BIOPENDIX
JURNAL BIOLOGI PENDIDIKAN DAN TERAPAN

ETHANOL EXTRACT OF RED DRAGONS (*Hylocereus polyrhizus*) IN PREVENTING DAMAGE OF THE ALVEOLUS OF MICE (*Mus Musculus*) EXPOSURE TO KRETEK SMOKE

Febi Rumangun[1], Th. Watuguly[2*], Ine Arini[3]

[1] Alumni of Biology Education Study Program, Pattimura University
[2, 3] Biology Education Study Program, Pattimura University

E-mail: twatuguly@gmail.com

Abstract

Background: The main toxins in tobacco such as tar, nicotine, and carbon monoxide can trigger the formation of free radicals. Red dragon fruit (*Hylocereus polyrhizus*) is one of the plants that can be used as a source of protein antioxidants in red dragon fruit that can increase body metabolism and maintain heart health, can lose weight, and vitamin C is able to maintain healthy skin.
Methods: This research is an experimental laboratory using a qualitative descriptive method. The data obtained from this study will be analyzed using a qualitative descriptive method by observing the histopathology of the alveoli in the lungs of mice.
Results: The results showed that the reduction of alveolar damage in mice at each treatment dose (250 mg/g BW, 500 mg/g BW and 750 mg/g BW) ethanol extract of dragon fruit before being exposed to kretek cigarette smoke for 30 days. This is because free radicals contained in kretek cigarette smoke are inhibited by flavonoids from dragon fruit which play a role in stopping chain reactions due to exposure to cigarette smoke, so that neutrophil activity can be balanced by antiproteases.
Conclusion: Administration of red dragon fruit ethanol extract at a dose of 750 mg/g BW gave a more effective effect in preventing alveolus damage in mice caused by free radicals from kretek cigarette smoke compared to a dose of 250 mg/g BB.

Keywords: *Red Dragon Fruit, Alveolus, Kretek Cigarette Smoke*

Abstrak

Background: Racun utama pada tembakau seperti tar, nikotin, dan karbonmonoksida dapat memicu terbentuknya radikal bebas. Buah naga merah (*Hylocereus polyrhizus*) merupakan salah satu tanaman yang dapat dijadikan sebagai sumber antioksidan protein dalam buah naga merah mampu meningkatkan metabolisme tubuh dan menjaga kesehatan jantung, dapat menurunkan berat badan, dan vitamin C yang mampu menjaga kesehatan kulit.
Methods: Penelitian ini bersifat eksperimental laboratorik dengan menggunakan metode deskriptif kualitatif. Data yang di dapat dari penelitian ini akan di analisa dengan metode deskritif kualitatif dengan mengamati histopatologi alveolus pada paru-paru mencit.
Results: Hasil penelitian menunjukkan bahwa penurunan kerusakan alveolus mencit pada tiap dosis perlakuan (250 mg/g BB, 500 mg/g BB dan 750 mg/g BB) ekstrak etanol buah naga sebelum dipapar asap rokok kretek selama 30 hari. Hal ini disebabkan karena radikal bebas yang terdapat pada asap rokok kretek dihambat pembentukannya oleh flavonoid dari buah naga yang berperan dalam menghentikan *chain reaction* akibat paparan asap rokok, sehingga aktivitas neutrofil dapat diimbangi oleh antiprotease
Conclusion: Pemberian ekstrak etanol buah naga merah dengan dosis 750 mg/g BB memberi pengaruh yang lebih efektif dalam mencegah kerusakan alveolus mencit akibat radikal bebas asap rokok kretek dibandingkan dosis 250 mg/g BB.

Kata Kunci : Buah Naga Merah, Alveolus, Asap Rokok Kretek

BACKGROUND

Cigarettes are processed products produced by tobacco that have been dried using or without the addition of certain ingredients (Bindar, 2000). Smoking has become a lifestyle of modern society, and every year the number of smokers tends to increase, especially in developing countries such as Indonesia. The type of cigarette that is much favored by the Indonesian people is kretek cigarette, which is a type of cigarette made from tobacco and has a mixture of clove aroma and taste. Kretek cigarettes have nicotine and tar content 2-3 times greater than white cigarettes (Hashim, 2005). Smoking can interfere with health caused by nicotine originating from mainstream smoke and side smoke, namely smoke that is spread into the air and inhaled by other people or passive smokers (Yueniwati and Ali, 2004).

