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Comparative Analysis of Mice Kidney Histology Prepared with Xylene and Sunflower Oil as Deparaffinization Agents

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ABSTRACT This study aims to evaluate and compare the effectiveness of xylene and sunflower oil (Helianthus annuus) as deparaffinization agents in preparing histological sections of mice kidneys. Deparaffinization plays a crucial role in histopathological analysis, but the use of xylene presents considerable health and environmental concerns. Thus, sunflower oil is examined as a safer alternative. Kidney tissue samples were divided into control (xylene) and treatment groups (sunflower oil) with deparaffinization durations of 5, 10, and 15 minutes at 50°C. Slides were assessed using histological scoring criteria focused on nuclear detail, cytoplasmic clarity, and staining uniformity. The results demonstrated that sunflower oil for 5 minutes yielded histological quality comparable to xylene, with no statistically significant difference in scoring outcomes. A Kruskal-Wallis test confirmed the similarity between agents at the optimal time point. These findings suggest that sunflower oil has strong potential to replace xylene without compromising staining quality. Moreover, the method supports improved laboratory safety due to reduced toxicity. Limitations of the study include variability in oil composition and the use of a single tissue type. Future research could explore its application on other tissues, cost-effectiveness, and long-term storage compatibility. In conclusion, sunflower oil serves as an eco-friendly and effective alternative for deparaffinization in histology, especially when applied for 5 minutes at 50°C.

INDEX TERM Deparaffinization, Sunflower oil, Xylene, Hematoxyllin, Eosin, mice.

I. INTRODUCTION

Histotechnology is a series of processes in making histology preparations from a tissue starting from the process of making tissue blocks to become preparations that are ready for the staining process[1]so that histology preparations can be observed under a microscope, the quality of histology preparations is influenced by all stages of tissue handling, one of which is deparafinization [2].Before staining a tissue preparation through the deparaffinization process, deparaffinization is an important process in the preparation of histology preparations which aims to remove paraffin from the tissue. Paraffin is a supporting medium in the process of making paraffin blocks[3], is hydrophobic and can inhibit the penetration of dyes into the tissue so that to dissolve the remaining paraffin which is hydrophobic, a non-polar solution is needed. The type of solution commonly used to remove residual paraffin is xylol[4]

Xylol is an aromatic hydrocarbon that is routinely used in histology laboratories xylol is biohazardous produces many toxic effects and is carcinogenic if exposed directly and continuously so it is dangerous both for health and the environment [5] However, despite its effectiveness, xylene poses substantial health and environmental risks. It is classified as a hazardous chemical, known for its neurotoxic, hepatotoxic, and nephrotoxic effects upon prolonged exposure. Inhalation of xylene vapors can lead to respiratory irritation, dizziness, and long-term organ damage, particularly among laboratory personnel frequently handling the solvent.[6] Moreover, its disposal contributes to ecological pollution, prompting regulatory agencies and researchers to explore safer alternatives.

In response to these concerns, various natural and less toxic substances have been investigated as potential substitutes for xylene. Among them, vegetable oils including coconut, olive, and sunflower oil have emerged as promising candidates due to their low toxicity, and availability[7]. Sunflower oil (*Helianthus annuus*), in particular, has garnered attention for its favorable chemical properties and potential compatibility with histological procedures. It possesses solvent like characteristics that may facilitate paraffin removal while preserving cellular integrity[8].

This study is focused on evaluating the potential of sun flower oil from became the deparafinization agent in kidney mice tissue processing because sunflower oil is a type of vegetable oil that made from sunflower seed and contains the content of unsaturated fatty acids, namely oleic acid, reaches 30%. besides that sunflower oil also contains high linoleic acid reaching 50%.[9] oleic acid that contain in the sun flower oil has the characteristics that oleic acid has one double bond in its carbon chain with a non polar and hydrophobic carbon atom chain bond, so sunflower oil is a type of compound that is non-polar [10] besides that the atomic chain structure of oleic acid does not have free electrons so that sunflower oil is a type of non polar solvent. Paraffin in the tissue needs to be removed using a non-polar

