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Comparison of 10% Sucrose Fixative and 10% NBF Fixative on Hepatic Histology of Mice Using He Staining at Variable Duration

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ABSTRACT NBF 10% is the most commonly used fixation solution in histopathological examinations, because this solution can maintain tissue quality as its initial state. The results of preparations from tissues fixed using 10% NBF have good quality when microscopic observation is carried out. However, routine exposure to NBF can have negative effects such as eye, nose, and throat disorders, NBF is also carcinogenic as stated by the Occupational Safety and Health Administration (OSHA). So, it is necessary to need an alternative to 10% NBF as a fixation substance to minimize the adverse effects. Based on research that has been done, it can be stated that natural sweeteners have the potential to be an alternative fixation solution. This study aims to evaluate 10% sucrose as an alternative to 10% NBF for hepatic tissue fixation. Histological quality was assessed based on the staining of the cell nucleus, cytoplasm and uniform color in the preparation. The type of research used was experimental. This research was conducted at the Farma Veterinary Center as a validation site and test animal provider, the Cytohistotechnology Laboratory of the Department of Medical Laboratory Technology as a research site, and the Faculty of Veterinary Medicine Unair as a place for excision of mice. The test material used was mice hepar with 6 replications per treatment group. After observation, the data were processed using Kruskal-Wallis and Mann-Whitney in the 10% sucrose group for 12 hours and 24 hours showed comparable results with 10% NBF for 24 hours because based on the mann whitney test, the value was obtained ($p > 0.05$). At 10% sucrose for 6 hours showed results that were not comparable to 10% NBF for 24 hours because the man whitney test obtained a value of ($p < 0.05$). So that 10% sucrose shows its potential as a safer alternative.

INDEX TERMS Fixation, 10% NBF, Sucrose, Slide Quality, Occupational Safety and health Administration (OSHA)

I. INTRODUCTION

Histopathology is a branch of science that studies body tissues, the purpose of histology is to determine any disturbances that occur in the cells or tissues tested, so that it can be started that this histopathological examination is the gold standard to determine neoplasm or non-neoplasm abnormalities, and determine the next therapy to be applied [1]. Test materials that are often used in the form of surgical tissue, biopsies taken from anesthesia procedures both in humans and live animals. Tissues that become preparations will be observed under a microscope. Tissue processing consists of several procedures from fixation, staining, and preservation of preparations with entellan or mounting reagents [2]. Preparations with good quality are needed by anatomical pathologists to find out the abnormalities that occur. The most important stage in making preparations is fixation [3].

Fixation is the first procedure in histotechnology or can be referred to as pre-analytical. Fixation One important step in tissue processing is to mature the tissue, The most common error factor caused in the fixation process is tissue that is not perfectly fixed. This causes the tissue to have poor color

intensity and morphology [2]. The purpose of fixation is to preserve the tissue, as well as prepare the tissue to enter procedures that use toxic reagents. The process of protein denaturation during fixation makes cells and their components resistant to further autolysis. Test material that is not perfectly fixed morphology can change and affect its coloring properties [4] fixation is to keep cells and other tissue components in a "life-like state" and inhibit the growth of decomposing bacteria, or decay, in tissue specimens is primarily caused by pre-existing microorganisms within the tissue itself [5].

A good fixative solution has a neutral pH between 6-8. Other factors that can affect the fixation results are the right combination of good penetration, sufficient fixative volume, appropriate concentration, sufficient immersion time, ideal temperature, and selection of fixative type [6]. The most commonly used fixation solution at the fixation stage is Neutral Buffer Formalin 10% (NBF 10%) [3]. The advantages of NBF 10% are a pH that is close to neutral, can fix the tissue in a relatively short time 12-24 hours, and has good color affinity when staining is performed. and the shelf

life in large quantities can be for a long time[7]. NBF 10% has good affinity for dyestuffs[8]. The drawback of this fixation solution lies in its long fixation power, which takes 12-24 hours. The high use of formalin can also have a negative impact on the health of officers. The carcinogenic nature of formalin can cause changes in the cells and tissues of the officer's body, formalin is irritating and corrosive, if formalin vapor is inhaled it is very dangerous for officers. Formalin also has a negative impact on the nervous system and other health problems [9].

