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Differences in diameter and thickness of the seminiferous tubules of male wistar rats (rattus norvegicus) after being exposed to e-cigarette smoke and conventional cigarettes

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Abstract

Background: Electric cigarettes and conventional cigarettes have a detrimental effect on their users. Cigarettes contain ingredients that reduce the quality of male reproduction.
Purpose: To determine the differences in diameter and thickness of the seminiferous tubules of male Wistar rats (rattus norvegicus) exposed to electric cigarettes compared to conventional cigarettes.
Method: This research is a true experimental research in the laboratory using a post test-only control group design. The treatment group consisted of group K- as the negative control group, group K+ which was included in the smoking chamber but not exposed to cigarette smoke, group P1 which was included in the smoking chamber and exposed to electric cigarette smoke, group P2 which was included in the smoking chamber and exposed to conventional cigarette smoke. The treatment was carried out for 30 days and then testicular samples were taken to calculate diameter and thickness of the seminiferous tubules. Data were analyzed with SPSS using descriptive statistics and One Way ANOVA and Kruskall Wallis tests.
Results: There was a decrease in the diameter and thickness of the seminiferous tubules in the treatment group which was significantly different from the control group. The results of the LSD post hoc test for differences in seminiferous tubule diameter between groups P1 and P2 were p=0.72 > 0.05. The results of the Mann Whitney test showed that the difference in seminiferous tubule thickness between groups P1 and P2 was p=0.06 > 0.05.
Conclusion: Exposure to electric and conventional cigarettes caused changes in the diameter and thickness of the seminiferous tubules from normal conditions. There was no significant difference in the impact of exposure to electric and conventional cigarettes, maybe because the damage they producedwas not different.

Keywords: Conventional Cigarettes; Electronic Cigarettes; Seminiferous Tubules.

INTRODUCTION

The electronic cigarette (e-cigarette) is considered by many to be a safer alternative to conventional cigarettes. The burning of tobacco is replaced by heating e-liquid in an e-cigarette, with some manufacturers claiming that the inhalation effects of e-cigarettes are less harmful than tobacco consumption. Other innovative features such as adjusting nicotine content and offering pleasant flavors have won the hearts of many users (Marques, Piqueras, & Sanz, 2021). However, based on research, e-cigarettes are still hazardous despite containing fewer toxic chemicals than conventional cigarettes (Centers for Disease Control and Prevention, 2022). E-cigarettes are battery-powered devices that produce aerosol by heating a liquid solution (with or without nicotine) with metal coils (Hod, Mohd Nor, & Maniam, 2022).

The prevalence of e-cigarettes is increasing worldwide. The current prevalence of e-cigarettes among adults is 11%, with 12% among men and 8% among women. Among adolescents, the prevalence is 25%, and among school students, it is 11%. The prevalence in America is 10%, in Europe 14%, in Asia 11%, and in Oceania 6% (Tehrani, Rajabi,

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Ghelichi-Ghojogh, Nejatian, & Jafari, 2022). The prevalence in some countries such as China is around 0.9%, Poland 3.1%, Malaysia 3.2%, and South Korea 7.6%. Among adolescents, the prevalence of e-cigarette use is related to smoking status (Hod et al., 2022; Jankowski, Gujski, Pinkas, Opoczyńska-Świeżewska, Krzych-Fałta, Lusawa, & Raciborski, 2021; Rahman, Yusoff, Mohamed, Naidu, Lim, Tee, & Aris, 2019; Tam, & Brouwer, 2021; Xiao, Yin, Di, Nan, Lyu, Wu, & Li, 2022). The highest current prevalence of e-cigarette use in Southeast Asia is in Indonesia (11.8%), while the lowest prevalence is in Thailand (3.3%). These differences may be due to varying e-cigarette regulations in these countries (Ling, Halim, Ahmad, Ahmad, Safian, & Nawi, 2023). The percentage of smokers in Indonesia is estimated to increase to 28.96% in 2021 (Central Bureau of Statistics, 2021). The most common types of cigarettes smoked by Indonesian adolescents are conventional cigarettes and electronic cigarettes, with kretek being the most popular, followed by white cigarettes, electronic cigarettes, hand-rolled cigarettes, and shisha (Ministry of Health of the Republic of Indonesia, 2018).

