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The Protective Effects of UV-blocking Spectacle Lenses and Class 1 UV-blocking Contact Lenses (Senofilcon A) on Superoxide Dismutase-2 and Catalase Expression in Lens Epithelial Cells Following UVB Radiation Exposure: An Experimental Study in Animal Model

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ABSTRACT

Introduction: To determine SOD2 and CAT expression in lens epithelial cells with the protection of UV-blocking spectacle lens and UV-blocking contact lenses after UVB radiation. **Methods:** 32 eyes of *Rattus norvegicus* mice were subjected to UVB radiation from Philips PL-S 9W/01 narrowband 311 nm lamps at a distance of 18 cm for 30 minutes. Divided into four groups: a control group, P1 group exposed to radiation, P2 group wearing UV-blocking spectacle lens, and P3 group wearing UV-blocking contact lens. The mice were euthanized three days later and had their eyes removed. Immunohistochemistry analysis was used to evaluate the expression of SOD2 and CAT. **Results:** P1 demonstrated a statistically significant SOD2 expression (p=0.002) compared to the control group. Neither P2 nor P3 exhibited significant differences in SOD2 expression (p=0.693; p=0.365). The SOD2 of the P1 group was significantly different compared to the P2 and P3 groups (p=0.007; p=0.023). No statistically significant difference between groups P2 and P3 (p=0.606). The CAT levels in the P1 group significantly differed from the control group (p=0.001). The differences between the P2 and P3 groups and the control groups were statistically insignificant (p=0.894; p=0.120). CAT P1 group had a significant difference with P2 and P3 groups (p=0.001; p=0.033). No statistically significant difference was observed between groups P2 and P3 (p=0.154). **Conclusions:** UV-B exposure decreases the expression of SOD2 and CAT. Both protection can provide equally good protection.

Keywords: SOD2, CAT, epithelial lens, UVB, UV-blocking spectacles, UV-blocking contact lens.

INTRODUCTION

Several risk factors are known to cause cataracts to appear sooner, one of which is UVB (ultraviolet B) radiation. UV radiation is emitted naturally by the sun, and almost everyone is exposed to it every day. A certain amount of UVB radiation can contribute to the development of cataracts. [1]

UV radiation initiates the creation of ROS (Reactive Oxygen Species), leading to lens epithelial cell death and, eventually, lens opacification. ROS are free radicals characterized by their instability and possession of unpaired electrons. Oxidative stress can arise from an imbalance between the generation of ROS and the body's defense mechanisms against them, resulting in potential cell damage. This damage includes lipid peroxidation and DNA fragmentation (Deoxyribonucleic Acid), leading to apoptosis and cell death. To reduce the development of cataracts, individuals can shield themselves from UVB radiation by wearing UV-blocking spectacles or UV-blocking contact lenses. [2,3,4]

Oxidative stress induced by ROS has been documented in various medical conditions, especially in age-related conditions like cataracts. The lens contains numerous antioxidant enzymes that directly interact with low molecular weight ROS for detoxification and carry out enzymatic reactions.[5] Eukaryotic organisms depend on crucial antioxidant enzymes, such as catalase (CAT) and superoxide dismutase-2 (SOD2), to convert reactive oxygen species (ROS) into less reactive substances such as water and oxygen via biochemical process. These enzymes are crucial in reducing oxidative stress by enhancing the defense mechanisms of antioxidants that regulate levels of ROS through specific ROS-reducing agents, effectively removing surplus free radicals and thus protecting the lens's structural integrity. [6,7]

This study shows that the effectiveness of UV-blocking eyewear and class I UV-blocking contact lenses (Senofilcon A) is manifested through the modulation of SOD2 and CAT expression in lens epithelial cells. The impact of these interventions was evaluated by contrasting the expression levels of SOD2 and CAT in lens epithelial cells of normal (negative control) *Rattus norvegicus* mice. Furthermore, a comparative analysis was conducted to assess the protective capacity of UV-blocking

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spectacle lenses in comparison to class I UV-blocking contact lenses (Senofilcon A).

