

The Effect of Red Ginger Ethanol Extract on Blood MDA Level of White Male Wistar Strain Rats Induced with Cigarette Smoke

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Abstract. Cigarette smoke causes lipid peroxidation and results in an increase of malondialdehyde (MDA) level in blood that could finally lead to decreased lung function and respiratory disease. Herbs and spices like red ginger are known to have beneficial compounds for health. The aim of this study is to prove the effect of red ginger ethanol extract on blood MDA level of white male Wistar strain rats induced with cigarette smoke. 32 white male rats were divided into 4 groups. Group 1 was the negative control group, group 2 was the positive control group, group 3 was the one induced with cigarette smoke and 250 mg of red ginger ethanol extract and group 4 was induced with cigarette smoke and 500 mg of red ginger ethanol extract. Blood samples were taken on the baseline (day 0) and day 7 to be analyzed. MDA levels were measured using spectrophotometer. One Way ANOVA test and post hoc Tukey test were used to compare with the negative group. The study show significant differences ($p=0,000$) and the results were $KN=1,55$; $KP=4,45$; $J1=2,81$; $J2=1,65$ (nmol/ml). Both doses of red ginger ethanol extract decrease blood MDA levels after 7 days treatment. Finally, it concludes that antioxidant content in red ginger ethanol extract can decrease MDA level.

Keywords: *red ginger, antioxidant, cigarette smoke, malondialdehyde*

1. INTRODUCTION

Smoking and cigarette smoke are known to have bad impact to immune system. World Health Organization has estimated that tobacco use is responsible for the death of about six million lives across the world each year including 600,000 people who die from the effect of sidestream cigarette smoke [1]

Cigarette smoke contains over 5000 types of chemicals, consisting of gas components (nitrosamine, formaldehyde, vinyl chloride, etc) and solid components (naphthalene, fluoranthene, nicotine, arsenic, nickel, etc). Each puff of cigarette smoke contains 1014-16 Reactive Oxygen Species (ROS) [2], [3]. The amount of gas-phase and particle-bound ROS concentration, determined using 2', 7' -dichlorodihydrofluorescein diacetate (DCFH-DA) for sidestream smoke from regular and light cigarettes, is 60-90 nmol and 30-70 nmol [4].

ROS is chemical constituents in cigarette smoke that can cause respiratory problems [5]. ROS is very reactive and could cause oxidative stress. If ROS interferes with lipid, it will eventually cause a reaction of lipid peroxidation that has an important role in biological cell and human health [6]. MDA is one of lipid peroxidation biomarkers [7]. MDA and acetaldehyde (AA) concentration increase in subjects that smoke or are exposed to cigarette smoke [8], [9]. Mainstream and sidestream cigarette smoke may cause lung structure alteration that lead to lung damage such as pulmonary edema [5].

Naturally, body produces antioxidants to inhibit oxidation process. However, if there are too many free radicals entering the body, antioxidants cannot effectively inhibit the oxidation process. In this condition, the body needs non enzymatic antibody such as red ginger. Red Ginger has many chemical components such as pungent principles, gingerols and shogaol [10]. Ginger has been used for respiratory therapy [11], because it has several compounds which function as antioxidants, anti-inflammatory, anti-microbial and anti-fibrotic agents [12]–[14]. The aim of this study is to prove the effect of red ginger ethanol extract on blood MDA level of white male Wistar strain rats induced with cigarette smoke.

2. METHODS

This study applied true experiment approach. The design of the study was Randomized Post Test Only Control Group Design. The samples used were 32 male strain wistar rats which were divided into 4 groups with inclusion criteria of healthy males, 8 weeks old and having 150 - 200 grams of weight. During the study, samples were kept at Animal Laboratory of Food and Nutrition Study Center of Gadjah Mada University, Yogyakarta. The study has passed the ethics of Health Research Ethics Committee of Faculty of Medicine of Universitas Sebelas Maret No. 415/UN27.6/KEPK/2019.

The samples were exposed with clove cigarette smoke with certain brands using smoking chamber. The dose of cigarette given to the samples was 2 bars / day for each group of KP, J1, and J2. Cigarette smoke exposure was given one hour after a dose of red ginger ethanol extract was given. The cigarette smoke exposure was conducted for 15 minutes in each treatment groups (KP, J1 and J2).

Red ginger was extracted with 96% ethanol. Red ginger ethanol extract was fed to the samples using enteral feeding. The doses that were given to each sample groups were 250 mg/kg weight for J1 and 500 mg/kg weight for J2. The extract was given before the cigarette smoke was induced during the 7 days of the experiment.

The MDA blood levels of the sample groups were collected before the treatment and after 7 days of treatment. MDA levels were measured using spectrophotometer at 532 nm and the results of the MDA levels were compared to the negative control group.

The data were tested for normality using Shapiro-Wilk test ($p > 0.05$) and then the homogeneity was tested using Lavene test ($p > 0.05$). One-way ANOVA was used to determine whether there were any effects of giving red ginger ethanol extract among the sample groups ($p < 0.05$) and to find out which groups have the most significant difference with Post-hoc Tukey test.

