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Effect of Exposure to E-Cigarette Vapor and Cigarette Smoke on Seminiferous Tubules Diameter and Spermatozoa Quality of Mice (*Mus musculus)*

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ABSTRACT

This study aims to compare the quality of spermatozoa (motility and viability) and the diameter of seminiferous tubules of mice (*Mus musculus*) by exposing to cigarette smoke and electronic cigarette vapor. This study used 28 male mice divided into 7 groups. Treatment group C was exposed to air, group A1 given 1 cigarette/day, group A2 given 2 cigarettes/day, group A3 given 3 cigarettes/day, group B1 given 0.7 ml/day, group B2 given 1,3 ml/day, group B3 given 2 ml/day. The treatments were carried out for 36 days. The result of this study showed significant difference ($p<0.05$) in spermatozoa motility percentage between C, A1, A2, A3, B3, but in B1 showed no significant difference ($p>0,05$) with B2. Spermatozoa viability percentage showed significant difference ($p<0.05$) between control and all treatment groups. Diameter of seminiferous tubules showed significant difference ($p<0,05$) between C, A1, A2, A3, B1, B3, but in B2 showed no significant difference ($p>0.05$) with A1 and A2. In conclusion, this study showed that both cigarette smoke and e-cigarette vapor exposure can decrease the quality of spermatozoa and diameter of seminiferous tubules. E-cigarette vapor exposure groups showed better quality of spermatozoa and greater diameter of seminiferous tubules compared to cigarette smoke exposure groups.

Key words : *Reproductive health, Cigarette smoke, E-cigarette vapor, Spermatozoa motility, Spermatozoa viability, Seminiferous tubules diameter.*

Introduction

Every year, about 2,25,700 people in Indonesia die from smoke or other diseases associated with tobacco. Indonesia is a country that ranks as the country with the seventh-highest number of smokers in the world, at 39.5% (WHO, 2018). Each cigarette burned, it will produce around 4000 kinds of chemicals. About 400 of these compounds are toxic, such as carcinogens, tar, nicotine, nitrosamines, carbon monoxide, Polycyclic Aromatic Hydrocarbons (PAH) compounds, phenols, carbonyl, chlorine dioxins, and furans (Fowles, 2000).

Cigarettes are one of the causes of cell damage because cigarettes contain ingredients that can form free radicals. Cigarette smoke inhaled by a smoker contains gas components and these particles can generate free radicals, including carbon monoxide, carbon dioxide, and hydrocarbon compounds. Decrease in the quality of spermatozoa can be caused

by oxidative stress conditions caused by an excessive number of free radicals (Muliartha *et al.*, 2009). Excess production of free radicals or Reactive Oxygen Species (ROS) can damage spermatozoa (Sari, 2014).

Today, cigarettes have developed from tobacco cigarettes to e-cigarettes (vaporizers). Using a vaporizer is considered an alternative that can replace cigarettes (Santana, 2018). A vaporizer is a device that can deliver nicotine through the battery working system into the human body. Standard liquid products contain nicotine, propylene glycol, flavorings, and water (Indra *et al.*, 2015).

The vapor produced from the vaporizer contains less harmful substances in a smaller concentration, which is 450 times lower than conventional cigarettes (Varlet *et al.*, 2015). However, another study by the Food and Drug Administration (FDA) in 2009 found that the vaporizer contains toxic Tobacco Specific Nitrosamines (TSNA) and di-ethylene glycol (DEG), which is known as a carcinogenic substance. Up to now, there are no international benchmarks regarding the definitive limits on production, consumption, and the substance of a good standard for e-liquid or vaporizer (Hallagan, 2014). By analyzing and evaluating the results of seminiferous tubule diameter and spermatozoa quality (motility and viability) of mice (*Mus musculus*) exposed to cigarette smoke and electric cigarette vapor, the results will be obtained whether e-cigarettes are less harmful compared to conventional cigarettes or otherwise.

Materials and Methods

The total of 28 male mice (*Mus musculus*) were divided into 7 groups, each group consisting of 4 mice. Group C was exposed by suction machine only, group A1 exposed with 1 cigarette/day, group A2 exposed with 2 cigarettes/day, group A3 exposed with 3 cigarettes/day, group B1 exposed with 0.7 ml/day, group B2 exposed with 1.3 ml/day, group B3 exposed with 2 ml/day. The treatments were carried out for 36 days. Exposure of cigarette smoke and e-cigarette vapor was done in an exposure box measuring 50 cm x 25 cm x 25 cm with two holes, one hole for entering cigarette smoke into the box and the second hole for ventilation. Exposure to cigarette smoke uses a modified smoke suction machine to enter cigarette smoke into the box. Each cigarette smoke exposure used commercial cigarette with a nicotine level of 2.0 mg/stick and E-cigarette vapor exposure used commercial e-liquid with a nicotine level of 3 mg/ml. On day 37th, all mice were sacrificed by cervical dislocation and then dissected to collect the testes and cauda epididymis to examine the percentage of spermatozoa motility and viability and measure the diameter of seminiferous tubules.

