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¹Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Indonesia

²Department of Anatomy Histology and Pharmacology, Faculty of Medicine, Universitas Airlangga, Indonesia

³Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Indonesia

⁴Department of Toxicology, Advanced Medical and Dental Institute USM, Sains@Bertam, 13200 Kepala Batas, Penang, Malaysia

Corresponding author. Arifa Mustika, Department of Anatomy Histology and Pharmacology Faculty of Medicine Universitas Airlangga Indonesia. Address: Mayjen Prof. Dr. Moestopo No.47 Surabaya East Java 60132 Indonesia. Phone: +62878-5154-0939. E-mail address: arifa-m@fk.unair.ac.id. ORCID ID: <https://www.orcid.org/0000-0001-6461-5782>.

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Comparison of UVA vs UVB Photoaging Rat Models in Short-term Exposure

Elly Mayangsari^{1,3}, Arifa Mustika², Nurdiana Nurdiana³, Nozlina Abdul Samad⁴

ABSTRACT

Background: Prolonged exposure to sunlight is known to induce photoaging of the skin, leading to various skin changes and disorders, such as dryness, wrinkles, irregular pigmentation, and even cancer. Ultraviolet A (UVA) and ultraviolet B (UVB) radiation are particularly responsible for causing photoaging. **Objective:** This study aims to identify and compare photoaging rat models exposed to UVA and UVB. **Methods:** This research method compared macroscopic (scoring degree of wrinkling) and microscopic (histology) signs and symptoms on skin samples of rat exposed to UVA and UVB for 4 weeks at a radiation dose of 840mJ/cm². **Results:** The results of this study indicated that the degree of wrinkling was highest in rat skin exposed to UVB rays by 51% (p<0.05). UVB histological results showed that the epidermis layer (40 µm, p<0.05) was thickened and the dermis layer (283 µm, p<0.05) was thinned in the skin of mice exposed to UVB light. The UVB group, showed the density of collagen in the dermis with a mean value of 55% (p<0.05). **Conclusion:** Our results suggest that short-term exposure to UVB radiation (in the acute, subacute or sub-chronic phase) induces more rapid and pronounced damage to rat skin when compared to UVA radiation exposure.

Keywords: Disease, Photoaging model, Short-term exposure, UVA, UVB. Adipocytokines.

1. BACKGROUND

The most important benefit of ultraviolet light lies in its role in facilitating the synthesis of vitamin D within the human body. Nonetheless, prolonged exposure to UV light can have detrimental effects on the structural integrity, physiological functionality, and barrier properties of the skin, ultimately leading to the process of photoaging. Photoaging is a skin aging process in the form of photodamage induced by sun exposure. Skin aging is a complex biological process that cannot be avoided and affects the appearance of the skin due to a decrease in the ability to restore normal skin function [1,2]. Basically there are two processes of skin aging, namely intrinsic aging and extrinsic aging. Intrinsic aging (genetics, race, variations in skin anatomy in certain areas, and hormonal changes) is a natural skin aging process that occurs with age and progresses slowly [3]. Extrinsic aging is triggered by exposure to sunlight containing ultraviolet (UV) light, known as photoaging. About 80% of facial skin aging is related to sun exposure [4].

According to research conducted in Australia, approximately 72% of men and 42% of women below the age of 30 encounter photoaging effects [5]. Being a tropical country with year-round exposure to the sun's ultraviolet rays, Indonesia has a population highly susceptible to photoaging. The prevalence of photoaging symptoms on the face were most commonly found in Javanese ethnicity (30.5%), age group 30-39 years (59.1%), non-smoking behavior (35%), indoor work such as laboratory assistants, employees and teachers (71%), duration of sun exposure > 34 hours/week (62%), Fitzpatrick IV skin type (moderate brown skin) (65%) and lentigo symptoms (28%) [6,7].

