Effects of Nicotine-Containing E-Cigarette Smoke on Testicular Histopathology in Albino Rats

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ABSTRACT

This study aims to assess the level of damage from histopathology of seminiferous tubules of testicular organs of albino rats affected by exposure to e-cigarette vapor. The 20 albino rats used were divided into 5 groups, which are the control group (C) not given exposure to e-cigarette vapor and the treatment group given exposure to e-cigarette vapor at different doses. Treatment groups T1 (0.3 mg/ml), T2 (3 mg/ml), T3 (12 mg/ml), T4 (36 mg/ml). All treatment groups were given perinhaled and the treatment was carried out for 14 days. At the end of treatment, albino rats were euthanized and then necropsied and testicular organs were preserved to make histopathological preparations using Hematoxilin-Eosin staining. The results of the histopathological picture of the seminiferous tubules of the testes were observed at 400x magnification and the thickness of the epithelium and the diameter of the seminiferous tubules. The results showed a significant change in the thickness of the seminiferous tubule epithelium in each treatment which decreased, but there was no significant change in the diameter of the seminiferous tubules. This study states that exposure to e-cigarette vapor in rats can cause a decrease in spermatogenesis.

Keywords: e-cigarette, nicotine, testis

Received: May 7, 2025 **Revised:** August 10, 2025 **Accepted:** November 29, 2025

INTRODUCTION

Electronic cigarette (e-cigarrete) is a battery-powered device designed to deliver nicotine in the form of vapor or smoke that can be inhaled into the respiratory system (En *et al.*, 2020). Electronic cigarettes do not use tobacco but instead utilize a liquid containing nicotine, other chemicals, and flavors that have toxic properties. In Indonesia, as of 2017, approximately 2.1% of people were electronic cigarette users, with 47% of the sample aged between 25-45 years (Elsa and Nadjib, 2019). The continuous use of electronic cigarettes containing nicotine will have adverse effects on the body's health, including the lungs, heart, liver, nervous system, and reproductive system (Abdel-Kareem *et al.*, 2022). Nicotine is a basic component of electronic cigarettes and can be quickly absorbed through the oral mucosa, respiratory system, and skin (Mishra *et al.*, 2015). Alegantina (2018) study stated that smokers who have smoked for years were found to have high levels of nicotine in their blood and urine. Nicotine in the blood will spread throughout the body, including the reproductive system.

The reproductive system in animals is divided into two types: the male reproductive system and the female reproductive system. The testis is one of the male reproductive organs that functions as the site of sperm production and hormone secretion. The

process of sperm formation is called spermatogenesis, which occurs in the seminiferous tubules. On the walls of the seminiferous tubules, there are germ cells and Sertoli cells that produce reproductive hormones. One of the cases affecting male reproductive organs is infertility. Infertility is closely related to the process of sperm formation or spermatogenesis (Aprini, 2019).

Many studies reveal that 60-65% of men have lower sperm quality due to smoking habits (En *et al.*, 2020). Exposure to cigarette smoke can inhibit the spermatogenesis process, as indicated by a decrease in cells that contribute to the spermatogenesis process (Aras dkk., 2023). The use of nicotine can lead to a decrease in the average levels of serum FSH (Follicle Stimulating Hormone) and testosterone, where an imbalance in the reproductive hormone mechanism affects the spermatogenesis process in the testes (Oyeyipo *et al.*, 2013; Nugraha, 2022). This study aims to investigate whether exposure to electronic cigarette vapor at different doses affects the histopathological features of epithelial thickness and seminiferous tubule diameter in the testes of albino rats.

METHODS

Experimental design

This study is experimental, using a completely randomized design (En Y.S. *et al.*, 2019). The sample size was determined using the Federer formula (Meutia *et al.*, 2023). Each group was required to have at least four replications. The rats were randomly selected and divided into five groups. This study used 20 male rats, with one control groups and four treatment groups. Each group consisted of four rats.

Experimental animal preparation

The experimental animals were 20 male albino rats of the Sprague Dawley strain, aged four months, weighing 130-150 grams, and in healthy condition. These animals were obtained from UD Tiput Abadi Jaya, a test animal farm in Yogyakarta. The experimental animals were placed in test cages equipped with tubes and aerators to direct electronic cigarette vapor into the cages. Each treatment cage housed four albino rats, which were marked on their tails with ink for identification. All treatments and animal adaptations were conducted at the Feed and Nutrition Laboratory, FIKKIA, Airlangga University, Banyuwangi. The treatments were carried out after obtaining an ethics test certificate No. 2.KE.004.01.2019.

