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Original Research Paper

Effectiveness of Artificial Wistar Rat Cage Size in Accelerating The Occurrence of Dysplasia in The Process of Formaldehyde Induction

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Abstract: Cage is a medium used in placing experimental animals in experimental-based studies to serve as learning instruments related to studying the cause and effect to be investigated. The size of the cage can affect several health factors in experimental animals, such as the social level and stress level of the animal. Formaldehyde is a carcinogenic compound that can cause malignancies if exposed continuously to living things. Therefore, the purpose of this study was to determine whether cage size can affect the degree of dysplasia in Wistar rats induced by 40 ppm formaldehyde. This study is a quantitative study that uses a true experimental research design with post-test only control group design. This study used male Wistar rats (Rattus norvegicus) with a total of 15 rats divided into 3 groups, namely, a small cage group with a size of (27.5 x 21.5 x 9) cm3, a medium cage group with a size of (28 x 22.5 x 10) cm3, and a large cage group with a size of (30 x 24 x 11.5) cm3 with a total of 4 treatment rats (formaldehyde 40 ppm) and 1 control rat (untreated) in each group. The treatment given to rats was carried out 6 hours per day and lasted for 12 weeks until all rats were terminated at the same time. The rats were terminated and histopathological observations were made to assess the degree of dysplasia that occurred. The results of the Kruskal-Wallis statistical test showed a significant relationship ($p < 0.05$). This study shows that there is a relationship between the size of artificial cages and the degree of dysplasia in Wistar rats induced by 40 ppm formaldehyde.

Keywords: Cage, degree of dysplasia, formaldehyde, wistar rats.

Introduction

Cancer or malignancy is one of the diseases that is considered very deadly among the public (Roy and Saikia, 2016; Yin et al., 2021). Malignancy is a non-communicable disease (NCD) (Rosita et al., 2021). This disease is a disease in which cell growth grows abnormally, this growth can be seen from the smallest part of a living body, namely cells that develop and reproduce abnormally and continuously (Rahayuwati et al., 2020; Gadaleta at al., 2022). According to data uploaded by (WHO) World Health Organization (2020), related to the "International Agency for Research on Cancer" it was recorded that 946,088 people out of 273,523,621 Indonesians had malignancy-related diseases during 2015 - 2020.

There are several habits that can be an initial trigger for malignancy. Some habits such as poor diet, history of obesity,

consumption of alcoholic beverages, active-passive smoking, physical activity, and environmental factors (Mena et al., 2019; Rotzinger et al., 2021). This research will focus on the causes of malignancies from the "environment" side. The environment is a place around humans that can affect the development of human life either directly or indirectly (Yulianti et al., 2016; Sijm-Eeken et al., 2023).

There are several studies that have reviewed the impact of the living environment on the occurrence of malignancies. One of them is research conducted by Makin et al., (2021) who researched with the title "Using Free-Range Laboratory Mice to Explore Foraging, Lifestyle, and Diet Issues in Cancer" in 2021. This study used subjects in the form of C57BL/6 laboratory mice, in this study also used 2 different methods, namely by releasing C67BL/6 laboratory mice to roam freely in the vivarium and then by looking at measurements of tumor growth rates from mice placed under standard laboratory conditions. However, this study found that tumor growth in mice living in the vivarium (outdoor) was slower than tumor growth in mice living in the laboratory (indoor).

In a study conducted by Kershaw et al. (2013) which examined "Environmental enrichment does not impact on tumor growth in mice". This study used subjects in the form of laboratory mice where this study also used 2 different methods, namely dividing the study into 2 different groups. Group 1 is mice placed in an environment enriched by games while group 2 is smaller than the size of the group 1 cage. This study produced insignificant results between the growth of B16F10 subcutaneous tumors in group 1 and group 2.

There were studies that examined the effect of housing on the incidence of malignancy. However, none of these studies included models of cages that were ventilated on the sides of the cage. In addition, no studies have compared three different cage sizes on the incidence of malignancies. Due to the high incidence of malignancy, researchers are interested in researching "The Effectiveness of Artificial Wistar Rat Cage Size in Accelerating the Occurrence of Dysplasia in the Formaldehyde Induction Process". The benefit that will be provided in this research is as knowledge related to whether cage size affects the degree of dysplasia in male Wistar rats induced by formaldehyde.

Material and Method

Time and place

This study took 3 months until all groups of rats were terminated for data collection and data analysis. This study was conducted in two different laboratories at the University of Mataram, namely:

a) Preparation and dilution of formaldehyde was carried out at the Pharmacochemistry Laboratory, Pharmacy Study Program, University of Mataram.
b) Formaldehyde induction was carried out at the Drug Testing Laboratory, University of Mataram

Variable

The independent variable in this study is artificial cages and the dependent variable is the degree of dysplasia.

Data analysis

Data analysis in this study used univariate analysis and bivariate analysis. In univariate analysis using descriptive data analysis to describe the results in presentation form, while bivariate analysis uses non-parametric tests with unpaired categorical-categorical data groups with the number of groups more than 2. Therefore, the bivariate analysis used is Kruskal-Wallis.

Method

This study used 15 Wistar rats which were divided into 3 treatment groups and 1 control group. The control group was divided based on different cage sizes including small cage (27.5 x 21.5 x 9) cm^3, medium cage (28 x 22.5 x 10) cm^3 and large cage (30 x 24 x 11.5) cm^3, while the control group used medium-sized cage but without any treatment. Formaldehyde compound was taken using a micropipette as much as 40 ppm induced in Wistar rats.

Result and Discussion

Univariate analysis

The data presented is a comparison of histopathology of nasopharyngeal tissue of Wistar rats induced by 40 ppm formaldehyde in small, medium and large cages. The degree of dysplasia in small cage samples shows the results of severe dysplasia, while in the sample group of medium and large cages shows the same results, which is dominant in showing the results of moderate dysplasia with some mild dysplasia results.