Data on motility, viability, and seminiferous tubules diameter were analyzed using the Statistical Product and Service Solution (SPSS) program, using the Analysis of Variance (ANOVA) test and followed by Duncan's test to determine the differences between treatments.

Results

The result on the effect of cigarette smoke exposure and e-cigarette vapor on spermatozoa quality (motility and viability) and seminiferous tubules diameter can be seen in Table 1.

Table 1. Mean and ± SD of spermatozoa motility and viability percentage and seminiferous tubules diameter in control and treatment groups.

Group	Means ± SD		
	Spermatozoa Motility (%)	Spermatozoa Viability (%)	Seminiferous Tubules Diameter (µm)
C	75.4 ± 4.6^{f}	82.7 ± 2.7^{g}	206.2 ± 6.5^{f}
A1	53 ± 1.8^{d}	59.8 ± 1.1^{d}	177.1 ± 2^{d}
A2	48 ± 3.2^{c}	49.8 ± 2.2^{c}	170.3 ± 0.9^{c}
A3	28.3 ± 4.5^{a}	32.6 ± 4.2^{a}	153.1 ± 3.2^{a}
B1	64.1 ± 2.9^{e}	70.3 ± 3.2^{f}	182.1 ± 0.9^{e}
B2	59.9 ± 2.3^{e}	64 ± 1.2^{e}	173.5 ± 0.7^{cd}
B3	38.3 ± 2.5^{b}	40.4 ± 2.7^{b}	159.7 ± 1.7^{b}

C: Control group, suction machine exposure; A1: exposed one cigarette smoke one daily; A2: exposed two cigarette smokes daily; A3: exposed three cigarette smokes daily; B1: exposed e-liquid vapor 0.7 ml daily; B2: exposed e-liquid vapor 1,3 ml daily; B3: exposed e-liquid vapor 2 ml daily. Different superscripts in each column showed significant differences ($p < 0.05$).

Spermatozoa Motility

Table 1 above showed that the highest mean percentage of spermatozoa motility was 75.4^{f} ± 4.6 which was obtained by the control group (C) and showed significant differences ($p < 0,05$) in each treatment group (A1, A2, A3, B1, B2, B3). In A1 group showed significant differences ($p < 0.05$) with

C, A2, A3, B1, B2, B3. In A2 group showed significant differences (p < 0,05) with C, A1, A3, B1, B2, B3. In A3 group showed significant differences (p < 0.05) with C, A1, A2, B1, B2, B3. In group B1 showed significant differences (p < 0,05) with C, A1, A2, A3, B3. B1 group showed no significant difference with B2 group. In group B2 showed significant differences (p < 0.05) with C, A1, A2, A3, B3 and showed no significant difference with B1 group. In group B3 showed significant differences (p < 0.05) with C, A1, A2, A3, B1, B2.

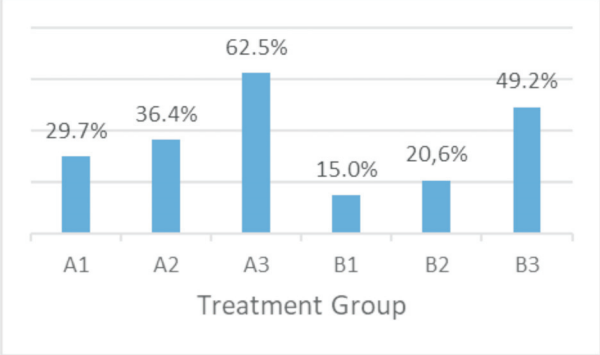

Fig. 1. Histogram of the percentage decrease in spermatozoa motility of treatment group compared to the control group.

Figure 1 showed the percentage decrease of spermatozoa motility compared to the control group. Group A1 showed a decrease of 29.7%, group A2 showed a decrease of 36.4%, and group A3 showed a decrease of 62.5%. Group B1 showed a decrease of 15,0%, group B2 showed a decreased of 20.6%, and group B3 showed a decrease of 49.2%. The figure showed that the decrease percentage of spermatozoa motility in group A (A1, A2, A3) is bigger than group B (B1, B2, B3).

Spermatozoa Viability

Table 1 above showed that the highest mean percentage of spermatozoa viability was $82.7^{g} \pm 2,7$ which was obtained by the control group (C) and showed significant differences (p < 0.05) in each treatment group (A1, A2, A3, B1, B2, B3). In A group (A1, A2, A3) showed significant differences (p < 0,05) with C, B1, B2, B3. In B group showed significant differences (p < 0,05) with C, A1, A2, A3.

