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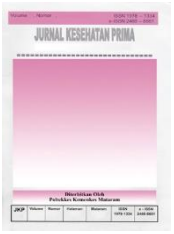

Effect of Solo Garlic Extract (*Allium Sativum*) on Malondialdehyde (MDA) in Rats (*Rattus Norvegicus*) Exposed to Cigarette Smoke

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Abstract

Oxidative stress can develop various non-communicable diseases (NCDs) which experienced by 41 million people globally. Cigarette smoke is one of the oxidant sources which cause stress oxidative. Cigarettes contain more than 4500 gases and chemicals that have negative effect on human body. Stress oxidative is marked by various parameters, one of which is malondialdehyde (MDA). As natural plant, solo garlic has a potential as antioxidant because contain a lot of active compounds. This study determines the effect of solo garlic on MDA level of Rattus norvagicus which exposed to cigarette smoke. This study is experimental animal study which use randomized posttest only control group design. Twenty-five rats were included and divided into five groups, such as negative control, positive control, and the treatment groups. Solo garlic administrated at doses of doses of 2.5, 5, and 10 (mg/200gBW/day) respectively. After the treatments, MDA level for each group is C- group 4.206 ± 0.772; C+ group 6.132 ± 0.860; T1 group 5.467 ± 0.434; T2 group 4.573 ± 0.588; and T3 group 4.458 ± 0.386. The statistical result showed that solo garlic extract can reduce the MDA level of treatment groups (p=0.000). This study concluded that solo garlic extract can reduce the MDA levels of rats to cigarette smoke. The significant effect was obtained at a dose of 10 mg/200gBW/day.

Keywords: *Allium sativum;* Cigarette Smoke; Garlic Extract; Malondialdehyde; Rats

INTRODUCTION

Oxidative stress is as a crucial factor in the initiation and development of various non-communicable diseases (NCDs). It is estimated that as many as 41 million people succumb to NCDs each year, equivalent to 74% of all global deaths. In 2030, the global mortality rate associated with NCDs is predicted to reach 55 million (Arifin dkk., 2022). Oxidative stress occurs when the levels of oxidants, particularly reactive oxygen species (ROS), surpass the antioxidant capacity to neutralize them. ROS trigger oxidative damage through the activation of redox-sensitive transcription factors, protein kinase activation, opening of ion channels, lipid peroxidation, protein modification, and DNA oxidation (Juan dkk., 2021). These processes lead to cellular and tissue death, subsequently manifesting as various diseases such as cancer, cardiovascular disease, neurological disorders, metabolic conditions, rheumatic autoimmune diseases, among others (Martemucci et al., 2023). Elevated levels of malondialdehyde (MDA), recognized as a biomarker resulting from lipid peroxidation process, effectively indicate oxidative stress in those various health conditions, highlighting the associated oxidative damage (Dharmajaya & Sari 2022).

One of the most common causes of oxidative stress is cigarette smoke. Cigarettes contain more than 4500 gases and chemicals, including carbon monoxide,

nicotine, oxidants, delicate particulate matter, and aldehydes (Meles dkk., 2021). These components trigger the formation of free radicals through the uncoupling of endothelial nitric oxide synthase (eNOS), activation of xanthine oxidase, and the mitochondrial electron transport chain (METC). The accumulation of free radicals, especially reactive oxygen species (ROS) from cigarette smoke, creates an imbalance between oxidants and antioxidants in the body, leading to oxidative stress (Ardiana dkk., 2021).

