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The Comparison of Hind Limb Mass Loss in House Mice Preserved with Advanced Fixative Solutions of Ethanol - Glycerin and 4% Formaldehyde

Ria Margiana[1,2,3,*], Amanda Natalie Wijaya[4], Sasanthy Kusumaningtyas[1,2,3], Deswaty Furqonita[1,2,3], Rahmadini[1,2,3]
[1]Department of Anatomy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Integrative Laboratory of Department of Anatomy, Biology, and Histology Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[3]Developmental Biology Laboratory of Department of Anatomy, Biology, and Histology Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[4]Undergraduate Programme in Biomedical Sciences Faculty of Medicine Universitas Indonesia.
corresponding author: margianaria@gmail.com

Abstract

Cadaver preservation usually uses formalin as a fixative solution. However, formalin has many negative effects on health and the environment. To reduce these negative effects, an alternative fixative is needed to be explored. Ethanol-glycerin (EG) can potentially be an alternative to formaldehyde as a fixative solution. Therefore, this study aims to compare the hind limb mass loss in mice treated with advanced preservation of EG and 4% formalin. This research method used adult male house mice (Mus musculus) aged 3-6 months with body weight ranging from 20-30 grams. The research group consisted of the control group using 10% of formalin, the EG group using ethanol-glycerin, and the formalin 4% group using 4% of formalin as a fixative solution. Hind limb mass and total were observed after 6 weeks post burial. The results showed a hind limb mass loss after 6 weeks for each group. Hind limb The hind mass loss in the EG fixative group had a significant mass loss compared to the 4% formalin fixative group on a weekly basis.

Keywords: Ethanol-glycerin, formalin, cadaver, hind limb, decomposition

BACKGROUND

The use of cadavers in medical science, especially in studying anatomy, is still very much needed, because it optimizes the use of the five senses (1). Cadavers need to be preserved before use. The use of preservatives for cadavers generally consists of 10% formalin/formaldehyde

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fixative solution (2). Cadaver preservation carried out in the anatomy department of FKUI uses a lower percentage of formalin, namely 4% formalin. The use of this lower percentage of formalin can reduce the level of formalin toxin. Apart from being safer, it has also been proven in previous studies that it has a faster decomposition effect when the body is buried (3).

The use of formaldehyde or formalin as a fixative aims to maintain the integrity of the cadavers, reduce and prevent the rate of decomposition. Formalin, has the ability to form a cross-link between proteins so as to prevent this process. Besides the optimal results, formalin is also easy to obtain and economical (4). However, in its use, formalin has many negative effects on health and the environment (5). Use of formalin and the effect of exposure to formalin can cause a burning sensation on the nose, eyes and skin. At a high dose, the use of formalin can irritate mucous membranes to the lower airways, causing bronchitis, pneumonia, and pulmonary edema. The International Agency for Research on Cancer classifies formaldehyde, as a carcinogenic substance (6). Use and exposure to formalin for a long time can cause leukemia and brain cancer (5). Formalin also has a negative effect on the environment, namely it can cause groundwater pollution. When a cadaver is buried, formalin will also be decomposed (6) and oxidized into formic acid and carbon monoxide which are dissolved in the groundwater, causing acid rain phenomenon (7,8). When a cadaver is about to be buried, formalin can become a problem because the use of formalin will also slow down the decomposition process.[4] This problem is related to disease outbreaks because there are many active decomposers at the burial site, namely bacteria and fungi, thus the environment could be polluted (7). Therefore, necessary alternative fixative materials to address all these issues are needed. One of the fixatives which is being extensively researched is the ethanol-glycerin (EG) combination (4,9,10). The combination of these two ingredients works as a good preservative by reversibly denaturing proteins, thus preventing the autolysis process as well as acting as an anti-infective agent against bacteria and other microbes. This has also been proven by the research done by Hammer, et al. EG can replace formalin as a fixative in the preservation process [9].

As has been reported in the research mentioned above, it is hoped that there will be advanced preservative agents such as EG which can make the to-be-buried cadavers experience faster decomposition. One sign of decomposition that is easy to observe is mass loss. Whether the use of EG as an advanced preservative provides another advantage, namely that when the cadaver that has been preserved for burial has a faster rate of mass loss, has not been

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investigated**.** Therefore, this study aimed to compare the hind limb mass loss in mice treated with advanced preservation of EG and 4% formalin.

METHODS

Place of Research

This research took place at the Department of Anatomy, Faculty of Medicine, Universitas Indonesia.