Despite the significantly lower toxic chemical content in e-cigarette aerosol compared to conventional cigarettes, there are concerns about its potential impact on male and female reproduction. Environmental factors and lifestyle choices, including smoking, are known to have adverse effects on gamete quality and can lead to reproductive disorders. Infertility is a public health issue affecting 186 million people worldwide (Boivin, Bunting, Collins, & Nygren, 2007; Gore, Chappell, Fenton, Flaws, Nadal, Prins, & Zoeller, 2015; Mascarenhas, Flaxman, Boerma, Vanderpoel, & Stevens, 2012; Montjean, Pagé, Bélanger, Benkhalifa, & Miron, 2023; Segal, & Giudice, 2019). Both e-cigarette and conventional cigarette smoke contain free radicals. Conventional cigarette smoke contains basic gases, including CO, CO2, NO, NO2, NH3, HCN, and specifically, metals, nicotine, and tar. Nicotine content causes adrenal medullary stimulation, leading to the release of catecholamines that affect the central nervous system. As a result of this mechanism, there is disruption in the feedback loop between the hypothalamus, anterior pituitary gland, and testes, causing disturbances in spermatogenesis. Additionally, e-cigarettes contain PAHs, VOCs, and TSNAs that trigger free radicals. The metal content in e-cigarettes triggers Reactive Oxygen Species (ROS) (Amandasari, Basuki, Ratnaningrum, & Kartikadewi, 2021).

This study aims to determine the differences in diameter and thickness of seminiferous tubules due to exposure to e-cigarette and conventional cigarette smoke. It is hoped that the results of this study can provide information regarding the effects of exposure to e-cigarette and conventional cigarette smoke on the diameter and thickness of seminiferous tubules, serving as a reference for further research and education regarding the effects of e-cigarette and conventional cigarette smoke on society.

RESEARCH METHOD

This study is a laboratory experimental study with a post-test only control group research design. There were four treatment groups: the K- group (negative control group, not subjected to treatment), the K+ group (positive control group, exposed to a smoking chamber but not to cigarette smoke), the P1 group (exposed to electronic cigarette smoke inside a smoking chamber), and the P2 group (exposed to conventional cigarette smoke upon entering a smoking chamber).

The study population consisted of Wistar strain rattus norvegicus rats. The rats were placed in an experimental animal laboratory at the Faculty of Medicine, Ciputra University. The inclusion criteria for the samples were male gender, aged 2.5 - 3 months, weighing between 150 - 250 grams, and being in good health, indicated by normal anatomical structure, agile movement, non-aggressive behavior, smooth skin and fur, absence of wounds, bright eyes, and no abnormal feces/urine discharge. Sample exclusion criteria included diseases, anatomical abnormalities, physical injuries, and death before or during the treatment. The number of samples used was 24 rats, with each group consisting of 6 rats. Randomization was performed using random sampling techniques.

The research tools included rat cages, a smoking chamber aquarium, aquarium filters, 20cc syringes, rat drinking bottles and containers, electronic scales, small surgical instruments, gloves, sample pots,

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glass slides, cover slips, Olympus CX 31 microscope, Olympus DP 22 digital camera, Cell Sens software. The research materials involved rats, vapes, kretek cigarettes, pellet food, mineral water, ketamine, 10% formalin, 70-96% graded alcohol, xylene solution, paraffin, Harris hematoxylin, and eosin.

The electronic cigarettes used were vape models with a liquid containing 9 mg of nicotine, while conventional cigarettes were kretek cigarettes with a nicotine content of 2.2 mg per stick. Exposure to electronic cigarette smoke was conducted for 30 days in the experimental animal laboratory, Faculty of Medicine, Ciputra University. After 30 days, termination was carried out. Histological preparation was performed in the Anatomy Pathology Laboratory, Faculty of Medicine, Airlangga University, and the data processing involved reading the results conducted in the Histology Laboratory, Faculty of Medicine, Ciputra University. The diameter and thickness of the seminiferous tubules were measured using a microscope and Cell Sens software in µm units. The obtained data were processed and analyzed using SPSS.

This research has obtained the Ethical Approval Certificate from the Research Ethics Commission, Faculty of Medicine, Ciputra University No. 025/EC/KEPK-FKUC/VIII/2022.