Cigarette smoke contains very high amounts of free radicals because one puff of a cigarette is estimated to contain 1,014 free radical molecules that enter the body. The main toxins in tobacco such as tar, nicotine, and carbon monoxide can trigger the formation of free radicals (Gondodiputro, 2007). Free radicals, which are produced in normal amounts, have an important role in the life of cells such as white blood cells that produce H2O2 to kill several types of bacteria (Hariyatmi, 2004). The lungs are organs that are susceptible to interference, this is because the respiratory system is included in the open channel group, meaning that the respiratory tract is directly related to the external environment (Aspinall, 2004). Based on research conducted by Kristianti (2004), exposure to subchronic cigarette smoke causes damage to the cilia on the epithelial surface of the bronchi and bronchioles, epithelial metaplasia, gland hyperplasia and an increase in inflammatory cells. In addition, free radicals contained in cigarette smoke inactivate 1-antrypsin (Menantara, 2013). 1-antrypsin is a serum protein produced by the liver and normally found in the lungs to inhibit the destructive action of the neutrophil elastase enzyme on lung tissue. A decrease in the level of 1-antrypsin to less than 35% of the normal value (150-350 mg/dL) causes reduced protection of lung parenchyma tissue, destruction of adjacent alveolar walls, and ultimately pulmonary emphysema. (Seersholm et al, 1994).

Physiologically, the body actually prepares itself to ward off free radicals with the availability of antioxidants in the intracellular membrane system, extracellular fluid, cytoplasm and membrane lipoproteins (Sies and Murphy, 199). However, with the increasing number of free radicals in the body, it is necessary to take additional antioxidants. Antioxidants are compounds that can inhibit oxidation reactions, neutralize or capture free radicals (Murray et al, 2000) and protect the tissues that make up organs from damage caused by free radicals.

Normally, free radicals are already present in the body. The body naturally has antioxidants that work to inhibit oxidation by reacting with reactive free radicals to form more stable free radicals. Antioxidants can delay or prevent the occurrence of free radical oxidation reactions in fats. However, if there are too many free radicals, natural antioxidants will not be able to overcome them. In this condition the body requires intake of antioxidants from the outside, for example antioxidants contained in red dragon fruit (Nurliyana et al., 2010). Research conducted by Rebecca et al

(2010) showed that red dragon fruit contains the most polyphenols compared to other species, namely 86.13 ± 17.02 mg in 0.50 g of dried red dragon fruit extract.

Red dragon fruit (Hylocereus polyrhizus) is one of the plants that can be used as a source of antioxidants. According to Subagja (2013), the protein in red dragon fruit can increase the body's metabolism and maintain heart health, can lose weight, and vitamin C is able to maintain healthy skin. Based on the description above, it is necessary to conduct research to determine the benefits of red dragon fruit as a source of antioxidants to prevent the formation of free radicals against the risk of oxidative damage in the body by looking at the number of alveolar macrophages. Therefore, a study was conducted to determine the antioxidant effect of red dragon fruit extract (Hylocereus polyrhizus) on the alveolar macrophages of rats exposed to cigarette smoke in neutralizing free radicals by kretek cigarette smoke, so as to prevent damage to the alveoli of mice.

MATERIALS AND METHODS

This research was carried out on April 1-30 2021 at the Zoology laboratory of the Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon. The subjects in this study were mice (Mus musculus) with healthy conditions, male type Balb/c and the object in this study was the damage to the alveoli of mice exposed to cigarette smoke after being given red dragon fruit extract. The data obtained from this study will be analyzed using a qualitative descriptive method by observing the histopathology of the alveoli in the lungs of mice.

