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Effect of Electronic Cigarette on Brain Prefrontal Cortex of Male Wistar Rats

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Abstract

The user of electronic cigarette continues to increase every year. Electronic cigarette smoke exposure can lead to neurotoxic effects on the brain. The purpose of this study to determine the effect on the prefrontal cortex of the brain white male rats (Rattus norvegicus Wistar strain) on electronic cigarette smoke exposure. This research is true experimental with post-test only control group design. Male rats were used as many as 24 rats were divided into 3 groups. Group P0 as a negative control (without exposure to an electronic cigarette). P1 group was exposed to electronic cigarette smoke with 0 mg of nicotine. P2 group was exposed to electronic cigarette smoke with 3 mg of nicotine. Exposure of electronic cigarettes were done twice a day in the period of 30 days. Then making preparations with Hematoxylin-eosin and counting necrosis of nerve cells in five fields of view using a microscope 400x magnification. Based on the results of microscopy observations obtained the amount of pyramidal necrosis cells in P0 groups are 7.85, P1 groups are 20.82 and P2 groups are 25.62. Data were analyzed using normality test obtained the results of data not normally distributed ($p < 0.005$) in the negative control group. Then data were analyzed using Kruskal Wallis test with $p = 0.000$ concluded that there is a significant difference ($p<0.005$) in all treatment groups. In the post hoc Mann Whitney test between groups I and II and groups I and III showed $p = 0.001$ concluded that there are significant differences ($p<0.005$) between the control group and the treatment group. At the Mann Whitney test between groups II and III showed $p = 0.006$ concluded that there are no significant differences ($p>0.005$) between the treatment groups. Based on the Linear Regression test results obtained nicotine in electronic cigarettes that were approved 46.4% of the increase in the number of pyramidal necrotic cells and there is an average increase in brain pyramidal necrosis cells of 1.6 cells by administering 1 mg of nicotine of electronic cigarettes. The conclusion of this study is exposure to electronic cigarette gives effect to an increase in necrosis of nerve cells in the prefrontal cortex of the brain.

Keywords: E-cigarette vapor, Prefrontal cortex, Pyramidal cells, Necrosis, Wistar rats.

INTRODUCTION

An Electronic Cigarette is a tool that can transform the chemicals into an aerosol and then inhale towards to the lungs by using electronic power from the battery. According to The National Center for Health Statistics (NCHS), in 2014 on the use of electronic cigarettes in the United States, it was found that 12.6% of the adult population had consumed electronic cigarettes, with a percentage of 14.2% of men and 11.2% of women. About 3.7% of the adult population of the United States currently consumes electronic cigarettes every day. When grouped by sex, 4.1% are male and 3.4% are female. And when grouped by age, the most users are aged 18-24 years by 5.1%, ages 25-44 years by 4.7%, ages 45-64 years by 3.5% and ages over 65 years by 1, 4%. There has been an increase in consumption of electronic cigarettes since 2011 until now, especially in the teens, 18-24 years [1].

Based on data on awareness of the existence of electronic cigarettes in Indonesia, the results reached 10.9%, with 2.5% being electronic cigarette users. Men who tend to know about electronic cigarettes are 16.8% compared to women which are 5.1%. If the percentage is differentiated by age, electronic cigarette consumers at the age of 15-24 years are 14.4% and at ages 25-44 are around 12.4%. Most electronic cigarette consumers in Indonesia are aged 15-24 years [2].

Most people believe that electronic cigarettes are harmless. About 19.4% of the American population thinks that electronic cigarettes are harmless, more than half the population, which is 53.8% considers that electronic cigarettes are not too dangerous and 26.8% consider electronic cigarettes as very dangerous [3].

The content in electronic cigarette liquids is glycol or glycerin, water, nicotine, flavorings, ammonia, arsenic, cadmium, NNK or 4 (methylnitrosamino) - 1- (3-pyridyl) - 1-butanone and NNN or N-nitrosonornicotine. Some of the substances contained in the electronic cigarette liquid some have changed when be heated in the e-cigarettes machine so that it becomes aerosol. In electronic cigarette

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aerosol, contained harmful substances, namely carbonyl, aromatic amines, tobacco-specific nitrosamines, volatile organic, ammonia, polyaromatic hydrocarbons, nicotine, and carbon monoxide in the form of hazardous substances in the human body [4].