Sucrose is an organic compound that belongs to the carbohydrate group. Sucrose is a disaccharide group consisting of two types of simple sugars, namely glucose and fructose [10]. The high sucrose content will hydrolyze into fructose and glucose. In this acidic atmosphere fructose and glucose will turn into aldehydes. aldehydes will form cross-links with amino acids present in the tissue, this binding process will then fixate the tissue. The fixation mechanism is similar to how formaldehyde or 10% NBF works, where aldehyde is key in the process of tissue preservation [11]. Based on the results of trials conducted by [11], it was found that a 10% sugar solution can be an alternative to a fixative solution.

Fixation time is also a success factor in tissue preservation. In general, fixation is carried out in the range of 6-24 hours. In general, 12 hours is a common time used as fixation because 10% NBF is effective at 6-24 hours. Fixation that is carried out for more than 24 hours will make the tissue texture too hard and if it is less than 6 hours the tissue texture is still fragile [12]. As for research conducted by [13] the best fixation results are on day 1 with good HE staining quality. Based on the background description above, Although NBF 10% has a fairly harmful effect on the body, it is still used as the gold standard fixative for histological tissue. Based on previous research, sucrose 10% can be an alternative fixative fluid, but the optimal time for tissue immersion is still unknown. Therefore, the researcher wants to determine the optimal time for fixation using sucrose 10%. The evaluation parameters to be assessed include the quality of color absorption in the cell nucleus, cytoplasm, and color uniformity. The purpose of this research is to :

1. Analyze the quality of mice hepatic preparations that have been fixed with 10% sucrose solution with a time variation of 6 hours, 12 hours, and 24 hours.
2. Comparing the quality results of 10% NBF fixation preparations and 10% sucrose solution.

II. METHOD

The type of research using a quantitative approach with an experimental research to determine the ratio of fixation solution between 10% NBF and 10% sucrose solution with time variations of 6 hours, 12 hours, and 24 hours on the quality of mice hepatic histology preparations stained using the Hematoxylin Eosin (HE) method. The mice hepatic test material was collected at the Faculty of Veterinary Medicine, Universitas Airlangga, located at Mulyorejo Campus C UNAIR Surabaya. Meanwhile, the research will be conducted at the Cytotechnology Laboratory of the Department of Medical Laboratory Technology of the Surabaya Ministry of Health Polytechnic which is located at Jl. Karangmenjangan

No. 18a, Gubeng District, Surabaya. This research will be conducted from January 2025 to March 2025.

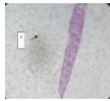
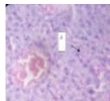
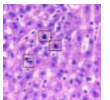
The independent variable used in this study is the use of time variations in the process of fixation of mice hepatic tissue (*Mus musculus*), namely 6 hours, 12 hours, and 24 hours using 10% sucrose solution as an alternative to 10% NBF. The dependent variable used in this study is the quality of histology preparations of mice hepar (*Mus musculus*) that have been stained with Hematoxylin Eosin (HE) method and microscopically.

The samples used in this study were mice hepatic organs (*Mus musculus*). This study required six mice, and tissue sampling was performed by UNAIR veterinary staff. The criteria of healthy and non-deformed mice, mice weighing between 18-35 grams, with male sex, mice can move actively, routine feeding is done for 7 days[14]. The experimental treatment in this study amounted to 4 groups consisting of 3 fixation treatments using 10% sucrose solution with variations in time of 6 hours, 12 hours, and 24 hours and 1 fixation group using 10% NBF which was used as a fixation control. The number of replications obtained is 6. So that the 6-hour fixation group of 10% sucrose solution will be replicated as many as 6 preparations, the 12-hour fixation group of 10% sucrose solution will be replicated as many as 6 preparations, the 24-hour fixation group of 10% sucrose solution will be replicated as many as 6 preparations, as well as the 10% NBF fixation group which is replicated as many as 6 preparations.

Hepar in the body acts as a detoxifier and body homeostasis so that the liver is very vulnerable to substances that are toxic[15]. Mice liver tissue consists of hepatocyte cells. Hepatocytes are large cuboidal or polyhedral epithelial cells, with a large, round, central nucleus and eosinophilic cytoplasm rich in mitochondria. Mitochondria have eosinophilic properties. Liver tissue rich in mitochondria has the ability to absorb eosin color strongly, resulting in a smaller color intensity compared to the nucleus [4].