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solvent because the nature of paraffin is hydrophobic [11] so that the high content of oleic acid makes sunflower oil that can dissolve only in non-polar solvents and cannot dissolve in water, from the non-polar and hydrophobic properties that make sunflower oil meet the criteria as a paraffin solvent in the deparaffinization process which is useful for removing residual paraffin in the tissue before staining with the help of a temperature of 50ºC which increases the ability of sunflower oil to become a deparaffinization solution because the temperature of 50ºC is the melting point of paraffin [10]. Therefore, a study was conducted using sunflower oil as an alternative material in the deparaffinization process with a temperature of 50ºC with varying times of 5 minutes, 10 minutes and 15 minutes to analyze the potential of sunflower oil as an alternative deparaffinization material and the best temperature and time Contributions of this study include:

1. Analyze the quality of mice kidney histology preparations using xylol in the deparaffinization process including cell nuclei, cytoplasm and color uniformity.
2. Analyze the quality of mice kidney histology preparations using sunflower oil at 50ºC for 5 minutes, 10 minutes and 15 minutes in the deparaffinization process including cell nuclei, cytoplasm and color uniformity.
3. Comparing the differences in the quality of mice kidney histology preparations using xylol and sunflower oil at 50°C for 5 minutes, 10 minutes and 15 minutes in the deparaffinization process.

This paper is organized as follows: Section II reviews related works and theoretical foundations; Section III delineates the research methodology; Section IV presents the results and their analysis; and Section V discusses the implications, limitations, and potential future directions of the study.

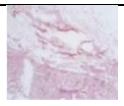
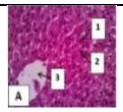
II. METHOD

In this study using a quantitative approach with an experimental research design that aims to determine the cause and effect relationship between research variables [12] and data in this study were obtained through laboratory tests to analyze differences in the quality of histology preparations of mice kidneys in the control group using xylol as a deparaffinization solution and the treatment group using sunflower oil as a deparaffinization solution.

The population used in this study is mice, Mice are considered one of the best and most commonly used experimental animals in laboratory research, particularly for studies related to mammalian biology [13] Additionally, mice are easy to breed due to their short gestation period, which lasts approximately three weeks [14]. Their abundance is supported by the fact that mice reach sexual maturity at around 8 weeks of age and can live for 2 to 3 years. Mice also exhibit variation in body weight, with normal adult males typically weighing between 20–40 grams and females between 25–40 grams [15] In this study, kidney tissue was selected as the experimental organ in mice due to its high susceptibility to toxic substances and its relatively firm consistency[13]. These characteristics make it an ideal histological target, facilitating easier sectioning and staining, thereby enhancing the overall effectiveness and efficiency of the research process.The group in this study is divided into two main groups which control group that using xylol and treatment group that using sun flower. The treatment group was further divided into 3 groups, namely group 1 which was deparaffinized with sunflower oil 50ºC for 5 minutes, group 2 which was deparaffinized with sunflower oil 50ºC for 10 minutes and group 3 which was deparaffinized with sunflower oil 50ºC for 15 minutes. the deparaffinization process was carried out through two immersions in the deparaffinization solution, with each immersion lasting 5 minutes, resulting in a total deparaffinization time of 10 minutes[2].

Accordingly, the use of sunflower oil at a temperature of 50°C for a duration of 5 minutes was selected as one of the treatment conditions, as it represents the time required for a single immersion phase. The 10-minute duration corresponds to the overall time typically required for the complete deparaffinization process, while the 15-minute treatment was designed to extend beyond the standard duration. This consideration was based on the nature of sunflower oil as a natural substance with high viscosity, which may necessitate a longer exposure time to enhance its efficacy in the deparaffinization process. This evaluation was conducted in a blinded manner, whereby the anatomical pathology validator at the Veteriner Farm Center assessed the quality of the histological preparations without being informed of whether the specimens had undergone deparaffinization using xylene or sunflower oil. This approach was implemented to prevent bias and ensure that the assessments were not influenced by any preconceived assumptions regarding the use of an alternative deparaffinization agent. The primary data served by score