RESEARCH RESULT

Table 1.Seminiferous Tubule Diameter After Exposure to Cigarette Smoke (N=24)

Group	n	Average Diameter of Tubules Seminiferous (µm)	Saphiro-Wilk Test	Homogeneity Test	p-value
K-	6	30.55	0.324	0.621	0.000
K+	6	31.15*	0.402		
P1	6	27.39	0.984		
P2	6	27.06**	0.413		

*:Highest value **: Lowest value

In Table 1, it was shown that the largest average diameter of the seminiferous tubules was in the K+ group (group placed in the smoking chamber without exposure to cigarette smoke), which was 31.15 µm, and the lowest was in the P2 group (group exposed to conventional cigarette smoke), which was 27.06 µm. The data's normality test indicated that the testis weights were normally distributed: K- at 0.324, K+ at 0.402, P1 at 0.984, and P2 at 0.413, where the normality test results with p-value > 0.05, and the homogeneity test (Levene's Test) showed a result of 0.621 with p-value > 0.05. Hence, the analysis was performed using the One Way ANOVA test with a p-value result of 0.000 < 0.05.

Table 2. Seminiferous Tubule Diameter Test Results (N=24)

Group	K-	K+	P1	P2
K-	-	0.510	0.002*	0.001*
K+	0.510	-	0.000*	0.000*
P1	0.002*	0.000*	-	0.717
P2	0.001*	0.000*	0.717	-

*:significantly different (p <0.05)

In Table 2, the results of the After this LSD test found that the K- group and the K+ group had a value of 0.510, the K- group and the P1 group had a value of 0.002, the K- group and the P2 group had a value of 0.001. Furthermore, the K+ group and the P1 group had a value of 0.000, the K+ group and the P2 group had a value of 0.000. Meanwhile, the P1 group and the P2 group had a value of 0.717. There was a significant difference in the

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diameter of the seminiferous tubules between the K- group and the P1 group, as well as between the K- group and the P2 group, but there was no significant difference between the P1 group and the P2 group.

Table 3. Seminiferous Tubule Thickness After Exposure to Cigarette Smoke (N=24)

Group	n	Average diameter of tubules seminiferous (µm)	Saphiro-Wilk Test	Homogeneity Test	Kruskall-Walis
K-	6	7.55	0.304	0.036	0.002
K+	6	8.51*	0.042		
P1	6	6.76	0.058		
P2	6	6.11**	0.578		

*:Highest value **: Lowest value

In Table 3, it was indicated that the largest average thickness of the seminiferous tubules was in the K+ group (group placed in the smoking chamber without exposure to cigarette smoke), measuring 8.51 µm, and the lowest was in the P2 group (group exposed to conventional cigarette smoke), measuring 6.11 µm. The normality test of the data showed that the thickness data of the seminiferous tubules in the K- group was 0.304, K+ group was 0.042, P1 group was 0.058, and P2 group was 0.578. There was a result indicating non-normal distribution in the K+ group with a p-value of 0.042 < 0.05, and the homogeneity test (Levene's Test) showed that the data was not homogeneous with a p-value of 0.036 < 0.05, thus the Kruskall Wallis test was conducted with a p-value result of 0.002 < 0.05.

Table 4.Mann Whitney Seminiferous Tubule Thickness Test Results (µm)

Group	K-	K+	P1	P2
K-	-	0.055	0.200	0.037*
K+	0.055	-	0.004*	0.004*
P1	0.200	0.004*	-	0.055
P2	0.037*	0.004*	0.055	-

*:significantly different (p <0.05)

In Table 4, the results of the Mann-Whitney test indicated that the K- group and the K+ group had a value of 0.055, the K- group and the P1 group had a value of 0.200, the K- group and the P2 group had a value of 0.037. Furthermore, the K+ group and the P1 group had a value of 0.004, the K+ group and the P2 group had a value of 0.004. Meanwhile, the P1 group and the P2 group had a value of 0.055. There was a significant difference in the thickness of the seminiferous tubules between the K- group and the P2 group, the K+ group and the P1 group, as well as the K+ group and the P2 group, but there was no significant difference between the P1 group and the P2 group.

