Male_White_Mice_of_Wistar_Strain_Exposed_to_Cigarette_Smoke.pdf

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The Use of Ethanol Extract of Gorek Seeds to Lower the Level of Lipid Peroxidation in Male White Mice of Wistar Strain Exposed to Cigarette Smoke

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Abstract— *Cigarette smoke causes an imbalance between free radicals and antioxidants, causing oxidative stress followed by an increase in lipid peroxidation. Gorek seeds (Caesalpinia bonducella) contain high phenolic, antioxidant capacity, flavonoids, and tannins that can capture free radicals. This experimental study aims to prove that the ethanol extract of Gorek seeds can reduce lipid peroxidation levels in mice exposed to cigarette smoke. The decrease in lipid peroxidation was indicated by a decrease in isoprostane F2 in mice urine. The research used a pre-test post-test control group design, with 2 groups of mice as the control group and the treatment group, exposed to cigarette smoke for 14 days. Besides being exposed to cigarette smoke, the treatment group was also given ethanol extract of Gorek seeds at a dose of 250 mg/kgBB. The results showed that the F2 isoprostane level of the treatment group decreased significantly from an average of 4.68±0.71 ng/mL to 3.52±0.44 ng/mL ($p<0.05$), while the F2 isoprostane level of the control group increased significantly from a mean of 4.75±0.42 ng/mL to 5.04±0.34 ng/mL ($p<0.05$). These results indicate that the ethanol extract of Gorek seeds can significantly reduce lipid peroxidation levels.*

Keywords— *Gorek seeds, F2 Isoprostan, oxidative stress, cigarette smoke, Wistar white mice.*

I. INTRODUCTION

Although people know that smoking is one of the causes of death, smoking is still widely practiced throughout the world. In Indonesia, about 36.1% of the Indonesian population smoke both regular tobacco cigarettes and smokeless cigarettes. Indonesia has the highest percentage of smokers in Southeast Asia and ranks fifth highest in tobacco consumption since 2004 (Kosen et al., 2011). Cigarette smoke contains gas and particulate components that have the potential to generate free radicals (Colagar et al., 2007). Free radicals in large quantities in cigarette smoke can generate lipid peroxidation due to damage to cell membranes and reduce antioxidant level, causing oxidative stress (WHO, 2013). Jana (2012) added that it can also reduce the working mechanism of antioxidants causing damage to cellular organs and enzymes, and increase lipid peroxidation and cause insulin resistance. It has been shown that oxidative stress is a crucial event in diseases such as lung cancer, oral cancer, and chronic obstructive pulmonary disease (Burlakova et al., 2010). If these factors can be avoided, the premature aging process can certainly be prevented, slowed down, and even inhibited and the quality of life can be maintained (Pangkahila, 2007).

Antioxidants are compounds that can inhibit or even prevent oxidation. The way antioxidant compounds work is to react with reactive free radicals to form relatively stable unreactive free radicals, by complementing the electron deficiency of free radicals (Utami et al., 2009). Antioxidants are often found in medicinal plants. The important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolics (Edeoga et al., 2005). One of the medicinal plants is *Gorek* seeds (Caesalpinia bonducella), whose ethanol extract contains high levels of phenolic which can bind free radicals, breaking chain reactions. From the results of phytochemical tests at the Laboratory of the Faculty of Agricultural Technology, Udayana University Bali, a *Gorek* seed contains flavonoids of 170,24 mg/L QE, phenol of 746.01 mg/L GAE, the antioxidant capacity of 689.9 mg/L GAEAC, and tannins of 586.35 mg/L (Aska, 2015). There is a close relationship between antioxidant activity and phenolic content, where phenolic is the main contributor of antioxidant activity by inhibiting DPPH, hydroxyl radicals, nitric oxide (NO), superoxide anion, and hydrogen peroxide activity, compared to the use of standard ascorbic acid (Shukla et al., 2009). On the other hand, tannins are high molecular weight phenolics that consist of gallic acid esters or flavan-3-ol polymers. Tannins stimulate antioxidant activity where tannins will capture free radicals kinetic (Riedl et al., 2002).