Hematoxylin Eosin is a gold standard stain used in histology examinations. The College of American Pathologists (CAP) and the National Society for Histotechnology (NSH) determined that Hematoxylin Eosin staining has good quality as a coloring agent, especially in cell nuclei and cytoplasm. Hematoxylin Eosin consists of two types of dyes, namely hematoxylin and eosin. Hematoxylin is a natural dye that can bind to the cell nucleus so that it is colored blue with a weak bond. Eosin is a dye that functions to color the cytoplasm red[16]. Hematoxylin has basic properties, where this substance colors basophilic tissue elements, or tissues that have color affinity to basic dyes such as cell nuclei, because the cell nucleus is rich in nucleic acids (DNA and RNA) which are negatively charged (acidic) so that the cell nucleus is very basophilic. Eosin is acidic so it will color acidophilic tissue components or tissues that have an affinity for acidic dyes. Cytoplasm Most of the proteins have positively charged groups (bases), so the cytoplasm will bind acidic dyes such as eosin and produce a red color [17]. So that the total number of preparations in this study is 24. The score assessment used is as follows:

TABLE 1
Histology Preparation Quality Assessment.

Slide Quality Assessment Criteria				
Score	Cyto Plasma	Nucleus	Colour	Picture
1 Bad	Blue color is not clearly visible	Red color is not clearly visible	The color produced is not uniform and unclear	
2 Good enough	Blue color appears good enough	Red color appears good enough	The color produced is uniform good enough	
3 Good	Blue color appears good	Red color appears good	The color produced is uniform good	

A. PROCESS OF MAKING A 10% SUCROSE SOLUTION

To obtain a concentration of 10% in 500 ml. Therefore, 50 grams of sucrose and 500 ml of distilled water are required. Weigh 50 grams of sucrose, then dissolve it in distilled water and stir until completely mixed. Ensure that the pH of the sucrose solution is 6-7 [18].

Preparations that have passed the staining process will be observed and assessed, to determine the comparison of the quality of preparations fixed using NBF10% for 24 hours and 10% sucrose solution for 6 hours, 12 hours, and 24 hours this study uses a scoring method with an interval scale and ordinal scale. Determination of good preparation quality is based on the cytoplasm and nucleus that have contrasting colors, as well as color uniformity in the preparation. The following is a table of treatment controls for the research to be carried out:

TABLE 2
Treatment Control

Number	group	Treatment
1	A	Tissue Fixation Using 10% NBF for 24 Hours
2	B	Tissue Fixation Using 10% Sucrose Solution for 6 Hour
3	C	Tissue Fixation Using 10% Sucrose Solution for 12 Hour
4	D	Tissue Fixation Using 10% Sucrose Solution For 24 Hour

Data were obtained from the results of microscopic examination of the preparations and scoring was carried out on the quality of the preparations, data in the form of ordinal and nominal scales with the criteria 1 not good, 2 quite good, and 3 very good. Then a statistical test will be carried out using Kruskal Wallis because the data distribution is not normal and the homogeneity between group data is not homogeneous. If the Asymp.Sig result or significant value in the Kruskal Wallis test (<0.05) indicates that there is a significant difference

between the four treatments, it will be continued with the Mann-Whitney test.

The Man-Whiney test is used to determine which groups have differences, by comparing between treatments. If the Asymp. Sig. (2-tailed) value (>0.05) then it indicates there is no difference.

III. RESULTS

Based on research conducted in April 2025 at the Faculty of Veterinary Medicine, Universitas Airlangga, Farma Veterinary Center and Cytohistotechnology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Surabaya regarding the comparison of fixation with 10% sucrose solution and 10% NBF on the quality of mice hepatic preparations with time variations using the Hematoxilin Eosin (HE) staining method. The results were obtained through scoring the criteria of the preparation. Assessment of the quality of preparations in this study is seen based on the blue color that appears on the inticelles, red cytoplasm, and color uniformity in the preparation of preparations. The test material used was the liver of male mice (*Mus musculus*), weighing 18-35 grams, not deformed and healthy, and acclimatized for 7 days. Liver organs are obtained by dissection or necropsy in mice. After the hepatic organs were successfully taken, fixation was immediately carried out using 10% NBF as a standard for 24 hours, and 10% sucrose as a treatment group for 24 hours, 12 hours, and 6 hours.

