
PROTECTIVE EFFECT OF TROPICAL FRUIT JUICE ON HISTOPATHOLOGICAL IMAGE OF RATS LUNG EXPOSED TO CIGARETTE SMOKE

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Abstract

Indonesia is a tropical country that has diversity of fruit. Fruit contain an array of antioxidant substances that give benefit for human health. This research purpose is to analyse the protective effect of antioxidant-rich tropical fruits juice on the lung of Wistar rats that exposed to cigarette smoke. Adult male Wistar rats (n 35) were divided into five groups of seven each, as follows: control group; cigarette smoke group; cigarette strawberry, papaya juice; cigarette+strawberry, red guava juice; cigarette+strawberry, apple juice. Rats exposed to cigarette smoke 20 minutes/day for 40 days. Three ml of mixed fruit juice was given orally one hour after exposed. We analyse the structural damage of alveolus lumen and membrane, along with connection between alveolus. The results show that the structural damage of alveolus lumen and connection between alveolus of all fruit juice groups were significantly decrease ($P < 0,05$) compared with cigarette smoke without fruit juice group. Strawberry & apple juice shows the best protective effect on the lung of Wistar rats that exposed to cigarette smoke.

Keywords: tropical fruit juice, lung, cigarette smoke

Background

Indonesia is one of the eight centers of plant genetic diversity in the world, especially tropical fruits. There are various types of tropical fruits grow in Indonesia, including papaya, guava, orange, banana, mango and others. According to Febrianti *et al.* (2015) Indonesian tropical fruits contain many important antioxidant compounds, such as ascorbic acid, beta carotene, phenols, anthocyanins, and flavonoids. The results also show that the tropical fruits have free radicals scavenger activity.

Fruits are potential source of natural antioxidants. Natural antioxidants from fruits is readily available and affordable to the people. According to Xu and Luke (2012) natural antioxidants are generally safer for consumption and can improve the health of the body than synthetic antioxidants. Antioxidants have the power to scavenge and neutralize free radicals that are harmful for the body, one of the free radical source is cigarette smoke.

Smoking is one of the biggest problems in the world's population today. Indonesia is one of the ten countries with the highest number of smokers in the world. According to WHO (2009) smoking is the biggest cause of death in the world. Smoking causes the death of one person every six seconds around the world and is a risk factor for six of the eight largest disease cause of death. The impact of the dangers of smoking is not only felt by smokers themselves (active smoker), but also felt by people exposed to cigarette smoke (passive smoker/secondhand smoker). According to WHO (2009), people who are exposed to cigarette smoke in the room (secondhand smoker) has caused more than 600,000 premature deaths per year and in 2004, causing 28% of children deaths.

The respiratory system is an organ system that is most vulnerable to the negative impacts cigarette smoke exposure. The development of pulmonary diseases, such as pulmonary emphysema, pulmonary fibrosis, and pulmonary cancer is known to be a risk of a smoking habit. Febrianti & Widayati (2014) examined the erosion of bronchial

mucosal epithelial cells and thickening of the septum interalveolaris in mice exposed to cigarette smoke. Histological changes in the form of abnormal proliferation in the epithelium of the trachea, the destruction of the cilia, and hyperplasia of the connective tissue of the lung alveoli obtained by Shraideh & Najjar (2011) which gave the treatment of cigarette smoke.

Various studies have used various types of fruits to overcome organ damage due to cigarette smoke exposure. Febrianti and Widayati (2014) found that the administration of papaya fruit juice to mice exposed to cigarette smoke causes its pulmo structure back to normal. Marianti (2013) and Imam & Sudarsono (2006) found that tomato juice also has a positive effect on preventing pulmonary damage and improving sperm motility in mice exposed to cigarette smoke due to its high antioxidant activity. Fruit mixture with high antioxidant content in juice form is expected has a synergistic effect that increases their antioxidant capability. This study aimed to analyze the protective effect of a mixture of tropical fruit juice on pulmonary histopathological image of rats exposed by cigarette smoke.

Method

Collection of fruit

Fruits in the study was obtained from a variety of places. Strawberries, papaya, and guava derived from plantations in Ketep, Magelang, Central Java. Apples obtained from one of the supermarkets in the city of Yogyakarta.