Table 1.
Histology Preparation Quality Assessment

Elements of the preparates quality				
Score	Colour	Cyto plasma	Nucleus	Picture
1 Bad	the color produce is not uniform and unclear	The red color of cytoplasm a isn't obvious	The Blue color of nucleus isn't obvious	 [16]
2 Good enough	the color produced is uniform enough	The red color of cytoplasm a is obvious enough	The Blue color of nucleus is obvious enough	 [4]
3 Good	the color produced is uniform	The red color of cytoplasm a is obvious	The Blue color of nucleus is obvious	 [4]

The definitions of the ratings 'good', good enough, and not good were based on the staining quality observed in specific tissue components, namely the cytoplasm and cell nuclei, as well as the overall color uniformity. These criteria were adapted from previous studies that established standardized parameters for histological evaluation [17][16]. The sample size in this study was determined based on the number of treatments (t) and the number of replications (r), applying the Federer formula. The number of treatments (t) in this research was 4, consisting of one treatment using xylene as the gold standard

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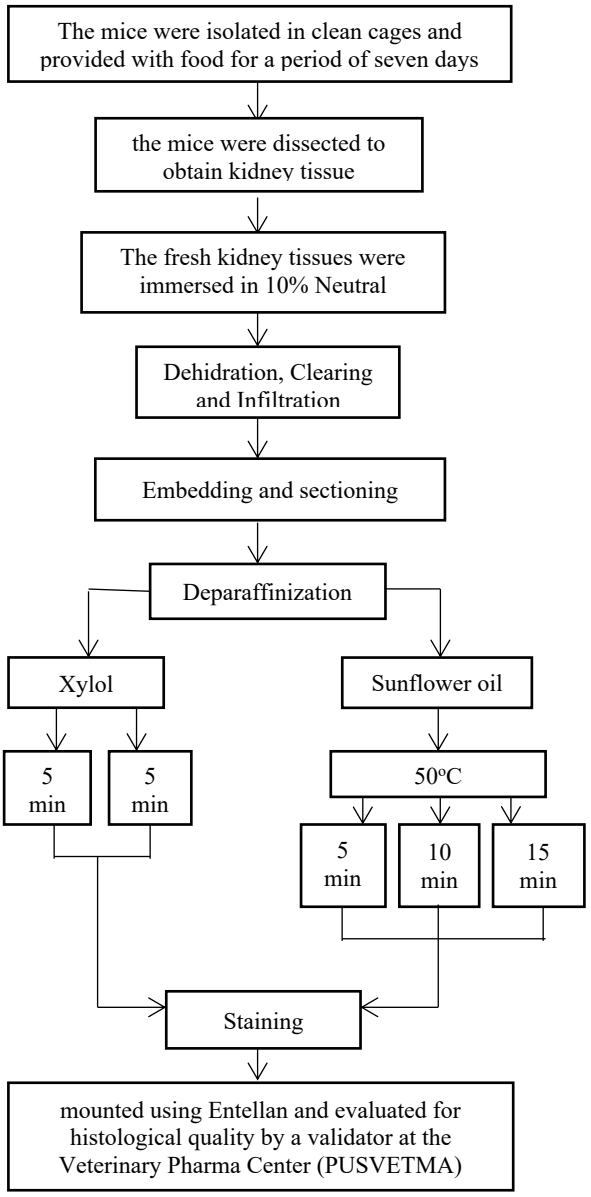
deparaffinization method, and three treatments using sunflower oil at 50°C for durations of 5, 10, and 15 minutes.

Table 2.
Flowchart of study



$$(t-1)(r-1) \geq 15$$
$$(4-1)(r-1) \geq 15$$
$$3(r-1) \geq 15$$
$$3r-3 \geq 15$$
$$3r \geq 18$$
$$r \geq 6$$

Based on this calculation using the Federer formula, the number of replications (r) was determined to be 6 for each treatment group. This means each group consisted of 6 histological preparations: the control group included 6 preparations deparaffinized with xylene, while the treatment groups each included 6 preparations deparaffinized with sunflower oil at 50°C for 5, 10, and 15 minutes respectively This research was conducted at the Faculty of Veterinary Medicine, Universitas Airlangga, located at Campus C Airlangga University, Mulyorejo Subdistrict, Surabaya, Farma Veterinary Center, located at Jl. Ahmad Yani No 68 Surabaya and Cytohistotechnology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Surabaya, located at Jl. Pucang Jajar Tengah No 56 Surabaya This research was conducted from January to May 2025. All procedures conducted in this study were reviewed and approved in accordance with ethical standards for animal experimentation. The dissection of mice was performed by certified animal facility personnel from Airlangga University, following protocols specifically designed to minimize pain and discomfort to the animals. The resulting kidney histological preparations were subsequently validated by a veterinary pathologist from Veterinary Pharma Center to ensure the quality and diagnostic accuracy of the histological specimens analyzed.

