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Analysis of malondialdehyde and superoxide dismustase levels after exposure of electric cigarette in rats

Lisdiana*, Nugrahaningsih WH and I Nufus

Biology Department, Faculty of Mathematics and Science Universitas Negeri Semarang, Indonesia

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

Abstract. Electric cigarette (e-cigarette) is cigarette that operate on battery power to burn liquids and to produce a steam. One of the contents of an electric cigarette is nicotine. It is a chemical compound that can cause addiction and trigger oxidative stress. This study aims to analyze the levels of malondialdehyde and superoxide dismutase in the blood of rats that exposed to nicotine from e-cigarettes. The study was conducted on 30 male Wistar rats which divided into 5 groups, control group there are negative and positive and treatment group with nicotine 0,25 mg, 0,5 mg, 0,75mg with exposure to cigarette smoke for 30 days. Malondialdehyde and superoxide dismutase levels measurements using the TBARs method, the results that can be known using a spectrophotometer with a wavelength of 532 nm. SOD levels were measured by the calorimetry method. The One Way Anova analysis showed that malondialdehyde and superoxide dismutase levels in the control group were significantly different from all groups. The conclusion is that nicotine has an effect on increasing malondialdehyde and decreasing superoxide dismutase levels.

1. Introduction

The prevalence of smoking in Indonesia is very high in various levels of society, especially men ranging from children, adolescents, and adults. The increase in the number of smokers occurred due to an increase in population that has doubled in the last 50 years. Based on this latest data, the number of smokers worldwide has increased by almost 250 million people [1].

In this era there has been progress with the emergence of electric cigarettes or vape which are believed to be able to reduce the harmful effects of tobacco cigarettes. Electric cigarettes as if they might look safer and non-toxic than tobacco -cigarettes. Even though e-cigarettes do not use tobacco, e-cigarettes still contain nicotine and other chemicals that are potentially harmful to the body. Electric cigarettes are cigarettes that operate using battery power which will then heat up the amount of liquid stored in the cartridge to produce smoke that will be sucked by the user [2].

The most popular solvents used in e-cigarettes are glycerin (VG), propylene glycol (PG), or a combination of glycerin and propylene glycol by comparison with certain ratios [3]. In this case the liquid in question is nicotine, because e-cigarettes also contain nicotine as in tobacco cigarettes. Nicotine is an addictive substance, nicotine can make users want to consume continuously or commonly called addiction. In an e-cigarette contains propylene glycol, nicotine, glycerin, and flavoring. Whereas tobacco cigarettes contain Tar, CO, Nicotine, and heavy metals.

One of the contents of an electric cigarette in tobacco cigarettes is nicotine. Nicotine is an alkaloid compound found in tobacco leaves in addiction to anabacin and other alkaloid compounds. Nicotine has the chemical formula C10H14N2 with a molecular weight of 162.23 gr/mol and the concentration of nicotine is usually around 5% by weight of tobacco. Nicotine is what makes a person addicted to smoking. Although contained in one cigarette about 10 mg, but the right one is absorbed into the body as much as 1-2 mg, the rest is thrown into the air [4].

If the electric cigarette is continuously consumed, the vapor produced into CO in the form of smoke will accumulate in the body. In this situation, the body can experience oxidative stress [5-7]. A marker of oxidative stress is high levels of malondialdehyde and decreased superoxide dismutase activity due to excessive lipid peroxidation process in cells [8]. This purpose of the study is to analyze the levels of malondialdehyde and superoxide dismutase in the blood of rats that exposed to nicotine from e-cigarettes.

2. Methods

This research is an experimental laboratory study with a "the post-test only group" study design with a sample of 30 male Wistar strain rats aged 2-3 months, with a weight of 200-300 grams, healthy and not deformed. In this study rats were divided into 5 groups consisting of negative groups (K-), control groups (K +), treatment group 1 (P1) with nicotine content of 0.25 mg, treatment group 2 (P2) with levels of 0,5 mg and treatment group 3 (P3) with nicotine levels 0.75 mg for 30 days.

Measurement of malondialdehyde levels using the method: TBARs, samples used blood plasma. The analysis results were show by spectrophotometer with a wavelength of 532 nm.

Table 1. Procedure for measuring malondialdehyde levels

	Sample (µl)	Standard (µl)	Blank (µl)
H_3PO_4	750 -	750µl	750µl
TBA	250 -	250µl	250µl
Sample	50 -		
Standard		50µl	
aquabides			50µl
Aquabides	450µl	450µl	450µl

Measurement of superoxide dismutase levels in the calorimetry method. The sample used in this measurement is serum. The procedure for measuring superoxide dismutase activity can be seen in Table 2.

Table 2. Procedure for measuring superoxide dismutase levels

	Sample	Blank 1	Blank 2	Blank 3
Whole blood	20 -	-	20 µl	-
ddH_2O	-	20 µl	-	20 µl
Reagent solution WST (*Water-Soluble Tetrazolium)*	200 -	200 µl	200 µl	200 µl
Buffer diluent solution	-	-	20 µl	20 µl
Enzyme reagent solution	20 -	20 µl	-	-

All solutions were homogenized and incubated at 370C for 20 minutes, then absorbance measurements were carried out at a wavelength of 450 nm using a microplate reader.

3. Results and Discussion

Malondialdehyde levels were measured by the TBARs method using blood. Then read with a spectrophotometer with a wavelength of 532 nm. Measurement of superoxide dismutase levels using the calorimetry method, using blood serum. Then the absorbance measurement at 450 nm wavelength was measured using a microplate reader.

