

POTENTIAL OF GREEN TEA INFUSION IN REDUCING LUNG MDA LEVELS OF BALB/C MICE DUE TO EXPOSURE TO ELECTRIC CIGARETTE VAPOR

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ABSTRACT

This research aims to evaluate the potential of green tea infusion in reducing Malondialdehyde (MDA) levels in the lungs of mice exposed to electronic cigarette vapor. The research employed an experimental method with a Randomized Post-Test Only Control Group design. The research sample consisted of 20 male mice aged 3-4 months with an average weight of 30 grams, divided into four groups: normal group (CN), negative control (NC), treatment 1 with a dosage of green tea 0.03 g (T1), and treatment 2 with a dosage of green tea 0.06 g (T2). After the treatment, an analysis of MDA levels in the mice's lungs was conducted by TBARS test. The average levels of MDA in mice were obtained as follows: CN=12.669 μ M, NC=23.940 μ M, T1=10.997 μ M, T2=14.395 μ M. Statistical analysis using the One-Way ANOVA test revealed significant differences ($p < 0.05$) between CN and NC, NC and T1, and NC and T2. These findings indicate that green tea infusion has the potential to reduce MDA levels in the lungs of mice exposed to electric cigarette vapor. Thus, this research aims to provide a foundation for further studies regarding optimal dosage and the mechanism of action of green tea infusion in combating the negative effects of exposure to electronic cigarette vapor on respiratory organs.

Keywords : Green Tea., Electric Cigarette., Malondialdehyde

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi potensi infusa teh hijau dalam mengurangi kadar MDA (Malondialdehyde) pada paru-paru mencit yang terpapar uap rokok elektrik. Metode penelitian yang diterapkan adalah eksperimental dengan desain Randomized Post-Test Only Control Group. Sampel penelitian terdiri dari 20 ekor mencit jantan berumur 3-4 bulan dengan berat rata-rata 30 gram, yang terbagi menjadi empat kelompok penelitian, yaitu kelompok normal (CN), kontrol negatif (NC), perlakuan 1 dengan dosis teh hijau 0.03 g (T1), dan perlakuan 2 dengan dosis teh hijau 0.06 g (T2). Setelah perlakuan, dilakukan analisis terhadap kadar MDA pada paru mencit dengan uji TBARS. Didapatkan rata-rata kadar MDA mencit yaitu, CN=12.669 μ M, NC=23.940 μ M, T1=10.997 μ M, T2=14.395 μ M. Hasil analisis statistik menggunakan uji One-Way ANOVA menunjukkan adanya perbedaan bermakna antara CN dengan NC, NC dengan T1, dan NC dengan T2. Temuan ini mengindikasikan bahwa infusa teh hijau memiliki potensi dalam mengurangi tingkat MDA pada paru mencit yang terpapar uap rokok elektrik. Dengan demikian, penelitian ini bertujuan untuk memberikan dasar untuk penelitian lebih lanjut terkait dosis yang optimal dan mekanisme aksi infusa teh hijau dalam melawan dampak negatif paparan uap rokok elektrik pada organ pernapasan.

Kata Kunci : Teh hijau., Rokok Elektrik., Malondialdehid

INTRODUCTION

According to the World Health Organization (WHO) data, there are approximately 225,700 deaths annually in Indonesia due to smoking and other tobacco-related diseases. With this high mortality rate, it can be assessed that there is still no precise solution to reduce these elevated death rates. The emergence of electronic cigarettes or vapes has added to the number of active smokers, contributing to the overall increase in both active and

passive smokers. Based on the results of the Global Adults Tobacco Survey (GATS) in 2021, there are approximately 69.1 million active smokers and 120 million passive smokers. This is expected to lead to more diseases and ultimately result in fatalities^{1,2}.

A vape is an electronic cigarette that does not burn tobacco but produces vapor by heating a special liquid^{3,4}. The liquid contains several chemicals, such as nicotine, propylene glycol, glycerin, water, and certain flavors, which create a taste in the

inhaled vapor⁵. The chemical content in the inhaled liquid, including nicotine, toxic flavors, and others, will naturally settle in the respiratory tract, causing damage to the respiratory system⁶.

The use of e-cigarettes has been proven to increase MDA levels, indicating that e-cigarettes cause an increase in free radicals in the body. Malondialdehyde (MDA) is the final compound resulting from the body's lipid peroxidation. MDA levels are used as a marker for the presence of free radicals that damage tissue⁷⁻¹⁰. This increase can result in an imbalance of antioxidants and free radicals, leading to oxidative stress in the body¹¹. The factors that can play a role in the pathogenesis of various diseases such as COPD (Chronic Obstructive Pulmonary Disease), premature aging, cancer, etc¹²⁻¹⁴.

