

Comparison of effects and differences in duration between exposure to conventional cigarette smoke and electronic cigarette vapors on changes in the number of hippocampal pyknotic pyramidal cell

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ABSTRACT

Background: Cigarettes contained addictive substances that may have adverse effects on health, such as memory impairment. The hippocampus is a part of the brain that plays a role in regulating memory. This study aimed to compare the effects of conventional cigarette smoke and e-cigarette vapor on hippocampal pyramidal cells in animal models.

Method: Experimental study used 30 male Sprague Dawley white rats aged 10-12 weeks. Rats were divided into 5 groups consisting of 1 control group and 4 treatment groups and exposed to conventional cigarette smoke and electronic cigarette vapor for 2 weeks and 4 weeks. The amount of exposure given was 12 times each day. After two weeks and four weeks of exposure, rats were necropsied, the Hippocampus was processed and stained using Hematoxylin and eosin. Pyramidal cell nuclei were viewed with a biological microscope CX-33 microcamera DP2 with 400x magnification in 3 fields of view. The average number of pyknotic nucleus pyramidal cells collected was statistically analyzed using the Mann-Whitney test.

Result: The hippocampal pyknotic pyramidal cells number in each treatment group was higher compared to the control group. A correlation was found between 2 weeks groups and 4 weeks groups of treatment. Conventional cigarette smoke groups showed higher means of pyknotic pyramidal cells than electronic cigarette vapor and were statistically significant in both 2 weeks groups and 4 weeks groups of treatment ($p < 0.05$).

Conclusion: The type of cigarette and the duration of cigarettes exposure affects the number of pyknotic pyramidal cells. Conventional cigarette causes more significant changes than electronic cigarettes, and the exposure duration of conventional cigarettes affects pyramidal cells in the hippocampus.

Keywords: Conventional cigarettes, electronic cigarettes, hippocampus, pyramidal cells, pyknotic.

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INTRODUCTION

Smoking habit is a phenomenon that is still popular throughout the world. Cigarette addiction can occur because of a substance called nicotine. Addiction and easy access to cigarettes play a role in increasing cigarette users every year.¹

According to the Indonesian Ministry of Health, smokers from 2013 to 2018 increased by 1.9%.² Today, people are starting to switch from consuming conventional cigarettes to electronic cigarettes (e-cigarettes), assuming that e-cigarettes are safer and have fewer side effects. E-cigarettes are cigarettes that

no longer use tobacco but instead use liquid that contained less nicotine that is converted into vapor.³

The hippocampus, which plays a role in regulating Ca^{2+} , is part of the brain affected by nicotine. According to Meashack, 2015, the effect of nicotine on the brain changes necrotic hippocampal pyramidal cell numbers which are characterized by the appearance of pyknotic pyramidal cells.³ Very few studies have compared the effects of nicotine between conventional and electronic cigarettes to histomorphological changes in cells, especially the brain. Therefore, this study was conducted to determine whether there are differences

between conventional cigarettes and e-cigarettes on histomorphological changes in the hippocampus by looking at pyramidal cells number changes.⁴

MATERIAL AND METHODS

This research is an experimental study with a post-test-only control group design. This research was conducted at the Experimental Laboratory of Universitas Katolik Indonesia Atma Jaya, Pluit, North Jakarta. This study used thirty male *Rattus norvegicus*, Sprague Dawley strain, aged 10-12 weeks with a body weight of 150-250 grams. First of all, the rats were

acclimatized for one week to adapt to the new environment. Rats were divided into five groups (6 rats in each group). Group 1 was the control group without intervention, group 2 was given conventional cigarette smoke for two weeks, group 3 was given electronic cigarette vapor for two weeks, group 4 was given conventional cigarette smoke for four weeks, and group 5 was given electronic cigarette vapor for four weeks, all treatments groups were given twelve smoke or vapor blows a day. Rats have necropsied at the end of two weeks or four weeks of treatment in Atma Jaya All-Purpose Laboratory.

Rat's brain tissue was removed surgically under ketamine and xylazine anesthesia. The hippocampus was taken, histologic preparations were made stained with Hematoxylin and Eosin staining, (Figure 1). Fixation using 10% buffered neutral formalin, trimmed, dehydrated, embedded, cut, and stained with Hematoxylin and eosin (H&E). Tissue slide brought to Pathologic Anatomy Laboratory of Universitas Katolik Indonesia Atma Jaya to be examined. Pyknotic pyramidal cell counted on hippocampus area with 400x



Figure 1. Coronal plane of rat brain. hippocampus.

magnification and 3 power fields for each tissue using Olympus Biological CX33 Microscope and Olympus Micro Camera DP22. Statistical analysis was done with the SPSS program. Data were analyzed using Kruskal-Wallis, Mann-Whitney, and Pearson tests. The results are expressed with the mean and p-value.

