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Effectivity of uv-light exposure on bacterial and fungal growth in *Channa striata* collagen-chitosan composite dressing for wound healing

Ary Andini[1*], Endah Prayekti[1], Nur Indah Kamaliyah[1], Nur Halimah[1]

[1]Medical Laboratory Technologist Study Program, Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia, 60237;

*Corresponding author:
Ary Andini;
Medical Laboratory Technologist Study Program, Universitas Nahdlatul Ulama Surabaya;
aryandini@unusa.ac.id

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ABSTRACT

Introduction: *Channa striata* collagen-chitosan wound dressing (3:1 v/v) was founded contaminate by bacterial and fungal. Therefore, to suppress bacterial and fungal contamination in *Channa striata* collagen-chitosan composite dressing, it needs several efforts such as sterilization under Ultra-Violet Light Exposure. The study aimed to determine UV-light exposure duration to supress bacterial and fungal growth on *C. striata* collagen-chitosan composite dressing.
Methods: *C. striata* collagen was extracted from skin and scales using 2% HCl for 48 hours, then neutralized using NaOH 1 M until collagen fibers appeared. Chitosan powder dissolved in 2% acetic acid. Afterward *C. striata* collagen and chitosan liquid (1:3 v/v) were mixed and formed until wound dressing was reached. Wound dressing was sterilized under UV exposure for 0, 5, 10, 15, and 30 minutes. Chitosan dressing was used as a negative control.
Results: The results of the study showed that the total number of bacteria and fungi decreased significantly with increasing time of exposure of UV light on composites dressing with a value of p=0.001 for bacterial growth and a value of p= 0.005 for fungus growth. The UV exposure on collagen-chitosan dressing to reduce bacterial and fungal growth proved effective in 10 minutes.
Conclusions: The UV light exposure treatment can reduce bacterial and fungal contamination on collagen-chitosan composites on each addition duration exposure descriptively.

Keywords: Collagen; Chitosan; *Channa striata*; Wound Dressing; Ultra Violet.
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INTRODUCTION

Management and wound care treatment have a critical role to ensure the wound healing process run well.[1] According to the duration of wound or trauma, wound divided into 2 categories such as acute wound which suddenly occurs due to surgical or accident injury, and chronic wound which happened protractedly due to failure progress through healing normal process.[2,3] An acute wound could be a chronic wound due to bacterial infection.[3] Therefore, wound care is needed to prevent and protect the wound from infections of germ, bacterial, and fungal such as wound dressing. Wound dressing is categorized into traditional dressing including cotton wool, bandages, plasters, lint's, gauzes, and modern wound dressing including foams, composites, films, hydrogels hydrocolloids.[1,2,4] Selected biomaterial should be considered as a component wound dressing to ensure wound healing gong faster. Collagen and chitosan are known as excellent biomaterial to produce a various biomedical application.

Snakehead fish (*Channa striata*) has known as traditional food with high protein, amino acid, and other essential nutrients that good for the healing process. *C. striata* contains amino acids such as glycine, glutamine, and arginine that has play role in wound healing. Glycine has a role in connective tissue to collagen synthesis. Glutamine has a main function in the inflammation and proliferation phase of wound healing process. Arginine is required to modulate immune function and give an effect on endothelial function.[5] C. striata collagen from skin and scales, included collagen type-I that is widespread use for wound treatment due to its biocompatibility and biodegradability to achieve faster healing.[6] Collagen from fish is a halal product with some advantages such as Foot and Mouth Disease (FMD), Bovine Spongioform Encelopathy (BSE), and Transmissible Spongioform Encephalopathy (TSE).[4,6,7]

