International Journal of Research and Review
Vol. 10; Issue: 5; May 2023
Website: www.ijrrjournal.com
Original Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Green Tea Kombucha Prevents Increased Malondialdehyde and Decreased Endothelial Nitric Oxide Synthase (eNOS) in Male Rats Exposed to Nicotine E-Cigarette Vapor

Agnes Lindrasari Jonathan[1], I Putu Eka Widyadharma[2], I Gusti Ayu Artini[3], Luh Putu Ratna Sundari[4], Ni Made Linawati[5], I Gusti Made Gde Surya Candra Trapika[6]

[1]Master Program in Biomedical Science, Anti-Aging Medicine Concentration, [2]Department of Neurology, [3,6]Department of Pharmacology, [4]Department of Physiology, [5]Department of Histology, Faculty of Medicine, Udayana University, Bali, Indonesia

Corresponding Author: Agnes Lindrasari Jonathan

DOI: https://doi.org/10.52403/ijrr.20230564

ABSTRACT

Background. Increased ROS accumulates oxidative stress which can be measured by increasing Malondialdehyde (MDA) and decreasing eNOS level. Green tea (*Camellia sinensis*) kombucha contains antioxidants, especially polyphenols that reduce ROS and prevent endothelial damage. This study aimed to determine the benefits of green tea kombucha in preventing increasing MDA and decreasing eNOS in male Wistar rats exposed to nicotine electric cigarette vapor.

Methods. A randomized pretest-posttest control group study was conducted in male Wistar rats (*Rattus norvegicus*) aged 2-3 months, weighing 150-200 grams. Thirty-five rats were randomly divided into negative, control, and three treatment groups that were given 0.5ml, 1 ml and 2 ml of green tea kombucha (1 hour before exposure to cigarette vapors). Before treatment, MDA and eNOS level were measured. After 14 days, posttest MDA and eNOS were tested and a comparative analysis were performed.

Results. Pre-post MDA levels showed significant differences ($p<0.05$). Meanwhile, the results of eNOS comparative test showed the average in the posttest group had a significant difference ($p<0.05$). Post Hoc test analysis showed treatment group 1 and 2 had significantly different mean eNOS levels compared to the control group ($p<0.05$), indicating 1ml green tea kombucha prevents increasing MDA levels and 0.5 and 1 ml prevents decreasing eNOS in male Wistar rats exposed to nicotine electric cigarette vapor.

Conclusions. Administration of green tea kombucha prevents an increase in MDA and decrease in eNOS in male Wistar rats exposed to nicotine e-cigarette vapor.

Keywords: Camellia sinensis, Kombucha, eNOS, Malondialdehyde, electric cigarette.

INTRODUCTION

Several studies have demonstrated an increased risk of oxidative stress from e-cigarettes.[1] The most commonly used biomarker of oxidative stress is malondialdehyde (MDA). MDA is the end product of lipid peroxidation due to the breakdown of fatty acid chains, which are toxic to cells. As a result of constant lipid peroxidation reactions, it can cause various diseases and accelerate the aging process. Low MDA levels are often associated with good antioxidant status, with normal plasma MDA levels ranging from 0.83 to 1.01 mol/L.[2,3]

The primary weapon of endothelial cells against vascular disease is endothelial nitric oxide synthase (eNOS), an enzyme that

produces the vasoprotective molecule nitric oxide (NO-).[4,5] However, risk factors such as cigarette smoking, hypertension, high blood sugar, or high blood lipids can damage endothelial cells.[6–8]

Green tea infusion contains many polyphenols, flavanols, flavonoids, and phenolic acids. Kombucha is one of the beverages that may protect against cell damage due to its antioxidant properties.[9] Sukrama (2105) conducted a study to reduce uric acid levels in rats by inhibiting xanthine oxidase and reducing MDA levels in rats that received 1 ml of kombucha daily for 14 days. The study showed significant results between the treatment group and the control group.[10] Aloulou et al. (2012) administered up to 5 ml of kombucha per kg of body weight to rats suffering from diabetes mellitus. They showed a positive effect on α-amylase and lipase inhibition.[11]

Understanding the basic concept of anti-aging medicine, we conclude that one way to prevent aging caused by free radicals is to administer antioxidants.[12] Considering the high content of active antioxidants in green tea kombucha, the authors intend to demonstrate the effect of green tea kombucha in preventing increased MDA levels and decreased eNOS levels after exposure to e-cigarette vapor as a source of free radicals.

