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Inhibition Inflammation Process on Cigarette Smoke Induced Rats by Extract of Manggosteen Peel (*Garcinia mangostana* L) Based on Oxidant-antioxidant Profile

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Abstract: Smoking, either active or passive was widely demonstrated inflamed respiratory organs through changing ratio oxidant anti-oxidant that causes inflammation. Herbal-based antioxidant therapy reduces inflammatory process, but the mechanism is still unclear. This study mainly aims the potencyof manggosteen peel extract to normalize the oxidant-antioxidant ratio and repair functional structure of epithelial bronchi on cigarette smoke induced rats. This study used cigarette smoke induced rats (*Rattus norvegicus*) 3-month-old male were divided into 5 groups. Group 1 was healthy rats (negative control group), group 2 was cigarette smoke induced rats (positive control group); groups 3, 4 and 5 (theraphy groups) were cigarette smoke induced rats also administered with mangosteen peel extract (*Garcinia mangostana* L) with dose of 150 mg / kg BW, 300 mg / kg BW, and 600 mg / kg BW, respectively. Inflammation was observed by epithelial histopathology structure of bronchioles and the level of oxidant antioxidant measured based on MDA level and SOD activity using spectrophotometry. The results showed that the extract of mangosteen peel (*Garcinia mangostana* L) significantly reduced the levels of MDA ($p < 0.05$) and increased the SOD activity. The extract of mangosteen peel (*Garcinia mangostana* L) was able to improve epithelial histopathology structure of bronchi on cigarette smoke induced rats. We concluded that the extract of mangosteen peel (*Garcinia mangostana* L) could reduce the levels of MDA, increase SOD activity and repair damaging of bronchial tissue.
Key words: Cigarette smoke induced rats, MDA; SOD, oxidant antioxidant level, bronchi histopathology.

Introduction

Smoking is one of the biggest causes of death in the world. Cigarette consumption in Indonesia is about 6.6 percent of cigarette consumption in the world. Indonesia is one of the developing countries that most of the people take up smoking [1]. The cigarette smoke contains about more than 4000 compounds, such as benzo-[a]-pyrene and other aromatic or poly-aromatic from cigarette burning productc, which able to lead the inflammation followed by lung cancer [2,3]

Exposures to cigarette smoke continuously cause bronchitis. Smoke absorption in the body will interact with cells, especially cells in the respiratory tracts, and the active substances in cigarettes can cause the formation of free radicals are reactive oxygen species (ROS) and nitric oxide (NO) [4]. ROS have been shown

to play an important role in numerous form of inflammation. Free radicals are formed will cause lipid peroxidation of cell membranes so will damage the cell membrane organization. Many enzymes are involved in

protection against the injurious effects of ROS, inhibiting activity of malondialdehyde (MDA), one of enzymes is superoxide dismutase (SOD). Molecular mechanism of airway inflammation as a result of smoking and the role of herbal therapy as exctract of Manggosrteen peel (*Garcinia mangostana* L) has not been determined.

Manggosteen peel (*Garcinia mangostana* L) contains xanthones which are antioxidants [5]. Alpha-mangostin and gamma-mangostin are the most content of the xanthone compounds. Antioxidant is a compound having a molecular structure which can provide electrons to the free radical molecules and can break the chain reaction of free radicals.

Material and Methods

Animals Models Preparation

Twenty five male rats were adapted for seven days before exposure cigarette smokes by feeding in the form of basal ration and drink *ad libitum*. The composition of the basal diet prepared by standard of AOAC (2005) that lack the carbohydrates, proteins, fats, minerals, and vitamins. The use of rats were approved by the Ethical Committee Brawijaya University No: KEP-183-UB. 3-month-old male with an average body weight of 150-200 grams were purchased from Animal Research Unit, Gajah Mada University, Indonesia.

The rats were assigned randomly to one of five groups: Group 1 was healthy rats (negative control group), group 2 was cigarette smoke induced rats (positive control group); groups 3, 4 and 5 (theraphy groups) were cigarette smoke induced rats also administered with mangosteen peel extract (*Garcinia mangostana* L) with dose of 150 mg / kg BW, 300 mg / kg BW, and 600 mg / kg BW, respectively. Manggosteen peel extract was given for 21 days by stomach gavage as much as 1 mL / rat / day. After 21 days, the rats were sacrified and the the bronci were removed and cut into two parts. One part was fixed with 4 % Paraformaldehyde (PFA), and the last part store in Phosphate Buffer Saline (PBS) buffer at – 70^{o}C until it was analyzed.

