Effectiveness of Cages Inducing Nasopharyngeal Dysplasia in Wistar Rats (Rattus norvegicus)

Gede Rama Hardy Nugraha1*, Hamsu Kadriyan2, Anak Agung Ayu Niti Wedayani3, Eka Arie Yuliyani4
1Medical Student, Faculty of Medicine, University of Mataram, Mataram, West Nusa Tenggara, Indonesia;
2Department of Ear, Nose, Throat, Head-neck Surgery, Faculty of Medicine, University of Mataram, Mataram, West Nusa Tenggara, Indonesia;
3Department of Pharmacology, Faculty of Medicine, University of Mataram, Mataram, West Nusa Tenggara, Indonesia;
4Department of Ear, Nose, Throat, Head-neck Surgery, Faculty of Medicine, University of Mataram, Mataram, West Nusa Tenggara, Indonesia;

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*Corresponding Author: Gede Rama Hardy Nugraha,
Medical Student, Faculty of Medicine, University of Mataram, Mataram, West Nusa Tenggara, Indonesia;
Email: rahadi4448@gmail.com

Abstract: The cage is a place used to put experimental animals in experimental studies to study the mechanism of the disease and its response to therapy. Formaldehyde as a carcinogenic compound can increase the occurrence of chronic inflammation which has the potential to increase the growth of cancer cells in experimental animals due to the stress experienced while in the cage. Therefore, the purpose of this study was to compare the effectiveness of large and small cages induced by formaldehyde 30 ppm against nasopharyngeal dysplasia in Wistar rats. This research is an experimental study with a true experimental design with the type of post-test only control group design. This study used 12 male Wistar rats (Rattus norvegicus) which were divided into two groups, namely the group of Wistar rats which were placed in large cages measuring (30 x 24 x 11.5) cm3 of 6 individuals, and in small cages (27.5 x 21.5 x 9) cm3 of 6 individuals induced by 30 ppm formaldehyde with a span of 6 hours per day for 16 weeks. The rats were terminated and retro-orbital blood samples were taken to measure MDA levels and nasopharyngeal tissue to assess the degree of dysplasia. The results of this study showed that there was a statistically significant difference (p <0.05) between the degrees of nasopharyngeal dysplasia (p=0.003) and MDA levels (p=0.003) in Wistar rats in small cages and large cages. In this study the effectiveness of small cages was greater than large cages in causing dysplasia in the nasopharyngeal tissue of Wistar rats.

Keywords: Cages, wistar rats, formaldehyde, MDA, nasopharyngeal dysplasia.

Introduction

The use of cages as a place for experimental animals is generally adjusted to the needs of the treatment. Conditions of temperature, density, cage system, and cage bedding were used as parameters in studying the response of the immune system, tumor growth, infection and inflammation. Cage conditions that can increase chronic stress in rats are also influenced by the type of cage, cage size, temperature, lighting, and cage bedding system (Hylander et al., 2022). The lighting cycle in the cage was adjusted to the laboratory light-dark cycle with a standard time of 12 hours each (Eraslan et al., 2023). The optimal temperature for experimental animal cages varies depending on the type of experimental animal. Types of experimental animals, such as mice range in temperature range of 20°-26°C (Garber, 2010).
The small size of the cage affects the stress level experienced by rats. The tumor growth rate in rats housed in laboratory cages was relatively greater compared to rats housed in the vivarium. This can be caused by several factors, such as activity level, stress, food intake, diet, weather changes, and other stimuli (Makin et al., 2021). Tumorigenesis induced by adrenergic stress played by β2-adrenergic receptors activates protein kinase A (PKA) to increase ROS resulting in DNA damage (Eng et al., 2014). Placement of the cage indoors aims to minimize sound stress which must be supported by providing adequate ventilation for adequate air and oxygen circulation. Ventilation also functions to dilute gas contaminants and pathogens in the air adjusted to room temperature (Mutiarahmi et al., 2021).

High intensity exposure to factors that trigger the growth of cancer cells has an impact on a decrease in the immune system. The mechanism of formaldehyde entering the body can be through inhalation, ingestion, and skin retention. Formaldehyde has an irritating effect on the eyes and upper respiratory organs when exposed directly indoors. Exposure to formaldehyde at a concentration of 0.25-3.0 ppm can cause irritation to the eyes, nose and throat with mild eye irritation at 1 ppm (Dan et al., 2020). The mechanism for the entry of carcinogenic substances into the body such as through the inhalation process from formaldehyde exposure increases tumorigenesis which can then become cancer cells (Salthammer, 2019). Increased Oxidative Stress (OS) causes an imbalance between oxidant and antioxidant content.

