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The effect of long exposure to uvb rays on histological features of wistar rats (*Rattus norvegicus)* in photoaging model

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ABSTRACT

Introduction: Indonesia is a tropical country with exposure to UV rays from the sun throughout the year, so the Indonesian population is very vulnerable to skin aging, especially extrinsic skin aging due to prolonged exposure to UV rays. This study aimed to determine the effect of long exposure to UVB rays on the thickness of the epidermis, the thickness of the dermis, and the number of blood vessels and sunburn cells in (*Rattus norvegicus*) Wistar strain.
Methods: This research was a post-test-only control group design using 27 rats which were divided into 3 groups, namely: control group K was untreated rats, and treatment groups P1 and P2 were rats exposed to UVB radiation for 3 weeks and 6 respectively exposure weeks. Test the effect on the study using the One-Way ANOVA test and Tukey's Post Hoc Test.
Results: The results indicated an increase in epidermal thickness of groups P1 (44.87 µm) and P2 (56.90 µm) (significant $p<0.05$). A decrease in dermis thickness occurred in groups P1 (726.91 µm) and P2 (559.40 µm) (significant $p<0.05$). In the photoaging area, there was an increased amount of sunburn cells in group P1 (6.40) and P2 (11.40) (significant $p<0.05$). There was a difference in the number of blood vessels between groups K and P2 and groups P1 and P2 ($p<0.05$).
Conclusion: There was an effect of long exposure to UVB rays on histology in rats (*Rattus norvegicus*) of the photoaging model including the thickness of the epidermis layer and dermis layer, the number of blood vessels, and sunburn cells.

Keywords: *Photoaging, epidermal thickness, dermis thickness, sunburn cells, blood vessels*.
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INTRODUCTION

Skin aging is a complex biological process due to intrinsic factors (from within the body such as genetics) and extrinsic factors (from outside the body such as the environment).[1] Skin aging involves various layers of the skin, the most visible changes being in the dermis and epidermis layers.[2] The most important extrinsic factor as a cause in accelerating the skin aging process is exposure to sunlight which contains ultraviolet (UV) rays so extrinsic skin aging is often referred to as photoaging. Indonesia is a tropical country with exposure to UV rays from the sun throughout the year, so the Indonesian population is very vulnerable to the occurrence of skin aging, especially extrinsic skin aging due to long-term exposure to UV rays.[3]

Research conducted by Choi et al., (2017) with the research title Far-infrared suppresses skin photoaging in ultraviolet B-exposed fibroblasts and hairless mice. This study used 15 shared rats into 3 groups, consisting of group control (P1) without treatment, group treatments (P2), and (P3) were exposed to different doses. Rats from all treatment groups were exposed to UVB rays. Rats were exposed to UVB radiation of 100 mJ/cm^2 (one minimal erythema dose = 100 mJ/cm^2) five times per week for the first week and then 200 mJ/cm^2 three times a week for 6 weeks. Research conducted results exposure to UVB rays for 3 weeks caused an acute effect on the skin of rats with symptoms of skin redness, and exposure to UV-B rays for 6 weeks caused a chronic effect on the skin of rats with symptoms of dry and wrinkled skin.[4] Research conducted by Adrianta and Putra, (2018) regarding photoaging with exposure to UVB rays for 3 weeks can reduce the amount of collagen and damage synthesis collagen by sustainability so that occur change in thickness in the dermis.[5] Another study by Fan et al., (2015) entitled An Experimental Model Design for Photoaging. This study used 2 groups consisting of a control group and a treatment group where each group used 7 mice. The treatment group was exposed to UVB rays for 6 weeks at a dose between 290 to 320 mJ/cm^2. The results of this study indicate a significant thickness change in the epidermis and dermis skin layers of the group that was given exposure to UVB rays for 6 weeks.