Extraction of Manggosteen peel (*Garcinia mangostana* L)

Mangosteen peel (*Garcinia mangostana* L) were washed using tap water then separated from the fruit. It was then cut to the size of 0,5x1cm^2, dried overnight at a temperature of 45^{o}C. Then it was destroyed with blander dried to make powder. The powder then was macerated using ethanol to obtains yellow viscous liquid, followed by distilation proces to separate ethanol and water in order to obtain extract of manggosteen peel [4,6].

Cigarette Smoke Exposure in Rats

Cigarette smoke exposures on rats were conducted for a month, every day rats were exposed to two cigarettes [4]. Cigarette smoke exposure on rats were done by using a 60 mL syringe and then tipped with yellow tip. The plastic hose prepared in accordance with the size of the cigarette, then lit cigarettes and cigarette smoke will be drawn using the syringe fitted with a yellow tip ends. Cigarette smoke was then transferred into a special cage for exposure. This was conducted repeatedly until finished cigarette.

Histopathologycal Analysis of Bronchi.

The bronchi were collected in 4% Paraformaldehyde (PFA) for preparing the section slide. The section slides were stained with Hematoxylene Eosine for histopathologycal examination, and were observed microscopically.

Malondialdehyde (MDA) Standard Curve

100 µL of solution of malondialdehyde assay kit (Sigma, MAK085) with vary concentration (0; 1.0; 2.0; 3.0; 4.0; 5.0; 6.0; 7.0; and 8.0 mg/mL) was pipetted in microtube and each was dissolved with 550 µL of distilled water. Each of tube was added with 100 µL of 100%), trichloroacetic acetic, 250 µL of 1 M hydrochloric acid, and 100 µL of 1 % sodium thiobarbiturate. The solutions were centrifuged at 500 rpm 4^{o}C for 15 min, and incubated at waterbath (100^{o}C) for 30 min. Then it was left at room temperature, then the absorbance was measured on 533 nm to obtain MDA standard curve.

Malondialdehyde (MDA) Samples Measurement

Bronchial tissue was taken as much as 0.1 grams, cut into small pieces and then crushed in a mortar in cold conditions. The homogenate then was added with 1mL of 0.9% NaCl, then it was transferred into a microtube and centrifuged at 8000 rpm, 4^{o}C for 20 min and the supernatant was collected. 100ìL of supernatant then was added with 550 mL of distilled water, 100ìL TCA, 250ìL of 1N HCl, and 100ìL of Na-Thio. Each addition of reagent the solution was vortexed. It was then centrifuged at 500 rpm, 4^{o}C for 15 min. The Supernatant were separated and transferred into a new microtube, incubated in a water bath at a temperature of 100^{o}C for 30 min, then left at room temperature. The absorbance were measured at ë max 532 nm test and plotted on the standard curve to calculate the concentration MDA samples.

Superoxide Dismutase (SOD) Activity Measurement

The bronchi were rinsed in ice - cold PBS (pH 7.0 - 7.2) to remove excess blood and weighed before homogenization. It were minced homogenized in 5 - 10mL of PBS with a glass homogenizer on ice. The resulting suspension was sonicated with an ultrasonic cell disrupter. After that, the homogenates were centrifugated for 5 minutes at 5000×g. The supernatants are collected and used as sample for Enzyme Linked Immunosorbent Assay (ELISA).

The SOD ELISA kit (LsBio,K335) wells were filled with 100 ìL standards, blank and sample test and incubated for 2 h, at 37^{o}C. The liquid on each well was removed out without washing and added with 100 ìL detection reagent A, the was incubated 1 h at 37^{o}C. The solution was aspirated and wash 3 times with 350 ìL washing solution. The liquid from wells were removed completely. Then the plate was inverted and blotted with absorbent paper. After that, it was added with 100 ìL detection reagent B, then incubated for 30 min at 37 ^{o}C. The aspiration and washing process was repeated with 350 ìL washing solution. Then 50 ìL of stop solution was added then solution turn to yellow. The last, the microplate reader was run to conduct the measuremet at ë max 450 nm immediately.

Results and Discussion

Profile of MDA Level on Cigarette Smoke Induced rats after adminitration of Manggosteen peel extract

Manggosteen peel extract can reduce level of MDA on cigareete induced rats (Table 1). Cigarette smoke induced the increasing level of MDA. MDA which is the product of lipid peroxidation can be used as an indicator for lipid disorder on cell membrane.

