The Potential Effect of Citrus Sinensis Extract on Malondialdehyde (MDA) Levels in Rat Induced by Cigarette Smoke

Ika Setyawati¹,*, Nunki Indah Hidayati²

¹Department of Biochemistry, School of Medicine, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia
²School of Medicine, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia
*Corresponding author. Email: ikasetyawati.dr@umy.ac.id

ABSTRACT
Cigarette smoke contains numerous substances containing free radicals and can prompt oxidative pressure. The buildup of free radicals cannot be neutralized by antioxidants for essential cancer prevention agent supplements from outside. In relation to that, the peel of citrus sinensis is expected to contain cell reinforcements that may eliminate free radicals. This study aims to determine the potential effect of Citrus sinensis peel extract on MDA levels in rats induced by cigarette smoke. This investigation is an experimental study with pre and post-test control groups. The 25 subjects of Rattus Norvegicus were divided into five groups: normal group, positive group, Treatment 1 (37.5 mg/kg BW Citrus sinensis peel extract), Treatment 2 (75 mg/kg BW Citrus sinensis peel extract) and Treatment 3 (112.5 mg/kg BW Citrus sinensis peel extract). Analysis of statistics resulted in a huge distinction between pre-test and post-test with p-value <0.05 (Wilcoxon test). Consequently, the Kruskal-Wallis test and post-test were applied to distinguish the expansion of MDA levels pre-test and post-test. The investigation showed that the concentration was in the most significant levels in the positive group (7.71 nmol/mL), the least in the negative group (0.90 nmol/mL), and 37.5 mg/kg Citrus sinensis peel extract group (0.96 nmol/mL) was the most reduced among the treatment groups. There are huge contrasts in all treatment groups with p = 0.000 (p <0.05). The investigation inferred that the concentrate of Citrus sinensis could lessen levels of MDA in Rattus Norvegicus prompted by cigarette smoke.

Keywords: cigarette smoke, Citrus sinensis, MDA, Rattus Norvegicus

1. INTRODUCTION

Smoking causes death and a danger to the whole population since it averagely slaughters six million individuals every year. Out of those numbers, more than five million are smokers and ex-active smokers, while 600,000 are passive smokers or non-smokers [1]. Smoking behavior in Indonesia keep increasing within age 15 from 34.2% (2007) to 36.3% (2013), and active smokers consist of 64.9% men and 21.1% women [2]. The level of smoking behavior dan knowledge for most respondents had a negative attitude, with 63.3% [3]. The Global Adult Tobacco Survey (GATS) stated that cigarette users in Indonesia reached 67.4% for men and 4.5% for women or equivalent to 61.4 million adult smokers [4].

Cigarette smoke contains oxidants and prooxidants that hideously produce free radicals and cause oxidative stress [5]. Free radicals must be eliminated by the body's antioxidants, but due to excessive production of free radicals or the hindered antioxidants, it undoubtedly disrupt the balance between free radicals and antioxidants, and this condition is called oxidative stress [6] [7].

The accumulation of radical compounds eventually damages the biomolecules such as lipids, proteins, or DNA, characterized by malondialdehyde (MDA), lipid hydroperoxides (LOOH), and 4-hydroxy-2-nonenal for lipids, carbonyl protein (CPs) and 8-OH to measure damage protein and DNA [8].

Phenolic compounds, especially flavonoids, are the main contributors of antioxidants in the skin of the fruit [9] [10]. Citrus sinensis contains calcium, vitamin C, liminoids, magnesium, pectin, folacin, hesperidin flavonoids, potassium, polyphenols, thiamine, synephrine, and niacin that have an essential role in preventing increasing cholesterol levels, high blood pressure, cancer, arteriosclerosis, stomach ulcers and kidney stones [11].

2. METHODS AND MATERIALS

2.1. Experimental Design
The citrus sinensis peel extract was administered on a single dose per day. The rats were then divided into 5 groups (n = 5 rats/group): normal rats, positive control, Treatment 1: 37.5 mg/kg BW/day Citrus sinensis peel extract + cigarette smoke. Treatment 2: 75 mg/kg BW/day Citrus sinensis peel extract + cigarette smoke, Treatment 3: 112.5 mg/kg BW/day Citrus sinensis peel extract + cigarette smoke.

MDA levels were determined by the method of Thiobarbituric acid-reactive substances (TBARs). MDA blood tests were through a method described by Wurystuti (1996) in Indonesian food and nutrition progress, 2000 Vol. 7
no. 2. A total of 0.75 mL of phosphoric acid was put into a polypropylene tube with 0.25 mL of thiobarbituric acid (TBA) solution.

2.2. Material

Sweet orange (Citrus sinensis) peel was purchased from the local grocery. Sweet oranges were then washed, peeled, and after that, the orange peel was dried at a temperature of 40°C. Next, the dried orange peel was blended with Simplicia. One hundred grams of Simplicia was mixed with a solvent in the form of 70% ethanol (800 mL) and maceration for 48 hours while occasionally stirred. For the next step, it was filtered until two substances were obtained, the pulp and mase rat. The results of maceration or mased rat were put into a rotary evaporator and became a thick extract.

2.3. Samples of MDA Levels

The sample for the MDA level test was serum. The serum was obtained by allowing the blood to clot for 1 hour at room temperature, then messed around at 3000 rpm for 10 minutes.

