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Meta-analysis

Difference Of Fetal Weight Between Conventional vs Electric Smoke Exposure in Mice (*Mus Musculus*)

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ABSTRACT

Background: The increase in tobacco excise rates (CHT) or cigarette excise in early 2020 is a reason for smokers to switch to using e-cigarettes. WHO reports that about 64.9% of adult men in Indonesia are smokers, while in women it is 2.1%. This shows that Indonesia is ranked first in the world. Exposure to e-cigarettes and conventional cigarettes will affect pregnancy and the fetus it contains. However, in reality, most people have the perception that e-cigarettes are safer than conventional cigarettes because their nicotine content is much lower than conventional cigarettes. The purpose of this study was to analyze differences in exposure to e-cigarettes and conventional cigarettes on fetal weight in mice (mus musculus).
Subjects and Method: This study used a true experimental design which was divided into two groups, namely 16 female mice as a group given exposure to e-cigarettes and 16 female mice as a group being exposed to conventional cigarettes. The dependent variable is the weight of the fetus. The independent variables were exposure to e-cigarettes and conventional cigarettes. The instrument in this study used a digital gold scale with an accuracy of 0.01 gram. Data were analyzed using the Mann Whitney test.
Results: On average, exposure to e-cigarettes had a greater fetal weight (Mean= 1.61; SD= 0.64) than exposure to conventional cigarettes (Mean= 1.48; SD= 1.19), and this result was statistically significant ($p < 0.001$).
Conclusion: There are differences in exposure to e-cigarettes and conventional cigarettes on fetal weight in mice (Mus musculus).

Keywords: E-cigarettes, Conventional cigarettes, Fetal weight, Mice (Mus Musculus)

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BACKGROUND

Smoking is one of the biggest threats to health that has become a habit that is difficult to break. According to data from WHO (2017), it is reported that around 64.9% of adult males in Indonesia are smokers, while in females it is 2.1%. The number of smokers in Indonesia is the number one smoker in the world with a percentage of about 57% of whom are men. The second place after Indonesia is Papua New Guinea, Russia and China (Kemenkes RI, 2018; Atlas, 2015).

Many conventional smokers have switched to using e-cigarettes due to the increase in tobacco product excise rates (CHT) in early 2020. Initially, e-cigarettes were designed as an effort to help tobacco smokers to stop smoking slowly because the content in e-cigarettes consists of a mixture of water, propylene glycol, flavor enhancers, tobacco aroma, and other compounds that do not contain tar, tobacco or other substances. other toxic substances that are not found in conventional cigarettes (Brown et al., 2020; Blount et al., 2020).

E-cigarette users increased by 78%, high school teens and 48% junior high school teens in one year from 2017 to 2018. EVALI cases reported by the CDC (2020) occurred in 48 states in the United States. Approximately 70% of the 889 EVALI patients were male consisting of 80% aged <35 years, 16% aged <18 years, and 21% aged 18-20 years. Patients using e-cigarettes were reported to have died from 15 states, namely 18 patients out of 578 patients with an age range of 27 to 71 years. The patient was reported to have used e-cigarettes for three months before developing symptoms. The United States has designated this disease as an epidemic and this has resulted in a number of states in the United States banning the circulation of e-cigarettes (Cullen et al., 2018; Nancy, 2019; Siegel, 2019).

E-cigarettes currently circulating contain nicotine which is an addictive substance and can affect brain development, increase the frequency of coughing and wheezing, heart rate, and asthma exacerbations. At a sufficiently high level of exposure, e-cigarettes can cause carcinogenic substances and other toxic substances that can cause inflammation and irritation. E-cigarette smoke tends to cause more complex respiratory problems than smoke from tobacco cigarettes (Siegel, 2019; Eaton, 2018).