The process of oxidative stress can be halted to prevent cellular damage through the use of antioxidants, whether synthetic or natural. One of potent natural antioxidant which substantiated by several studies is garlic. Garlic (*Allium sativum*) is herb that is commonly used in food and natriceuticals. It goes through two different bulbing processes. In the first type, lateral buds form in the axils of some of the youngest leaves, typically leading to the development of multi-clove bulbs, often alongside the formation of inflorescence. In the second type, the lateral bud transition into storage leaves, and the sprout enclosed within the storage leaf gradually becomes dormant as the storage leaf grows. This process typically results in the production of single-clove garlic bulbs or known as solo garlic (Shang dkk., 2019). Solo garlic contains the main phytochemicals content such as peptides, steroids, saponin, terpenoids, flavonoids, and phenols. It also has organosulfur compounds of S-allyl-L-cysteine sulfoxides (alliins) which will be converted into allicin as the precursor of various allyl sulfides such as diallyl disulfide (DADS), diallyltrisulfide (DATS), diallyl sulfide (DAS), E-ajoene, Z-ajoene, thioacroleins and vinyldithiins. Compared with multi clove garlic, solo garlic extract had a higher phenolic compounds and sulfide content, thus showed stronger antioxidant activity (Januarti, Taufiq & Sulistyaningsih, 2019). These bioactive compounds in solo garlic play a role as a chain-breaking antioxidant for scavenging free radicals include intracellular ROS. It also increase superoxide dismutase, catalase, glutathione, and glutathione-s-transferase as antioxidant enzyme (Dinu dkk., 2023).

Previous studies have evaluated the antioxidant effects of solo garlic. Naji dkk. (2017) found that, in rabbits with hepatic damage induced by carbon tetrachloride (CCl4), solo garlic increased antioxidant capacity and more pronounced elevation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) levels compared to conventional garlic. Another experimental study on mice fed a high-fat diet suggested that solo garlic could be a potential treatment to ameliorate oxidative stress by increasing antioxidant enzymes such as superoxide dismutase (SOD) and decreasing pro-inflammatory cytokines such as TNF-α (Arifah dkk., 2020). In rat model exposed to cigarette smoke, the ethanol extract of garlic demonstrated the ability to decrease leucocytes and inflammatory cells infiltration thus prevent lung damage (Savira dkk., 2023). This study aimed to determine the effect of solo garlic extract on malondialdehyde level in rat exposed to cigarette smoke.

METHOD

1. Experimental Design

This study has obtained ethical approval by Health Research Ethics Committee in Faculty of Medicine, Universitas Airlangga (Ethical Clearance No. 270/EC/KEPK/FKUA/2020). A randomized posttest only control group design was used in this animal experimental study. The rats were divided into the following five groups: negative control group (C−) without any treatment; positive control group (C+) with exposure of three cigarettes smoke per day; and treatment groups (T1, T2, and T3) with exposure of three cigarettes smoke per day plus administration of black garlic extract at doses of 2.5, 5, and 10 (mg/200gBW/day) respectively (Khaerunnisa dkk.,

2019; Arifah dkk., 2020). Exposure to cigarette smoke and extract administration were carried out for 14 days. On the 15th day, all rats in each group were sacrificed. The blood samples were collected via cardiac puncture for examination of serum MDA levels.

2. Materials and Tools

This research utilized male wistar strain rats obtained from the experimental animal unit at the Laboratory of Biochemistry, Faculty of Medicine, Universitas Airlangga. The rats were 3 months old with a body weight ranging from 150 to 200 grams. The sample size was determined using the federer formula, resulting in a total of twenty-five rats distributed into five groups. The rats were housed in standard cages, maintained under 12 h light and dark cycles in the room temperature of 25–30ºC, and received a standard diet and water ad libitum.

Each rat was exposed to three conventional filtered cigarettes per day at different times (morning, noon, and afternoon) using smoke pump (Khaerunnisa dkk., 2019). The cigarette smoke exposure system consisted of a glass box with several holes, a rubber tube with three-way iron pipe, and a 50cc syringe which were interconnected. Briefly, a cigarette placed on the iron pipe was sucked and pumped into the glass box repeatedly until burned out using a syringe which is connected to glass box by rubber tubes. One cigarette take about 5 minutes of pumping process.

Solo garlic (*Allium sativum* L.) was purchased from UPT Materia Medica, Batu, East Java, Indonesia. Solo garlic was extracted using the maceration method, wherein it was initially dried, subsequently peeled, and then pulverized into powder. Dry powder was macerated in 70% ethanol solution for 72 h. This solution was filtered dan evaporated using a rotatory evaporator and thereafter using a waterbath (Abubakar & Haque, 2020). The obtained extract was dissolved in a 0.9% NaCl saline solution and administered to the rats via oral gavage.