Redox imbalance due to increased levels of oxidants triggers DNA chain termination induced by ROS (Ghelli et al., 2021). The carcinogenic properties of formaldehyde at a concentration of 15 ppm can increase inflammation and apoptosis in the respiratory tract (Kang et al., 2022). The mechanism of formaldehyde in inducing cancer is induced by oxidative stress, DNA damage, chromosomal aberrations, and apoptosis (Guo et al., 2018). Increased oxidative stress can be assessed based on malondialdehyde (MDA) levels (Susilawati et al., 2022). This study will review the comparison of the effectiveness of large and small cages induced by inhalation of 30 ppm formaldehyde against the occurrence of nasopharyngeal dysplasia in Wistar rats.

**Material and Method**

This research is a quantitative research with a true experimental research design with the type of post-test only control group design and was carried out after obtaining approval from the Health Research Ethics Commission, Faculty of Medicine, University of Mataram with registration number 190/UN18.F8/ETIK/2023. This study used 12 male Wistar rats (*Rattus norvegicus*) aged 8-12 weeks weighing 150-200 grams which were divided into two groups of 6 each, namely the Wistar rat group which was placed in a large cage and a small cage. Prior to treatment, the 12 rats were acclimatized for seven days in large and small cages by being fed SB11 and drinking ad libitum with 6 rats in each cage. The cage conditions were adjusted to light-dark cycle laboratory lighting for 12 hours each. The temperature of the cage was maintained at 25°-27°C.

Formaldehyde with a concentration of 10% was prepared from a 37% stock solution to obtain a solution of 100 ml. The induction process was carried out by taking formaldehyde from a solution of 30 ppm using a micropipette and then dripping it on cotton as an induction medium in each cage. This process takes place 6 hours once a day for 16 weeks. The cages were prepared from large plastic boxes (30 x 24 x 11.5) cm³ and small ones (27.5 x 21.5 x 9) cm³. After that, make ventilation diagonally between the front and back of the cage and the roof which is closed using a wire net. During the induction process, the roof of the cage is covered with a plastic-coated wire mesh. Nasopharyngeal samples were examined to determine the degree of dysplasia, while blood samples were taken retro-orbitally to check MDA levels. The data that has been obtained is followed by univariate and bivariate analysis using IBM SPSS Statistics 25. Mann Whitney U statistical test for the analysis of the degree of dysplasia, while the Independent T statistical test for the analysis of MDA levels.
Result and Discussion

Result
After induction of 30 ppm formaldehyde for 16 weeks, samples of the nasopharyngeal tissue of Wistar rats were examined using a binocular light microscope stained with hematoxylin eosin (H&E) at 40X magnification. The results of the examination can be seen in Figure 1 and Figure 2.

![Figure 1. Wistar rat nasopharyngeal normal tissue (HE 40X)](image)

![Figure 2. Wistar rat nasopharyngeal tissue with severe dysplasia (HE 40X)](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>Degree of Dysplasia</th>
<th>Large Cage</th>
<th>Small Cage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moderate dysplasia</td>
<td>Severe dysplasia</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mild dysplasia</td>
<td>Severe dysplasia</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>Moderate dysplasia</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>Severe dysplasia</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Normal</td>
<td>Severe dysplasia</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Normal</td>
<td>Severe dysplasia</td>
<td></td>
</tr>
</tbody>
</table>

The degree of dysplasia in the large cage sample number 1 indicates moderate dysplasia, number 2 indicates mild dysplasia, and numbers 3 to 6 indicate normal nasopharyngeal tissue of Wistar rats, while the small cage sample number 3 indicates moderate dysplasia, and number 1, 2, 4, 5, and 6 show severe dysplasia. The distribution of data can be seen in table 1.

The results of data analysis related to the comparison of dysplasia degrees (Table 2) and MDA levels (Table 3) in each large and small cage group showed that the $p$ value <0.05 where the value in the comparison of dysplasia degrees showed a $p$ value = 0.003 and the value in comparison of levels MDA shows a value of $p = 0.003$. The $p <0.05$ statistically indicated a significant difference between the two groups so that it could be interpreted that there was a significant difference in the degree of nasopharyngeal dysplasia between the large cage Wistar rat group and the small cage Wistar rat group induced with 30 ppm formaldehyde.

