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Effect of Thai banana (*Musa* AA group) in reducing accumulation of oxidation end products in UVB-irradiated mouse skin

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ABSTRACT

Scope: Chronic UVB exposure causes skin disorders and cancer through DNA strand breaks and oxidation of numerous functional groups of proteins and lipids in the skin. In this study, we investigated the effects of Thai banana (*Musa* AA group, "Khai," and *Musa* ABB group, "Namwa") on the prevention of UVB-induced skin damage when fed to male ICR mice.
Methods and Results: Mice were orally fed banana (Khai or Namwa) fruit pulps at dose of 1 mg/g body weight/day for 12 weeks. The shaved backs of the mice were irradiated with UVB for 12 weeks. The intensity dose of UVB-exposure was increased from 54 mJ/cm^2/exposure at week 1 to 126 mJ/cm^2/exposure at week 12. A significant increase in skin thickness, lipid peroxidation, protein oxidation end products, and expression of MMP-1 was observed in UVB-irradiated mouse skin. A reduction in the accumulation of oxidation end products was found in the skin of UVB-irradiated mice receiving Khai. This occurred in conjunction with a reduction in MMP-1 expression, inhibition of epidermal thickening, and induction of γ-GCS expression.
Conclusion: The dietary intake of Khai prevented skin damage from chronic UVB exposure by increased γ-GCS expression and reduced oxidation end products included carbonyls, malondialdehyde and 4-hydroxynonenal.

1. Introduction

The action of ultraviolet radiation on the skin, in particular UVB, which penetrates up to the outer dermis [1], creates reactive species such as oxygen and nitrogen radicals [2], which cause DNA strand-breaks, protein cross-linking, and the oxidation of numerous functional groups of proteins and lipids in the skin [3]. Ultimately, these chemical pathologies lead to premature skin aging and carcinomas [4,5]. The oxidation products include protein carbonyls, malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE); these reactive aldehydes can further interact with other biological molecules [6]. UVB also promotes keratinocyte and fibroblast inflammation, which leads to extracellular matrix disorganization via collagenases such as matrix metalloproteinase-1 (MMP-1) [7]. However, free radicals can activate cellular defenses [8,9] via Nrf2 and the expression of glutathione, which are involved in cellular defense mechanism against free radicals [10,11]. Moreover, the skin contains endogenous substances, such as L-ascorbic and α-tocopherol [12], which have the capacity to reduce oxidized elements. Nevertheless, the overproduction of free radicals that results from excessive exposure to external stimuli, including UVB, can overcome the capacity of endogenous antioxidant defense, which leads to skin disorders characterized by inflammation, dermal connective tissue degradation, and abnormal accumulation of the oxidized products.

The fortification of tissue protection and repair can be developed by a dietary intake that includes antioxidants. Plant polyphenols, which are bioavailable antioxidants, contained in fruits and vegetables, can make an important contribution to this [13,14]. Furthermore, plant antioxidants promote the expression of endogenous antioxidant substances, such as glutathione [15,16], which reduce the generation of diseases resulting from oxidative stress [17].

The nutritional effectiveness of the banana is somewhat neglected, and we are unaware of any research into its protection against UVB-induced skin pathologies. Previously, two types of Thai banana, *Musa suerier* (AA group or Khai) and *Musa sapientum* (ABB group or Namwa) were tested for their UVB-protection efficacy in mice. The oral administration of Khai (1 mg/g body weight/day) or L-ascorbic acid (50 mg/day) prevented both the loss of dermal connective tissue and skin glutathione after exposure to chronic UVB radiation, whereas Namwa was not notably effective. Therefore, we thought that the higher

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phenolic and β-carotene contents of Khai would result in lower oxidative stress. To demonstrate this, in the present study, we fed mice with L-ascorbic acid and fruit pulps of Khai and Namwa, and measured the levels of MDA, 4-HNE, protein carbonyls, and MMP-1 in UVB-irradiated mouse skin. Moreover, the expression of γ-glutamylcysteine synthetase (γ-GCS), as a surrogate for Nrf2 release, was also measured.

2. Materials and Methods

2.1. Collection of Banana Fruits

Fresh ripe bananas [*Musa* AA group (*var.*), Khai (Kamphaeng Phet) in Thai and *Musa* ABB group (*var.*), Namwa (Mali-Ong) in Thai] with low browning or few dark spots on the skin were purchased from local markets in Phitsanulok province, Thailand, between February and May 2015 (dry season).