Sunlight emits ultraviolet (UV) rays, which can be categorized into three types: ultraviolet A (UVA) with a wavelength of 320-400 nm, ultraviolet B (UVB) with a wavelength of 280-320 nm, and ultraviolet C (UVC) with a wavelength of 100-280 nm. UVA, characterized by its long wave, constitutes 95% of the UV rays reaching the Earth's surface, allowing it to penetrate deep into the dermis and subcutaneous layers. UVA can induce the production of reactive oxygen species (ROS) which causes photoaging. UVB has short waves and only about 5-10% can reach the surface and can be absorbed by the epidermis and part of the dermis. UVB radiation can cause

skin redness, photoaging, DNA damage and immunosuppression [2]. Based on the description above, although there have been many studies explaining the impact of UV light exposure, but no one has compared the making of photoaging models from experimental animals in short-term exposure, so further research is needed.

2. OBJECTIVE

This study aims to identify and compare photoaging rat models exposed to UVA and UVB in short-term exposure.

3. MATERIAL AND METHODS

Study group

At the start of the experiment, rat were randomly divided into three groups, with nine rats in each group. Control or normal rat group were only shaved dorsal skin from time to time, as were the rat of other groups. UVA rat group were the dorsal skin of rats was UVA exposure with a total dose of 840 mJ/cm². UVB rat group were the dorsal skin of rats was UVB exposure with a total dose of 840 mJ/cm².

Experimental design

This research is a true experimental study using a post test only control group design.

Sample collection

Thirty male rats of the Wistar strain aged 10-12 weeks with a body weight of 150-250 grams were used in this study from the Pharmacology Laboratory of Brawijaya University. Rat were randomly divided into 3 treatment groups, with 9 rats in each group. Group Control/Normal (rat without UVA and UVB irradiation), group UVA (rat exposed to UVA) and group UVB (rat exposed to UVB). All rats were maintained under standard environmental conditions, consisting of a temperature of 25 ± 2°C, with a relative humidity of 50 ± 5%, and a 12 h light/dark cycle, and were given standard food and water ad libitum. Rat were adapted for one week before treatment. In this study, the dorsal surface of the rat skin was shaved with a razor over an area of 5 × 5 cm² and was hairless during the experimental period. The frequency of exposure was determined three times a week (Monday, Wednesday and Friday) for four weeks. Radiation exposure was carried out using a Nomoy Pet 25W UVA lamp with a wavelength of 320-400 nm and a Philips TL 20W/12 RS SLV/25 UVB lamp with a wavelength of 290-320 nm. The details of the exposure dose were 50 mJ/cm² in the first and second weeks, 70 mJ/cm² in the third week and 80 mJ/cm² in the fourth week, bringing the total dose to 840 mJ/cm². Throughout the experimental period, the skin of the rat's back was photographed 2 times per week. Wrinkle formation was evaluated and measured via ImageJ 1.53e software. Rat were sacrificed using cervical dislocation and skin tissue was collected for histological analysis.

Histology analysis

Hairless mouse dorsal skin tissue was collected, fixed with 10% formalin neutral buffer solution (Sigma-Aldrich), embedded in paraffin, and cut into 4 μm-thick packets, deparaffinized with xylene, and rehydrated via graded alcohol. Hematoxylin eosin (HE) staining is used for histological observations of skin structure, thickness of the epidermis and dermis. HE staining was carried out in several stages starting with deparaffinization, hydration, hematoxylin staining, eosin staining, and finally dehydration. The preparations were analyzed at

10 random locations per slide using an Olympus Cx21 light microscope with 1000x magnification. Each specimen is photographed under a camera with 48MP. Histological changes and collagen fiber density were evaluated and measured using ImageJ 1.53e software.

Statistical analysis

Results are presented as mean ± standard deviation. Differences between groups was analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey test analysis using SPSS software [SPSS, Version 21.0]. The difference was considered statistically significant when the p-value < 0.05.

