

Effects of *Allium sativum* Linn. (Solo Garlic) Extract in Increasing Superoxide Dismutase (SOD) Levels in *Rattus norvegicus* Strain Wistar Exposed by E-Cigarette

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ABSTRACT

Background: The popularity of E-Cigarette has increased significantly over the last decade, and has potential to replace tobacco cigarettes. Aerosol which is produced by E-Cigarette, contains reactive carbonyl and free radicals, which is inducing oxidative stress. This oxidative stress can be seen through the Malondialdehyde (MDA) levels in the body, and reduced by antioxidant activity in Solo Garlic extract through Superoxide Dismutase (SOD) enzyme activity.

Materials and Methods: The aim of this study is analyzing the differences levels of SOD in four treatment groups. Twenty eight white rats were divided into four groups, three of them were exposed by E-Cigarette, and another one was a control group. Two treatment groups were given solo garlic extract dose 0,05 gram/day and 0,10 gram/day. The average of SOD levels in these four treatment groups was analyzed using One Way ANOVA test.

Result: This study showed that the lowest SOD levels was found in group which was exposed by E-Cigarette only, amount 88,431 U/ml. SOD levels can be increased to 93,814 U/ml by the lowest dose of solo garlic extract (0,05 gram/day), which is higher than the control group (92,669 U/ml).

Conclusion: It can be concluded, that using solo garlic extract less than 0,05 gram/day can restore SOD levels due to E-Cigarette exposure to a normal state.

Keywords: E-Cigarette, Superoxide Dismutase (SOD), and *Allium sativum* Linn. (Solo Garlic).

1.0 Introduction

Electronic Cigarette (E-Cigarette) is a kind of vapor product consisting of battery-powered device, heating element, and solution in the cartridge or commonly called E-Liquid (Flora, *et.al.*, 2016). E-Liquid contains nicotine, water, propylene glycol, glycerin, and fluxes derived from tobacco. E-Liquid also contains chemicals flavoring to make good smell of the smoke which is produced by E-Cigarette. The heating element in E-Cigarette vaporizes E-Liquid in the cartridge, and produces aerosols in both liquid and gas phase. Aerosols which is produced by E-Cigarette contains flavor and nicotine, and the users used to inhale and exhale of this aerosols, called "vaping".

One of major health concerns using E-Cigarettes is the toxic emission content in E-Cigarettes named carbonyl compounds such as formaldehyde, acetaldehyde, and acrolein. The other E-Cigarettes contents reported as free radical specimens are polyaromatic hydrocarbons, carcinogenic nitrisamines, and some toxic metals such as cadmium (Cd), nickel (Ni), and plumbun (Pb) (Taylor, *et.al.* 201). Iskandar, *et.al.* (2016) explains that aerosols exposure causes epithelial pseudostratification and goblet cells development that produce mucus, ciliary cells, and basal cells. It is based on studies of in vivo airway epithelial cell caused by E-Cigarette exposure which is containing aerosols. These results can be used to identify the toxicity mechanism due to aerosols exposure, which the respiratory epithelial cells response the stimulant (aerosols exposure) and produce various mediators that can affect the airway of epithelial cell system (Iskandar, *et.al.* 2016).

Propylene glycol (PG) and glycerol (GLY) which are solvents in E-Liquid, are also capable for producing high concentration of free radicals in E-Cigarette aerosols. Aerosols contain reactive carbonyl and free radicals which can induce oxidative stress and cause inflammation, proliferation, and cellular damage (Bitzer, *et al.* 2017). Oxidative stress is an imbalance condition between the present of free radicals and antioxidants in the body (Werdhasari, 2014). This oxidative stress can be seen by Malondialdehyde (MDA) levels in the body (Bhandari, 2012).

High concentrations of MDA in the body can be decreased through antioxidant activity that can fight free radicals. *Allium sativum* Linn. (solo garlic) contains more than one hundred secondary metabolites and is biologically useful, such as an antioxidant (Divya, *et.al.* 2017). Flavonoids and organo sulfur compounds are chemical components contained in solo garlic, and it works as an antioxidant. Solo garlic has better antioxidant power than the other species of garlic (Prasonto, *et.al.*, 2017). Secondary metabolites in solo garlic, form a complex chemical system as a self-defense mechanism from damage caused by microorganisms and other external factors (Hernawan & Setyawan, 2003).

