ISSN 1978-2071 (Print); ISSN 2580-5967 (Online)
Jurnal Ilmiah Kedokteran Wijaya Kusuma 7(2) : 149-157, September 2018

# Pengaruh Ekstrak Bawang Putih Tunggal dalam Penurunan Kadar Malondialdehid (MDA) akibat Pemaparan Rokok Elektronik

**Aliefia Meta Duwairoh[1]*, Bambang Wirjatmadi[2], Merryana Adriani[3]**
Master Programme of Public Health Nutrition Department, Faculty of Public Health, Airlangga University, Surabaya[1]
Public Health Nutrition Department, Faculty of Public Health, Airlangga University, Surabaya[2,3]
Jl. Dr. Ir. H. Soekarno, Mulyorejo, Surabaya, East Java (60115)
* e-mail: aliefiameta.d@gmail.com

**Abstrak**

Popularitas Rokok elektronik telah meningkat secara signifikan selama dekade terakhir, dan memiliki potensi untuk menggantikan rokok tembakau. Aerosol yang diproduksi oleh Rokok elektronik mengandung karbonil reaktif dan radikal bebas, menginduksi stres oksidatif dan menyebabkan peradangan, proliferasi, dan kerusakan sel. Stres oksidatif ini dapat dilihat melalui tingkat malondialdehid (MDA) di dalam tubuh. Tingkat MDA mungkin dikurangi oleh aktivitas antioksidan dalam ekstrak bawang putih tunggal. Tujuan dari penelitian ini adalah untuk menganalisis perbedaan tingkat MDA empat kelompok perlakuan. Dua puluh delapan tikus putih dibagi menjadi empat kelompok dan tiga diantaranya dipapar oleh Rokok elektronik. Penelitian ini menunjukkan bahwa tingkat MDA tertinggi (7,179 nmol / ml) ditemukan pada kelompok yang dipapar oleh Rokok elektronik tanpa pemberian ekstrak bawang putih tunggal. Tingkat malondialdehid dapat dikurangi hingga 3,715 nmol / ml oleh aktivitas antioksidan dari kelompok yang dipapar oleh Rokok elektronik bersama ekstrak bawang putih tunggal dengan dosis terkecil. Rata-rata tingkat MDA dalam kelompok dengan dosis ekstrak bawang putih tunggal terkecil lebih rendah daripada kelompok kontrol. Jadi, dapat disimpulkan bahwa menggunakan ekstrak bawang putih tunggal kurang dari 0,05 gram / hari dapat memulihkan tingkat MDA ke keadaan normal (4.210 nmol / ml).

**Kata Kunci**: Rokok elektronik, Malondialdehid (MDA), Bawang putih tunggal

# *The Effect of Solo Garlic Extract in Decreasing Malondialdehyde (MDA) Levels due to E-Cigarette Exposure*

***Abstract***

*The popularity of E-Cigarette has increased significantly over the last decade, and has potential to replace tobacco cigarettes. Aerosol produced by E-Cigarette contains reactive carbonyl and free radicals, inducing oxidative stress and causes inflammation, proliferation, and cell damage. This oxidative stress can be seen through the malondialdehyde (MDA) levels in the body. The MDA level might be reduced by antioxidant activity in solo garlic extract. The aim of this study was to analyze the differences of MDA levels four treatment groups. Twenty-eight white rats were divided into four groups and three of them were exposured by E-Cigarette. This study showed that the highest MDA level (7,179 nmol/ml) was found in the group which exposed by E-Cigarette without solo garlic extract administration. Malondialdehyde level can*

*be reduced to 3,715 nmol/ml by antioxidant activity of the group which exposed by E-Cigarette along with smallest dose of solo garlic extract administration. The average of MDA level in the group with smallest dose of solo garlic extract was lower than the control group. So, it can be concluded that using solo garlic extract less than 0,05 gram/day may restore MDA levels to a normal state (4,210 nmol/ml).*

***Keywords:*** *E-Cigarette, Malondialdehyde (MDA), and Solo Garlic.*

## 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, 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 which 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 speciments 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. Based on the 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, when 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).