RESULTS AND DISCUSSION

Results

The results of photomicrograph observations of the alveoli of mice using Hematoxylin-Eosin (HE) staining showed that the group of mice that were not given ethanol extract of dragon fruit and were not exposed to kretek cigarette smoke for 30 days (Figure 1) did not appear to have any damage to the alveoli, including inflammatory cell infiltration, thickening of the walls. alveolus and widening of the alveolar lumen. Alveolar photomicrographs of mice that were not given dragon fruit ethanol extract and exposed to kretek cigarette smoke for 30 days (Figure 2) showed alveolar damage which included inflammatory cell infiltration, thickening of the alveolar walls and widening of the alveolus lumen.

Photomicrograph depiction of the alveolus of a group of mice given ethanolic extract of red dragon fruit at a dose of 250 mg/gBW before being exposed to kretek cigarette smoke for 30 days (Figure 3), a group of mice given ethanolic extract of dragon fruit at a dose of 500 mg/gBW before being exposed to kretek cigarette smoke for 30 days (Figure 4) and the group of mice given ethanol extract of dragon fruit at a dose of 750 mg/gBW before being exposed to kretek cigarette smoke for 30 days (Figure 5) showed a decrease in alveolar damage compared to the photomicrograph picture of the alveoli in the group of mice exposed to kretek cigarette smoke. for 30 days without giving dragon fruit ethanol extract.

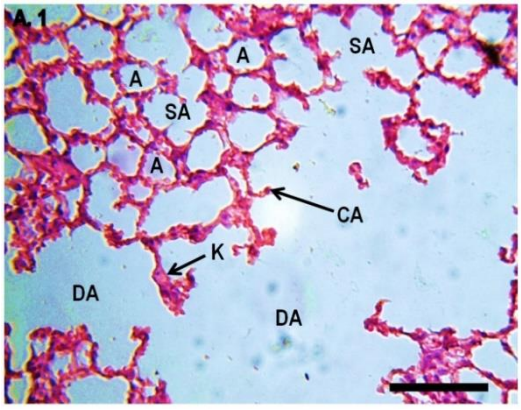


Figure 1. Mice that were not given dragon fruit ethanol extract and were not exposed to kretek cigarette smoke for 30 days

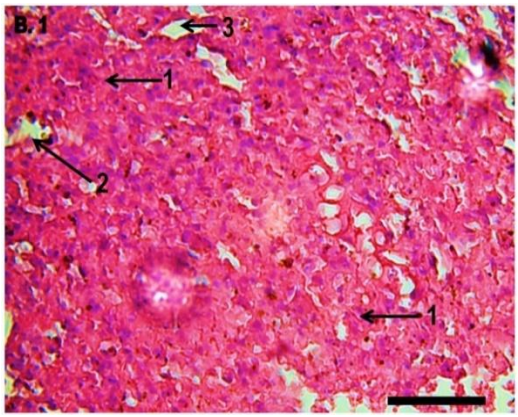


Figure 2. Mice that were not given dragon fruit ethanol extract and were exposed to kretek cigarette smoke for 30 days

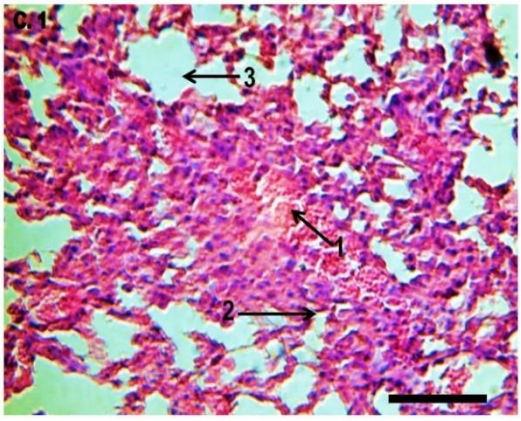


Figure 3. Mice given dragon fruit ethanol extract at a dose of 250 mg/g BW before being exposed to kretek cigarette smoke for 30 days