Research Yano et al., (2004) stated that exposure to UVB radiation on the 8th day of irradiation with a radiation dose of 200 mJ/cm^2 caused the vascularization of blood vessels in the upper dermis to greatly increase. The acute effect of exposure to UVB radiation causes a significant increase in the mean number of

blood vessels, however, occur drop vessels blood due to cell apoptosis endothelium.[6] Research Ivic (2008) stated that UVB rays cause sunburn cells after 8 to 12 hours after exposure, the result of this study is damaged DNA in keratinocytes and melanocytes.[7]

The purpose of this study was to determine the effect of long exposure to UVB rays on the histological features of Wistar rats *(Rattus norvegicus)* photoaging model including the thickness of the epidermis and dermis layer, number of sunburn cells, and number of blood vessels.

METHODS

Study Design

This research was a true-experimental-research using a post-test-only control group design used 27 rats which were divided into 3 groups, consisting of the control group (K), which were untreated rats, and treatment groups, P1 and P2, which were rats exposed to UVB radiation for 3 weeks and 6 weeks. The rats used were male rats aged 10-12 weeks obtained from the Experimental Animal Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya, and had gone through a period of acclimatization (adaptation) for one week before treatment. During the acclimatization process, the rats were trained with the use of exposure cages.

UVB exposure or photoaging model

Ultraviolet B rays were exposed to P1 and P2 groups in order to obtain rats with a photoaging model. The UVB exposure source is in the form of Ultraviolet B Broadband TL lamps Philips brand TL 20W/01RS series with an exposure distance of 30 cm, during exposure, the rats still move freely in the cage and exposed UVB to the skin that has been shaved on the dorsal part by 1x1 cm^2. The dose of UVB exposure given to each treatment group was different. The first three weeks of irradiation were carried out 3 times a week with a gradually increasing dose (1^{st} week = 120mJ/cm^2 for 11 minutes/day; 2^{nd} week = 240mJ/cm^2 for 22 minutes/day; 3^{rd} week = 360mJ/cm^2 for 33 minutes/day). The next three weeks irradiation was only done 2 times a week with a total dose of irradiation for 6 weeks was 4.2 J/cm2 for 44 minutes/day.

Histopathology analysis

Rats which already received different UVB irradiation exposure treatments in each group were completely anesthetized by inserting rats into the jar containing an ether solution. After the rats were completely anesthetized, all groups of rats would have their skin tissue removed using a minor surgical instrument. After the tissue is removed, the skin tissue was stretched across the carton was then stamped and put into a sample jar containing 10% formalin for further preparation of histology with HE staining at the Anatomical Pathology Laboratory, Faculty of Medicine, Airlangga University, Surabaya. Observation of the preparations aims to observe the thickness of the epidermis, the thickness of the dermis, the number of sunburn cells, and the number of vessels blood. The preparations were observed using a microscope with a magnification of 40x on one field of view using the Cellseen application.

Data analysis

This study's output was gathered, edited, entered into a computer, coded, and cleaned. The Statistical Product for the Social Sciences (SPSS) data format version 20.0 (SPSS, Inc., Chicago, Illinois) was used to enter the obtained data. Because there were fewer than 30 samples in each group and Levene's Test was used for the homogeneity test data, the normality test with the Shapiro-Wilk test was the statistical test that was used. If the data were homogeneous and regularly distributed, perform a parametric test using the One-Way ANOVA test. If the results are significant (p 0.05), perform the Tukey's Post Hoc test.

RESULTS

Effect of exposure to UV-B rays on the thickness of the epidermis and dermis

In order to ascertain the impact of exposure to UVB rays on the thickness of the epidermis and dermis, a parametric test called a One-Way ANOVA was conducted on the normally distributed and homogenous thickness of the epidermis and dermis in this study. This study found that following exposure to UVB rays, there was a significant difference in the thickness of the epidermis and dermis between groups (p 0.05 and 0.05, respectively). The Tukey's Post Hoc test was used to determine how the thicknesses of the epidermis and dermis varied between groups. Based on the Tukey's Post Hoc test, there was no difference in the thickness of the epidermis and dermis between groups K and P1, while there was a difference in the thickness of the epidermis and dermis between groups K and P2 and groups P1 and P2 ($p<0.05$).