4. RESULTS

Macroscopic assessment

The process of wrinkle formation can be assessed using grading scale for evaluation of skin wrinkles (macroscopic visual on photoaging) [8]. The macroscopic appearance of skin wrinkling in rat in the final week of the study for each group was photographed, recorded, and displayed [Figure-1(a)]. Wrinkle formation was quantified using ImageJ 1.53e analysis software [Figure-1(b)]. During the study period, the control (normal) group showed light wrinkling of 10% (p<0.05), which corresponds to the age of 10–12 weeks (young adults) rat. The UVA group showed little damage to the surface of the rat skin, the skin looked dry with rough wrinkles by 31% (p<0.05). The UVB group showed dry skin and rougher wrinkles than the other groups, which was 51% (p<0.05).

Microscopic assessment

Assessment of epidermal thickness

Epidermal thickness is one of the histological parameters that reflects skin damage due to ultraviolet light. HE staining was used to analyze the histological effect of rat skin from ultraviolet light exposure [Figure-2(a)]. The different mean skin thickness values were calculated by measuring 10 selected sites for each section [Figure-2(b)]. HE staining showed that

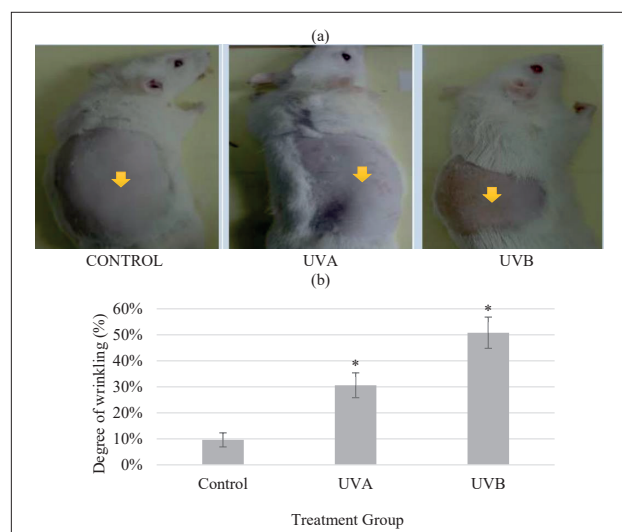


Figure 1. Wrinkle formation on rat skin exposed to ultraviolet light. Rat were radiated with UVB (840 mJ/cm²) three times a week for four weeks. (a) Skin macroscopic appearances at the end of the experiment period, (b) The quantitative analysis of wrinkle (grading macroscopic visual rat skin exposed to ultraviolet light). Data are presented as mean ± SD. Data with different notation in the same chart implied a significant difference (p < 0.05).

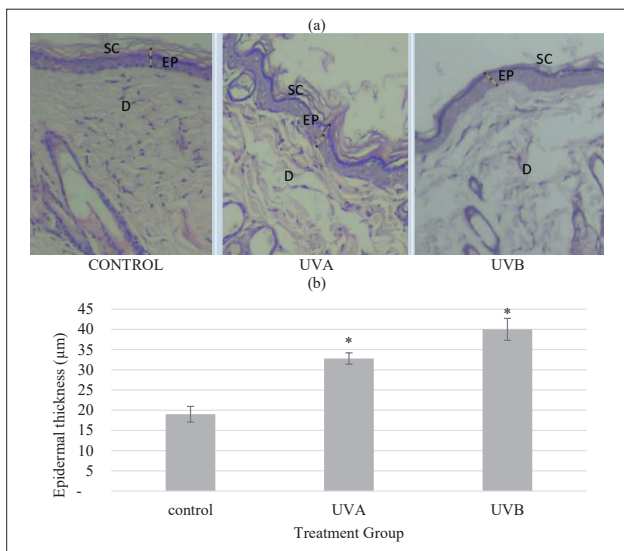
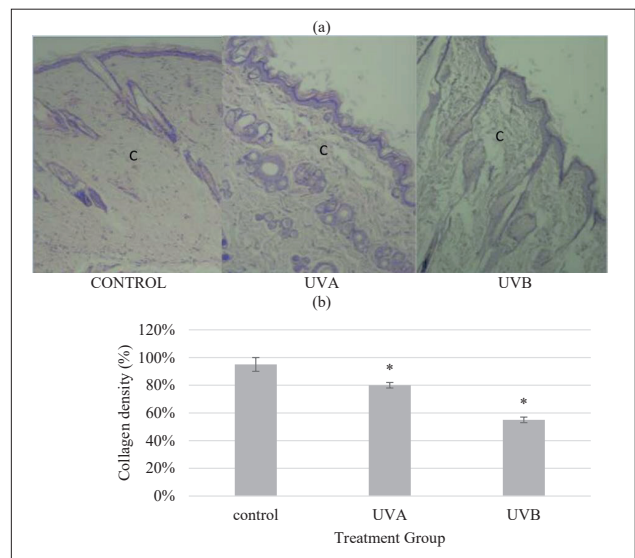