Antioxidants are compounds that can reduce and neutralize free radicals, thereby preventing tissue damage^{15,16}. One plant that contains high antioxidants is green tea¹⁷. The antioxidant content in green tea infusion, namely polyphenols, one of which is catechin. Polyphenolic compounds can neutralize free radicals by providing one of their free electrons, stabilizing the free radical compound, and preventing damage to cells in the body¹⁸. Based on this, green tea has the potential to be a compound that can reduce MDA levels resulting from exposure to electric cigarette vapor. Therefore, this study examines the potential of green tea infusion in reducing lung MDA levels due to electric cigarette vapor.

MATERIALS & METHOD

The research design employed in this study is an experimental design known as a randomized post-test-only control group design. Observations were conducted after the treatment to assess the effectiveness of the independent variable in influencing the dependent variable. Changes in the untreated group (normal group/CN) were compared with the group exposed to electronic cigarette vapor and only given distilled water (negative control group/NC), the group exposed to electronic cigarette vapor and administered green tea infusion at dose 1 (0.03g green tea) (treatment group 1/T1), as well as the group exposed to electronic cigarette vapor and administered green tea infusion at dose 2 (0.06g green tea) (treatment group 2/T2). Subsequently, the data were analyzed using the TBARS method to assess the lung MDA levels.

The experimental animals utilized in this study were mice (*Mus musculus*), specifically male, aged 3-4 months. The research samples consisted of the lungs of male mice. The sample size used is based on the resource equation method formula as follows:

$$E = \frac{\text{Total number of test animals} - \text{Total number of experimental groups}}{\text{Total number of experimental groups}}$$

With E needing to be in the range of 10-20. The research subjects are divided into 4 groups: normal control group (CN), negative control group (NC), treatment group 1 (T1), and treatment group 2 (T2). According to this method, 5 mice per group are used, totaling 20 male mice. This research was conducted at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, consisting of the Histology and Cell Biology Division and the Biochemistry and Molecular Biology Division located on Jl. PB. Sudirman, Sudirman Campus. The activities included in the study involve the maintenance and adaptation of experimental animals for 7

days, the preparation of green tea infusion, the experimental treatment involving the exposure of mice for 30 days, and the collection and analysis of research data. The research was conducted starting from February - Juni 2023.

The statistical analysis of MDA levels was performed using the Statistical Package for the Social Sciences (SPSS) computer program. The normality of the data was assessed using the Shapiro-Wilk test, followed by a test for homogeneity using the Levene statistic. If the data were both normal and homogeneous, the data analysis would involve the One-Way ANOVA test to determine the significance of each group. Subsequently, the Least Significant Difference (LSD) test would be conducted. The statistical tests were conducted with a confidence level of 95% ($p < 0.05$). This research has been approved by the Research Ethics Commission of the Faculty of Medicine, Udayana University, through ethical clearance with letter number 959/UN14.2.2.VII.14/LT/2023.

RESULT

Based on the research results regarding the potential of green tea infusion in reducing lung MDA levels due to electronic cigarette vapor, numerical data were obtained. The data were collected from the TBARS test on the lung proteins of mice. The obtained data will be categorized into four groups according to the research groups, namely Normal Control (CN), Negative Control (NC), Treatment 1 (T1), and Treatment 2 (T2). Five data sets were collected for each research group, resulting in a total of 20 research data sets (Table 1).

Table 1. MDA levels (μM)

No	CN	NC	T1	T2
1	13.279	25.444	12.246	8.311
2	6.393	29.650	8.656	10.672
3	13.180	24.855	6.443	18.126
4	14.509	28.093	14.557	16.192
5	15.981	11.656	13.082	18.673
Mean	12.669	23.940	10.997	14.395

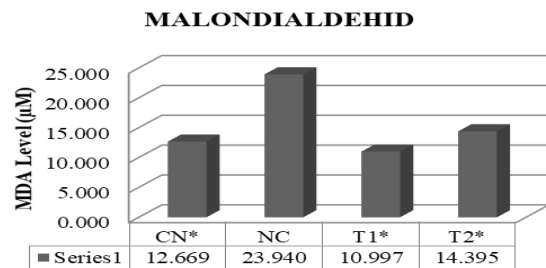


Figure 1. The graph illustrating the mean results of the MDA levels (μM). *The MDA levels are significantly different from the NC group.

