

Protective Effect of Mixed Tropical Fruit Juice on Histopathological Image of Rat's Trachea Exposed to Cigarette Smoke

Novi Febrianti^{1*}, Rosa Fahmi¹ and Haris Setiawan²

¹*Biology Education Department, Faculty of Teacher Training & Education, Universitas Ahmad Dahlan, Jalan Ring Road Selatan, Tamanan, Bantul, DIY, 55166 Indonesia*

²*Biology Department, Faculty of Science & Technology, Universitas Ahmad Dahlan, Jalan Ring Road Selatan, Tamanan, Bantul, DIY, 55166 Indonesia*

Smoking is considered as a worldwide phenomenon. Cigarette smoke is actually very dangerous to body's organs, including trachea. Indonesia is a tropical country that has fruit diversity. Fruit contain many types of antioxidant substances that give benefit for human health. The purpose of this research was to analyse the protective effect of antioxidant-rich tropical fruits juice on the trachea of albino rats that exposed to cigarette smoke. Adult male Wistar rats (n=35) were divided into five groups, as follows: control group (K); cigarette smoke group (K-); strawberry+papaya group (P1); strawberry+red guava group (P2); strawberry+apple group (P3). Rats exposed to cigarette smoke 20 minutes/day for 40 days. Three ml of mixed fruit juice was given orally one hour after the cigarette smoke exposed. Results show that there were some structural changes of epithelial trachea including, cellular atrophy, cilia destruction, and increasing number of goblet cell. Treatments by mixed tropical fruit juice can reduce the structural changes of the epithelial trachea. Strawberry & red guava juice (P2) shows the best protective effect to the trachea of albino rats that exposed to cigarette smoke.

Keywords: tropical fruit juice; trachea; cigarette smoke

I. INTRODUCTION

Smoking is a harmful activity for health. Respiratory system is an organ system that is most vulnerable to the negative impacts of cigarette smoke exposure. Disorders of the respiratory system are caused by the exposure of free radicals from cigarette smoke. Some respiratory diseases, such as emphysema, pulmonary fibrosis, and respiratory cancer are known as big risk of a smoking habit. According to Febrianti and Suryati (2014) cigarette smoke can damage epithelial tissue in rat's trachea. Histological changes, such as abnormal proliferation in the epithelium of trachea, destruction of the cilia, and hyperplasia of the connective tissue of the lung alveoli obtained by Shraideh & Najjar (2011) that gave cigarette smoke treatment to albino rats.

Indonesia is a tropical country that has an abundance of natural resources. One of the abundant natural resources is

tropical fruits species. People usually take advantage of fruits as a source of vitamins and minerals. Fruits have health benefits because contain rich natural antioxidants that are beneficial for the body. Some tropical fruits which are consumed in Indonesia often are red guava, papaya, grapefruit, strawberry, dragon fruit, mango, apple, watermelon, melon, and apple.

According to Wulandari (2010), apples contain high antioxidant compounds. The main antioxidants in apples are quercetin, epicatechin, and prosoianidin D that can prevent cancer. Santi (2013) explained that guava fruit contain antioxidants such as carotenoids, polyphenols and flavonoids. Juansah (2013) reported that strawberries have high antioxidant activity because it contains quercetin, ellagic acid, anthocyanins and kaempferol. Meanwhile, Ramdani

*Corresponding author's e-mail: novifebrianti@pbio.uad.ac.id

(2013) mentioned that papaya fruit contains antioxidants such as vitamin C and β -carotene.

Antioxidants in fruit play an important role in protecting body from free radicals, especially free radicals from cigarette smoke. Febrianti and Suryati (2014) proved that antioxidants in red guava fruit juice affect histopathological images in trachea of mice exposed to cigarette smoke. The fruits reduced epithelial tissue's damage in the mice trachea. 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. In this research, we analysed the protective effect of antioxidant-rich tropical fruits juice on the trachea of albino rats that exposed to cigarette smoke.

II. MATERIALS AND METHOD

A. Fruit Collection

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

B. Fruit Juice Preparation

Papaya and apples are washed, 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.

C. 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. We

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.

D. Histopathological Examination

After 40 days of treatment, the rats were sacrificed by ether anaesthesia and cervical dislocation. Trachea 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.