F2 Isoprostanes (F2-IsoPs) are prostaglandin-like compounds produced from the esterification of arachidonic acid in tissues by non-enzymatic catalyzed reactions of cell-generated free radicals or lipoprotein phospholipids (Kaviarasan et al., 2008). The properties of the F2 Isoprostan molecule are stable, strong, and can be detected through various body fluids such as urine, plasma, or cerebrospinal fluid (Milatovic and Aschener, 2009). Many studies use urine samples because the sampling method is simple and non-invasive (Cracowski and Baguet, 2003). However, F2 Isoprostan has a weakness that is a short half-life, less than 20 minutes (Roberts and Milne, 2009). However, if the exposure to cigarette smoke continues, the F2 Isoprostane level will persist in the urine.

The role of F2 isoprostanes is important for the measurement of lipid peroxidation and oxidative stress (Janssen, 2001). The advantage of measuring F2 Isoprostan as a biomarker of lipid peroxidation is to monitor disease and response to therapy, its potential function as a mediator of

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oxidative stress. Lipids are the main target of the free radical attack that causes lipid peroxidation. Lipid peroxidation can cause atherosclerosis where the increase in lipid peroxidation can be stopped by the administration of antioxidants (Jay et al., 2010).

In a study conducted by Jana et al., (2011), it was found that the hydro-methanol extract of *Gorek* seeds (Caesalpinia bonducella) was able to inhibit the formation of lipid peroxidation under in vitro conditions. Meanwhile, this study wanted to find out whether the ethanol extract of *Gorek* seeds could also inhibit the formation of lipid peroxides. As the research sample, male white mice (*Rattus norvegicus*) of Wistan strain were used. To measure the level of lipid peroxidation reaction, F2 Isoprostane was used, which has important implications for biological markers. Measurement with F2 Isoprostane is widely used because it is easier and stable so it can be relied upon to assess oxidative stress status *in vivo*. This examination can be done through plasma and urine (Halliwell and Gutteridge, 2007).

II. Materials and Methods

This experimental study used the pre-test post-test control group design method (Pocock, 2008). As the research material, male white mice of the Wistar strain, aged 2.5-3 months, weighing 190-200 grams and in good physical condition, were exposed to cigarette smoke as the population. Mice with isoprostane F2 levels > 2 ng/ml were taken randomly and grouped into two as samples. Based on the results of preliminary research (Aska, 2015), the mean F2 Isoprostane pre-test = 4.36 ng/ml, with standard deviation = 0.21 ng/ml while the post-test F2 Isoprostan mean = 4.02 ng/ml. By using a = 0,05 dan β = 0.10, according to Pocock's (2008) formula, each group of mice contains at least 8.01 mice. To anticipate sample dropout, 10% was added, so that it became 8.81 mice and rounded up to 9 mice.

In the first group, the control group, mice were exposed to smoke within 3 hours every day for 14 days and given 2 cc of distilled water 1 hour earlier. While in the second group, the treatment group, mice were exposed to cigarette smoke within 3 hours every day for 14 days and were given orally an ethanol extract of *Gorek* seeds diluted with distilled water to 2cc, once every day for 14 days, with exposure to administration 1 hour after administration of the extract. The independent variable in this study was the ethanol extract of *Gorek* seeds given to mice at a dose of 250 mg/kgBW mice, while the dependent variable was the level of F2 Isoprostan, and the control variables were the age of the mice, the type of mice, and their food and drink.

The research method is diagrammatically illustrated in Figure 1. For 7 days, 30 mice were adapted to the research environment. For the next 7 days, all mice were exposed to cigarette smoke. On the 8th day, the mice's urine that had been stored for 24 hours was measured for urine F2 Isoprostane level as a pretest. After that, the mice were randomly divided into two groups. On the following days, exposure to cigarette smoke was given for 3 hours every day for 14 days. Afterward, the levels of F2 Isoprostane in mice's urine were checked using the 8-iso-PGF2α enzyme immunoassay kit (EIA) from the assay design as a post-test. Urine collection was carried out by collecting the urine of each mouse for 24 hours in a special container with a filter. Urine specimens were taken twice, namely before treatment and after treatment. During the study, the mice's food intake was maintained, i.e., food containing 20-25% protein, 5% fat, 45-50% carbohydrates/starch, 5% crude fiber, and 4-5% ash. The amount of food per day for a mouse is 12-20 grams (Smith et al., 1988). The mouse feed used came from PT. Pokphan. Food and drink were provided *ad libitum*. Likewise, the cleanliness and comfort of the cage were also maintained. After all the required data were collected, the next step was data analysis.