The prefrontal cortex is located in the frontal lobe, in the anterior part of the motor cortex and premotor cortex. The brain's prefrontal cortex plays a role in cognitive function, regulating behavior and memory. The neurotoxic effects on the brain's prefrontal cortex can cause disturbance to this important function of the prefrontal cortex [5].

Various substances contained in e-cigarettes are toxic, carcinogenic and substances that can induce oxidative stress. This has a devastating effect on almost all organs of the body and increases the risk of chronic diseases, including affecting brain health and brain development [3].

Consumption of electronic cigarettes can affect brain function, such as cognitive decline, memory loss, mood disorders and drug dependence on humans and also animals. Electronic cigarettes can cause direct damage to neurons and cause muscle spasms and tremors [6].

Nicotine contained in electronic cigarettes can affect brain function, which can cause addiction and interfere with brain development at the age of children and adolescents [6].

Based on the results of previous studies, exposure to electronic cigarette smoke can lead to memory disorders and behavioral changes [6,7]. Memory and behavioral functions are functions of the prefrontal cortex, so this time the researcher wants to observe cell damage from the prefrontal cortex which is exposed to electronic cigarette smoke.

The urgency of this study is related to the increasing number of users of electronic cigarettes lately among young people who are classified as productive age, so the consumption of electronic cigarettes that have an impact on the brain will certainly reduce the productivity in the adolescent [1,2]. Public awareness of the dangers of electronic cigarettes tends to be low, people consider electronic cigarettes to be harmless because the smoke looks clean and does not smell sharp [3]. This research can also be a reference for the further development of research and education.

Based on the background described above, the purpose of this study is to determine the effect of e-cigarettes on increasing the number of necrotic cells (pyramidal cells) in the brain prefrontal cortex of the male Wistar rats (Rattus norvegicus strain Wistar). The hypothesis of this study is electronic cigarettes give effect to an increase in pyramidal necrosis cells in the brain prefrontal cortex of male Wistar rats.

MATERIALS AND METHODS

Research Design

The type of this research is an experimental study with Post Test Only Control Group Design method by counting the number of pyramidal cell necrosis on the brain prefrontal cortex after being treated.

Research Samples

The sample of this study was white male rats (Rattus norvegicus strain Wistar) with a sampling technique using simple random sampling. The inclusion criteria of the sample are bodyweight 150-200 grams, ages 8-12 weeks, and health conditions that characterized by active movements. Exclusion criteria were mice that deformed before being treated, mice that had given previous treatment, and mice that failed during the acclimatization. The drop out criteria in this study were mice that were sick and died during the treatment process.

Research Groups

The total sample of this study used male Wistar rats as many as 24 rats. The determination of the samples takes from the formula of Arifin WN & Zahirudd in WM (2017). The samples divided into 3 groups consisting of 8 mice in each group [8].

Group P0 as a negative control group (without exposure to an electronic cigarette). P1 group was exposed to 3 ml liquid of electronic cigarette smoke containing 0 mg of nicotine. P2 group was exposed to 3 ml liquid of electronic cigarette smoke containing 3 mg of nicotine.

Research Procedure

The independent variable of the study was exposure to electronic cigarette smoke and the dependent variable was the number of pyramidal necrotic cells in the brain prefrontal cortex of male Wistar rats. The study was conducted in a biomedical laboratory in University of Muhammadiyah Malang for 30 days. This research declared to be ethically appropriate in accordance with seven WHO 2011 standards by the health research ethics committee the University of Muhammadiyah Malang.

First, animals are acclimatized for 7 days. During this process, experimental animals are given BR-1 food and drink water. Then, on the 8th day, the process of electronic cigarette exposure in groups P1 and P2 begins, while the P0 group did not exposure to electronic cigarette smoke. Electronic cigarettes smoke exposure twice a day in a period of 30 days. Once exposure used 3 ml liquid of e-cigarettes in each group. Then in a day exposure used 6 ml liquid of e-cigarettes in each group [9].