Most E-Liquid (99%) contain nicotine, so “vaping” exposes its users to the effects of nicotine toxicity such as cardiovascular disease, activating carcinogenic pathways including proliferation, angiogenesis, apoptotic suppression, and promotion of cell motility (Alexander *et al,* 2015). Aerosols that contain reactive carbonyl and free radicals, are also capable in the inducing oxidative stress such as inflammation, proliferation, and cellular damage (Bitzer *et al*, 2017).

Oxidative stress is an imbalance condition between the number of free radicals and antioxidants in the body (Werdhasari, 2014). Oxidative stress can be seen through the concentration of Malondialdehyde (MDA) in the body (Bhandari, 2012).

MDA is an aldehyde compound derived from lipoperoxidation and produce additional covalent substances that can damage proteins, and its accumulation will also damage tissue (Traverso *et al,* 2004). Oxidative damage in proteins can occur through an indirect mechanism involving the production of aldehydes which is derived from lipoperoxidation such as MDA and 4-hydroxynonenal (HNE). These aldehydes can spontaneously bind proteins, modify and produce fluorescent additions. Glycation is another spontaneous protein modification, which begins from a nonenzymatic binding to reduce sugar to free amino. This process is known as the first step in the occurrence of the Maillard series that causes changes in proteins (such as flux development, functional deficiency, fragmentation, or aggregation). MDA fluctuations indicate oxidative damage to proteins that permit metal and ROS absorption

High concentrations of MDA in the body can be reduced through antioxidant activity which 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 work as an antioxidant. Solo garlic extract that acts as an antioxidant can also protect cells against Reactive Oxigen Species (ROS) through the activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Superoxide Dismutase (SOD) enzyme.

Organo sulfur compounds are also capable for lowering lipid levels in the blood, and working as antithrombotic, blood anticoagulation, antihypertensive, anticancer, antioxidant, and antimicrobial (Rahman *et al,* 2012). The most well-known antioxidant from organo sulfur compounds in solo garlic is Allicin, which is produced by crushed solo garlic. Allicin is an active biologic component produced by the interaction between non-protein amino acids (Alliin) and amino acid enzyme (Allinase) (Rahman *et al,* 2012). Allicin and its derivative compounds will be metabolized into 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 also play an important role as an antioxidant (Werdhasari, 2014). Allicin and its derivatives are the result of degradation

of Alliin (cysteine sulfoxide), when garlic is cut or otherwise processed, Alliin breaks out of its compartment and interacts with the Allinase enzyme to form Allicin.

## MATERIAL 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 Wista*r* as research sample. This study will analyze the differences of Malondialdehyde (MDA) levels in each group as a parameter of free radical due to the E-Cigarette exposure which is observed after 28-day using solo garlic extract (Suryadinata, 2016). This research was conducted in the Biochemistry Laboratory, Department of Biochemistry, Faculty of Medicine, Airlangga University, Surabaya, and this study took place from May 2nd to June 4th 2018. Samples used in this study were wistar white rats that were kept in the Biochemistry Laboratory, Faculty of Medicine, Airlangga University, Surabaya. Samples used in this study were 28 white rats between aged 3-4 months and weights 180-200 grams.

Measurement of Malondialdehyde (MDA) levels was conducted at the Biochemistry Laboratory, Faculty of Medicine, Airlangga University, Surabaya. MDA levels were measured using a spectrophotometer on a UV-VIS spectrophotometer with a maximum wavelength 535 nm. MDA levels showed in nmol/ml using a calibration curve and expressed as a percentage based on comparison of MDA levels with the control group. The maceration method is used to make solo garlic extract as an antioxidant to decrease MDA levels. This method is generally used for materials that is heat unresistant like solo garlic, and it won't eliminate the main contents of solo garlic as an antioxidant. This maceration method uses 96% ethanol solvent to make solo garlic extract.