RESULT

The characteristic of the animals used in this study was described in Table 1. This study found that the amount of hippocampal pyknotic pyramidal cells was higher in the exposure group compared to the control group (Table 2). The pyknotic pyramidal cell is shown as a cell with a darker appearance in the CA1 region of the hippocampus in each group indicating neurodegeneration. Control shows minimal pyknotic pyramidal cells, while other groups showed a higher amount of pyknotic pyramidal cells (Figure 2).

The hippocampal pyknotic pyramidal cells number in groups 2 and 4 was higher compared to the control group, as seen from the mean number and statistically significant results ($p < 0.05$) with the Mann-Whitney test (Table 3). Using the Pearson test, we found a positive correlation between the duration of 2 weeks and 4 weeks of exposure to conventional cigarettes groups with $p < 0.05$ (Table 4). There was also a significant difference in the increase in hippocampal pyknotic pyramidal cells between the control groups, groups 3 and 5 using the Mann-Whitney test (Table 3). With Pearson's test, we saw a positive correlation in duration between 2 weeks and 4 weeks of exposure to electronic cigarette vapor with $p < 0.05$ (Table 4).

There were no significant differences between the two weeks conventional

cigarette group and electronic cigarettes group, and there were also no significant differences in the number of pyramidal cells with pyknotic nuclei of the two groups (Table 5). While a significant difference was found between the 4 weeks' treatment of conventional cigarettes and electronic cigarettes (Table 5).

DISCUSSION

Increase in the Number of Pyknotic pyramidal Cells Due to Conventional Cigarette Smoke and Electronic Cigarette Vapors exposure

Cigarettes can affect the hippocampus by administering dangerous substances such as nicotine. Nicotine could decrease the expression of polysialylated-neural cell adhesion molecules (PSA-NCAM). PSA-NCAM is present through the hippocampus region and a decrease in PSA-NCAM played a role in the downregulation of neurogenesis which explains why there is an increase in the pyknotic pyramidal cell.⁵ Pyknotic cells could be identified by seeing darker cells with nucleus shrinkage as a sign of pyknosis in the pyramidal layer of the CA1 hippocampus.⁶ Pyramidal cells are located dominantly in the CA1 region which serves as one hippocampus function in memory.⁷ In this study, when compared with the control group, there was an increase in the number of pyramidal pyknotic nuclei in all groups (groups 2,3,4, and 5) with a p-value < 0.05 . Based on these data, it can be concluded that exposure to e-cigarette vapor and conventional cigarette smoke increases the number of pyramidal cells with pyknotic nuclei in the rat hippocampus. These results are consistent with the study of Abrous et al., which stated that both e-cigarettes and conventional cigarettes contain nicotine which can

Table 1. Characteristic of animals (Weight and Condition).

Group	Average Weight (Gram)				Condition
	Week 1	Week 2	Week 3	Week 4	
Group 1	227.50	250.68	-	-	Healthy
Group 2	216.33	235.50	-	-	Healthy
Group 3	220.00	239.17	-	-	Healthy
Group 4	210.00	230.00	255.50	277.50	Healthy
Group 5	225.50	245.33	274.67	289.50	Healthy

Notes: Group 1 (Control), Group 2 (2 weeks of conventional cigarette smoke), Group 3 (2 weeks of electronic cigarette vapor), Group 4 (4 weeks of conventional cigarette smoke), and Group 5 (4 weeks of electronic cigarette vapor).

Table 2. Means of hippocampus pyknotic pyramidal cell.

Group	n	Mean \pm SD	CI 95%
Group 1	6	2.16 \pm 1.16	0.93 – 3.39
Group 2	6	6.83 \pm 3.37	3.29 – 10.37
Group 3	6	5.16 \pm 0.75	4.37 – 5.95
Group 4	6	16.50 \pm 6.47	9.70 – 23.29
Group 5	6	7.83 \pm 10.17	5.49 – 10.17

Notes: Group 1 (Control), Group 2 (2 weeks of conventional cigarette smoke), Group 3 (2 weeks of electronic cigarette vapor), Group 4 (4 weeks of conventional cigarette smoke), and Group 5 (4 weeks of electronic cigarette vapor).