After fixation with a predetermined time, then proceed with grossing the hepar as a test material and inserting the hepar in the tissue cassette. The tissue is processed on the Tissue Possessing tool consisting of dehydration which aims to remove water in the tissue, clearing to remove residual alcohol due to the dehydration process, then the infiltration process keeps the tissue from shrinking due to the clearing process. After the Tissue Possessing tool is followed by embedding by placing the tissue on the base mold and then filled with liquid paraffin and allowed to stand until the paraffin solidifies, sectioning is done with a microtome and the tissue is sliced 3 µm -5 µm thick. Staining is done with hematoxylin eosin paint and then the preparations are mounted using entelan before reading on a

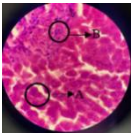
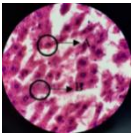
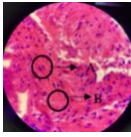
TABLE 3
The Results of The Comparative Assessment Of The Quality Of Tissue Histology Preparations Resulting From The Fixation Of 10% Sucrose Solution And 10% NBF With Variations In Time.

Replication	Slide Quality Assessment			
	10% NBF Solution 24 hours	10% Sucrose Solution 6 hours	10% Sucrose Solution 12 hours	10% Sucrose Solution 24 hours
1	3 (Good)	1 (Bad)	2 (Good enough)	3 (Good)
2	3 (Good)	2 (Good enough)	2 (Good enough)	2 (Good enough)
3	2 (Good enough)	2 (Good enough)	3 (Good)	3 (Good)
4	2 (Good enough)	2 (Good enough)	2 (Good enough)	3 (Good)
5	3 (Good)	2 (Good enough)	2 (Good enough)	3 (Good)
6	3 (Good)	2 (Good enough)	2 (Good enough)	2 (Good enough)

microscope. The following is the data from the research experiments conducted and has been validated by veterinarians from the Farma Veterinary Center.

The assessment results in table 2 were obtained from 24 samples of mice hepatic tissue preparations, which were divided into 4 treatment groups, namely tissue fixed with 10% NBF as group 1, fixation with 10% sucrose solution for 6 hours as group 2, fixation with 10% sucrose solution for 12 hours as group 3, fixation with 10% sucrose solution for 24 hours as group 4. The preparations were stained with Hematoxylin Eosin paint so that when observed under a microscope had cell staining results in the form of cell nuclei appearing blue and cytoplasm appearing red. The following is a description of the results of the assessment of the coloring quality of samples observed under a Microscope.

TABLE 4
Microscope View of The Results.

Score	Description	Picture
1 (Bad)	- A: Cell nucleus does not look blue - B: Cytoplasm does not appear red in color - The whole is not evenly colored	
2 (Good Enough)	- A: The cell nucleus is clearly blue in color - B: The cytoplasm is quite clearly colored red - The whole is colored quite evenly	
3 (Good)	- A: The cell nucleus is clearly blue in color - B: Cytoplasm is clearly colored red - The whole is evenly colored	

Each treatment group was replicated 6 times. The treatment is divided into 4 groups so that the total number of preparations obtained is 24. Based on table 3, treatment group 1 has 2 preparations with a score of 2 (quite good) and 4 preparations with a score of 3 (good). Treatment group 2 there was 1 preparation with a score of 1 (not good) and 5 preparations with a score of 2 (quite good). Treatment group 3 there were 5 preparations with a score of 2 (quite good) and 1 preparation with a score of 3 (good). treatment group 4 there were 2 preparations with a score of 2 (quite good) and 4 preparations with a score of 3 (good).

TABLE 6
Frequency Distribution of Research Results.

Group Treatment	Frequency of Assessment Score			Total
	1	2	3	
A		2	4	6
B	1	5		6
C		5	1	6
D		2	4	6

The frequency distribution of the research results in table 4.3 is determined based on the color quality of the cell nucleus, cytoplasm, and color uniformity in the preparation.