2.4. Animals

This study used 25 physically healthy white male rats (Rattus norvegicus) aged between 2 - 3 months with weight ± 200 g. The rats were adapted for three days at Central Inter-University Building, Laboratory of the Center for Food and Nutrition Studies, Gajah Mada University with adequate feeding with standard pellet rats diet, drinking, and lighting at room temperature. Consequently, this study abode all proper ethics.

2.5. Induction of Cigarette Smoke

Cigarette smoke was obtained from kretek cigarettes without side flow filters induced at a dose of 2 cigarettes a day.

2.6. Data Analysis

The data were processed using the Kruskal-Wallis test followed by the Mann Whitney U test to compare differences in MDA levels between treatment groups in the administration of sweet orange peel extract. The value is significant if p < 0.05.

3. RESULTS AND DISCUSSION

The data distribution was tested using the Shapiro-Wilk normality test, and the results of the data analysis were not normally distributed. The significance of the differences in pre-test and post-test MDA levels was determined by using the Wilcoxon statistical analysis test. The statistical analysis of differences in MDA levels pre-test and post-test can be seen in table 1.

Table 1. The Difference of Mean MDA Levels of Rattus norvegicus Pre-test and Post-test Induced By Cigarette Smoke and Given Sweet Orange Peel Extract (Citrus sinensis)

<table>
<thead>
<tr>
<th></th>
<th>Wilcoxon test</th>
<th>Valid N</th>
<th>Levels of MDA (nmol/mL) ± SD (Pretest)</th>
<th>Levels of MDA (nmol/mL) ± SD (Protest)</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>5</td>
<td>1.08 ± 0.08</td>
<td>1.17 ± 0.13</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>5</td>
<td>1.18 ± 0.19</td>
<td>8.90 ± 0.33</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td>Treatment 1</td>
<td>5</td>
<td>1.21 ± 0.12</td>
<td>7.37 ± 0.26</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>Treatment 2</td>
<td>5</td>
<td>1.16 ± 0.16</td>
<td>4.30 ± 0.29</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>Treatment 3</td>
<td>5</td>
<td>1.44 ± 0.18</td>
<td>2.40 ± 0.13</td>
<td>0.043</td>
<td></td>
</tr>
</tbody>
</table>

The difference in MDA levels was positive in all groups (p <0.05). Table 1 shows that the positive group experienced the highest average increase in MDA levels compared to the other groups with 7.71 nmol/mL. The increase in MDA levels in the positive group indicates that exposure to cigarette smoke causes oxidative stress, which then triggers lipid peroxidation and increases MDA production in the body [12]. The plasma MDA levels are higher in smokers than in non-smokers. This study was conducted on 100 subjects with 50 healthy subjects and 50 chronic or heavy smokers and obtained significant results with p-value = 0.0001 (p <0.05) [13]. Other studies have also shown increased levels of MDA in rats exposed to cigarette smoke as many as 40 cigarettes per day (p <0.05) [14].

In the normal group, MDA levels were lower than the treatment group because the MDA levels were very dependent on the amount of oxidative stress and could only be neutralized by antioxidants, while under normal conditions, MDA levels could be formed at low levels. When normal conditions, lipid peroxidation in the body can still be overcome by natural antioxidants (endogenous antioxidants), namely catalase, superoxide dismutase (SOD) and glutathione peroxidase [15].

Table 2. Results of The Inter-Group Comparison Test

<table>
<thead>
<tr>
<th></th>
<th>Mann Whitney Test</th>
<th>Inter Group</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>Positive control</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment 1</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment 2</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment 3</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>Treatment 1</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment 2</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment 3</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Treatment 1</td>
<td>Treatment 2</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment 3</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Treatment 2</td>
<td>Treatment 3</td>
<td>0.009</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows that the results of the mean MDA levels in Treatment group 1, 2, and 3 experienced a significant decrease when compared to the positive group (p=0.009). The decrease in MDA levels (when compared to the positive group) indicates that the orange peel extract contains the main
phenolic compounds, namely hydroxycinnamic acid (HCA) and flavonoids, which act as antioxidants [16]. Orange peel extract can reduce MDA levels (p=0.001) in the heart and liver tissue [17]. Other research suggests orange peel extract does a potent hepatoprotective effect because it prevented oxidative damage in mice induced with paracetamol [18].

Orange peels contain good radical antioxidative potential and a high antioxidation concentration compared to the pulp [10][16]. Antioxidants stabilize radicals by complementing the lack of electrons that free radicals have and inhibiting chain reactions for the formation of free radicals resulting in cell damage [19].

The action mechanism of flavonoids as antioxidants was to suppress ROS formation (Reactive Oxygen Species) by inhibiting enzymes in the formation of ROS and increasing regulation and protection of antioxidants. Flavonoids can also protect membrane lipids from oxidative damage so that lipid peroxidation can be inhibited and increased levels of Malondialdehyde (MDA) can be prevented [20][21].

4. CONCLUSION

The administration of orange peel extract (Citrus sinensis) can reduce MDA levels in animals induced by cigarette smoke.

ACKNOWLEDGMENTS

The research teams appreciated the Research, Publication and Community Service Institute (LP3M) for the research funding through internal university grants.

REFERENCES