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The Impact of Conventional Cigarettes, Nicotine-containing, and Nicotine-free Electronic Cigarettes on Testicular Weight of Male Wistar Rats

Hanna Tabita Hasianna Silitonga
Medicine, Ciputra Surabaya University, Indonesia
hanna.silitonga@ciputra.ac.id

Etha Rambung *
Medicine, Ciputra Surabaya University, Indonesia
etha.rambung@ciputra.ac.id
Corresponding author

Cempaka Harsa Sekarputri
Medicine, Ciputra Surabaya University, Indonesia
cempaka.harsa@ciputra.ac.id

Elizabeth Sulastri Nugraheni
Medicine, Ciputra Surabaya University, Indonesia
elizabeth.sulastri@ciputra.ac.id

Mellyanawati
Medicine, Ciputra Surabaya University, Indonesia
mellyanawati@ciputra.ac.id

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***Abstract*—**E-cigarettes are gaining increasing popularity worldwide. The World Health Organization (WHO) estimates that there are nearly one billion (984 million) smokers globally, accounting for approximately one-fifth of the adult population. The use of e-cigarettes in Southeast Asian countries has also seen a rise since 2015, although at a slower pace compared to high-income countries. Presently, Indonesia has the highest prevalence of e-cigarette use in Southeast Asia at 11.8%, while Thailand has the lowest prevalence at 3.3%. Testes play a crucial role in male reproductive function. Decreased testicular size can lead to disruptions in testicular function, resulting in infertility. Testicular size is significantly correlated with sperm density in both fertile and infertile men. The objective of this study is to investigate changes in testicular weight due to exposure to conventional cigarettes, nicotine-containing, and nicotine-free e-cigarettes. This experimental research utilizes a post-test control only design. The sample comprises 24 male Wistar rats divided into four groups: Group A (control), Group B (exposed to conventional cigarettes), Group C (exposed to nicotine e-cigarettes), and Group D (exposed to non-nicotine e-cigarettes). Exposure to cigarettes lasts for 30 days, after which the rats undergo surgery to remove their testes. The research findings indicate a decrease in testicular size due to exposure to conventional cigarettes, nicotine e-cigarettes, and non-nicotine e-cigarettes. There is no significant difference observed among the three types of exposure. In conclusion, conventional cigarettes, nicotine-containing, and nicotine-free e-cigarettes elicit similar effects in reducing testicular weight in male Wistar rats.

***Keywords*—** Conventional Cigarettes, Nicotine E-Cigarettes, Non-Nicotine E-Cigarettes, Vape, ENDS, ENNDS

I. Introduction

An electronic cigarette, commonly referred to as an e-cigarette or vape, utilizes an electronically powered coil to heat the e-liquid solution contained within the device, so that producing vapor or aerosol. There are two main types of e-cigarettes: electronic nicotine delivery systems (ENDS), also known as nicotine-containing e-cigarettes, and electronic non-nicotine delivery systems (ENNDS), also known as nicotine-free e-cigarettes (WHO, 2020; Dahal, Bhattarai and Adhikari, 2022; Ling *et al.*, 2023).

The formation and composition of aerosols during the use of nicotine-containing and nicotine-free e-cigarettes are influenced by several factors: the composition of the e-cigarette liquid, the materials utilized in the device's construction, the electronical power (wattage) employed to heat the e-liquid, and the inhale and exhale patterns of the user (WHO, 2020).

E-liquid typically contains a carrier liquid (humectant), nicotine, and flavorings. Propylene glycol and glycerol are the primary carriers in e-liquid, and they may undergo partial decomposition upon contact with the heating coil, generating various toxins, including carbonyls (WHO, 2020).

The heating coil in both nicotine-containing and nicotine-free e-cigarettes is typically composed of resistance wires made from metals like nickel or combinations of metals such as nichrome (a blend of chromium and nickel). Metal components of the device are occasionally soldered with tin (WHO, 2020).

When activated, both nicotine-containing and nicotine-free e-cigarettes utilize the electronic current from the battery to pass through the coil, heating and converting the e-liquid into aerosol. The temperature attained depends on the electronical power produced, which is contingent upon the energy supplied by the battery and the resistance of the coil. Lower resistance results in higher electronicity flow and increased heat generation within the coil. Typically, under standard operating conditions, e-liquid temperatures range between 100–350 °C (WHO, 2020).

