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THE ABILITY OF INFUSED GREEN TEA (*Camellia sinensis*) IN REDUCING OXIDATIVE STRESS LEVELS IN THE BLOOD OF BALB/C MICE EXPOSED TO ELECTRONIC CIGARETTE SMOKE

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ABSTRACT

Electronic cigarettes allow Reactive Oxygen Species (ROS) in their emissions, and ROS induce oxidative stress. Oxidative stress can be mitigated with antioxidants. Green tea can act as an exogenous antioxidant that can limit the development of free radicals by binding to ROS. This research aims to determine the potential of green tea infusion (*Camellia sinensis*) at doses of 0,03 g/head/day and 0,06 g/head/day in reducing the levels of malondialdehyde (MDA) oxidative stress in the blood of BALB/c mice exposed to electronic cigarette smoke. The research design is an *in vivo* experimental study with a Randomized Post Test Only Control Group design using 20 male BALB/c mice, divided into four groups: normal control group (KN), negative control group (K-) (exposed to electronic cigarette smoke), treatment group 1 (P1) (exposed to electronic cigarette smoke and given green tea infusion at a dose of 0,03 g/head/day), and treatment group 2 (P2) (exposed to electronic cigarette smoke and given green tea infusion at a dose of 0,06 g/head/day). The results showed a significant and meaningful difference in the mean comparison of malondialdehyde (MDA) levels in the blood of mice in all four groups, K- (14,706 µm/L) with KN (8,907 µm/L), P1 (7,069 µm/L), and P2 (6,654 µm/L). This study revealed that the administration of green tea infusion at doses of 0,03 g/head/day and 0,06 g/head/day is effective in reducing the levels of oxidative stress in the blood of mice exposed to electronic cigarette smoke.

Keywords : electronic cigarettes., green tea., oxidative stress., malondialdehyde

INTRODUCTION

Smoking is a familiar issue in society, and every year, the number of smokers worldwide continues to rise. According to data from the World Health Organization (WHO), it is estimated that there are about 6 million deaths per year due to smoking, and this number is projected to increase annually until reaching 8 million deaths per year by 2030.[1] Alongside the changing times, the use of electronic cigarettes has become an epidemic in society, both among teenagers and adults.[2]

Electronic cigarettes are devices created to reduce and stop cigarette use. It is important to note that electronic cigarettes have unclear physicochemical properties and emission toxicity, allowing the presence of Reactive Oxygen Species (ROS) in their emissions.[1–4]

Oxidative stress can occur due to an imbalance between antioxidants and free radicals entering the body.[3,5,6] Increased oxidative stress can pose potential health risks. A common biomarker used as an indicator of oxidative stress is malondialdehyde (MDA) because its levels are directly proportional to oxidative stress and can be measured using various existing methods, providing accurate results.[7,8] As exposure to electronic cigarettes triggers excessive ROS production, leading to oxidative stress, the body requires additional antioxidants, which can be sourced from green tea.

Green tea (*Camellia sinensis*) contains bioactive components called flavonoids, specifically catechins. Catechins are antioxidants that can reduce free radicals, which trigger oxidative stress.[9–12] Flavonoids are well known for their capacity to act as powerful antioxidants, providing strong protection against ROS.[11–13] Therefore, researchers are interested in conducting a study to demonstrate the ability of green tea infusion to reduce oxidative stress levels in BALB/c mice exposed to electronic cigarette smoke by checking the MDA levels in the mice's blood.

MATERIALS AND METHOD

This research employed an analytical, experimental design using the randomized posttest-only control group method. The study was conducted from April to June 2023 at the Integrated Biomedical Laboratory of the Division of Histology and Cell Biology and Biochemistry and Molecular Biology, Faculty of Medicine, Udayana University. Ethical clearance was obtained with the approval number 800/UN.14.2.2.VII.14/LT/2023 from the

Ethics Commission of the Faculty of Medicine, Udayana University.

The sample size was determined using the resource equation method, resulting in 20 mice. These mice were divided into four groups: one normal control group (KN) that received no treatment and three groups induced with electronic cigarette smoke. The induced groups included a negative control group (K-) exposed only to electronic cigarette smoke and two groups given green tea infusion concurrently with exposure to electronic cigarette smoke, with doses of 0,03 g/head/day (P1) and 0,06 g/head/day (P2). Afterward, the four groups were analyzed using the Thiobarbituric Acid Reactive Substance (TBARS) method to determine the blood MDA levels. The independent variables in this study were green tea infusion doses of 0,03 g/head/day (P1) and 0,06 g/head/day (P2), while the dependent variable was the blood MDA levels in mice. Control variables included gender, age, weight, diet, and the mice's environment.The test subjects were BALB/c mice, specifically males aged 3-4 months and weighing 30 grams. They were housed in cages measuring 35 × 30 × 15 cm, provided with 594-type feed, and bedded with autoclaved rice husks. The environment was maintained at a temperature of around 25-27 ℃ with 70% humidity. Before the commencement of the treatments, the mice were allowed to acclimate for one week.