MATERIALS AND METHODS

This research represents a legitimate experimental laboratory study conducted on male *Rattus norvegicus* mice procured from locally accredited breeders. The mice were aged 6-8 weeks, weighing 250-300 grams, and were in good health with normal body and ocular conditions. The dropout criteria consist of mice who are sick, deceased, or experiencing infectious issues after therapy. The mice were subjected to treatment in adherence to the guidelines established by the Association for Research in Vision and Ophthalmology (ARVO) for the Use of Animals in Ophthalmic and Vision Research. Intramuscular administration of ketamine hydrochloride at a rate of 5 mg/kg and xylazine at a rate of 20 mg/kg were utilized to induce anesthesia. After 15 minutes, two doses of 1% tropicamide eye drops were utilized to dilate the left eye (Cendo mydriatil, Cendo, Bandung-Indonesia). A hole drape completely covered the mice, leaving only the right eye uncovered, while the left eye was shielded by a patch. The exposure of the subjects to UV-B radiation from Philips PL-S 9W/01 narrowband 311 nanometer UV lamps was quantified using a UV-340A ULTRON radiometer at a wavelength of 310 nanometers. Radiation reaches the eye from a distance of 18 cm for 30 minutes, during which the average intensity of the radiation is 0.361 mW/cm2 (7.2 times the maximal sunlight exposure on the cornea). As a consequence, the mean energy is 0.650 J/cm2.

Subjects were assigned to experimental groups by a randomization process. A total of 32 mice were utilized, with five mice designated to group K0, and nine mice each to the P1, P2, and P3 groups. Group K0 served as the negative control, comprising mice that were not subjected to UVB radiation. The mice in Group P1 were exposed to UVB radiation without any protective measures. Group P2 consisted of mice that were exposed to UVB radiation while equipped with UV-blocking spectacles (Crizal Easys, Essilor), where the UV-blocking lens was placed on the right eye using a wire support, positioned 1 cm from the eye. For Group P3, the mice were exposed to UVB radiation while wearing protective contact lenses (Acuvue OASYS; Johnson & Johnson). The use of contact lenses altered the size of the mice's eyeballs. The contact lenses were crafted using a 5 mm diameter puncher under a microscope during the aseptic cutting process. The mice were euthanized three days after exposure to UVB light, and their eyes were harvested. Eye tissue samples were collected and preserved in a 10% buffered formalin solution. Immunohistochemistry analysis is planned to assess the expression levels of SOD2 and CAT, using the HRP/DAB UltraVision Detection System Anti-Polyvalent IHC reagent from Thermo Scientific. For solution preparation, CAT will be diluted in diluent at a ratio of 1:100, requiring a volume of 5cc, and SOD2 will be diluted in the same manner.. The Faculty of Veterinary Medicine at Airlangga University in Surabaya served as the site for both animal research and preparation of the necessary substances. The Immunohistochemistry (IHC) analysis was conducted within the Department of Anatomical Pathology, which is associated with Airlangga University's Faculty of Veterinary Medicine. The commencement of animal experiments took place in March 2023, while the immunohistochemistry analysis was carried out from April to August 2023.

Ethical Approval

The ethical approval for the feasibility study was granted by the Research Ethics Commission of the Faculty of Veterinary Medicine Animal Care and Use Committee (ACUC) at Airlangga University Surabaya On March 3, 2023, with the reference number 2.KEH.028.03.2023.

Statistical Analysis

Data pertaining to the expression scores of SOD2 and CAT were obtained utilizing the modified Remmele technique, which computes the Immuno Reactive Score (IRS) through the multiplication of the percentage of cells or areas displaying positive immunoreactivity by the color intensity score assigned to these cells/areas. The standardized representation for the percentage of positive cells is delineated as follows: 0 indicates the absence of positive cells; 1 denotes less than 10% positive cells; 2 indicates positive cells ranging from 11% to 50%; 3 represents positive cells spanning from 51% to 80%; and 4 signifies more than 80% positive cells. Attainment of a color reaction intensity score of 3 indicates a robust color reaction, while a score of 0 signifies the absence of any color reaction. The dataset for each specimen is composed of the mean IRS value calculated from five fields of view at 400x magnification using a Nikon H600L light microscope fitted with a 300-megapixel DS Fi2 digital camera and Nikon Image System software.

The normality of the data was assessed by applying the Shapiro-Wilk test. A homogeneity test also utilized to assess the homogeneity of the data. After analyzing comparative data regarding the expression of SOD2 and CAT using the Kruskal-Wallis test, the post hoc Tukey's multiple comparison test was applied. The descriptive data was analyzed and displayed as the mean ± standard deviation using the binomial test. The significance level of a p-value is set below 0.05. Statistical analysis was performed using SPSS 25.