Chitosan is a biopolymer that widely uses for wound healing and artificial muscles. It has excellent bio properties such as non-toxic, bacteriostatic, biodegradable, and biocompatible.[8] Generally, it is isolated from crustacean shells such as shrimp, lobster, crab, prawn, clams, oysters, and squid.[9] Chitosan is one of the best biomaterial choices to develop tissue engineering due to immune-potentiating and promote wound healing.[9] Chitosan is also known as a biomaterial for inflammatory diseases treatment (asthma, sepsis, arthritis, inflammatory bowel disease), drug delivery and trigger in modulating immune responses.[10] Andini and Prayekti (2019) had studied about chitosan as antifungal and antibacterial in *C. striata* collagen-chitosan composite

in a various concentration such as 25% collagen - 75% chitosan, 50% collagen - 50% chitosan, and 75% collagen- 25% chitosan, as a control group, they used film chitosan. Based on results study showed that on wound dressing with a concentration combination of 25% chitosan and 75% collagen was obtained any bacterial and fungal growth. Therefore, to enhance antibacterial and antifungal of *C. striata* collagen-chitosan wound dressing, it needs several efforts such as sterilize it by using Ultra-Violet Light Exposure.[4,11,12] UV light could be used as surface disinfection by removing germicidal.[13]

In the present research, we focused to enhance the quality of wound dressing with 75% *C. striata* collagen - 25% chitosan compound as antibacterial and antifungal dressing by sterilizing it under UV-light exposure with wavelength 280 nm for 0 minute, 5 minutes, 10 minutes, 15 minutes and 30 minutes. The study aimed to determine UV-light exposure duration to supress bacterial and fungal growth on *C. striata* collagen-chitosan composite dressing.

METHODS

Materials

Skin and scales of *C. striata* that obtained from fish seller at the Karah fish market, Karah Village, Jambangan District, Surabaya, East Java Province, Indonesia. Hydrochloric acid technical grade reagents, Natrium Hydroxide technical grade reagents, paper filter Whatman No. 93 with porous 10 µm. Chitosan for making collagen-composite dressing was isolated from black shrimp that collected from Monodon (Marine Natural Product), chitosan powder sized 100 mesh with food and medical grade, Acetic acid technical grade reagents. Sterilization UV device with UV Lamp EVACO 10 Watt build by CV. Kasriani Rahayu. Total bacterial count used Nutrient Agar (NA) Merck1.05450.0500 and Total fungal count used Potato Dextrose Agar (PDA) HI media M096-500G. Bacterial and fungal growth count used Electric bacteria colony counter (H-EBCC) Health, autoclave All American Electric 75X Volume 39 Liter.

Extraction of *Channa striata* Collagen

Collagen extracted by macerating *C. striata* skin and scales in hydrochloric acid 2% (1:8 w/v) for 48 hours. Afterward, residue and filtrate were separated by using paper filter. The filtrate was neutralized by Natrium Hydroxide 1 M until collagen fibers appear (approximately until pH-7). Collagen was filtered and stored in the fridge until ready for use.[4,12]

Fabrication of *Channa striata* Collagen-Chitosan Composite Dressing

Chitosan powder was dissolved in acetic acid 1% by using a magnetic stirrer. Afterward, collagen liquid mixed into chitosan liquid by using a stirrer until homogeneous. Collagen-chitosan liquid molded and covered using gauze, then dried at room temperature.[12] Furthermore, *C. striata* collagen-chitosan divided into 6 groups such as C- as a negative control group which containing pure chitosan, positive control (C+) group for collagen-chitosan dressing without UV light exposure, C1 group for dressing with UV light exposure for 5 minutes, C2 group for dressing with UV light exposure for 10 minutes, C3 group for dressing with UV light exposure for 15 minutes, C4 group for dressing with UV-light exposure for 30 minutes.

Antibacterial assay of composite dressing

Antibacterial assay was determined based on Total Plate Count method. As growth bacteria media, we used nutrient agar (NA) Merck1.05450.0500. NA media made by 20 gr NA powder which was dissolved in 1 L aquadest, then stirring on hotplate until homogenous achieve. NA media sterilization was done by using autoclave for 15 minutes at 121 °C.

Chitosan dressing and *C. striata* collagen-chitosan dressing were weighed about 0,1 gram and dissolved in 9,9 ml saline water 0,85 %. Afterwards, dressing liquid was taken about 1 ml and poured into petri dish, then added 15 ml NA media on it. Furthermore, it should be incubated for 24 hours at temperature 37°C, and finally total bacterial counted by using colony counter.

Antifungal assay of composite dressing

Antifungal assay on wound dressing used Total Plate Count Methods. As preparation, Potato Dextrose Agar (PDA) HI media M096-500G liquid made by PDA powder 39 gram which was dissolved in aquadest 1 L and stirred on hotplate until homogenous obtained. PDA liquid was sterilized in autoclave for 15 minutes at 121°C.