E. Histopathology observation

Trachea histopathology images observation was performed using a microscope with a magnification of 100x and 400x. The parameter of damages were cell erosion, cilia erosion, necrosis, and number of goblet cells. . The formula of percentage of damage is in box below:

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

F. Statistical Analysis

Quantitative data were analysed using The Statistical Package of Social Science (SPSS). The significance of differences between groups was evaluated by one-way analysis of variance (ANOVA) followed by Least Significance Different (LSD) test.

III. RESULT AND DISCUSSION

In this research, we observe the effect of mixed tropical fruit juice on histopathological image of epithelial trachea that exposure to cigarette smoke. There was many damages to

the cells such as atrophy and erosion in epithelial cells and/or cilia that allegedly due to an increase in free radicals from the cigarette smoke exposure. Basically, free radicals are formed naturally in the body through metabolic processes, the negative effects of the antioxidants can be mitigated by cellular antioxidants. But free radicals can also be increased due to the external conditions outside the living body, such as cigarette smoke. Cellular antioxidant individually can not work without the support of the secondary antioxidant intake from food. Fruits are the best natural sources of antioxidants from the outside body that give benefit for human health.

The structure of the albino rat trachea exposure to cigarette smoke demonstrated that there were some damages of cells in the trachea, including cell atrophy and erosion (epithelial cells and cilia). There were improvements in the structure of the treatment groups (P1, P2 and P3) due

to the administration of mixed tropical fruit juice. Figure 1 shows the effect of mixed tropical fruit juice on structure of epithelial tissue in the lining of the trachea. The most repaired of trachea structure was in P2 group (strawberries and papaya fruit juice).

We also observed the rat's body weight to determine the effect of mixed tropical fruit juice on the weight gain of rats. Based on the the data we know that most groups of rats gained weight during the 40 days of the treatment. P1 has the highest weight gain equal to 57.67 grams. But based on the ANOVA test there was no significance different of the rat's weighth between the groups after 40 days treatment ($p > 0.05$) indicating no effect of a mixed fruit juice treatments.

Number of goblet cells in the trachea showed that there was a difference between the control with the treatment differences. P1 group has the highest number of goblet cells, whereas K group has the lowest (Figure 2).