Table 1. **MDA Level of Bronchi Rats**

Group	MDA (iL /mL)		
	Value	Increase (%)	Decrease (%)
Healthy Rats	0.1768 ± 0.245^{a}		
Cigerette smoke induced rats	O.6574± 0.035 ^{d}	271.83	
Therapy dose of 150 mg/kg BW	0.3967 ± 0.024 ^{c}		39.67
Therapy dose of 300 mg/kg BW	0.2186± 0,0124 ^{b}		66,75
Therapy dose of 600 mg/kg BW	0.1854± 0.0228 ^{a}		71,80

SOD Activity on Cigarette Smooke Induced Rats After adminitration of Manggosteen peel extract

The result indicate that mangosteen peel extract therapy increase SOD activity of bronchi on cigarette smoke induced rats (Table 2). The result give positive affect for increasing SOD activity after administration of manggosteen peel extract on Cigarette smoke induced rats.

Table 2. SOD Activity of Bronchi Rats

Group	SOD activity U/mL		
	Value	Decreas activity (%)	Increase (%)
Healthy Rats	58,68 ± 1,47[c]		
Cigerette smoke induced rats	25,54 ± 1,53 [a]	56,48	
Therapy dose of 150 mg/kg BW	39,67 ± 2,06 [b]		55,32
Therapy dose of 300 mg/kg BW	56,38± 2,34 [c]		120,75
Therapy dose of 600 mg/kg BW	54,89± 2,28 [c]		112,92

Repairing Functional Structure of Epithelial Bronchi on Cigarette Smooke Induced Rats after Adminitration of Manggosteen peel Extract

Histopathological examination of the results of this study indicate that there is inflammation in bronchi (bronchitis) caused by exposure to cigarette smoke. This bronchitis condition causes damage to cell structures due to the inflammatory process indicated the presence of inflammatory cells (Fig. 1). In healthy conditions or negative controls (Fig 1a) indicated the presence of coating on the walls of the airways with the pseudostratified ciliated epithelium.

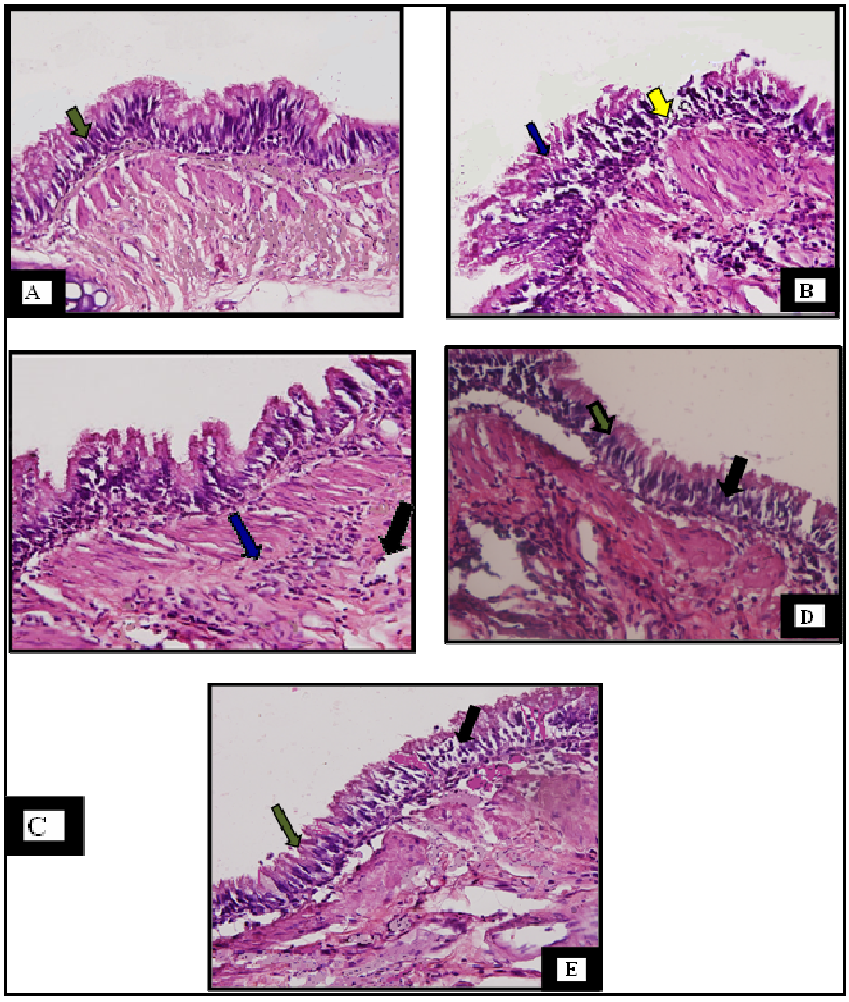

Figure 1. Histopathology of Bronchial Tissue Rats (*Rattus norvegycus)* with Haematoxilen-Eosine (HE) Staining (400x)

