Skin damage in animal model using the UVB-box

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Nowadays, an *in vivo* animal model has been widely used to investigate the biological activities of an interested substance. In this study, a mouse model was utilized for the assessment on the protective effect of the interested natural product(s) on UVB-induced skin damage. Hence, UVB-box model was developed to modify the experimental procedures to prevent blindness and minimize the distress of mice during UVB radiation. Our model has been under approval from Center for Animal Research (no.600707), Naresuan University. The designed number of hair-shaved mice on the dorsal back was anesthetized and laid down in the UVB box. The mice's head was jutted around the box. The inside UVB-box area was W×L×H; 20×72×22(cm). It consisted of 4 UVB lamps (alpha type, 18W) which has wavelength 280-320 nm. The UVB-box was modified with wooden material that can prevent from UVB spreading. Measurement of UVB intensity was performed by UV meter to calculate the exposure time. After UVB radiation, dorsal mice skin tissues were collected and analyzed by histological techniques. And their structural morphology changes were observed under microscope to determine the hyperplasia as compare to the non-UVB irradiated mice (control group). This model can compromise the UVB exposure directly to avoid the blindness of the mice. Also, they were exposed to constantly stabilized UVB irradiation.

1. Introduction

Ultraviolet (UV) radiation is a risk factor from external environment that can cause sunburn or skin disorder which leading to skin cancer. Especially, UVB (290-320 nm) can penetrate through human skin and generate free radical such as reactive oxygen species (ROS).[1] They are capable to destroy DNA and disrupt the mechanism of skin cells. Chronic UVB exposure stimulates the over-proliferation of keratinocytes in the epidermis layer to protect skin damage which resulting in epidermal hyperplasia.[2]

Nowadays, natural product has been widely used to prevent the effects of chronic UVB exposure. Mango (*Mangifera indica*) is the one of tropical fruit that commonly found in various regions of Thailand. It enriches with the mangiferin (MGF) which can be isolated from the leaves and bark.[3] MGF (1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside) is a bioactive ingredient which belongs to xanthan group. It possesses the effective anti-oxidant and anti-inflammatory activity which againsts skin-related disorders.[4]

2. Materials and Methods

2.1 Materials

Reagents; Sodium Sulfide x-hydrate, Technical grade (Lot0001113437, PanReac AppliChem, Spain), Deionized water (LabScan Asia, Thailand), Mangiferin Mangifera indica (Lot#SLBV4993, Sigma-Aldrich, Co., St. Louis, USA), Poly-L-Lysine solution 0.1% (w/v) in H2O (Lot#SLBX1450, Sigma-Aldrich, Co., St. Louis, USA), Frozen Section Compound (Lot#121117, Leica, USA), Cyotome (Leica, Milton Keynes, UK), Permount Mounting Medium (Lot181130, Fisher Chemical, Belgium), Histological stain for Nuclear staining Heamotoxylin and Eosin

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stain (Lot.NOV.2018, C.V.Laboratories, Thailand), Acetone (RCI Labscan, Thailand), Xylene (RCI Labscan, Thailand), Light microscope and camera (Axio Observer Z1, Carl Zeiss Microscopy Ltd., Cambridge, UK).

2.2 Animal Treatment

The animal protocol was approved by Naresuan University Animals Ethics Committee (code: NU-600707). Male ICR mice (3 weeks old) were purchased from Nomura Siam International Co., Ltd., Thailand. They were received food and water for 1 week in a temperature–controlled room (22±1°C), %relative humidity (55±10%) and light (12h light:12h dark). Two days before starting of experiment, they were shaved dorsal hair in the sized of 4×4 cm² by 8% sodium sulfide x-hydrate solution. Then, mice were separated randomly into 3 groups (5 mice per group) including non-UVB-irradiated mice (control group), UVB-irradiated group and Mangiferin-treated (MGF) group. All groups were weighed daily before oral administration. Both of untreated UVB-irradiated group and UVB-irradiated group were fed water at dose of 1 ml/body weight/day. While, Mangiferin-treated group was fed mangiferin at dose of 19.4 mg/body weight/day treatments for 16 weeks.