Figure 2 showed the percentage decrease of spermatozoa viability compared to the control group. Group A1 showed a decrease of 27.2%, group A2 showed a decrease of 39.7%, and group A3 showed

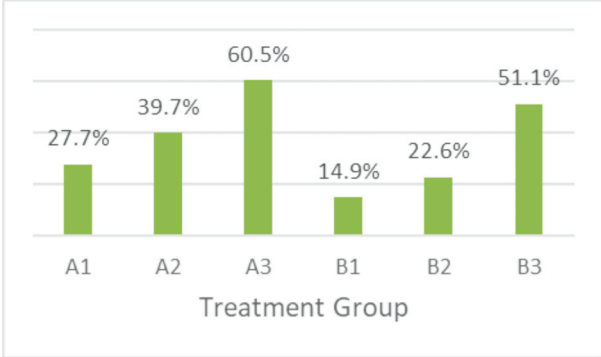

Fig. 2. Histogram of the percentage decrease in spermatozoa viability of treatment group compared to the control group.

a decrease of 60.5%. Group B1 showed a decrease of 14.9%, group B2 showed a decrease of 22.6%, and group B3 showed a decrease of 51.1%. The figure showed that the decrease percentage of spermatozoa viability in group A (A1, A2, A3) is bigger than group B (B1, B2, B3).

Seminiferous Tubules Diameter

Table 1 above showed that the highest mean percentage of spermatozoa viability was $82.7^{g} \pm 2,7$ which was obtained by the control group (C) and showed significant differences (p < 0.05) in each treatment group (A1, A2, A3, B1, B2, B3). In A1 group showed significant difference (p < 0.05) with groups C, A2, A3, B1, and B3, A1 group showed no significant difference (p > 0.05) with B2 group. In A2 group showed significant difference (p < 0.05) with groups C, A1, A3, B1, and B3, group A1 showed no significant difference (p > 0.05) with group B2. In A3 group showed significant difference (p < 0.05) with groups C, A1, A2, A3, B1, B2, and B3. In B1 showed

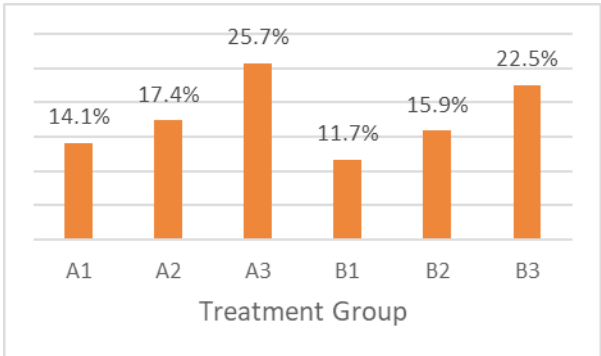

Fig. 3. Histogram of the decrease in seminiferous tubules diameter of treatment group compared to the control group.

significant difference ($p < 0.05$) with groups C, A1, A2, A3, B2, and B3. In B2 group showed significant difference ($p < 0.05$) with groups C, A3, B1, and B3, B2 group showed no significant difference ($p > 0.05$) with groups A1 and A2. In B3 group showed significant difference ($p < 0.05$) with groups C, A1, A2, A3, B1, and B2.

Figure 3 showed the percentage decrease of seminiferous tubules diameter compared to the control group. Group A1 showed a decrease of 14.1%, group A2 showed a decrease of 17.4%, and group A3 showed a decrease of 25.7%. Group B1 showed a decrease of 11.7%, group B2 showed a decrease of 15.9%, and group B3 showed a decrease of 22.5%. The figure showed that the decrease percentage of seminiferous tubules diameter in group A (A1, A2, A3) is bigger than group B (B1, B2, B3).

Discussion

Spermatozoa Motility

The decrease in the percentage of spermatozoa motility in this study can be caused by free radicals or ROS contained in cigarette smoke, thereby increasing the amount of lipid peroxidation and causing damage and a decrease in the integrity of spermatozoa membranes resulting in a decrease in spermatozoa motility (Negoro, 2016). Free radicals can also reduce the frequency of tail movement of spermatozoa because free radicals cause low mitochondrial ATP production (Susmiarsih, 2012). Excessive production of ROS will cause oxidative stress, Oxidative stress can cause loss of motility, viability, and plasma membrane integrity of spermatozoa (Tooy *et al.*, 2016).