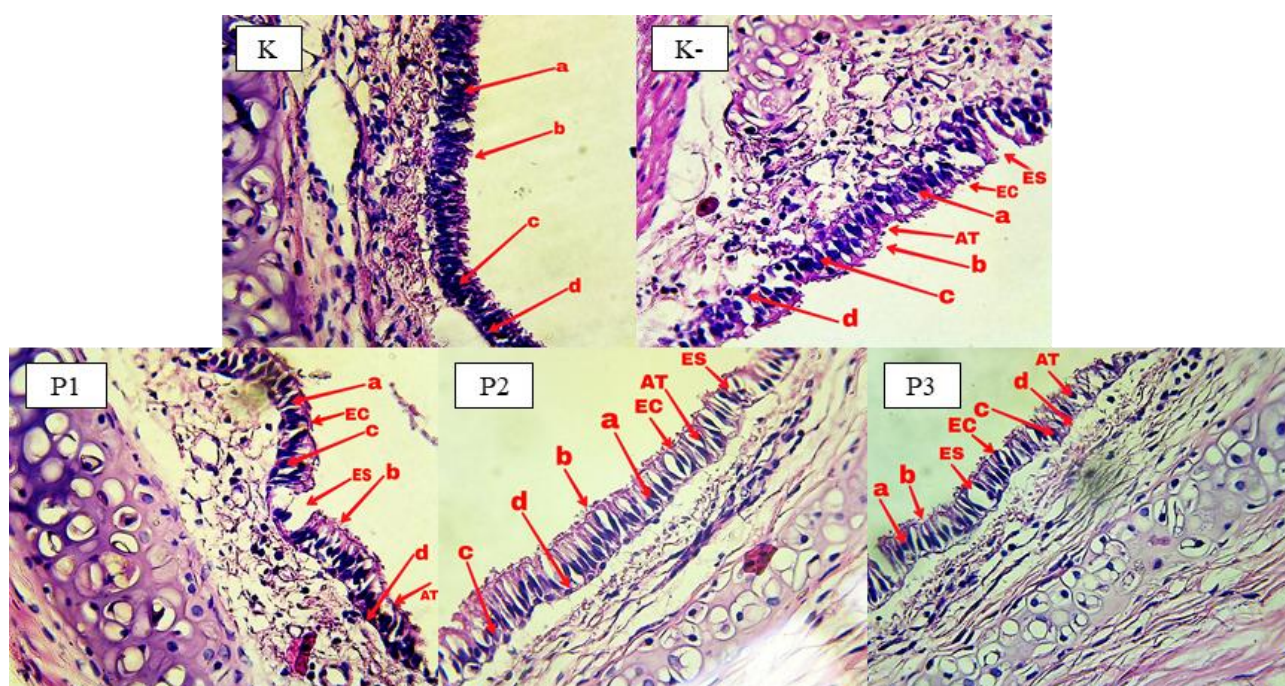


Figure 1. Histopathological structure of albino rat trachea due to the provision of tropical fruit juice after being exposed to cigarette smoke (K: Control without treatment; K-: negative control; P1: strawberry + guava fruit juice; P2: strawberry + papaya fruit juice; P3: strawberry + apple Pictures at 400x magnification and HE staining. a. Epithelial cells; b. cilia; c. Goblet cells; d. Basement membrane; AT: Atrophy; ES: Cell erosion; EC: Cilia erosion)

The body has the ability to repair or replace damaged cells, this is done to maintain cell survival. Cell erosion in this study is a form of attempt to replace damaged cells. Erosion is marked by the release of epithelial cells from the basal membrane. Erosion cells causing reduced cell number, thus

causing the cells to experience a condition known as atrophy. Atrophy is a form of cell adaptation to the environment is reversible. This event marked by shrinking the size of the cells due to loss of cell substance or a reduced number of cells. One cause of atrophy is reduced supply of blood or

oxygen (Vegad, 2007). Atrophy and erosion of cilia showing their body's response to the presence of foreign substances that can interfere with the body. Gultom (2011) explains that

atrophy and erosion of the tracheal epithelial cells can be caused by irritation of the mucous lining of the trachea.

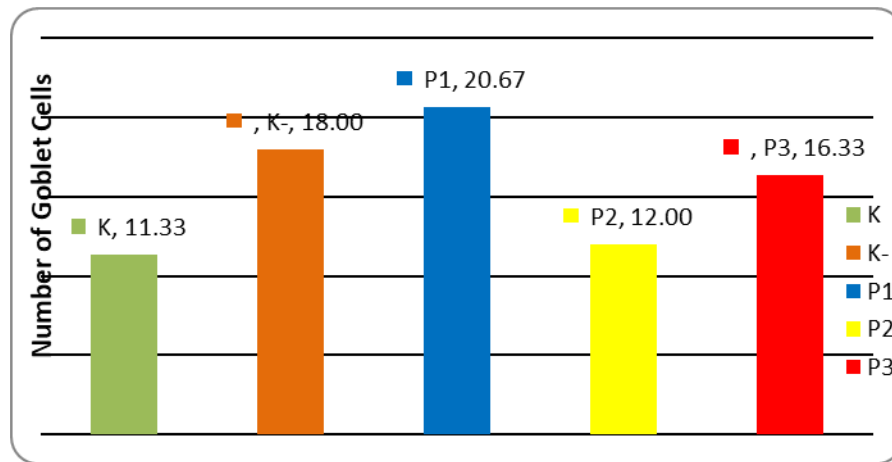


Figure 2. Diagram of the number of goblet cells of white rats as a result of the provision of tropical fruit juice after being exposed to cigarette smoke (K: Control without treatment; K-: negative control; P1: strawberry + guava fruit juice; P2: strawberry + papaya fruit juice; P3: strawberry + apple

Infections can occur in the epithelium of the trachea because of exposure to secondhand smoke is composed of various irritants that trigger irritation. Irritation of the mucosal lining of the trachea will further eroding the tracheal mucosa lining cells.

In this study, the condition of reduced supply of oxygen is very likely to occur. This is due to the presence of carbon monoxide (CO) in cigarette smoke may decrease the supply of oxygen to the cells, because a stronger affinity towards CO Hb than O₂ to CO in the slightest amount can bind to haemoglobin in a large percentage. In the treatment group P1, P2 and P3, in which rats were given a mixture of juice of tropical fruit showed good effects on the epithelial lining of the trachea, although still found erosion and cell atrophy. Effective influence seen in P2 (strawberry and papaya juice). The diverse content of antioxidants in this fruit juice gives the maximum effect in counteracting free radicals that can minimise cell damage.