2.3 Modified UVB-radiated box

In this study, the modified UVB-box with wooden material was used for UVB-irradiated protocol that can prevent from UVB spreading. The inside area of box was W×L×H; 20×72×22 (cm^3). It consisted of 4 UVB lamps (alpha type, 18W) which has wavelength of 280-320 nm. The designed number of hair-shaved mice on the dorsal back were anesthetized with mixture of ketamine (40 mg/kg) and xylazine (2 mg/kg) and laid down in the UVB box. The mice's head was jutted around the box. UVB-irradiation group and MGF-treated group were received UVB radiation three times per week. The period of exposure time was 6 to 15 minutes on week 1–4, 54 mJ/cm^2; weeks 5–8, 72 mJ/cm^2; weeks 9–12, 108mJ/cm^2; and weeks 13–16, 126mJ/cm^2, respectively.[5]

2.4 H&E staining and Measurement of Epidermal Thickness

After 16 weeks, all mice were euthanized with thiopental at the concentration of 100 mg/kg. The dorsal skin was collected in the sized of 4×4 cm² immediately and frozen using frozen section compound followed by liquid nitrogen. The frozen dorsal skin were cut in thickness of 8 µm using cyotome. Then, section tissue was placed on poly-L-lysine coated slides and stained with hematoxylin and eosin. Structural morphology and epidermal thickness were measured by light microscope and AXIO software.

3. Results & Discussion

3.1 Appearance of skin morphology

After 16 weeks, the appearance of skin morphology in both of UVB-irradiated group and MGF-treated group was changed obviously as compared to control group (Figure 1). The skin morphology of control group was smooth and wrinkleless, while both of UVB-irradiated group and MGF-treated group were appeared with non-smooth and wrinkles on dorsal skin which according to the repeated-UVB exposure.

3.2 Hematoxylin & Eosin (H&E) staining and Measurement of Epidermal Thickness

H&E staining is usually used in histopathology. This method typically uses 2 stain colors which are hematoxylin and eosin. The hematoxylin is blue color for staining nucleus, and eosin is pink color for the extracellular matrix and cytoplasm.[6] After 16 weeks, the results found that the thickness of epidermis layer in control group was 16.31±2.03 µm, while UVB-irradiated group and MGF-treated group were 35.52±2.75 and 30.00±1.51 µm, respectively (Figure 2). The increase of epidermis layer thickness was the cause of over-proliferation of keratinocyte in epidermis layer as called epidermal hyperplasia. Keratinocyte plays an important role to respond the external stimuli that causes skin disorders. UVB

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radiation or photodamage can deteriorate connective tissue and collagen in skin cells and also generate free radical which stimulating keratinocyte to secrete pro-inflammatory mediators leading to skin wrinkles and finally become to chronic skin damage.[5,7] However, the thickness of epidermis layer in MGF-treated group was thinner than UVB-irradiated group significantly (p<0.01). From the result can be implied that MGF has the ability to reduce the oxidative stress from free radicals in the skin cells and decrease stimulating pro-inflammatory activities, which is caused over-proliferation of keratinocyte.[8]

4. Conclusion

In this study, mice dorsal skin was exposed to repeated-UVB irradiation resulting in the increase of epidermal thickness which leading to the risk of skin damage. MGF-treated group can delay the over-proliferation of keratinocyte as compared to UVB-irradiated group. This can be implied that MGF possessed the active compounds that responsible for anti-oxidant and anti-inflammatory activity. In addition, the animal model was applied to represent the methodology to clarify the efficacy of the interesting bioactive compound using the modified UVB-box which mimic to UVB exposure in human. For using animal model have to concern the safety that can be occurring during experiment. Thus, the modified UVB-box was developed to reduce the blindness and minimize the distress of mice and also monitor UVB intensity by UV meter to control the exposure time in the UVB irradiation method.