The most well-known organosulfur compounds in solo garlic is Allicin, which is produced when solo garlic is crushed. Allicin is an active biologic component produced by the interaction between non-protein amino acids (Alliin) and enzyme amino acid (Allinase) (Rahman, *et al.*, 2012). Allicin and its derivative compounds will be metabolized to Alil methyl sulfide (AMS) which is an active metabolite. Allicin derivatives such as ajoene, z-ajoene, 3-vinyl-1,2-dithiin, diallyl sulfide, and diallyl trisulfide which also act as an antioxidant by increasing Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPX) activity (Werdhasari, 2014).

Antioxidant in solo garlic extract can also protect cells against Reactive Oxygen Species (ROS) through the activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Superoxide Dismutase (SOD) enzyme. Superoxide Dismutase (SOD) is an intracellular antioxidant defense mechanism that plays an important role in catalyzing the reduction of superoxide to oxygen and hydrogen peroxide. Non-stressful cells will conduct to form and remove O_2^- in a balance way, but under stress conditions, ROS can damage body's defense system and make Superoxide Dismutase (SOD) as the front-line defense against ROS (Alscher, et al., 2002).

Based on the co-factor metal used in the enzyme, SOD can be classified into three groups: FeSOD, MnSOD, and CuZnSOD. These whole types of SOD regulate various normal physiological aspects simultaneously, so an abnormal SOD can lead to the pathology of some diseases. Various studies mention that SOD plays an important role in cancer cells development. The activity of SOD is able to protect cells from O_2^- which can damage cells. O_2^- can increase cell's ability to divide and causes cells abnormal growth (Alscher, et al., 2002). SOD essentially evolves to protect cells from damage caused by O_2^- .

Low levels of SOD in the blood, damages cells growth that are also affected by high levels of ROS. Cancer cells development is also caused by an excessive adaptation of high levels of ROS in the blood, and it causes irregular cellular proliferation. Normal cellular proliferation is strictly regulated by redox cycle due to SOD activity. In an abnormal conditions, disregulated enzymes damage normal cell cycle and it causes cancer potentially (Case, 2017). SOD enzyme measurements can be performed by using gel activity test or enzymatic test to determine the activity of SOD, CAT, and GPX which is used to understand the biological effects of antioxidant enzyme activity (Weydert & Cullen, 2009).

2.0 Materials and Methods

This study is an experimental research using Randomized Controlled Trial (RCT) with Post Test Control Group Design on white rats (*Rattus norvegicus strain Wistar*) as research sample. This study will analyze the differences of Superoxide Dismutase (SOD) levels in each group as a parameter of free radical due to E-Cigarette exposure which is observed after 28-day using solo garlic extract (Suryadinata, 2016). This research was conducted in Biochemistry Laboratory, Department of Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, and took place from May 2nd to June 4th 2018. Samples used in this study were 28 white rats aged 3-4 months and weights 180-200 grams.

Solo garlic extract as an antioxidant to increase SOD levels, was made by maseration method. This method is generally used for heat unresistant materials such as solo garlic. Maseration method won't eliminate main active content as antioxidants. It also uses 96% ethanol solvent to make solo garlic extract. SOD levels measurement is done at Biochemistry Laboratory, Faculty of Medicine, Airlangga University, Surabaya. The measurement of total plasma SOD enzyme activity started by preparing SOD stock solution. SOD activity was measured by the rate of inhibition of ferisitoxrome c reduction by superoxide anions produced through xanthine oxidase. Xanthine was oxidized to uric acid, while the superoxide anion which is formed subsequently reduces ferisitochrome c. The reduction of ferisitochrome c was observed based on increasing of absorbance at 550 nm wavelength.

The data of Superoxide Dismutase (SOD) levels that have been collected, then analyzed to see the role of solo garlic extract as an antioxidant. One Way Anova test was used to see the differences of Superoxide Dismutase (SOD) levels between four treatment groups (control group, E-Cigarette exposure group, solo garlic extract group dose 0,05 gram/day and solo garlic extract group dose 0.10 gram/day). To see the difference of SOD levels in each group, could be analyzed using Least Significance Difference (LSD).