III. RESULTS

A. *HISTOLOGICAL QUALITY ASSESSMENT RESULTS*

The results of the preparation quality assessment are validated at veteriner farma center based on several qualitative parameters, including Nuclear Detail Clarity, Cytoplasmic Integrity and Staining Uniformity [17] and were evaluated by pathologist at vetenerary pharma center based on the criteria at table 1 and the results were categorized into qualitative scores as follows

Table 3.
Results of Histology Preparation Quality Assessment

Replication	The result of histology preparate			
	Xylol	Sunflower oil in 5 minutes	Sunflower oil in 10 minutes	Sunflower oil in 15 minutes
1	3 (good)	3 (good)	3 (good)	2 (good enough)
2	2 (good enough)	2(good enough)	2 (good enough)	3 (good)
3	3 (good)	2(good enough)	2 (good enough)	3 (good)
4	3 (good)	3 (good)	3 (good)	2 (good enough)
5	3 (good)	3 (good)	2 (good enough)	2 (good enough)
6	3 (good)	3 (good)	3 (good)	2 (good enough)

Assessment of the quality of the preparation is the result of microscopic observations at 100x lens magnification with oil immersion and Each slide was scored across six replicates per group (xylol and sunflower oil at 5, 10, 15 minutes). Summary of observations are the control group which xylol consistently scored 3 showing obvious nuclear and cytoplasma clarity, the sunflower oil 5 minutes at 50ºC achieved a score of 3 indicating comparable histological quality to xylol In contrast, sunflower oil treatments applied for 10 and 15 minutes predominantly yielded preparations that were assigned a score of 2, indicating moderate histological quality with reduced clarity and preservation compared to the optimal duration. These findings are illustrated in the form of a bar chart to facilitate visual comparison across treatment groups.

Based on Table 4 we can conclude that The quality of mice kidney histology preparations deparaffinized using xylol obtained 5 preparations (83.3%) with a score of 3 which is good and 1 preparation (16.7%) with a score of 2 which is quite good, in the treatment of deparaffinization

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using sunflower oil for 5 minutes obtained 4 preparations (66.7%) with a score of 3 which is good and 2 preparations (33.3%) with a score of 2 which is quite good, In the treatment of deparaffinization using sunflower oil for 10 minutes, 3 preparations (50%) with a score of 3 which is good and 3 preparations (50%) with a score of 2 which is quite good, and in the treatment of deparaffinization using sunflower oil for 15 minutes, 2 preparations (33.3%) with a score of 3 which is good and 4 preparations (66.7%) with a score of 2 which is quite good. Table below presents the frequency distribution of histological preparations across control and treatment variables, illustrating the comparative outcomes based on scoring criteria

Table 4.
Results of Histology Preparation Quality Assessment

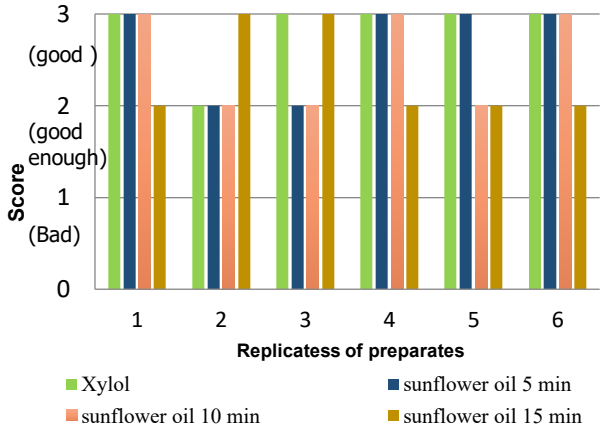

Table 5.
Frequency Distribution of Research Results

Deparaffinization method	Frequency score			Total
	Score 1	Score 2	Score 3	
Xylol		1	5	6
Sunflower oil in 5 minutes		2	4	6
Sunflower oil in 10 minutes		3	3	6
Sunflower oil in 15 minutes		4	2	6

Each prepates replicatess six time in total there is twentyfour preparates in this study this sample quantity was obtained from federer formula to determine whether there is a difference in quality between the control group and the treatment group and whether there is a difference between the control group and each treatment group, data analysis is carried out.

