

PAPER • OPEN ACCESS

The analyze of lung's GSH number in rats exposed by cigarette smoke and induced by rambutan peel extract

To cite this article: Lisdiana 2018 *J. Phys.: Conf. Ser.* **983** 012179

View the [article online](#) for updates and enhancements.

Related content

- [Effects of Rambutan Peel Extract to The Number of Erythrocytes and Haemoglobin in Rats Exposed to Cigarette Smoke](#)
Lisdiana and F K Dewi
- [Development of nitroxide radicals-containing polymer for scavenging reactive oxygen species from cigarette smoke](#)
Toru Yoshitomi, Kazuhiro Kuramochi, Long Binh Vong et al.
- [Exhaled carbon monoxide in airway diseases](#)
Réka Gajdócsy and Ildikó Horváth



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

The analyze of lung's GSH number in rats exposed by cigarette smoke and inducted by rambutan peel extract

Lisdiana^{1,*}

¹Department of Biology, Universitas Negeri Semarang, Semarang, Indonesia

*Corresponding author: lisdiana@mail.unnes.ac.id

Abstract. The cigarette smoke is one of the pollutants in human and environment. It contains free radical compounds which cause oxidative stress. In the oxidative stress condition, the free radical causing peroxidation of cell membrane lipid as well as damages the cell membrane. One of the biomarkers of oxidative stress happens the number of GSH. The purpose of this study was to analyze the amount of rat's GSH which exposed by cigarette smoke as well as inducted by rambutan pell extract. This study applied to 25 male rats of Wistar which divided into five groups; K1 (control), K2 (negative), K3, K4, and K5 were the treatment groups of rambutan peel extract with various dosage; 3, 6, 12 mg/200 gramBB and cigarette smoke exposure along 30 days. The number of GSH measured by the DTNB of lung tissue. To know the difference of GSH number of each group did the data analysis with one way ANOVA test and LSD advance test. The result of statistic analysis showed that there was a significant difference between the control group and treatment group. The conclusion of this study was the rambutan peel extract with 3 mg/200 gramBB dosage could increase the number of lung's GSH of rats exposed to cigarette smoke.

1. Introduction

Cigarette smoke contains more than 4,000 harmful substances. These components are the sources of harmful free radicals which having potency as oxidants and causing a stress response. Heavy stress is known to cause oxidative stress. Oxidative stress is an imbalance between the number of free radicals and the antioxidants of the body [1,2]. Under conditions of oxidative stress, there is an increase in the number of reactive oxygen species (ROS) in the body [3]. Each ROS formed can initiate a chain reaction that continues until the ROS was eliminated by another ROS or antioxidant system. ROS is a highly reactive oxidant which can damage cell components [4, 5]. When the ROS level rises beyond the endogenous defense capability, it will cause lipid peroxidation of the cell membrane and destroy the cell membrane organization [6]. One of the biomarkers of lipid peroxidation in membranes due to oxidative stress is the decrease in GSH (Glutathione) levels. This decrease is because ROS will oxidize GSH to disulfide form, thereby decreasing the amount of GSH [7].

Rambutan (*Nephelium lappaceum* L.) is an attractive tropical fruit plant and commonly found in Southeast Asia. Rambutan has consumed because of its fresh tastes and contains antioxidants such as high vitamin C [8]. While the peel of the fruit has useful compounds as antioxidants that have not utilized yet in the pharmaceutical field. The average of vitamin C content in the rambutan peel is 36.4 mg /100g [8]. The ethanol extract of rambutan peel had catching activity of free radical



comparable to vitamin C and much higher than grape seeds [9, 10]. Rambutan peel found has the biology effects such as antioxidant activity, antibacterial [10,11], antiproliferation [12], obesity [13] and antihyperglycemic [14].

