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Teratogenic effect of electric mosquito repellents on mouse (*Mus musculus*) fetuses

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Abstract

Introduction: This study aims to determine the teratogenic effects of electric mosquito repellents on mouse (*Mus musculus*) fetuses. The electric mosquito repellent used contains the active ingredient dimefluthrin 0.566% (4.2 mg/matt) which was inhaled into pregnant mice during the organogenesis period of 6-15 days of gestation.

Material and Methods: A total of 30 pregnant mice were divided into five groups with six repetitions, namely C: Control group (without exposure to electric mosquito repellent), T1: exposed to electric mosquito repellent for 4 hours/day, T2: exposed to electric mosquito repellent 6 hours/day, T3: exposed to electric mosquito repellent 8 hours /day, and T4: exposed to electric mosquito repellent 10 hours/day. Parameters tested including fetal weight and length were analyzed using One-Way Anova and then continued with Duncan test. Live fetuses (%), dead fetuses (%), and morphological abnormalities (%) were analyzed using the Kruskal-Wallis test and continued with the Mann-Whitney test.

Results: This study found that there were no gross morphological abnormalities, however hemorrhage was found on the skin surface. The results showed that there were significant differences ($p<0.05$) in the weight and length of the fetus, live fetus (%), dead fetus (%), and hemorrhage (%).

Discussion: The highest rates of fetal death and hemorrhage were shown in the T4 treatment (exposed to electric mosquito repellents 10 hours/day). Dimefluthrin enters the body of pregnant mouse as a substance that forms reactive oxygen species (ROS). Blood vessel cells that are exposed to ROS and have oxidative stress will be damaged and even die so that the blood vessels become brittle and break easily.

Keywords: Electric mosquito repellent; Dimefluthrin; Mouse fetus; Teratogenic; Pesticide stress

1. Introduction

Indonesia is a tropical country which makes it a good place for mosquitoes to breed [1]. High rates on mosquito breeding encourage people to overcome the problem and drive the mosquitoes away, one of which is by using mosquito repellents that contain insecticides with several chemical compounds [2]. Insectides are classified as pesticides that is included as toxic substances [3].

Mosquito repellent contains active ingredients such as d-allethrin, transfluthrin, bioallethrin, dimefluthrin, pralethrin, dphenothrin, cypenothrin or esbiothrin, which are derivatives of pyrethroids [4]. Pyrethroid chemical substances are classified as organic insecticides that can cause immobilization of insects by poisoning the nervous system. Mosquito

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repellents available at the market are spray mosquito repellents, mosquito coils, lotion mosquito repellents, or electric mosquito repellents [5].

Excessive use of anti-mosquito insecticides will be harmful if it is not balanced with sufficient knowledge. The negative impacts can affect humans, the environment, and the target insects themselves. Previous research conducted by Almahdy et al. [2] and Rahayuningsih [5] stated that exposure to mosquito coils containing transfluthrin to pregnant mice could lead to abnormalities on its fetuses and a decrease in body weight of the pregnant mice. This shows that mosquito repellents have a negative impact when exposed to pregnant individuals, because pregnancy is a period that is sensitive to the effects of chemical compounds [6].

Mice as experimental animals can be used for teratogenesis test, which is a test to obtain information on fetal abnormalities that occur due to exposure to mosquito repellents during organ formation [7]. Based on a research by Almahdy et al. [2] and Rahayuningsih [5], the researchers are interested in conducting further research on the teratogenic effects of other mosquito repellent preparations with the active ingredient dimefluthrin on body weight, body length, and morphological abnormalities of the mouse (*Mus musculus*) fetus. Inhaling insecticides continuously causes toxicity and leads to hematological, biochemical, cytokine disturbances and mutagenic damage to tissues [8].

2. Material and methods

This study used 30 pregnant mice aged 2-3 months with body weights ranging from 25-30 grams [2] obtained from the Veterinary Farma Center (PUSVETMA) Surabaya, Indonesia.

2.1. Ethics approval

This research has received ethical approval from the animal ethics commission, Faculty of Veterinary Medicine, Airlangga University No: 1.KE.127.11.2021.

