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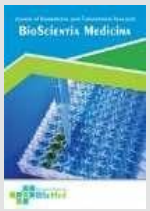

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# The Effect of UVB Exposure at Collagen Density and Dermal Thickness of Wistar Rats: A Pilot Study

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**A B S T R A C T**

**Background:** Skin aging has been characterized by decreasing skin regeneration and loss of structure and function. The most significant cause of extrinsic skin aging is exposure to ultraviolet (UV) radiation (80%). UVB radiation causes DNA damage which leads to dermal thickness reduction. This study aimed to compare the features of collagen density and dermal thickness of rats before and after UVB exposure. **Methods:** This experimental study was conducted at the experimental animal laboratory of Universitas Setia Budi, Surakarta, from May to June 2022. The male Wistar rats were epilated and given UVB exposure, and a back skin biopsy was performed before and after the total dose of UVB exposure. The UVB exposure dose was 50 mJ/cm$^2$ in the first week, 60 mJ/cm$^2$ in the second week, and 70 mJ/cm$^2$ in the third and fourth weeks. The UVB tool used was the Kernel® UV Phototherapy KN-4003BL UVB lamp. **Results:** The collagen densities before vs. after total dose UVB exposure are 61.9% vs. 50.3% for 4x magnification and 63.8% vs. 49.6% for 10x magnification. The dermal thickness also reduced from 1422.07 ± 1165.96 µm before the total dose of UVB exposure to 1049.52 ± 1018.97 µm after the total dose of UVB exposure. **Conclusion:** UVB exposure can induce decreased collagen density and dermal thickness in Wistar skin rats, similar to photoaging.

## 1. Introduction

Skin aging has been characterized by slower skin regeneration and eventual loss of skin structure and functions.[1] Multifactorial processes can induce skin aging, such as intrinsic factors (genetic factors and age-related) and extrinsic factors.[2] Intrinsic aging is the process that can be seen in skin thinning, dry skin, fine wrinkles, and gradual dermal atrophy. Meanwhile, extrinsic aging is carried on by environmental factors like air pollution, smoking, poor nutrition, and sun exposure, which causes coarse wrinkles, loss of elasticity, laxity, and a rough-textured appearance. The most significant cause of extrinsic skin aging accounts for around 80% of facial age, is exposure to UV radiation.[3] Ultraviolet-B (UV-B) radiation can cause changes that are primarily apparent in the epidermis, but it can also be seen in the upper dermis. Ultraviolet-B radiation can generate photoaging through direct DNA damage and reactive oxidative stress (ROS).[4]

Reactive oxidative stress (ROS) induced the activation of the mitogen-activated protein kinase (MAPK) pathway and activated the activator protein 1 (AP-1) and nuclear factor-kappa-beta (NF-kβ), which were the main causes of DNA damage and induced matrix metalloproteinases (MMP) overproduction,

enzymes that break down collagen and another extracellular matrix (ECM) proteins, leading to the loss of skin elasticity and firmness.[5,6] Due to decreased collagen, there is less mechanical engagement between fibroblasts and the ECM, which impairs fibroblast function and further reduces the amount of dermal collagen.[7,8]

The previous in-vitro experimental study reported UVB exposure could induce destruction of the growth of fibroblast culture by expressed reactive oxygen species (ROS) and direct DNA damage.[9] Reactive oxygen species induce activation of mitogen-activated protein kinase (MAPK), nuclear factor-kB (NF-kB), and activator protein-1 (AP-1). It leads to increased transcription of matrix metalloproteinase (MMP), especially MMP-1. An increase in MMP-1, the major MMP mediator of the collagen cycle, causes collagen fragmentation. On the other hand, another study showed that UV-B exposure was able to induce histopathological changes and altered expression of cleaved caspase-3 and Ki-67 in rat skin, which could lead to the risk of skin carcinogenesis.[10] Previous studies also evaluated the role of UVB radiation in producing photoaging in the mouse model by use of 3100 mJ/cm$^2$ of UVB for 5 weeks in the Wistar rat. This study used Masson trichrome staining and trans-epidermal water loss (TEWL) to evaluate collagen density and skin hydration.[11] This study aimed to compare the features of collagen density and dermal thickness of rats before and after UVB exposure.