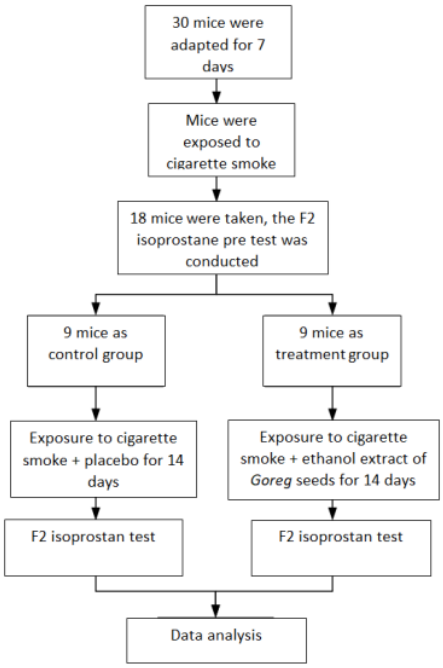

Fig. 1. Research flowchart

To facilitate the implementation of this research, several research tools and equipment were used, i.e., a mouse cage made of wood and wire netting equipped with food and drink containers, urine collection containers, scales, gloves, 3cc injection syringe, gastric probe, measuring cup, filter paper, oven, evaporator, freeze dryer, enzyme immunoassay kit consisting of microwell precoated with Anti-15-isoprostane F2t reagent, 15-isoprostane F2t Standard, wash buffer, dilution buffer, TMB substrate, 15-isoprostane F2t HRP Conjugate, pipette reagents, microplate shaker, Microplate reader with 450 nm, and digital camera.

III. Results and Discussion

Results

At the end of the experiment, the urine Isoprostane F2 data both before and after treatment were tested for normality and

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homogeneity. The results of the Shapiro-Wilk test showed that the data were normally distributed ($p>0.05$), and the results of Levene's test showed that the data was homogeneous ($p>0.05$). The test of the average comparison of urine F2 Isoprostane in each group between before and after being given treatment (i.e., in the form of aquadest and ethanol extract of *Gorek* seeds) showed the results as shown in Table I below.

TABLE I. Comparison of Urine F2-Isoprostane levels before and after treatment

Group	Urine F2-Isoprostan Mean		p
	Before treatment (ng/ml)	After treatment (ng/ml)	
Control	4,75±0,42	5,04±0,34	0,058
Treatment	4,68±0,71	3,52±0,43	0,001

Table I shows that in the control group there was no significant decrease in the mean of urinary F2 Isoprostane ($p>0.05$), while in the treatment group, there was a significant decrease in the mean of urinary F2 Isoprostane ($p<0.05$), from 4, 68(0.71 ng/ml) to 3.52(0.43 ng/ml). The results indicate that lipid peroxidation can be significantly reduced by using ethanol extract from *Gorek* seeds.

Discussion

Based on the results of the study above, it was found that in the treatment group there was a 24.8% decrease in urinary F2 Isoprostane, which also significantly decreased lipid peroxidation compared to the control group. This is because the *Gorek* seeds contain high phenolic content of 746.01 mg/L GAE, 170,24 mg/L QE of flavonoids, 586.35 mg/L of tannins, and antioxidant capacity of 689.93 mg/L GAEAC, which is useful as an antioxidant (Aska, 2015).

Cigarette smoke contains various kinds of free radicals, some of which have been proven to be carcinogens and mutagens consisting of nicotine, lead (Pb), carbon monoxide (CO) gas, tar (Fowles and Bates, 2000). Free radicals and other radicals in large quantities in cigarette smoke can cause lipid peroxidation, due to cell membrane damage, and reduce antioxidant levels, causing oxidative stress (WHO, 2013).