The process of exposure to electronic cigarette smoke is done by putting experimental animals in a box (size 60x60x15 cm3). The box connected with a hose leading to a pump that serves to suck electronic cigarette smoke.

After 30 days of e-cigarettes exposure, the rats will be anesthetized before surgery with chloroform 0,67ml. Brain organs are taken surgically and organ fixation using formalin 10%. And then rats will be collected into one and buried. The histopathology preparation of the brain prefrontal cortex used Hematoxylin-eosin staining in Kessima Laboratory.

Histopathology preparation observed with microscope in magnification of 400x. Pyramidal cell necrosis characterized by a pyknotic core cell (solid, and angular) and stained eosinophilic cytoplasm. Calculated the presence of pyramidal cell necrosis in each field of view, then counted the average of necrosis cells in 5 fields of view. Then made the average calculation necrotic cells in each group [10,11].

Statistical Analysis:

Analysis of the data will be processed using SPSS 24. The data tested by the normality test with the level of statistical significance was set at $p>0.005$. The Kruskal Wallis test, the post hoc man-Whitney test with the level of statistical significance was set at $p<0.005$, and the linear regression test.

RESULTS

There were 3 groups at the end of the study, with the number of samples taken in each group was 8 male Wistar rats. No dead mouse at the end of the study. The picture of prefrontal cortex pyramidal necrosis cells in the brains of male white rats in this study appears in the following figure

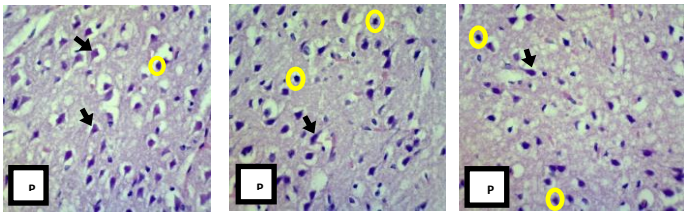

Figure 1: pyramidal necrosis in brain prefrontal cortex of male Wistar rats

The yellow circle is a normal pyramidal cell. The black arrows are pyramidal necrotic cells, characterized by pycnotic nuclei (dense and angular) and eosinophilic cytoplasm. Figure P0, the negative control group. Figure P1, the group exposed to electronic cigarettes with 0 mg of nicotine. Figure P2, which is the group exposed to electronic cigarettes with 3 mg of nicotine.

The average number of pyramidal necrosis cells in the brain prefrontal cortex of male Wistar rats with exposure to electronic cigarette smoke in each group looks like the graph below

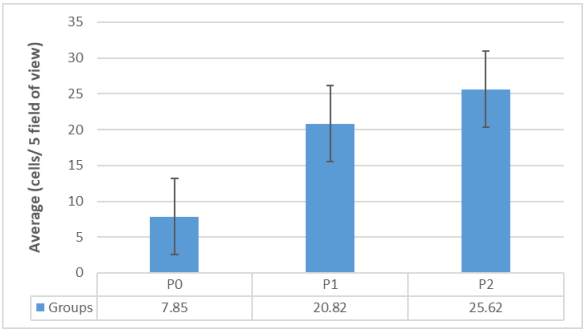

Figure 2: Graph of average pyramidal necrosis cells in the brain prefrontal cortex of male Wistar rats (Rattus norvegicus strain Wistar) in each group

The first data analysis used in this study was the normality test. Obtained the results of data not normally distributed (p <0.05) in the negative control group.

Table 1: Result of Normality test

	Groups	P Value
Average of pyramidal necrosis cells	P0 (Negative control)	0,002
	P1 (E-cigarettes with 0 mg nicotine)	0,456
	P2 (E-cigarettes with 3 mg nicotine)	0,631

Then proceed with the Kruskal Wallis test to find out whether there is a significant difference from the average necrosis cell between groups. The result obtained p = 0.005, so it can be concluded that there is a significant difference (p <0.005) in the increased pyramidal necrosis cells in the brain prefrontal cortex of the rats after exposure to electronic cigarette smoke.