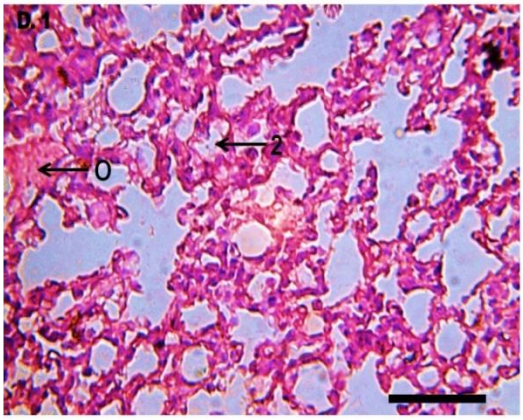
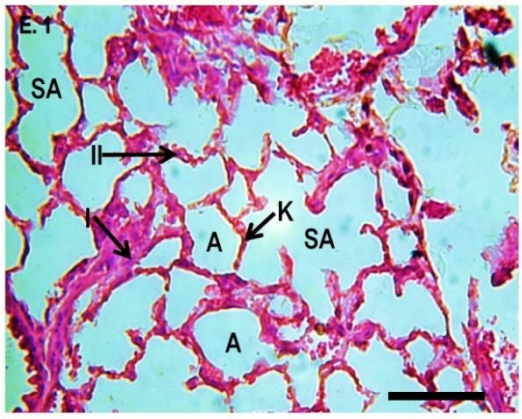


Figure 4. Mice given ethanol extract of dragon fruit at a dose of 500 mg/g BW before being exposed to kretek cigarette smoke for 30 days



Gambar 5. Mencit yang diberikan ekstrak etanol buah naga dengan dosis 750 mg/g BB sebelum dipapar asap rokok kretek selama 30 hari

Discussion

The results of photomicrograph analysis of the alveoli of mice with Hematoxylin-Eosin (HE) staining in the group of mice exposed to kretek cigarette smoke for 30 days without dragon fruit ethanol extract (Figure 2) showed that there was damage to the alveoli of mice which included inflammatory cell infiltration, thickening of the alveolar walls and widening of the alveolar lumen. This shows that kretek cigarette smoke causes damage to the alveoli in the lungs. The cause of alveolar damage is because kretek cigarette smoke contains many chemicals including carbon monoxide (CO), tar and nicotine as well as free radical compounds.

The photomicrograph of the alveolus of the group of mice given the ethanolic extract of dragon fruit at a dose of 250 mg/gBW and exposed to kretek cigarette smoke for 30 days (Figure 3) showed the same damage to the alveoli as the photomicrograph of the alveolus of the group of mice that was not given the ethanolic extract of dragon fruit and exposed to cigarette smoke. kretek for 30 days. This is due to the binding of some free radicals that enter along with cigarette smoke by the antioxidants contained in dragon fruit. However, the ability of antioxidants contained in dragon fruit to prevent damage to the alveoli of mice is not comparable to the amount of free radicals in kretek cigarette smoke.

The photomicrograph of the alveolus of a group of mice that was given ethanol extract of dragon fruit at a dose of 500 mg/g BW before being exposed to kretek cigarette smoke for 30 days (Figure 4) showed that there was less damage to the alveoli, including lumen widening and edema, the decrease in alveolar damage was thought to be due to the antioxidants present. in the ethanolic extract of dragon fruit is greater than the antioxidant content of the ethanolic extract of dragon fruit at a dose of 250 mg/gBB, so that the alveolar damage is less than the alveolus damage of the mice group that was not given dragon fruit ethanol extract and exposed to kretek cigarette smoke for 30 days and the mice group that was given ethanol extract of dragon fruit dose of 250 mg/gBW and exposed to cigarette smoke for 30 days against alveolar damage. The photomicrograph of the alveolus of the mice given dragon fruit ethanol extract at a dose of 750 mg/gBW and exposed to kretek cigarette smoke for 30 days (Figure 5) showed no damage to the alveoli of the mice compared to the two previous groups, namely the group of mice that were not given dragon fruit ethanol extract. and exposed to kretek cigarette smoke for 30 days and a group of mice given ethanol extract of dragon fruit at a dose of 250 mg/gBW and exposed to cigarette smoke for 30 days. This is due to the high antioxidant content contained in the ethanol extract of dragon fruit at a dose of 750 mg/gBB. An indication of high levels of dragon fruit antioxidants that play a role in preventing alveolar damage due to free radicals from kretek cigarette smoke can be seen from the photomicrograph image of the alveoli of mice given dragon fruit ethanol extract at a dose of 750 mg/gBW which has the same photomicrograph image of the alveoli of mice as the group of mice that did not. given dragon fruit extract and dragon fruit ethanol extract and not exposed to kretek cigarette smoke. This proves the effect of dragon fruit as a source of antioxidants in preventing alveolar damage caused by free radicals from cigarette smoke.