MATERIALS & METHODS

Study design and experimental animals

This is an experimental study with a randomized pre-posttest control group design. The research was carried out at Udayana University. Kombucha used in the study was made at a local kombucha production site located in Mengwi, Badung, Bali. The experiment was carried out for eight weeks:

One week for preparations of tools and materials.

One week for mice adaptation.

Two weeks for pretest MDA and eNOS testing and treatment of the rats.

Four weeks to examine MDA and eNOS levels after treatment and analyze research data.

The sample needed in this experiment was at least 30 male Wistar rats (n=6), aged 2 to 3 months old, weighing 150-200 grams from the Pharmacology Laboratory of Udayana University. To anticipate dropout, 10% of the total sample was added, with a total amount of 35 rats which were divided into five groups (n=7).

Intervention Procedure

Thirty-five male Wistar rats, used as samples, were adapted for seven days. The cage used for the experiment was 40x30x20 cm and made of plastic. The top of the cage was covered with wire mesh, and the bottom was covered with rice husks to absorb the rats' feces. Holes were made on the side of the cage for ventilation and to insert the ventilation tube connected to the e-cigarette. The rats were kept under 12 hours of light and 12 hours of darkness (12-12h dark-light cycle). The temperature of the cage was maintained at 28-32°C.

After a seven-day adaptation period, 1 ml of blood was drawn from the medial orbital sinus on the eighth day to examine the pretest MDA and eNOS levels. The rats were then divided into five random groups with the same number of samples in each group. From day eight, group P0 (control) was administered a placebo in 1 ml of distilled water for 14 days. Groups P1, P2, and P3 received 0.5, 1, and 2 ml of green tea kombucha by gavage one hour before exposure to nicotine e-cigarette vapor. The naive/negative group was left without any treatment or exposure. The four groups of rats were placed in a cage to be exposed to nicotine e-cigarette vapor. Exposure was for 20 minutes each. After 14 days of treatment, blood samples were collected from the rats to check post-test MDA and eNOS levels. Rats were anesthetized with 10% ketamine at a dose of 50 mg/kg body weight and 2% xylazine at 20 mg/kg body weight and injected intramuscularly into the thigh. The

rats were then returned to the Udayana integrated biomedical laboratory.

STATISTICAL ANALYSIS

Statistical analysis using SPSS version 26 for Windows. One-way test ANOVA was performed to compare the different results between groups. Post-hoc test using Bonferroni method was used to detect specific differences between groups when ANOVA analysis was significant. The significance level between groups was adjusted with a p value <0.05 and a confidence interval of 95%.

RESULT

The normality test results using the Shapiro-Wilk done on MDA and eNOS levels for each group show that the variables in each treatment group are normally distributed (p>0.05). In order to compare pre-and post-differences in the treatment groups, a comparison test using one way ANOVA test was carried out. Meanwhile, to compare pre-and post-MDA and eNOS levels in each group, the t-dependent test was used. Homogeneity test on MDA and eNOS levels was tested using Levene's test. Based on the test, it is concluded that all variables were homogenous (p>0.05).

Significance analysis was tested using one way ANOVA test. Meanwhile, to compare the pre-post results of each treatment group, the t-dependent was used. The comparability test of MDA level between groups is presented in Table 1, and the pre-post Post Hoc test is in Table 2. Comparability test of eNOS level are shown in Table 3. and was continued using the Post Hoc test due to the significant result found inspire-post Post Hoc test (p<0.05). The results of the pre-post Post Hoc test are shown in Table 4.

