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Development of skin health test efficacy modules *in vivo*

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Abstract

Sunlight contains ultraviolet (UV) light that causes sunburn and makes the skin age faster, leading to more wrinkles as older. The UV light can come from the natural and artificial sources. Moreover, UV light has shorter wavelengths than the visible light. Therefore, people's eyes can't see UV, but people's skin can feel it. In this study, the *in vivo* skin health test efficacy modules have been established via the detection of skin's moisture retention (%), skin's cytokine expression levels, enzymatic expressions in the skin, the expression levels of hyaluronic acid (HA), collagen type I, melanin, and malondialdehyde (MDA) in the skin, and the experimental mice' skin thickness and lesions via histo-pathologic examination. According to the results, the clinical behavior observation indexes of Institute of Cancer Research (ICR) mice in each group were normal during the experiments. Moreover, all ICR mice were survival until the end of the experiments. The moisture retention (%) of skin in ICR mice in UVB group was significant decrease after D1, D3, and D5 of UVB irradiation compared to the normal control group. Based on the IL-1 β , IL-6 and TNF- α analysis expressions, both IL-1 β and IL-6 expressions in UVB group were significantly increase than the control group, while there was no significant difference in the TNF- α expression between the groups. ICR mice' skin enzymatic expressions in each group presented that catalase (CAT) expression and superoxide dismutase (SOD) activity in UVB group were significantly lower than the control group. The MDA expression in UVB group were significantly higher than the control group. The HA and collagen type I expressions in UVB group were significantly lower than the control group. However, the melanin expressions in UVB group and the control groups were not significantly different. The matrix metalloproteinase 2 (MMP-2) expressions in UVB group was significantly higher than the control group. The skin epidermal thickness in UVB group was significantly thicker than the control group. The dermal thickness in two groups was not significantly different. The number of sunburn cells in the derma in UVB group was significantly increase than the control group. The solar elastosis in the derma in two groups was not significantly different. Based on the above results, we have successfully established *in vivo* skin health test efficacy modules to evaluate the status of skin health. We hope the modules should be provide for the research and development (R&D) of the effective treatment included drugs and therapeutic strategies.

Keywords: Development; *In vivo*; Modules; Skin health test efficacy

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1. Introduction

Sunlight contains ultraviolet (UV) light that causes sunburn and makes the skin age faster, leading to more wrinkles as older. The UV light can come from the natural and artificial sources. Moreover, UV light has shorter wavelengths than the visible light. Therefore, people's eyes can't see UV, but people's skin can feel it. Sunlight is the nature source of UV contains UV radiation, which major consists of three types of rays. According to the electromagnetic spectrum wavelength, UV rays are classified as UVA (longest wavelength; 320-400 nm), UVB (medium wavelength; 280-320 nm), and UVC (shortest wavelength; 100-280 nm). UV radiation is a form of electromagnetic energy. The energy levels were increasing sequentially as UVA, UVB, and UVC. Effects of the skin cells were in the dermis to epidermis of skin by UVA, UVB, and UVC irradiation. The short-time effects of UVA, UVB, and UVC respectively caused premature aging / wrinkles / some skin cancers, skin cancer / premature aging, and skin cancer / premature aging. The long-time effects of UVA, UVB, and UVC respectively caused immediate tanning / sunburn, delayed tanning / sunburn / blistering, and redness / ulcer lesions / severe burns [1-5].

The UV rays in the sun are the main cause of sunburn. The shorter wavelength of UV possesses the stronger UV energy and the more greatly tissue damage. However, but the penetration ability is opposite to the wavelength of UV. The main causes of tanning and sunburn are UVA and UVB. UVA mainly causes skin tanning, aging, and wrinkles because its strong penetrating ability, that can penetrate the glass, plastic, etc. UVB has the strong energy that can mainly make the skin sunburn and it is also more likely to cause skin cancer. Although UVC has the strongest energy between UVA, UVB, and UBC, it can hardly reach people's skin surface [5-8].