The results showed that free radicals from cigarette smoke can cause damage to the alveoli in the lungs through the mechanism of cigarette smoke causing oxidative stress that damages the alveoli of the lungs. Oxidative stress that occurs continuously results in alveolar damage which includes inflammatory cell infiltration, thickening of the alveolar walls, widening of the alveolus lumen and edema.

The occurrence of alveolar damage in the form of inflammatory cell infiltration (Figures 2 and 3) is thought to be due to free radicals from kretek cigarette smoke which trigger increased neutrophil sequestration and 1-AT inactivation caused by oxidative stress. Increased neutrophil sequestration and inactivation of 1-AT causes inflammation of cells in the alveoli and damage to alveolar tissue which results in narrowing of the alveolar septum so that the circulation process of O2 and CO2 does not run normally. This is supported by Seersholm et al, 1994; MacNee, 2005; Larasati; 2010; Lenzatti et al, 2011 which stated that cigarette smoke free radicals activate neutrophils in the alveolar septum and inactivate 1-AT which causes an increase in proteases and causes inflammation and lung tissue damage which is characterized by infiltration of inflammatory cells in the alveolar septum.

The accumulation of inflammatory cells in the alveolar walls causes thickening of the alveolar wall structures (Figs 2, 3 and 4). These results are supported by research by Aditama, 2003 which states that the thickening of the alveolar septum is caused by osmotic pressure on endothelial cells due to the chemotaxis process, namely the withdrawal of neutrophils to the area of inflammation. In addition, cigarette smoke can increase airway resistance and increase capillary endothelial permeability, causing protelysis with alveolar microvascular fluid and deposited in tissues and causing pulmonary edema (Aditama, 2003). The occurrence of widening of the alveolus lumen in the alveolus is thought to be the result of high levels of ROS in the alveolus, causing 1-AT deficiency. These results are in accordance with the opinion of PDPI Quoted by Nisa, 2011 which states that free radicals from kretek cigarette smoke cause 1-AT deficiency which causes widening of the lung alveoli and causes leakage in the lung walls.

Cigarette smoke contains high amounts of free radicals so that it can act as a cause of respiratory tract damage. Free radicals of cigarette smoke reduce the function of intracellular antioxidants in lung cells through mechanisms associated with oxidative stress (Arief, 2002). Oxidative stress conditions caused by free radicals contained in cigarette smoke are related to the inactivation of proteinase inhibitor enzymes, damage to the airway epithelium, increased neutrophil sequestration in the pulmonary microvasculature and the expression of proinflammatory genes (Marwan, 2005).

This can be seen when the study for each treatment group of 3 mice exposed to clove cigarette smoke 1 stick per 5-10 minutes for 1 hour every day experienced stress. This was observed in the behavior of mice every time they were exposed to kretek cigarette smoke. At the beginning of the exposure process, the mice still looked active and aggressive, after a while, the mice were observed overlapping each other in the corner, possibly to get oxygen from the gap in the corner of the smoking chamber. After ±1 hour, the mice looked weak and no longer aggressive.

Research on the effect of free radicals from kretek cigarette smoke on alveolus damage has been widely studied, namely the antioxidant activity test of the ethanol extract of the wild-buas leaf (Premna cordifolia Linn.) on the histopathological features of the lungs of Wistar Males (Rattus norvegicus) after exposure to cigarette smoke (Kristina, 2014), showing the potential of plant antioxidants in binding free radicals that have the potential to damage tissues. In addition, research by Susantiningsih et al, 2014 showed that the administration of plant extracts that have the potential as antioxidants can counteract free radicals and prevent chain reactions caused by toxic compounds that can damage the lungs..