The data of Malondialdehyde (MDA) 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 Malondialdehyde (MDA) levels between four treatment groups (control group, E-Cigarette exposure group, solo garlic extract group 0,05 gram/day and solo garlic extract group 0.10 gram/day). Further analysis was done to see the differences of each group by using Least Significance Difference (LSD).

## RESULT

The average of Malondialdehyde (MDA) levels in this study can be seen in the distribution table below:

**Table 1:** The Average of Malondialdehyde (MDA) Levels

| Group | Mean ± SD | Max Levels (nmol/ml) | Min Levels (nmol/ml) |
|---|---|---|---|
| I | 4,210 ± 0,102 | 4,313 | 4,057 |
| II | 7,179 ± 0,150 | 7,346 | 6,984 |
| III | 3,715 ± 0,101 | 3,837 | 3,552 |
| IV | 3,222 ± 0,102 | 3,314 | 3,029 |

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

Based on the table 1 above, it can be seen that the highest average of Malondialdehyde (MDA) level (7,179 nmol/ml) was found in group II, and the lowest one was found in group IV (3.222 nmol/ml). The first statistical test used was Test of Homogeneity of Variance which to test the variance of the homogeneous data using Lavene Test. Based on the Test of Homogeneity of Variance, it showed that the value of significance or p (0.625) > α (0.05), so we can conclude that Ho is accepted. It means that the data variance of Malondialdehyde (MDA) 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.

Based on One Way ANOVA test, it showed that the value of significance or p (0,000) < α (0,05). So it can be concluded that Ho is rejected which means there is at least one different treatment groups. Post Hoc Multiple Comparisons Test was used to see different levels of Malondialdehyde (MDA) in all groups. The result showed that the ratio of Malondialdehyde (MDA) levels as a whole has differences in each treatment group. So, it can be concluded that there is difference MDA levels between group I and the other groups in this study.

## DISCUSSION

The aim of this study was to analyze the role of antioxidant contents in Allium sativum Linn. (Solo Garlic) in lowering Malondialdehyde (MDA) levels. The result showed that the highest average of Malondialdehyde (MDA) levels was in group II (7,179 nmol/ml), and the lowest one was in group IV (3,222 nmol/ml). The results of this study also indicate a significant difference in Malondialdehyde

(MDA) levels between four treatment groups.

Based on the comparative statistical test between group I and group III, it was found that there were differences of MDA levels in both groups. The results showed that using solo garlic extract with the smallest dose had significant effect on decreasing MDA levels. The average comparison for both groups showed that MDA levels in group III (3.715 nmol/ml) was lower than in group I (4,210 nmol/ml). Solo garlic extract lower than 0.05 gram/day to restore MDA levels as normal as in group I (control group).

The results are in accordance with the theory that the active ingredients contained in E-Cigarettes can induce levels of Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), and acetaldehyde compounds commonly called free radicals. Free radicals have very unstable characteristics, and are able to interact in a complex biological system to trigger oxidative stress when neutralizing or antioxidant molecules cannot fight free radicals (Bello *et al,* 2017). Reactive Oxigen Species (ROS) that trigger oxidative stress are formed in the human body in: cytosol, mitochondria, lysosomes, peroxisomes, and plasma membranes that eventually lead to neural and cellular damage (Khajehnasiri *et al,* 2013).

Khajehnasiri (2013) also explained that the occurrence of oxidative stress due to free radical activity that cannot be resisted by antioxidants in the body is characterized by high concentrations of Malondialdehyde (MDA) in the blood. Malondialdehyde (MDA) is produced by free radicals that undergo lipid peroxidation. This process destroys membrane, then causes some damage such as: DNA damage, cell damage, and neurological disorders (Khajehnasiri *et al,* 2013). Based on these analyzes, it can be concluded that the activity of free radicals due to E-Cigarette exposure causes oxidative stress and characterized by high levels of Malondialdehyde (MDA) in the blood. This is indicated by the highest levels of MDA in group II, this group is only exposured by E-Cigarette smoke without solo garlic extract.