UV light causes the sunburn symptoms include red and painful skin, redness or blisters, mild headache, and fever. The sunburn symptoms do not appear immediately and usually begin gradually after 4 hours of UV light exposure. The pain of sunburn usually begins on 1st day after UV light exposure. The sunburn symptoms are most severe from 6 hours to 2nd day of UV light exposure and the overall sunburn symptoms are most severe around 2 to 3 days-UV light irradiation, and usually recover gradually after 3 to 5 days-UV light irradiation [9-12].

Therefore, the most important treatment after a sunburn-caused skin injury is to take care of the injured skin and avoid continued exposure to the sun and excessive stimulation. In addition, there is no quick and effective treatment for sunburned skin tissue, currently. Establishment of the skin health test efficacy modules *in vivo* for the research and development (R&D) of the effective treatment included drugs and therapeutic strategies will be very important and need.

2. Material and methods

2.1 Chemicals and Reagents

Phosphate-buffered saline (PBS; Cat. No. P3813, Sigma-Aldrich, MO, United States), ethanol (Cat. No. 493511, Sigma-Aldrich), saline (Cat. No. 100-120-1101, Taiwan Biotech Co., LTD, Taipei, Taiwan), Zoletil 50 (50 mg/mL; Cat. No. 5TK3, Virbac Laboratories, Carros, France), isoflurane (Cat. No. B506, Aesica Queenborough Ltd., Queenborough, UK), hyaluronic acid (HA) ELISA kit (Cat. No. E4626, BioVision, CA, United States), mouse matrix metalloproteinase 2 (MMP-2) ELISA kit (Cat. No. CSB-E04676m, Cusabio, TX, United States), mouse IL-1 beta ELISA kit (Cat. No. ELM-IL1b-1, RayBiotech, GA, United States), mouse TNF-alpha ELISA kit (Cat. No. ELM-TNFa-1, RayBiotech), mouse IL-6 ELISA kit (Cat. No. ELM-IL6-1, RayBiotech), mouse catalase (CAT) ELISA kit (Cat. No. CSB-E14190m, Cusabio), malondialdehyde (MDA) ELISA kit (Cat. No. E4601, BioVision), superoxide dismutase (SOD) assay kit (Cat. No. KA6030, DojinDo, MD, United States), melanin assay kit (Cat. No. S311, Abnova, Taipei, Taiwan), collagen type I ELISA kit (Cat. No. E4618, BioVision), and T-PER™ Tissue protein extraction reagent (Cat. No. 78510; Thermo Fisher, Taipei, Taiwan) were applied in this experiment.

2.2 Experimental Animals and Experimental Design

Adult male 16 Institute of Cancer Research (ICR) mice [8 weeks old; Body weight (BW) between 32-35 g] with specific pathogen-free conditions were used for this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). The environment was maintained room temperature (24-27°C) and 60%-70% humidity with a photoperiod of 12-hr light/12-hr dark cycle. The study will begin after a week acclimation. The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC-110047 approved by the IACUC ethics committee. The male ICR mice were divided respectively into as the normal control group (n = 8) and UVB-treated group (30 mJ/cm² for 10 minutes once per day for 5 days, continuously) (n = 8). All ICR mice were fed with standard laboratory diet (No. 5001, LabDiet®, PMI Nutrition

International, St. Louis, MO, USA) and were administrated with distilled water ad libitum during the experimental period. The clinical behaviors and ICR mice' body weight (BW) were monitored during the experiment.

2.3 Detection of the Moisture Retention (%)

During the experiment, ICR mice were anesthetized with 2-3% isoflurane. Moisture retention was detected by using the moisture analyzers (Halogen Moisture Analyzer HX204, Mettler Toledo, Greifensee, Switzerland).

2.4 Detection of the Cytokine Expression Levels

At the end of the experiment, ICR mice were euthanasia with Zoletil 50. Their sera were collected and processed for the further studies. The sera were detected the expression levels of cytokines (IL-1 β , TNF- α , and IL-6) by using the commercial ELISA kits as mouse IL-1 beta ELISA kit (RayBiotech), mouse TNF-alpha ELISA kit (RayBiotech), and mouse IL-6 ELISA kit (RayBiotech). The standard detection schedule was according to the manufacture' protocols.