Each dressing was weighed about 0,1 gram and dissolved in 9,9 ml saline water 0,85% until homogeny and samples liquid obtained. Furthermore, samples 1 ml poured into petri dish and PDA liquid 15 ml poured also onto samples. Afterwards, samples should be incubated for 2-3 days at temperature 37°C and fungal growth total counted using colony counter.

Data analysis

Data were analyzed statistically by using SPSS with Kruskal Wallis test and Mann Whitney test, also the results were presented as mean ± standard deviation (SD). When *p*-value less than 0.05 was indicated any significant differences.

RESULT

Antibacterial activity

Total bacterial count on chitosan dressing (C-) was zero bacterial contamination, but on dressing collagen-chitosan without UV-light exposure (C+) had the highest bacterial growth about 4.26 ± 0.14 Log CFU gr^{-1}. Hereafter, collagen-chitosan dressing with UV light exposure for 5 minutes (C1) about 4.19 ± 0.17 Log CFU gr^{-1}, 10 minutes (C2) about 3.98 ± 0.16 Log CFU gr^{-1}, 15 minutes about 4.17 ± 0.05 Log CFU gr^{-1}, and 30 minutes about 4.16 ± 0,04 Log CFU gr^{-1}. Descriptively, the lowest bacterial contamination was *C. striata* collagen-chitosan dressing sterilized under UV-Light exposure for 10 minutes. Furthermore, the data obtained was analyzed statistically using the Kruskal Wallis Test (Table 1). Kruskal Wallis test results showed a significant difference for each group with a *p*-value of 0.005. The total number of bacterial growths which statistically analyzed using Mann Whitney test showed a significant

Table 1. Mean and Standard Deviation (SD) of total bacterial count on each wound dressing group.

Groups	N	Mean (Log CFU gr^{-1})	SD (Log CFU gr^{-1})	*p*-value*
C-	5	0.00	0.00	
C+	5	4.26	0.14	
C1	5	4.19	0.17	
C2	5	3.98	0.16	0.005[a]
C3	5	4.17	0.05	
C4	5	4.16	0.04	

**p*-value for Kruskal Wallis test at each group
[a]Statistically significant

Table 2. Mann Whitney test of total bacterial count between groups.

	C-	C+	C1	C2	C3	C4
C-	-	0.005[a]	0.005[a]	0.005[a]	0.005[a]	0.005[a]
C+		-	0.602	0.047[a]	0.249	0.175
C1	-	-	-	0.059	0.600	0.602
C2	-	-	-	-	0.116	0.117
C3	-	-	-	-	-	0.832
C4	-	-	-	-	-	-

[a]Statistically significant

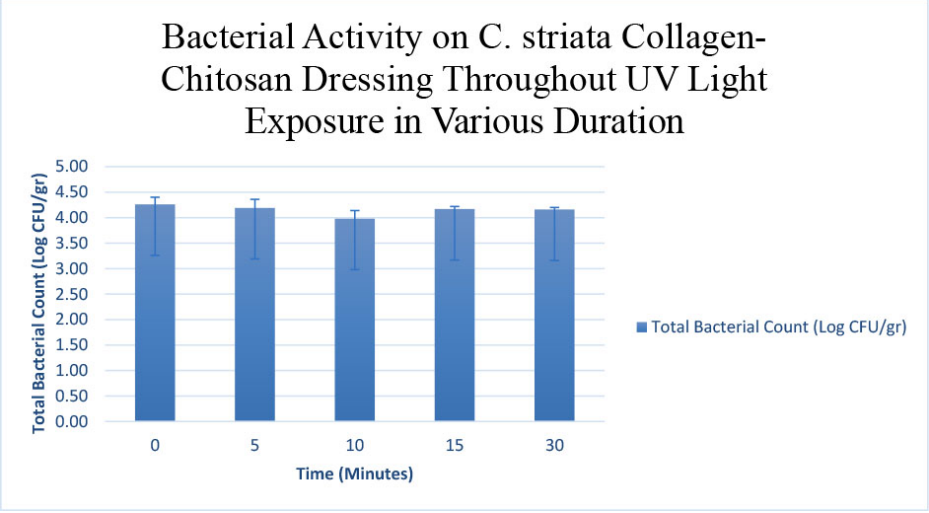

Figure 1. Total bacterial count on each group of wound dressing.

difference between C- group and others group. However, only C2 group had a significant difference with C+ due to p = 0.047 (p<0.05). The other group were no significant differences with C+ due to p ≥ 0.05 (Table 2).