The data analysis results indicate that the data are normally distributed and homogeneous. The One-Way ANOVA test results show significance or a significant difference in the data among the research groups, with a p-value of 0.003. In the Least Significant Difference (LSD) test, significant differences were found between the Normal

Control (CN) group and Negative Control (NC) with $p=0.002$, Negative Control (NC) and Treatment 1 (T1) with $p=0.001$, and Negative Control (NC) and Treatment 2 (T2) with $p=0.007$ (Figure 1).

DISCUSSION

Based on the One-Way ANOVA statistical test results, there is a significant difference among the normal control group, negative control group, treatment 1 (with a dose of 0.03 g), and treatment 2 (with a dose of 0.06 g) in reducing lung MDA levels due to exposure to electronic cigarette vapor, with a p -value of <0.05 . This result indicates that the administration of green tea infusion overall has a significant impact in reducing oxidative stress in the lungs of mice exposed to electronic cigarette vapor. Furthermore, the Least Significant Difference (LSD) test shows that the administration of green tea infusion at doses 1 and 2 significantly lowers lung MDA levels compared to the negative control. This suggests that the doses of green tea used in this study were sufficient to create a suppressing effect on the increase in MDA levels.

These findings can be explained by the abundant antioxidant content in green tea, such as epigallocatechin gallate (EGCG) and other catechins, which have been proven to possess free radical-fighting properties. With the presence of abundant antioxidants, green tea helps neutralize free radicals that may enter the lungs, preventing an imbalance between free radicals and antioxidants. Thus, green tea infusion plays a role in inhibiting oxidative stress, which, in turn, prevents lipid degradation and an increase in MDA levels¹⁸. This notion is corroborated by Agnes (2023), who found that administering green tea kombucha prevents the elevation of MDA and the reduction of eNOS in male Wistar rats exposed to nicotine vapor from electronic cigarettes¹⁹.

The significance of these antioxidants becomes clearer when looking at the results of the Least Significant Difference (LSD) test, which indicates a significant difference between the normal control group and the negative control group. This finding suggests that exposure to electronic cigarette vapor can increase free radical levels in the body, causing an imbalance that triggers oxidative stress. Without additional antioxidants, the body undergoes lipid degradation due to oxidative stress, reflected in the increase in MDA levels corresponding to the amount of free radicals entering the body. Consistent with the study by Husfa *et al.*, which showed that green tea could reduce MDA levels in mice serum induced by alloxan²⁰.

Meanwhile, treatment group 1 and treatment group 2, given green tea infusion, had lower MDA levels compared to the negative control group, as shown in Table 1. There is a significant difference between the negative control group and both of these groups. These groups received antioxidant intake from green tea infusion, allowing them to neutralize incoming free radicals. Looking at Table 1, the MDA levels of treatment group 1 are even lower than the normal control group. However, this is not the case with treatment group 2, which has slightly higher MDA levels than the normal control, although not significantly different.

Based on this, green tea infusion with a dose of 0.03 g can effectively reduce MDA levels compared to green tea infusion with a dose of 0.06 g, although the difference is not significant. This finding suggests that a low dose of green tea may already be sufficient to provide a maximum antioxidant effect, while a higher dose does not offer significant additional benefits.

CONCLUSION AND SUGGESTION

Based on the analysis of the research findings, it can be concluded that the administration of green tea infusion inhibits the increase in lung MDA levels due to exposure to electronic cigarette vapor. Based on the results of this research, the recommendation is that further studies are needed to explore the potential of green tea in reducing or investigating its impact on diseases related to MDA.