Effect of exposure to UVB rays on sunburn cells

Sunburn cells in this study were normally distributed and homogeneous, so a parametric test was carried out One-Way ANOVA to determine the effect of exposure to UVB rays on the increase in sunburn cells in the rat epidermis. This study showed that there was a significant difference in the number of sunburn cells ($p<0.05$) after being exposed to UVB rays. Difference enhancement of sunburn cells between groups was evaluated with the Tukey's Post Hoc test. Based on Tukey's Post Hoc test, there was no difference in enhancement sunburn cells between groups K and P1 ($p>0.05$), while there was a difference in the increase in sunburn cells between groups K and P2 and groups P1 and P2 ($p<0.05$).

Effect of exposure to UVB rays on blood vessels

This study also evaluated the number of blood vessels in the group with UVB exposure for 3 weeks and 6 weeks and the group without UVB exposure. The results showed that the blood vessels were normally distributed and homogeneous, so a parametric test was performed One-Way ANOVA to determine the effect of exposure to UVB rays on blood vessels blood. This study shows there is a significant difference in the amount of vessels blood ($p<0.05$) after given exposure to UVB rays. The different amount of blood vessels between groups is known with the Post Hoc Tukey test was performed. Based on the Post Hoc Tukey test, there was no difference in the number of blood vessels between groups K and

Table 1. Comparison of the thickness of the epidermis and dermis between groups

Group	Epidermis Thickness (mean)	Dermis Thickness (Mean ± S D)
K	33.19	728.64 ± 161.35
P1	44.23	726.91 ± 115.83
P2	56.78	559.40 ± 75.12
p-value (ANOVA)	0.0 00 *	0.010 *

*Analysis was carried out using a One-Way ANOVA test. Results were considered significant if the p-value≤0.05.

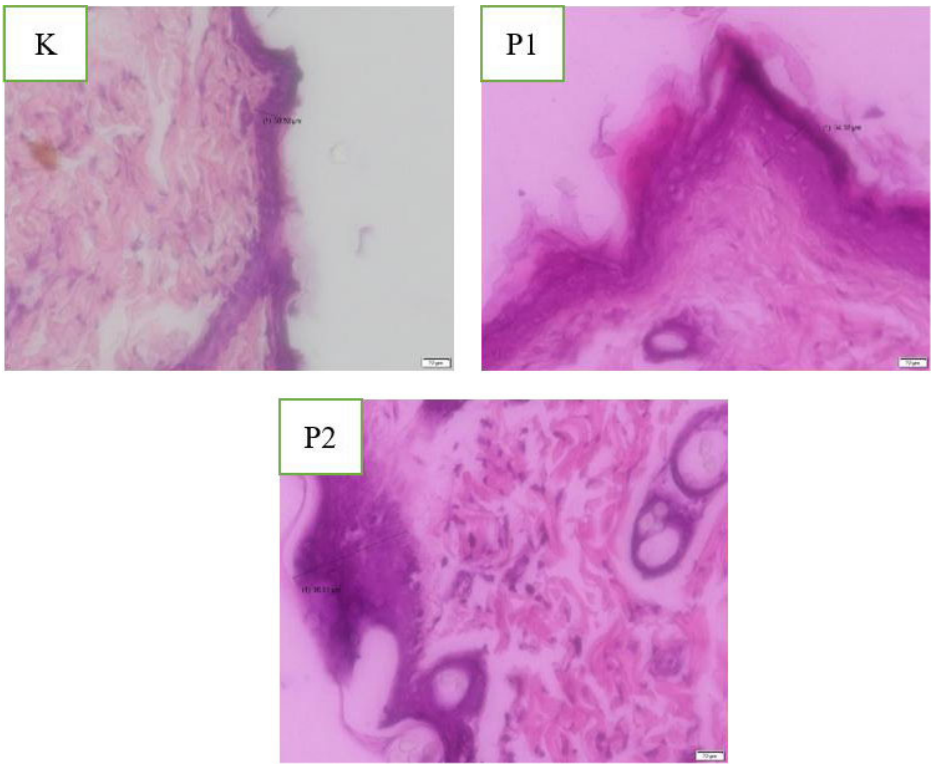

Figure 1. The thickness of the epidermis of groups K, P1, and P2 with a magnification 40x microscope.