Antifungal activity

Chitosan dressing (C-) had zero fungal contamination. However, collagen-chitosan dressing on C+ group had the highest fungal growth about 3.56 ± 0.11 Log CFU gr^{-1}, followed on C1 group about 3.47 ± 0.08 Log CFU gr^{-1}, C2 group about 3.19 ± 12.00 Log CFU gr^{-1}, C3 group about 3.36 ± 0.14 Log CFU gr^{-1}, and C4 about 3.16 ± 0.49 Log CFU gr^{-1}. Based on Kruskal Wallis test showed p=0.001 which was proven to a significant difference of each group. Furthermore, Mann Whitney test showed any differences between C- and another group. However, there were differences between C+ with C2 (p=0.009), C3 (p=0.046) and C4 (p=0.047). The best one was C2 due to also had a significant difference with C- and C+ group (p=0.009). Mann Whitney test results of the total fungal count could be seen in table 4.

DISCUSSION

Wound healing is always associated with several aspects, one of which is a microbial imbalance which can cause long-term inflammation caused by the results of microbial metabolites. The use of wound dressing needs to pay attention to the cleanliness and sterility of the wound dressing so as not to cause irritation and allergies. Research on natural-based composites has been done to create wound dressings that can prevent allergic reactions due to chemicals. Collagen and chitosan are natural biopolymers that have the ability as cell scaffolding with biocompatible properties.[14] Collagen has biological activity that is able to form a coagulum, activate neutrophils and fibroblast cells.[15] Whereas chitosan has non-toxic biodegradable properties, and has antibacterial and fungal properties.[16,17] Research has proven that chitosan has antifungal properties that can inhibit mycelium extension and alter the morphology of the mycelium.[18] Chitosan-collagen composites have also been shown to play a role in the process of wound healing in rats strain Wistar by increasing the number of fibroblasts and new blood vessels.[11]

In this study, collagen derived from snakehead fish (*C. sriata*) was used because this fish is one of the freshwater fish that is easily obtained and has an amino acid content that can reduce pain and speed up the healing process.[5] Collagen extraction is carried out aimed at removing unwanted fat, protein and getting acid-soluble collagen.[6] Chitosan as wound dressing biomaterial could promote adhesion, homeostasis, and re-epithelialization of wound healing.[3]

Based on result study in table 1 and table 3 on chitosan dressing (C0) showed zero growth bacterial, and fungal. Chitosan has a polycationic structure that is capable of being an anti-microbial agent by electrostatic interactions between the polycationic structure and the dominant anionic component of microorganisms.[19] Basically, polycationic activity can be influenced by molecular weight, degree of substitution, concentration, type of microbe, and type of functional group in chitosan.[20]

Table 3. **Mean and Standard Deviation (SD) of total fungal count of wound dressing group.**

Group	N	Mean (Log CFU gr^{-1})	SD (Log CFU gr^{-1})	*p*-value*
C+	5	0.00	0.00	
C-	5	3.56	0.11	
C1	5	3.47	0.08	
C2	5	3.19	0.12	0,001[a]
C3	5	3.36	0.14	
C4	5	3.16	0.49	

**p*-value for Kruskal Wallis test at each group
[a]Statistically significant

Table 4. **Mann Whitney test of total fungal count between groups.**

	C-	C+	C1	C2	C3	C4
C-	-	0.005[a]	0.005[a]	0.005[a]	0.005[a]	0.005[a]
C+	-	-	0.115	0.009[a]	0.046[a]	0.047[a]
C1	-	-	-	0.009[a]	0.115	0.173
C2	-	-	-	-	0,066	0,248
C3	-	-	-	-	-	0,916
C4	-	-	-	-	-	-