2.2. Material

The tools used in this study consisted of 5 experimental animal cages measuring 45 cm x 30 cm x 15 cm, consisting of 2 rooms partitioned off with wire, places to eat and drink, disecting kits, gloves, petri dishes, opaque paper, surgical board, and pins, scales, millimeter block paper, loupe, tweezers and camera. The materials used in this study consisted of an electric mosquito repellent with the active ingredient dimefluthrin 4.2 mg/pcs, PMSG 5 IU and hCG 5 IU, and alcohol 70, feed and mineral water, and sawdust.

2.3. Animal mating

The experimental animals were adapted for seven days before mating. The estrus synchronization was carried out with female mice aged 3 months with a body weight of 25-30 grams, injected with PMSG 5 IU 0.1 cc, 48 hours later injected with hCG 5 IU 0.1 cc intraperitoneally. Female mice in estrus were mated with male mice aged 5 months with a ratio of 1:1 in the afternoon and separated the next morning. In the morning a vaginal plug examination was performed. Vaginal plug indicates that the mice have had copulation and are on day 1 of gestation.

2.4. Exposure to electric mosquito repellents

Thirty pregnant mice were divided into 5 groups and each group consisted of 6 replicates. The groups were divided as follows: control group (C) (without exposure to electric mosquito repellents), T1 (exposure to electric mosquito repellents for 4 hours per day), T2 (exposure for 6 hours per day), T3 (exposure for 8 hours per day), and T4 (10 hours of exposure per day). Exposure began on the 6th to 15th day of gestation or at the organogenesis stage. Pregnant mice were exposed to electric mosquito repellents by inhaling Whole body in a special glass box measuring 38 cm x 28.5 cm x 22.5 cm, equipped with a ventilation connected to an electric mosquito repellent [9].

2.5. Mice Surgery

On the 18th day of gestation, the mice were killed by cervical dislocation. The surgery was carried out by caesarean technique, that is dividing the abdomen in an upward direction so that the uterus containing the fetus was visible. The fetus was obtained by cutting the placenta in the uterus. The placenta and mucous membranes covering the fetuses were then cleaned.

2.6. Fetal Examination

The fetus which had been removed from the uterus was then observed whether it was alive, dead, or underwent resorption. External abnormalities in the fetus were observed using a magnifying glass on the outer body part of fetus. Fetal body weight was measured with an analytical balance. The length of the fetus was measured with a ruler in centimeters, the measurement began from the forehead to the base of the tail by projecting the length of the fetus' body on millimeter block paper. Documentation was carried out with a camera.

2.7. Statistical analysis

The research data were analyzed statistically using the analysis of variance (ANOVA) method, and if there was a significant difference between the treatments, Duncan Multiple Range Test (DMRT) was carried out to obtain quantitative data including fetal body weight and fetal body length. Data on live fetuses, dead fetuses, and morphological abnormalities were analyzed using the Kruskal-Wallis test and if there was a significant difference, the Mann-Whitney test would be performed.

3. Results

3.1. Live and dead fetuses

The mean of live fetuses and dead fetuses were analyzed using the Kruskal-Wallis test and followed by the Mann-Whitney test to see differences between groups. Statistical tests showed significant differences in the mean of dead fetuses and live fetuses between groups ($p<0.05$). The mean and standard deviation results of live and dead fetuses are presented in Table 1.

Table 1 The mean of live fetuses and dead fetuses in various treatment groups in pregnant mice exposed to electric mosquito repellents from the 6th to the 15th day of gestation