2.2. Determination of Total Phenolic Content

The blended pulp (30 g) was mixed with a 1:1 mixture of ethanol and water to give a total volume of 100 mL, stirred in dim light for 10 min [18,19], and centrifuged at 14,000 rpm at 4 °C for 5 min. The total phenolic content in the resultant supernatant was measured by using the Folin-Ciocalteu method [20]. Briefly, the sample (20 μL) was mixed with deionized water (130 μL) and Folin-Ciocalteu reagent (10 μL). Sodium bicarbonate (7% Na_2CO_3; 100 μL) was added, and the mixtures were incubated at room for 30 min. The sample absorbance was measured at 750 nm with a microplate reader (BioStack Ready, BioTek Instruments, Vermont, USA). Blank samples contained 50% ethanol plus Folin-Ciocalteu reagent and the standards contained 0.015–0.3 mg/mL of gallic acid solution (POCH S.A., Gliwice, Poland). The total phenolic content was expressed in gallic acid equivalents (mg GAE per 100 g of fresh pulp). All determinations were made in triplicate.

2.3. Determination of β-Carotene Content

The method of β-carotene content analysis was as used described in previous studies with some modifications [14,21]. Briefly, the blended pulp (5 g) was placed in a Soxhlet chamber, followed by the addition of freshly prepared 10% L-ascorbic acid (10 mL) (POCH, Sowinskiego, Poland) and 2 M ethanolic potassium hydroxide (50 mL) (Ajex Finechem Pty, Ltd., Auckland, New Zealand). The extraction mixture was refluxed in a boiling water bath. After 1 h of reflux, the resultant extract was cooled to room temperature and partitioned with hexane (70 mL). The yellowish hexane layer was transferred to a separating funnel that contained 5% KOH solution (50 mL), and then 10% NaCl (100 mL) was added. The upper layer was evaporated in a rotary evaporator under vacuum at 37 °C. The residue was dried with N_2 gas, and re-dissolved in methanol. The β-carotene content was measured in 96-well plates by using a microplate reader at an absorbance 450 nm along with 100–700 μg/mL of β-carotene standard (Sigma-Aldrich, Co., St. Louis, USA). The amount of β-carotene was expressed as μg per 100 g of fresh pulp. Determinations were conducted in triplicate.

2.4. Animal Treatment

The study protocol was approved by Naresuan University Animal Ethics Committee (Approval No. NU-AE570513). Four-week-old male ICR mice (25–30 g) were purchased from the National Laboratory Animal Centre (Mahidol University, Nakhon Pathom, Thailand), housed at 22 ± 1 °C and 55 ± 10% relative humidity and fed ad libitum for 1 week at the Center for Animal Research, Naresuan University. Sodium sulfite (8%) (Solon, Ohio, USA) was applied to a 4 × 4 cm^2 area of dorsal skin, and the hair was removed with wet cotton without damaging the skin. The 25 mice were randomly separated into five groups of five animals; two groups were fed freshly blended bananas (Khai or Namwa) at dose of 1 mg/g body weight/day [19] in 0.4 mL water for 12 weeks via intragastric tubes, the UVB + Khai and UVB + Namwa groups, respectively. A third group was fed 50 mg/day of L-ascorbic acid [19] in 0.4 mL water (the UVB + L-ascorbic acid group), and the fourth group (UVB + water group) received 0.4 mL water only. The mice were weighed daily before feeding. After 1 h of feeding, the denuded skin was irradiated with UVB. The final group, the non-UVB (control) group, received no treatments and no UVB irradiation.

2.5. UVB Irradiation

The irradiation protocol used was described previously [19] with some modifications. In brief, mice were anesthetized with ketamine (40 mg/kg) and xylazine (2 mg/kg), and the denuded skin was irradiated by UVB radiation (wavelength range: 280–315 nm) from sunlamps (FL8BLB, Toshiba, Japan) positioned at a distance of 30 cm from the animal, three times per week. To prevent initial skin burning, the radiant energy was progressively increased over the 12-week period by the adjustment of the exposure period from 15 min to 40 min: weeks 1–4, 54 mJ/cm^2; weeks 5–7, 72 mJ/cm^2; weeks 8–10, 108 mJ/cm^2; and weeks 11–12, 126 mJ/cm^2.

2.6. Histology and Measurement of Epidermal Thickness

After 12 weeks, the mice were euthanized with a pentobarbital overdose (100 mg/kg). The irradiated skins (approximately 3 × 3 cm^2) were quickly removed, fixed in freezing medium (Tissue-Tek, Sakura Finetek USA, Inc., California, USA), and then frozen. The skins were sliced into 8-μm-thick transverse sections, placed on poly-L-lysine coated slides, and stained with hematoxylin and eosin. Epidermal thicknesses were measured using AXIO software (Carl Zeiss Microscopy Ltd., Cambridge, UK).