E-cigarettes are experiencing a surge in popularity worldwide. According to the World Health Organization (WHO), there are nearly one billion (984 million) smokers globally, constituting approximately one-fifth of the adult population. This statistic has remained relatively stable since 2000. Approximately 80% of smokers reside in low- and middle-income nations, with China, India, and Indonesia collectively accounting for 46% of smokers (United Nations, 2019; Jerzyński and Stimson, 2023; Ling *et al.*, 2023).

In the United States, there has been a dramatic 900% increase in e-cigarette usage between 2011 and 2015, with approximately 11% of young adults aged 18-24 years using e-cigarettes in 2021. Additionally, 24% of 12th-grade students reported vaping in the last 30 days (Ling *et al.*, 2023; Sun *et al.*, 2024).

In contrast to high-income countries, several countries in Southeast Asia have experienced a slower increase in e-cigarette use since 2015 (Institute for Public Health, 2018; Ling *et al.*, 2023). Currently, Indonesia reports the highest prevalence of e-cigarette usage at 11.8%, while Thailand has the lowest prevalence at 3.3%. These variations could stem from the diverse e-cigarette regulations in place within these countries. For instance, Thailand imposed a ban on electronic cigarette imports in 2014, followed by a prohibition on their sale and service in 2015. Conversely, Indonesia, like some other Asian countries, has not ratified the WHO Framework Convention on Tobacco Control (FCTC) and does not ban e-cigarette sales. In Malaysia, e-cigarette regulations fall under state jurisdiction, with only four states (Johor, Kelantan, Terengganu, Pahang) prohibiting their sale (Chotbenjamaporn *et al.*, 2017; Tan and Dorotheo, 2021; Bigwanto *et al.*, 2022; Ling *et al.*, 2023). Reports indicate that e-cigarette usage among Malaysian adolescents surged by over 700%, from 1.2% to 9.8% (Institute for Public Health, 2018; Ling *et al.*, 2023).

Presently, the e-cigarette industry has set its sights on the Southeast Asian region due to its sizable smoking population and the continuous expansion of the e-cigarette market. In 2019, e-cigarette sales in six Southeast Asian countries amounted to $595 million, with projections indicating an increase to $766 million by 2023. Marketing strategies in Southeast Asia often target young people through a variety of flavors, trendy designs, and point-of-sale promotions. Despite these efforts, policy responses across Southeast Asian countries vary widely, ranging from stringent bans to minimal or partial regulations. In countries with lax e-cigarette regulations, widespread use, especially among young individuals, may occur. Although Southeast Asia holds potential for the future growth of the e-cigarette industry, research on e-cigarettes in this region remains scant (Chan *et al.*, 2021; van der Eijk *et al.*, 2022; Ling *et al.*, 2023).

There are six key factors influencing e-cigarette use: sociodemographic characteristics, childhood trauma experiences, influence of peers and parents, knowledge and perceptions about e-cigarettes, substance use, and e-cigarette accessibility (Ling *et al.*, 2023). Sociodemographic factors, such as gender, age, ethnicity, school location, academic performance, and sexual experience, play significant roles in adolescent e-cigarette use. Multiple studies consistently indicate that adolescent boys are more likely to use e-cigarettes. This trend could be attributed to male adolescents perceiving e-cigarettes as less harmful and having greater exposure to e-cigarette advertising online compared to their female counterparts (Amrock *et al.*, 2015; Richardson, Ganz and Vallone, 2015; Ling *et al.*, 2023).

Among adults aged 18 years and older, the prevalence of e-cigarette use stands at 4.5%, with the highest usage observed among individuals aged 18–24 years at 11.0% (Kramarow and Elgaddal, 2023). E-cigarette users predominantly consist of males, younger individuals, and dual users of conventional cigarettes, alcohol, and vaping marijuana. They typically perceive e-cigarettes as less harmful than traditional cigarettes and use them either to aid smoking cessation or for recreational purposes (U.S. Department of Health and Human Services, 2016; Mayer *et al.*, 2020; Patten *et al.*, 2021; Dahal, Bhattarai and Adhikari, 2022). Currently, there is no conclusive evidence regarding whether prolonged e-cigarette use among smokers (dual users) affects morbidity or mortality compared to exclusive conventional cigarette smokers. However, recent findings suggest that dual users of e-cigarettes and conventional cigarettes exhibit higher levels of oxidative stress, potentially exacerbating health risks associated with smoking (WHO, 2020).