Table 3. Average results of malondialdehyde and superoxide dismutase levels

Treatment	Superoxide dismutase Level (%)	Malondealdehyd Level (nmol/ml)
Normal	85.12 ± 1.94^{a}	3.92
3 Clove Cigarette	22.34 ± 3.98^{b}	27.50
E- Cigarette with nicotine 3 mg	76.32 ± 3.98^{c}	9.08
E- Cigarette with nicotine 6 mg	51.77 ± 4.59^{d}	15.50
E- Cigarette with nicotine 9 mg	39.00 ± 4.59^{e}	21.50

Note: Numbers followed by letters in the same column show differences in each treatment group with a level of accuracy $p < 0.05$.

Whereas for electric cigarettes with 9 mg nicotine levels can produce malondialdehyde levels higher than other nicotine variation groups because of the continuous consumption of nicotine which causes damage to the lipid membrane and nicotine can increase dopamine production in the brain which causes an addiction and the emergence of a lot of radicals. Free radicals that accumulate in the body can cause oxidative stress. The increase in a free radical in the body is characterized by high levels of malondialdehyde in the body [8]

Malondialdehyde is one of the aldehyde compounds resulting from lipid peroxidation. The occurrence of lipid peroxidation is caused by the oxidation of polyunsaturated fatty acids in cell membranes by free radicals and high and low levels of malondialdehyde highly dependent on the high and low levels of endogenous antioxidants in the body [9]. One of the contents of electric cigarettes that are still found as in tobacco cigarettes is nicotine. Nicotine is the result of secondary metabolism included in the group of true alkaloids derived from the synthesis of nicotinic acid. If the consumption of nicotine is done continuously it will cause addiction.

Nicotine can enter the body through the respiratory tract, digestive tract, and skin. From the way someone smokes, nicotine enters the body through the respiratory tract. To get into the blood, nicotine in through the pulmonary circulation and will then be carried to the brain. In the brain there are nicotine receptors called Nicotinic Cholinergic Receptors (nicotinic acetylcholine receptors or nAChRs. The nicotine bonding on the surface between the 2 subunits in this receptor will open a pathway that allows sodium or calcium ions to enter, the entry of these 2 ions will activate the calcium channel tension which makes more calcium intake. The effect caused by the entry of calcium ions is the release of neurotransmitters. And dopamine is one of the released neurotransmitters. Before dopamine is released, nicotine activates glutamine at first, which functions as a neurotransmitter that works in assisting the release of dopamine and the release of y-aminobuteric acid (GABA) which can inhibit the active dopamine. Nicotine can increase dopamine levels in the brain. If the consumption of cigarettes has decreased, automatically there is also a decrease in nicotine levels in the body so the smoker will experience anxiety to be able to consume cigarettes continuously [10].

Nicotine that enters the body will also be a metabolized in the liver. Nicotine will be converted to cotinine through a transformation involving 2 ways, first mediating from cytochrome P450 which can produce nicotine, the ion-equilibrium with 5-hydroxynicotine. And the second is catalysis from cytoplasmic aldehyde oxidase. Imminium metabolism can occur through the electron transport pathway

with a redox cycle that will produce a radical. Cationic metabolism arises from several pathways, including nicotine oxidation itself and protonation of myosmine derived from nor nicotine through the demethylation of nicotine. This metabolism requires the hydrolysis of nicotine immotine to ketoamine with an open chain so that it will undergo nitrosation to form a toxic nitrosamine. Furthermore nitrosamine functions as a DNA alkylator and causes various oxidative damage and radical pathways to activate [11].

The nicotine in electric cigarettes has an effect on decreasing superoxidase dismutase levels in the body. Whereas for e-cigarettes with 9 mg nicotine levels can produce lower superoxidase dismutase levels than the nicotine variation group in other e-cigarettes because of the continuous consumption of nicotine so, there is a decrease in endogenous defences in the body and nicotine can increase dopamine production in the brain. can cause an addiction so that more free radicals are produced. And free radicals can cause oxidative stress. Increased free radicals in the body is characterized by decreased levels of superoxidase dismutase in the body.

Superoxide dismutase is an enzymatic antioxidant that protects tissues from oxidative damage caused by free radicals. superoxidase dismutase catalyzes the reaction of the radical reduction of superoxide anions to hydrogen peroxide (H2O2) and oxygen (O2). superoxidase dismutase enzyme is an endogenous defense to protect any free radicals that enter the body. So, when a smoker consumes an electric cigarette with a nicotine level of 9 mg, a smoker will be more addicted to wanting to consume continuously and cause an increase in free radical build-up. From the accumulation of free radicals that continue to experience an increase in superoxidase dismutase enzyme activity becomes disrupted which makes the process of imbalance of antioxidants and oxidants in the body. Groups that are exposed to clove cigarettes continue to produce a decrease in low superoxidase dismutase levels because in clove cigarettes not only contain nicotine but there are still other compounds that make dangerous free radicals produced and enter the body. Nicotine is an amine (a group of compounds that contain nitrogen). Nicotine binds to the nicotine acetylcholine receptor abbreviated as nAChRs. Nicotine stimulates the receptor to start a reaction that results in further release of neurotransmitters (chemical messengers that move between nerves, muscles, or glands) [12].

Reactive Oxygen Species (ROS) are very reactive oxidants. In this case free radicals are oxidants which are very reactive. The free radicals produced not only come from clove cigarettes but can also come from electric cigarettes [13]. The negative impact of these compounds arises because of its activity, so that it can damage cell components that are very important to maintain cell integrity. Each ROS that is formed can start a chain reaction that continues until the ROS is removed by another ROS or antioxidant system [14][15]. Based on research, it is known that even though e-cigarettes do not contain tobacco, there is still nicotine which is influential in increasing malondialdehyde levels and decreasing superoxidase dismutase levels.

4. Conclusion

The nicotine in electric cigarettes has an effect on increasing malondialdehyde levels and decreasing superoxidase dismutase levels in the body.

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