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THE EFFECT OF FRANGIPANI FLOWER ETHANOL EXTRACT ON SUPEROXIDE DISMUTASE (SOD) LEVELS IN MALE RATS EXPOSED TO CIGARETTE SMOKE AND VAPE

Abstract

Background: Both types of cigarettes (traditional and electronic) have negative health effects, and public interest remains high. Based on several studies, antioxidants are known to counteract the effects of ROS caused by cigarettes. One of them is frangipani (*Plumeria alba*), which has phenols and flavonoids.

Objective: To find out the antioxidant effects of frangipani flower extract on test animals exposed to cigarette and vape smoke.

Methods: An Experimental method with a randomized post-test control group design was used in this study. The sample consists of healthy male Wistar rats divided into five sample groups, which received intervention for 3 3-week duration. The One-Way ANOVA test and the Post hoc test analysed the data.

Results: This study found that both cigarettes and vaping lowered SOD levels (P1 and P2 groups; 3,381 and 4,462). Meanwhile, the groups with frangipani flower extract, who were exposed to cigarettes and vaping, had a higher level of SOD (P3 and P4; 6,999 and 8,681).

Conclusions: The administration of frangipani flower extract containing antioxidants has a positive effect on SOD levels.

Keywords : Cigarette, Vaping, SOD, Frangipani Flower, Antioxidant

INTRODUCTION

14 The use of electronic cigarettes (vapes) as a substitute for traditional cigarettes is currently on the rise. Vapes are also said to have negative effects that can be similar to those of traditional cigarettes (1). Cigarettes are known to contain chemicals such as nicotine, tar, and carbon monoxide, which can also trigger reactive oxygen species (ROS) in the body, thereby hurting health(2,3). In the lungs, cigarettes cause an obstructive disease called Chronic Obstructive Pulmonary Disease (COPD), which causes shortness of breath in sufferers. In addition to the lungs, cigarettes also have negative effects on blood vessels and the heart, increasing the risk of cancer (mouth, larynx, and tongue cancer), as well as dental and oral health (3,4).

9 Although both types of cigarettes have negative health effects, public interest remains high. Both government programs and independent initiatives have launched numerous smoke-free movements. Based on several studies, antioxidants are known to counteract the effects of ROS caused by cigarettes (5,6).

9
13 Antioxidants are currently used as a preventive measure against various types of diseases because they are believed to donate electrons to free radical compounds (6). Antioxidants in the body are categorized into two main groups: enzymes and non-enzymes. One of the antioxidant enzymes that is often used as a parameter is superoxide dismutase (SOD). Many natural ingredients have been extensively tested for their antioxidant activity. One of them is frangipani (*Plumeria alba*) (7). The phenol, flavonoid, and carotene content in this flower shows antioxidant activity. Previous studies have shown that frangipani flower extract can reduce malondialdehyde (MDA) levels in rats(7-9). Although frangipani extract is known to contain phenolic compounds, its effect on enzymatic antioxidants under cigarette and vape exposure conditions has not been extensively evaluated. Based on the above, researchers sought to investigate the antioxidant effects of frangipani flower extract on test animals exposed to cigarette and e-cigarette smoke.

MATERIALS AND METHODS

Material

7 The research sample consisted of 25 healthy male Wistar rats aged 3 months and weighing 180-200 grams. The dropout criteria were death or illness during the study.

The Frangipani flower extract used in this study was obtained from frangipani flowers, extracted using a 96% ethanol solvent. This extract was made using the maceration method and then evaporated using an evaporator.

The vape used was a MOD type, specifically the Centaurus G80s. The vape liquid used had a nicotine content of 3mg/ml.

Method

The mice were adapted or acclimatized for 7 days and divided into 5 sample groups with the following conditions:

- 1] P0: Control (no exposure and no extract)
- 2] P1: Treatment 1 (cigarette smoke exposure, 30 minutes, twice/day)

- 3] P2: Treatment 2 (exposure to 3mg/ml vape smoke, 30 minutes, twice daily)
- 4] P3: Treatment 3 (cigarette smoke exposure, 30 minutes, twice/day, and administration of 3% frangipani flower extract)
- 5] P4: Treatment 4 (vape smoke exposure 3mg/ml, 30 minutes, twice/day, and administration of 3% frangipani flower extract)

Rats were treated with standard treatment, which includes standard food, a standard rat cage that consists of 3-5 rats each. Control group, P1, and P2 received 1 ml Aquabides as a placebo, meanwhile the other, P3 and P4 received 1 ml of 3% extracts ethanol of frangipani flower.