The biochemical and pathological processes of free radicals play an important role in the development of some damages such as DNA damage, cell damage, and neurological disorders caused by oxidative stress. This process can be controlled by antioxidants that have three central enzymes in the oxidative stress pathways. They are Superoxide Dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Sahu *et al,* 2013). Antioxidants are divided into two systems:

enzymatic and non-enzymatic systems. The enzymatic system involves those enzymes which are produced by the organism itself, such as Superoxide Dismutase (SOD) and catalase (CAT). SOD acts as a defense system while CAT acts on hydrogen peroxide (H2O2) (Sahu *et al,* 2013).

Based on these analyzes, high levels of Malondialdehyde (MDA) due to the E-Cigarette exposure can be neutralized by antioxidant activity in Allium sativum Linn. (solo garlic) extract. Antioxidants in Allium sativum Linn. (solo garlic) such as: SOD, CAT, and GPX are able to block reactions in free radical chains when overproduced or when cellular antioxidant defense systems break down. These three enzymes can reduce activity of free radicals that can create cellular and neurological damage such as: Reactive Oxigen Metabolite (ROM), super anion oxide (O2-), hydroxyl radicals (0OH), and hydrogen peroxide (H2O2) (Sahu *et al,* 2013).

Solo garlic has the lowest IC50 value, compared with other types of garlic. This explains that 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 and Setyawan, 2003). The most well-known organosulfur compounds in solo garlic is Allicin, which is produced when this substance 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, also act as an antioxidant by increasing SOD, CAT, and GPX activity (Werdhasari, 2014).

An antioxidant activity capable on fighting free radicals was demonstrated by group IV in this study, where the group was induced by E-Cigarette and solo garlic extract dose 0,10 gram/day. This group had the lowest levels of Malondialdehyde (MDA) compared to the other groups. Meanwhile, using the smallest dose of solo garlic extract was able to decrease the MDA levels almost the same as the control group. Based on these results, it can be concluded that high levels of Malondialdehyde (MDA) due to E-Cigarette exposure can be decreased by antioxidant activity found in Allium sativum Linn. (solo garlic) extracts such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPX).

## CONCLUSION

From this study can be conducted that e-Cigarette exposure increases Malondialdehyde (MDA) levels and is shown by group II (E-Cigarette exposure without solo garlic extract), having the highest Malondialdehyde (MDA) levels (7,179 nmol/ml). Malondialdehyde (MDA) levels can be decreased by antioxidant activity in Allium sativum Linn. (solo garlic) extract and is shown by group III (E-Cigarette exposure and solo garlic extract 0,05 gram/day), whereby MDA levels can already be lowered to 3,715 nmol/ml with this smallest dose. The average of MDA levels in group III was smaller than those in group I (control group), so using solo garlic extract less than 0,05 gram/day could restore MDA levels to normal state as in group I (4,210 nmol/ml).

## ACKNOWLEDGEMENT

The Authors are very thankful to the colleague, Public Health Nutrition Department, Faculty of Public Health, Airlangga University and Biochemistry Laboratory, Department of Biochemistry, Faculty of Medicine, Airlangga University, Surabaya for their valuable support research assistance and cooperation.