3.0 Result

3.1 The Average of Superoxide Dismutase (SOD) Levels

The average of Superoxide Dismutase (SOD) levels in this study can be seen in the distribution table below:

Table 1: The Average of Superoxide Dismutase (SOD) Levels

Group	Mean \pm SD	Maximum Levels (U/ml)	Minimum Levels (U/ml)
I	92,669 \pm 1,436	94,158	90,378
II	88,431 \pm 1,261	90,034	86,598
III	93,814 \pm 1,392	94,845	91,065
IV	94,616 \pm 1,369	96,220	92,784

Note:

Group I : control group (without E-Cigarette exposure and solo garlic extract)

Group II : E-Cigarette exposure without solo garlic extract

Group III : E-Cigarette exposure and solo garlic extract dose 0,05 gram/day

Group IV : E-Cigarette exposure and solo garlic extract dose 0,10 gram/day

The average diagram of Superoxide Dismutase (SOD) levels in each group can be seen from the figure below:

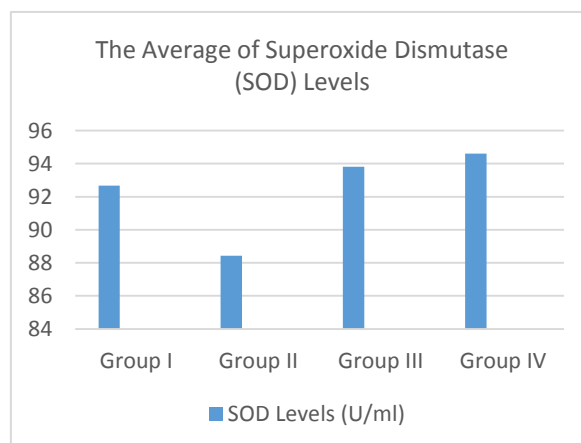


Figure 1: The Average of Superoxide Dismutase (SOD) Levels

Based on the diagram in figure 1 above, it can be seen that the highest SOD level (94,616 U/ml) was found in group IV, and the lowest one was found in group II (88,431 U/ml).

3.2 Test of Homogeneity of Variance

The first statistical test used was Test of Homogeneity of Variance to test the variance of homogeneous data using Lavene Test. It showed that the value of significance or p (0,930) $> \alpha$ (0,05), so we can conclude that H_0 is accepted. It means that the data variance of Superoxide Dismutase (SOD) level is homogeneous, and can be proceed using One Way Analysis of Variance (One Way ANOVA) test to determine the differences between the four treatment groups.

3.3 One Way ANOVA Test

The results of One Way ANOVA test in all groups showed that the value of significance or p (0,000) $< \alpha$ (0,05), so it can be concluded that H_0 is rejected which means there is at least one different treatment groups.

3.4 Multiple Comparisons Post Hoc Test of Superoxide Dismutase (SOD) Levels

Post Hoc Multiple Comparisons Test was used to see different levels of Superoxide Dismutase (SOD) in all groups, and the results can be seen below:

Table 2: Multiple Comparisons Post Hoc Test of Superoxide Dismutase (SOD) Levels

Group	Value of Significance (p) in Each Group			
	I	II	III	IV
I	-	0,00	0,162	0,023
II	0,00	-	0,00	0,00
III	0,162	0,00	-	0,321
IV	0,023	0,00	0,321	-

To determine the difference levels of Superoxide Dismutase (SOD) in each group, that is indicated by significance value or $p < \alpha$ (0,05). Based on the data above, it can be seen that there is difference SOD levels between group I and the other groups in this study. Post Hoc Multiple Comparisons test also explains that there are some groups having significance value or $p > \alpha$ (0,05). It indicates that there is no difference in SOD levels between two groups which are compared. Based on the result in this study, we find out that there is no difference SOD levels between group I and group III. It also occurs in the comparison between group III and group IV, where the whole groups have significance value or $p > \alpha$ (0,05).