Other chemicals found in e-cigarettes, namely cigarette aerosols, such as formaldehyde and acrolein. Both of these substances can cause DNA damage, which can cause health problems and have long-term risks, namely cancer (Eaton, 2018). In some trademarks e-cigarettes contain formaldehyde that exceeds the recommended limit for humans. Research conducted by the Public Library of Science Journal shows that the vapors in some brands of e-cigarettes contain high levels of benzene. According to research conducted by dr. Nauki Kunugita in one of the e-cigarettes was found to have 10x higher levels of carcinogens than one cigarette of tobacco (Bekki et al., 2014).

E-cigarettes can also cause burns as a result of exploding e-cigarettes in the user's mouth. Based on the problems that arise from e-cigarettes, the authors are interested in conducting research on "Comparison of Effects of Exposure to E-cigarettes and Conventional Cigarettes on Fetal Weight in Mice (*mus musculus*)".

SUBJECTS AND METHOD

1. Study Design

This was a randomized controlled trial. The sample was divided into two groups, namely the group given exposure to e-cigarettes and the group given exposure to conventional cigarettes. This study was conducted to determine the difference between exposure to e-cigarettes and conventional cigarettes on fetal weight in mice (*mus musculus*).

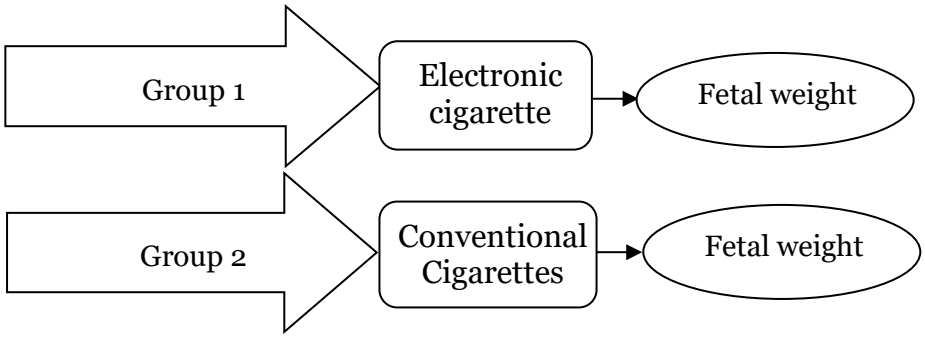

Figure 1. Research Design

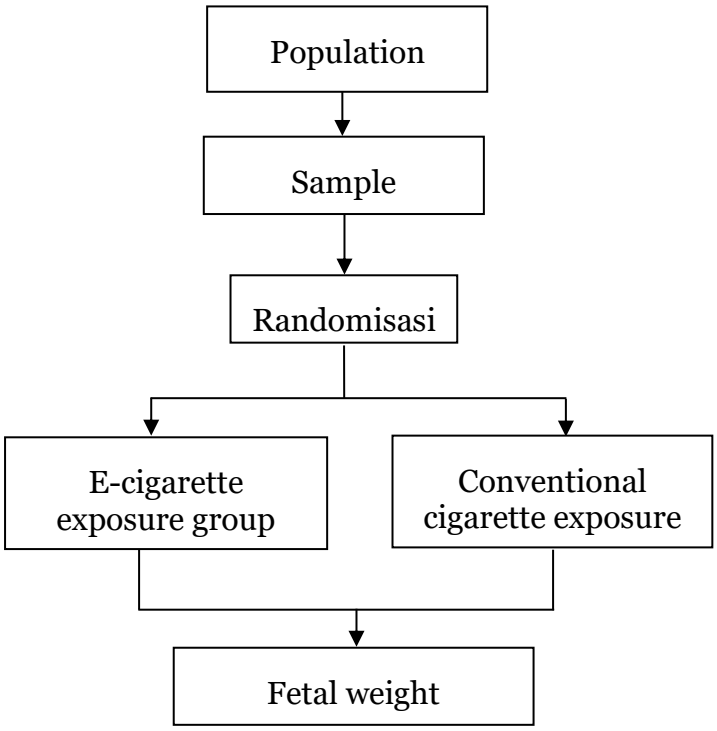

Figure 2. Research Flow

This study was conducted in a rental house in the Bugel area, Karawaci, Tangerang, from June 7, to August 18, 2021. The temperature and humidity of the rental house during maintenance were under controlled conditions.