## REFERENCE

Alexander LEC, Vyas A, Schraufnagel DE, and Malhotra A, 2015. Electronic Cigarettes: The New Face of Nicotine Delivery and Addiction. *Journal of Thoracic Disease*. 7(8): E248-51

Bello HA, Dandare A, and Danmaliki GI, 2017. Effects of Cigarette Smoking On Lipid Peroxidation and Serum Antioxidant Vitamins. *IOSR Journal of Pharmacy and Biological Sciences*. 44(5): 725-738

Bhandari, Prasan R, 2012. Garlic (Allium sativum L.): A Review of Potential Therapeutic Applications. *International Journal of Green Farmation*. 6(2): 118-129

Bitzer ZT, Goel R, Reilly SM, Elias RJ, Foulds JT, Muscat JT, and Richie JP, 2017. Effect of Flavouring Chemicals on Free Radical Formation in Electronic Cigarette Aerosols. *Journal of Free Radical Biology and Medicine*. 120: 72-79

Divya BJ, Suman B, Kumar, LL, Venkataswamy M, Eswari B, and Thyagaraju K, 2017. The Role of Allium sativum (Garlic) in Various Disease and Its Health Benefits: A Comprehensive Review. *International Journal of Advanced Research*. 5(8): 592-602.

Flora JW, Wilkinson CT, Wilkinson JW, Lipowicz, PJ, Skapars JA, Anderson A, and Miller, John H, 2016. Method for the Determination of Carbonyl Compounds in E-Cigarette Aerosols. *Journal of Chromatographic Science*. 55(2): 142-148

Hernawan UE., dan Setyawan AD, 2003. Review: Senyawa Organosulfur Bawang Putih (Allium sativum Linn.) dan Aktivitas Biologinya. *Jurnal Biofarmasi*. 1(2): 65-76

Iskandar AR, Gonzalez-Suarez I, Majeed S, Marescotti D, Sewer A, Xiang Y *et al,* 2016. A Framework for in Vitro Systems Toxicology Assesment of E-Liquid. *Journal of Toxicology Mechanism and Methods*. 2(6): 392-416

Khajehnasiri F, Mortazavi SB, Allameh A, Akhondzadeh S, and Hashemi H, 2013. Resarch Article: Total Antioxidant Capacity and Malondialdehyde in Depresssive Rotational Shift Workers. 120: 1-5

Prasonto D, Riyanti E, dan Gartika, M. 2017. Uji Aktivitas Antioksidan Ekstrak Bawang Putih (Allium sativum). *ODONTO Dental Journal*. 4(2): 122-128

Rahman MM, Fazlic V, and Saad NW, 2012. Antioxidant Properties of Raw Garlic (*Allium sativum*) Extract. *International Food Research Journal*. 19(2): 589-591

Sahu A, Varma M, and Kachhawa Kamal, (2013). A Prognostic Study of MDA, SOD, and Catalase in Breast Cancer Patients. International *Journal of Sciene and Research*. 4(5): 157-159

Suryadinata, Rivan V, 2016. Peranan Ekstrak Curcumis melo LC dalam Menurunkan Kadar Malondialdehyde dan Hiperplasia Sel Goblet pada Dinding Bronkus Paru dengan Paparan Asap Rokok. *Tesis*. Fakultas Kesehatan Masyarakat Universitas Airlangga

Taylor M, Carr T, Oke O, Jaunky T, Brehemy D, Lowe F, and Gaca M, 2016. E-Cigarette Aerosols Induce Lower Oxidative Stress inVitro When Compared to Tobacco Smoke. *Journal of Toxicology Mechanism and Method*. 26(6): 465-476

Traverso N, Menini S, Maineri EP, Patriarca S, Odetti P, Cottalasso D, Marinari, UM, and Pronzato MA, 2004. Malondialdehyde, A Lipoperoxidation-Derived Aldehyde, Can Bring About Secondary Oxidative Damage to Proteins. *Journal of Gerontology Biological Science*. 59(9): 890-895

Werdhasari, Asri, 2014. Peran Antioksidan Bagi Kesehatan. *Jurnal Pusat Biomedis dan Teknologi Dasar Kesehatan Balitbangkes, Kemenkes RI*. 3(2): 59-68.