## 2. Methods

This study is an experimental animal study with a post-test control group design. The samples were four male Wistar rats obtained from laboratory experimental animals that had undergone adaptations and also matched the inclusion criteria. The inclusion criteria were male Wistar rats, white and healthy hair, active movement and normal behavior, age 7-8 weeks, and body weight during treatment 180-200 grams. The exclusion criteria were anatomical abnormalities such as physical disability. The study was conducted at the experimental animal laboratory, Universitas Setia Budi, Surakarta, from May to June 2022. The rats were epilated and then given UVB exposure, and a back skin biopsy was performed before and after the total dose of UVB exposure. The UVB exposure dose given was 50 mJ/cm$^2$ in the first week, 60 mJ/cm$^2$ in the 2$^{nd}$ week, and 70 mJ/cm$^2$ in the 3$^{rd}$ and 4$^{th}$ weeks. The UVB tool used was the Kernel® UV Phototherapy KN-4003BL UVB lamp.

Skin tissue samples were obtained from the back skin of male Wistar rats with a size of 10 mm which were then stained with hematoxylin-eosin (HE) and Mason-trichrome (MT) staining at the anatomical pathology laboratory, Faculty of Medicine, Universitas Sebelas Maret. Hematoxylin-eosin (HE) staining was used to assess the dermal thickness, and MT staining was used to assess collagen density. Assessment of dermal thickness was then measured with image raster software, and assessment of collagen density was measured using Image-J software. Observation of anatomical pathology preparations was carried out using an Olympus CX21 light microscope (Tokyo, Japan) connected to OptiLab Advance and OptiLab Viewer 2.2 (Jebres, Surakarta). Observations were made at 40x and 100x magnification at 5 visual fields by two experts in anatomical pathology. Microscopic observation be done by observing the dermal thickness and collagen density before and after UVB exposure.

## 3. Results

Epidermal and dermal structure before and after total dose UVB exposure in both 4x and 10x magnification using Mason trichrome (MT) staining was shown in Figure 1. Collagen density was determined by averaging the 50 photos taken automatically for each skin sample to obtain the average collagen density. The result showed that the collagen densities before total dose UVB exposure are 61.9% for 4x magnification and 63.8% for 10x magnification (Figure 2A and 2C). After being exposed to a total UVB dose, collagen densities decreased to 50.3% for 4x magnificent and 49.6% for 10x magnification (Figure 2B and 2D).

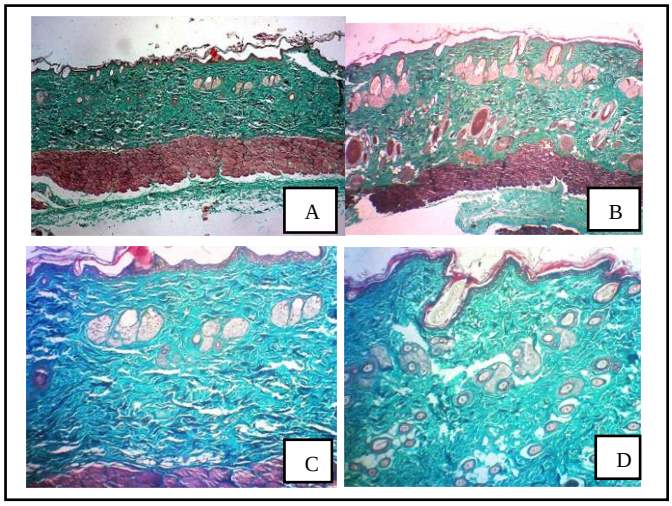


Figure 1. Mason trichrome (MT) staining before and after UVB exposure; (A and C) Structure of the epidermis and dermis of rat skin before UVB exposure (4x and 10x magnification); (B and D) Structure of the epidermis and dermis after a total dose of UVB exposure (4x and 10x magnification).