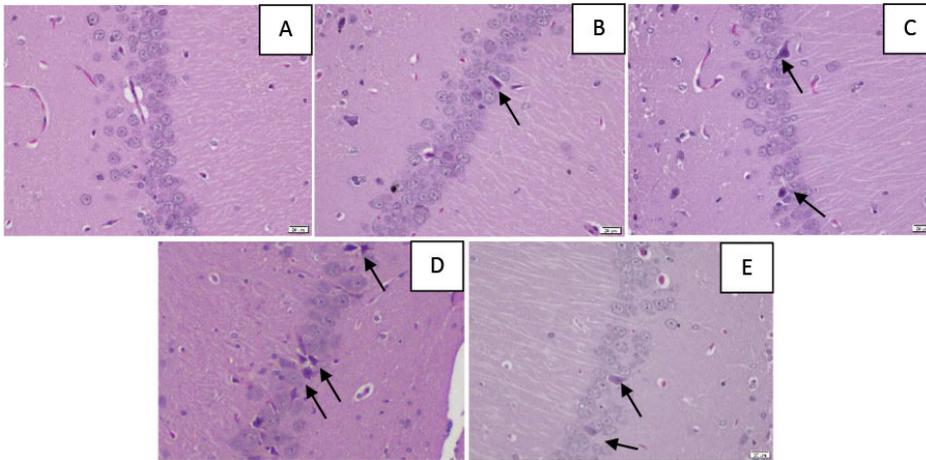


Figure 2. Image of Sprague Dawley rats hippocampus CA1 region (x400) with H&E staining showed pyramidal cells in each treatment group. (A) Control, (B) 2 weeks of conventional cigarette smoke, (C) 2 weeks of electronic cigarette vapor, (D) 4 weeks of conventional cigarette smoke, and (E) 4 weeks of electronic cigarette vapor. Black arrow = Pyknotic pyramidal cell.

Table 3. Hippocampal pyknotic pyramidal cell in CA1 region, control group versus treatment group.

No.	Mean (n=6)		p
	Control	Group	
1	2.16 \pm 1.16	6.83 \pm 3.37	0.008
2	2.16 \pm 1.16	5.16 \pm 0.75	0.004
3	2.16 \pm 1.16	16.50 \pm 6.47	0.004
4	2.16 \pm 1.16	7.83 \pm 10.17	0.004

Notes: Hippocampal pyknotic pyramidal cell in CA1: (1) Group 1 (Control) versus Group 2 (2 weeks of conventional cigarette smoke), (2) Group 1 (Control) versus Group 3 (2 weeks of electronic cigarette vapor), (3) Group 1 (Control) versus Group 4 (4 weeks of conventional cigarette smoke), (4) Group 1 (Control) versus Group 5 (4 weeks of electronic cigarette vapor).

reduce the expression of PSA-NCAM in the hippocampal formation, thereby reducing the plasticity of neurons in the hippocampus by forming more pyramidal cells of the pyknotic nucleus.^{4,8-10}

Previous research conducted by Samuel et al. showed that exposure to cigarette smoke for five weeks increased the number of hippocampal pyramidal

cells with pyknotic nuclei than controls. In this study, the duration used was two weeks and four weeks to see the effect of the length of exposure that could affect the pyramidal cells.¹¹ Another study by Eser et al. stated that smoking could induce apoptosis in CA1, CA2, CA3, and dentate gyrus of rabbits exposed to cigarette smoke for four weeks.¹²

Correlation of the Duration of Exposure to Electronic Cigarette Vapor and Conventional Cigarette Smoke to the Number of Pyramidal Cells in the Hippocampal Pyknotic Nucleus

The results showed a positive relationship between the length of exposure to conventional cigarette smoke and the number of pyknotic pyramidal cells, which could be seen from the results of the Pearson correlation ($p < 0.05$). The average result shows that the number of pyknotic pyramidal cells increases with exposure duration. The difference in the duration of exposure can affect the number of pyknotic pyramidal cells, which the accumulation of cigarette nicotine may cause. These results are similar to Samuel et al. research, which is carried out within five weeks with an exposure time of 20 minutes for four days in 1 week (if calculated exposure in 1 week is 80 minutes). In this study, the exposure time of 6 minutes (12 puffs) for seven days with a total of 42 minutes in 1 week has shown a difference in the average number of pyknotic pyramidal cells.¹¹ This shows that exposure to conventional cigarette smoke in just two weeks can increase the number of pyknotic pyramidal cells compared to the control group.