The three groups (K-, P1, P2) were exposed to electronic cigarette smoke by placing them in a smoking box, while KN was not subjected to electronic cigarette smoke exposure. Over 30 days, the groups were exposed once daily for 30 minutes per exposure. Following the exposure period, P1 and P2 groups received oral gavage of green tea infusion. The green tea infusion prepared using dried green tea simplicial in powdered form, involved steeping the green tea powder in distilled water at 90℃ for 15 minutes. After 30 days, blood samples were collected from the mice via the intraorbital route, and then the levels of MDA in the blood were checked using the TBARS test.

The results of the study were processed using Statistical Product and Service Solutions (SPSS), employing one-way Analysis of Variance (ANOVA) for the initial analysis, followed by post-hoc analysis using the Least Significant Difference (LSD) test.

RESULT

Table 1. Blood MDA level measurement data (µm/L)

Sample Number	KN	K(-)	P1	P2
1	6,169	11,478	8,140	9,607
2	4,854	10,719	4,096	2,275
3	8,949	16,043	8,292	6,725
4	14,283	24,118	8,949	10,062
5	10,820	11,174	5,865	4,601
Mean	**8,907**	**14,706**	**7,069**	**6,654**

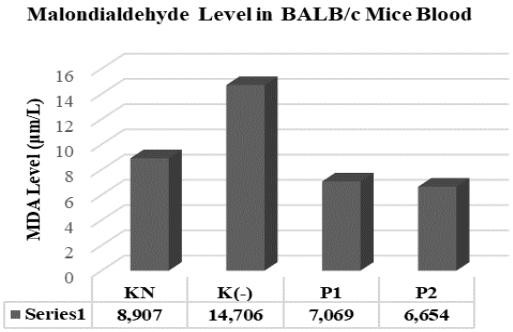

Figure 1. Graph of average MDA level

Based on the TBARS test results, the mean blood MDA level in mice given only distilled water without exposure to electronic cigarette smoke (Normal Control, KN) was 8,907 µm/L. In the negative control group (K-), which received distilled water and exposure to electronic cigarette smoke, the mean blood MDA level was 14,706 µm/L. In the treatment group 1 (P1), which received oral gavage of green tea infusion at a dose of 0,03 g/head/day and exposure to electronic cigarette smoke, the mean blood MDA level was 7,069 µm/L. In treatment group 2 (P2), which received oral gavage of green tea infusion at a dose of 0,06 g/head/day and exposure to electronic cigarette smoke, the mean blood MDA level was 6,654 µm/L. These results are presented in Table 1.Based on the Levene statistic for homogeneity and the Shapiro-Wilk test for normality, it was determined that the data distribution is homogeneous and normally distributed. Proceeding with the parametric one-way ANOVA test, the results indicate a significant difference in the means between and within the groups for blood MDA levels in mice. The F statistic is 4,51, and the p-value is 0,018 ($p<0,05$), signifying a statistically significant difference among the groups.

Table 3. *Post-hoc* LSD test result

(I) Group Name	(J) Group Name	Sig.
KN	K (-)	,032
	P1	,467
	P2	,375
K (-)	KN	**,032**
	P1	**,007**
	P2	**,005**
P1	KN	,467
	K (-)	,007
	P2	,869
P2	KN	,375
	K (-)	,005
	P1	,869

Based on the results of the post-hoc LSD test, it is observed that the negative control group (K-), exposed only to electronic cigarette smoke without green tea infusion, differs significantly from the normal control group (KN), treatment group 1 (P1), and treatment group 2 (P2) with p-values of 0,032; 0,007; and 0,005, respectively. However, there is no significant difference between the normal control group (KN) and treatment groups 1 (P1) and 2 (P2) with p-values of 0,467 and 0,375, respectively.