Research Subjects

This research has received approval from the ethics committee. The study used adult male house mice (*Mus musculus*) aged 3-6 months with body weight ranging from 20-30 grams as research subjects. The research groups was divided into three with a total of 18 mice. The first group consisted of 6 mice in the control group that were not preserved, the second group comprised 6 mice treated with formalin fixative, and the third group included the remaining 6 mice treated with ethanol-glycerin (EG).

Treatment of the research groups

The mice were sacrificed/euthanized by fixation with 10% formalin. Prior to fixation, the mice were given anesthetic treatment, namely by injecting 100 mg/kg ketamine and 10 mg/kg xylazine. After the fixative entered the mice's tissues, the hind limbs of the mice were taken and stored in a secondary fixative solution of EG or 4% formalin at 4°C for one week. After the preservation process was complete, the mice were buried for 1.5 months in standardized soil. Observations were carried out every week, where the mice were re-excavated to assess and measure the hind limb mass loss and the total mass of mice. Total observations were done for six weeks.

RESULTS AND DISCUSSION

This research on mass loss in the preservation process with formalin and ethanol-glycerin (EG) solutions has many benefits. The use of formalin as a cadaver preservative has been used for a long time. Preservation with formalin has been proven to provide a good cadaver preservation effect. However, the use of formalin also has many negative effects for health and the environment. EG is used as an alternative substitute for the use of formalin. Therefore, this study has obtained comparative results on the results of preservation with formalin and EG. Based on the results of the study, after burial for 6 weeks, it was found that each experimental group experienced a decrease in lower leg mass in mice (Figure 1).

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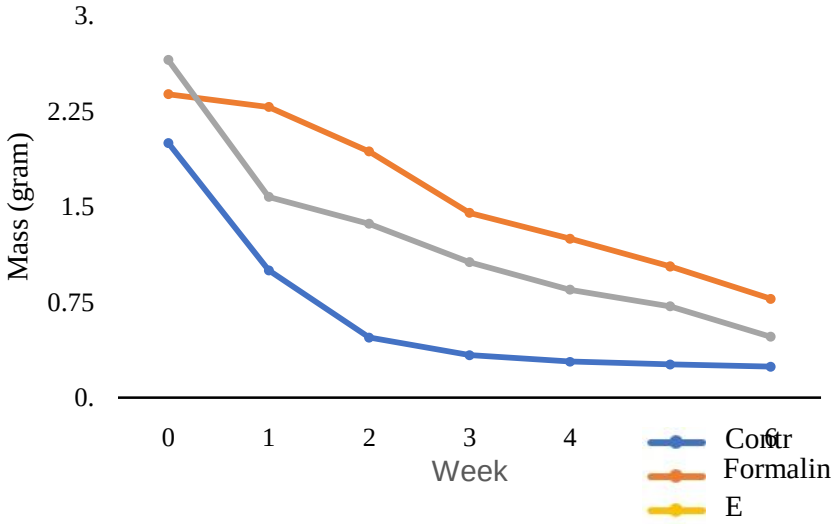

Figure 1. Hind Limb Mass against Time of Burial

The largest mass loss was seen in the control group. During the first 2 weeks of burial, the hind limb mass of the mice decreased significantly, while the other two variable groups decreased gradually. To compare the mass loss of each group, the following shows the data on the increase in the difference in mass of the hind limbs of mice per week to the initial mass and the percentage (Figure 2)

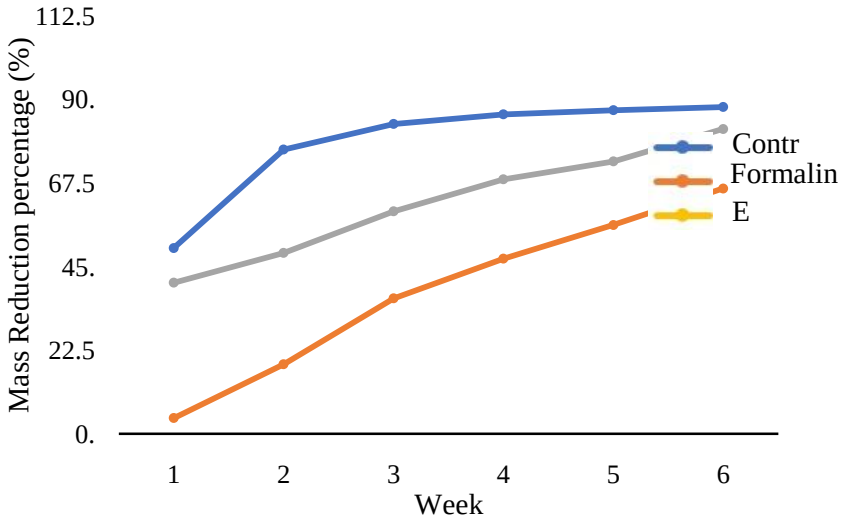

Figure 2. Comparison of Hind Limb Mass Loss Percentage in Mice per Week

The EG variable group had a higher mass loss percentage than the 4% formalin group. The difference between them was quite significant, in contrast to the EG and control variables of which the mass loss values were quite close together, especially in the first and the sixth weeks. It could be seen that the two fixative solution groups have a constant mass difference increase rate, unlike the control group.