B. DATA ANALYSIS

Data anaylis methode that used in thus study is non – parametric named kruskal – wallis using statistic aplication SPSS. The Kruskal-Wallis test was applied without conducting tests for normality and homogeneity, as the data used in this study are categorical in nature. Therefore, classical assumption tests for normality and homogeneity are not required in this context the purpose of the Kruskal-Wallis non-parametric test is to determine the difference between control and treatment [18] p – value in this study is P = α (α = 0.005) and the conclusions in decision making in the Kruskal-Wallis non-parametric test are :

1. Ho is rejected and H1 is accepted if P < α (α = 0.005) which means there is a significant difference between the control group and the treatment group.
2. Ho is accepted and H1 is rejected if P > α (α = 0.005), which means that there is no significant difference between the control group and the treatment group.

This is the result of data from preparate quality score that processed with non parametric test kruskal wallis using SPSS.

Table 6
The result of kruskal wallis test

Group of Treatment	Kruskal Wallis		Conclusion
	N	P	
Xylol	6	0,350	no significant difference
Sunflower oil in 5 minutes	6		
Sunflower oil in 10 minutes	6		
Sunflower oil in 15 minutes	6		

A Kruskal-Wallis test was performed to evaluate differences among four deparaffinization treatment groups: xylol, sunflower oil for 5 minutes, sunflower oil for 10 minutes, and sunflower oil for 15 minutes. The analysis result p-value 0.350 which P > 0,05 therefore Ho is accepted and H1 is rejected , indicating that the differences observed among the treatment groups were not statistically significant. Based on these findings, it can be concluded that The treatment group has the same quality as the control group so that sunflower oil has the potential to be an alternative material to replace xylol.

IV. DISCUSSION

In this study, natural ingredients are used as an alternative to xylol, namely non-polar vegetable oils that can be an alternative material for deparaffinization[19], one type of vegetable oil is sunflower oil and from the results based on histological validation conducted by Veteriner Farma Center, the treatment group utilizing sunflower oil for 5 minutes demonstrated the highest overall tissue quality. Specifically, 4 out of 6 preparations were rated with a score of 3 (good), while the remaining 2 received a score of 2 (good enogh). In the 10-minute treatment group, 3 preparations were scored 3 and 3 scored 2, whereas the 15-minute group yielded inferior results, with 4 preparations scoring 2 and only 2 scoring 3. These findings suggest that a 5-minute deparaffinization duration using sunflower oil at 50°C is the most effective in preserving the morphological integrity of mice kidney tissues. Longer exposure times of 10 and 15 minutes appear to diminish tissue clarity and staining quality, potentially due to over-treatment or tissue desiccation.

This proves that sunflower oil 50°C in 5 minutes can be an alternative to xylol in the deparaffinization process because have simillar qualitiy with xylol. This can be attributed to the heating of the oil at 50°C[20], which enhances its penetration into tissue structures. Importantly, this temperature corresponds to the melting point of paraffin, allowing the sunflower oil to effectively dissolve and remove residual wax from within the tissue spaces[17]. However, prolonged exposure beyond this duration tends to over-clear the tissues, rendering them excessively

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transparent and diminishing the visibility of cellular components under microscopic examination. Moreover, extended deparaffinization may compromise tissue morphology, leading to structural damage and failure in achieving proper histological visualization[21]. Therefore, a deparaffinization duration of 5 minutes using sunflower oil heated to 50°C represents the most appropriate and effective timeframe, as it ensures sufficient paraffin removal while preserving tissue morphology This study supports research previously conducted by using other types of vegetable oil, namely virgin coconut oil at 50ºC and 60ºC, the results of the quality assessment of preparations with a temperature of 50ºC gave better results while research conducted by [21] using *cedarwood oil* as a natural alternative to xylene in histopathological tissue processing. The researchers found that cedarwood oil demonstrated comparable clearing efficacy to xylene, particularly in nuclear and cytoplasmic staining quality, with statistically significant results in certain tissue types also previous study that highlighted coconut oil as a viable biofriendly substitute for xylene in the deparaffinization process, demonstrating its effectiveness in maintaining tissue integrity and staining quality during routine histological preparations.