2.5 Detection of the Enzymatic Expressions

At the end of the experiment, ICR mice' sera were collected and processed for the further studies. The sera were detected the enzymatic expressions of CAT, MMP-2, and SOD by using mouse matrix metalloproteinase 2 (MMP-2) ELISA kit (Cusabio), mouse catalase (CAT) ELISA Kit (Cusabio), and SOD Assay Kit (DojinDo). The standard detection schedule was according to the manufacture' protocols.

2.6 Detection of the Expression Levels of HA, Collagen Type I, Melanin, and MDA

At the end of the experiment, ICR mice' sera were collected and processed for the further studies. The sera were detected the enzymatic expressions of HA, melanin, collagen type I, and MDA by using hyaluronic acid (HA) ELISA kit (BioVision), melanin assay kit (Abnova), and collagen type I ELISA kit (BioVision), and malondialdehyde (MDA) ELISA kit (BioVision), respectively. The standard detection schedule was according to the manufacture' protocols.

2.7 Detection of ICR Mice' Skin Thickness and Lesions via Histo-Pathologic Examination

During the experiment, the skin thickness of ICR mice were observed and recorded with a veterinarian. At the end of the experiment, the ICR mice were euthanasia with Zoletil 50. Their skins were collected and processed for the further studies. The skin tissues were fixed, sectioned, and H&E stained. The histopathologic examination for the epidermal and dermal thicknesses were performed by a senior pathologic veterinarian with a light microscope.

2.8 Statistical Analysis

The data were expressed as mean \pm SD. All comparisons were made by one-way ANOVA (Analysis of Variance). All significant differences are reported at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3. Results

3.1 Expression of the Moisture Retention in All Groups

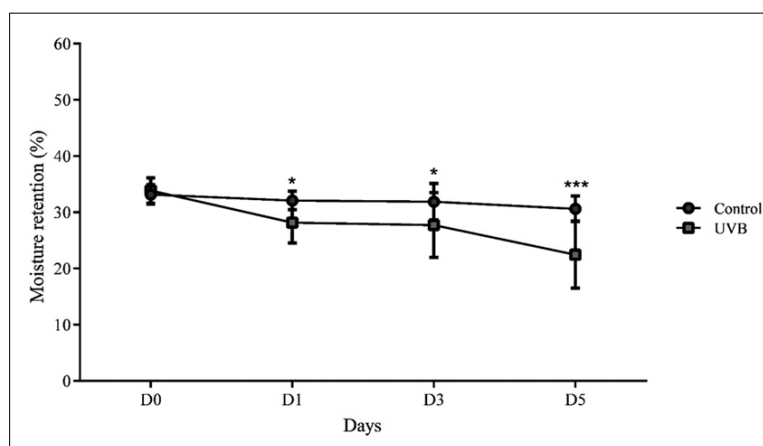


Figure 1 Comparison of moisture retention (%) in two groups with or without UVB irradiation. * $p < 0.05$; *** $p < 0.001$

ICR mice in UVB group were irradiated with UVB light during the experiment. At day 0 (D0), D1, D3, and D5, the moisture retention (%) in two groups were detected by using the moisture analyzers. In this study, the clinical behavior observation indexes of ICR mice in each group were normal during the experiments. During the experiments, the ICR mice in each group had smooth hair, normal hair color, and the normal activity. Moreover, all ICR mice were survival until the end of the experiments. The survival percentage of ICR mice was 100% (16/16) (data not shown). The moisture retention (%) of skin in ICR mice in UVB group was significant decrease after D1, D3, and D5 of UVB irradiation compared to the normal control group ($p < 0.05$ - $p < 0.001$) (Figure 1).