Tretment	Number of Pregnant Mice	Number of implant-ation	Mean of Fetus Number (%) ± SD		Mean of Fetus Weight (g) ± SD	Mean of Fetus Length (cm) ± SD	Mean of Hemorrhage Fetus Number (%) ± SD
			Live Fetus	Dead Fetus			
C	6	53	96.33 ± 5.68^{c}	3.67 ± 5.68^{a}	1.44 ± 0.04^{b}	2.62 ± 0.03^{c}	0.00 ± 0.00^{a}
T1	6	49	90.67 ± 11.97^{bc}	9.50 ± 12.02^{ab}	1.34 ± 0.17^{b}	2.63 ± 0.01^{c}	4.00 ± 6.32^{ab}
T2	6	49	75.33 ± 22.68^{ab}	24.67 ± 22.68^{bc}	1.30 ± 0.05^{b}	2.52 ± 0.05^{b}	7.50 ± 8.71^{ab}
T3	6	53	72.00 ± 20.58^{ab}	26.00 ± 22.90^{abc}	0.96 ± 0.19^{a}	2.51 ± 0.11^{ab}	16.00 ± 15.11^{bc}
T4	6	51	60.00 ± 16.49^{a}	40.17 ± 16.46^{c}	0.92 ± 0.15^{a}	2.43 ± 0.10^{a}	22.17 ± 11.01^{c}

Notes: Different superscripts in the same column shows significantly different results with ($p<0.05$). C: Control group (without exposure to electric repellents), T1: exposed to electric repellents for 4 hours/day, 6 hours/day (T2), 8 hours/day (T3), 10 hours/day (T4)

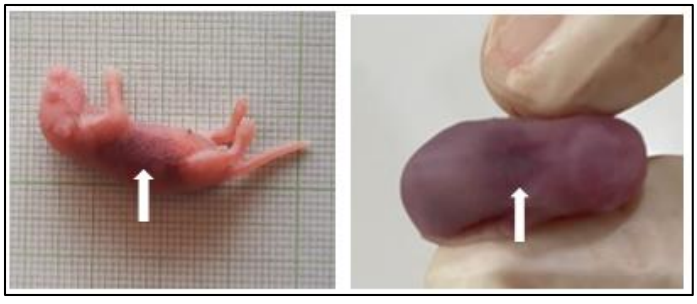

Figure 1 Collection of hemorrhagic fetuses (white arrows) from various treatment groups exposed to electric mosquito repellent containing the active ingredient dimefluthrin from the 6th to the 15th day of gestation

Significant differences in fetal death were shown by T2 group with the mean of dead fetus of 24.67 ± 22.686% and it rose when the duration of exposure to electric mosquito repellents was longer. The highest percentage of deaths was found in the T4 treatment group with the mean of dead fetus of 40.17 ± 16.46%. This is in line with a research conducted

by Damayana et al. [6] which stated that there was an increase in the percentage of dead fetuses and a decrease in live fetuses due to the combination of transfluthrin and d-Alletrin found in mosquito repellents along with the duration of exposure.

4. Discussion

Fetal death can be caused by the accumulation of the active ingredient of the electric mosquito repellent, dimefluthrin which is classified as pyrethroid which can trigger the formation of free radicals causing oxidative stress [10]. Oxidative stress is an imbalanced condition between oxidants and antioxidants that has the potential to cause damage. The presence of free radicals in the blood can damage cell biochemical components such as deoxyribunucleic acid (DNA), ribonucleic acid (RNA), carbohydrates, fats, proteins and micronutrients (vitamins and minerals) [1]. This causes the pregnant mice's metabolism to be disrupted which will affect the distribution of blood to the fetus. A fetus with a lack of blood supply has the potential to have growth inhibition, fail to develop, and even die. However, the death that occurs in the mouse fetus is not only caused by the active ingredient dimefluthrin, but can also be caused by other factors such as environmental and genetic factors. Fetal death did not occur in all pregnant mice. This is because each pregnant mouse has a different ability to metabolize chemical compounds that enter its body, even though it is in the same strain and to the same teratogenic compound [11].

The One-Way ANOVA statistical test showed that there was a significant difference in mean of fetal body length and fetal body weight between groups ($p < 0.05$) then Duncan test was performed. The lowest mean on fetal body weight was found in the T4 treatment group (exposed to electric repellents 10 hours per day) with body weight mean of 0.92 ± 0.15 grams, while the highest mean of body weight was found in group C (without exposure to electric mosquito repellents) with body weight mean of 1.44 ± 0.03 grams. The highest mean on fetal body length was found in the control group with body length mean of 2.62 ± 0.02 cm, while the lowest was found in the T4 group with body length mean of 2.43 ± 0.10 cm.