Fruit juice preparation

Papaya and apples are washed, then peeled and taken part flesh. For strawberries and guava taken skin and flesh. Those part is then blended without adding water. The mixture fruit juice for groups treatment are strawberry+red guava (P1), strawberry+papaya (P2), and strawberry+apple (P3). Each type of fruit is given as much as 1.5 ml, so that the total volume given 3 ml/mice/day.

Treatment of tobacco smoke and tropical fruit juice

Rats were divided into five groups, K (rats were fed drinking as usual), K- (exposed to cigarette smoke, not given fruit juice), P1 (exposed to cigarette smoke, strawberry+guava juice), P2 (exposed to cigarette smoke, strawberry+papaya juice), P3 (exposed to cigarette smoke, strawberry + apple juice). Exposure to cigarette smoke was in the smoking chamber measuring 50x50x50cm. Cigarettes are paired in reverse under fumigation enclosure and then burned. Once curing is used three cigarettes within \pm 20 minutes. Exposure to cigarette smoke was done at 08:00 pm five days a week, for 40 days. Tropical fruit mix juice was given 1 hour after curing. Provision of tropical fruit juices using 6 mL disposable syringe with a replaceable tip cannula. Juice was given orally about 3 mL/mice/day, 5 days a week, for 40 days.

Histopathological Examination

After 40 days of treatment the rats were sacrificed by ether anesthesia and cervical dislocation. Pulmonary organ was gently dissected out, washed well with normal saline (0.9% NaCl) then fixed in 10% saline formalin buffer for at least 24 hrs. Dehydration was achieved by passing tissues through a graded series of alcohol followed by two changes of xylene. Tissues were embedded in paraffin wax after infiltration in paraffin wax. 5 μ thick of sections were obtained by microtome. At the final, sections were mounted on glass slides and stained with Hematoxylin-Easin. Sections were examined and photographed by microscope.

Histopathology observation

Lung histopathology images observation was performed using a microscope with a magnification of 100x and 400x. The parameters of pulmonary alveolar damage are widening of alveoli lumen, distance between alveoli, and alveolar membrane disorder. The formula of percentage of damage is in box below.

$$\text{Percentage of damage} = \frac{\text{Number of damage in the parameter}}{\text{Total cells in the parameter}} \times 100\%$$

Results and Discussion

Fruits are the natural food that give benefit for human health. From a variety of research get results that fruit consumption can reducing cancer risk.. In this research we analyse the effect of tropical juice mixture on the histopathological image of *rattus norvegicus* that exposure to cigarette smoke. We observe the alveoli of pulmo.

Cigarette smoke contains 7,000 chemical compounds that more than half of them can damage the body, especially the respiratory system Cigarette smoke is inhaled will pass through the respiratory tract into the alveoli. Alveoli are tiny air sacs of the lungs with simple squamous epithelium which make it very susceptible to damage from cigarette smoke exposure.

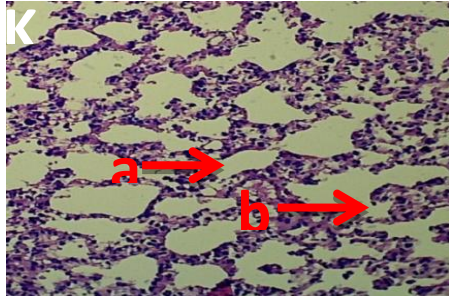
In this study the K- group exposed to secondhand smoke experienced a widening of the alveoli lumen of the alveoli with in a score of 3.2. These scores showed that only about 25-50% lumen alveoli are rounded to the proportional size. Relations between the alveoli in the group of K- shows score of 2.93., shows nearly 25-50% alveoli have closed relation. Alveolar membrane in group K- also shows the highest percentage of damage and scoring, with score 2.77. (Fig. 1-4).

The results of this study are similar with Widodo *et al.* (2007) who get histopathological changes in the airway and alveolar of white mice that exposed to cigarette smoke. Pneumonia seen an increase in interstitial and an increasing number of pneumatosit type II in the group exposed by smoking. According to the Surgeon General (2010), cigarette smoke reaches the alveoli will make the alveoli can not expand and return to its original shape. Ziad *et al.* (2013) obtain thickening of the alveolar wall, alveolar failure and bleeding in rats exposed by cigarette smoke. These things make the alveoli become weak and wide to affect the volume of oxygen that can be accommodated and the volume of carbon dioxide released.