3.2 The Cytokine Expression Levels in All Groups

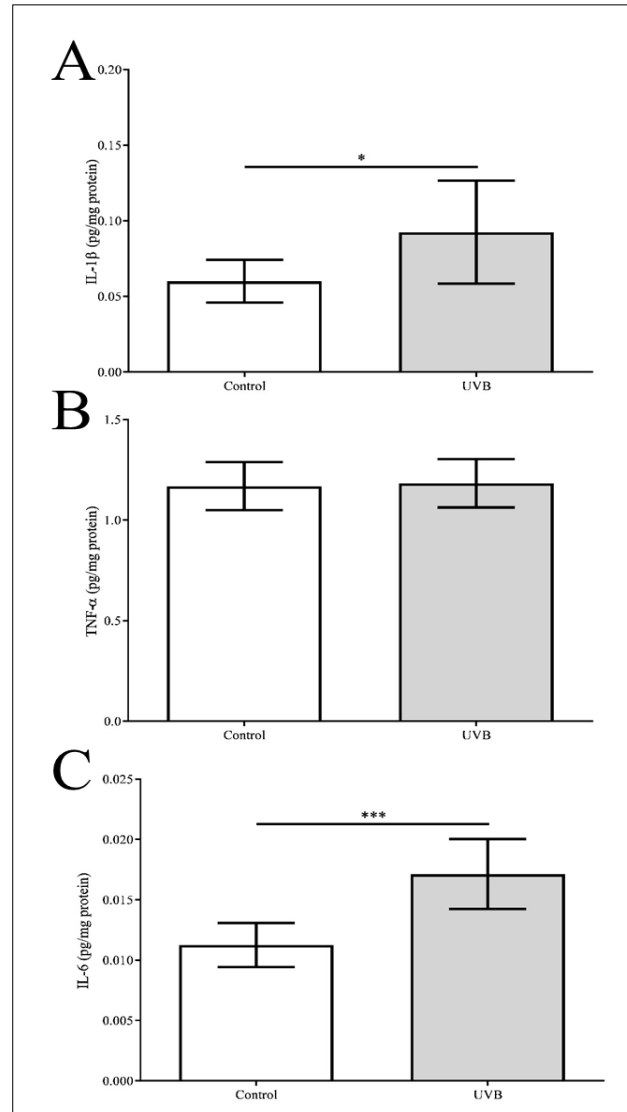


Figure 2 The expression levels of cytokines in two groups with or without UVB irradiation. (A) IL-1 β (B) TNF- α (C) IL-6. All data are expressed as mean \pm SD. * $p < 0.05$; *** $p < 0.001$

Except for the control group, UVB group was irradiated with UVB light (30 mJ/cm²) for 10 minutes per day for 5 days. At the end of the experiment, all ICR mice were sacrificed and their skin tissues were collected. Based on the IL-1 β , IL-6 and TNF- α analysis expressions, both IL-1 β and IL-6 expressions in UVB group were significantly increase than the control group ($p < 0.05$ - $p < 0.001$) (Figure 2A, 2C), while there was no significant difference in the TNF- α expression between the groups ($p > 0.05$) (Figure 2B).

3.3 The Enzymatic Expressions in All Groups

ICR mice' skin enzymatic expressions in each group were measured in the end of the experiment. The CAT expression and SOD activity in UVB group were significantly lower than the control group ($p < 0.01$) (Figure 3A-B).