(A) healthy rats (negative control group); (B) cigarette smoke induced rats (positive control group); (C,D, E) cigarette smoke induced rats also administered with mangosteen peel extract (*Garcinia mangostana* L) with dose of 150 mg / kg BW, 300 mg / kg BW, and 600 mg / kg BW, respectively. Green arrow pointing to compact epithel, blue arrow pointing to damage epithel, yellow arrow pointing to missing epithel, black arrow pointing to repairing epithel

Discussions

Antioxidant contained in manggosteen peel extract decrease MDA level of bronchi cigarette smoke induced rats. Statistically analysis also showed a significantly difference between treated rats ($p<0.05$). Antioxidants that contained in manggosteen peel extract acts as a radicals scavenger so that the free radical chain can be interrupted, confirmed by decreasing of MDA level (Table 1). One potential mechanism of inflammation in the respiratory tract is the production of free radicals, followed by the process of oxidative stress. It shows on the positive control group which showed the highest level of MDA. Oxidative stress is a term denoting an imbalance between the production of oxidants and the respective defense systems of an organism. High Level of MDA in cigarette smoke induced rats indicate high level of oxidative stress on cells. Manggosteen extract therapy could reduce MDA level on rat bronchi. The higher dose the lower of MDA level was obtained. Dose of 600 mg/kgBW reached the optimum yield to reduce MDA level as healty rats.

Manggosteen peel contains xanthones which are natural polyphenolic compound and have antioxidant effects [7]. The mechanism of these compounds is by inhibiting the production of intracellular ROS significantly [8,9]. The ethanol extract of mangosteen peel able to inhibit 50% of radical formation and also reduce the production of ROS by inhibiting superoxide radical (O2-) by capturing the hydroxyl radical (OH), but its more powerful in inhibiting superoxide radical [10].

Cigarette smoke contains two populations of free radicals derived from tar phase and gas phase. Tar phase is a substance that trapped when the smoke stream passed through glass-fiber filters that can save 99.9% particle with size of 0.1 μm^2. While the gas phase are molecules or particles that can pass through this filter. The gas phase containing 10 radical / puff of a cigarette which is a small oxygen-radical and carbon-centered radicals with a short life span is much more reactive compare with free radicals contained inside tar phase. The SOD activity cigarette smoke induced rats decrease 56,48 % comparing to the healthy one. The positive result is the manggosteen peel extract increase up to 120,75 % compare of control group. This result suggests that cigarette smoke contains many free radicals that suppress endogenous antioxidant enzymatic, as SOD activity of bronchi. Antioxidants contains in mangosteen peel extract can increase the SOD activity in order to stabilize the bonding of free radicals in the cells.

In the positive control group or cigarette smoke induced rats (Fig. 1b) demonstrated the bronchus epithelial damage and abnormal cilia condition. Bronchial tissue damage in cigarette smoke-induced rats caused by free radicals from cigarette smoke contains many types of oxidants. In contrary, on theraphy group showed the repairing structural damage of bronchi. It is believed due to the role of antioxidants from manggosteen peel extract can increase the activity of SOD enzyme, which can improve the ratio of oxidant-antioxidant (MDA and SOD). Bronchial epithelial damage in the positive control group is believed to be due to the presence of inflammatory cell mediators, such as eosinophils are released throughout the process of inflammation, mucus hypersecretion and accumulation of free radicals. Eosinophils release of proteolytic enzymes such as Major Basic Protein (MBP) which can damage the epithelial bronchi because it can cause the destruction of the epithelium, while lymphocytes release lymphokines (IL5) as proinflammatory mediators

Conclusion

In conclusion, we have demonstrated a possible protective effect of manggosteen peel extract againts to free radicals that induced by cigarette smoke on the basis of oxidant-antioxidant confirmed by MDA level and SOD activity of bronchi and functional structure of epithelial bronchi by HE staining.

Conflict of interest

There is no conflict of interest.

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