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The Potency of Red Guava*(Psidium Guajava L.)*Extractas Antioxidant on Cigarette Smoke Exposure in Mice lungs

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ABSTRACT

Cigarette smoke can cause to the formation of an oxidative stress, resulting in increase in concentration of free redicals and decrease antioxidant. Psidium guaya Lextracthas shown to possess powerful antioxidant properties. In the present study, we investigated the impact of Psidium guaya Lextract in against Cigarette smoke -induced lung damage in mice. The 28 male mice were divided into: negative control (mice were exposed with fresh air daily 1 h twice a day for 30 day) ; positive control (Mice were exposed with cigarette smoke 1 h twice a day for 30 days), and the treatment group (mice were given the Psidium Guajava Lextract 0.35 ppm, 0.70 ppm orally once in a day for 34 days and on 4th day, were exposed with cigarette smoke 1 h twice a day for 30 days. On day 34,the mice were sacrificed, and lung tissues were collected to histological evaluations.The cigarette smoke significant induced necrosis inlung cell. However, treatment with the Psidium Guajava Lextract extract significantly improvedlung cell damage.From the results, it is concluded that the Psidium Guajava Lextractare a potent antioxidantin against cigarette smoke-induced lung damge in mice.

Keywords : *Psidium Guajava L*, Antioxidant, Lung, Cigarette smoke

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INTRODUCTION

Cigarette smoke (CS), with its over 4700 different substances (Genbacev-Krtolica, 2005), most of which are described as toxic, mutagenic and carcinogenic (Czekaj et al., 2002), is involved in the development of various chronic pulmonary, cerebro vascular and cardio vascular diseases, cancers and several others (Pryor,1997). Harmful influences of cigarette smoke can be mainly associated with its ingredients like nicotine, cadmium, benzopyrene, oxidants, acetone, mercury and inducers of reactive oxygen species (ROS) like nitric oxide (NO), nitrogen dioxide (NO2), peroxynitrite and nitro samines.The number of ROS, especially O2-, hydroxylradical. (OH) and hydrogen peroxide (H2O2) increases after CS exposure in cells (Rahman, 2005). Reactive oxygen species have a considerable role in smoking related diseases by initiating lipid peroxidation (LPO) and protein oxidation (Reznick et al.,1992) as well as causing DNA strand breaking (Kodama et al., 1997). One puff of cigarette smoke includes 1015 oxidant radicals (Mc Cusker andHoidal, 1990).

Reactive oxygen species are suppressed orscavenged by cellular antioxidant defense systems

consisting of enzymatic mechanisms such as super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) as well as non-enzymatic mechanisms such as reduced glutathione (GSH), vitamins A, C and E. Preventive role of antioxidants was reported in modifying the major diseases related to Cigarette smoke (Anbarasi et al., 2005). When ROS increased excessively, antioxidant deficiency occurs. This inadequacy may be enhanced by usually insufficient intake of both supplementary and dietary antioxidants (Zondervanet al., 1996). One of the materials being used as antioxidant source to eliminate free radicals in CS are plant extracts (Chang et al., 2010).

Antioxidant was mostly in plant, and one of natural antioxidant sources was red guava (*Psidium guaya L)*which was very nutritious because it contained ascorbic acid (50-3000mg)/100g fresh weight), three until six times higher rather than orange (Agustina, 2009). Red guava (*Psidium Guajava L*) was a fruit with vitamin C and ascorbic acid even *flavonoid* compound which had antioxidant that could slow down aging process and damage of the membrane. The objective of the present study was to investigate the antioxidant activity of the *Psidium Guajava L*extract on cigarette smoke-induced lung cell damage in mice.

MATERIAL AND METHODS

Preparation of ethanol extract of *Psidium Guajava L*

Plant material and extract preparation of *Psidium Guajava L* were collected from Surabaya, Indonesia. *Psidium Guajava L* materials were cleaned with running tap water and chopped into pieces. They were dried under shade at ambient temperature for 5 days, and the air dried *Psidium Guajava L*was then ground to powder for extraction. The powdered *Psidium Guajava L* (1 kg) was macerated with ethanol 96% (5 L) for 1 week at 37°C. The supernatant was then collected and filtered through Whatman No. 1 filter paper in a Buchner funnel under vacuum. The filtrate was concentrated by evaporation with a vacuum rotary evaporator at 45°C . Further more, the extract was freeze dried to get ethanol-free powder.

Experimental animal

Male mice weighing approximately 24-30 g (2.5-3 months) were obtained from Airlangga University, Surabaya, Indonesia for experimental purpose. They were housed in plastic cages in an air-conditioned room with a temperature maintained at 26 ± 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 (mice were exposed with fresh air daily 1 h twice a day for 30 day) ; positive control (Mice were exposed with cigarette smoke 1 h twice a day for 30 days), and the treatment group (mice were given the *Psidium Guajava L*extract 0.35 ppm, 0.70 ppm orally once in a day for 34 days and on 4th day, were exposed with with cigarette smoke 1 h twice a day for 30 days. On day 34,the mice were sacrificed, and lung tissues were collected to histological evaluations. The tissue of lung was fixed in a 10% neutral buffered formal in solution, embedded in paraffin and used for histopathological examination with hematoxylin and eosin (H&E) stain.

RESULT AND DISCUSSION

Histological observations on the negative control showed that in lung cell is observable and they appear normal architecture of the lung cell. In the positive control, mice were administered with cigarette smoke showed lung cell damage (necrosis). In the mice, treated with *Psidium Guajava L*

extract, the number and morphological integrity of lung cells are being preserved. Observations indicate that the damage effects cigarette smoke was reduced by *Psidium Guajava L*extract [Figure 1].