Treatment group A fixation using NBF 10% 24 hours as a control obtained 2 preparations (33.3%) with a score of 2 meaning quite good and 4 preparations (66.7%) with a score of 3 meaning good. Treatment group B which was fixed using 10% sucrose solution for 6 hours obtained 1 preparation (16.7%) with a score of 1 meaning not good and 5 preparations (83.3%) with a score of 2 meaning quite good. Treatment group C which was fixed using 10% sucrose solution for 12 hours obtained 5 preparations (83.3%) with a score of 2 meaning quite good and 1 preparation (16.7%) with a score of 3 meaning good. Treatment Group D which was fixed using 10% sucrose solution for 24 hours obtained 2 preparations (33.3%) with a score of 2 meaning quite good and 4 preparations (66.7%) with a score of 3 meaning good. In this study the data used is nominal scale data, so it does not require normality and homogeneity tests. This test is used to determine whether there is a difference or not between control and treatment[19]. This test is included in non-parametric testing, with the basis for making decisions as follows:

- if the value of Asymp. Sig < 0.05 then H₀ is rejected, and H₁ is accepted, so it can be stated that there is a significant difference between control and treatment.
- if the value of Asymp. Sig > 0.05 then H₀ is accepted, and H₁ is rejected, so it can be stated that there is no significant difference between control and treatment

TABLE 7
Kruskal-Wallis Statistical Test Results.

Treatment Group	Kruskal-wallis		Description
	N	Asymp. Sig	
A	6	0,024	There is a Difference
B	6	0,024	
C	6	0,024	
D	6	0,024	

From table 6, the Asymp. Sig. value obtained is 0.024. Since this value is less than 0.05, it can be concluded that there is a significant difference in preparation quality between the control group and the treatment group. The difference indicates that H₀ is rejected and H₁ is accepted. Identification of significant group differences was performed using the Mann-whitney test.

TABLE 5
Mann-Whitney Test Results

Treatment Group	Mann-Whitney		Description
	N	Asymp. Sig (2 tailed)	
A and B	12	0.018	There is a Difference
A and C	12	0.093	There is no Difference
A and D	12	1.000	There is no Difference

The Mann-Whitney test is a further test conducted after the Kruskal-Wallis test. This test is used to determine the difference by comparing the treatment group with the control group. So as to determine which group has a significant difference[20]. The conclusion of this test is:

- If the value of Asymp. Sig. < 0.05 H₀ is rejected and H₁ is accepted so that it can be stated that there is a difference between the treatment group and the control group.

b. If the value of Asymp. Sig. > 0.05 H_0 is accepted and H_1 is rejected so that it can be stated that there is no difference between the treatment group and the control group.

Based on table 7, it is known that the fixation group that has the same quality as the control is in :

- Group C with 10 % sucrose solution fixation for 12 hours with Asymp. Sig. (2 tailed) 0.093
- Group D with 10 % sucrose solution fixation for 24 hours with Asymp. Sig. (2 tailed) 1.000

Both treatments have an Asymp. Sig. (2 tailed) > 0.05 which indicates that the group has the same quality as the control.

IV. DISCUSSION

Histology is a gold standard examination because this examination can determine abnormalities that occur in cells, both neoplasm and non-neoplasm disorders and can determine the therapy that will be given next to the patient[21]. Fixation aims to maintain the integrity of cell morphology or tissue structure like the initial condition, and prevent decay due to microorganisms [22]. The most commonly used fixation solution is 10% NBF where the repeated use of this solution will cause poor potential for health, based on the grouping by the Occupational Safety and Health Administration (OSHA) formaldehyde is included in class 1 carcinogens. [23].

The alternative solution used to minimize the harmful effects of 10% NBF is a 10% sucrose solution, using a variable soaking time of 6 hours, 12 hours and 24 hours. Based on research [24] sucrose has the potential to be a substitute for fixatives because sucrose when hydrolyzed will break down into fructose and glucose. In an acidic atmosphere, the hydrolysis results will turn into aldehydes. These aldehydes will crosslink with amino acids in the tissue. The bond can ripen or fix the tissue. Because this mechanism is in accordance with the fixation mechanism carried out using formaldehyde or NBF 10%.