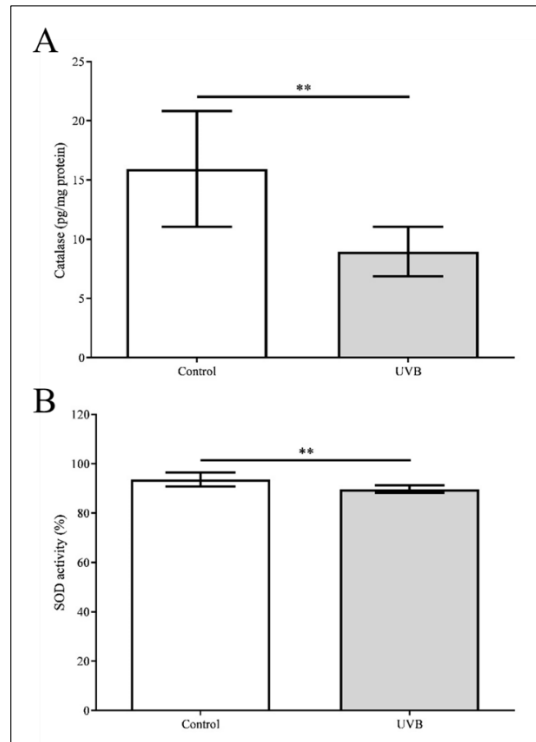


Figure 3 The expression levels of enzymes in two groups with or without UVB irradiation. (A) catalase (B) superoxide dismutase (SOD) activity. All data are expressed as mean \pm SD. ** $p < 0.01$

3.4 The Levels of Lipid Peroxidation in All Groups

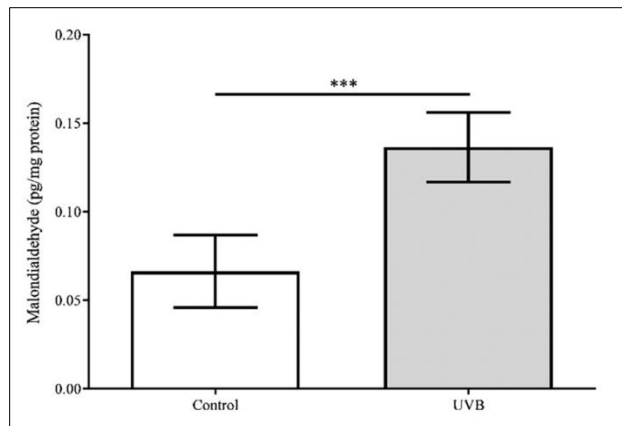


Figure 4 The levels of lipid peroxidation in two groups with or without UVB irradiation. All data are expressed as mean \pm SD. *** $p < 0.001$

ICR mice' skin levels of lipid peroxidation in each group were measured in the end of the experiment. The MDA expression in UVB group were significantly higher than the control group ($p < 0.001$) (Figure 4).

3.5 Expression Levels of Hyaluronic Acid, Collagen Type I, and Melanin in All Groups

ICR mice' skin levels of HA, collagen type I, and melanin in each group were measured in the end of the experiment. The HA and collagen type I expressions in UVB group were significantly lower than the control group ($p < 0.01$ - $p < 0.001$) (Figure 5A-B). However, the melanin expressions in UVB group and the control groups were not significantly different ($p > 0.05$) (Figure 5C).