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The normality test was carried out on the data obtained, namely the difference in mass per week against the initial mass (amount of mass loss) and the percentage. The test method used was Shapiro-Wilk test and it was found that all data had normal values, namely $p>0.05$. However, when looking at the histogram graph, the data distribution does not appear normal. This is due to the small number of samples. The data obtained had abnormal values, so data processing was continued with the Kruskal-Wallis H test for significance screening for data that had more than 2 variables. All data has a p value<0.05, so it is said to be significant. Finally, the analysis was continued with the Mann-Whitney U nonparametric test. Based on the results of the Mann-Whitney U test, a significant difference was found in the comparison of the mass loss percentage between the 4% formalin group and EG, control and EG, as well as 4% formalin and control. This showed that the mass loss percentages between each variable group are significant, especially between 4% formalin and EG fixative solutions. There were two data that showed insignificant results, namely the comparison of the mass difference between the control and EG groups at week 3 and 4, but in the percentage comparison, both of them show significant results. Apart from the hind limb mass of mice, changes in the total mass of mice were also recorded for 6 weeks. The to

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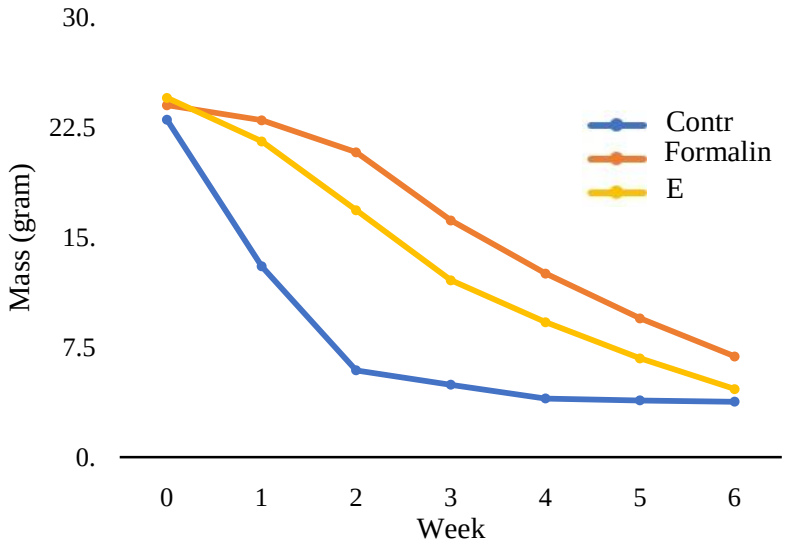

Figure 3. Total Mass of Mice against Time of Burial

The largest change in total mass was experienced by the control group in the first two weeks, while that of the EG and 4% formalin groups decreased gradually, having a constant trend. At week 0, namely the initial mass of mice before being euthanized, the EG group had the highest mass. After the first week, the position was surpassed by the 4% formalin group which indicated

a slower mass loss than the EG group. At week 6, it was seen that the total mass of mice for the control and EG groups was almost close to each other even though initially they had quite different rates of mass loss. To compare the decrease in the absolute total mass of each group, the data is presented as the difference between the total mass per week against the initial mass as well as the percentage. This differences in mass loss can be caused by differences in the way the two preservative solutions work. Formalin is able to prevent the decomposition process by forming a cross-link between proteins through the insertion of methylene groups between the nitrogen bonds so as to provide a more durable tissue fixation effect (4). EG solution is an organic solution. EG solution can stop protein denaturation and stop cell metabolism and autolysis (9,11). EG solution concentration in cadaver tissues can be decreased with the passage of time. The reversibility effect with a decrease in EG concentration is the main indicator in the comparison of mass loss in mice. Mass loss occurs more easily in EG than in 4% formalin, so EG can be an alternative substitute for formalin as a fixative solution. The use of EG as an alternative to 4% formalin can be proven by its fixative ability and more environmentally friendly trait.

CONCLUSION

Hind limb mass loss in mice was observed weekly for six weeks. The hind limb mass loss of the mice treated with advanced preservation of fixative using ethanol-glycerin was significantly higher than that of 4% formalin each week.

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