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THE INFLUENCE OF GUAVA EXTRACT (*PSIDIUM GUAJAVA* L.) ON HISTOPATHOLOGICAL FEATURE OF WHITE RAT (*RATTUS NORVEGICUS*) EXPOSED CIGARETTE SMOKE

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ABSTRACT

The purpose of this study was to determine the effect of giving guava extract (*Psidium guajava* L.) to the histopathological feature of the lung of white rats (*Rattus norvegicus*) exposed cigarette smoke. This study used 25 male rats with an average body weight of 150-200 gr per head. The negative control group (K-) was given CMC Na 0.5% 2 mL/head without exposure to cigarette smoke. P0 group was given 3 cigarettes and CMC Na 0.5% 2 mL/fish per day. P1, P2 and P3 groups were given 3 cigarettes as much exposure to cigarettes and 3.78 mg / 200g BW, 7.58 mg / 200g BW, 11.34 / 200g BW, respectively. Provision of cigarette smoke and guava extracts carried out for 45 days. The results showed that the administration of guava extract (*Psidium guajava* L.) can repair congestion damage and thickening of white rat alveoli at the highest dose of 11.34 mg/200g BW. Based on the results of the Kruskal Wallis statistical analysis p = 0.000 which shows there are significant differences, further tests using Mann Whitney show there are differences in each group. The conclusion of this study is the administration of guava extract can improve congestion damage and thickening of white rat septa alveoli which are exposed to cigarette smoke.

KEY WORDS : Guava extract, Congestion, Thickening the alveoli septa, Cigarette smoke.

INTRODUCTION

Cigarettes are consumed by various levels of society, various ages, and various economic status. Although they are aware of the dangers of smoking, the pleasure they feel causes many people to forget the dangers (Winarsih, 2007). At present there are an estimated 1.3 billion smokers in the world. The number of deaths due to cigarette consumption is 5.4 million people per year, 1 person every 6 seconds and 1 in 10 adult deaths worldwide. If consumption patterns continue, the number of deaths will approach 8 million people per year by 2030 (World Health Organization, 2006).

Cigarettes contain more than 4,000 hazardous substances, including tar, arsenic, formaldehyde, and benzo (a) carcinogenic pyrene. In cigarette smoke also contains carbon monoxide (CO), hydrogen cyanide, nitrogen oxides, and ammonia (Cancer Research UK, 2006).

In guava fruit there are chemicals that can affect antioxidant activities, such as flavonoid compounds, a combination of saponins with oleanolic acid, guaijavarin and quercetin (Paniandy *et al.*, 2000). The content of vitamin C per 100 grams of ripe guava is 150.5 mg, optimal ripe 130.13 mg and too ripe 132.24 mg (Parimin, 2006). When it is ripe, the vitamin C content can reach 3-6 times higher than oranges (Dalimartha, 2003).

This varied antioxidant content is a great potential to be able to capture free radicals, especially free radicals from cigarette smoke (O'Dell, 1999). Guava fruit contains various substances that function as inhibitors of various types of diseases, including types of flavonoids, tannins, essential oils, and there are also saponins (Dweck, 2001).

There have been no studies linking the role of guava fruit extract in lung disease, but research using the bitter extract in lung disease has been done (Oktavia, 2016). Based on the above background, the study of the effect of guava fruit extract (*Psidium guajava* L.) on histopathological features of

congestion, and thickening of septa alveoli in rats exposed to cigarette smoke was carried out.

MATERIALS AND METHODS

Research design and procedure

This study used 25 male rats (*Rattus norvegicus*) about 200 gr BW were divided into 5 groups so that each group consisting of 5 heads was adapted for 7 days. In the negative control group (K-) no smoke exposure was given and only CMC Na 0.5% 2 mL/ head. On the 8th day the treatment began with 3 cigarettes per day in cigarette P0, P1, P2 and P3 groups then the P0 group was only given CMC Na 0.5%, guava fruit extract was given with 3 different doses for P1, P2 and P3 namely amounted to 3.78 mg/200gBW, 7.58 mg/200gBW, 11.34 mg/200gBW per day. Exposure to cigarette smoke is done by putting each group of rats in the smoking box connected by a hose, cigarettes are burned and smoked using a 60cc syringe, a syringe containing cigarette smoke is injected into the box, a total of 1 cigarette is burned as much as 32 suction and 1 cigarette spend about 5 minutes. Exposure to cigarette smoke and administration of guava fruit extracts was carried out for 45 days and on the 46th day all mice were sacrificed and their lungs were removed and histopathological preparations were made to check for congestion and septa thickening.

Assessment of research variable

Congestion and thickening of the septa alveoli, congestion is the accumulation of red blood cells in the lumen of the alveoli and thickening of Septa Alveoli is apparently infiltration of red blood cells, leukocytes and neutrophils, inflammatory cells, and proliferation of alveolar macrophages through hematoxylin-eosin staining with 400x magnification.