Treatment

This study began with a 7-day adaptation period for the test animals, during which they were provided with food and water ad libitum. The test animals were considered to have successfully adapted if their body weight remained stable or increased. On the 8th day, the treatment phase began. The treatment was conducted for 14 days according to the respective treatment groups. The control group was not exposed to electronic cigarette vapor, while the treatment groups were exposed to electronic cigarette vapor twice a day using 1 ml of e-liquid with varying nicotine doses: T1 with a nicotine dose of 0.3 mg/ml, T2 with 3 mg/ml, T3 with 12 mg/ml, and T4 with 36 mg/ml. (Goniewicz *et al* 2015) The e-cigarette, loaded with 1 ml of e-liquid, was activated and automatically switched off after 10 seconds, then placed inside the treatment chamber for 4 minutes to allow optimal vapor dispersion.

Slide preparation and histopathological examination

On the 15th day, the animals were euthanized by first weighing the final body weight, then euthanized using ketamine HCl and xylazine HCl peritoneally. Mice that have been euthanized are then necropsied on the abdomen and testicular organs are taken and macroscopic observations are made, after which they are fixed in a 10% formalin buffer solution for histopathology (Abdel-Kareem *et al.*, 2022). Making histopathology with paraffin method. The fixed organs were then dehydrated using graded alcohol (70%, 90%, 100%) and cleaned with xylene. Infiltration with paraffin at 60℃ and followed by embedding. Paraffin blocks were cut with a thickness of 4-5 µm using a microtome and then stained using Hematoxyline-Eosin (HE). Observation of histopathological preparations was carried out using a trinocular microscope and NIS (Nikon Imaging Software) application at 400x magnification and observations were made on the thickness of the seminiferous tubule epithelium and the diameter of the seminiferous tubules.

Data analysis

Data from observations on seminiferous tubule epithelial thickness and seminiferous tubule were first checked for normality using the Shapiro–Wilk test and for homogeneity of variance. For comparisons between groups, a one-way ANOVA was performed. To account for the repeated measurements of multiple seminiferous tubules per rat, the mean value per animal was calculated and used as the experimental unit. Post hoc comparisons were conducted using Duncan's multiple range test. All statistical analyses were performed using SPSS, and p-values <0.05 were considered statistically significant. Results are reported as mean ± standard deviation (SD). However, if the data were normally distributed, a one-way ANOVA test was conducted to determine differences between group means and continued with post hoc analysis using the Duncan test to compare each treatment group (Purba and Simanjuntak, 2012).

RESULT AND DISCUSSION

The results of the observation of histopathological preparations of testicular seminiferous tubules are in the control group significantly different from the other four treatments, but in each treatment group is not significantly different but there is a decrease in the diameter of seminiferous tubules that can be seen in Table 1 and Figure 1. Treatment T4 with nicotine administration of 36mg/ml showed a decrease in the average diameter of the seminiferous tubules the smallest of the other treatment groups. The results in the control group were significantly different from other treatments, and the T1 treatment group was significantly different from other treatments, but the T2, T3, and T4 treatment groups did not differ significantly between each treatment, but there was a decrease in the thickness of the seminiferous tubule epithelium which can be seen in Figure 2. The T4 treatment group with nicotine administration of 36mg/ml decreased the average thickness of seminiferous tubule epithelium and in T4 obtained the smallest epithelial thickness among other treatments.

The results of this study can be seen in that e-cigarette smoke vapor given per inhalation with nicotine dose produces a decrease in the diameter and thickness of the seminiferous tubular epithelium. The control treatment was significantly different from the other treatments where the control group was not given exposure to e-cigarette smoke

vapor so the results of the average diameter and thickness of the seminiferous tubule epithelium had the highest or largest value of the other treatments. In the variable diameter of seminiferous tubules, treatments T1, T2, T3, and T3 did not differ significantly between the four treatments, but it can be seen that the average diameter of seminiferous tubules for each treatment decreased until the smallest average diameter in T4 which had been exposed to a dose of 36mg/ml. In the variable of seminiferous tubule epithelium thickness, the T1 treatment group is significantly different from T2, T3, and T4, while the T2, T3, and T4 treatment groups are not significantly different between the three treatments, but it can be seen that the average thickness of the seminiferous tubule epithelium in the treatment groups decreases until the smallest average is in T4 which has been exposed to a dose of 36mg/ml.