Table 1. Comparability Test of MDA Level Between Groups

MDA Level (nmol/mL)	Pretest	Posttest	ΔPre-Post	P[a]
Naive	0.27±0.05	0.31±0.04	0.04±0.02	0.175
Control	0.18±0.01	0.50±0.05	0.31±0.04	0.001*
P1	0.39±0.03	0.55±0.08	0.15±0.06	0.045*
P2	0.34±0.05	0.41±0.06	0.07±0.02	0.032*
P3	0.32±0.02	0.46±0.07	0.14±0.05	0.047*
P[b]	0.089	0.111	0.012*	

a= analysis using t-dependent; b= analysis using one-way ANOVA

Table 2. ΔPre-posttest Post Hoc Test of MDA Level (nmol/mL) Between Groups

MDA Level	Control (Reff.)	P1	P2	P3
Control (Reff.)	1.000	0.179	0.010	0.106
P1	0.179	1.000	1.000	1.000
P2	0.010	1.000	1.000	1.000
P3	0.106	1.000	1.000	1.000

Table 3. Comparative Test of eNOS Level Between Groups

MDA Level	Pretest	Posttest	ΔPre-Post	P[a]
Naive	23.52±0.97	19.81±1.90	-3.71±1.62	0.071
Control	28.15±0.51	12.78±0.79	-15.36±0.90	<0.001*
P1	22.98±0.85	14.33±1.23	-8.65±1.50	0.002*
P2	21.64±0.92	11.38±0.14	-10.25±0.84	<0.001*
P3	23.25±0.91	10.60±0.88	-12.64±0.55	0.004*
P[b]	0.121	<0.001*	<0.001*	

a= analysis using t-dependent; b= analysis using one-way ANOVA

Table 4. ΔPre-posttest Post Hoc Test of eNOS Level (pg/mL) Between Groups

eNOS Level	Control (Reff.)	P1	P2	P3
Control (Reff.)	1.000	0.001*	0.011*	1,000
P1	0.001*	1.000	1.000	0,007
P2	0.011*	1.000	1.000	0.665
P3	0.429	0.068	0.665	1.000

DISCUSSION

This study showed that MDA level significantly increased in the pretest and posttest of the control group, followed by a decrease in eNOS after 14 days of exposure to e-cigarette vapor, proving that exposure to nicotine e-cigarette vapor increases MDA level and decreases eNOS level. The result is consistent with the study that e-cigarette nicotine vapor causes oxidative stress characterized by the production of ROS and lipid peroxidation markers, significantly increased MDA.[13] In a study by Suryadinata et al. (2019), exposure to nicotine e-cigarette vapor for 10 minutes per day for two weeks significantly increased serum MDA levels in Wistar rats.[14]

Nicotine e-cigarette vapor contains superoxide and other reactive substances that react with NO to form peroxynitrite (ONOO-). This oxidative stress leads to increased degradation of NO, lack of endothelial defense, and endothelial dysfunction.[7,15] Decreased NO bioavailability is a central mechanism in the pathophysiology of endothelial dysfunction. Endothelial nitric oxide synthase (eNOS) is an enzyme responsible for NO production in endothelial cells so the eNOS level may represent the availability of NO in endothelial cells.[16–18] This result is consistent with the findings of He et al. (2017), showing a significant decrease in eNOS levels in endothelial cell cultures exposed to cigarette smoke, which also decreased eNOS gene and protein expression, resulting in endothelial cell dysfunction. The eNOS-reducing effect depends on the duration of cell exposure. The longer the exposure, the lower the eNOS level.[15]

This study shows that 1 ml green tea kombucha administration prevented increased MDA levels ($p>0.05$) in male Wistar rats exposed to nicotine e-cigarette vapor. These results are similar to a study conducted by Sukrama (2015) in which the administration of 1 ml of kombucha tea to Wistar rats with hyperuricemia for 12 days prevented an increase in MDA. Administration of 0.5 ml green tea kombucha in this study may not have significantly affected MDA levels because 0.5 ml green tea kombucha did not contain antioxidants.[10]