Figure 2. Assessment of epidermal thickness. (a) Dorsal skin sections were stained with hematoxylin and eosin (H&E) (microscope magnification 1000×). (b) The quantitative analysis of epidermal thickness. Data are presented as mean ± SD. Data with different notation in the same chart implied a significant difference ($p < 0.05$). SC: stratum corneum; EP: epidermis; D: dermis; yellow line: epidermal thickness



Assessment of collagen density

Density of collagen fibers and irregular arrangement of collagen fibers is a manifestation of skin damage due to ultraviolet light [Figure-4(a)]. In the control group (normal), it shows the density of collagen in the dermis with a mean value of 95% [Figure-4(b)]. The UVA group showed the density of collagen in the dermis with a mean value of 80% ($p < 0.05$). The UVB group, showed the density of collagen in the dermis with a mean value of 55% ($p < 0.05$).

5. DISCUSSION

The most important benefit of ultraviolet light is that it helps the synthesis of vitamin D in the body. However, long-term exposure to ultraviolet (UV) light can damage the structural integrity and physiological function and barrier properties of the skin resulting in photoaging. Photoaging is a skin aging process in the form of photodamage induced by sun exposure. Interest in photoaging has grown with people's increased awareness of skin aging. Various efforts have been made to prevent skin aging, and many studies have been conducted and dedicated to skin health and beauty. Skin aging factors can be classified as intrinsic or extrinsic. Extrinsic aging is caused by external factors, such as exposure to ultraviolet radiation, food and chemicals (cigarettes), thus increasing skin damage such as sagging, wrinkle formation, and skin roughness. This study aims to identify and compare photoaging rat models exposed to UVA and UVB.

The appearance of wrinkled, rough, sagging skin is an aging process due to direct exposure to ultraviolet light which is also related to the thickness of the epidermis, dermis thickness, and collagen fibers. In this study, we demonstrated the effect of ultraviolet radiation on the control group, UVA group and UVB group. Epidermal thickness is one of histological parameter used to determine the extent of skin damage due to exposure to ultraviolet light. On macroscopic assessment, exposure to UVB rays shows that the degree of wrinkles on the skin surface is more severe than UVA rays. These results are in accordance with the research of Feng et al (2014) and Wang et al (2019) which showed that UVB rays have a higher energy so that UVB rays can cause wrinkles earlier [9,10]. In the interim, the exposure to UVA rays necessitates an extended duration of