2. Population and Sample

The population and samples used in this study were female mice (mus musculus) of the DDY strain 2-3 months old and weighing 25-30 grams. The sample in this study used 32 female mice selected at random and then divided into 2 groups, namely 16 female mice as treatment group 1 (P1) were given exposure to e-cigarettes and 16 female mice as treatment group 2 (P2) were given exposure to conventional cigarettes.

The sampling technique was carried out using random sampling. The inclusion criteria in this study were female mice that were not pregnant and healthy. The exclusion criteria in this study were sick and dead female mice. The technique in taking the sample uses random sampling with the federer formula.

The samples in this study were 32 samples which were divided into 2 groups, namely 16 mice exposed to e-cigarettes and 16 mice exposed to conventional cigarettes. In this study also used 9 male mice aged 2-3 months weighing 25-35 grams for natural mating with female mice.

Preparation phase. The mouse cage was in the form of a litter box measuring 36 x 26 x 12 cm with a ram wire cover which was given a husk base to absorb mouse droppings. The cages were divided into breeding tanks and rearing tanks for each treatment group. Feed mice using Hi-Pro-Vite CP 512B which is high in protein, carbohydrates, fats, minerals and vitamins. Drinking water for mice, which is refilled water that is available at any time by using a bottle with a hole with an aluminum pipe. Data was collected by adapting (acclimatizing) mice for 1 month and the last day of the adaptation process being given worm medicine (Kaufman, 1992). The following is a picture of the adaptation process of mice.

Figure 3. Mice adaptation process

Stage of exposure to cigarette smoke. Female mice were exposed to e-cigarettes and conventional cigarettes for 15 days and separated between 16 female mice in cages labeled with e-cigarettes and 16 female mice in cages labeled with conventional cigarettes. Conventional cigarettes used are mild types of cigarettes, namely Sampoerna Mild with tar content of 14 mg and nicotine 1.0 mg which is given 1 stick/day. Liquid in e-cigarettes with 9 mg nicotine levels exposed for 15 minutes/day. The following are the stages of exposure to e-cigarette smoke and conventional cigarettes.

Figure 4. Mice with electric cigarette exposure

Figure 5. Mice with conventional cigarettes exposure

Mating stage. Female mice were in estrus in a cage with male mice in a mating cage for 7 days with a ratio of 4 female mice and 1 male mouse in 1 cage with their respective labels. If the next day there is a vaginal plug or residual sperm in the vagina of female mice, then that day is day 0 of pregnancy. Then separated in a separate cage during pregnancy. The mouse cage used was a plastic box cage measuring 40 x 30 x 18 cm for 5 animals (1 male and 4 female) lined with wood shavings. The frequency of changing the cage mat is done 2-3 times a week.

Figure 6. Mice adaptation process

Observation stage. At the age of 18 days of gestation, the mice were subjected to neck dislocation (euthanasia). Then surgery was performed from the lower abdomen and the right and left fetuses were isolated. The isolated fetuses were then cleaned of

adhering tissues and washed with NaCl. Fetuses that have been cleaned are weighed one by one. Here are pictures of the right and left fetuses that have been isolated.

Figure 7. Right and left fetuses

3. Study Variables

The dependent variable is reproductive health. The independent variables are electronic and conventional cigarettes.

4. Operational Definition of Variables

Fetal weight is the weight of the fetus on the 18th day of gestation in the right and left uterus in female mice. The measuring instrument uses a digital gold scale with an accuracy of 0.01 grams. The measurement results are in the form of fetal weight in grams with a numerical measurement scale.

Exposure to e-cigarettes is exposure to modern cigarettes in the form of electronic devices that function to convert chemical substances which are a mixture of substances, such as nicotine and propylene glycol into vapor and drain it into the lungs. Exposure to conventional cigarettes is exposure to cigarettes made from dried tobacco leaves containing carbon monoxide (CO), tar, and nicotine consumed by burning at the base and then sucking it through the oral cavity.