[a]Statistically significant

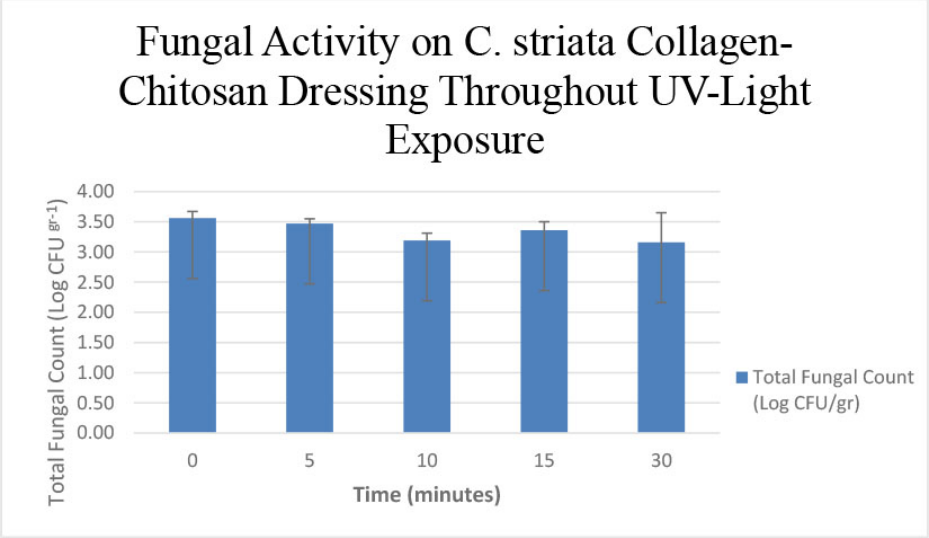

Figure 2. Total fungal count on each group of wound dressing.

Collagen-chitosan composites with comparison 75%:25% component had high potential for contamination. This is caused by the high content of collagen compared to chitosan. Andini and Prayekti's research in 2019 showed that higher collagen content compared to chitosan caused higher contamination.[11] The less chitosan content will reduce the antimicrobial properties it has. Collagen itself does not have antimicrobial properties and it can be broken down by protease enzymes. If collagen is contaminated with bacteria and fungi that produce proteases, bacteria and fungi can grow in the presence of collagen.[21]

Uncontrolled or excessive use of UV-light can cause ineffectiveness of UV-light. Therefore, the distance and duration of exposure to UV light must be in accordance with the material to be sterilized.[22] The time of UV exposure in reducing the number of bacteria and fungi in a material needs to be determined to optimize the sterilization process. The duration of UV exposure tested in this study was 5 minutes, 10 minutes,15 minutes and 30 minutes at a wavelength of 280 nm. The results of this study indicate that UV sterilization of 75% collagen-chitosan composites, was able to inhibit the growth of bacteria and fungi in collagen-chitosan composites. The results of this study indicate that UV sterilization in 75% collagen-chitosan composites, was able to inhibit the growth of bacteria and fungi in collagen-chitosan composites (Figure 3). The best time to reduce bacteria and fungus in this study is 10 minutes (Figure 1 and Figure 2).

Based on the research results, it was found that there was a decrease in the number of bacteria after sterilization of exposure to UV light with a different number of bacteria in each treatment. In C2 group there was a decrease in the number of bacteria that was quite a lot, while in C3 and C4 treatment there was a slight decrease. This could happen due to the interaction between UV light and genetic material in bacteria and fungi depends on the wavelength and the length of time of exposure. Low exposure to UV rays could cause irreparable biological damage to bacterial cells. But excessive exposure to UV rays can also cause a decrease in the ability of UV rays.[23]

Apart from the difficulty of using good UV rays to inhibit bacterial growth, UV rays are also able to damage collagen content in the form of damage to collagen synthesis by reducing the process of controlling gene expression and breaking the collagen chain.[24,25]

The results study showed that the optimum time for UV light exposure to inhibit bacterial and fungal growth were 10 minutes. These results are in line with research conducted by Ariyadi and Dewi (2009) and Rahayu (2017) which explain that exposure to UV light for 10 minutes can inhibit bacterial growth.[26,27]