Not only contains the antioxidants of vitamin C, but rambutan peel is also known contains ellagic acid, corilagin, and geranin which is a phenolic compound. The phenolic compound is strongly antioxidant, having an aromatic ring with more than one hydroxyl group. As an antioxidant, phenolic can suppress oxidative stress so can protect the body cells from the danger of free radicals by binding the free radicals [11]. As a plant which containing antioxidants, rambutan peels can inhibit the occurrence of oxidative damage and can reduce levels of GSH in the body [10]. However, there is no publication yet in related to the number of rat's GSH which exposed by cigarette smoke as well as inducted by rambutan pell extract.

2. Methods

2.1. Preparation of rambutan peel extract

The rambutan used was Sekaran Cultivar Rambutan which obtained in the area of Gunungpati, Semarang. The rambutan peel was washed with water and dried at room temperature for 2-3 hours. The peel then was cut thinly and dried at room temperature for 2-4 days. A mixer grinder smoothed the dry peel and filtered with a mesh 1 mm in size. The dry powder was extracted by maceration method with 96% ethanol solvent with ethanol, and dry powder ratio was 1:1 for 24 hours at room temperature. The suspension was filtered through a filter paper. The filtrate was collected and dried using a rotary evaporator and then converted to be a crude extract from a frozen dryer.

2.2. Exposure to cigarette smoke

The test animals were 25 male Wistar strains, acclimatized for seven days. The rats were divided into five groups at random for each group of 5. The feeding of rambutan peel extracts from a feeding tube for 30 days. The exposure to cigarette smoke was given with a dose of 3 cigarettes per day in a particular smoking chamber according to the group. The disclosure of cigarette smoke did by kretek cigarette with a syringe that had been modified and was carried on until the cigarette spent.

2.3. The measurement of GSH lung level

The level of glutathione (GSH) of the lung determined by the DTNB method. Took some lung homogenates which given a solution of precipitation included the metaphosphoric acid solution. The precipitation solution: 1.67 g glacial acid metaphosphoric (mixture between HPO_3 and NaPO_3), 0.2 g disodium ethylenediamine tetraacetic acid (EDTA) and 30 g sodium chloride per 100 mL distilled water. DTNB reagent is 40 mg 5'5-Dithio-bis-(2-nitrobenzoic acid) (Wako Pure Chem Indust., Ltd.) in 100 mL of 1% trisodium citrate solution.

The 2.0 mL sample (pulmonary homogeneous precipitate) with 2.0 mL of 4 mM Na_2HPO_4 had mixed with 1.0 mL DTNB reagent. After had mixed, a 412 nm absorbance was recorded on the Spectrophotometer (UV-160 SHIMADZU UV-Visible Recording Spectrophotometer) with standard glutathione solution (Wako Pure Chem, Indust., Ltd.). The pulmonary glutathione levels are expressed in $\mu\text{g}/\text{gram}$ of tissue.

2.4. Data analysis

To know the difference between GSH level of the lung in each group was analyzed data with one way ANOVA test and continued with Least Significant Differences (LSD) analysis.

3. Results and discussion

The results showed that each group indicated variations in GSH levels. Based on the results of one way ANOVA test showed that rambutan peel extracts able to decrease GSH level of lung exposed by cigarette smoke. The further LSD test result explained that the control group had a significant effect on the other treatment groups. The mean of GSH levels of the lung shown in Table. 1.

Table 1. The mean of GSH levels of rat lung was exposed by cigarette smoke and given rambutan peel extract

Groups	Treatment	GSH levels ($\mu\text{g}/\text{gram}$)
Control	Normal	$75,82 \pm 2,95^a$
K1	3 cigarettes	$29,71 \pm 3,95^b$
K2	3 cigarettes + rambutan peel extract 3mg / 200g BB	$62,97 \pm 3,99^c$
K3	3 cigarettes + rambutan peel extract 6mg / 200g BB	$53,71 \pm 3,72^d$
K4	3 cigarettes + rambutan peel extract 12mg / 200g BB	$45,22 \pm 2,65^e$

Description: The number followed by the letters in the same column shows the differences in each treatment group with the level of accuracy $p < 0.05$

The exposure of cigarette smoke can cause damage to lung organ. The damage to the lungs as the main target directly affected by cigarette smoke can be explained by the exposure of chemical agents in cigarette smoke [2][15]. However, the effects that cause chronic disease in other organ systems are likely to be indirectly exposed result [1][16]. There are two phases of cigarette smoke, namely the gas phase and the particle phase.