RESULTS

Figure 1 displays the expression of SOD2. The expression of SOD2 in group P1 was the lowest compared to the other groups. Groups P2 and P3 had decreased SOD2 expression in comparison to group K but elevated levels in comparison to group P1. The expression in P2 and P3 was analogous. The IRS reported that the mean expression of SOD2 was 4.40 in group K, 2.00 in group P1, 4.11 in group P2, and 3.73 in group P3.

The SOD2 significant difference test findings indicated that treatment group P1 exhibited a statistically significant difference compared to the control group (p=0.002), while P2 and P3 had no significant difference with the control group (p=0.693; p=0.365). A notable distinction was observed between the control group and the P1 group (p=0.002). There was a significant difference between P2 and P3 compared to the P1 group (p=0.007; p=0.023). The P2 group was not significantly different from the P3 group (p=0.606). Figure 2 displays the boxplot for the SOD2 real difference test.

Figure 3 displays the CAT expression. The P1 group had the lowest CAT expression microscopically compared to the other groups. Groups P2 and P3 had robust expression levels, nearly equivalent to the control group. Group P3 exhibits an expression that is more potent than group P1 but less powerful than group P2. The IRS reported that the average CAT expression was 6.96 in group K, 3.18 in group P1, 6.82 in group P2, and 5.38 in group P3.

The outcomes derived from the CAT significant difference test indicated a significant differentiation between treatment group P1 and the control group in a statistically significant manner (p=0.001), while treatment groups P2 and P3 did not display any significant variance from the control group (p=0.894; p=0.120). The control group revealed a marked difference with the P1 group (p=0.001). Furthermore, both P2 and P3 presented a significant differentiation from the P1 group (p=0.001; p=0.033). A statistically insignificant difference did not exist between groups P2 and P3 (p=0.154). Figure 4 displays the boxplot for the CAT real difference test.

DISCUSSION

SOD2 is an enzymatic antioxidant that provides a defense that works by converting O2- into H2O2, where H2O2 can protect cell membranes from damage caused by ROS. However, decreased levels of SOD2 can

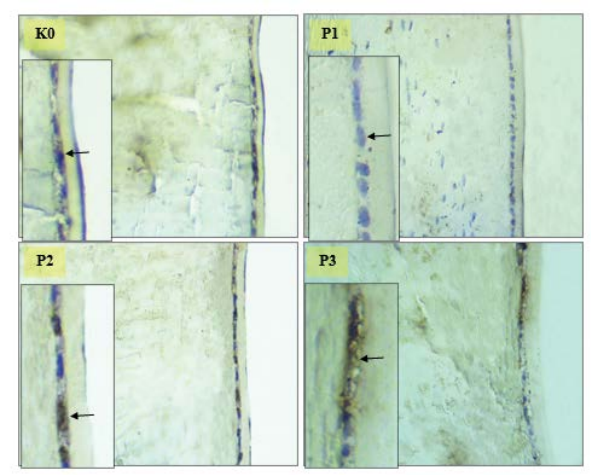

Figure 1 Comparison of SOD2 expressions. Comparative analysis of SOD2 gene expression. Arrows show lens epithelial cells expressing SOD2. Group P1 appeared to be the weakest compared to other treatment groups. (Immunohistochemical staining, objective lens 40x; bar = 50 microns; Eclipse E-i microscope; DS Fi2 300 megapixel camera).

