,78	
K2 (5)	238,68 ±25,18	0,000*
K3 (5)	576,77 ±26,96	

Table 1. Glutathione levels in the smoker rat model after 40 days. Keterangan: KN= Control group; K1=Smoker group; K2=Smoker and Allium sativum L. group*=signifikan

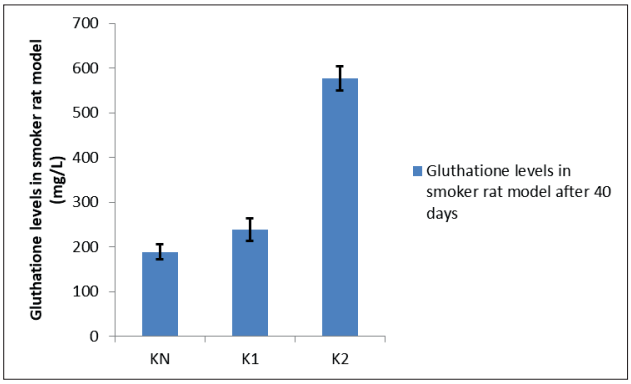

Figure 1. Gluthatione levels in smoker rat model after 40 days, ┬= standar deviation (SD).

were significant difference in glutathione levels between the treatment groups (K2) with the control group (KN) and the smokers group (K1) ($p < 0.05$).

The LSD (Least Significant Difference) test was then used to determine the differences between each group, the results of which can be seen in figure 1

5. DISCUSSION

In this study, it was found that glutathione levels in the group of smokers who were given Allium sativum extract showed a significant increase compared to the control group and smokers ($p<0.05$). The presence of the main organosulfur compounds found in Allium sativum L., namely the polar amino acids -glutamyl-S-Allyl-L-cysteines and Aliin (S-Allyl-L-cysteines sulfoxide) which are volatile, was responsible for the increase in GSH levels in the group given Allium sativum L and also other sulfur compounds such as diallyl disulfide (DADS), diallyl trisulfide (DATS) and other compounds. 82% of the total content of Allium sativum L. which is a sulfur-containing compound (20). as well as other sulfur compounds such as diallyl disulfide (DADS), diallyl trisulfide (DATS), and others. 82% of the total content of Allium sativum L., a sulfur-containing compound (21). It is known that cysteine, GSH, and H2S are closely related in metabolic processes, the active ingredients contained in Allium sativum L. have the potential to increase endogenous H2S levels. Previous research has shown that the active ingredient DATS in Allium sativum L. has the ability to increase H2S levels in mice blood and myocardial tissue, where it is known that H2S regulates the Keap1-Nrf2 pathway, which is the main regulator of the cytoprotective response to oxidative stress, resulting in increased expression of ARE, where Nrf2-ARE is the only pathway that controls enzymes responsible for GSH production, such as glutamate- cysteine ligase (GCL). Because it can activate Nrf2, the S-allylcysteine (SAC) compound from Allium sativum L. is known as a Nrf2 activator because it can increase the activation of this pathway, thereby increasing the production of antioxidant products (22). In inflammatory reactions, the Nrf2 pathway has also been shown to suppress the NF-k pathway (23). The allicin content contained in Allium sativum L also inhibits the expression of NFκβ by preventing the decrease of the inhibitor of kappa B (Iκβ) which is an inhibitor of NFκβ (24). ntigens from cigarette smoke that are recognized by receptors initiate inflammatory signaling cascades via activation of nuclear factor kappa B (NF-B), which causes an increase in tumor necrosis factor (TNF-) and its receptor, Interleukin (IL)-1, IL-6, and IL-8, and granulosite macrophage-stimulating factor (G-CSF) colonies and initiates oxidative stress, which causes a decrease in GSH levels (25). As a result, obstructing the NF-kB pathway reduces oxidative stress and raises GSH levels.

6. CONCLUSION

It is clear that garlic ethanol extract administration can increase gluthatione levels and prevent oxidative stress in smoker rat model.

- **Author's contribution**: MS contributed to conception, design of the study and manuscript preparation. DKM performed data acquisition and experimental laboratory works. YM and SSW contributed to data analysis. AJAU, SI, JS and PP were involved in article drafting. All authors have approved the final version of the manuscript.
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