Table 1. Diameter and thickness of seminiferous tubule epithelium of albino rats

Treatment Groups	Mean ± SD	
	Diameter	Epithelium Thickness
C	394.25^{b} ± 47.74	66.78^{c} ± 8.67
T1	341.78^{a} ± 27.60	56.38^{b} ± 4.21
T2	336.19^{a} ± 24.47	52.19^{a} ± 5.58
T3	334.30^{a} ± 32.56	51.54^{a} ± 5.11
T4	332.99^{a} ± 25.16	49.23^{a} ± 4.39

Different alphabets in the same column indicate significant differences ($p<0.05$).

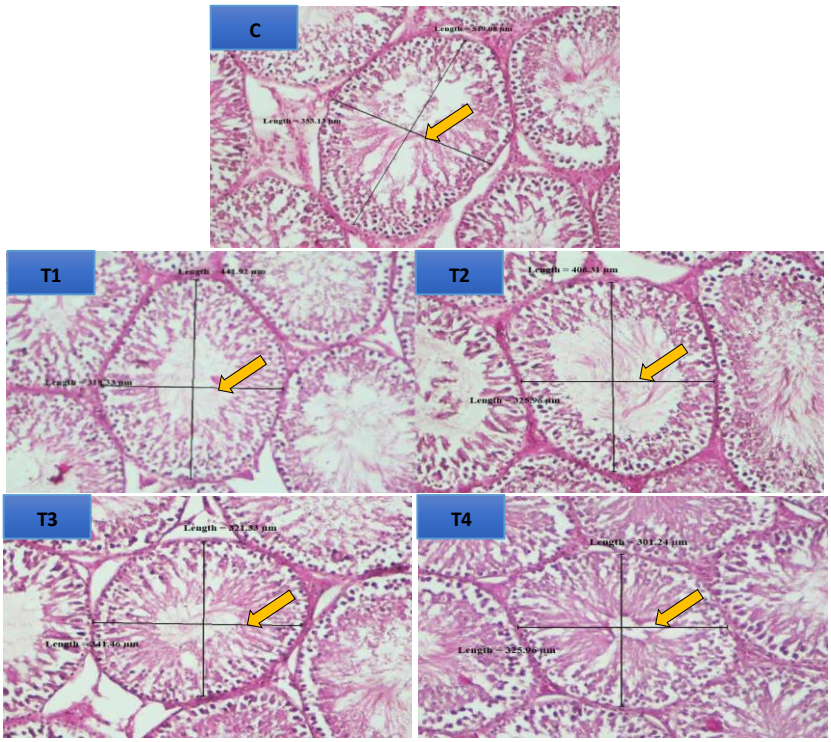

Figure 1. Microscopic images representing the diameter (⟸) of seminiferous tubules of albino rats in each treatment after 14 days of exposure to perinhaled e-cigarette smoke vapour. 400x magnification.