DISCUSSION

Electronic cigarettes allow Reactive Oxygen Species (ROS) emissions, particularly in the gas phase. Exposure to electronic cigarette smoke increases the synthesis of Nitrite Oxide (NO), elevating antioxidative mechanisms within the body, ultimately inducing oxidative stress. This elevated oxidative stress increases blood Malondialdehyde (MDA) levels.[14–16] The data analysis results indicate a significant increase in MDA in the normal control group compared to the negative control group (p=0,032). This substantial increase in MDA levels demonstrates that electronic cigarettes effectively induce oxidative stress in the blood of mice. This finding aligns with prior research by Hikmah and Paramita Sari (2021) and Rohmani (2018), asserting that electronic cigarette exposure's ability to increase ROS is comparable to that of conventional cigarette exposure.[15,17]

Five treatment groups were utilized in a study by Hikmah and Paramita Sari (2021) on the effect of lemon water infusion on MDA levels in the placenta of pregnant rats exposed to electronic cigarette vapor for 18 days of exposure with each exposure lasting 7 minutes and 30 seconds. It states that exposure to electronic cigarette vapor significantly increases placental MDA levels, consistent with the current study. However, the exposure duration in the present study was longer, spanning 30 days, with each exposure lasting 30 minutes.

The MDA levels in serum serve as an indicator of cellular damage caused by free radicals. MDA is a robust biomarker for assessing oxidative stress, with increased MDA formation directly correlating with elevated oxidative stress. Higher levels of free radicals lead to increased MDA formation, highlighting the impact of oxidative stress on cellular integrity.[7,14]

The significant increase in MDA levels, as demonstrated in the comparison between the normal control group (KN) and the negative control group (K-) in the Post-hoc test results, prompted the use of exogenous antioxidants to reduce blood MDA levels.

In this study, green tea demonstrated significant results in reducing oxidative stress, indicated by a decrease in blood MDA levels in the P1 and P2 groups. The parametric One-Way ANOVA analysis yielded a significance level (Sig.) of 0.018 ($p < 0.05$). The post-hoc LSD test revealed that the negative control group (K-) significantly differed from the normal control group (KN), treatment group 1 (P1), and treatment group 2 (P2), with p-values of 0.032; 0.007; and 0.005, respectively.

Green tea infusion was chosen due to its content of flavonol catechins, known for their antioxidative properties that reduce oxidative stress. Green tea's flavonoid content exhibits anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties. Therefore, this compound can potentially treat various diseases, including cancer, inflammation, and cardiovascular dysfunction, and mainly prevent injuries caused by free radicals through its widely recognized ability as an antioxidant.[12,18] This assertion is supported by various studies, such as the research by Susmiarsih *et al.* (2018) on the effects of green tea extract on the motility, morphology, concentration, and speed of spermatozoa in rats exposed to conventional cigarette smoke. The study concluded that green tea extract can enhance the quality and motility of rat spermatozoa due to the potent antioxidants present in green tea polyphenols, which prevent oxidative damage.[9,19] It is also supported by Agnes (2023), who found that administering green tea kombucha prevents an increase in MDA and a decrease in endothelial nitric oxide synthase (eNOS) in male Wistar rats exposed to nicotine electronic cigarette vapor.[20] Additionally, Alihar (2018) conducted a study on the influence of green tea leaf extract on the morphology of spermatozoa in male mice exposed to cigarette smoke.[21] This study found that green tea leaf extract at doses of 0,025; 0,0125; and 0,05 g/head/day could improve spermatozoa morphology significantly, showing a notable difference from the negative control group. This suggests that the antioxidant content in green tea can effectively alleviate oxidative stress in mice. Comparing the present study with previous research, the green tea doses fall within the same range and even higher than those used by Alihar (2018). Both doses of green tea infusion demonstrated significant efficacy in reducing oxidative stress in mice exposed to electronic cigarette smoke, with the lower dose of 0,03 g/head/day already showing a significant reduction in blood MDA levels.

CONCLUSION AND RECOMMENDATIONS

Based on the results of the research data analysis, it is concluded that the administration of green tea infusion at doses of 0,03 g/head/day and 0,06 g/head/day can significantly reduce the level of oxidative stress in the blood of mice exposed to electronic cigarette smoke. This suggests that green tea, with its antioxidant content, has the potential to act as a reducer of oxidative stress induced by electronic cigarettes.

Further in-depth research is recommended, particularly regarding the optimal, maximum, or toxic dose of green tea infusion administration in mice to reduce oxidative stress levels. This information will be beneficial for understanding the limitations and safety of using green tea as an antioxidant agent.

ACTKNOWLEGDEMENT
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