ANALYSIS OF GSH LEVELS IN THE LUNG EXPOSED TO E-CIGARETTE SMOKE ON THE MOUSEMODEL

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Abstract: Electric cigarette, also known as Vape, is a cigarette modification in the form of a liquid-filled device and battery operated. Electric cigarettes containpro pilinglycol, diethyl leneglycol, glycerol, distilled water, flavorings and nicotine solvents or nicotine plus plaster. Which will turn into nitronism when exposed to heat and is toxic to the body. The principle of working electric cigarettes is to change the chemicals contained in liquids into steam. Steam from the heating of the battery will be sucked and flow into the body of the e-cigarette user. This study aimed to analyze pulmonary GSH levels in rats exposed to e-cigarettes. The subjects of the study were 30 male Wistar rats aged 2-3 months, weighing 200-300 grams, healthy conditions. The sample was divided into 5 groups, namely the control group, the group exposed to tobacco cigarettes and the group exposed to electric cigarettes with nicotine levels of 3.6 and 9mg. Exposure to 3 cigarette tobacco cigarettes a day exposure to e-cigarettes 3 times a day for 30 days, with 5 ml. Data on GSH levels were analyzed by Anova test with LSD further testing. The results showed that there were significant differences between GSH levels of rats without exposure to smoke and GSH levels of rats exposed to e-cigarette smoke with the lowest GSH levels in the group with 9 mg of nicotine.

Keyword: E-cigarette, GSH, lung

INTRODUCTION

Indonesia is a developing country with the third largest smoker population in the world after China and India. (WHO, 2013). Smoking is a health problem in Indonesian society and a big threat to health in the world. According to the WHO survey (2008) one third of the world's population, especially adults, are smokers. The number of smokers every year tends to increase along with the increase in cigarette consumption. The death rate in the world due to smoking reaches 500 million people every year.

Along with the development of knowledge about the hazard of smoking, currently developing also in society which is cigarettes without tobacco or known as e-cigarettes. Electric cigarettes are modified cigarettes in the form of a liquid-filled device and are battery-operated. Electric cigarette liquid contains propylene, diethylene glycol and, glycerin which will turn into nitrotism when exposed to heat. The principeof working electric cigarettes is to change the chemicals contained in liquids, and due to the heating of the liquid battery into steam. Steam from the heating will flow to the body of the e-cigarette user. Electric cigarettes do not contain carbon monoxide as in tobacco cigarettes which can cause an imbalance of cells in the body called free radicals. However, the results of combustion of chemical components contained in e-cigarettes such as nitrosamines are toxic components that are very potential as free radicals and harmful to the body.

Free radicals are molecules that had unpaired electrons, making them unstable. Free radicals can be dangerous and cause pathological damage such as inflammation, metabolic disorders, cellular aging, carcinogens and other damage. Free radicals can originate from process processes that occur in the body, from inflammatory processes, and from outside the body, such as the use of drugs and also from pollutants and radiation processes. Exposure to smoke or steam from combustion is classified as exposure originating from the outside of the body, generally because of the large amount of water and exposure that cannot be avoided. Free radicals from outside the body are called exogenous free radicals. One exogenous free radical is a compound contained in cigarette smoke (Rohmatussolihat, 2009).

Free radical activity in the body is balanced with an endogenous defense mechanism, at least the body will produce antioxidants that had an effect as an anti-free radical. One of the endogenous antioxidants is superoxide dismutase, which is the first body defense system against ROS activation. When the level of ROS increases due to exposure to exogenous free radicals, such as vapors from combustion of liquids on e-cigarettes or cigarette smoke exceeding endogenous defense capabilities, oxidative instability or oxidative stress occurs. In conditions of oxidative stress, free radicals will cause cell membrane lipid peroxidation and will damage cell membranes, resulting in cell damage (Hu *et al.* 2014).