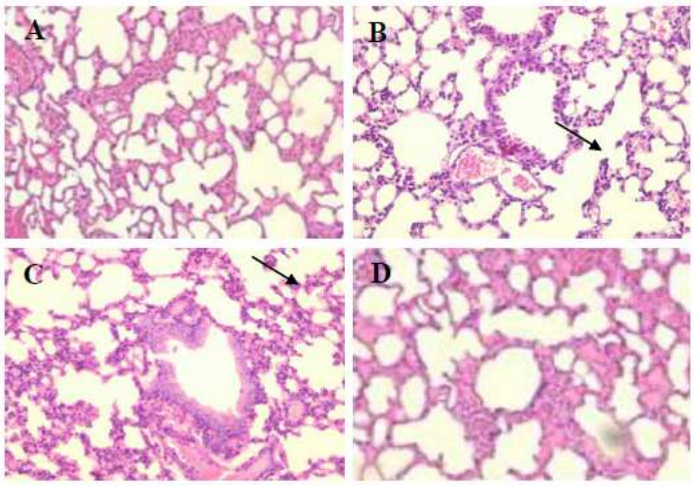

Figure 1: Histological study of treatment with *Psidium Guajava L* extract on cigarette smoke-caused lung damage. Normal morphology of lung sections in the negative control group (A). The cigarette smoke treated group showed necrosis (indicated by black arrows) (B). Mice treated with *Psidium Guajava L* extract0.35 ppm and 0.75 ppm showed necrotic changes (C and D). H&E ×400

DISCUSSION

Cigarette smoke includes many oxidants and pro-oxidants that generate free radicals and increase the oxidative stressin lung tissues (Park et al., 1998). Thus, cigarette smoke is a constant oxidant stress on the respiratory tract.A cell could protect itself against oxidative stress by the use of endogenous and exogenous antioxidants.Depending on this data, smokers are usually directed to use antioxidants (Smith and Hodges, 1987). Current study was realized to determine whether *Psidium Guajava L*extract would be an effective antioxidant against smoking-mediated oxidative damage.

Cigarette smoke exposure was a trigger of necros is based that was influenced by the duration of smoking, the number of cigarettes smoked per day, the age and depth of smoking.Cigarette smoke could lead the entry of free radicals in the body and cause necrosis on epithelium. The process of necrosis began with the weakening of blood vessel wall, inflammation response, the formation of plaque that was foam cells and the formation of blood clots.Cigarette smoke exposure for a month aimed to provide allergen exposure directly to the main target cause the occurrence of bronchitis. Normal respiratory tract wall waslayered by ciliated pseudo stratified. The condition of bronchitis caused cell changes and structural abnormalities due to inflammation. Through histopathologic examination on bronchus, it was obtained damage of epithelial bronchus with cillia abnormalities and smooth muscle hypertrophy.

Necrosis could be recognized due to the changes either macroscopically or microscopically. Necrosis caused nucleus cell changes that consistedof 3 processes of picnosis$_1$, which was core contraction that was occurred due to homogenizing cytoplasm and the increase of eosinophil, and then, DNA condensed into a dense mass. After that, it was occurred cariorexis$_2$, which was a pignotic fragmented nit state (divided into fragments), then, it was occurred cariolisis$_3$, which was the fading of basophil chromatin due to DNA se activity (Lestari and Mulyono 2011).

Psidium Guajava L. as an antioxidant contained various substances that had a function as an inhibitor of various diseases, including flavonoids, tannins, oil and also Saponin. Flavonoid was an alcohol in the form of Phenol. Meanwhile, polyvenol compounds of red guava were quercetin, guajavarin, gallic acid, leukocyanidi 0.1% hexahidroksidiven ester in the form of glycoside 0.1%, elagat acid (Agustina, 2009).

Flavonoid could prevent LDL oxidation 20 times stronger rather than vitamin C. Flavonoids were proven to have very strong biological effects as anti-oxidants that stimulated the production of nitrite that could widen blood vessels. Free radicals in the body affected the work of endogenous antioxidants. Continuous cigarette smoke exposure could increase free radicals in the body that would trigger an imbalance between free radicals and endogenous antioxidants that caused oxidative stress. Flavonoid in red guava extract (Psidium guajava L) could increase endogenous antioxidant activity (SOD, Catalase, GPx), thus, it could stabilize free radical bonds in the body, and the IgI decreased. The improvement process of epithelial was occurred by the decrease of epithelial damage when the free electrons from cigarette smoke exposure were tied to flavonoid antioxidant of red guava extract (Psidium guajava L) in the form of OH cluster. Red guava extract (Psidium Guajava l) would trigger SOD, Catalase and GPx activity in the body in order to stabilize free radicals. Nevertheless, the results showed administration of Psidium Guajava L extract could improve lung tissue damage (inflammation, oedema, necrosis) that was caused by cigarette smoke exposure.

CONCLUSION

The results of the present study indicate that cigarette smoke-caused lung damage (necrosis) might be related to oxidative stress. The administration of Psidium Guajava L extract lessened the effects of cigarette smoke-induced lung tissue necrosis possibly by inhibiting free radical-mediated process. Further investigation of these promising protective effects of Psidium Guajava L extract against cigarette-induced lung cell damage may have a considerable impact on developing clinically feasible strategies to treat patients with cigarette smoke-induced lung damage.

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