3. MDA Measurement

The measurement of MDA levels was conducted using the Thiobarbituric Acid Reactive Substances (TBARS) assay as described by De Leon & Borges (2020) with modifications. A volume of 0,5 ml blood sample was added to 4.5 ml concentrated solution. The supernatant formed was added to 1 mL of 15% TCA solution. Subsequently, 1 ml of a 0.37% TBA solution in 0.25 N HCl was added. The solution was heated in a water bath at 80ºC for 15 minutes and then cooled to room temperature for 60 minutes. After cold, it was centrifuged at 3000 rpm for 15 menit. The absorption of the MDA supernatant was measured using a spectrophotometer at $\lambda = 532$ nm.

4. Statistical Analysis

Statistical Analysis was performed using IBM SPSS Statistics 25 software. All data were presented as mean ± standard deviation. The normality assumption for the entire dataset was evaluated using the Shapiro-Wilk test, while the homogeneity of variances was examined through Levene's Statistics. One-way ANOVA test and post-hoc Tukey HSD were used for comparative analysis with significance level of p value < 0.05.

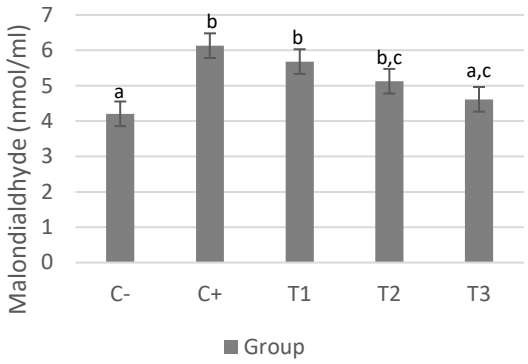
RESULT AND DISCUSSION

This study compared the serum malondialdehyde (MDA) levels in wistar rats among the negative control, positive control, and three treatment groups. The MDA levels of 25 rats, divided into 5 groups, were descriptively analyzed by calculating the mean and standard deviation, as presented in Table 1. The lowest serum MDA level was observed in the negative control group without treatment, while the highest serum MDA level was found in the positive control group exposed to 3 cigarettes per day. The groups treated with exposure to 3 cigarettes per day and garlic extract administration at different doses exhibited sequential means from high to

low, namely treatment 1 (2.5 mg/200gBW/day), treatment 2 (5 mg/200gBW/day), and treatment 3 (10 mg/200gBW/day).

Table 1. The results MDA level of the groups after cigarette smoke exposure and garlic extract treatment

Gro	n	MDA (nmol/ml) (Mean ± SD)	One-Way ANOVA
C-	5	4.206 ± 0.772[a]	
C+	5	6.132 ± 0.860[b]	
T1	5	5.467 ± 0.434[b]	p = 0.000
T2	5	4.573 ± 0.588[b, c]	
T3	5	4.458 ± 0.386[a, c]	



Picture 1: Average MDA Levels by Group after cigarette smoke exposure and garlic extract treatment. C-: negative control group, C+: positive control group, T1: treatment group which reveived 2.5 mg/200gBW/day, T2: treatment group which reveived 5 mg/200gBW/day, T3: treatment group which reveived 10 mg/200gBW/day

Table 2. The results of post-hoc one-way ANOVA test (*p* value) between groups. **p*<0.05

Group	C-	C+	T1	T2	T3
C-	-	0.001*	0.013*	0.028*	0.156
C+		-	0.317	0.184	0.009*
T1			-	0.321	0.027*
T2				-	0.098*
T3					-

To conduct parametric statistical tests, the data must be normally distributed and homogeneous. Normality test was performed using the Shapiro-Wilk test, resulted in p<0.05, indicating normal data distribution. Meanwhile, homogeneity test using the Levene test resulted in p>0.05, signifying homogeneous data. Comparative statistical analysis was carried out using the One-Way ANOVA test, followed by the Tukey HSD post hoc test to determine significant differences between groups. The ANOVA test resulted a p=0.000. The significance values between groups from the Tukey HSD post hoc test are presented in Table 2. The direct comparison of MDA levels in each group is also presented as a diagram (Picture 1 and table 2).