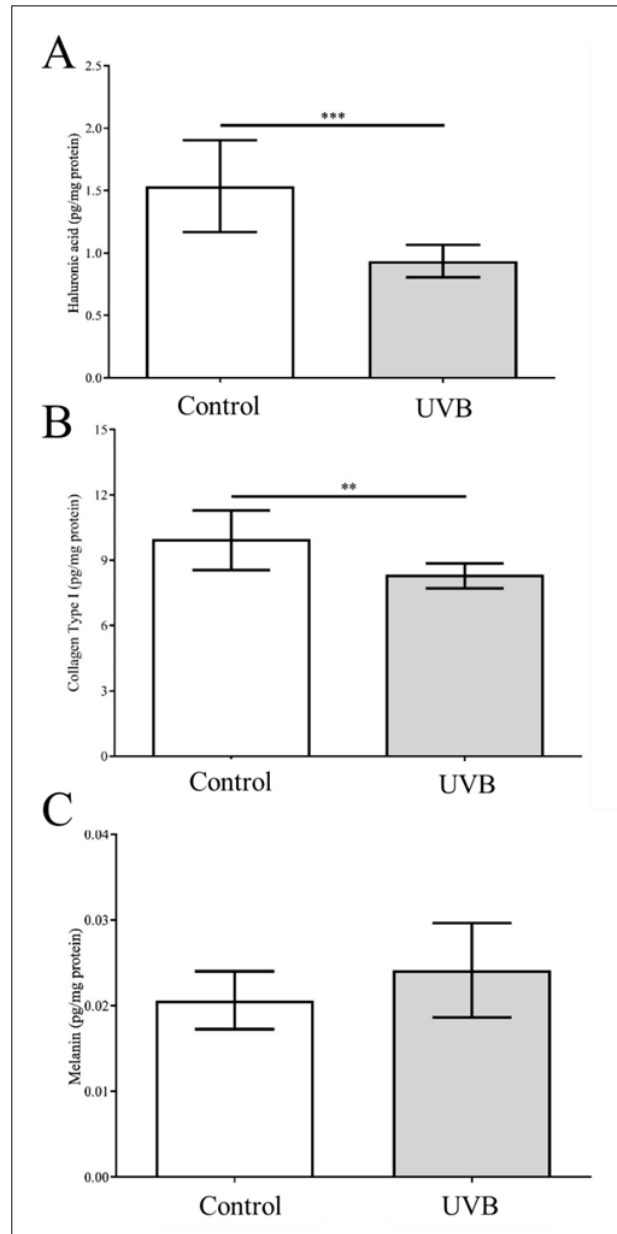


Figure 5 The expression levels of hyaluronic acid, collagen type I, and melanin in two groups with or without UVB irradiation. (A) hyaluronic acid (B) collagen type I (C) melanin. All data are expressed as mean \pm SD. ** $p < 0.01$; *** $p < 0.001$

3.6 Expression Levels of MMP-2 in All Groups

ICR mice' skin levels of MMP-2 in each group were measured in the end of the experiment. The MMP-2 expressions in UVB group was significantly higher than the control group ($p < 0.05$) (Figure 6).

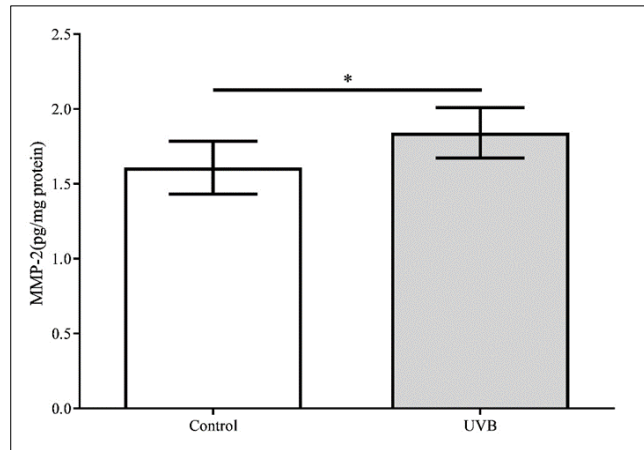


Figure 6 The expression level of MMP-2 in two groups with or without UVB irradiation. All data are expressed as mean \pm SD. * $p < 0.05$

3.7 Histopathologic Examination of ICR Mice' Skin Thickness, the Number of Sunburn Cells, and Solar Elastosis in All Groups