Lungs are organs in the respiratory system that are the first to have the chance to get exposure to exogenous radicals that enter through inhalation such as exposure to steam from burning electric cigarettes. One indicator of damage to the lung organs is GsH (gluthation). Therefore a study related to the effect of vapor exposure to e-cigarettes on pulmonary GSH levels was carried out.

METHOD

This research is an experimental study conducted in vivo. The study was designed with a randomized post-test control group with research subjects Wistar strain white rats, aged 2-3 months with a weight of 200-300 grams, 30 healthy mice and grouped into 5 groups randomly consisting of negative control groups (K), the positive control group treated tobacco cigarettes (KP), the treatment group with the treatment of electric cigarettes with 3mg nicotine content (KP1). (KP2) by treating electric cigarettes with 6mg and KP3 nicotine content with 9mg nicotine content. Each group was given standard chicken BR2 feed and drank ad libitum.

How to group and how to treat can be seen in Table 1. Below

Table 1. Provision of research treatment

Group	Treatment		Information
		Vol	
K- KP	- Tobacco cigarettes	3 times a days	Lungs and blood Lungs and blood
KP1	Tobacco cigarettes with nicotin 3 mg	5ml	Lungs and blood
KP2	Tobacco cigarettes with nicotin 6 mg	5ml	Lungs and blood
KP3	Tobacco cigarettes with nicotin 9 mg	5 ml	Lungs and blood

The treatment was carried out by exposing tobacco cigarette smoke to group 2 and vape combustion from vape in groups 3,4 and 5 with nicotine levels 3,6 and 9 mg, exposure was carried out for 30 days. The treatment was carried out in a cage for experimental animal care at the Biology Laboratory of Semarang State University. GSH analysis was carried out at the UGM Food and Nutrition Study Center laboratory.

Measurement of pulmonary GSH levels.

GSH levels measured are GSH levels in the lung organs. The GSH level was determined by the DTNB method, with a Spectrophotometer (UV-160 SHIMADZU UVVisible Recording Spectrophotometer). The method of measurement by taking a number of homogeneous lungs given a precipitation solution including metaphospor acid solution. Precipitation solutions: 1.67 g glacial acid metaphospor (mixture of HPO3 and NaPO3), 0.2 g disodium ethylenediamine tetraacetic acid (EDTA) and 30 g sodium chlorid per 100mL of distilled water.

The DTNB reagent consisted of 40 mg 5 '5 dithiobis (2-nitrobenzoic acid) (Wako Pure Chem. Indust., Ltd.) in 100 mL of 1% trisodium citrate solution. 2.0 mL sample (pulmonary homogenate precipitate) with 2.0 mL 4 mM Na2HPO4 mixed with 1.0 mL DTNB reagent. After mixing, the absorbance of 412 nm was recorded on the spectrophotometer (UV-160 SHIMADZU UV Visible Recording Spectophotometer) with a standard glutathione solution (Wako Pure Chem. Indust., Ltd.). lung glutathione levels can be expressed in µg / gram tissue.

RESULTS AND DISCUSSION

The study of the effect of exposure to e-cigarettes on GSH levels in lung organs in mice has been carried out for 8 months starting in May to October 2018. Measurements of GSH levels were carried out at the UGM Central Food and Nutrition Study Laboratory. Table 2

Table 2. Data on GSH Lung Levels

Number of animal /Group	1 (u/mg)	2 (u/mg)	3 (u/mg)	4 (u/mg)	5 (u/mg)	6 (u/mg)	Average
K (control -)	84,89	83,35	85,19	84,50	82,15	82,90	83,83±1,10
KP (tobacco cigarettes)	32,41	30,10	33,95	36,27	30,87	29,32	32,15±2,38
KP 1 (nic 3mg)	78,71	76,40	74,86	82,57	77,94	84,12	79,10 ±3,27
KP 2(nic 6mg)	58,65	61,74	55,56	52,48	50,93	56,33	55.95±3,61
KP 3(nic 9mg	42,44	46,30	44,76	50,16	41,67	54,79	46,69±4,55

The data in Table 2. show the highest average GSH levels seen in the negative control group and the lowest average seen in the group with tobacco cigarette exposure. The results of the analysis with ANOVA showed significant differences between the control group and all treatment groups with a f value of 4.22> 0.05. Low levels of GSH in the exposed group of cigarettes and the group exposed to vape with the highest nicotine level (6mg). This result is because there are naturally immune responses in three categories, namely the first line immune response mediated by the physical structure, the second line

immune response mediated by immunocompetent cells such as neutrophils, NK cells. The last immune response is the humoral and cellular immune response.

