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Protective Effect of *PsidiumGuajava L.)*on SuperoxideDismutaseof Cigarette Smoke Exposure in Mice

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ABSTRACT

Background: *Cigarette smoke that was accumulated on blood vessel membrane would constrict smokers' blood vessels. Free radicals which were from cigarette smoke would cause lipid peroxidation of cell membranes, then, it would damage cell membrane organization. If free radicals in the body increased continuously,it was needed enzymes in greater numbers to neutralize superoxide dismutase (SOD). Antioxidants were substances that gave their electrons to free radical molecules and could break the chain reaction of free radicals. Psidiumguaya L extract was a source of anti-oxidants because they contained phenolic and ascorbic acid.* ***Aim****: To analyse the effect of giving extracts of Psidiumguajava l as an antioxidant for the protection of cell membrane damage due to cigarette smoke exposure.* ***Method****: This research was experimental laboratory by using healthy male mice which aged 3-4 weeks, had weightin 25-40gr, were adapted for a week and then, they were divided into 4 groups treated by cigarette smoke exposure and guava extract (PsidiumGuajava L.). Afterwards, it was checked SOD activity levels of mice' blood.* ***Results****: The givingextract of Psidiumguajava L as an antioxidant was proven to give real effect to cell membrane damage due to cigarette smoke exposure that was the increase of the content of Superokside Dismustase (SOD)activity in mice.* ***Conclusions****: Psidiumguaya L extract possesses antioxidative properties against cigarette smoke via augmentation of SOD activity*

Keywords : *Psidiumguajava l*, Antioxidative, SOD,cigarette smoke.

INTRODUCTION

Cigarette smoke exposure continuously caused bronchitis. Cigarette smoke absorption in the body would interact with cells and active substances in cigarettes could cause free radical formation that was *Reactive Oxygen Species* (ROS) and *Nitric Oxide* (NO). The influx of free radicals in the body was caused by the oxidation products when it was exposed by smoke. Free radical which was formed would cause lipid peroxidation of cell membranes, then, it would damage cell membrane organization. If the free radical in the body increased continuously, it would be needed enzymes in greater numbers in order to neutralize *superoxide dismutase enzyme* (SOD). In this moment, SOD enzyme whichhad already existed in the body would be used, and continuously it would be drained. When this enzyme was continuously used, it could cause death. (Cantin, 2010)

This condition led to an excess of free radicals, which would react with fats, proteins, nucleic acids cell, then, it would cause local damage and specific organ dysfunction. If oxidative stress was prolonged, it would cause cells or tissue damage, which in turn was the cause of malignancy, inflammation, atherosclerosis, aging, and ischemia (Chan et al., 2009).

An impact of free radical increase in the body caused body reaction which it was occurred body respond by the increase of antioxidant enzyme activity.

According to Qian(2004), antioxidant was a compound that had a molecular structure which could give its electrons to free radical molecules and could break the chain reaction of free radicals. Antioxidants were often found in fruits and one of them was *PsidiumGuajava L.*

PsidiumGuajava L extract was a fruit that contained vitamin C and beta-carotene which was rich of antioxidants that could slow aging process and cell membrane damage due to free radical attack. Guava fruit contained some chemicals such as quercetin, guajaverin, gallic acid, leucocyanidin and ellagicacid. Antioxidant was a compound that could inhibit the oxidation of other molecules. One of natural antioxidant sources was guava (*Psidiumguajava L*) and it was very nutritious because it contained ascorbic acid (50-3000mg) / 100g fresh weight), three to six times higher than in oranges (Thaipong, 2006)

The purpose of this study was to analyse the effect of *Psidiumguajava L* extract as an antioxidant for protectingSOD due to cigarette smoke exposure that was exposed to mice.

MATERIALS AND METHOD

Experimental Animal

Male BALB/c mice of 6 to 8 weeks oldwere obtained from Airlangga University, Yogyakarta, Indonesia for experimental purpose. They were housed in plastic cages in an air-conditioned room with a temperature maintained at 26^{o}C ± 2^{o}C and 12 h alternates light and dark cycles. The mice were given *ad libitum* with tap water and fed with standard commercial rat chow.

Experimental Design

The sample used 28 male mice were divided into 4 groups: negative control (rats were given daily with aquadest) ; positive control (mice were given daily with cigarette smoke once in a day for 30 days); and the treatment group (mice were given *Psidiumguajava L* extract400 mg; 800 mg/kg BW orally once in a day and were given cigarette smoke one hour after *Psidiumguajava L* extractadministration for 30 days). On day 30 measured levels of Blood Superoxide Dismutase (SOD). On day 30, blood samples were taken by cardiac puncture into tubes and centrifuged at 3000 rpm for 20 minutes; then sera were stored at -85 ^{0}C until the analyzes of antioxidant enzymes (SOD).

Statistical Analysis

Data were presented as means ± standard deviation. One-way ANOVA was carried post hoc tests, and the statistical comparisons among the groups were performed with an LSD test using a statistical package program (SPSS version 17.0)

RESULT AND DISCUSSION

Positive control (cigarette smoke treated mice) showed significant (p<0.05) decrease in the level of SOD compared with negative control. Groups pretreated with *Psidiumguajava L* extract400 mg; 800 mg/kg BW showed a significant (p<0.05) increase in the level of SOD compared with lead

acetate treated rats towards the normal level and close to the negative control (Table. 1).