In this study, the benchmark is the quality of the preparation which is assessed from the bright blue cell nucleus, pink cytoplasm (eosin), and uniformity of color that is evenly distributed on the preparation. Sucrose crystals will be made a solution with distilled water to a concentration of 10% then the tissue will be fixed with different time variations of 6 hours, 12 hours, and 24 hours. After fixation according to the treatment group, the mice hepatic tissue will go through a histotechnical process from tissue processing (dehydration, clearing, impregnation) to staining using hematoxylin eosin. Preparations that have gone through the process of staining and mounting their cell integrity will be observed microscopically. Each treatment group will be replicated 6 times, the number of tissue preparations that will be observed is 24 preparations. Observations were made using a 100 times objective lens to determine the cell quality of mice hepatic tissue.

Fixation is generally done within 6-24 hours. fixation time also depends on the type of fixative used [12]. In the first 6 hours, the 10% NBF solution will diffuse into the cells and form methylene hydrate. This methylene hydrate will bind to cell protein chains to form reactive hydroxymethyl chains. This chain formation process takes 24 hours. Therefore, the recommended fixation time is 24 hours.

Fixation that exceeds the recommended time can cause irreversible cross-reactions, which will eventually make the tissue hardened and difficult to cut [25].

Based on the observations that have been made, the tissue fixed using 10% sucrose solution with a time variation of 6 hours, 12 hours, and 24 hours shows a difference. Good fixation groups are found in the 12-hour and 24-hour groups. Both groups have the same quality of preparation with NBF 10% fixation 24 hours as gold standard. While the 6-hour group has a different quality with 10% NBF fixation 24 hours as gold standard. This is obtained from the assessment of preparation quality criteria that have been determined. The fixation group of 10% sucrose solution variation time of 24 hours produces quality preparations equivalent to fixation NBF 10% 24 hours as gold standard.

The 10% sucrose solution used as an alternative in this study was effective at 12 hours and 24 hours. This is supported by the results of the quality of the preparations that have been observed. Based on research conducted by [11] tissues fixed with 10% granulated sugar solution for 24 hours have the same quality as tissues fixed using 10% NBF for 24 hours, so 10% granulated sugar solution can be used as an alternative to 10% NBF. Granulated sugar itself consists of 97.1% sucrose. In this study using pure sucrose, and obtained effective results for 12 hours and 24 hours fixation. To produce histology preparations with clear results on the cytoplasm and cell nucleus requires a fixation time of 24 hours [22].

The fixation group of 10% sucrose solution for 6 hours showed a difference with the fixation of 10% NBF for 24 hours as a gold standard, there was 1 preparation with a value of 1 (not good), the cytoplasm and cell nucleus were not all colored, the cells looked shrunken. The difference is because sucrose is included in organic substances so that it requires a longer time to diffuse into the tissue, as well as due to errors when sectioning which is too thick can cause cells to accumulate so that uniformity in the preparation is lacking. Thick tissue cutting causes the cytoplasm and color uniformity to be less obvious[26]. According to[22] fixation carried out under 6 hours will cause the tissue to not be colored perfectly and can experience shrinkage.

The results of scoring the quality of mice hepatic preparations that have been observed microscopically will proceed to data processing using SPSS. The tests used are Kruskal-Wallis statistical test and Mann-Whitney statistical test. The data is processed using the Kruskal-Wallis test because it is included in non-parametric, in this study the data obtained is nominal data so there is no need to test normality or homogeneity. The function of the Kruskal-Wallis test is to determine if there is a difference in all treatment groups, if a difference is found then the statistical test is continued using Mann-Whitney, to find out which groups have significant differences or similarities.

Based on the results of the Kruskal-Wallis test that has been carried out, tissue fixation using 10% NBF for 24 hours and 10% sucrose solution with a time variation of 6 hours, 12 hours, and 24 hours gets an Asymp. Sig. 0.024, this indicates that in these treatments there is a difference in quality. value Asymp. Sig < 0.05 , so H_0 is rejected and H_1 is accepted. The existence of these differences, the statistical

test must be continued using the Mann-Whitney test in order to find out which group has the most significant difference.