In the lungs of mice exposed to vapor vapors, the first component that changes changes in surface epithelial tissue containing mucous fluid. The fluid of the epithelial layer consists of a heterogeneous mixture of mucus, cells, proteins, and antioxidants with low molecular weight. Basically, epithelial wall fluid provides a physical barrier to many inhaled oxidants and is an important component of host defense against immunogen compounds. In addition to functioning as a physical barrier, in the epithelial wall fluid high-concentration antioxidants are needed which are intended to detoxify exogenous or endogenous oxidants. One of these antioxidants is GSH which is concentrated in epithelial wall fluid 10-100 times more in plasma (Gould et al. 2011). GSH is a tripeptide composed of amino acids glutamate (Gla), cysteine (Cys), and glycine (Gly). Even though it is not an enzyme, it is a substance for the glutathione peroxidase enzyme. Therefore, GSH also invites antioxidants. As an antioxidant, this tripeptide is facilitated by the sulfhidryl group of cysteine (Lushchak 2012)

GSH can function as an antioxidant through various mechanisms. The compound can chemically react with singlet oxygen, superoxide radicals, and hydroxyl and can directly act as a free radical scavenger. When GSH is oxidized, it forms GSH disulfide (GSSG), and can be reduced again with a specific enzyme, glutathione reductase (Ghezzi 2011). The amino acid cysteine (constituent of GSH) is an amino acid that has a -SH (sulfhidryl) group. This compound is very susceptible to oxidation reactions, even to air. Oxidized cysteine will form disulfide bonds. This bond is the result of condensation of 2 molecules of cysteine through the -SH group. Disulfide bonds are also the result of condensation of reduced form glutathione (GSH) and oxidized form glutathione (GSSG). In this case, glutathione acts as the cosubtrat of the glutathione reductase enzyme. The change in the form of glutathione is thought to be due to the displacement of GSSG from cells into the plasma. Oxidized GSH into GSSG in cells is evidence that in the body there is an increase in the number of free radicals. GSH has a role as an antioxidant by directly reducing free radicals or as a cofactor of antioxidant enzymes such as glutathione peroxidase and glutathione transhydrogenase. The main function of GSH is detoxifying drugs, xenobiotics or pesticides catalyzed by the GSHStransferase enzyme. GSH also plays a role in maintaining thiol groups (-SH) in essential proteins, by reducing disulfide bonds in proteins, which are catalyzed by thiol transferase enzymes (Safyudin&Subandrate 2015). During the process of oxidative phosphorylation, oxygen will be reduced to water with the addition of 4 electrons. In this reduction reaction superoxide anion radicals (O2 • -) are formed, which are then converted to hydrogen peroxide (H2O2) by the superoxide dismutase enzyme. GSH is widely used as a substrate by glutathione peroxidase (GSH-Px) reducing hydrogen peroxide (H2O2) or organic peroxide (ROOH or LOOH in the case of lipid peroxide) with GSSG production. GSSG produced from the use of GSH can also be restored again by the action of glutathione reductase (Breen & Murphy 1995; Lushchak 2012).

CONCLUSION

Based on the discussion above, it can be concluded that electronic cigarette vapor exposure for 30 consecutive days with a nicotine dose of 3.6.9 mg in mice can cause a decrease in glutathione levels. The lowest glutathione level was found in rat lungs with exposure to electric cigarette vapor with a nicotine level of 6 mg.

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