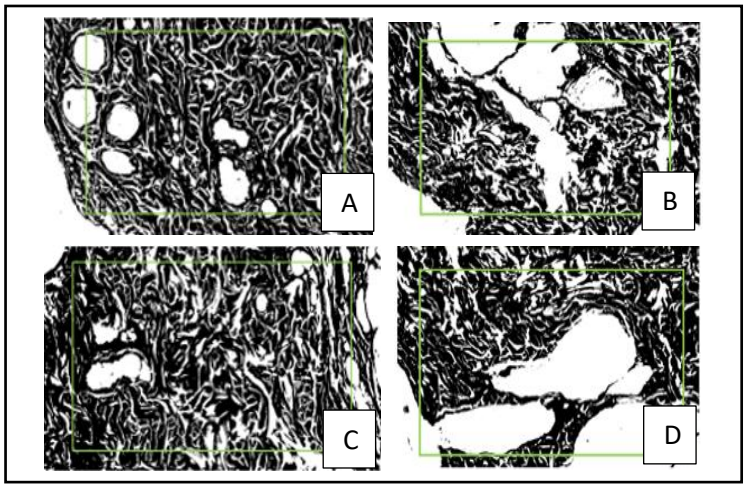


Figure 2. Assessment of collagen density with Image-J software before and after UVB exposure; (A and C) Images of mouse skin collagen density before UVB exposure of 61.9% and 63.8% (4x and 10x magnification), respectively; (B and D) Collagen density images after exposure to UVB total dose of 50.3% and 49.6%, respectively (4x and 10x magnification).

The dermal thickness was measured at three standard points along the width of one picture for a total of six measurement sites and was defined as the region between the upper dermal layer and the junction between the dermal layer and subcutaneous tissue. The reduction was also found in dermal thickness, which is 1422.07 ± 1165.96 μm for 4x magnification before total dose UVB exposure (figure 3A) and 1049.52 ± 1018.97 μm for 4x magnification after total dose UVB exposure (Figure 3B).

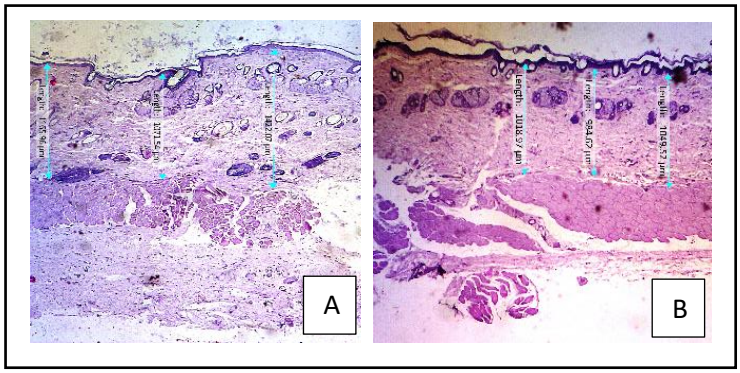


Figure 3. Hematoxylin-Eosin (HE) staining for assessment of dermal thickness with Image Raster software before and after UVB exposure; (A) The dermal thickness of rat before UVB exposure is 1422.07 ± 1165.96 µm (4x magnification); (B) The dermal thickness of rat after UVB exposure is 1049.52 ± 1018.97 µm (4x magnification).