The composition and quantity of potentially harmful substances in the aerosol emitted by ENDS/ENNDS vary significantly and are influenced by product characteristics (including device and e-liquid features) and user behavior. Nonetheless, under typical usage conditions, pure ENDS/ENNDS generally release lower amounts and concentrations of potentially toxic substances compared to tobacco smoke, except for certain metals. Metals such as chromium, nickel, and lead, along with carbonyls like formaldehyde, acetaldehyde, acrolein, and glyoxal, constitute the primary substances in aerosols that pose health risks (WHO, 2020).

The ENDS/ENNDS device itself and the vapor produced from the e-liquid have the potential to induce health issues. These impacts may manifest as neurological disorders (including disruptions in learning, mood regulation, and impulse control), respiratory disorders, cardiovascular complications (including arterial stiffness, changes in vascular endothelium, and the development of atherosclerotic plaque), and oral health ailments (such as periodontal disease, dental caries, and oral carcinoma). Chemical components present in e-liquids, such as nicotine and flavorings, are typically dissolved in propylene glycol and vegetable glycerin(Javed *et al.*, 2017; Blagev *et al.*, 2019; Irusa, Vence and Donovan, 2020; Wiener, 2023).

Infertility represents a significant public health concern, with approximately 15% of couples of reproductive age encountering fertility challenges. Male factor infertility contributes to around 30 to 50% of these cases (Lee *et al.*, 2023). The causes of male infertility encompass various factors, broadly categorized based on common underlying etiologies. These include endocrine disorders, predominantly hypogonadism, accounting for an estimated 2% to 5% of cases; sperm transport disorders, such as vasectomy, affecting around 5% of cases; primary testicular defects, encompassing abnormal sperm parameters without identifiable causes, observed in 65% to 80% of cases; and idiopathic cases, where infertile men exhibit normal sperm and semen parameters, occurring in 10% to 20% of cases (Leslie, Soon-Sutton and Khan, 2024). Furthermore, numerous risk factors contribute to male infertility, spanning biological, behavioral, environmental, and socio-demographic domains. Biological factors linked to male infertility include genetic factors, urogenital infections, and varicocele. Behavioral factors encompass habits like smoking, alcohol consumption, inappropriate body mass index, sexual behavior, and drug exposure. Environmental factors that can lead to male infertility include exposure to chemicals, various pesticides, and mycotoxins. Increasing age is a socio-demographic factor frequently associated with male infertility (Okonofua *et al.*, 2022).

Male infertility often arises from deficiencies in semen parameters, highlighting the pivotal role of the testes in sperm production. Within the testes, the seminiferous tubules, spanning over 80% of their size, are primarily responsible for this intricate process. Hence, the size of the testicles bears a direct relationship with their function in sperm production. The measurement of testicular size stands out as a straightforward, rapid, and non-invasive procedure. This assessment holds profound significance as deviations from typical dimensions have been observed in a significant portion of men experiencing infertility, accounting for approximately 64% of cases. Notably, individuals grappling with infertility and oligospermia, characterized by a low sperm count, typically exhibit reduced testicular volume. Moreover, the size of the testicles serves as a valuable indicator of the severity of infertility, showcasing a noteworthy correlation with sperm density in both fertile and infertile men. Therefore, the meticulous measurement of testicular size represents a critical parameter in the initial evaluation of male infertility (Tijani *et al.*, 2014; Bellurkar *et al.*, 2020).

This study endeavors to explore alterations in testicular weight among male Wistar rats following exposure to conventional cigarettes, as well as nicotin-containing and nicotine-free e-cigarettes. Understanding the effects of conventional cigarettes, as well as nicotin-containing and nicotine-free e-cigarettes on testicular weight could provide valuable insights into its role in male infertility, paving the way for more informed approaches to mitigate its adverse effects.

II. Methods

This study is an experimental laboratory investigation employing a post-test only control group research design. Prior to commencement, ethical clearance was obtained from the Health Research Ethics Commission, Faculty of Medicine, Ciputra University (Certificate No. 025/EC/KEPK-FKUC/VIII/2022).

In terms of equipment, the research utilized a rat cage, an aquarium smoking chamber, an aquarium filter, a 20cc syringe, drinking bottles, a rat food container, electronic scales, and hand scoons. The materials employed in the study comprised mice, vape devices, clove cigarettes, pellet food, mineral water, and ketamine.