Decreased body weight and body length of the fetus was aligned with the duration of exposure to electric mosquito repellents. Dimefluthrin is an active ingredient in the pyrethroid class which can enter the body and be detected as a xenobiotic. The more dimefluthrin that enters the body, the higher the oxygen consumption. This reflects the rapid use of oxygen (respiratory burst) and the formation of large amounts of reactive oxygen species (ROS) which can trigger a chain reaction that forms lipid peroxidation. Fat peroxidation in erythrocyte cells can reduce the integrity of the cell membrane and cause erythrocyte lysis. Decreased production or increased destruction of red blood cells causes a decrease in hemoglobin levels [1]. A decrease in hemoglobin levels causes the supply of oxygen to the body's tissues, including to the fetus, to become insufficient. If the fetus has a shortage of substrate levels in the growth process, both nutrients and oxygen, it will change its metabolic activity in order to survive which affects the growth of the fetus. Decreased growth rates lead to fetal weight which is lower than normal weight [12].

Sinha et al. [13] stated that mosquito coils and electric mosquito repellents cause hypoxia in treated mice. Hypoxia is a lack of oxygen in the body's tissues. Hypoxia was caused by decreased oxygen levels in the air around the mice room due to the presence of mosquito coils and electric fumes. Low oxygen in the blood results in inhibition of nutrient intake from the mother to the fetus and can ultimately result in stunted fetal growth, including fetal body weight and length. The normal body weight of a mouse fetus with a range of 6-15 off-springs per head is 0.5-1.5 grams. The decreased body weight of the fetus compared to the body weight of the control group is still relatively normal so that it is possible for the fetus to grow and survive.

Observation of morphological abnormalities was carried out by observing abnormalities that occurred on the outer body parts of the fetus. The results of morphological observation did not reveal any abnormalities in the head, eyes, ears, extremities, fingers or tail shape. This shows that exposure to electric mosquito repellent containing the active ingredient dimefluthrin has no effect on the gross morphology of the mouse fetus. However, in this study, hemorrhage was found on the surface of the fetal skin (Table 1 and Figure 1).

The highest rate on hemorrhagic abnormalities was found in the T4 treatment group with a percentage of 22%, that is, with exposure for a maximum of 10 hours per day, this is in line with a research conducted by Rahayuningsih [5] that hemorrhage will increase with the duration of exposure to mosquito repellents, due to longer time of exposure so the more smoke from mosquito repellents was inhaled by the pregnant mice. Hemorrhage is a condition in which blood is coming out of the vascular system accompanied by accumulation in body tissues [14]. Dimefluthrin, which is the active ingredient of mosquito repellents classified as pyrethroid, enters the body of pregnant mouse as a substance that forms reactive oxygen species (ROS). In the body, it not only damages the erythrocytes but also other cells, including those

that make up the blood vessels of the fetus. Blood vessel cells that are exposed to reactive oxygen species (ROS) and have oxidative stress will be damaged and even die so that the blood vessels become brittle and break easily [15]. If this occurs to the blood vessels in the subcutis, there is potential for hemorrhage to occur on the surface of the skin, as was found in the treatment group exposed to mosquito repellents. A research by Retna [15] stated that mosquito repellents containing the active ingredient pyrethroid with a concentration of 7.8% in 1 gram/pcs can cause teratogenic effects in the form of morphological abnormalities with an exposure of 4 hours and 8 hours/day. Exposure to electric mosquito repellent containing the active ingredient dimefluthrin can increase fetal death in mice (*Mus musculus*) and decrease fetal body weight and length as well as cause hemorrhage on the surface of the fetal skin. However, it does not cause gross morphological abnormalities

5. Conclusion

The highest rates of fetal death and hemorrhage were shown in the exposed to electric mosquito repellents10 hours/day (T4 treatment). Dimefluthrin enters the body of pregnant mouse as a substance that forms reactive oxygen species (ROS). Blood vessel cells that are exposed to ROS and have oxidative stress will be damaged and even die so that the blood vessels become brittle and break easily.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

This research has received ethical approval from the animal ethics commission, Faculty of Veterinary Medicine, Airlangga University No: 1.KE.127.11.2021.