P1 (p>0.05), while there was a different amount of blood vessels between groups K and P2 and groups P1 and P2 (p<0.05).

DISCUSSION

Aging skin caused by exposure to UV rays from the sun called photoaging.[8] Photoaging is characterized by fine and rough wrinkles on the skin, depigmentation, changes in skin texture, and loss of elasticity. UVB rays have high energy with short wavelength characteristics so that they are able to pass through the epidermis and penetrate the upper dermis and are the main cause of photoaging.[9]

Study by Djawad et al., (2020) states that the group of mice (*Rattus norvegicus)* exposed to UVB rays experienced an increase in epidermal thickness compared to the group without exposure.[10] Skin damage after being exposed to UVB radiation causes polarity cells in the epidermis to disappear so that the epidermis will become thicker.[11] UVB rays increase free radicals which result in the keratinocyte process resulting in hyperplasia of the epidermis which causes a visible layer of the epidermis thicker. Changes in epidermal histopathology is characterized by the occurrence of hyperkeratosis (thickening of the stratum corneum), spongiosis (fluid-filled edema in the intercellular tissue), vesicles, and the most severe is cell damage and even necrosis.[12]

Research conducted by Adrianta and Putra, (2018) states that exposure to UVB rays for 3 weeks can reduce the amount of collagen and damage collagen synthesis on an ongoing basis, resulting in changes in thickness in the dermis. The thickness of the epidermis and dermis of rats with UVB exposure for 6 weeks was statistically significant when compared to rats with UVB exposure for 3 weeks and rats without UVB exposure (p<0.05).[5] The results of this study are similar to those of Fan et al., (2015) who showed an increase in epidermis thickness and a decrease in dermis thickness after being exposed to UV-B light for 6 weeks.[13] This study agreed with research conducted by Wasliati *et al.,* (2019) which states that rats exposed to UVB radiation for 6 weeks caused a decrease in dermis thickness through changes in collagen pathway in two ways, namely stimulating the breakdown of collagen into several fragments and inhibiting procollagen biosynthesis.[14] Choi et al., (2019) stated the effect of exposure to UV-B radiation in mice for 8 weeks with an exposure dose of 100 mJ/cm^2 every day in the first week and continued with an exposure dose of 200 mJ/cm^2 three times a week starting from 2 to 8 got results drop thickness the dermis layer is caused by the damage structure collagen.[4] Another study conducted by Hachiya et al., (2009) found that UVB radiation for 6 weeks at a dose of 165 mJ/cm^2 caused a decrease in dermis thickness through an increase in the accumulation of abnormal elastin fibers that induce MMP by fibroblasts.[15]

Differences in exposure time to UVB rays also cause a different average number of sunburn cells between groups. Sunburn cells are dyskeratotic keratinocytes scattered due to acute UVB exposure indicating irreversible cellular DNA damage and apoptosis keratinocytes epidermis which can cause cancer.[16] Studies show enhancement amount of sunburn cells in the group exposure UVB rays for 6 weeks was compared with the UVB exposure group for 3 weeks and the no-exposure group. The results of this study agree with the research conducted by Tedesco, (1997) that the longer the mouse gets exposed to UVB rays the more many amounts of sunburned cells.[12] The results of this study are in accordance with