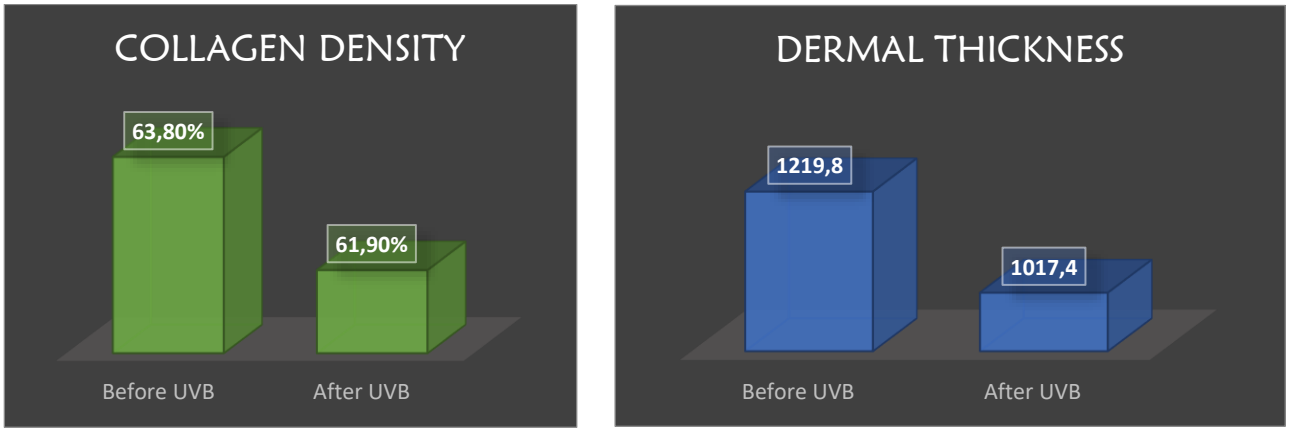


Figure 4. Collagen density and dermal thickness before and after UVB exposure.

**4. Discussion**

There was collagen density reduction found in rat skin after UVB exposure compared to before UVB exposure, indicating that UVB exposure decreased collagen density in the dermis of rat skin. Long-term UVB exposure increased reactive oxygen species (ROS) and caused direct DNA damage.[5] Nuclear factor-B (NF-B) and activator protein 1 (AP-1) are two transcription factors that are induced by reactive oxygen species (ROS) that are produced throughout the photoaging process. Due to increased matrix metalloproteinase (MMP) expression and decreased transforming growth factor (TGF) signaling caused by this activation, collagen is fragmented, and collagen production is reduced.[12] Collagen reduction prevents fibroblasts from mechanically interacting with the extracellular matrix (ECM), which in turn causes cutaneous fibroblast size to decrease. A positive feedback loop that speeds up dermal aging is created when older fibroblasts produce more ROS, which in turn causes MMP expression to rise and TGF-ß signaling to be inhibited. The production of MMP-12 by fibroblasts and macrophages is essential for the growth of solar elastosis and the loss of functional elastic fibers.[7]

There was also dermal thickness reduction found in rat skin after UVB exposure compared to before UVB exposure, indicating that UVB exposure decreased the dermal thickness of rat skin. It is believed that a decrease in the amount of extracellular matrix (ECM), mainly collagen in the dermis, is one of the leading causes of dermal atrophy.[13] The primary cause for increased ECM degradation is an imbalanced

expression of matrix metalloproteinases (MMP)/tissue inhibitors of matrix metalloproteinases (TIMPs) in dermal fibroblasts.[6] The excess of MMP was caused by reactive oxygen species (ROS) and direct DNA damage due to UVB exposure with a similar mechanism to collagen density reduction.[5]

Several studies regarding the reduction of dermal thickness due to UVB exposure strengthen the previously mentioned mechanism. Murine skin exposed to UVB over time in a study showed decreased fibroblast proliferation, smaller collagen fibril diameter, and weaker, stiffer, less elastic skin compared to controls. DNA damage and oxidative stress can cause cutaneous fibroblasts to age prematurely.[14] Cultured human dermal fibroblasts underwent senescence following repeated exposure to sub-cytotoxic UVB levels. Zhou et al. also reported that human dermal fibroblasts exposed to UVB had significantly increased miR-34c-5p levels and decreased SA-gal activity.[15] MiR-34c-5p reduction by siRNA delayed fibroblast senescence. Due to UVB radiation, Blackstone et al. (2020) also reported that skin became weaker, less elastic, stiffer, and less malleable. Notably, these modifications persisted following a 5-week recuperation period. Dermal collagen fibrils' diameters dramatically decreased after UVB exposure, while the expression of the miR-34 family significantly increased.[14] Reduced collagen density and dermal thickness, as well as a decline in the synthesis and replacement of essential structural proteins, are all linked to skin aging. This results in the dermis finding lost pliability and integrity, which clinically shows as slack and wrinkled skin.[16] Histologically, skin aging appeared as epidermal and dermal thinning, loss rate ridges, decrease in the amount of collagen and elastin connective tissue, reduction of fibroblasts and ECM, also solar elastosis.[17] Further research in humans is urgently needed since this study only involves the Wistar rat. The other parameters besides collagen density and dermal thickness are also accounted for in the subsequent studies.

## 5. Conclusion

UVB exposure can induce decreased collagen density and dermal thickness in Wistar skin rats, similar to photoaging.

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