The research subjects were male Rattus norvegicus of the Wistar strain, meeting specific inclusion criteria: aged between 2.5 and 3 months, weighing between 150 and 250 grams, and exhibiting good health. Exclusion criteria encompassed the absence of diseases, anatomical abnormalities, physical injuries, and pre-existing death prior to or during treatment. A total of 24 rats were utilized, divided into 4 groups, each comprising 6 individuals. Simple random sampling was employed for randomization purposes.

The study consisted of four treatment groups: Group A received no treatment, Group B was exposed to conventional kretek cigarette smoke containing 2.2 mg of nicotine per stick, Group C was exposed to vape mod smoke containing liquid with a nicotine content of 9 mg, and Group D was exposed to vape mod smoke with nicotine-free liquid. Smoke exposure was administered over a period of 30 days, following which termination and surgery were conducted to extract testicular samples.

Data collected underwent processing and analysis utilizing the Statistical Package for the Social Sciences (SPSS).

III. Results and Discussion

In this study, it was concluded that there were changes in the average testicular weight of rats after exposure to conventional cigarettes, nicotin-containing and nicotine-free e-cigarettes, compared to the control group. Exposure to each type of cigarette resulted in a decrease in the average testicular weight of rats, but the lowest testicular weight occurred with exposure to conventional cigarettes compared to the control group, as seen in Figure 1.

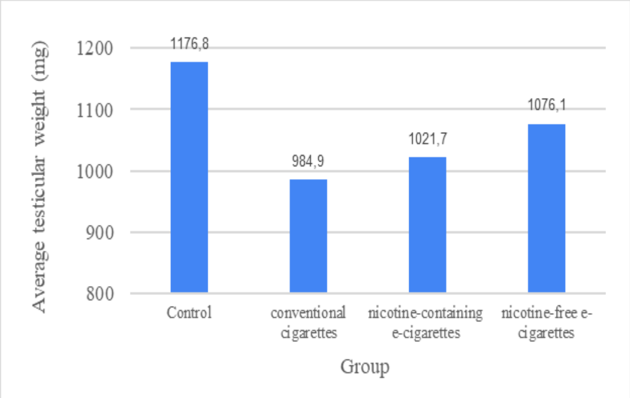

Figure 1. Average testicular weight after exposure to cigarette smoke

The evaluation results of data homogeneity and normal distribution indicate that the data exhibit homogeneity and normal distribution, thus ANOVA test is employed to assess the significance of the data. The ANOVA test results indicate significant differences in the obtained data, as presented in table 1.

Table 1. Homogeneity and normality test of testicular weight data

Group	n	Saphiro-Wilk Test	Homogenates Test	*ANOVA*
A	6	0.541	0.614	0.002
B	6	0.190		
C	6	0.181		
D	6	0.128		

After conducting post LSD statistical analysis, it was found that there were significant differences in the average changes in rat testicular weight due to exposure to conventional cigarettes, nicotine-containing electronic cigarettes, and non-nicotine e-cigarettes compared to the control group. The research results also revealed that there were no statistically significant differences in the average changes in rat testicular weight due to exposure to conventional cigarettes, electronic cigarettes whether containing nicotine or not, compared to the control group, as seen in table 2. This indicates that the effect of exposure to electronic cigarettes is not significantly different from exposure to conventional cigarettes in terms of changes in rat testicular weight. However, both types of cigarette exposure showed significant differences compared to the control group.

Table 2. Post Hoc LSD Test Results testicular weight (mg)

Group	A	B	C	D
A	-	0. 000*	0.002*	0. 033*
B	0. 000*	-	0. 415	0. 415
C	0.002*	0. 415	-	0. 232
D	0. 033*	0. 052	0. 232	-

*Significantly different ($p < 0.05$)

The results above indicate that conventional cigarettes, nicotine-containing electronic cigarettes and non-nicotine e-cigarettes have the same impact. All three types of cigarettes can lead to a decrease in rat testicular weight. This is possible due to the substances contained within them.