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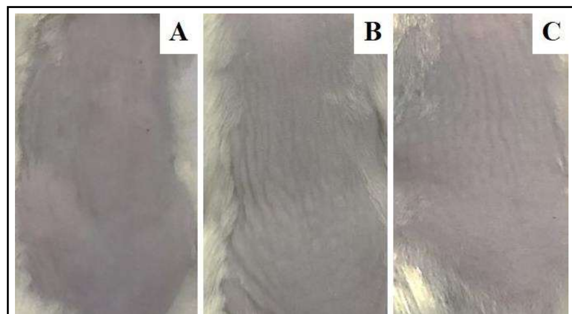
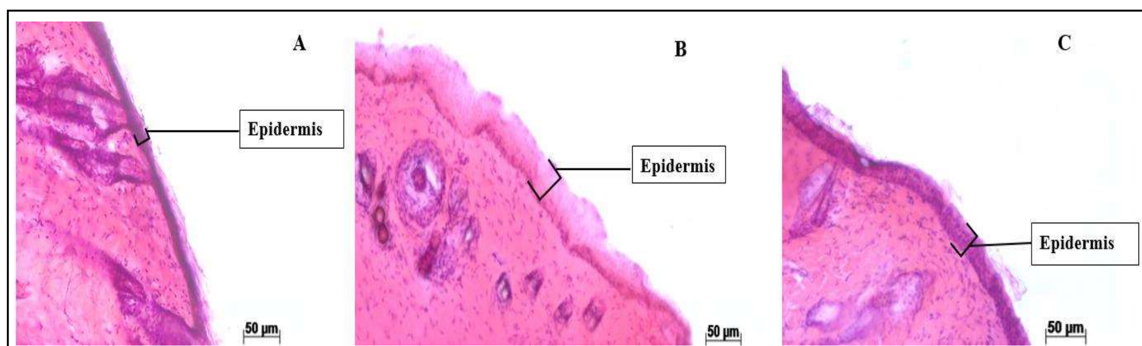


Figure 1. Skin morphology of control group (A), UVB-irradiated group (B) and MGF-treated group (C) after 16 weeks.



(D)

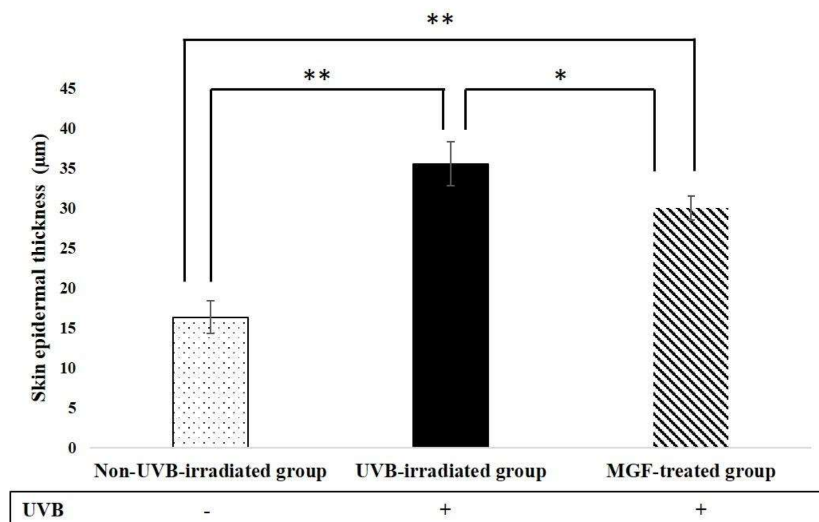


Figure 2. H&E staining dorsal skin sections shown epidermal thickness after 16 weeks under microscope (at magnification, 20x). Non-UVB-irradiated mice (A), UVB-irradiated group (B), MGF-treated group (C). Corresponding measurements of dorsal skin tissues thicknesses (panel D). Bars are mean±SD; n = 5 mice. Control is Non-UVB-irradiated mice. Student's t-test presents statistically significant differences between groups. *p<0.01,

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