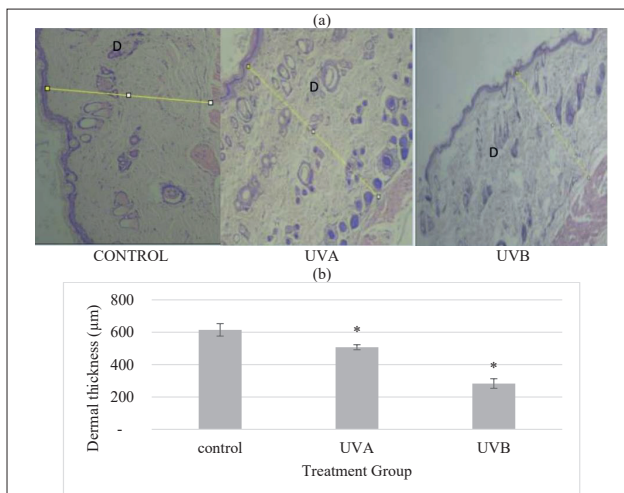


Figure 3. Assessment of dermal thickness. (a) Dorsal skin sections were stained with hematoxylin and eosin (H&E) (microscope magnification 400×). (b) The quantitative analysis of dermal thickness. Data are presented as mean ± SD. Data with different notation in the same chart implied a significant difference ($p < 0.05$). D: dermis; yellow line: dermal thickness.

the control group (normal), showed epidermal thickness with a mean value of 19 µm. The UVA group showed epidermal thickness with a mean value of 33 µm ($p < 0.05$). The UVB group showed epidermal thickness with a mean value of 40 µm ($p < 0.05$).

Assessment of dermal thickness

Dermal thickness is also shown in Figure-3(a). HE staining of the dermis layer showed that the control group (normal) showed dermal thickness with a mean value of 615 µm [Figure-3(b)]. The UVA group, showed dermal thickness with a mean value of 508 µm ($p < 0.05$). The UVB group, showed dermal thickness with a mean value of 283 µm ($p < 0.05$).

exposure and a considerably greater dosage of radiation (10-100X) to result in more severe damage.

The assessment of the epidermal thickness within 4 weeks (840 mJ/cm²) showed that exposure to UVB light can cause thickening of the epidermal layer of the skin faster than exposure to UVA light. These findings align with the research conducted by Kim et al. (2016) and Wang et al. (2019) which showed that exposure to UVB rays had a higher amount of radiation in the epidermis and some of the dermis [10,11]. Meanwhile, UVB rays possess higher energy levels and exhibit greater carcinogenic potential, thereby leading to premature damage in the skin exposed to these rays.

In the assessment of the dermis thickness within 4 weeks (840 mJ/cm²) showed that exposure to UVB rays can reach the dermis layer and can cause damage to the dermis layer faster than UVA rays. These results are consistent with research by Hidayati et al (2015) and Maeda (2018) which showed that low-dose acute UVB exposure can change skin immunity and activate keratinocytes to secrete IL-10 so that UVB rays can cause immunosuppression that affects the structure of the dermis layer [12,13]. UVB exposure can penetrate the dermis layer and only requires a dose of 30-50% of the total UV dose needed to cause damage. Meanwhile, UVA exposure requires a longer time and a larger dose of radiation to cause the same effect.

Skin aging caused by exposure to ultraviolet light increases collagen breakdown and decreases collagen synthesis, resulting in an overall reduction in collagen levels. Collagen is the main component of the extracellular matrix (ECM) which is responsible for maintaining tensile strength, wrinkle formation, skin resilience and can be directly degraded by exposure to ultraviolet light. The assessment of collagen density shows that exposure to UVB light can affect collagen regularity more than UVA light in the dermis layer. These results are in accordance with research by Hidayati et al (2015), Maeda (2018) and Wang et al (2019) which showed that exposure to UVB rays can penetrate up to the dermis layer and can cause collagen degradation [10,12,13]. Meanwhile, exposure to UVB rays can penetrate up to a thickness of 300 µm.

6. CONCLUSION

During a consistent time frame of four weeks and under the identical radiation dose of 840 mJ/cm², diverse UV wavelengths can elicit varying effects on the skin of rats exposed to ultraviolet light. Compared to UVA light exposure, the skin of rats shows an earlier onset of damage when exposed to UVB light within a brief timeframe. Consequently, UVB light exposure can serve as a valuable reference for developing photoaging models during short-term exposure (acute, subacute, and subchronic phases)..

- **Ethical approval:** The experimental animal protocol was approved by the Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, Indonesia.
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