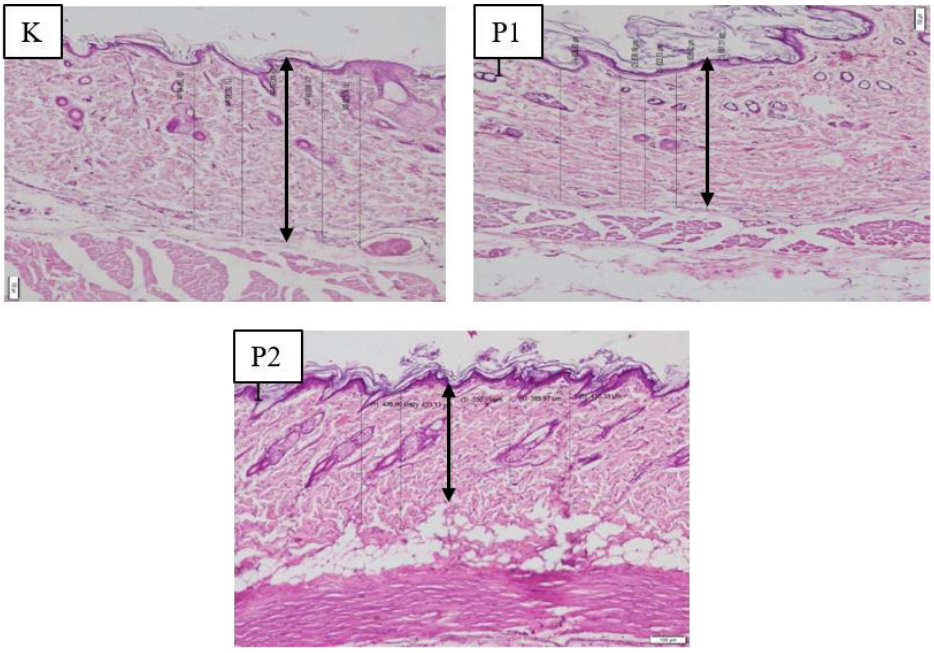

Figure 2. The thickness of the dermis of groups K, P1, and P2 with a magnification 40x microscope.

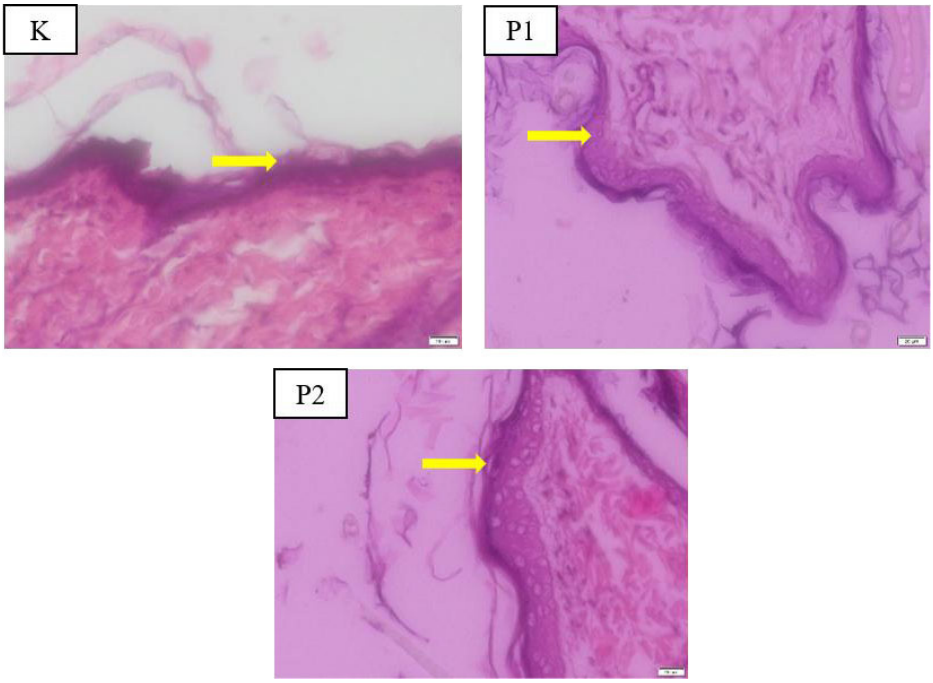

Figure 3. Overview of sunburn cells in rat's epidermis with magnification 40x microscope (shown arrow).