Next, is the post hoc Mann Whitney test to find out whether there are significant differences in the mean necrosis cell counts between the two groups. The results obtained as in the following table:

Table 2: Result of Post hoc Mann- Whitney test

Groups	Comparison Groups	P value	Interpretation
P0 (Negative control)	P1 (E-cigarettes with 0 mg nicotine)	0,001	p value <0.005 indicates that there is a **significant difference**
	P2 (E-cigarettes with 3 mg nicotine)	0,001	
P1 (E-cigarettes with 0 mg nicotine)	P2 (E-cigarettes with 3 mg nicotine)	0,006	p value >0.005 indicates that there is a **no significant difference**

Based on the Linear Regression test results obtained nicotine in electronic cigarettes that were approved 46.4% of the increase in the number of pyramidal necrotic cells.

Based on the correlation value, which is 0.681 is included in the strong category. Then the strength of the relationship between nicotine in e-cigarettes with the number of necrosis cells is strongly categorized.

The form of the regression equation that connects nicotine in electronic cigarettes with an increase in pyramidal necrosis cells is listed as follows:

Y = A + B(x)
Y = 20,825 + 1,6(x)

Information:

Y = Average cell necrosis
x = Dosage of nicotine in electronic cigarettes (mg)

Based on these equations, there is an average increase in brain pyramidal necrosis cells of 1.6 cells by administering 1 mg of nicotine of electronic cigarettes.

DISCUSSION

This study aims to determine the effect of exposure to electronic cigarette smoke on increasing the number of pyramidal necrotic cells in the prefrontal cortex of male white rat's brain. Cell necrosis is obtained by observing the brain's prefrontal cortex preparations of hematoxylin-eosin staining through a 400-magnification microscope. Calculated the presence of pyramidal cell necrosis in each field of view, then counted the average of necrosis cells in 5 fields of view. Then made the average calculation necrotic cells in each group.

The results then performed statistical tests. From the Kruskal-Wallis test there was a significant difference in the administration of electronic cigarettes to the average pyramidal necrosis cells between each group.

Statistical results prove a significant effect on exposure to electronic cigarette smoke to an increase in the number of pyramidal necrosis cells in the brain prefrontal cortex. This is because the content of harmful substances in electronic cigarettes has the potential to activate NADPH and then stimulate oxidative stress. Smoking can trigger the activation of NADPH (nicotinamide adenine dinucleotide phosphate oxidase) resulting in an increase in superoxide (O2-) levels in the body. Superoxide (O2-) will react with NO and produce peroxynitrite (ONOO). Then peroxynitrite (ONOO-) will increase the oxidation of BH4, causing

BH4 deficiency. While BH4 (pteridine tetrahydrobiopterin) is a cofactor for NOS (Nitric oxide synthase). The release of bonds in eNOS results in a decrease in NO endothelial production, which will result in endothelial dysfunction [12].

Nitric Oxide (NO) plays a role in the maintenance and regulation of blood vessel pressure. NO is produced by endothelial cells, and the release of NO will trigger vascular smooth muscle relaxation. Decreased eNOS activity causes vasoconstriction [13].

Decreased Nitric oxide will cause vasoconstriction resulting in cerebral hypoperfusion which results in decreased blood flow and decreased glucose supply. Decreased blood flow will cause oxygen supply to fall. Glucose and oxygen which are important components in the metabolic process of the brain and when it decreases in number will cause metabolic dysfunction. The metabolic activity of the brain is classified as high, so if there is a metabolic disorder it will trigger nerve cell death [14].

The nicotine contained in electronic cigarettes can also cause a decrease in nitric oxide, causing nerve cell death (necrosis). Nicotine in the acute phase can cause addiction by stimulating the nicotinic acetylcholine receptors (nAchRs) so that dopaminergic transmission increases. These conditions will stimulate the reward system that has the effect of increasing mood. Long-term consumption of nicotine will cause GABA neurons to desensitize and eliminate the effects of dopamine inhibition resulting in addictive effects [15].

Reactive Oxygen Species (ROS) will initiate lipid peroxidation. The result of lipid peroxidation is malonic dialdehyde (MDA) which has a small size so that it easily penetrates DNA. These conditions can damage DNA strands, especially mitochondrial DNA (mtDNA), resulting in mitochondrial damage and a decline in ATP production. The body's metabolic process requires ATP, if there is a decrease in the amount of metabolic dysfunction there will be a result of cell death [16].

