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). Skin aging involves various layers of the skin, the most visible changes being in the dermis and epidermis layers. 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.

Research conducted by Choi et al., (2017) with the research title Far-infrared exposure to UV rays. 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 (72.91 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) in 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.


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

Nadia Hidayati1, Noor Aulia Hatikhah1, Winawati Eka Putri2*, Meidyta Sinantryana Widyaswari2, David Sajid Muhammad3, Nadia Nisaussholihah1, Deny Febriwijaya Romadhani1

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 (72.91 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.

blood vessels, however, occur drop vessels blood due to cell apoptosis endothelium. 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.

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. 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.

**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 cm². 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 (1st week = 120mJ/cm² per 11 minutes/day; 2nd week = 240mJ/cm² for 22 minutes/day; 3rd week = 360mJ/cm² for 33 minutes/day). The next three weeks irradiation was only done 2 times a week with a total dose of 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 was performed with the use of a microscope and 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).
**Table 1. Comparison of the thickness of the epidermis and dermis between groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Epidermis Thickness (mean)</th>
<th>Dermis Thickness (Mean ± S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>33.19</td>
<td>728.64 ± 161.35</td>
</tr>
<tr>
<td>P1</td>
<td>44.23</td>
<td>726.91 ± 115.83</td>
</tr>
<tr>
<td>P2</td>
<td>56.78</td>
<td>559.40 ± 75.12</td>
</tr>
<tr>
<td>p-value (ANOVA)</td>
<td>0.00 *</td>
<td>0.010 *</td>
</tr>
</tbody>
</table>

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

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 of 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). 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. 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. Choi et al., (2019) stated the effect of exposure to UV-B radiation in mice for 8 weeks with an exposure dose of 100 ml/cm² every day in the first week and continued with an exposure dose of 200 ml/cm² three times a week starting from 2 to 8 got results drop thickness the dermis layer is caused by the damage structure collagen. Another study conducted by Hachiya et al., (2009) found that UVB radiation for 6 weeks at a dose of 165 ml/cm² caused a decrease in dermis thickness through an increase in the accumulation of abnormal elastin fibers that induce MMP by fibroblasts.

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. 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 amounts of sunburned cells. The results of this study are in accordance with

**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. 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.

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. Skin damage after being exposed to UVB radiation causes polarity cells in the epidermis to disappear so that the epidermis will become thicker. 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.
UVB exposure induces increased VEGF mRNA expression and decreased TSP-1 mRNA expression in the epidermis, resulting in a shift towards a proangiogenic environment. The acute effect of UV exposure stimulates skin angiogenesis. The number of blood vessels decreases in skin damaged by chronic UV exposure due to regression by endothelial cell apoptosis. 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. 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. 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 apply these findings into applicable usages.

FUNDING

The authors declare no funding in this study.
Table 3. Number of blood vessels of groups K, P1, and P2

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Blood Vessels (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>3.33 ± 0.71</td>
</tr>
<tr>
<td>P1</td>
<td>2.67 ± 0.50</td>
</tr>
<tr>
<td>P2</td>
<td>1.33 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>0.00*</td>
</tr>
</tbody>
</table>

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/Committee, the Universitas Nahdlatul Ulama Surabaya with No: 293/EC/KEPK.

AUTHOR CONTRIBUTION
All authors contributed equally to this study.

REFERENCES


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.