Based on the research findings and statistical tests, the serum malondialdehyde (MDA) levels in the positive control group were significantly higher compared to the negative control group ($p < 0.05$). This indicates that exposure to three cigarettes per day for 14 days can increase the serum MDA levels in rats. In line with this study, Levinna, Suyono, & Chiuman (2023) found that exposure to three cigarettes per day in rats for 14 days increased MDA levels, reduced superoxide dismutase (SOD) enzyme activity, and caused lung tissue damage characterized by septal destruction, inflammatory cell infiltration, and pulmonary edema. Exposure of cigarette smoke for 28 days in rats has also been shown to increase serum MDA levels and carotid artery intima-media thickness (cIMT) as a marker of atherosclerosis (Khaerunnisa dkk., 2019). In a further study using a different method, exposure to forty cigarettes for ten rats over four weeks led to an increase in MDA levels, a decrease in SOD activity, a decrease in endothelial nitric oxide synthase (eNOS), an increase in cIMT, and the disorganization and vacuolation of smooth muscle cells in aortic tissue (Ardiana dkk., 2021).

Cigarette smoke is a source of exogenous free radicals. The two primary components of cigarette smoke, tar and gas phase, have been shown to contain high concentrations of oxidants that can initiate the

generation of ROS, which are reactive molecules that are constantly formed by the enzymatic reactions within the body cell (Meles at al., 2021). ROS compounds are produced by several oxidase enzymes, including nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, uncouple endothelial nitric oxide synthase (eNOS), cyclooxygenase (COX), glucose oxidation, lipoxygenase, and mitochondrial electron transport (Ahmadkhaniha, Yousefian, & Rastkari, 2021). A variety of ROS and reactive nitrogen species (RNS) has been found to be involved in the disease pathology mechanism including radical superoxides (O^{-}_{2}), radical hydroxyl (OH.), hydrogen peroxide (H2O2), heme ferryl species protein, radical peroxyl (ROO), radical peroxyl nitrite ($ONOO^{-}$), nitrite oxide (NO), and radical nitrogen dioxide (NOO-) (Caliri, Tommasi, & Besaratinia, 2021).

The imbalance between oxidants and antioxidants inside the human body has the potential to cause damage through a process known as oxidative stress. It is a cellular, organic, or organism-wide metabolic condition that is characterized by an oxidative excess. These oxidants or free radicals especially ROS can cause oxidative damage by triggering lipid peroxidation, DNA modification, protein degradation, and enzymatic antioxidant disorders in cells which causes cell death (Juan dkk., 2021). A lipid peroxidation process occurs when free radicals attack lipids that have double bonds between carbon and carbon in their structure. When unsaturated fatty acids are converted to lipid peroxide in the phospholipids of the cell membrane, MDA is the result (Dharmajaya & Sari, 2022). Malondialdehyde (MDA) is one of oxidative stress biomarker whose levels will increase as free radicals increase in the body. This marker has often been identified in patients with atherosclerosis, certain types of cancers, neurodegenerative diseases, and lung illnesses, especially those due to inflammation (Martemucci et al., 2023).

In this study, the administration of solo garlic extract in three different doses is expected to act as an antioxidant, effectively reducing the serum MDA levels in rats exposed to cigarette smoke. According to our results, as showed in Table 2 and Picture 1, the serum MDA levels in the treatment groups, specifically T3 group, were significantly lower than the positive control group (p<0.05). Meanwhile, the MDA levels in the T1 and T2 groups did not show significantly different values compared to the positive control. This indicates that the administration of solo garlic extract in rats exposed to three cigarettes per day for 14 days can decrease serum MDA levels. The dosage that exhibited a significant effect commenced at 10 mg/200gBW/day. These findings align with the research conducted by Proverawati & Jehloh (2023), which stated that the administration of solo garlic extract in rats exposed to E-cigarette for 28 days could restore MDA levels to a normal state. Naji dkk. (2017) also observed that in rabbits with hepatic damage induced by CCl4, solo garlic extract exhibited antioxidant activity, as evidenced by the improvement in liver profiles and oxidative stress markers such as a decrease in MDA and peroxidase (POx), along with an increase in catalase (CAT) and superoxide dismutase (SOD). Meanwhile, in type 1 diabetic rats with a nephropathy model induced by streptozotocin (STZ), aqueous garlic extract was found to ameliorate kidney damage based on histopathological parameters of kidney tissue and levels of glucose, urea, and uric acid. This is presumed to occur through the antioxidant and anti-inflammatory mechanisms of garlic, as evidenced by improvements in the MDA profile, total oxidant status (TOS), total antioxidant capacity (TAC), nitrite oxide (NO), and tumor necrosis factor-alpha (TNF-a) (Nasiri dkk., 2017).