In this study, there was a positive correlation between the duration of exposure of 2 weeks and four weeks in the group of rats exposed to e-cigarette vapor ($p < 0.05$). The mean of pyknotic pyramidal cells in these three groups also tended to increase in pyknosis with more prolonged exposure. The results of this study indicate that the use of e-cigarettes for a long time can increase the damage that occurs to the hippocampus due to the accumulation of nicotine in e-cigarettes.^{8,10}

Significant Differences of Pyknotic Pyramidal Cells numbers between Conventional Cigarette Smoke and Electronic Cigarette Vapor Exposure

Mann-Whitney test showed no significant differences ($p > 0.05$) for two weeks of exposure to the conventional cigarette smoke group and e-cigarette vapor group. While Mann-Whitney test comparing four weeks exposure of conventional cigarette smoke group and e-cigarette vapor group showed a significant difference in the number of hippocampal

Table 4. Pearson Correlation between each duration.

Control		Mean (n=6)		r	p
		Intervention			
		2 weeks	4 weeks		
2.16 ± 1.16	1 (Conventional)	6.83 ± 3.37	16.50 ± 6.47	0.837	0.000
2.16 ± 1.16	2 (Electronic)	5.16 ± 0.75	7.83 ± 10.17	0.858	0.000

Notes: Pearson Correlation. (1) Conventional Cigarette Smoke Correlation between Control, 2, and 4 weeks duration. (2) Electronic Cigarette Intervention Correlation between Control, 2, and 4 weeks duration.

Table 5. Hippocampal pyknotic pyramidal cell in CA1, Conventional versus Electronic Cigarette.

Duration (Weeks)	Mean (n=6)		P
	Conventional	Electronic	
2	6.83 ± 3.37	5.16 ± 0.75	0.217
4	16.50 ± 6.47	7.83 ± 10.17	0.012

pyramidal cells with pyknotic nuclei with $p < 0.05$. Although there was no statistically significant difference, the mean of pyknotic pyramidal cells in the conventional cigarette groups was higher compared to the electronic cigarette groups. These results explain show that conventional cigarettes cause more pyramidal cell damage than electronic cigarettes.

According to Dong et al., nicotine is known to have neuroprotective functions, but in excessive doses, it can cause cell damage. Conventional cigarettes contain more nicotine than e-cigarettes¹³, which naturally suggest more significant hippocampal damage.¹⁴ The amount of nicotine in e-cigarettes is around 0.5-15.4 mg, while conventional cigarettes contain 17 mg of nicotine. Dopamine is a neurotransmitter that gives a relaxing sensation that can cause addiction, hence lower nicotine in an e-cigarette is meant to reduce dopamine levels compared to a conventional cigarette and in the end reduce the addiction of smoking, making harmful substance administration lower.^{14,15} Meashack et al., shows the formation of pyknotic pyramidal cells as the effect of nicotine on the histomorphological changes of pyramidal cells throughout the CA1 hippocampus region.³ The difference in pyknosis cells number is higher in the conventional cigarette group, indicating that conventional cigarettes are more harmful than e-cigarettes. However, this does not mean that e-cigarettes are safer and not dangerous at all since our study results showed an increase in the average

number of pyknotic pyramidal cells.

CONCLUSION

An increase in hippocampal pyknotic pyramidal cells was found in rats exposed to conventional cigarette smoke and e-cigarette vapor for 2 and 4 weeks. There is a relationship between the administration of conventional cigarette smoke and e-cigarette vapor for two weeks and four weeks on pyramidal cells with pyknotic nuclei numbers. There was no significant difference in hippocampal pyramidal cells with pyknotic nuclei number between rats exposed to conventional cigarette smoke and e-cigarette vapor with an exposure duration of 2 weeks. However, there was a significant difference in hippocampal pyramidal cells with pyknotic nuclei number between rats exposed to conventional cigarette smoke and e-cigarette vapor with an exposure duration of 4 weeks.

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DISCLOSURE

Conflict of Interest

There is no conflict of interest regarding this article.

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Ethical Consideration

The methodology of this study was approved by the Ethical Clearance Commission (No: 01/07/KEP-FKIKUAJ/2021).

Author Contribution

All authors contributed equally to this study.

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