REFERENCES

1. Safitri IA, Suryawan A, Wicaksono B. Hubungan antara Tingkat Paparan pada Perokok Pasif dengan Volume Oksigen Maksimal (VO₂max) pada Remaja Usia 19-24 tahun. *Nexus Kedokt Komunitas*. 2016;5(1):69–78.
2. Putra AI, Hanriko R, Kurniawaty E. Pengaruh Efek Paparan asap Rokok Elektrik Dibandingkan Paparan Asap Rokok Konvensional Terhadap Gambaran Histopatologi Paru Mencit Jantan (*Mus musculus*). *Majority*. 2019;8(1):90–4.
3. Fitriani K, Mustafa Z. Penggunaan Rokok Elektrik (Vape) di Kota Makassar Perspektif Hukum Islam. *J Ilm Mhs Perbandingan Maz*. 2020;1(2):113–35.
4. Damayanti A. Penggunaan Rokok Elektronik di Komunitas Personal Vaporizer Surabaya. *J Berk Epidemiol*. 2016;4(2):250–61.
5. Diva Widyantari D, Lestari R. Dampak Penggunaan Rokok Elektrik (Vape) terhadap Risiko Penyakit Paru. *Lomb Med J*. 2023;2(1):34–8.
6. Aprilia Nurul Aini TH. Pengaruh Paparan Asap Rokok Elektrik (Vapour) dengan Berbagai Variasi Dosis terhadap Gambaran Histologi Bronkiolus Mencit (*Mus musculus*). *J Prodi Biol*. 2018;7:590–7.
7. Mulianto N. Malondialdehid sebagai Penanda Stres Oksidatif pada Berbagai Penyakit Kulit. *Cermin Dunia Kedokt*. 2020;47(1):39–44.
8. Ananda Savitri IDAA, Sugiritama IW, Arijana IGKN, Linawati NM. Pengaruh Pemberian Ekstrak Etanol Kulit Buah Naga Merah (*Hylocereus polyrhizus*) Terhadap Kadar MDA (Malondialdehyde) Liver. *J Med Udayana*. 2023;12(1):40–6.
9. Zaetun S, Kusuma Dewi LB, Rai Wiyadna IB, Srigede L. Profil Kadar Mda (Malondialdehyde) Sebagai Penanda Kerusakan Seluler Akibat Radikal Bebas Pada Tikus Yang Diberikan Air Beroksigen. *J Anal Med Biosains*. 2019;4(2):63–8.
10. Nufus I, Lisdiana, Marianti A, Peniati E. Pengaruh Nikotin dalam Rokok Elektrik Terhadap Kadar MDA dan SOD pada Darah Tikus. *Life Sci*. 2020;9(2):161–70.
11. Bangsa FM, Retnoningrum D, Bhima SKL. Pengaruh Inhalasi Cairan Rokok Elektrik Terhadap Kadar Malondialdehid Serum Tikus. *J Kedokt Diponegoro*. 2019;8(3):970–8.

12. Suryadinata RV. Pengaruh Radikal Bebas Terhadap Proses Inflamasi pada Penyakit Paru Obstruktif Kronis (PPOK). *Amerta Nutr.* 2018;2(4):317–24.
13. Iskandar B, Tartilla R, Lukman A, Leny L, Surboyo MDC. Uji Aktivitas Anti-aging Mikroemulsi Minyak Nilam (*Pogostemon cablin Benth.*). *Maj Farmasetika.* 2022;7(1):52–64.
14. Mulia K, Hasan AEZ, Suryani. Total Phenolic, Anticancer and Antioxidant Activity of Ethanol Extract of Piper retrofractum Vahl from Pamekasan and Karang Asem. *Curr Biochem.* 2016;3(2):80–90.
15. Arnanda QP, Nuwarda RF. Penggunaan Radiofarmaka Teknisium-99M Dari Senyawa Glutation dan Senyawa Flavonoid Sebagai Deteksi Dini Radikal Bebas Pemicu Kanker. *Farmaka.* 2019;17(2):236–43.
16. Pratiwi H A., Yusran, Islawati, Artati. Aalisis Kadar Antioksidan pada Ekstrak Daun Binahong Hijau *Anredera cordifolia* (Ten.) Steenis. *J Biol Makassar.* 2023;8(2):66–74.
17. Wibowo NK, Rudyanto M, Agus Purwanto D. Antioxidant Activity of Green Tea and Black Tea. *Clin Pharm Anal Pharm Community J.* 2022;1(2):48–55.
18. Nugraheni ZV, Rachman TM, Fadlan A. Ekstraksi Senyawa Fenolat dalam Daun Teh Hijau (*Camellia sinensis*). *Akta Kim Indones.* 2022;7(1):69–76.
19. Jonathan AL, Widyadharma IPE, Ayu Artini IG. Green Tea Kombucha Prevents Increased Malondialdehyde and Decreased Endothelial Nitric Oxide Synthase (eNOS) in Male Rats Exposed to Nicotine E-Cigarette Vapor. *Int J Res Rev.* 2023;10(5):547–52.
20. Husfa RK, Rustam E, Hasmiwati. Pengaruh Teh Hijau Terhadap Kadar Gula Darah dan MDA Serum Mencit Diabetes. *J Ilmu Kesehat Indones.* 2020;1(2):93–9.