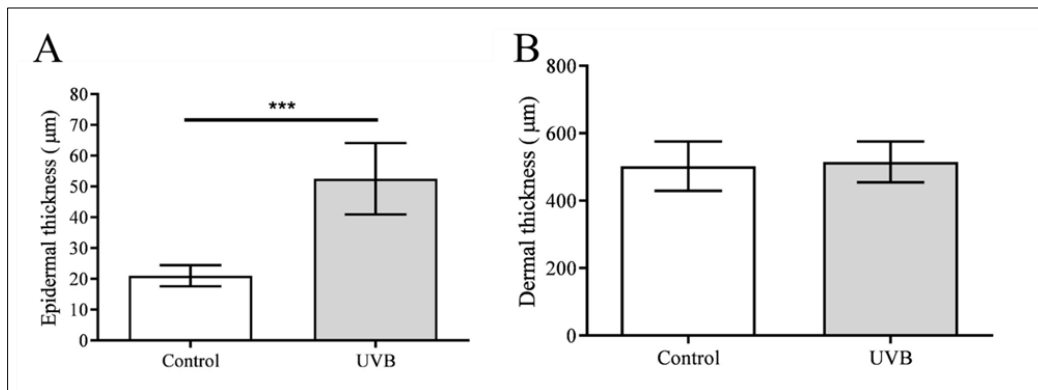


Figure 7 The skin thickness in two groups with or without UVB irradiation. (A) the epidermal thickness (B) the dermal thickness. All data are expressed as mean \pm SD. *** $p < 0.001$

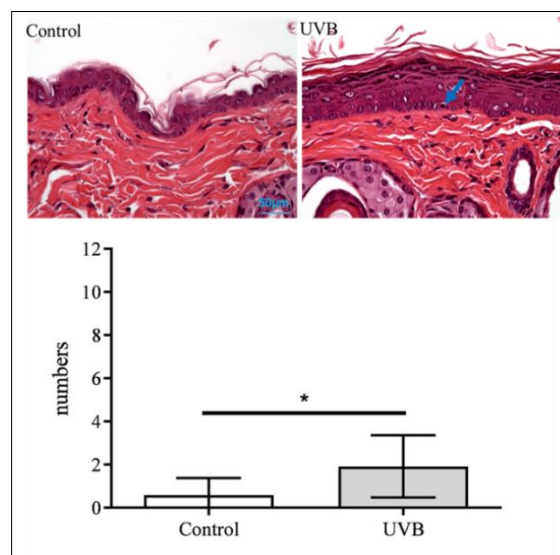


Figure 8 The measurement of sunburn cells in the derma by the histopathologic examination in two groups with or without UVB irradiation. All data are expressed as mean \pm SD. A blue arrow presented sunburn cells in the derma. The bar is 50 μ m. * $p < 0.05$

ICR mice' skin epidermal and dermal thicknesses in each group were measured in the end of the experiment. The skin epidermal thickness in UVB group was significantly thicker than the control group ($p < 0.001$) (Figure 7A). The dermal thickness in two groups was not significantly different ($p > 0.05$) (Figure 7B).

The number of sunburn cells and solar elastosis in the derma in two groups were measured in the end of the experiment. The number of sunburn cells in the derma in UVB group was significantly increase than the control group ($p < 0.05$) (Figure 8). The solar elastosis in the derma in two groups was not significantly different ($p > 0.05$) (Figure 9)

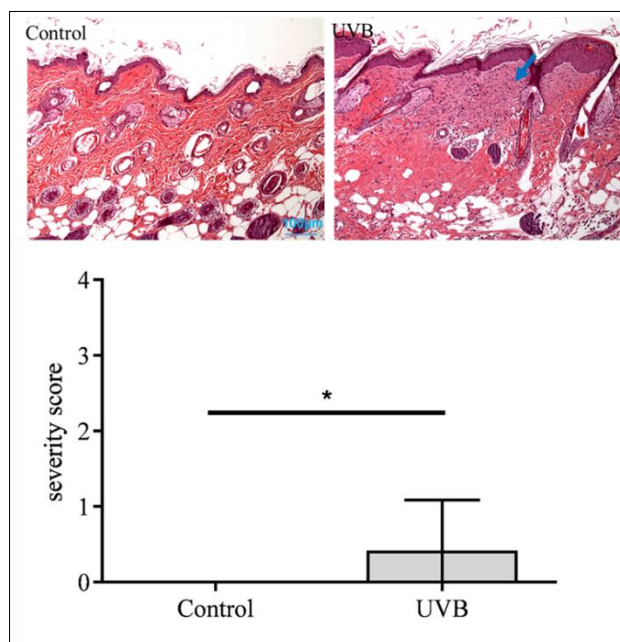


Figure 9 The measurement of solar elastosis in the derma by the histopathologic examination in two groups with or without UVB irradiation. All data are expressed as mean \pm SD. A blue arrow presents solar elastosis in the derma. The bar is 100 μ m. * $p < 0.05$

4. Discussion

The degree of skin damage depends on the intensity of UV rays and the length of UV light exposure time. When you live where the sun is strong year-round, UV light exposure level and risk are increase. UV light exposure is a powerful attack on the skin that creates skin damage from premature wrinkles to dangerous skin cancer. Damage from UV light exposure is cumulative and increases your skin cancer risk over time [13-15].