The conventional cigarettes and nicotine-containing e-cigarettes utilized in this investigation contain nicotine components. Nicotine, being a toxic alkaloid, swiftly enters the brain within about 10 seconds. It traverses the blood-brain barrier and disperses across brain cells, subsequently circulating throughout the body within 15-20 minutes after the last inhalation. Nicotine present in cigarette smoke triggers the adrenal medulla to release catecholamines, impacting the central nervous system and disrupting the feedback mechanism involving the hypothalamus, anterior pituitary, and testes. Consequently, the synthesis of testosterone hormone and spermatogenesis is disturbed (Putra, 2014). The reduction in sperm production leads to a decrease in the diameter of the seminiferous tubules, which, being the primary component of testicular mass, contributes to the reduction in testicular weight (Sripratiwi, 2019). This corroborates Iring's findings (2023) indicating that exposure to conventional cigarettes and e-cigarettes results in a decline in the diameter and thickness of testicular seminiferous tubules (Iring, Rambung and Winarso, 2023).

The presence of nicotine in cigarette smoke, whether from e-cigarettes or conventional cigarettes, prompts an escalation in reactive oxygen species (ROS). Elevated ROS levels instigate cellular, tissue, or organ damage within the body (Tooy, Tendean and Satiawati, 2016b). When ROS attack the lipid membrane, it leads to lipid peroxidation (LPO). This process results in nicotine targeting unsaturated fatty acids to form acid peroxides. These peroxides react with fat molecules, inducing oxidation on the spermatozoa membrane. The extensive oxidation reactions due to high levels of unsaturated fatty acids result in damage to the spermatozoa membrane. Ultimately, lipid peroxidation produces malondialdehyde (MDA), a mutagenic compound that indirectly damages DNA (Fitria *et al.*, 2013; Pramudita *et al.*, 2020). Furthermore, heightened ROS formation triggers apoptosis, leading to an increase in pro-apoptotic cytokines and subsequently causing elevated sperm DNA damage, disrupted spermatogenesis, and decreased sperm production, ultimately resulting in testicular organ degeneration (Tooy, Tendean and Satiawati, 2016a; Omolaoye *et al.*, 2021). Exposure to cigarette smoke induces alterations such as smaller seminiferous tubules, tubular atrophy and degeneration, as well as a reduction in the spermatogenic cell layer, culminating in decreased testicular weight (Iring, Rambung and Winarso, 2023).

Additionally, the constituents found in cigarette smoke, specifically Polynuclear Aromatic Hydrocarbons (PAH), contribute to testicular atrophy, impede spermatogenesis, and compromise sperm morphology. Testicular atrophy primarily affects the seminiferous tubules, the predominant structures comprising the testes. Should these tubular cells incur damage or atrophy, disruptions in the spermatogenesis process may arise, resulting in diminished sperm quality and a reduction in testicular weight (Kurniati and Nugraheni, 2019). Sripratiwi's investigation reveals that a decline in sperm production leads to a reduction in the diameter of the seminiferous tubules. This reduction subsequently influences testicular weight, given that the seminiferous tubules constitute the bulk of the testes' mass (Sripratiwi, 2019). These substances, particularly nicotine found in both conventional cigarettes and nicotine-containing e-cigarettes, likely contributed to the observed decrease in testicular weight among mice in this study.

Even though nicotine-free e-cigarettes are marketed as a safer alternative, they are not without risks. While they may lack nicotine, they still contain a plethora of chemicals, flavorings, and solvents, all of which can pose significant health hazards. Research suggests that these ingredients have not been proven safe for inhalation, indicating potential dangers associated with their use (Gaiha *et al.*, 2022). Among the ingredients found in nicotine-free e-cigarettes are propylene glycol, vegetable glycerin, aldehydes, volatile organic compounds, polycyclic aromatic hydrocarbons, metals, silicate particles, and various heavy metals (Pisinger and Døssing, 2014; Gaiha *et al.*, 2022). These ingredients are included in the list of dangerous and potentially harmful chemicals in tobacco products issued by the US Food and Drug Administration (FDA) (Esteban-Lopez *et al.*, 2022; Gaiha *et al.*, 2022). Aldehydes, for instance, have been linked to an elevated risk of cancer, while flavoring agents have been associated with inflammation and cellular dysfunction (Fetterman *et al.*, 2018; Muthumalage *et al.*, 2018; Gaiha *et al.*, 2022). Additionally, propylene glycol and vegetable glycerin have been shown to disrupt cellular metabolism and airway function (Ghosh *et al.*, 2018; Woodall *et al.*, 2020; Gaiha *et al.*, 2022). It's noteworthy that heating glycerol, a common component in e-cigarette liquids, can produce acrolein, a constituent found in tobacco smoke (Alawam, 2020). Moreover, both nicotine-free and nicotine-containing e-cigarettes have been implicated in causing oxidative stress, heightened inflammation, and damage to blood vessels, akin to the injuries observed in individuals with lung injuries (Chichger *et al.*, 2024).