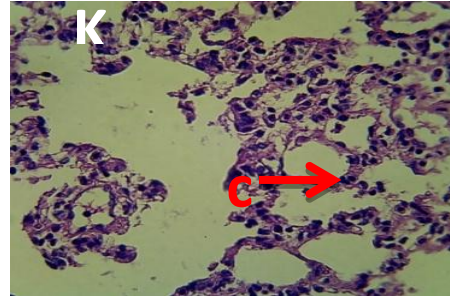
Tropical fruit juice mixture treatment generally may decrease alveolar membrane damage. In this experiment we used strawberries, guava, papaya and apples. Those fruits were selected because they contain a wide variety of antioxidant compounds that will act as a natural antioxidant. According Febrianti *et al.* (2016) these fruits contain high ascorbic acid, 66,65; 49,86; 49,57; 48,4 (mg / 100g) respectively for strawberry, guava, apple, and papaya. Febrianti *et al.* (2015) also getting beta-carotene in guava as much as 278.48; papaya 52.79; strowberi aand pple as much as 21.12 and 5.69 (mg / 100g).

In this study, we mix tropical fruit juices to determine whether there is a synergistic effect between the tropical fruit to repair the alveoli that exposed to cigarette smoke. Strawberries are used as main ingredients on three treatment groups. Febrianti (2015) report that strawberry contains high ascorbic acid, anthocyanins and phenolic. Scavenge ability of fruit to free radicals also higher than other tested tropical fruits. Generally all treatment groups give positive effects on improvement of pulmonary alveolar rat that exposed to cigarette smoke. Between the treatment groups, P3 group (mixture of

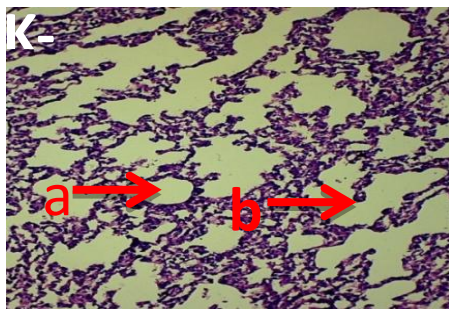
strawberry + apple juice) had a lower score than the other treatments. Apple contain quercetin that has powerful antioxidant activity. According to Akdemir (2016) quercetin has special activity to protect the tissue damage caused by free radicals and oxidative substances.