SOD was measured by the ELISA method on the blood samples of the rats. The blood was collected from the rat's eyes before it was collected and analysed. Data analysis was performed descriptively and analytically. Descriptive analysis was employed to identify the characteristics of the research data, while the analytical test used was the one-way ANOVA test with a significance level of 95% (p < 0.05). This study has obtained ethical approval from the Ethics Committee Unit of the Faculty of Medicine and Health Sciences, Warmadewa University, with the number 143/Unwar/FKIK/EC-KEPK/XII/2024.

RESULTS AND DISCUSSION

RESULTS

Superoxide dismutase enzyme levels were tested in five sample groups, yielding different results as presented in Figure 1 below. SOD testing was performed on each sample in the group, and the average was calculated. The results of this study show that the SOD levels in the control group (SOD 7.971) were almost the same as the average SOD levels in P3 and P4 groups, which were exposed to cigarette smoke, vape smoke, and frangipani flower extract. The lowest SOD levels were found in P1 group, which was exposed to cigarette smoke only, followed by P2 group, which was exposed to vaping. The SOD level comparison between groups can be seen in Figure 1 below.

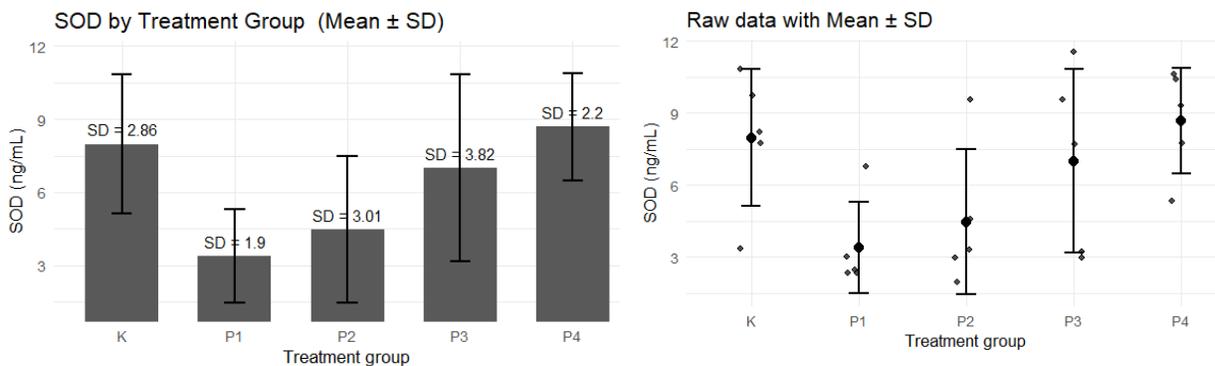


Figure 1. SOD levels among groups.

After conducting descriptive analysis, the Levene test for homogeneity was performed, and the results indicated that the data were homogeneous ($p = 0.469$). Next, the significance test was continued with the One-Way ANOVA test, with the results showing a significant difference between group averages ($p = 0.033$, $p < 0.05$), as presented in Table 1.

Based on the significant results of the one-way ANOVA ($p < 0.05$), the test was followed by a post hoc test, specifically the Bonferroni test, which revealed that the most significantly different groups were groups 1 and 4 ($p < 0.05$). The results are presented in Table 2.

Table 2. Uji Post Hoc Bonferroni

Comparison	p (Bonferroni)	Sig
P1 vs P4	0.023	*
P0 vs P1	0.105	
P2 vs P4	0.183	
P0 vs P2	0.392	
P1 vs P3	0.409	
P2 vs P3	0.769	
P3 vs P4	0.904	
P1 vs P2	0.955	
P0 vs P3	0.989	
P0 vs P4	0.991	

*Keterangan: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Bonferroni-adjusted).*

DISCUSSION

Based on the results of this study, the superoxide dismutase enzyme (SOD) value in the control group (P0) had an average that was nearly the same as that of the treatment groups with extracts (P3 and P4). These values were higher than the SOD values in P1 and P2 groups (Table 1). SOD is a first-line enzyme that plays a role in fighting free radicals and superoxide (5). Generally, acute exposure to cigarette smoke will cause ROS accumulation and SOD depletion as a response. SOD works as a catalyst for the dismutase reaction of superoxide anions to hydrogen peroxide (H₂O₂) and oxygen (O₂). However, long-term exposure will cause an increase in SOD enzyme as a compensatory mechanism for free radicals and excess ROS in the body (3,10).