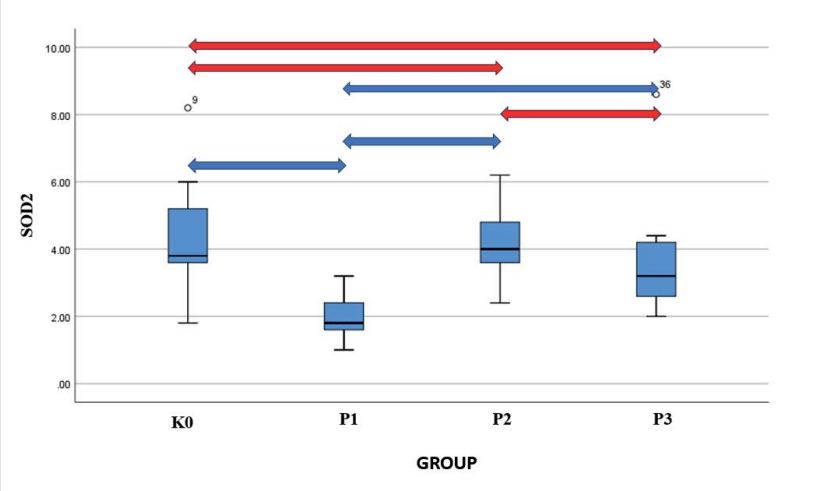

Figure 2 Boxplot comparison of SOD2 expressions. The red line denotes a statistically non-significant difference, while the blue line indicates a significant difference. Group P1 exhibited a notable distinction from the control, P2, and P3 groups. There was no significant difference between the P2 and P3 groups.

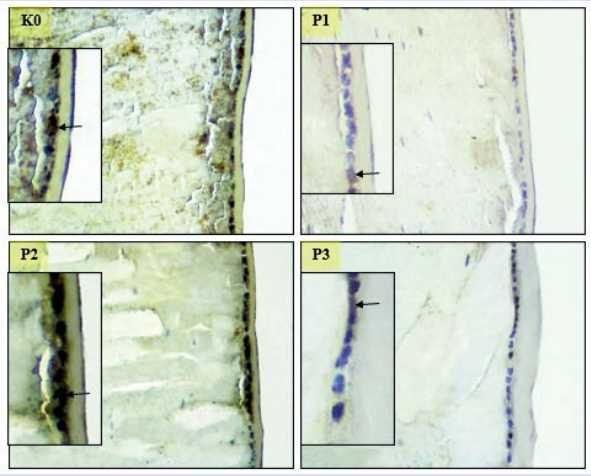

Figure 3 Comparison of CAT expression. The arrows point to lens epithelial cells that contain CAT. The P1 group appeared to be the weakest compared to the other treatment groups.

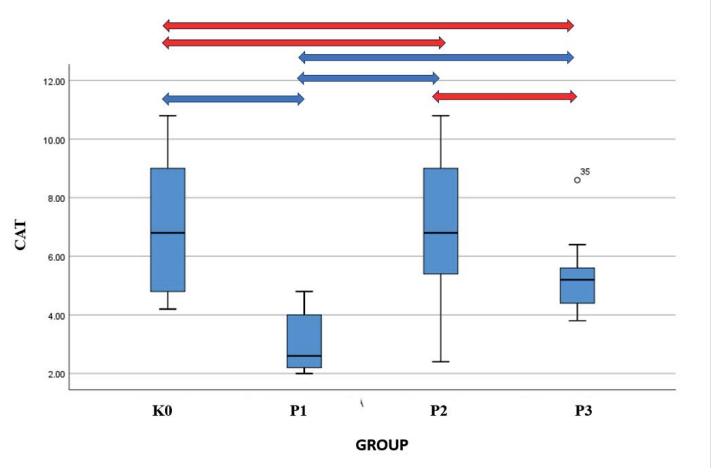

Figure 4 Boxplot comparison of CAT expression. The red line represents non-significant variation, whereas the blue line represents significant variation. Group P1 showed a notable distinction compared to the control, P2, and P3 groups. There was no significant difference between the P2 and P3 groups.

lead to increased lipid peroxidation, resulting in cellular stiffness and deformability. [8,9]

This study showed that UVB radiation for 30 minutes decreased the SOD2 expression of rat lens epithelial cells twofold on the 3rd day after exposure, which was 2,000 ± 0.686. Comparison of SOD2 expression of lens epithelial cells given UVB exposure was significantly lower than those without UVB exposure with a p-value of 0.002 ($P < 0.05$). This result supports previous studies. In a study by Kaur et al., it was said that the level of the antioxidant enzyme SOD2 decreased significantly in cataract patients compared to controls after UVB radiation exposure treatment. (J. Kaur et al., 2012) Similar results were also found in a study by Tsai et al., where SOD2 was found to be significantly lower in lens damage caused by oxidative stress.[8,10,11]