The gas phase of cigarette smoke proved to initiate in vitro autoxidation of Polyunsaturated fatty acids (PUFAs) resulting in lipid peroxidation. The gas phase of cigarette smoke can contain up to 1014 free radicals and reactive substances per cigarette smoke. Free radicals and oxidants present in the gas phase of cigarette smoke have short half-lives, but they can enter the bloodstream and cause oxidative damage to macromolecules [17]. The gas phase of cigarette smoke also contains saturated and unsaturated aldehydes which are more stable than free radicals and hydrogen peroxide. Such compounds may enter the bloodstream and produce ROS through interaction with the NADPH enzyme. Consequently, not only lung tissue is subjected to oxidative stress, but distant tissue from the lung can also increase oxidative stress [18].

The particle phase of cigarette smoke contains a hydrocarbon complex which will react with nitrogen oxide (NO) and form another radical compound [19]. No found in cigarette smoke can initiate PUFA and result in lipid peroxidation. The particle phase of cigarette smoke has a longer half-life than the gas phase. Particle phase contains metal ions that can produce hydroxyl radicals from hydrogen peroxide. Such radicals can penetrate cell membranes and can induce oxidative stress [20]. The free radicals will bind the most susceptible molecules to cell membranes [21]. In the gas phase, oxidants are found in the form of O_2 and NO. These compounds quickly form the peroxynitrite molecule (ONOO^-). The free radicals in the particle phase are semiquinone which can react with O_2 to form superoxide radical and H_2O_2 [22].

The results of the study in Table 1. showed a decrease in GSH levels in groups of rat exposed by cigarette smoke differed significantly from the normal group and the treatment group with rambutan peel extract. GSH is an intracellular tripeptide shaped Gamma-Levo-glutamyl-L-cysteine-glycine, with various uses such as detoxification, antioxidant and cell proliferation modulation. GSH is widely used as a cosubstrate by glutathione peroxidase (GSH-Px) reducing hydrogen peroxide (H_2O_2) or organic peroxide (ROOH or LOOH in the case of lipid peroxide) by GSSG production. GSSG produced from GSH use can also be restored by the action of glutathione reductase.



This decrease in GSH levels will lead to an increase in free radicals. The condition of imbalance between the number of free radicals present with the number of antioxidants in the body will trigger the occurrence of oxidative stress. The antioxidants contained in the rambutan peel can play a role as

preventative of the occurrence of oxidative stress, so the damage to the cell membrane and the other cell functions can be inhibited [23].

Various of original natural resources of Indonesia contains many antioxidants with various active ingredients [24]. Rambutan peel extract contains active compounds of alkaloids, phenolics, steroids, terpenoids, vitamin C (ascorbic acid) that can work as an antioxidant [8]. In the Hernández *et al* [10]. explained that ethanol extract from rambutan peel has high free radical-scavenging activity, comparable to vitamin C and much higher than grape seed. Giving antioxidants and polyphenol compound components in conditions where free radicals increase, the antioxidants can capture free radicals and reduce oxidative stress. Flavonoids also protect cells from the damaging effects of ROS, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite [12, 25].

4. Conclusion

The exposure of cigarette smoke can increase the free radicals that result in lowering lung's GSH levels. The pulmonary GSH decrease can be eliminated with rambutan peel extract. The feeding of rambutan peel extract which doses 3 mg/200 gBB able to increase the levels of lung's GSH rat exposed by cigarette smoke. It is necessary to analyze the levels of GSH in other organs in rat exposed by cigarette smoke and get additional rambutan peel extract.