The SOD results in P1 and P2 groups are in line with the above explanation, where group P1 represents the SOD value in the cigarette exposure group and group P2 represents the SOD value in the vape exposure group. Both types of cigarettes can cause an inflammatory response in the body and a decrease in SOD in the acute phase. Previous research also shows that toxic substances in cigarettes can spread systemically and cause

7 a decrease in most low-molecular-mass antioxidants in plasma. (11) The results of this study also prove that both traditional cigarettes and electronic cigarettes (vapes) have negative effects on the body because they are free radicals(12). Several studies on nicotine-free flavored and scented vape liquids have shown an increased risk of lung diseases such as COPD, emphysema, and bronchial asthma, as well as systemic inflammatory effects and a decrease in malondialdehyde (MDA) levels (12–15).

In P0 group, which was the control group without exposure to cigarette smoke or vape, the SOD values were similar to those in P3 and P4 groups, which received 3% frangipani extract after exposure to cigarettes or vape (Table 1). In group P0, the SOD values were higher than those in P1 and P2 groups because they were not exposed to free radicals from vaping or cigarettes. This is supported by the explanation that endogenous antioxidant regulation works normally without acute exposure to free radicals (16). The antioxidant defense mechanism comprises both enzymatic and non-enzymatic (endogenous antioxidants) as well as several types of low-molecular-weight molecules commonly obtained from food sources (exogenous antioxidants) (10, 17).

19 The groups of P4 and P3, which were given frangipani flower extract, had higher SOD values than P1 and P2 groups (without extract). These higher SOD values were attributed to the presence of exogenous antioxidants in the frangipani flower extract. Frangipani flowers are known to possess antioxidant activity and contain bioactive compounds, including polyphenols, flavonoids, alkaloids, and terpenoids (7,18,19). The high antioxidant activity in frangipani flowers, supported by their bioactive content, is believed to have a defensive effect against free radicals originating from cigarette smoke exposure.(20) Thus, in this study, the higher SOD values in P3 and P4 indicate the antioxidant activity induced by the extract, mainly by polyphenols and flavonoids effects.

10
15 Several studies with similar findings support the above results. One al. (2022) shows the antioxidant levels in the blood plasma of cigarette smokers. It was found that most types of antioxidants, such as vitamin C, beta carotene, vitamin A, and vitamin E, were lower in the smoking group than in the non-smoking group (21). For example, the vitamin C content in smokers was found to be 0.77 ± 0.23 mg/dL, while in non-smokers it was 1.73 ± 0.28 mg/dL. There are also several studies that support the results that external antioxidant supplementation can provide benefits for cardiovascular and respiratory health (22,23). Flavonoid supplementation was found to improve inflammatory and cardiovascular conditions in healthy smokers (22). Vitamin E and vitamin C supplementation also showed improvement in inflammatory conditions, improved vascular endothelial function, and reduced inflammatory mediators such as TNF- α in smokers (21,23). Several studies on the administration of antioxidant-rich diets to smokers have also obtained results in line with this study, finding improved lung function and a reduced risk of lung cancer in smokers who consume antioxidant-rich diets such as fruits and vegetables (24,25). Research on systemic antioxidants found that the administration of morel berry leaf extract can prevent a decrease in SOD levels and increase serum malondialdehyde (MDA) in mice exposed to cigarette smoke (15).

8 Based on the supporting research results above, SOD levels can increase due to the effects of antioxidants in frangipani flower extract on exposure to cigarette smoke and

vaping. The high antioxidant activity in frangipani flowers and their bioactive components can work effectively to counteract free radicals from cigarette smoke and vaping.

CLINICAL IMPLICATION

3 The clinical implications of this study are to provide knowledge about the in vivo preclinical effects of exposure to cigarette and vape smoke on the body's antioxidants. In addition, the results of this study also show the effects of administering a natural ingredient, namely frangipani flower extract, on the levels of antioxidant in the body exposed to cigarette and vape smoke. This can serve as a preliminary study to determine the effects of frangipani flower extract on the body exposed to the risk factors of cigarette and vape smoke.

LIMITATIONS

The limitations of this study are that the exposure time is still moderate and the sample consists of laboratory rats. In addition, the dosage of the extract and smoke exposure can also be further modified, as well as the varied biomarkers that should be analysed.

CONCLUSIONS

Based on the study results, it was concluded that exposure to cigarette smoke and vaping has a similar effect on SOD levels in rats. Administration of frangipani flower extract in cigarette smoke and vape exposure treatments showed benefits for the body's antioxidants, with higher SOD levels found in the extract treatment compared to the non-extract treatment. These results conclude that administration of 3% *ethanol extract of Plumeria alba* attenuated the reduction of SOD levels caused by acute cigarette and vape exposure in male Wistar rats.

CONFLICT OF INTEREST

2 There are no conflicts of interest of this research.

AUTHOR CONTRIBUTIONS

1 All authors contribute to this experiment and manuscript.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

5 The authors used Grammarly to assist in improving the language and grammar of the manuscript. The authors reviewed and verified the content to ensure accuracy and integrity.

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