In this study used strawberries as a main ingredient a mixture of fruit juice because of the strawberries have all types of antioxidants found in other fruits such as anthocyanins, ascorbic acid and also has a fairly complete nutritional content. Besides strawberries also used guava, papaya and apples which are known antioxidants and

vitamin C is high enough. Febrianti *et al.* (2016) showed that ascorbic acid content in fruits in Yogyakarta and Central Java region which has the highest rank in avocados, oranges, strawberries, guava, apples and papayas. The highest phenol content was mango, apple, guava, tamarind, papaya and strawberries, respectively. For betacaroten highest content is guava then avocado, papaya, oranges, mangoes, apples, tamarind and strawberries. While for most high anthocyanin content is strawberries, then papaya, guava, tamarind, mango, apple, avocado and last is orange. From these results it is used to study the fruit is strawberries, guava, papaya and apples.

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 the 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.

IV. 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.

V. ACKNOWLEDGEMENT

The authors are thankful to the Directorate General of Higher Education Department of Research, Technology and Higher Education RI for a competitive grant to funding for this research.

VI. REFERENCES

- Akdemir, FNE, Ilhami, G, Berna, K & Recep 2016, 'Quercetin protects rat skeletal muscle from ischemia reperfusion injuri', *Journal of Enzyme Inhibition and Medicinal Chemistry*, ISSN: 1475-6366.
- Febrianti, N & Suryanti RY 2014, 'Pengaruh jus buah jambu biji merah (*Psidium guajava*L.) terhadap gambaran histopatologik trakea mencit (*Mus musculus*) strain swiss yang dipaparkan asap rokok', *Jurnal Bioedukatika*, vol. 2, no. 1, pp. 16-18.
- Febrianti, N, Yunianto, I & Dhaniaputri, R 2016, 'Kandungan antioksidan asam askorbat pada buah-buahan tropis', *Bio Wallacea Jurnal Ilmiah Ilmu Biologi*, vol. 2, no. 1, Januari 2016, pp. 1-5.
- Gultom, HL 2011, Pengaruh Paparan Asap Rokok Terhadap Gambaran Histopatologik Saluran Pernapasan Bagian Konduksi Mencit (*Mus musculus*) Jantan Galur SD, Skripsi, Bandar Lampung: Fakultas Kedokteran Universitas Lampung.
- Imam, D & Juwono 2006, 'Pengaruh pemberian jus buah tomat (*Lycopersicum esculentum* Mill) terhadap Motilitas spermatozoa mencit balb/c jantan yang diberi paparan asap rokok', *Karya Tulis Ilmiah*, Fakultas Kedokteran Universitas Diponegoro, Semarang.
- Juansah, J, Ariyanti, RK & Akhirudin 2013, 'The potential optical methods for estimating the anthocyanin content of black mulberry and strawberry fruit', *Biophysical Journal*, vol. 9, no. 1, pp. 22-30.
- Marianti, A 2009, 'Aktivitas antioksidan jus tomat pada pencegahan kerusakan jaringan paru-paru mencit yang dipapar asap rokok', *Biosaintifika*, vol. 1, no. 1, pp. 1-10.
- Ramdani, Fitria Apriliani, Gebi Dwiyantri & Wiwi Siswaningsih 2013, 'Determination of antioxidant activity papaya fruit (*Carica papaya* L.) and other dairy products candied papaya form', *Journal of Chemical Science and Technology*, vol. 4, no. 2, pp. 115-124.
- Santi, DA 2013, 'Effect fruit juice guava (*Psidium guajava* Linn) on impaired glucose tolerance in male rats (*Rattus norvegicus*) due dexamethasone side effects', *Student Scientific journal Universitas Surabaya*, vol. 2, no. 1.
- Shraideh, ZA & Najjar, HN 2011, 'Histological changes in tissues of trachea and lung alveoli of albino rats exposed to the smoke of two types of narghele tobacco products', *Jourdan Journal of Biological Sciences*, vol. 4, no. 4, pp. 219-224.
- Vegad, JI 2007, *A Textbook of Veterinary General Pathology*, India: International Book Distributing Co.
- Widodo, E, Priosoeryanto, BP, Estuningsih, S, Agung priyono, DR & Utji, R 2007, 'Effect of glove cigarette exposure on white rat: special emphasis on the histopathology of respiratory tract', *Med. J. Indonesia*, vol. 16, no. 4, pp. 212-218.
- Wulandari, EPWS 2010, 'Characteristics of stirred yoghurt mango (*Mangifera indica*) and apple (*Malus domestica*) during storage', *Journal of Animal Science*, June 2010, vol. 10, no. 1, pp. 14-16, Padjadjaran University Faculty of Animal Science.