Decreased spermatozoa motility of mice also occurred in mice exposed to e-cigarette vapor. The decreased motility of spermatozoa exposed to e-cigarette vapors was caused by the free radical content found in e-cigarette vapor (Mandasari, 2019). The content of propylene glycol, glycerin, nicotine and flavoring substances in e-liquid triggers the formation of free radicals in e-cigarette vapor. Nicotine in cigarette smoke or e-cigarette vapor can stimulate the adrenal medulla to release catecholamines that can affect the central nervous disrupting the hypothalamus's feedback mechanism, anterior pituitary, and testicles (Anita, 2004). E-cigarettes cause less oxidative stress due to the absence of tar and other toxicants content compared to conventional cigarettes which causes greater disruption of the spermatogenesis process. Based on research conducted by Goniewicz *et al.* (2014) about levels of selected carcinogens and toxicant in vapor from e-cigarette showed that the levels of the toxicants were 9 to 450 times lower than in cigarette smoke.

Spermatozoa Viability

Exposure to cigarette smoke decreased in the percentage of spermatozoa viability due to damage to the plasma membrane. When spermatozoa are exposed to hypo osmotic conditions, water will enter the spermatozoa to achieve osmotic balance, as a result, the volume of spermatozoa increases and the plasma membrane is damaged (Anggraini, 2019). The damage to the plasma membrane that results in a decrease in the percentage of spermatozoa viability is caused by the presence of free radicals or ROS contained in cigarette smoke and e-cigarette vapors (Martaningtyas, 2015). Free radicals also damage the DNA integrity in the nucleus of spermatozoa so that both of these things will induce cell apoptosis. Excessive levels of free radicals can cause oxidative stress. Oxidative stress can cause loss of motility, viability, and plasma membrane integrity of spermatozoa (Tooy *et al.*, 2016).

Decreased spermatozoa viability of mice also occurred in mice exposed to e-cigarette vapor. The nicotine content in e-cigarette liquid can act as a spermatoxic agent on maturing or matured spermatozoa (Oyeyipo *et al.*, 2013). ROS from e-cigarette vapor will increase the amount of lipid peroxidation, cause damage and decrease the integrity of spermatozoa membranes, thereby reducing spermatozoa viability and quality (Cocuzza *et al,* 2007). E-cigarette vapor can contain some of the toxicants present in tobacco smoke, but at levels which are much lower (Hajek *et al*., 2014). An early study by Westenberger (2009) found nitrosamines and tobacco-specific impurities 'at very low levels' and diethylene glycol in one of the cartridges. An analysis of e-liquid samples from 11 manufacturers found nitrosamine concentrations about 1,000 times lower than those in tobacco cigarette products (Kim *et al.*, 2013). The low toxicants content of e-cigarette vapor can lead to less disturbances in spermatozoa quality.

Seminiferous Tubules Diameter

The decrease in the diameter of the seminiferous tubules can be caused by a decrease in the quantity

and quality of spermatozoa in the seminiferous tubules (Anindita, 2012). Nicotine administration causes degenerative changes in the seminiferous tubules, manifested by changes in general architecture and a reduction in the number of diameters and thicknesses of the epithelium proportional to the dose (Nesseim *et al.*, 2011). Cigarette smoke is a source of free radicals that can cause increased oxidative stress in the body, causing testicular atrophy, damaging the seminiferous tubules, and interfering with the activity of the reproductive organs in producing spermatozoa (Tias, 2019). The testes are organs with low levels of endogenous antioxidants so that oxidative stress can easily damage the seminiferous tubules and spermatogenic cells (Ishii *et al.*, 2005).

Decreased diameter of seminiferous tubules of mice also occurred in mice exposed to e-cigarette vapor. Nicotine in e-liquid can cause disruption of spermatogenesis in the seminiferous tubules. Nicotine affects the work of the central nervous system by inhibiting the work of GnRH so that the formation of FSH and LH is inhibited. With the inhibition of the formation of FSH and LH, spermatogenesis runs abnormally (Wawryk-Gawda *et al.*, 2019). Oxidative stress, membrane lipid peroxidation, and decreased FSH secretion affect the production of spermatogenic cells thus affect the diameter of the seminiferous tubules (Kalsum, 2013). E-cigarette deliver lower levels of nicotine than cigarettes (Nides *et al.*, 2014). E-cigarette vapor can contain some of the toxicants present in tobacco smoke, but at levels which are much lower. Previous studies by Farsalinos (2014) detected the presence of aldehydes, especially formaldehyde, in the e-cigarette vapor, but at levels much lower than in cigarette smoke.

Conclusion

Exposure to e-cigarettes vapor was less decreased the motility and viability percentage also seminiferous tubules diameter of mice *(Mus musculus)* than exposure to cigarette smoke.

Conflict of Interest

Authors declare that they have no conflict of interest.

Acknowledgments

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Authors' Contribution

MAZ designed the study, interpreted the data, and drafted the manuscript. HAH, SU, TH and SKwere involved in collection data and also contributed in manuscript preparation. MAZ, EPH and EML took part in preparing and critical checking of this manuscript.

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