Solo garlic exerts protective effects against many chemical agent induced oxidative stress damage. This may be attributed to the presence of numerous bioactive compounds that scavenge free radicals and restore or enhance the activity of antioxidant defence system thus reducing MDA contents in serum and organs (Proverawati & Jehloh, 2023). Solo garlic has a remarkable potency to lowering of free radicals by some mechanism such as impaired initiation and propagation of the peroxidative process, act as a chain breaking antioxidant for scavenging free radicals, and donate electrons to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction (Farhat dkk., 2023; El-Saber Batiha dkk., 2020).

The main bioactive compound in solo garlic include phenolic compounds, flavonoid, and organosulfur compounds such as alliin, allicin, and its derivatives (Shang dkk., 2019). Investigation of garlic extracts for total phenolic content and antioxidant capacity showed that solo garlic has greater result when compared to multi clove or conventional garlic (Naji dkk., 2017). The antioxidant activity of phenolic compounds is mainly due to their redox properties, allowing them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Flavonoids found in solo garlic act to capture ROS against hydroxyl radicals, superoxide anions, peroxyl and alkoxyl radicals, and as metal chelators (Januarti, Taufiq & Sulistyaningsih, 2019). In addition, the saponin content is also quite effective in reducing the level of lipid peroxidation and increasing the antioxidant defense system in the serum, liver, and pancreas. Allicin derivatives such as ajoene, z-ajoene, diallyl sulfide, diallyl trisulfide, and s-allyl cysteine sulfoxide act as an antioxidant by increasing SOD, CAT, and glutathione peroxidase (GPX) activity (El-Saber Batiha dkk., 2020). These first line defence antioxidants are able to block reactions in free radical chains when overproduced or when cellular antioxidant system break down. It also can reduce the activity of free radicals that can create cellular damage directly such as ROS, hydrogen peroxide (H2O2), and others oxidant thus prevent lipid peroxidation (Sharifi-Rad dkk., 2020).

Previous studies support our findings that solo garlic is a good natural antioxidant source which protects cellular membranes and lipoproteins against peroxidation caused by oxidative damage. This is substantiated by the decrease in the serum MDA as an oxidative stress biomarker in rats exposed to cigarette smoke and administered solo garlic extract. The limitation of this study is the short duration of treatment and the limited parameters, preventing a comprehensive evaluation of the effects of cigarette smoke exposure and solo garlic extract administration. A longer treatment duration is required to observe changes in various laboratory parameters and organ histopathology resulting from oxidative stress induction and the antioxidant effects provided. However, this study is the first to discover the antioxidant effect of solo garlic extract, particularly on MDA levels in rats exposed to cigarette smoke within specific dosage parameters.

CONCLUSION

Solo garlic extract (*Allium Sativum*) can reduce the serum MDA levels in rats exposed to cigarette smoke. The significant effect was obtained at a dose of 10 mg/200gBW/day. These findings demonstrate that solo garlic, with its bioactive compounds, exhibits antioxidant activity against oxidative stress. The use of solo garlic extract as an herbal antioxidant is suggested, given its potent effect in protecting the body from free radicals, such as those found in cigarette smoke. Further research is needed with a longer treatment duration and testing of laboratory biomarkers along with tissue histopathology to evaluate the antioxidant

effects of solo garlic comprehensively. Further research can be carried out by standardizing the phytochemical content in Solo Garlic extract and other oxidative stress parameters to produce more comprehensive research outcomes.

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