The similarity of sunflower oil with oil in previous studies namely virgin coconut oil, cedarwood oil and coconut oil is a type of vegetable oil[22]. Vegetable oils have the ability to dissolve paraffin because they contain oleic acid and are non-polar. Sunflower oil containing high oleic acid oleic acid is an unsaturated fatty acid with one double bond in the carbon chain, the non-polar and hydrophobic carbon atom chain bonds make oleic acid included in the type of non-polar compounds [10] and oils that have non-polar properties are able to remove the remaining paraffin in the tissue [23]

The limitations of sunflower oil in histological deparafinization are

1. Low Oxidative Stability
   The high content of unsaturated fatty acids namely oleic acid and linoleic acid in sunflower oil makes it prone to oxidation when exposed to heat. This leads to rancidity, which negatively affects the oil's quality. As a result, sunflower oil used as a deparaffinization agent is not suitable for repeated use. [8]
2. Chemical Compotition
   The component utilized in sunflower oil for its deparaffinization properties is oleic acid while oleic acid component in the sunflower oil is depends on the type of sunflower oil and the metode used so this variations influenced the efectivity [9][8]
3. Over-deparafinization
   Extended exposure to heated sunflower oil may cause paraffin in tissues to become over clean, reducing contrast and compromising microscopic visualization of cellular structures[21].

The deparaffinization process using sunflower oil requires a longer working process than xylol but sunflower oil does not have a harmful impact on the body so as to minimize the adverse effects on the health of medical laboratory technology personel [24] Moreover, sunflower oil is relatively more affordable compared to xylene and widely available in commercial markets, making it an accessible and practical option for laboratory use. The limitations of this study are that it only focuses on one type of tissue, namely the kidneys of mice, so that its deparaffinization ability cannot be generalized to other types of tissue, but this can be overcome by adding time to tissue types that have a larger size and thickness than the kidneys of mice because the time is adjusted to the type and size of the tissue [25] but this study has proven the potential of sunflower oil as a deparaffinization material that is more affordable and safer for health than xylol.

V. CONCLUSION

The deparaffinization process of histology preparations using xylol and sunflower oil at 50°C for 5 minutes, 10 minutes and 15 minutes overall gives the quality of preparations that are not significantly different from xylol so that sunflower oil can be an alternative material to replace the deparaffinization solution (xylol) but from these treatments, preparations deparaffinized using sunflower oil at 50°C for 5 minutes give better results and it is comparable to xylol. Future research is recommended using different tissue types to evaluate the efficacy of sunflower oil across various histological structures. Additionally, exploring variations in temperature and exposure duration could help determine the most optimal conditions for achieving high-quality staining outcomes. Future studies may also consider testing other plant-based oils with higher oleic acid content, which could potentially enhance staining performance and further reduce reliance on xylene in histotechnical procedures.

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DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

AUTHOR CONTRIBUTION

Juliana Christyaningsih supervised the implementation of the research, contributed to decision-making processes, and was involved in revising the manuscript. Adin Bhagawanti was responsible for designing the study, collecting data, conducting the research, and drafting the manuscript. Ratno Tri Utomo conceptualized the data collection process and participated in both the drafting and revision of the manuscript. Wisnu Istanto provided critical input during the

evaluation of research findings and contributed valuable feedback to the manuscript. All authors have reviewed and approved the final version of the manuscript and have agreed to be accountable for all aspects of the research to ensure the integrity and accuracy of the result in this study.

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/3375/KEPK-Poltekkes_Sby/V/2025] and the research process with experimental animals is carried out with experimental animal ethics together with professionals in the experimental animal cages of the veterinary faculty of airlangga university and using healthy mice declared on a health certificate with no 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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