Severe sunburn symptoms are usually caused by a large sunburn area that may be at risk of dehydration and may be accompanied by other symptoms such as headache, nausea and vomiting, high fever, dizziness, and chills. Additionally, the exposure to UV light damages the DNA in skin cells that produced genetic defects or mutations. Even, this damage can lead to skin and eyelid cancers, premature aging, eye damage (cataracts etc.). Evidences were showed that the UV rays that damage skin can also alter a gene that suppresses tumors, raising the risk of sun-damaged skin cells developing into skin cancer. UV light exposure that leads to sunburn has proven to play a strong role in developing melanoma that is the most dangerous of the three most common types of skin cancer. A majority of non-melanoma skin cancers (NMSC) and a large percentage of melanomas are associated with the exposure to UV light radiation from the sun. UV light radiation is a proven cause of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), which often appear on sun-exposed areas of skin. Fortunately, when discovered early enough, these common forms of skin cancer are almost always curable.

Some protection methods to avoid the sunburn included avoid of sunlight exposure, wearing sunscreen clothing, and using sunscreen products can be apply. The sunlight is strongest between 10:00 am and 4:00 pm in a day to avoid going out and stay in the shade as much as possible outdoors. The dark, long-sleeved, and tight-fitting clothing for better sun protection. When you spend a lot of daytime outdoor activities, you can buy sunscreen clothing with a UPF (ultraviolet protection factor) value greater than 30 or use UV absorbers on clothes to enhance the sunscreen effect of clothes. UPF refers to the multiples of sunscreen products that can prolong the time that the skin is sunburned. Generally, it is

recommended to choose products with UPF 15 or above for daily sun protection, and products with UPF 30 or above for daytime outdoor activities. The sunscreen products should be smeared to all exposed skin (including lips and ears) for 15 minutes before exposure to sunlight. The sunscreen products should be sufficient amount should be applied and reapplied regularly to achieve the indicated protection. The swimming and sweating will reduce the sunscreen ingredients that should dry the skin and then reapply. Other physical sunlight protection equipment included holding an umbrella, wearing a wide-brimmed hat, and wearing sunglasses [1-5, 16-18].

Some treatment will be suggested that UV light-caused skin blisters, it is best not to puncture it to avoid increasing the risk of infection and affecting healing. The sunburn area is too large or other severe symptoms occurred such as nausea, vomiting, and high fever, the medical attention is immediately need [19-21]. Currently, there is no quick and effective treatment for sunburned skin tissue, but some treatments can reduce the symptoms of sunburn as ice and sunburn ointment on the sunburn area, and aspirin, acetaminophen and other non-steroidal pain relievers, orally. In this study, the successful established *in vivo* skin health test efficacy modules will be evaluated the status of skin health. We also hope the modules should be provide for R&D of the effective sunburn treatment included drugs and therapeutic strategies.

5. Conclusion

Sunlight causes sunburn and makes the skin age faster. In this study, the *in vivo* skin health test efficacy modules have been successfully established via the detection of skin's moisture retention, skin's cytokine expression levels, enzymatic expressions in the skin, the expression levels of HA, collagen type I, melanin, and MDA in the skin, and the experimental mice' skin thickness and lesions via histo-pathologic examination. Based on the all results, the successful established *in vivo* skin health test efficacy modules will be evaluated the status of skin health. We also hope the modules should be provide for R&D of the effective sunburn treatment included drugs and therapeutic strategies.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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