5. Study Instruments

The instrument in this study uses a digital gold scale with an accuracy of 0.01 gram to measure the weight of the fetus.

6. Data analysis

Univariate analysis in this study describes the characteristics of the research sample, namely fetal weight. Bivariate analysis used statistical tests, namely the Mann Whitney test because the measuring scale used was numerical, there were two unpaired groups, and the distribution was not normal.

7. Research Ethics

This research has passed the ethical test by the research ethics committee of STIKes Yatsi Tangerang, No.071/LPPM-STIKES YATSI/IV/2021 on June 2, 2021.

RESULTS

1. Sample Characteristics

The univariate description of the study variables explains the general description of the research data for each research variable,

including exposure to e-cigarettes, exposure to conventional cigarettes, and fetal weight of mice.

The results of the description of e-cigarettes showed that there were 32 mice, 16 mice (50%) were given an electric cigarette exposure intervention (50%) and 16 mice (50%) were given conventional cigarette exposure interventions (Table 1).

Table 1. Description of Cigarette Exposure Variables

Intervention	Frequency (n)	Percentage (%)
Exposure		
Electronic cigarette	16	50 %
Conventional cigarettes	16	50%

2. Bivariate Analysis

The mean fetal weight of female mice exposed to e-cigarettes (Mean= 1.61; SD= 0.64) was greater than that of conventional cigarettes (Mean= 1.48; SD= 1.19), and this result was statistically significant (p< 0.001) (Table 2).

Table 2. Mann-Whitney Test Effect of Exposure to E-Cigarettes and Conventional Cigarettes on Fetal Weight in Female Mice

Variable Group	n	Mean	Median	SD (min-max)	p
Fetal weight					
Electronic cigarette	15	1.61	1.54	0.64 (1.52-1.69)	<0.001
Conventional cigarettes	15	1.48	1.43	1.19 (1.16-1.60)	

DISCUSSION

The results in the study showed that there was a significant difference between the e-cigarette treatment group and the conventional cigarette treatment group. In the e-cigarette treatment group, the fetal weight was heavier than the group given conventional cigarette treatment. In the e-cigarette treatment group, the mean value was 1.61, while the conventional cigarette was 1.48. The median value of fetal weight given exposure to e-cigarettes is 1.54, while conventional cigarettes are 1.43. The standard deviation of exposure to e-cigarettes is 0.64 and conventional cigarettes is 1.19. The significance value in this study was <0.001, so there was a difference between exposure to e-cigarettes and conventional cigarettes on fetal weight.

According to Febriyeni (2010), cigarette smoke contains more than 7000 chemicals and compounds found in tobacco, namely nicotine, hydrogen cyanide, formaldehyde, arsenic, ammonia, benzene, carbon monoxide (CO), carbon dioxide (CO_2), and nitrosami. These chemicals can cause various diseases and health problems, including reproductive health will also be disrupted (American Cancer Society, 2019). One of the reproductive health disorders is the disruption of hormone production which can cause women to find it difficult to get pregnant, impaired fetal growth and development, impaired oxygen to the fetus, and can even cause miscarriage during pregnancy. Disruption of hormone production in the body occurs due to decreased levels of the hormones estrogen and progesterone which results in the reproductive performance of female mice not running optimally (Febriyeni, 2010). Exposure to cigarette smoke also results in a high risk of ectopic pregnancy, failure in IVF because it affects the ovaries, oocyte count, fertilization process, and implantation of the placenta. Mothers and babies who are exposed to cigarettes during and after pregnancy will also be at risk of sudden infant death syndrome (SIDS)

(American Cancer Society, 2019). Factors that influence the impact of cigarette smoke, which depend on the dose, time, and type of exposure (Dechanet et al., 2011).