Table 2. Effects of *Psidiumguajava L* extract on cigarette smoke induced changes in SOD

Groups	Means ± SD SOD (U/ml)
Negative Control	99.16^{a} ± 3.05
Positive Control	77.68^{b} ± 4.72
Psidiumguajava L 50 mg/kg BW	92.53^{c} ± 3.42
Psidiumguajava L 100 mg/kg BW	87.02^{c} ± 2.89

Superscript within each column indicate significant difference between the means

($p< 0.05$)

DISCUSSION

This study aimed to determine the effect of giving *Psidiumguajava l* extract as an antioxidant toward*superoxide dismutase enzyme* (SOD) activity that was applied in mice. The burning cigarette could trigger oxidative stress. *Oxidative stress*was a condition that described production of free radicals that exceeded body immune system. The presence of free radicals could affect normal oxidation-reduction chains, then, it caused oxidative damage of tissue.

Reactive oxygen species or ROS was a derivative oxidizing compounds that were highly reactive and consisting of free radical and non-radical groups. Groups of free radicals included*superoxide anion* (O2), *hydroxyl radicals* (OH) and *peroxyl radical* (RO2). This reactive oxygen compounds produced in process of oxidative metabolism in body, for example food oxidation process into energy (Anand et al., 1996). ROS was produced in sufficient quantities in body such as leukocyte that produced H_2O_2 which had a function to kill several types of bacteria and fungi, but H_2O_2 was not capable of specifically attacking, thus,it could attack polyunsaturated fatty acids of cell membranes. Afterwards, it could cause a damage of structure and function (Anbarasi et al., 2003). The main endogenous antioxidant in body's cells was SOD (*Superoxide Dismutase*) enzyme. Antioxidants could inhibit the release of H_2O_2that was produced by neutrophils of experimental animals.

Cigarette smoke exposurecorrelated with variety of respiratory illnesses. Harmful substances such as sulphur dioxide, nitrogen oxide, and particles resulted from cigarette smoke exposure caused an increase in immunoglobulin E (IgE) with different inflammation mechanisms in respiratory tract. However, this was appropriate with conducted research by Morriet al(2008) who stated that air pollution and cigarette smoke were predisposes factors of the increase of IgE level. Cigarette smoke exposure as allergens would trigger *Antigen Presenting Cells* (APCs) and then, it wasdegraded into peptides which subsequently presented to lymphocyte T cells, or better known asTh cells. Cigarette smoke exposure for a month aimed at providing allergen exposure directly to the main target, the respiratory tract. Cigarette smoke exposure would activate $CD4^{+}$ and mast cells in respiratory tract. $CD4^{+}$would differentiate to be Th2. Differentiation processes would produce IL-4 and IL-5. Th2 that joined with IL-5 could activate and increase eosinophil production. Activation of mast cells would trigger the release of inflammatory mediators such as histamine, prostaglandins, leukotriene, and cytokines.

Smoke absorption in body would interact with cells and active subtances in cigarettes that could cause the form of free radical which was*Reactive Oxygen Species* (ROS) and *Nitric Oxide* (NO). The influx of free radicals in body was caused by the products of oxidation when it was exposed to smoke. The free radicals that were formed would cause lipid peroxidation of cell membrane, then, it would damage membrane cell organization. If the free radicals in the body continued to increase, then,it was needed enzymes in greater numbers in order to neutralize *superoxide dismutase* (SOD) enzyme. In this moment, SOD enzyme that had already existed in the body would be used, and continuously it would be drained. When this enzyme was continuously used, it could cause death. (Kode et al, 2008)

Cigarette smoke exposure that indirectly increased free radicals could reduce the content of SOD as an antioxidant enzyme in the body (endogenous anti-oxidant). From the findings, it could be seen that after being given extract of *Psidiumguajava)*, SOD.

According to Cantin (2010), antioxidant was a compound that had a molecular structure which could give its electrons to free radical molecules and could break the chain reaction of free radicals. Antioxidants were often found in fruits and one of them was guava.Antioxidants were compounds that had a function to prevent, decrease oxidation reaction, discontinue, impede, suspend, and stabilize free radicals (Prakash, 2001). The amount of free radicals in the body affected the endogenous antioxidants work. Exposure to cigarette smoke that was given continuously could increase free radicals in the body that would trigger an imbalance among free radicals.

The negative effects of free radicals were caused by oxidative stress in mice, but the effect could be prevented by vitamin C. Antioxidants reacted with a wide range of ROS in the blood or cells by donating electrons to free radicals such as superoxide radical damper which could ultimately strengthen the work of SOD enzyme in mice's body that was exposed to cigarette (Chan et al., 2009).

PsidiumGuajava L was a fruit that contained vitamin C and beta-carotene which was rich of antioxidants that could slow aging process and cell membrane damage due to free radical attack. Guava fruit contained some chemicals such as quercetin, guajaverin, gallicacid, leucocyanidin, and ellagicacid (Qian 2004). Vitamin C as well as a wide range of carotenoids increased with the participation of flavonoids from guava. The flavonoids werequercetin, kaempferol, and pelargonidin. More complete anitioxidants substances, more qualified its protective effect. Antioxidant wasa compound that was capable of inhibiting the oxidation of other molecules. Compounds that had potential as anti-oxidant were generally flavonoids, phenolic, and alkaloids. An anxiety of side effect of synthetic antioxidant became natural antioxidant as a selected alternative one. Antioxidants were compounds that could inhibit the oxidation of other molecules. One of natural antioxidant sources was guava (*Psidiumguajava L*) and it was very nutritious because it contained ascorbic acid, three to six times higher than in oranges (Thaipong, 2006).

CONCLUSION

Giving*Psidiumguajava L*extract as an antioxidant was proven to give real effect to cigarette smoke exposure. It was characterized by the increaseof *superoxide Dismutase* (SOD) enzyme activity in mice.

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