In contrast, the dose of 2 ml green tea kombucha did not significantly affect MDA levels, possibly because it was close to the dose that caused side effects in previous reports when converted to the human dose. A study conducted by Bellassoued et al. (2015) reported a protective effect of administering 1 ml of green tea kombucha with an antioxidant capacity of 955 mg/L GAEAC on liver and kidney function. The kombucha used in this study has a capacity of 976.2 mg/L GAEAC. In this study, although green tea kombucha at a dose of 1 ml prevented an increase in MDA, the increase still occurred, and at a dose of 0.5 and 2 ml, the increase was not prevented.[19] These results suggest that although kombucha has an antioxidant effect, the optimal dose and duration of administration should be further explored.

The study showed that administration of 0.5 and 1 ml of green tea kombucha prevented the decrease of eNOS level ($p > 0.05$) in male Wistar rats exposed to nicotine e-cigarette vapor. These results suggest that the antioxidant content in 0.5 and 1 ml green tea kombucha effectively prevents endothelial damage and dysfunction caused by exposure to nicotine e-cigarette vapor. These results are consistent with an in vitro study by Lobo et al. (2017), which found that kombucha NO scavenged radicals 1.4 times more efficiently than regular tea and had a higher metal chelating capacity.[20] Gharib (2009) studied male albino rats exposed to trichloroethylene (TCE) as an oxidative stress trigger and used kombucha tea as an antioxidant. The results showed a significant NO reduction in the population receiving kombucha tea (0.1 ml/100 g body weight) compared to the control group.[21]

In this study, a dose of two ml of green tea kombucha did not show the expected effect on preventing a decrease in eNOS levels. This is likely due to the production of

alcohol and acetic acid during kombucha fermentation, which are pro-oxidants. In this study, the administration of 1 ml green tea kombucha prevented an increase in MDA, and the administration of 0.5 and 1 ml green tea kombucha prevented a decrease in eNOS levels in male Wistar rats exposed to nicotine e-cigarette vapor. By administering kombucha at these doses, cells, and organs are protected to enhance the quality of life by slowing aging by preventing an increase in ROS and oxidative stress.

CONCLUSION

Administration of the right dose green tea (*Camellia sinensis*) kombucha prevents increase in Malondialdehyde decrease in Endothelial Nitric Oxide Synthase (eNOS) in male Wistar rats exposed to nicotine electric cigarette vapor.