References

[1] Niswah, C., Syarifah, and S. Fitria. 2018. The Effect of Burning Paper Anti-Mosquito Smoke on The Number of Erythrocytes in Mice (*Mus musculus*). Journal of Health Sciences. 2(1): 40-45.

[2] Almahdy, A., D. Dachriyanus, and M. Rosa. (2014): Test of Teratogenic Effects of Mosquito Repellent Containing Transfluthrin on White Mice Fetuses. Scientia Journal. 4(2): 46-50.

[3] Hayes, A. W. 2001. Principles and Methods of Toxicology. 4th ed. USA: Taylor & Francis Routledge.

[4] World Health Organization (WHO). 2006. Who Specifications and Evaluations for Public Health Pesticides. Geneva. Pp: l 1-20.

[5] Rahayuningsih, T. 2006. Teratogenic effects of mosquito coil on fetus of mice (*Mus musculus* L.) Balb-c strain during organogenesis period. Thesis. UNS. Surakarta

[6] Damayana, S., S. Wigati, and Elisma. 2019. Test of Teratogenic Effects of Transfluthrin and D-Alletrin Compounds in Mosquito Drugs Against Mice Fetus (*Mus musculus* L.). Indonesian Journal Pharmaceutical Science. 1(1), 26-35.

[7] Badan Pengawas Obat Dan Makanan (BPOM RI). 2014. Guidelines for in Vivo Nonclinical Toxicity Testing, BPOM RI, Jakarta.

[8] Al-Damegh, M. A. 2013. Toxicological Impact of Inhaled Electric Mosquito-Repellent Liquid on the Rat: A Hematological, Cytokine Indications, Oxidative Stress and Tumor Markers. Inhalation Toxicology. 25(5): 292-7.

[9] En, Y. S., P. Hardany, W. Widjiati, and E. M. Luqman. (2020): The Effect of Nicotine Per Inhalation on the Diameter and Epithelium Thickness of the Seminiferous Tubules of Rats. Iraqi Journal of Veterinary Sciences. 34(1): 209-215.

[10] Wijayanthi, R. N. 2011. The effect of giving antioxidants various vitamins (A, C, and E) on erythrocyte counts and hemoglobin levels in male white rats (Rattus novergicus) exposed to mosquito repellent smoke. [Thesis]. Malang: Universitas Negeri Malang.

[11] Weber, E. M. and I. A. S. Olsson. 2008. Maternal Behaviour in *Mus musculus* Sp.: An Ethological Review. Applied Animal Behaviour Science. 114(1-2): 1-22.

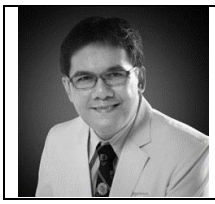
[12] Muhammad, S., H. Ridwan, and F. Z. Lubis. 2012. The relationship between anemia in pregnancy and the incidence rate of low birth weight babies at Moehammad Hoesin Hospital Palembang. Thesis. Fakultas Kedokteran Universitas Muhammadiyah Palembang. 2(2):1-5.

[13] Sinha C, Seth K, Islam F, Chaturvedi RK, Shukla S, Mathur N, N Srivastava N, and Agrawal AK. 2006. Behavioral and Neurochemical Effects Induced by Pyrethroid-Based Mosquito Repellent Exposure in Rat Offsprings During Prenatal and Early Postnatal Period. Neurotoxicology and Teratology. 28(4): 472-81.

[14] Widiyani, T. and M. Sagi. 2001. Effect of Aflatoxin B1 on the Growth and Development of the Embryo and Fetal Skeleton of Mice (*Mus musculus* L.). BioSmart Scientific Journal. 3(2): 28-35.

[15] Retna. 2012. The effect of exposure to d-allatrin anti-mosquito drug on body weight, body length, and morphological abnormalities of infants from female Wistar rats. [Tesis]. Universitas Diponegoro. Yogyakarta.

Authors short biography



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