Proteins that bind to ROS will also trigger protein oxidation which will damage enzymes, transporters, and alter calcium homeostasis (Ca). Changes in the membrane protein structure will cause membrane disintegration and increased membrane permeability which in turn can also lead to cell death [16].

The increasing number of ROS in the body triggers the active pathway c-Jun N-terminal Kinases (JNKs). JNKs are part of protein kinases that play a role in pathways that involve gene expression, regeneration, neuronal plasticity, cell death, and regulation of cell arrest. The JNKs pathway is activated via cytokines, growth hormones, and oxidative stress [17].

Active JNKs pathway will increase the phosphorylation of Tau in the brain. It is widely available in the central and peripheral nervous systems. Tau is a protein that connects and binds to the cytoskeleton and lipids. Tau protein is bound to the outside and inside of the microtubules which are part of the cytoskeleton. Tau ties and microtubules function to bind and stabilize microtubules. Phosphorylation is the addition of phosphate groups to proteins. Tau phosphorylation resulting from the activation pathway of JNKs will change the formation of tau so that it can release tau from microtubules [18]. The release of the tau bond with the microtubule will reduce the stability of the microtubule. These conditions cause interference with the transport of neuron signals [19].

Various conditions caused by oxidative stress in the body will cause damage to deep nerve cells causing death or nerve cell necrosis.

The Post hoc Mann-Whitney test obtained on average cell necrosis between the negative control group and the 0 mg nicotine electric cigarette group and the negative control nicotine 3 mg cigarette indicating a significant difference. While the results obtained on average cell necrosis between the 0 mg nicotine cigarette group and the 3 mg nicotine cigarette group indicating there were no significant differences.

The insignificant difference between the nicotine 0 mg and nicotine 3 mg electronic cigarette groups indicates that electronic cigarettes without nicotine and electronic cigarettes with nicotine both cause damage. This is due to the role of nicotine in causing pyramidal necrosis cells only through a decrease in nitric oxide [15]. While other harmful substances in electronic cigarettes besides nicotine can cause necrosis through three pathways, which are lipid peroxidation [16], protein oxidation [16], and activation of c-Jun N-terminal kinase [17]. So that the small difference in nicotine levels in electronic cigarettes, which are 0 mg and 3 mg does not cause a significant difference in the number of brain necrosis cells. The difference in nicotine levels is higher to get the results of a significant difference between electronic cigarettes without nicotine and electronic cigarettes with nicotine.

Based on the results of linear regression, it was found that nicotine in electronic cigarettes influences on increasing the number of necrotic cells. Damage caused by nicotine occurs through the mechanism of decreasing nitric oxide until vasoconstriction occurs [15]. This condition causes the supply of oxygen and glucose to decrease, resulting in metabolic dysfunction [14]. The brain does not get the energy to carry out its function so that there is death/necrosis of nerve cells.

The limitation in this research is the staining method used for necrosis cell observation is a basic staining method. Further research is needed to observe more specific cell damage.

CONCLUSION

1. Electronic cigarette smoke exposure has a significant effect (p = 0,000) on the increase in the number of pyramidal necrosis cells in brain prefrontal cortex of male Wistar rats.

2. Nicotine in electronic cigarettes has a 46.4% effect on the increase in the number of pyramidal necrosis cells in brain prefrontal cortex of male Wistar rats.

3. There is an increase in the average of pyramidal necrosis cells in the brain prefrontal cortex of male Wistar rats by 1.6 cells by administering 1 mg of nicotine to electronic cigarettes.

Conflict of interest

The authors declare no conflict of interest.