UV light sterilization is effective to kill contamination microorganisms on the surface of medical device through inhibiting bacterial proliferation and bacterial DNA mutation. Particularly, UVC could reduce of vegetative bacteria (99.9%) and *C. difficile* 99.8% sterilization.[28] Nowadays, UVC-LED model with 280 nm wavelength has been developed to inactivate the effect of microorganism such as *Bacillus subtilis, E.coli,* adenovirus, MS2, Qβ, and ϕX174 with log inactivation approximately 4, 4, 3, 4, 2, 1, 3, and 2 respectively accord to UV dose.[29] Furthermore, sterilization

methods by LED's emitting DUV with 280 nm wavelength was developed and proven has an effect to inactivation of pathogenic *E. coli* and *L. innocua*.[30] The use of UV light with an irradiation level at 254 nm can also be used for surface disinfection at hospital facilities, at 254 nm it takes 40 minutes to disinfect.[31]

According to Andini and Prayekti's research, it showed that 75% collagen with 25% chitosan composites had bacterial and fungal contamination, this was caused by a higher collagen content compared to chitosan.[11,12] The sterilization process on chitosan collagen composites is done to prevent contamination from microorganisms such as bacteria and fungi. UV exposure on composites is able to maintain material sterility.[4] UV spectrum divided into UVA with a wavelength of 400 nm to 315 nm, UVB with 315 nm to 280 nm, and UVC with 280 nm to 200 nm. UVC[32], UV spectrum entirely could inhibit or inactivation of microorganisms, but based on Martin et al (2008) showed UVC with 265 nm was an optimum wavelength as being the most germicidal effect. However, based on Walker et al (2013) UV light exposure with 254 nm could damage DNA microorganism by denaturalizing formation of pyrimidine dimmers that caused blocking DNA replication therefore inactivation microorganisms happened.[33] Besides, the mechanism of influence of UV light exposure on microorganisms also begins with absorption of UV rays by the nucleic acids of microorganisms without damaging the cell surface. UV energy that has been absorbed can cause the formation of bonds between thymine molecules into thymine dimers, causing disruption of the function of nucleic acids and cause the death of microorganisms.[22]

Walker et al (2013) had studied about disinfection of fish surgical tools by using UV light for three different periods, namely 2, 5 and 15 minutes. Based on their research showed that UV light exposure with wavelength 254 nm was effective to prevent *A. salmonicida*, *R. psychrophilum* and *R. salmonninarum* for some surgical tools.[33] Also, Ariyadi and Dewi's research showed that UV light exposure which can kill microbes were 10 minutes and 15 minutes.[34] However, based on Katara et al (2008) study showed that UV light exposure for 30 minutes at ≤ 8 feet could inactivation bacteria approx 4 log reduction.[13] The use of UV rays for disinfectants has also been tested in the pure culture of several types of pathogenic bacteria. The distance and duration of exposure to UV light affect the decrease in the number of pathogenic bacterial colonies and fungi tested. Multidrug bacteria tested in the study were MDR-*Pseudomonas aeruginosa*, MDR-*Acinetobacter baumannii*, *Methicillin-Resistant Staphylococcus aureus* (MRSA), *Vancomycin-Resistant Enterococcus faecium* (VRE), *Mycobacterium abscessus*. However, UVC exposure within 5-15 minutes at 1 m distance in vitro could kill *A. fumigatus* that causing human disease by reducing more than 3 log10 CFU/cm.[2,35]

CONCLUSION

This research made a composite for wound dressing using collagen from snakehead fish (*C. striata*) with a ratio of collagen:chitosan concentration at 75%: 25%. The UV light exposure treatment can reduce bacterial and fungal contamination on collagen-chitosan composites on each addition duration exposure descriptively. But, statistically antibacterial and antifungal effect were proved effective in 10 minutes. Significant difference (p-value <0.05) for decreasing bacterial and fungal contamination was seen in the treatment groups of 0 minutes and 10 minutes. As the future research, needs to explore the chemical stability and mechanical properties of the constituents of UV light exposure wound dressing conducted in vivo.

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AUTHOR CONTRIBUTION

All authors contributed to this study's conception and design, data analysis and interpretation, article drafting, critical revision of the article, final approval of the article, and data collection.

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CONFLICT OF INTEREST

There is no conflict of interest for this manuscript.

ETHICAL CONSIDERATION

Animal studies were approved by Health Research Ethics Committee Universitas Nahdlatul Ulama Surabaya (UNUSA).

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