3. RESULTS AND DISCUSSION

The results of study show that there are differences in the MDA blood levels from before and after treatments among the groups. The average results of MDA blood levels in each group are shown below (figure 1) and the codes of each group are: normal diet without cigarette smoke exposure (KN), normal diet with cigarette smoke exposure (KP), normal diet with cigarette

smoke exposure and red ginger ethanol extract 250 mg/kg weight (J1) and normal diet with cigarette smoke exposure and red ginger ethanol extract 500 mg/kg weight (J2).

Table 1. Means of MDA Blood Levels from Pre and Post Test and ANOVA result

Group	Pre-test		ANOVA Result	Post-test		ANOVA Result
	MDA Blood Level	Standart Deviation		MDA Blood Level	Standart Deviation	
KN	1,37 ± 0,24		$p = 0,376$	1,55 ± 0,25		$p = 0,000$
KP	1,35 ± 0,20			4,45 ± 0,57		
J1	1,34 ± 0,26			2,81 ± 0,50		
J2	1,18 ± 0,19			1,65 ± 0,22		

It can be seen from table 1 that the highest MDA blood level mean of 4.45 nmol/ml is found in KP group, and the lowest MDA blood level mean of 1.55 nmol/ml is found in KN group. The groups that were dosed with red ginger ethanol extract have decreased MDA blood levels along with the increase of red ginger ethanol extract doses of 2,81 nmol/ml (J1) and 1,65 nmol/ml (J2).

The normality test in this study was conducted using Shapiro-Wilk Test and the results show that all data are normal ($p > 0,05$), and the homogeneity test was performed using Lavene Test which show that all data in each group are homogenous. ANOVA test results found that the red ginger ethanol extract treatment has impact on MDA blood level with $p < 0,05$. There is a clinical decrease on the MDA blood level mean which is dependant to the dose of red ginger ethanol extract treatment. The results of post-hoc Tukey test on all groups can be seen in table 2. It shows that there are statistically significant differences on the means of MDA blood level in the post-test. However, there is no statistically significant difference between KN group and J2 group.

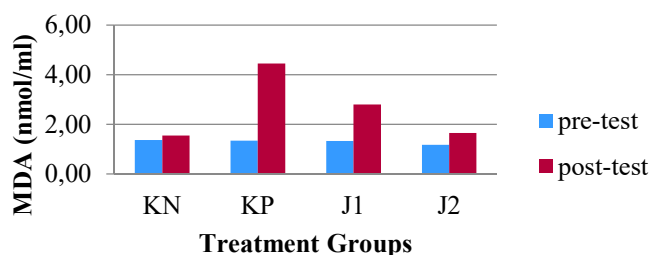


Figure 1. MDA Blood Level Differences among Pre-test and Post-test Groups

Table 2. The Post Hoc Test Result on MDA Blood Levels

Group	KN	KP	J1	J2
KN	-	0,000*	0,000*	0,970
KP	0,000*	-	0,000*	0,000*
J1	0,000*	0,000*	-	0,000*
J2	0,970	0,000*	0,000*	-

Cigarette smoke has bad impacts to health. This study proves that the lowest MDA level was found in the KN group which was not exposed with cigarette smoke and the highest MDA level was found in KP which was exposed with cigarette smoke and did not get red ginger ethanol extract treatment.

The results of this study conclude that cigarette smoke significantly increases MDA level because smokers' lung secretes pro-inflammatory cytokines when induced with cigarette smoke [9]. When a large amount of free radicals such cigarette smoke enters the body to the level where the body could not compensate, it could cause tissue damage. Cigarette smoke can decrease superoxide dismutase (SOD) activity level in healthy rats then increase MDA level [15]. Smoking and cigarette smoke can increase oxidative stress through the formation of ROS that can lead to lipid peroxidation, which is also closely related to cigarette consumption level and the frequency of cigarette smoke exposure. [16].

Ginger is considered as one of antioxidant sources because it has so many bioactive compounds such gingerol, shogaol, zingerone that can inhibit oxidase enzyme [17]. This study shows that J1 and J2 groups that were exposed to by cigarette smoke and given red ginger ethanol extract have MDA blood levels which are lower than the KP group that did not get red ginger ethanol extract treatment. The result of this study is in line with other studies which conclude that ginger can decrease MDA level when given to hypoxia rats [11]. Some researches have used ginger to prevent lipid peroxidation. 1 month of ginger supplementation and anti-tubercular treatment significantly lower MDA concentration compared to control group [18]. Another study shows that zingerone is a ginger component that suppresses oxidative stress and inflammation in nephrotoxicity rat model [19]. Grape skin extract [20] and extra virgin olive oil [21] are also associated with preventive and therapeutic resource for lung inflammation and oxidative stress caused by cigarette smoke.

4. CONCLUSION

This study concludes that antioxidant contained in red ginger ethanol extract could decrease MDA level and could be used as preventive measure for respiratory disease. The rate of MDA level decrease depends on the dose of red ginger ethanol extract given.

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