Table 2. Number of sunburn cells of groups K, P1, and P2

Group	*Sunburn cells* (Median)
K	2.00
P1	6.40
P2	11.60
p-value (ANOVA)	0.000*

research conducted by Levi, (2013) that duration exposure to longer UVB rays improves the number of sunburn cells in the epidermis.[17] Research by Ivic, (2008) states that UV-B rays cause sunburn cells 8 to 12 hours after exposure.[7]

UVB exposure induces increased VEGF mRNA expression and decreased TSP-1 mRNA expression in the epidermis, resulting in a shift towards a proangiogenic environment.[6] The acute effect of UV exposure stimulates skin angiogenesis.[18] The number of blood vessels decreases in skin damaged by chronic UV exposure due to regression by endothelial cell apoptosis.[6,19] This study showed a decrease in the number of blood vessels in the group that received UVB light exposure for 6 weeks compared to the group without exposure and the group with UVB exposure for 3 weeks. Sun exposure to the skin sun directly, the density of vessels blood reduces compared with areas that are not exposed to the sun.[6,18,20,21] A decrease in the number of blood vessels in the upper dermis, together with the flattening of endothelial cells, correlates with the severity of photoaging. Quantitative analysis of skin blood vessels in photoaging skin and intrinsically aging skin showed that the number of blood vessels in the sub-epidermal area of photoaged skin was significantly reduced.[20] This study still lacked control for a number of compounding variables that may have decreased the study's dependability.

CONCLUSION

This research shows the effect of long exposure to UV-B rays against the thickness of the epidermis, the thickness of the dermis, the number of sunburn cells, and the number of vessels blood. The results showed that exposure to UV-B rays for 6 weeks had a significant effect on increasing the thickness of the epidermis due to hyperplasia and causing inflammation and a drop in dermis thickness due to degradation of collagen, then increase amount sunburn cells and decrease amount of blood vessels compared to group with UV-B light exposure for 3 weeks and no exposure. Further studies are needed to validate these findings in order to applicate these findings into applicable usages.

FUNDING

The authors declare no funding in this study.