In the Mann-Whitney test, each treatment group of 6-hour, 12-hour, and 24-hour sucrose solution fixation will be compared with the 24-hour 10% NBF control to see the difference. If the value of Asymp. Sig. <0.05 , there is a difference between groups, but if the Asymp. Sig. >0.05 then there is no difference between treatment groups. Based on the test that has been done, the group that has no difference in quality with 10% NBF is the 10% sucrose solution fixation group for 12 hours with an Asymp. Sig. 0.093 and the fixation group of 10% sucrose solution 24 hours with Asymp. Sig. 1.000. The 24-hour 10% sucrose solution group has a value equivalent to the 10% NBF group. It can be concluded that fixation using 10% sucrose solution for 12 hours and 24 hours is effective to be an alternative to 10% NBF.

Sucrose 10% solution can be used as an alternative to NBF 10% as a fixation solution with a minimum immersion of 12 hours. The repeated use of sucrose for fixation does not cause harmful effects on its users, the price is affordable and easy to obtain because it can be found in granulated cane sugar. However, tissues fixed using sucrose do not have a long shelf life unlike NBF 10% which can be stored for years. Tissues fixed with sucrose solution can only last 1 to 2 days the rest of the tissue will be destroyed. Sucrose 10% solution is unstable and bacteria can appear in the solution. The presence of bacteria will make the tissue disintegrate.

In this study, there were many preparations with a score of 2 or quite good, this was due to the limited amount of xylol, so that it only used 1 chamber with 4 repetitions. This resulted in the remaining paraffin in the preparation not being maximally clean, so that the cell nucleus and cytoplasm were not maximally colored, and errors in sectioning that were too thick resulted in stacked cells and were difficult to observe the cell nucleus and cytoplasm.

V. CONCLUSION

Based on the research that has been done, mice hepatic tissue fixed using 10% NBF has good preparation quality when observed under a microscope. The cell nucleus appears blue, the cytoplasm is red, and the color uniformity is evenly distributed. This is because NBF 10% itself is the gold standard used in histopathological examination. Mice hepatic tissue fixed using 10% sucrose solution with a time variation of 6 hours, 12 hours, and 24 hours showed a difference. The best group is the fixation of 10% sucrose solution for 12 hours and 24 hours. The 10% sucrose fixation group for 6 hours has poor results because the cell nucleus and cytoplasm are not clearly visible, some cells are also not evenly colored. The group of preparations with 10% sucrose solution fixation treatment for 12 hours and 24 hours after being compared with 10% NBF for 24 hours as a gold standard has no difference, this is judged from the observation of cell nuclei that appear clear blue, clear red cytoplasm, and evenly distributed color uniformity, so that 10% sucrose solution is effective to replace 10% NBF as an alternative fixation with a minimum immersion time of 12 hours. However, if it is going to use 10% sucrose as a fixative solution with other organs, it must be

tested again to see its stability, because 10% sucrose is not the gold standard in histological examination.

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DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

AUTHOR CONTRIBUTION

Juliana Christyaningsih provides advice and directs fixation materials that are suitable for comparison with gold standards. Gabriella Jihan is responsible for the research that takes place, starting from the collection of test materials to data processing, as well as compiling the manuscript. Ratno Tri Utomo helped supervise the course of the research and helped compile the research flow. Evy Dyah helped compile the manuscript that was not right, and gave advice on this research. All authors have reviewed and approved the manuscript and agree to take responsibility for all aspects of the study to ensure its integrity and accuracy.

DECLARATIONS

ETHICAL APPROVAL

This study was conducted by ethical standards and has received approval from the Institutional Review Board (IRB) of Poltekkes Kemenkes Surabaya, Indonesia, with approval number [EA/ 3244 /KEPK-Poltekkes_Sby/V/2025] in this study using experimental animals in the form of mice, all nursing procedures and tissue collection were supervised by experts from the airlangga university of veterinary medicine and the mice used were certified healthy as stated by the certificate number 1021/F.4.A.3.2/04/2025 issued by the farma veterinary center (PUSVETMA). All procedures adhered to ethical guidelines for research involving animal testing.

CONSENT FOR PUBLICATION PARTICIPANTS.

Consent for publication was given by all participants

COMPETING INTERESTS

The authors declare no competing interests.

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