Antioxidants are compounds that are able to counteract or reduce the negative effects of oxidants in the body. The compounds in dragon fruit that act as antioxidants can work by donating one electron to compounds that are free radicals so that the activity of these radical compounds can be inhibited. Intracellular antioxidants in the alveoli are the enzyme Glutathione S-transferase (GST) which plays a role in the detoxification of various aromatic hydrocarbons (Ladina et al, 2002) and the enzyme Microsomal epoxide hydrolase (mEPHX) which acts as a xenobiotic metabolizer that converts reactive epoxides into more soluble dihydrodiol derivatives. in water so that it is more readily excreted from the body (Pare, 2002). However, the cellular antioxidants present in the alveoli cannot work without the support of secondary antioxidant intake from food. This enzyme requires compounds and minerals that can increase the ability of the body's system to inhibit free radicals such as flavonoids, saponins, Mg, Cu and Zn found in plants. Therefore, these compounds and minerals must be available in sufficient quantities in order to inhibit the occurrence of alveolar cell damage due to free radicals.

This study showed a decrease in alveolus damage in mice at each treatment dose (250 mg/gBW, 500 mg/gBW and 750 mg/gBW) ethanol extract of dragon fruit before being exposed to kretek cigarette smoke for 30 days. This is because the formation of free radicals in kretek cigarette smoke is inhibited by flavonoids from dragon fruit which play a role in stopping the chain reaction due to exposure to cigarette smoke, so that neutrophil activity can be balanced by antiproteases. This is in accordance with Pratiwi et al., (2006) which stated that the (OH) group was donated by flavonoids to reduce the alveolar septal environment so that the chain reaction was stopped, oxidants were directly proportional to antioxidants and proteases secreted by neutrophils were balanced with alveolar septal antiproteases. This condition causes the inflammatory process to decrease. This result is also supported by the opinion of Comalada (2006); Lenzatti (2011) which states that flavonoid compounds can be efficacious as antioxidants through their activities as scavengers and protease inhibitors. These flavonoids will inhibit the work of proteases to elasticize the lungs so that the lung tissue will experience improvement from the widening of the alveolus lumen. In addition, the role of flavonoids as antioxidants in reducing the degree of damage to lung alveoli can reduce the release of inflammatory cells such as alveolar macrophages and neutrophils. According to Elekofehinti (2012) saponins in Solanum anguivi have antioxidant activity through their activity as scavengers and according to NgTB et

al (2014) Saponins act as antioxidants because they have a role as protease inhibitors in inhibiting the release of cytokines such as TNF-α, IL-1beta, IL- 2 and interferon-gamma.

The body requires additional intake of antioxidants in sufficient quantities to be able to induce the work of the cellular antioxidant system in the body so as to suppress excessive cell damage and maintain cellular antioxidant status. In dragon fruit seeds there are many nutrients and antioxidants such as flavonoids and saponins that function as free radicals that damage cells.

CONCLUSION

Based on the results of the study, the following conclusions were obtained: Red dragon fruit has antioxidant potential that plays a role in preventing damage to the alveoli of mice (Mus musculus) exposed to kretek cigarette smoke. Administration of red dragon fruit ethanol extract at a dose of 750 mg/g BW gave a more effective effect in preventing alveolus damage in mice due to free radicals from kretek cigarette smoke compared to a dose of 250 mg/gBW.

BIBLIOGRAPHY

Abdelmohsen Usama Ramadan,Matthias Szesny, Eman Maher Othman, Tanja Schirmeister, Stephanie Grond , Helga Stopper and Ute Hentschel, Antioxidant and Anti-Protease Activites of Diazepinomicin from the Sponge-Associated *Micromonospora* Strain RVII5. Mar. Drugs, 2012. ISSN : 1660-3397. 10 : 2208-2221.

Aditama, Tjandra Y. 1993. The situation of several lung diseases in the community. Mirror of the World of Medicine No. 84, 1993. Jakarta. Pp.28-30.

Agestia R, and Sugriani A, 2009. Main Material of Marine Natural Organic Chemistry. Makassar. Faculty of Math and Science. Hassanudin University.

Akers R M, D M Denbow. 2008. Anatomy and Physiology of Domestic Animal.Iowa: Blackwall Publishing: 396-397.