Authors Contribution

Centaury Noor Kuncorowati [1]: Concept and design of study, drafting and revising article

Sofia Mawaddatul Urfah [2]: analysis and interpretation of data

Devanico Yuangga Duta Maulana [3]: Drafting and revising the article

Mochamad Bahrudin [4]: Final approval of the version to be published

REFERENCES

1. Schoenborn CA, Gindi RM. Electronic cigarette use among adults: United States, 2014. NCHS data brief, no. 217. Hyattsville (MD): National Center for Health Statistics; 2015
2. Palipudi KM, Mbulo L, Morton J, Bunnell R, Nelson GB, Kosen S, et al. Awareness and Current Use of Electronic Cigarettes in Indonesia, Malaysia, Qatar, and Greece: Findings From 2011–2013 Global Adult Tobacco Surveys. Nicotine & Tobacco Research 2015:1-7
3. U.S. Department of Health and Human Services. E-Cigarette Use Among Youth and Young Adults. A Report of the Surgeon General. Atlanta (GA): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2016
4. Flora JW, Meruva N, Huang CB, Wilkinson CT, Ballentine R, Smith DC, et al. Characteristic of Potential Impurities and Degradation Products in

Electronic Cigarette Formulation and Aerosol. Elsevier Regulatory Toxicology and Pharmacology 2016; 74(1) : 1-11
5. Akkoc RF, Ogeturk M. The Prefrontal Cortex: A Basic Embryological, Histological, Anatomical, and Functional Guideline. Journal Of Human Anatomy and Physiology 2017; 1(1): 1-4
6. Qasim H, Karim ZA, Rivera JO, Khasawneh FT, Alshbool FZ. Impact of Electronic Cigarettes on the Cardiovascular System. Journal of The American Heart Association 2017; 6(9): 1-14
7. Lamara AD. The Effect of Moringa Olifera Extract on Mus musculus exposed to cigarettes smoke [dissertation]. Surabaya: Airlangga Univ; 2017
8. Arifin WN, Zahiruddin WM. Sample Size Calculation in Animal Studies. Malays Journal Of Medical Science 2017; 24(5): 101-105
9. Tursinawati Y, Yazid N, Purnawati FW. Histopathology Left Ventricle of Rats Exposed by electric cigarettes and conventional cigarettes. Qanun Medika 2017; 1(2): 87-93.
10. Kristianingrum YP, Widyarini S, Kurningsih, Sutrisno B, Tabbu CR, Sugiyono. Histopatology Changes of the Rat Brain due to Trimethyltin Injection as Alzheimer's Disease Model. Journal Sain Veteriner 2016; 34(1): 84-91.
11. Ramadhani KZ. Effect of Papaya Leaf Extract (Carica papaya) on Histopathological of Pyramidal Cells on Cortex Cerebri and Memory Function of Male White Rat (Rattus norvergicus strain wistar) Induced by MSG [dissertation]. Malang: Muhammadiyah Malang Univ; 2018
12. Förstermann U, Xia N, Li H. Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. Circulation Research 2017; 120(4): 713-735.
13. Astutik P, Adriani M, Wirjatmadi B. The level of Superoxide (O2-), Nitric Oxide (NO) and fat intake in hypertensive and non hypertensive patients. Journal of Indonesia Nutrition 2014; 3(1): 1-6.
14. Toda N, Okamura T. Cigarette smoking impairs nitric oxide- mediated cerebral blood flow increase: Implication for Alzheimer's disease. Journal Of Pharmacological Science 2016; 131(4): 223-232.
15. Mishra A, Chaturvedi P, Datta S, Sinukumar S, Joshi P, Gorg A. Harmful effect of nicotine. indian Journal Of Medical and Paediatric Oncology 2015; 36(1): 24-31.
16. Dąbrowska N, Wiczkowski A. Analytics of oxidative stress markers. Advances in Clinical and Experimental Medicine 2017; 26(1): 155 - 166.
17. Yarza R, Vela S, Solas M, Ramirez MJ. c-Jun N-terminal Kinase (JNK) Signaling as a Therapeutic Target for Alzheimer's Disease. Frontiers in Pharmacology 2016; 6(321)
18. Wiryawan IGNS. Struktur Protein Tau Dan Perannya Pada Patogenesis [dissertation]. Denpasar: Udayana Univ; 2018
19. Chang RCC, Ho YS, Wong S, Gentleman SM, Ng HK. Nauropathology Of Cigarette Smoking. Acta Neuropathol 2014; 127(1): 53-69.