CAT is a 240 kDa tetrameric protein composed of four identical subunits and encoded by the ctt1 gene. It plays a crucial role in physiological processes as a primary antioxidant defense enzyme. Decreased CAT is associated with oxidative stress-induced DNA damage. CAT can decompose hydrogen peroxide into water and oxygen by catalytic activity. The catalase enzyme can also display peroxidase activity in the presence of low levels of hydrogen peroxide (H2O2). UVB rays can lead to elevated levels of ROS in keratinocytes and reduce the activity of the CAT enzyme. UVB light's impact on catalase was discovered to be influenced by pH and dependent on oxygen levels. [12,13]

This study showed that there was a two-fold decrease in CAT expression in lens epithelial cells that received UV-B exposure for 30 minutes on day 3 compared to the control, which amounted to 3,180 ± 1,088. Comparison of CAT expression of lens epithelial cells that were given UVB exposure was significantly lower than lens epithelial cells that were not given UVB exposure with a p-value of 0.001 ($P < 0.05$). This result is in accordance with previous studies. In the study by Tsai et al., the CAT antioxidant enzyme activity in exposed lenses was significantly lower ($p < 0.05$) than in the normal control group. [10]

The study demonstrates that the expression of SOD2 in lens epithelial cells exposed to UVB radiation while using UV-blocking eyeglass lens protection was measured at 4.110 ± 1.175. There was a significant difference in SOD2 expression between lens epithelial cells protected with UV-blocking eyeglass lenses and rat lens epithelial cells without protection, with a p-value of 0.007 ($p < 0.05$). SOD2 expression of lens epithelial cells given UV-blocking eyewear protection was not significantly different from lens epithelial cells without UVB exposure, with a p-value of 0.693 ($p > 0.05$).

Research by Rifai *et al.* in 2018 stated that standard clear eyeglass lenses can reduce UV radiation exposure by up to 31%. This study also compared the transmission of clear spectacle lenses with UV-blocking spectacle lenses, where UV-blocking spectacle lenses can reduce radiation by 93% of ordinary clear spectacles. [14,15]

Mouse lens epithelial cells exposed to UVB with UVB protection goggles showed CAT expression of 6,820 ± 2,878, which is 2.1 times higher than mouse lens epithelial cells exposed to UVB without protection (3,180 ± 1,088). This difference showed significant results in the LSD Multiple Comparison Test post-hoc analysis with a p-value of 0.001 ($p < 0.05$).

These results support previous research conducted by Backes *et al.* in 2018. This study describes the daily UV radiation rays that hit the facial, periorbital, and eye skin zones without protection and with the protection of UV-blocking spectacles. In the unprotected eye area, the total exposure to the cornea reached 1718.4 J/m2 on a cloudless summer day. With the protection of UV-repellent goggles, the exposure was reduced. In the case of large models of goggles, they can block almost all of the UV exposure received. With medium-sized UV protection goggles, the exposure to the ocular area is 290.8 J/m2. [16,17,18]

The SOD2 expression in lens epithelial cells increased to 3,730 ± 2,000 after exposure to UV-B radiation while using Senofilcon A contact lenses, which provide class 1 UV-blocking protection. These results showed a significant difference with the SOD2 expression of lens epithelial cells given UVB exposure without protection, which amounted to 2,000 ± 0.686 (p-value 0.023, $p<0.05$). When lens epithelial cells are exposed to UVB and protected by UV-blocking contact lenses, the expression of SOD2 is similar to that of control cells, with a value of 4,400 ± 1,905. Statistical analysis shows that this difference is not significant, with a p-value of 0.365 ($p> 0.05$).

The results of this study are in line with previous studies on UV-blocking contact lenses, one of which is an in vivo experimental study with albino rabbits by Giblin et al. (2011), who found that rabbit eyes exposed to UVB radiation for 30 minutes (wavelength 270- 360 nm, 1.7mW/cm2) showed extensive lens opacities in eyes that did not use contact lenses. Analyzed the UVB-induced harm to the rabbit lens epithelium in depth by transmission electron microscopy 48 hours after a 30-minute exposure. Significant injury was found in the central lens epithelium of eyes without contact lenses, indicating cell expansion, vacuole formation, nucleus fragmentation, and chromatin condensation. Senofilcon-treated rabbit eyes Contact lens protection

can effectively prevent UVB-induced lens opacification. Senofilcon with Class I UV-blocking capabilities Contact lenses were discovered to effectively prevent nearly all UVB-induced effects on rabbit eye tissues, such as corneal damage, lens epithelial damage, and cataracts. [5,19,20]

Lens epithelial cells' CAT expression increased to 5,380 ± 1,456 following UVB exposure when protected by class 1 UV-blocking contact lenses (Senofilcon A). The CAT expression score of lens epithelial cells exposed to UVB without protection was (3,180 ± 1,088). The figure displays a statistically significant difference with a p-value of 0.033 ($p < 0.05$). Lens epithelial cells, when exposed to UVB radiation and shielded by UV-blocking contact lenses, exhibited a 1.6-fold rise in CAT expression in comparison to control cells.