Table 1. Histopathology results of nasopharyngeal tissue of Wistar rats induced with 40 ppm formaldehyde sometimes small, medium and large fields

		Cage Size		
		Small	Medium	Large
Degree of Dysplasia	Normal	1	1	1
	Mild	0	1	1
	Moderate	0	3	3
	Severe	4	0	0
Number of Sample		5	5	5

Bivariate analysis

The results of the analysis of the table above related to the relationship between cage size and the degree of dysplasia show the results of the p value <0.05 where the value in the comparison of cage size shows a value of p = 0.012. The value of p < 0.05 statistically shows a significant difference between groups so that it can be interpreted that there is a significant difference in the degree of nasopharyngeal dysplasia between groups of Wistar rats in small, medium and large cages induced by 40 ppm formaldehyde.

Table 2. Kruskal-Wallis Test Results on the Relationship between Cage Size and Degree of Dysplasia

Kruskal-Wallis Test Cage Size	
Category	**Sig.**
Cage Size	0.012
Significance *p < 0.05* (p = 0.012)	

Discussion

Formaldehyde exposure

Formaldehyde exposure is an exposure to toxic compounds that are carcinogenic to the body of living things and can cause diplasia (Nugraha et al., 2023; Adamović et al. 2021). In this study using formaldehyde as much as 40 ppm in the treatment group which will be induced for 6 hours per day and the total research process lasts up to 12 weeks. Based on research conducted by Wedayani et al., (2023) related to the use of formaldehyde as much as 20 ppm, 30 ppm and 40 ppm can increase oxidative stress in the body so that it can interfere with cell growth and reproduction so that cells grow into abnormal cells or abnormal cells and can cause malignancy in the form of dysplasia stages if this is allowed continuously.

According to Karunagaran et al., (2022) formaldehyde is an organic compound that is easy to evaporate (Votalite Organic Compounds). Formaldehyde has a boiling point of -19 ° C so that with the normal temperature of artificial cages according to Rejeki et al., (2018) which ranges from 20 ° C - 25 ° C, the compound can work optimally to have an effect on rats in the cage and the effect will indirectly be in a long period of time in the cage because the compound is trapped by a plastic cage cover.

Formaldehyde exposure given to rats in this study was optimized with different cage sizes, namely sometimes small, medium and large cages with several additional ornaments such as cage covers in the form of wire sieves coupled with clear plastic, 2 air vents, and plastic cups to place cotton for formaldehyde induction of 40 ppm so that formaldehyde exposure induced to rats can work optimally to provide the desired results in this study.

Cage material

The media used in this study used artificial cage media with different sizes in each cage. For small cages have a size of 27.5 cm x 21.5 cm x 9 cm (5.3 liters), medium cages have a size of 28 cm x 22.5 cm x 10 cm (6.3 liters) and large cages have a size of 30 cm x 24 cm x 11.5 cm (8.3 liters). This study uses the same cage design as the cage design that has been recorded in HAKI by Dr. dr. Anak Agung Ayu Niti Wedayani, M. Sc and dr. Novia Andansari Putri Restuningdyah, Sp. Rad with the recording number 000473934 in 2023.

Rat cages are made of plastic base material, with some additional ornaments such as cage lids made of wire sieve coupled with clear plastic, as well as several air vents that have the same size placed on both sides of the cage with

the same material as the cage lid, namely made of wire sieve. On the ground floor of the cage or commonly referred to as bedding using materials that are smooth and comfortable for rats to carry out their activities (Mutiarahmi et al., 2021). Poor cage quality can cause rats to experience stressful conditions that can have an impact on rat health. When a living being experiences stressful conditions, whether physically, emotionally or environmentally, the body will produce more molecules called Reactive Oxygen Species (ROS). Too high ROS in the body can cause excessive oxidative stress. Oxidative stress can damage various cell components such as protein and fat, so that if oxidative stress is allowed to continue, it can interfere with the normal function of cells which causes cells to grow and divide abnormally so that it can lead to dysplasia growth (Jelic et al., 2021; Nakai and Tsuruta, 2021).

Cage Size

The cage size in this study has a different size in each cage group. However, according to Yusuf et al. (2022) the ideal cage for 5 to 7 rats is a cage measuring 40 cm x 30 cm x 18 cm. The difference in the three cage sizes in this study can affect the density and mobility of the rats in each cage group so that it can affect the results that will be obtained. Other studies conducted by Makin et al., (2021) and Manouze et al., (2019) support this research theory. In the research conducted by Makin et al., (2021) divided 2 groups, namely 1 group of rats placed in a vivarium and 1 group placed in a standard laboratory cage.

The study showed significant results regarding the relationship between cage size and tumor growth rate in mice. Tumor growth in mice placed in a vivarium showed slower tumor cell growth compared to mice placed in standard laboratory cages. However, in a study conducted by Manouze et al., (2019) by placing control male rats that were socially housed during the pretest and then transferred to individual cages for six weeks showed increased anxiety and biological stress markers compared to pretest values or rats that were socially housed for six weeks.

The results of this study indicate that several factors that can affect the results of the study such as exposure to toxic formaldehyde compounds, cage size that can affect the density and mobility of the rats and the number of rats in each cage can be a determining factor of the desired results, namely to see the effect of these factors with the growth of the degree of dysplasia.

Conclusion

In this study, there was a significant difference in the degree of nasopharyngeal dysplasia that occurred in the small cage, medium cage and large cage groups. This is due to the difference in cage size which shows that small cages are the most effective in accelerating the degree of dysplasia in the 40ppm formaldehyde induction process.

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