Algameta D. Elfara. 2009, Test of Antioxidant Activity of Effervescenr Dewandaru (Eugenia unifrora L.) and Sambiloto (Andrographis paniculata) Tablets on Glucose Loaded Rats: Thesis. Surakarta: Faculty of Pharmacy, University of Muhammadiyah Surakarta.

Anonymous. 2011. Literature Review. Accessed from http://receptory.usu.ac.id (29 January 2014, 15:45 WIT)

Arief S. 2002. Free Radicals. http://www.pediatrik.com/buletin/06224113752-x0zu61.doc. (18 February 2015).

Aspinall, R. 2004. Ageing and Immune System *In Vivo:* Commentary on the 16th Session of British Society for Immunology Annul Conggres Thoragante December 2004. Immunity and Againg 2005; 2;5

Bals R, DJ Weiner, JM Wilson. 1999. The Innate Immune System im Cystic Fibrosis Lung Disease. *J CLIN INVEST* 103: 303-307

Banks WJ. 1993. Applied Veterinary Histology 3rd Edition Missouri: Mosby,Inc. 390-400

Binder, Y. 2000. The Economy of Cigarettes and Its Consequences. Department of Chemical Engineering.http://www.angelfire/com/il/nalapralaya/rokok/html

Bitam F, Ciavatta ML, Carbone M, Manzo E, Mollo E, Gavagnin M. 2010. Chemical analysis of Flavonoid

Constituent of the Seagrass *Halophilla stipulacea:* fris of malonylated derivates of marine phanerogams. Biochemical Systematic and Ecology.

Christyaningsih J. Suandito, Sri Utari Purnomo. 2003. Effect of Vitamin E and C Supplementation on Superoxidase Dimustase (SOD) Enzyme Activity in Erythrocytes of Rats Exposed to Clove Cigarette Smoke. JPB Vol 5 No.3

Comalada M. Inhibition of pro-inflammatory markes in primary bone morrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure-activity relationship. Biochemical pharmacology. 2006; (72)1010-1021. Coville T, JM Bassert. 2002. Clinical Anatomy and Physiology for veterinary Technicians: Mosby, Inc 220-235

Dewi Rizqiana Marissa. 2010. Effect of Hepatoprotector Honey on Histopathological Damage to Mice (Mus musculu) Liver Cells Treated with Sodium Cyclamate. Surakarta: Faculty of Medicine, Sebelas Maret University.

Droge W. 2002. Free radical in the physiological control of cell fuction. *Physiol Rev.*82:47-95.

Effendi, 1998. Test of Anti-Inflammatory Power of Petroleum Ether, Ethyl Acetate and Water Faction of Belimbing Wuluh Leaves (Averhoa bilimbi L) in White Rats (Rattus novergicus). Essay. Faculty of Pharmacy UGM: Yogyakarta.

Elekoehinti OO, Adanlawo IG, Saliu, Sodehinda SA. Saponins from *Solanum anguivi* fruits exhibit hypolipidemic potential in Rattus novergicus. Der Pharmecia Lettre. 2012: 4 (3) 811-814.

Ghedolf N, Wang Xiao-Hong and Engeseth N, J, 2002. Identification and Qualifications of Antioxidant Components of Honeys from Various Floral Sources, *Journal of Agricultural and Food Chemisty.* 50, 5870-5877.

Gondodiputro S. 2007. Dangers of Tobacco and Tobacco Preparation Forms. Bandung: Padjadjaran University. Accessed from http://www.resources.unpad.ac.id (01 July 2014).

Nurliyana, R., Syed Zahir, I., Mustapha, S.K., Aisyah, M.R., and Kamarul, R.K. (2010). Antioxidant Study, of Pulps and Peels of Dragon Fruits: A Comparative Study. International Food Research Journal, 17, 367-375.

Rebecca, O.P.S., Boyce, A.N., and Chandra, S. (2010). Pigment Identification and Antioxidant Properties of Red Dragon Fruits (Hylocereus polyrhizus). African Journal of Biotechnology, 9 (10), 1450-1454.

Subagja, H.P. (2013). The Magic of Dragon Fruit and Pomegranate: Ward off Deadly Diseases. Yogyakarta: FlashBooks.