DISCUSSION

The research results indicated that exposure to electronic and conventional cigarette smoke led to a decrease in the diameter and thickness of the seminiferous tubule epithelium. Changes in these values, resulting from exposure to both electronic and conventional cigarette smoke, did not differ significantly statistically. This suggested that electronic and conventional cigarettes had similar impacts on the diameter and thickness of the seminiferous tubule epithelium, possibly because both cigarettes contained nearly the same dosage of nicotine.

Tobacco and electronic cigarettes contained nicotine as the main psychoactive agent. Nicotine acted as an agonist on nicotinic acetylcholine receptors (nAChRs), distributed throughout the brain

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and peripheral nervous system. When activated by nicotine binding, nAChRs underwent changes that opened internal pores, allowing the entry of sodium and calcium ions. On the postsynaptic membrane, nAChR activation could lead to increased action potential and modulation of gene expression through calcium-mediated secondary messenger systems. nAChRs were also localized on the presynaptic membrane, where they regulated neurotransmitter release (Changeux, 2018;Le Foll, Piper, Fowler, Tonstad, Bierut, Lu, & Hall, 2022; Wittenberg, Wolfman, De Biasi, & Dani, 2020). Nicotine increased the expression of p53 and caspase-3 and significantly reduced mRNA and protein levels of bcl-2, resulting in damage to spermatogenic cells and a decrease in seminiferous tubule thickness. Nicotine could also cause a decrease in testosterone levels (Mosadegh, Hasanzadeh, & Razi, 2017; Walker, & Cheng, 2005). Testosterone is necessary for the normal development and function of the testes and the male reproductive system. Low serum testosterone levels had been reported to affect the histological structure, weight, and function of the testes and epididymis (Oyeyipo, Adeyemi, & Abe, 2018).

Both conventional cigarette smoke and electronic cigarettes were known to cause an increase in reactive oxygen species (ROS), subsequently leading to oxidative stress development (Omolaoye, El Shahawy, Skosana, Boillat, Loney, & Du Plessis, 2022; Rambung, 2020). Increased reactive oxygen species (ROS) caused cellular, tissue, or organ damage due to lactate dehydrogenase activity, increased oxidative stress markers (lipid peroxidation, protein carbonylation), and reduced antioxidant enzyme activity (Tooy, Tendean, & Satiawati, 2016; Vivarelli, Canistro, Cirillo, Cardenia, Rodriguez-Estrada, & Paolini, 2019). The increased formation of ROS leads to apoptosis, where levels of pro-apoptotic cytokines increase, subsequently causing increased DNA damage in sperm cells, disruption of spermatogenesis, and reduced sperm production, resulting in testis degeneration (Omolaoye et al., 2022; Tooy et al., 2016). Cigarette smoke can also disrupt the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the anterior pituitary gland, reducing secreted testosterone and inhibiting Leydig cells from producing testosterone, thus reducing testosterone levels (Omolaoye et al., 2022; Sripratiwi, 2019). The deficiency of testosterone and FSH hormones is believed to cause seminiferous tubule atrophy. Reduced testosterone hormone inhibits the proliferation of spermatogonium cells, leading to incomplete sperm maturation and apoptosis. This results in a decrease in the number of spermatogenic cells, including sperm production, decreasing the diameter and thickness of the seminiferous tubule epithelium (Sripratiwi, 2019).

These results are in line with previous research that there is no significant difference in spermatozoa quality because the ingredients in e-cigarettes and conventional cigarettes have the same content, namely nicotine and (Tooy et al., 2016). Apart from that, other studies have found that exposure to electronic and conventional cigarette smoke has an effect on morphological and functional changes in the seminiferous tubule epithelium in the form of vacuolization, decreased spermatogenesis, increased apoptosis of spermatogonia and spermatocytes which causes a decrease in the thickness of the seminiferous tubule epithelium (Wawryk-Gawda, Zarobkiewicz, Chłapek, Chylińska-Wrzos, & Jodłowska-Jędrych, 2019; Tooy et al., 2016).

CONCLUSION

Exposure to electronic and conventional cigarette smoke leads to changes in the diameter and thickness of the seminiferous tubules from the normal condition. There were differences in the diameter and thickness of the seminiferous tubules due to exposure to electronic cigarette smoke and conventional cigarette smoke, but these differences were not statistically significant.

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