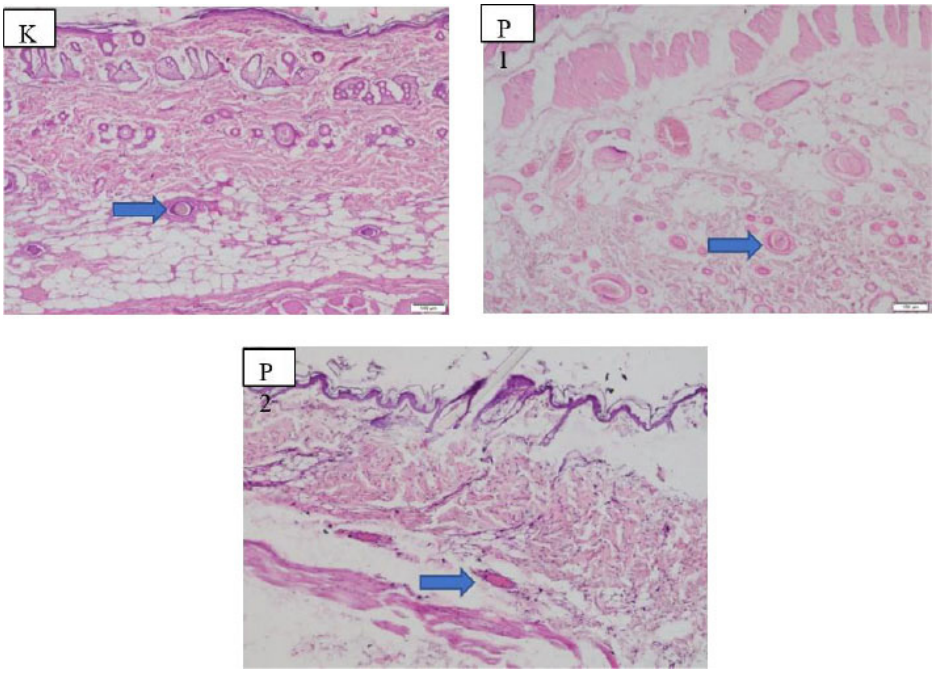

Figure 4. Overview of blood vessels of groups K, P1, and P2 with magnification microscope 100x (shown arrow).

Table 3. Number of blood vessels of groups K, P1, and P2

Group	Number of Blood Vessels (Mean ± SD)
K	3.33 ± 0.71
P1	2.67 ± 0.50
P2	1.33 ± 0.50
One-way ANOVA	0.000*

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

ETHICAL STATEMENT

This study has been declared ethically feasible by the Health Research Ethics Committee, the Universitas Nahdlatul Ulama Surabaya with No: 293/EC/KEPK/UNUSA/2021.

AUTHOR CONTRIBUTION

All authors contributed equally to this study.