Acknowledgement

The author would like to thank: Head of Biology Department Universitas Negeri Semarang and Head and technician of Biology laboratory Universitas Negeri Semarang.

References

- [1] Trihandini, I 2015 *Makara J. Health Res.* **19** 8
- [2] Lodovici M and Bigagli E 2009 *Int. J. Environ. Res. Public Health* **6** 874
- [3] Haliwell, B and John M C Gutteridge 2017 *Free Radicals in Biology and Medicine. Fifth Edition* (Oxford: University Press, Acta Cryst) pp. 384–385
- [4] Lee, J G, K Baek, N Soetandyo, and Y Ye 2013 *Nat. Commun.* **4** 1568
- [5] Vázquez, J, B González, V Sempere, A Mas, M J Torija, and G Beltran 2017 *Front Microbiol.* **8** 1066
- [6] Fusco G, Chen S W, Williamson P T F, Cascella R, Perni M, Jarvis J A, Cecchi C, Vendruscolo M, Chiti F, Cremades N, Ying L, Dobson C M and De Simone A 2017 *Science*, **15** 1440
- [7] Pillon, N J and C O Soulage 2012 *Lipid peroxidation: Lipid peroxidation by-products and the metabolic syndrome* (Croatia: InTech)
- [8] Haddouchi F, Chaouche T M, Ksouri R, Medini F, Sekkal F Z and Benmansour A 2014 *Chin. J. Nat. Med.* **12** 415
- [9] Fila W O, Johnson J T, Edem P N, Odey M O, Ekam V S, Ujong U P, and Eteng O E 2012 *Ann. Biol. Res.* **3** 5151
- [10] Hernández C, Ascacio-Valdés J, De la Garza H, Wong-Paz J, Aguilar C N, Martínez-Ávila G C, Castro-López C and Aguilera-Carbó A 2017 *Asian Pac. J. Trop. Med.* **10** 1201
- [11] Thinkratok A, Suwannaprapha P and Srisawat R 2014 *Indian J. Exp. Biol.* **52** 895
- [12] Khonkarn R, Okonogi S, Ampasavate C and Anuchapreeda S 2010 *J. Food Chem. Toxicol.* **48** 2122
- [13] Palanisamy U D, Teng L L, Manahatan T and Appleton D 2011 *J. Food Chem.* **127** 21
- [14] Palanisamy U, Manaharan T, Teng L L, Radhakrishnan A K C, Subramaniam T and Masilamani T 2011 *J. Food Res. Int'l* **44** 2278
- [15] Zhou G, Xiao W, Xu C, Hu Y, Wu X, Huang F, Lu X, Shi C and Wu X 2016 *Tob. Induc. Dis.* **19** 24
- [16] David M L, Howard M C, Wilson S J and Geier C F 2016 *J. Health Psychol.* **21**
- [17] Lee K H, Jeong J, Koo Y J, Jang A H, Lee C H and Yoo C G 2017 *J. Biol. Chem.* **14** 11970

- [18] Fitria, T R I N K R, J C Mangimbulude and F F Karwur 2013 *Sains Medika* **5** 113
- [19] Ghezzi P 2011 *Int. J. Gen. Med.* **4** 105
- [20] Shin H J, Sohn H U, Han J H and Park C H 2009 *J Food Chem Toxicol* **47** 192
- [21] Petersen, R C 2017 *AIMS Biophys* **4** 240
- [22] Lushchak V I 2012 *J. Amino Acids* **1** 26
- [23] Safyudin and Subandrate 2015 *Jurnal Kedokteran dan Kesehatan* **2** 277
- [24] Werdhasari A 2014 *Jurnal Biotek Medisiana Indonesia* **3** 59
- [25] Alam M B, M S Hossain, and M E Haque 2010 *Int. J. Pharm. Sci. Res.* **2** 303
- [26] Petersen, R C 2012 *Int. Res. J. Pure Appl. Chem.* **2** 247