The results of this study support previous studies that studied the protective effect of UV-blocking contact lenses. Research conducted by Walsh et al. in 2011 studied the protection of contact lenses against UV exposure. Contact lenses have been demonstrated to shield the eye's internal structures from all types of UV radiation, allowing their protective capabilities to be accurately assessed based on their spectral transmittance. The contact lens has a UV-blocking class 2 spectral transmittance, showing a highly effective protection against UV rays. The front part of the lens is exposed to significant UVB light that passes through the cornea, and this exposure has been linked through epidemiological studies to the development of cataracts. The research indicates that UVB radiation reaches the ocular surface quickly. The best way to prevent UV light damage is to completely block UV light in front of the cornea and shield the limbal stem cells and surrounding conjunctiva from all UV light sources. Ultraviolet radiation-blocking contact lenses are the primary means of safeguarding the eyes and should be used along with traditional methods to safeguard the eyelids and nonvisual ocular areas. [21,22]

This study evaluated the preventive impact of UV-blocking spectacles with Class I UV-blocking contact lenses (Senofilcon A) by looking at the expression of SOD2 in rat lens epithelial cells. SOD2 expression in the UV-blocking spectacles group showed 4,110 ± 1,175, while the UV-blocking class I contact lens group (Senofilcon A) showed SOD2 expression of 3,730 ± 2,000. SOD2 expression in both protection groups gave significantly different results from the UV-B exposure group without protection. The SOD2 expression of the UV-blocking spectacle group was not significantly different from the SOD2 expression of the UV-blocking class I contact lens group (Senofilcon A).

UV-blocking contact lenses and UV-blocking eye spectacles exhibited comparable efficacy in mitigating the development of cataracts caused by exposure to UVB radiation. This research represents the initial attempt to assess and juxtapose the protective advantages offered by contact lenses and UVB-resistant eye spectacles. Promoting both protective modalities can effectively mitigate the risk of senile cataract, an eye structure injury caused by UV-B exposure.

This study examined the protective efficacy of UV-blocking spectacles against class I UV-blocking contact lenses (Senofilcon A) by analyzing CAT expression of rat lens epithelial cells. CAT expression in the UV-blocking spectacles group showed a result of 6.820 ± 2.878, while the class I UV-blocking contact lens group (Senofilcon A) showed CAT expression of 5.380 ± 1.456. CAT expression in both protection groups gave significantly different results from the UV-B exposure group without protection. CAT expression in the UV-blocking spectacle group was not significantly different from CAT expression in the class I UV-blocking contact lens group (Senofilcon A). Class I UV-blocking contact lenses (Senofilcon A) and UV-blocking spectacles are both equally effective at preventing the accumulation of ROS, which can damage lens epithelial cells.

CONCLUSION

From the results of this study, it can be concluded that UVB radiation will cause a decrease in the number of antioxidant enzyme markers. This study demonstrates that both UV-blocking spectacles and contact lenses effectively protect lens epithelial cells against UVB radiation-induced ROS processes, making them suitable for cataract prevention. Future research can develop similar research models or use models that are more similar to the human lens to determine the protective effects of compounds that have the potential for cataract prevention, as well as other markers involved in the DNA damage process.

AUTHOR'S CONTRIBUTION

MD designed and performed the experiments, derived the models, and analyzed the data. NP and RN helped with the experiment. DL assisted in pathology anatomy analysis. MD wrote the manuscript in consultation with N and MF.

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CONFLICTS OF INTEREST

The authors declared that they have no conflict of interest.

FUNDING SOURCE

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ETHICAL CLEARANCE

Ethical eligibility was obtained from the Research Animal Ethics Commission of the Faculty of Veterinary Medicine Animal Care and Use Committee (ACUC) Airlangga University Surabaya, number 2.KEH.028.03.2023.

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