REFERENCES

1. Jusuf NK, Putra IB, Muslim M. Evaluation of dermoscopic photoaging score among multiethnic in Medan, Indonesia. Bali Med J. 2022;11(3):1919–23. DOI: https://doi.org/10.15562/bmj.v11i3.3810.
2. Nabila Ayu Safitri, Oktavia Eka Puspita VY. Optimasi Formula Sediaan Krim Ekstrak Stroberi (Fragaria x ananassa) sebagai Krim Anti Penuaan. Maj Kesehat FKUB. 2014;1(4):235–46.
3. Ahmad Z, Damayanti. Penuaan Kulit : Patofisiologi dan Manifestasi Klinis. Berk Ilmu Kesehat Kulit dan Kelamin – Period Dermatology Venereol. 2018;30(03):208–15. DOI: https://doi.org/10.20473/bikk.V30.3.2018.208-215.
4. Choi SH, Choi S Il, Jung TD, Cho BY, Lee JH, Kim SH, et al. Anti-photoaging effect of Jeju Putgyul (unripe citrus) extracts on human dermal fibroblasts and ultraviolet B-induced hairless mouse skin. Int J Mol Sci. 2017;18(10). DOI: https://doi.org/10.3390/ijms18102052.
5. Adrianta KA, Sunadi Putra IMA. Utilization of Artocarpus heterophylla Lamk. As UV Protector In mice exposed to UV-B. Maj Obat Tradis. 2018;23(3):112. DOI: https://doi.org/10.22146/mot.37407.
6. Yano K, Kajiya K, Ishiwata M, Hong YK, Miyakawa T, Detmar M. Ultraviolet B-Induced Skin Angiogenesis Is Associated with a Switch in the Balance of Vascular Endothelial Growth Factor and Thrombospondin-1 Expression. J Invest Dermatol. 2004;122(1):201–8. DOI: https://doi.org/10.1046/j.0022-202x.2003.22101.x.
7. Puizina-Ivić N. Skin aging. Acta Dermatovenerologica Alpina, Pannonica Adriat. 2008;17(2):47–54.
8. Trojahn C, Dobos G, Lichterfeld A, Blume-Peytavi U, Kottner J. Characterizing facial skin ageing in humans: Disentangling extrinsic from intrinsic biological phenomena. Biomed Res Int. 2015;2015. DOI: https://doi.org/10.1155/2015/318586.
9. Peng Z, Chen B, Zheng Q, Zhu G, Cao W, Qin X, et al. Ameliorative effects of peptides from the oyster (crassostrea hongkongensis) protein hydrolysates against UVB-induced skin photodamage in mice. Mar Drugs. 2020;18(6). DOI: https://doi.org/10.3390/md18060288.
10. Khairuddin Djawad, Siswanto Wahab, Olivia Wibisono, Christina Avanti, Astridani R. Putranti, Agussalim Bukhari IJP. Protective Effect of Mangosteen Pericarp Extract Cream Against UVB-Induced Erythema. J Clin Aesthet Dermatol. 2020;13(10):E59–E65.
11. Ma W, Wlaschek M, Tantcheva-Poór I, Schneider LA, Naderi L, Razi-Wolf Z, et al. Chronological aging and photoageing of the fibroblasts and the dermal connective tissue. Clin Exp Dermatol. 2001;26(7):592–9. DOI: https://doi.org/10.1046/j.1365-2230.2001.00905.x.
12. Tedesco AC, Martínez L, González S. Photochemistry and photobiology of actinic erythema: Defensive and reparative cutaneous mechanisms. Brazilian J Med Biol Res. 1997;30(5):561–75. DOI: https://doi.org/10.1590/s0100-879x1997000500002.
13. Fan Y, Jeong JH, You GY, Park JU, Choi TH, Kim S. An Experimental Model Design for Photoaging. J Craniofac Surg. 2015;26(6):e467–71. DOI: https://doi.org/10.1097/scs.0000000000001902.
14. Wasliati B, W P, A W. Krim Ekstrak Bawang Hitam (Allium sativum Linn) Mencegah Peningkatan Ekspresi MMP-1 dan Penurunan Jumlah Kolagen Dermis Kulit Tikus (Rattus norvegicus) Wistar Jantan yang Dipapar Sinar UV-B. Cermin Dunia Kedokt. 2019;46(8):487–90. DOI: https://dx.doi.org/10.55175/cdk.v46i8.444.
15. Hachiya A, Sriwiriyanont P, Fujimura T, Ohuchi A, Kitahara T, Takema Y, et al. Mechanistic effects of long-term ultraviolet B irradiation induce epidermal and dermal changes in human skin xenografts. Am J Pathol. 2009;174(2):401–13. DOI: https://doi.org/10.2353/ajpath.2009.070500.
16. Yuko Ibuki, Tatsushi Toyooka, Jisshou Shirahata TO, Rensuke G. Water Soluble Fraction of Solar-Simulated Light-Exposed CrudeOil Generates Phosphorylation of Histone H2AX in Human Skin Cells Under UVA Exposure. Environ Mol Mutagen. 2007 Jul;48(6):430-9. DOI: 10.1002/em.20292. PMID: 17372987.
17. Levi K. UV damage and sun care: Deciphering mechanics of skin to develop next generation therapies. J Mech Behav Biomed Mater. 2013;28:471–3. DOI: http://dx.doi.org/10.1016/j.jmbbm.2013.02.008
18. Chung JH, Eun HC. Angiogenesis in skin aging and photoaging. J Dermatol. 2007;34(9):593–600. DOI: https://doi.org/10.1111/j.1346-8138.2007.00341.x.

19. Gunin AG, Petrov V V., Golubtzova NN, Vasilieva O V., Kornilova NK. Age-related changes in angiogenesis in human dermis. Exp Gerontol. 2014;55:143–51. DOI: http://dx.doi.org/10.1016/j.exger.2014.04.010
20. Sawane M, Kajiya K. Ultraviolet light-induced changes of lymphatic and blood vasculature in skin and their molecular mechanisms. Exp Dermatol. 2012;21(SUPPL.1):22–5. DOI: https://doi.org/10.1111/j.1600-0625.2012.01498.x.
21. Kajiya K, Kim YK, Kinemura Y, Kishimoto J, Chung JH. Structural alterations of the cutaneous vasculature in aged and in photoaged human skin in vivo. J Dermatol Sci. 2011;61(3):206–8. DOI: https://doi.org/10.1016/j.jdermsci.2010.12.005.