Smoking can cause oxidative stress not only through the production of ROS in cigarette tar and smoke but also through a decrease in the antioxidant defense system. Smoking causes an imbalance of free radicals and antioxidants, resulting in oxidative stress followed by an increase in lipid peroxidation, oxidative DNA damage, and impaired enzymatic antioxidant defense. It has been shown that oxidative stress is a crucial event occurred in diseases like lung cancer, oral cancer, and chronic obstructive pulmonary disease (Burlakova et al., 2010).

The role of isoprostanes is important for the measurement of lipid peroxidation and oxidative stress (Janssen, 2001). The advantage of measuring F2 Isoprostan as a biomarker of lipid peroxidation is to monitor diseases and responses to therapy, its potential function as a mediator of oxidative stress. Lipids are the main target of the free radical attack that causes lipid peroxidation. Lipid peroxidation can be stopped by the administration of antioxidants (Jay et al., 2010).

The nature of the F2 Isoprostan molecule is stable, strong, and can be detected through various body fluids such as urine, plasma, or cerebrospinal fluid (Milatovic and Aschener, 2009). Many studies have used urine samples because the sampling method is simple and non-invasive (Cracowski and Baguet, 2003). However, F2 Isoprostane has a short half-life, less than 20 minutes (Roberts and Milne, 2009). Increased levels of F2 Isoprostane in various body fluids and tissues can be found in various disease conditions such as atherosclerosis, diabetes, obesity, neurodegenerative diseases, and various other diseases. Treatment for these diseases, including the use of antioxidant supplements, diabetes treatment, smoking cessation, and weight loss, can reduce the production of F2 isoprostanes (Roberts and Milne, 2009). The increase in F2 Isoprostane in the early pathological process of disease proves the occurrence of oxidative stress in these diseases (Jausette et al., 2009).

The ethanol extract of *Gorek* seeds contains a high level of phenolic which are able to bind free radicals, breaking chain reactions. There is a close relationship between antioxidant activity and phenolic content, where phenolic is the main contributor of antioxidant activity by inhibiting DPPH, hydroxyl radicals, nitric oxide (NO), superoxide anion, and hydrogen peroxide activity compared to the use of standard ascorbic acid. In addition, the ethanol extract can act as the superoxide of free-radical scavenging activity with the EDTA/NBT system (Shukla et al., 2009). In a study by Jana et al. (2011), it was found that the hydro-methanolic extract of Caesalpinia bonducella inhibited the formation of lipid peroxidation under *in vitro* conditions.

Administration of Caesalpinia bonducella extract orally at a dose of 250 mg/kgBW can reduce lipid peroxidation by improving the antioxidant activity of the enzymes SOD (superoxide dismutase) and CAT (catalase) in the liver, resulting in reduced reactive oxygen from free radicals (Jana et al., 2012). Likewise, the preliminary study of this research showed that the most effective dose was 250 mg/KgBB. If various factors that cause aging can be avoided, then the aging process can certainly be prevented, slowed down, even inhibited; and the quality of life can be maintained, so that life expectancy becomes longer with a better quality of life (Pangkahila, 2011). Provision of antioxidants in the form of ethanol extract of fried seeds can be used as a preventive measure against the aging process in passive smokers and active smokers, although it does not mean that ethanol extract of fried seeds can be used as medicine for smokers considering the dangers of smoking and this research is still limited to mice.

IV. Conclusion

Based on the results of the study above, the following conclusions were obtained: Oral administration of ethanol extract from *Gorek* seeds at a dose of 250 mg/KgBW reduced F2 Isoprostane levels in the urine of mice exposed to cigarette smoke. The decrease occurred significantly from a mean of 4.68±0.71 ng/mL to 3.52±0.44 ng/mL ($p<0.05$). Meanwhile, in the control group, there was a significant increase from the mean of 4.75±0.42 ng/mL to 5.04±0.34 ng/mL ($p<0.05$).

As a suggestion in this study, further research is needed on a more detailed mechanism of action of *Gorek* seed extract in

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reducing urinary F2 Isoprostan. In addition, a clinical trial is needed regarding extracts from *Gorek* seeds before they can be used in humans.

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