Table 2. Results of data analysis on the degree of dysplasia using the Mann-Whitney U statistical test

<table>
<thead>
<tr>
<th>Cage</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Cages</td>
<td>6</td>
<td>9.42</td>
<td>56.50</td>
</tr>
<tr>
<td>Large Cages</td>
<td>6</td>
<td>3.58</td>
<td>21.50</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymp. Sig. (2-tailed)</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Significance of $p < 0.05$ ($p = 0.003$)

Table 3. Results of analysis of data on MDA levels in large and small cage samples using the Independent T Test

<table>
<thead>
<tr>
<th>Cage</th>
<th>N</th>
<th>Mean Rank</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Cages</td>
<td>6</td>
<td>0.323</td>
<td>0.092</td>
</tr>
<tr>
<td>Large Cages</td>
<td>6</td>
<td>0.130</td>
<td>0.024</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Significance of $p < 0.05$ ($p = 0.003$)
Discussion

The quality of the cage is influenced by various factors, one of which is discussed in this study is the size of the cage. The size of the cage affects the space for rats to move. However, there is no specific formula for making cages based solely on body size and body weight. The natural nature of animals and their ability to survive are the main things that serve as an ideal guide in making cages. The ability of animals to maintain body postures without touching walls or barriers, to be able to turn around and to have access to food and drink is part of the animal's natural expression of survival (Garber, 2010). The size of the cages used in the study for large cages and small cages were 11.5 cm and 9 cm respectively. However, based on research by Garber (2010) it shows that the recommended size for cage height for rats weighing 100 g – 500 g is 17.8 cm. This significant difference in minimum size causes space for mice to be limited, thereby increasing stress (Mutiarahmi et al., 2021).

![Figure 3. A: Small Cage. B: Large Cage](image)

Exposure to toxic compounds through inhalation can also increase oxidative stress. In this study, the induction process of 30 ppm formaldehyde was carried out by inhalation with an induction time interval of 6 hours which was carried out once a day for 16 weeks. Based on the inhalation toxicity study guide by the OECD (2018) it states that rats as test animals in an inhalation study require 6 hours. In addition, rat inhalation in a small room can maximize the level of exposure to toxic compounds because it minimizes the re-breathing of exhaled air (OECD, 2018).

Toxic compounds such as formaldehyde have carcinogenic properties for the body which can cause dysplasia to carcinoma (Sulaksana and Kadriyan, 2019). In this study, induction of formaldehyde compounds with a concentration of 30 ppm placed in large cages and small cages caused dysplasia in the nasopharyngeal tissue of Wistar rats. In line with the research of Wedayani et al., (2023) regarding exposure to tobacco dust which is carcinogenic can increase oxidative stress in the body so that it can damage cells and tissues related to their structure and function so that they will enter the dysplasia stage which if allowed to continue can cause carcinoma in situ or malignancy. Other research that supports the results of this study was conducted by Susilawati et al., (2022) regarding exposure to formaldehyde with a concentration of 20 ppm for 16 weeks showing the occurrence of dysplasia in the nasopharyngeal tissue. Based on previous research conducted by Susilawati et al., (2022) showed that increased oxidative stress can be measured using MDA expression levels. The results of this study indicated a significant difference in the statistical test of MDA levels between large and small pens which could be due to differences in the effectiveness of exposure to formaldehyde in increasing oxidative stress. Exposure to formaldehyde with concentrations above 6 ppm causes epithelial damage, such as squamous metaplasia and hyperplasia (CDC, 2002).

Small cages have higher effectiveness compared to large cages. Formaldehyde exposure is more effective in small induction rooms due to slow airflow and high formaldehyde concentrations, thereby increasing subject exposure (OECD, 2018). The presence of a cage cover on the roof will prevent airflow from escaping through the roof of the cage. Diagonally positioning of the vents on both sides of the cage allows for low airflow which can increase formaldehyde inhalation. This is in line with the study of Teixeira et al., (2006) concerning the cage ventilation system for rat airflow in a Brazilian laboratory which stated that a cage ventilation system made diagonally could control airflow better than unidirectional airflow, so that the airflow entry into the cage can be maintained longer to all parts of the cage.

Conclusion

In this study there is a significant difference degree of nasopharyngeal dysplasia that occurred between groups large cage with
groups of small cages. This matter caused by differences in the size of the cage where small cages are more effective in optimizing exposure formaldehyde in the cage compared with large cage.

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