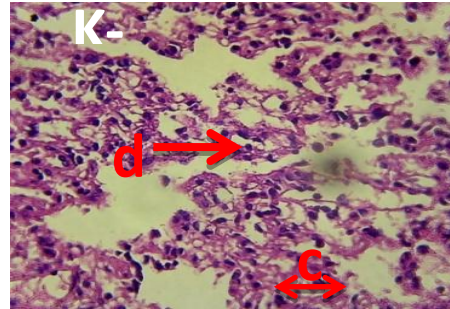
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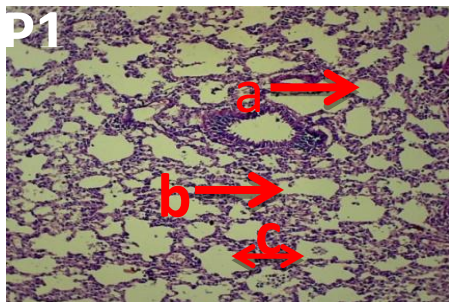
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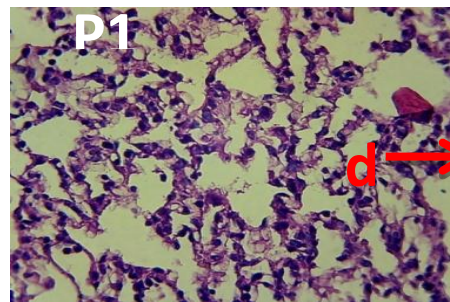
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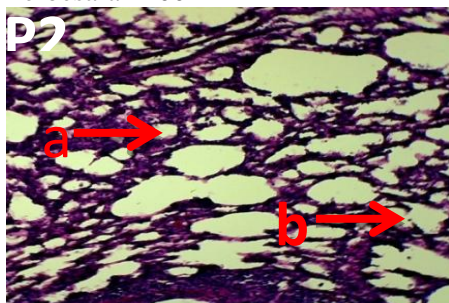
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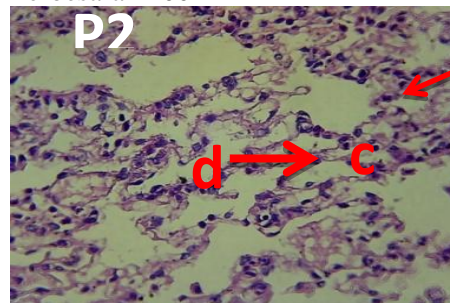
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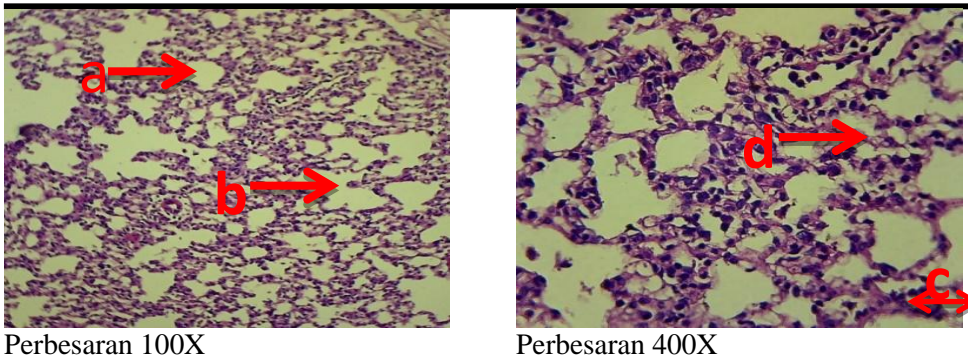


Figure 1. Histopathological images of alveoli in treatment groups (a) normal lumen, (b) widening lumen, (c) relationship between alveolus, (d) alveolus membrane damage