Declaration by Authors

Ethical Approval: Approved
Acknowledgement: None
Source of Funding: None
Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Khachatoorian C, Luo W, McWhirter KJ, Pankow JF, Talbot P. E-cigarette fluids and aerosol residues cause oxidative stress and an inflammatory response in human keratinocytes and 3D skin models. Toxicol In Vitro 2021;77:105234.
2. Khoubnasabjafari M, Ansarin K, Jouyban A. Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. BioImpacts BI 2015;5:123–7. https://doi.org/10.15171/bi.2015.20.
3. Salehi S, Sadeghi A, Karimi A. Growth performance, nutrients digestibility, caecum microbiota, antioxidant status and immunity of broilers as influenced by kombucha fermented on white sugar or sugar beet molasses. Ital J Anim Sci 2021;20:1770–80.
4. Erdogan M, Kulaksizoglu M, Tetik A, Solmaz S, Kucukaslan AS, Eroglu Z. The relationship of the endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) gene polymorphism in Turkish type 2 diabetic patients with and without diabetic foot ulcers. The Foot 2018;37:5–10. https://doi.org/10.1016/j.foot.2018.06.006.
5. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J 2012;33:829–37. https://doi.org/10.1093/eurheartj/ehr304.
6. Abdelghany TM, Ismail RS, Mansoor FA, Zweier JR, Lowe F, Zweier JL. Cigarette smoke constituents cause endothelial nitric oxide synthase dysfunction and uncoupling due to depletion of tetrahydrobiopterin with degradation of GTP cyclohydrolase. Nitric Oxide 2018;76:113–21.
7. Ardiana M, Pikir B, Santoso A, Hermawan H, Al-Farabi M. The effect of subchronic cigarette smoke exposure on oxidative stress parameters and endothelial nitric oxide synthase in a rat aorta. ARYA Atheroscler J 2021;17. https://doi.org/10.22122/arya.v17i0.2150.
8. Król M, Kepinska M. Human nitric oxide Synthase—Its functions, polymorphisms, and inhibitors in the context of inflammation, diabetes and cardiovascular diseases. Int J Mol Sci 2020;22:56.
9. Ahmed RF, Hikal MS, Abou-Taleb KA. Biological, chemical and antioxidant activities of different types Kombucha. Ann Agric Sci 2020;65:35–41.
10. Sukrama IDM. Xanthine oxydase inhibition of kombucha tea in hyperuricemia induced wistar rat: Decrease of uric acid, malondialdehyde, and 8-hydroxy-2'-deoxyguanosine. Bali Med J 2015;4:32–6.
11. Aloulou A, Hamden K, Elloumi D, Ali MB, Hargafi K, Jaouadi B, et al. Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. BMC Complement Altern Med 2012;12:1–9.
12. Silva S, Ferreira M, Oliveira AS, Magalhães C, Sousa ME, Pinto M, et al. Evolution of the use of antioxidants in anti-ageing cosmetics. Int J Cosmet Sci 2019;41:378–86. https://doi.org/10.1111/ics.12551.
13. Bjørklund G, Chirumbolo S. Role of oxidative stress and antioxidants in daily nutrition and human health. Nutr Burbank Los Angel Cty Calif 2017;33:311–21. https://doi.org/10.1016/j.nut.2016.07.018.
14. Suryadinata RV, Wirjatmadi B, Adriani M, Sumarmi S. The Effects Of Exposure Duration To Electronic Cigarette Smoke On Differences In Superoxide Dismutase And

Malondialdehyde In Blood Of Wistar Rats. Int J Curr Pharm Res 2019;11:13–6.
15. He Z, Chen Y, Hou C, He W, Chen P. Cigarette smoke extract changes expression of endothelial nitric oxide synthase (eNOS) and p16 (INK4a) and is related to endothelial progenitor cell dysfunction. Med Sci Monit Int Med J Exp Clin Res 2017;23:3224.
16. Bruno RM, Gori T, Ghiadoni L. Endothelial function testing and cardiovascular disease: focus on peripheral arterial tonometry. Vasc Health Risk Manag 2014;10:577.
17. Shu X, Keller TCS, Begandt D, Butcher JT, Biwer L, Keller AS, et al. Endothelial nitric oxide synthase in the microcirculation. Cell Mol Life Sci CMLS 2015;72:4561–75. https://doi.org/10.1007/s00018-015-2021-0.
18. Kumar G, Dey SK, Kundu S. Functional implications of vascular endothelium in regulation of endothelial nitric oxide synthesis to control blood pressure and cardiac functions. Life Sci 2020;259: 118377.
19. Bellassoued K, Ghrab F, Makni-Ayadi F, Pelt JV, Elfeki A, Ammar E. Protective effect of kombucha on rats fed a hypercholesterolemic diet is mediated by its antioxidant activity. Pharm Biol 2015;53: 1699–709.
20. Lobo RO, Dias FO, Shenoy CK. Kombucha for healthy living: evaluation of antioxidant potential and bioactive compounds. Int Food Res J 2017;24.
21. Gharib OA. Effects of Kombucha on oxidative stress induced nephrotoxicity in rats. Chin Med 2009;4:23. https://doi.org/10.1186/1749-8546-4-23.

How to cite this article: Agnes Lindrasari Jonathan, I Putu Eka Widyadharma, I Gusti Ayu Artini et.al. Green tea kombucha prevents increased malondialdehyde and decreased endothelial nitric oxide synthase (eNOS) in male rats exposed to nicotine e-cigarette vapor. *International Journal of Research and Review*. 2023; 10(5): 547-552.
DOI: *https://doi.org/10.52403/ijrr.20230564*

******