Histopathological changes of alveoli are observed microscopically at 400x magnification. Pulmonary histopathological damage with both dependent variables (alveolar congestion, and septa alveolar thickening) is performed by a scoring system according to Klopfleisch (2013)(Klopfleisch, 2013). Congestion:0 = there is no congestion, 1 = congestion <10% field, 2 = there is a congestion of 11-25% field, 3 = congestion 26 - 50% field, 4 = there is congestion 51 - 75% field, 5 = there is congestion> 75% field. Thickening of the Septa:0 = no septa thickening, 1 = thickening of septa <10% visual field, 2 = thickening of septa 11-25% visual field, 3 = thickening of septa 26 - 50% visual field, 4 = thickening of septa 51 - 75% visual field, 5 = thickening of septa> 75% visual field.

Data Analysis

Analysis of alveolar histopathological features was statistically analyzed by the Kruskal Wallis test to find out at least one treatment that had a high dose effectiveness compared to other treatments. Analysis continued with the Mann Whitney test for pairwise comparisons. All data were analyzed using the SPSS (Statistics Program and Service Solution) program (Dahlan, 2013).

RESULTS AND DISCUSSION

Based on the observation of histological preparations of the results of congestion in groups that were given cigarette smoke treatment by giving guava fruit extract at a dose of 3.78 mg / 200gBW, 7.56 mg/200gBW, 11.34 mb/200gBW could increase the amount of congestion compared to P0. Congestion in the white rat's lungs at P0 was more numerous and evenly distributed compared to other treatments. P1 at a dose of 3.78 mg/200gBW decreased the amount of congestion compared to P0 even though the amount of congestion distribution was not much different from P0 which was only given CMC Na 0.5%. P2 with a dose of 7.56 mg / 200gBW also experienced a decrease in congestion. At P3 at a dose of 11.34 mg/200gBW the decrease in congestion was almost close to the size at (K-).

Table 1. Alveoli congestion on the histopathological features of the lung of male white rats for each treatment.

Treatment	Means ± SD
K- (CMC Na 0.5%)	0.53^{b}± 0.05
P0 (CMC Na 0.5% + exposed to cigarette smoke)	4.03^{a}±0.36
P1 (3.78m mg/BW + exposed to cigarette smoke)	3.06^{a}±0.35
P2 (5.76 mg/BW + exposed to cigarette smoke)	2.13^{a}±0.49
P3 (11.34 mg/BW + exposed to cigarette smoke)	1.10^{b}±0.11

Note: Different superscripts in the same column show significant differences ($p < 0.05$).

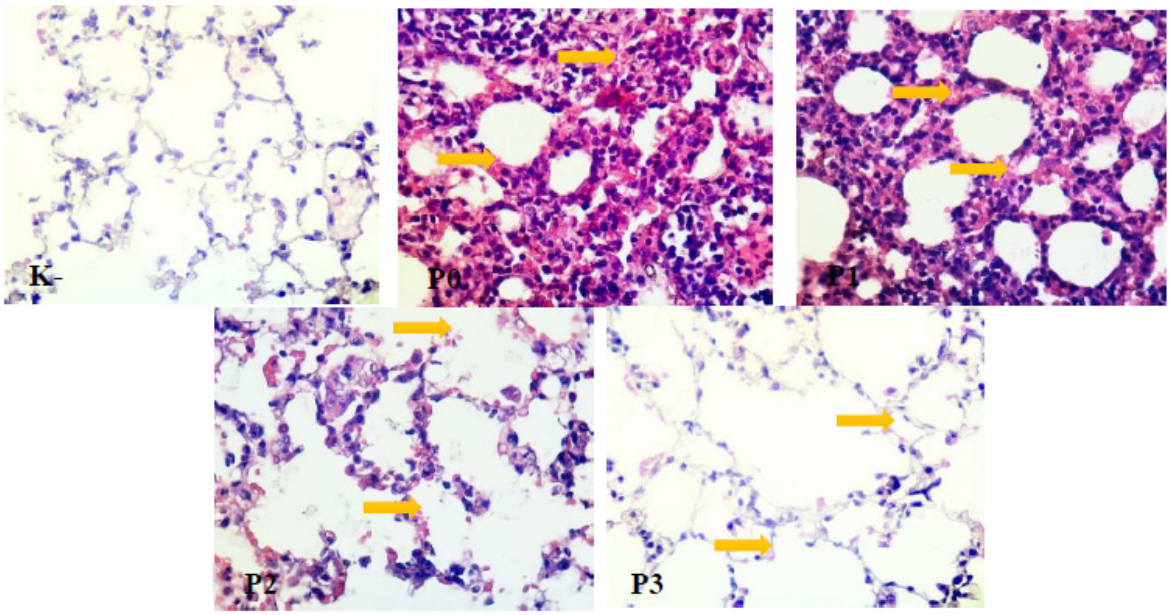

Fig. 1. Histopathological features of alveolar congestion with 400x magnification with HE staining in the negative control group (K-), Group (P0), Group (P1), Group (P2), Group (P3). Yellow arrow indicates congestion

The treatment of guava fruit extract dosage from a dose of 3.56 mg/200gBW, 7.56 mg/200gBW and 11.34 mg/200gBW significantly improved the decrease in congestion. By administering the highest dose of guava fruit extract, it can reduce ROS production and restore congestion close to the negative group treatment. Antioxidants contained in guava fruit extract can stabilize free radicals by completing the lack of electrons owned by free radicals and inhibit the chain reaction of the formation of free radicals that can cause cell damage. Thus damage to the cell membrane due to free radicals can be inhibited (Arsana, 2014).