Exposure to nicotine-free electronic cigarettes in this study may alter testicular weight due to the chemicals inducing oxidative stress. This results in structural disturbances in testicular tissue and reduces the weight of rat testes.The findings of this research indicate that there is no substantial variance in the impact of traditional cigarettes, nicotine-containing and nicotine-free e-cigarettes. These results are anticipated to assist governmental bodies in formulating policies to oversee the availability, accessibility, and promotion of ENDS/ENNDS, particularly concerning the younger demographic, including children and adolescents, who represent the future of the nation. Given that children and teenagers are at heightened vulnerability to the repercussions of traditional cigarettes as well as e-cigarettes, whether containing nicotine or not, there exists a possibility of imposing usage restrictions on this demographic. Such measures are deemed necessary to safeguard the health and well-being of the youth population, given their susceptibility to the adverse effects associated with tobacco and electronic nicotine products. By considering the potential risks posed by these products, regulatory interventions could be devised to mitigate their impact on the younger generation, thereby fostering a healthier environment for their growth and development (Yoong *et al.*, 2021). The mechanism for reducing testicular weight that may occur due to exposure to conventional cigarettes, nicotine- containing and nicotine-free e-cigarettes can be seen in figure 2.

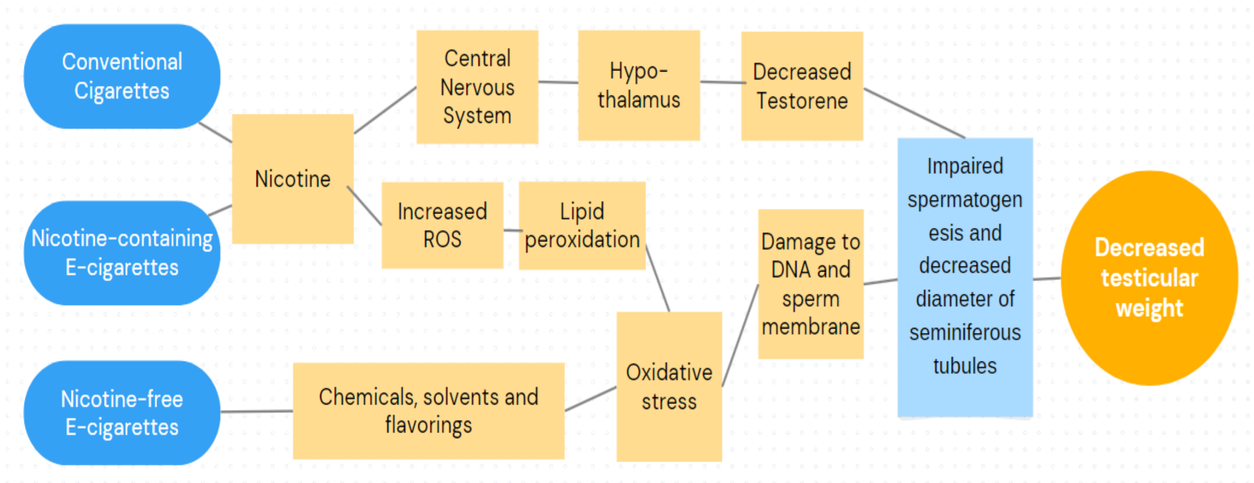

Figure 2. The mechanism for reducing testicular weight that may occur due to exposure to conventional cigarettes, nicotine- containing and nicotine-free e-cigarettes

IV. Conclusion

Conventional cigarettes, e-cigarettes with and without nicotine caused a decrease in testicular weight in mice which was significantly different from the control group. The decrease in testicular weight in mice due to exposure to conventional cigarettes, e-cigarettes and without nicotine was not significantly different. This may be because the ingredients contained in conventional cigarettes, nicotine and non-nicotine e-cigarettes can cause oxidative stress so that cells become atrophic and cause a decrease in testicular weight. This decrease in testicular weight will affect testicular function so that it can cause a decrease in spermatozoa production and cause infertility in men. It is hoped that these results can help the government in making policies that regulate the availability, accessibility and marketing of ENDS/ENNDS, especially for children and teenagers.

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