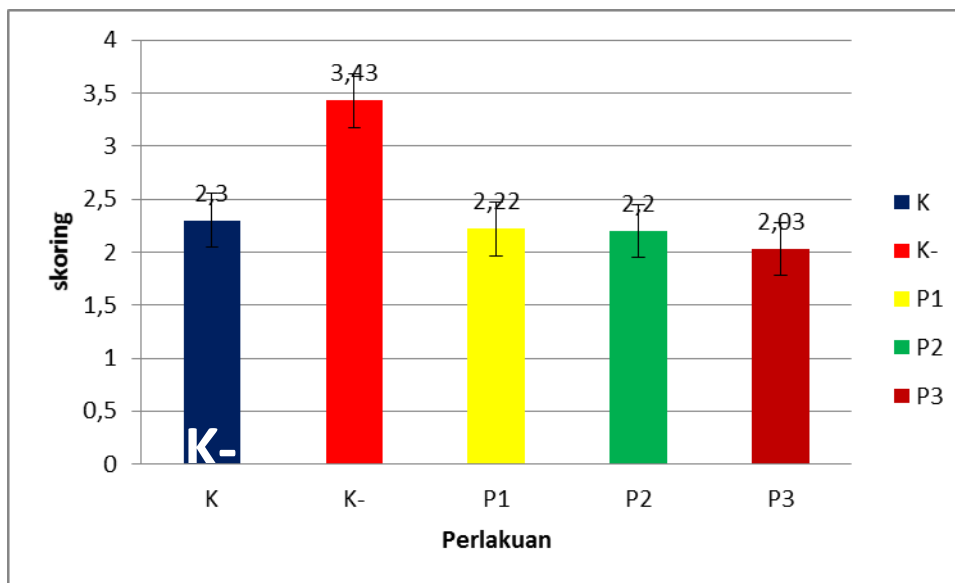


Figure 2. Widening of alveolar lumen in treatment groups

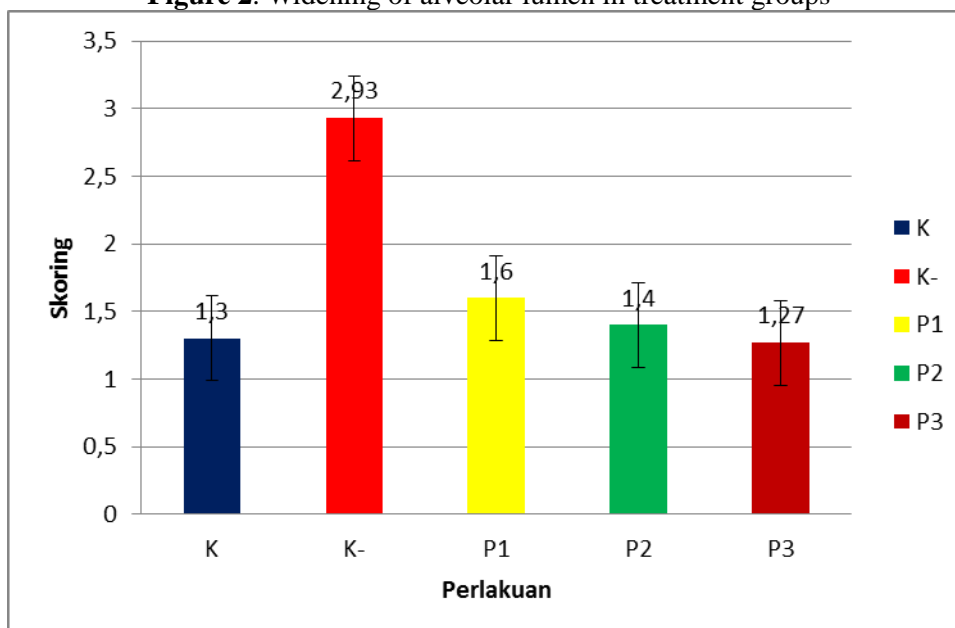


Figure 3. Relationship between alveolus in treatment groups

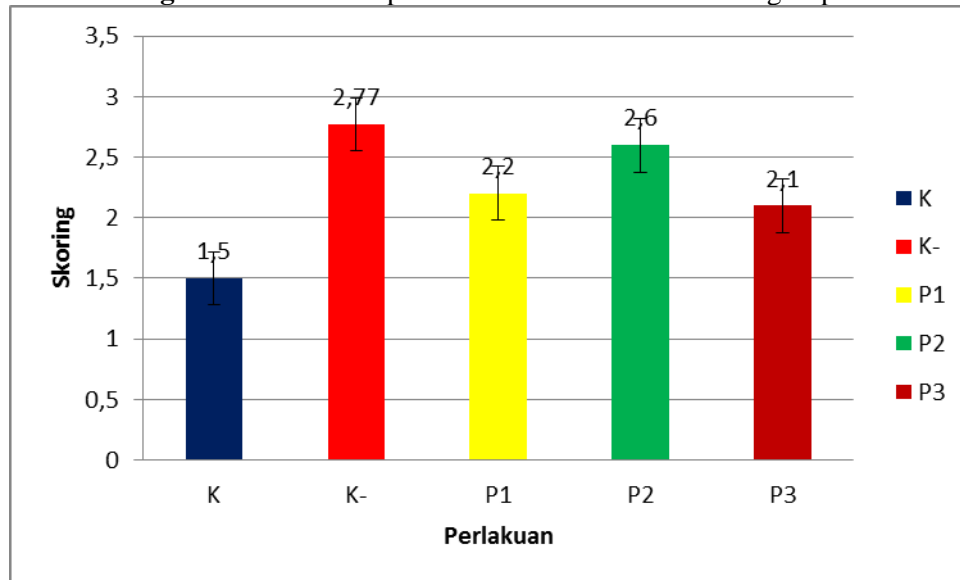


Figure 4. Alveolar membrane damage in treatment groups

Conclusion

Tropical fruit juice can protect rat pulmonary alveoli against damaged of cigarette smoke exposure. Strawberry+apple mix juice is the most effective juice in protecting rat alveoli from damage.

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