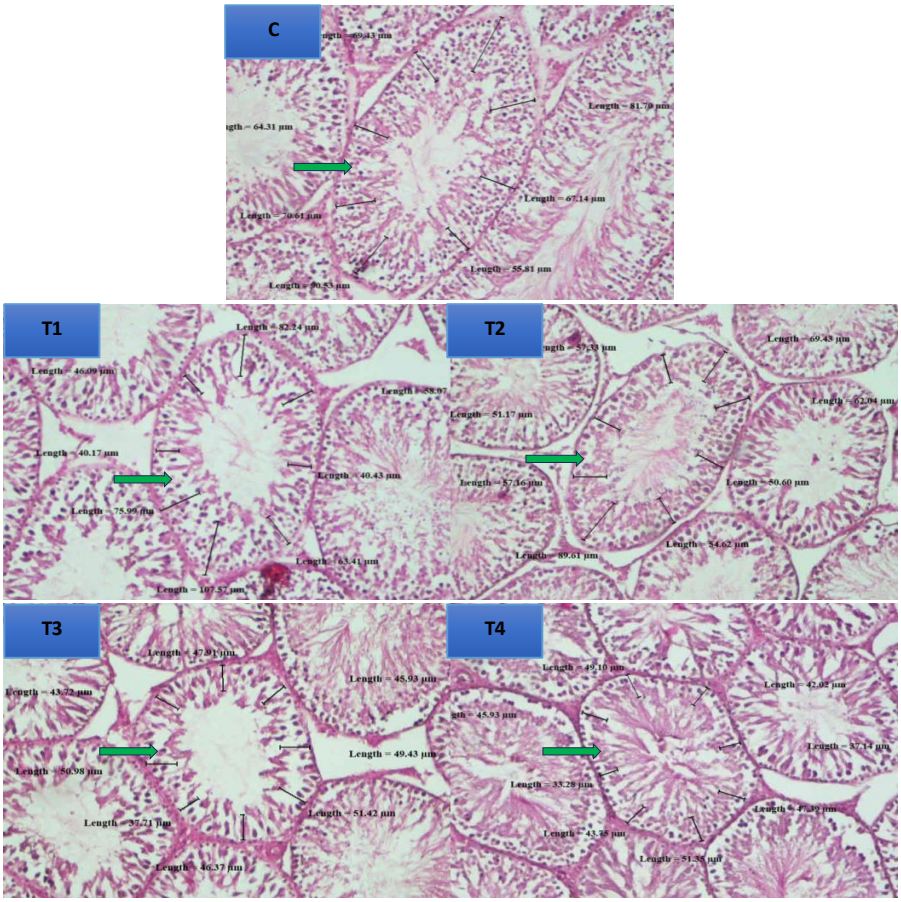

Figure 2. Microscopic images representing the thickness (⟹) of the seminiferous tubule epithelium of albino rats in each treatment after exposure to per inhaled e-cigarette smoke vapour for 14 days. 400x magnification.

The number of e-cigarette users has increased in recent years as many tobacco cigarette users have switched to e-cigarettes (Marthur and Dempsey, 2018). The change from tobacco cigarettes to e-cigarettes is due to several things, one of which is that e-cigarette liquids have many flavors (Glantz and Bareham, 2018). The flavors in this liquid contain ingredients such as aldehydes, benzyl alcohol, terpenes, pyrazine, menthol, menthone, and ethyl maltol (Wang *et al.*, 2020). Liquid in e-cigarettes also contains nicotine which differs from brand to brand, nicotine has a danger to brain development because nicotine is a psychoactive and addictive component (Le Foll *et al.*, 2022), besides nicotine, propylene glycol, and glycerin can also cause malfunction in male reproductive organs (Tooy *et al.*, 2016). Based on research from the FDA, substances contained in e-cigarette liquids have carcinogenic properties and can increase free radicals so that they can cause oxidative stress (Fajariyah *et al.*, 2021). Nicotine can affect the performance of the central nervous system by inhibiting GnRH so that the formation of the hormones FSH and LH is inhibited, if these hormones are inhibited, the spermatogenesis process will run abnormally (Wawryk-Gawda *et al.*, 2019). Oxidative stress and decreased secretion of these hormones will affect the production of spermatogenic cells and will affect the diameter and epithelium of the

seminiferous tubules (Kalsum *et al.*, 2013).

This insignificant result can occur due to several factors, in the study of Zulfikar *et al.* (2022) stated that the decrease in the diameter of the seminiferous tubules was due to differences in the amount of nicotine administration per day, the smallest seminiferous tubule size resulted from the administration of 2ml / day nicotine while the research conducted was only 1ml / day. Other factors can occur due to the length of exposure to cigarette smoke, research by Zulfikar *et al.* (2022) and En Y.S. *et al.* (2019) conducted research on e-cigarette smoke exposure for 20-35 days, and the intensity of administration also affects the results of seminiferous tubules.

In the present study, we measured only seminiferous tubule diameter and epithelial thickness to assess testicular changes following nicotine. We acknowledge that critical reproductive parameters, including sperm count, motility, hormone levels (testosterone, LH, FSH), oxidative stress markers, and Sertoli cell integrity, were not assessed. Therefore, while the observed reductions in tubule diameter and epithelial thickness suggest potential impairment of spermatogenesis, these findings alone do not provide a comprehensive evaluation of reproductive function.

This limited duration has been noted as a study limitation and should be considered when interpreting the findings.

CONCLUSION

Based on the results of this study, nicotine-containing e-cigarette exposure was associated with reductions in seminiferous tubule diameter and epithelial thickness; however, these changes were not statistically significant among the treatment groups T1, T2, T3, and T4. While the observed reductions may suggest potential effects on spermatogenesis, the underlying mechanisms, such as oxidative stress or hormonal disruption, were not directly measured in this study and therefore cannot be confirmed. Further studies assessing these parameters are needed to clarify the biological processes involved.

Acknowledgment

The authors acknowledge laboratory assisstants of pathology division in FIKKIA Unair for supporting facilities during this study.

Author Contribution

All authors participated to all aspects of this work, including preparation, research, data collecting and analysis, manuscript drafting, and publication approval.

Competing Interest

None.

Ethical Approval

The present study was approved by the Ethics Committee of Faculty of Veterinary Medicine, Universitas Airlangga.

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