Exposure to cigarette smoke which contains a mixture of toxic compounds that continuously to mice breeders causes the number of free radicals that enter the body exceeds the number of endogenous antioxidants produced by the body. The content contained in cigarette smoke, such as nicotine, CO and CO2 causes contraction of blood vessels. This will affect the blood flow from the fetus through the fetal umbilical cord in distributing nutrients. Nicotine that enters the bloodstream, either directly or indirectly, will inhibit the process of cell division. This can lead to failure of embryo implantation due to poor uterine conditions and damaged cells due to the administration of chemical compounds contained in cigarettes. Implantation failure can occur because the fertilized zygote cannot reach the uterus (Lovita et al., 2014). This is because the toxic substances found in cigarette smoke cause oxidative stress, thereby reducing reproductive quality (American Cancer Society, 2019).

The reproductive system is a process to produce offspring in order to maintain the survival of a living thing. Reproductive organ disorders are one of the health problems that can occur in human life. One of the external factors that can affect reproductive disorders is caused by cigarette smoke which can cause various types of diseases both in active smokers and passive smokers (American Cancer Society, 2019). Exposure to cigarette smoke can also cause a decrease in fetal weight. The decrease in body weight and length is the mildest form that occurs due to the effect of teratogenic agents and is a sensitive parameter.

Disorders of fetal development in the uterus cause abnormalities, including births with abnormal body weights. Body weight is an important parameter to determine the effect of foreign compounds on the fetus. The decrease in fetal weight is thought to be due to exposure to foreign compounds that enter the body. The growth and development of the fetus will affect the size of the tiller. Factors that affect fetal weight, namely the number of glomeruli, the presence of fat degeneration and hydropic degeneration. Foreign compounds that enter the body cause parts of the cell to lose volume regulation, so that the cell is disturbed. Changes in the state of cells will cause changes in organ weights. Disruption of energy metabolism in cells makes cells unable to pump sodium ions, so that cell size will change. Another factor that can cause changes in cell size is the presence of lipid deposits in cells, so that cells experience swelling (Widyaningsih et al., 2018; Santosa et al., 2011).

E-cigarettes have emerged since mid-2003 as an alternative in overcoming addiction to conventional cigarettes. In terms of the price of e-cigarettes, they are cheaper than conventional cigarettes, which in early 2020 experienced an increase in cigarette excise prices. They consider that e-cigarettes have less risk than conventional cigarettes because they do not burn tobacco. However, in reality e-cigarettes will also interfere with respiration and endanger health, including male and female reproductive health, although the effect for e-cigarettes is lower than conventional cigarettes (Stanton et al., 2018). This e-cigarette has a device consisting of a battery, an electric heating element (atomizer) and a cartridge that can be replaced or refilled using e-liquid. This e-liquid contains propylene glycol and/or glycerol, water, food flavours, nicotine, and other chemicals. Liquid (e-liquid) will turn into an aerosol when an e-cigarette is activated and inhaled into the lungs. E-liquid in e-cigarettes has many variants of

fruit flavors, candy, and refreshments, so they are more delicious to consume (World Health Organization, 2019). The nicotine content in e-cigarettes ranges from 0-18 mg per 1 ml of e-liquid, such as propellin glycol, glycerin, and natural or artificial flavors. Consumption for 1 pc of e-liquid cartridge (150 puffs) in e-cigarettes is comparable to 10 conventional cigarettes (Bahri et al, 2015; Prochnow, 2017).

AUTHOR CONTRIBUTION

Nurry Ayuningtyas Kusumastuti, Siti Haeriyah, and Yuni Susilowati developed a research design, providing exposure to electronic and conventional cigarette smoke in a group of mice, measuring fetal weight of mice, analyzing data, and writing a publication manuscript.

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CONFLICT OF INTEREST

There was an Emergency PPKM due to the increasing number of COVID-19 cases in May to July, so the research was not possible to be carried out in the STIKes Yatsi laboratory. The implementation location is carried out in a suitable rental house. Another obstacle due to emergency PPKM is that the coordination between team members is carried out online because it is not possible to do it offline, except for some things that cannot be done online, such as surveys of test materials.

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