Based on the observation of histological preparations resulting from thickening of septa alveoli in groups treated with cigarette smoke by giving guava fruit extract at a dose of 3.78 mg/200gBW, 7.56 mg/200gBW, 11.34 mg/200gBW can increase thickening in alveoli septa with P0. In alveoli, male white mice at P0 were seen to have thickened septa compared to treatment at (K-). The reduction in septa alveoli thickening in the treatment can result from a decrease in the amount of congestion. P1 at a dose of 3.78 mg/200gBW experienced an increase in congestion causing septa thickening compared to P0 although it did not have a much different thickness size than P0 which was only given a 0.5% CMC Na. P2 group with a dose of 7.56 mg/200gBW also experienced an increase in congestion causing thickening of the alveoli septa. At P3 at a dose of 11.34 mg/200gBW, the thickening of the alveoli septa is almost close to the thickness at (K-). The treatment of guava fruit extract dose from a dose of 3.56 mg/200gBW, 7.56 mg/200gBW and 11.34 mg / 200gBW can significantly reduce ROS production and improve the thickening of septa alveoli.

Quercetin compounds inhibit oxidative stress by

Table 2. Thickening of septa alveoli on the histopathological feature of the lung of male white rats (*Rattusnovergicus*) each treatment.

Treatment	Means ± SD
K- (CMC Na 0.5%)	0.40^{b}±0.93
P0 (CMC Na 0.5% + exposed to cigarette smoke)	4.20^{a}±0.10
P1 (3.78 mg/BW + exposed to cigarette smoke)	2.93^{a}±0.11
P2 (5.76 mg/BW + exposed to cigarette smoke)	2.26^{a}±0.05
P3 (11.34 mg/BW + exposed to cigarette smoke)	1.06^{b}±0.86

Note: Different superscripts in the same column show real differences ($p<0.05$).

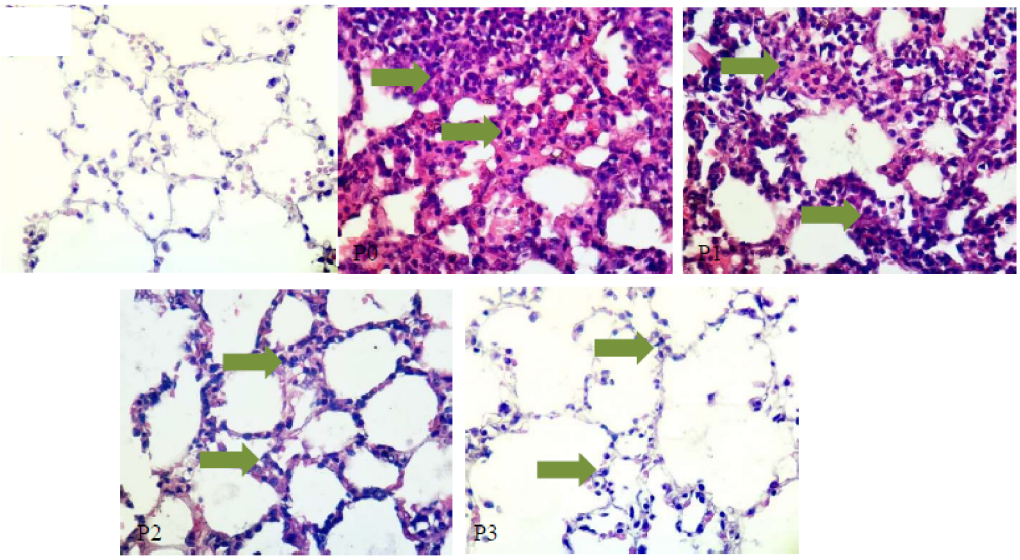

Fig. 2. Histopathological featureof thickening of septaalveoli with 400x magnification with HE staining in the negative control group (K-), Group (P0), Group (P1), Group (P2), Group (P3). Green arrow indicates thickening septa

regulating the balance between oxidants and antioxidants. Quercetin effectively protects cells from free radical damage by increasing endogenous antioxidant levels. Bioflavonoids act as reducing agents for hydrogen or electron agents that can inhibit or reduce free radicals and increase antioxidants in the body. Thus, quercetin can reduce oxidative stress including nicotine-induced ROS production from cigarette smoke exposure (Dong et al., 2018).

CONCLUSION

Based on research that has been done, it can be concluded that administration of guava fruit extract at a dose of 11.34 mg/200g BW per day in male white rats can improve congestion damage and thickening of rat septa lung exposed by cigarette smoke.

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