4.0 Discussion

The aim of this study is analyzing the role of antioxidant contents in solo garlic extract for increasing Superoxide Dismutase (SOD) levels. Based on the research that has been done in four treatment groups, showed that the highest average value of superoxide dismutase (SOD) levels was found in group IV, amount 94.616 U/ml. The lowest average value of Superoxide Dismutase (SOD) levels was found in group II, amount 88,431 U/ml.

This study also showed differences SOD levels between four treatment groups, except the comparison of SOD levels between group I and group III. Comparison between group III and group IV also showed that there is no difference SOD levels. Based on these results, it can be concluded that SOD levels in each group is different, although there are two pairs of groups that do not have significant differences in SOD levels.

Based on these results, it can be concluded that group I does not have significant differences in SOD levels with group III. It shows that using solo garlic extract dose 0.05 gram/day (smallest dose) can restore SOD levels as normal as group I. Comparison of SOD levels between these two groups, showed that SOD levels in group III (93,814 U/ml) was higher than in group I (92,669 U/ml), so using solo garlic extract less than 0.05 gram/day can restore SOD levels just as normal in group I amount 92,669 U/ml.

The results are consistent with the theory that Superoxide Dismutase (SOD) is an antioxidant enzyme which plays an important role in protecting cells from damage, caused by Reactive Oxygen Species (ROS) (Azadmanesh & Borgstahl, 2018). SOD performs a bioprotective role by converting superoxide to oxygen (O_2) and hydrogen peroxide (H_2O_2) by cyclic oxidation and reduction reaction to active metals (Azadmanesh & Borgstahl, 2018). Superoxide Dismutase (SOD) enzyme consists of four antioxidant enzymes such as FeSOD, MnSOD, CuZnSOD, and NiSOD. While the process of superoxide or free radical reduction in the mitochondria is performed by MnSOD via catalyst mechanism (Case, 2017).

Antioxidant activity is also found in *Allium sativum* Linn. (solo garlic), which have higher concentration of organosulfur compounds compared to other *Allium* species (Naji, *et al.*, 2017). Organosulfur compounds such as Alliin (S-alilsistein sulphoxide) and Allinase enzyme work together to produce Allicin which acts as an antioxidant enzyme, or better known as a hydroxyl radical inhibitor. These components can also increase antioxidant enzymes activity, such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathion Peroxidase (GPX), and Glutathione-s-Transferase which are useful for boosting immune system (Naji, *et al.*, 2017).

Based on this analysis, it can be concluded that solo garlic extract can increase antioxidant enzyme activity such as SOD, CAT, and GPX through organo sulfur compounds contained as active substance in solo garlic. This was demonstrated by increasing levels of superoxide dismutase (SOD) in group III (using smallest dose of solo garlic extract) compared to group II (E-Cigarette exposed only). Whereas the lowest SOD levels was found in group II (E-Cigarette exposed without solo garlic extract).

5.0 Conclusion and recommendation

Allium sativum Linn. (solo garlic) contains high concentration of active substances that can increase Superoxide Dismutase (SOD) enzyme activity which acts as an antioxidant to fight free radicals. This was demonstrated by SOD levels in group III (the smallest dose) was higher than in group II (E-Cigarette exposed only). Group III had an average SOD levels amount 93,814 U/ml, whereas group II had an average SOD levels amount 88,431 U/ml.

Group I as a control group, did not have significant difference of SOD levels compared to group III, so using the smallest dose of solo garlic extract (0,05 gram/day) can restore SOD levels in normal state. The mean comparison of SOD levels between these two groups, showed that the average SOD levels in group III (93,814 U/ml) was higher than in group I (92,669), so using solo garlic extract less than 0,05 gram/day was recommended to restore SOD levels as normal as control group.

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The ethical clearance of this research had been accepted and proof with ethical approval certificate from Health Research Ethics Comittee Faculty of Public Health, Airlangga University.

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Declaration

Author(s) declare that there's no conflict of interest in this research.

Authors contribution

Author 1: Concept, idea, data collection, statistical analysis, and manuscript writing

Author 2: Concept, idea, literature review and manuscript writing

Author 3: Concept, idea, literature review, and manuscript writing

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