2.7. Immunofluorescence Staining

To quantify lipid peroxidation end-products, including malondialdehyde (MDA), 4-hydroxynonenal (HNE), and matrix metalloproteinase (MMP-1), frozen skin sections were placed on poly-L-lysine coated slides, fixed in cold methanol for 15 min and washed with PBS. Tissues were permeabilized by 0.2% Triton-X in Tris-buffered saline for 10 min and washed twice. Nonspecific antibody binding was blocked by incubation of the sections in 5% bovine serum albumin (BSA) with 0.1% Triton-X in Tris for 30 min. Sections were incubated at 4 °C overnight in a humidified chamber with either polyclonal rabbit anti-MDA (AB6463, Abcam plc., Cambridge, UK), polyclonal rabbit anti-4-HNE (AB46545), or polyclonal rabbit anti-MMP1 (AB137332) primary antibodies at a 1:200 dilution with 1% BSA in Tris. The tissues were washed 3× with Tris and incubated for 1 h with goat anti-rabbit polyclonal antibodies conjugated with FITC (AB6717) or Cy5® (AB6564) at 1:100 dilution with 1% BSA in Tris. The tissues were washed again with Tris, mounted in Fluoromount (Diagnostic BioSystems, California, USA), and observed by fluorescence microscopy (Axio Observer Z1, Carl Zeiss Microscopy Ltd., Cambridge, UK). The fluorescence intensities were semi-quantitatively measured by using digital imaging software (ImageJ; Rasband 1997–2016) [22].

2.8. Immunohistochemical Staining

To detect protein carbonyl adducted protein, the sectioned skin tissues were placed on poly-L-lysine coated slides and fixed (30 mL methanol, 15 mL chloroform, and 5 mL of glacial acetic acid) for 30 min at room temperature. Protein carbonyls (indicative of oxidative stress) were detected with OxyIHC™ Oxidative Stress Detection Kit (S7450, MERCK, Darmstadt, Germany), in accordance with the manufacturer's instructions. Sections were counter-stained with hematoxylin, dehydrated, and mounted with mounting media before observation

under light microscope (Axio Observer Z1, Carl Zeiss Microscopy Ltd., Cambridge, UK). Immunohistochemical staining was semi-quantitatively measured by using digital imaging software (ImageJ; Rasband 1997–2016) [22].

2.9. Western Blot Analysis

Total protein was extracted from the collected skin tissues using 'T-Per' protein extraction solution (QC214313, Thermo scientific, Illinois, USA). The collected protein extracts (total protein, 50 μg per lane) were loaded on to gels, separated by SDS-PAGE (Cat. 161-0301, Bio-rad Laboratories Inc., USA), and transferred (PierceG2 Fast blotter, Thermo scientific, Inc., Illinois, USA) onto a polyvinylidenefluoride (PVDF) membrane (Pall Corporation, Florida, USA). The membrane was incubated in Tris-T buffer [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween 20] containing 5% skimmed milk powder (VL-321563-126, Merck, Darmstadt, Germany) overnight in a refrigerator. After washing, the membranes were incubated with either anti-γ-GCS polyclonal antibody (AB41463, Sigma-Aldrich, St. Louis, Missouri, USA; 1:1000 dilution), or anti-β-actin monoclonal antibody (A5441, 1:2000 dilution) for 2 h in Tris-T/milk powder. The membranes were then incubated for 1 h with goat anti-rabbit-HRP conjugated secondary antibody (Millipore, Massachusetts, USA) in Tris-T/milk powder (1:2000 dilution) for 1 h. Afterward, the blots were exposed to 3,3″,5,5″-tetramethylbenzidine (TMB) substrate Luminata™ Forte (Millipore, Billerica, Massachusetts, USA). The chemiluminescence was measured with the Chemi-Doc™ XRS + system and Image Lab™ Software (BIO-RAD, California, USA). β-Actin was used as the loading control.

2.10. Statistical Analysis

The data for each condition were compared by Student's unpaired *t*-test. All quantitative data are expressed as means for each treatment. Values of $p < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1. Total Phenolic and β-Carotene Contents

Only a few studies have quantified nutrients in fresh banana pulp [23]. It has been reported that the Khai variety has higher total phenol, vitamin E, and β-carotene contents than other varieties [14,24]. In the present study, the total phenolic content was found to be higher in Khai (38.39 ± 1.39 mg GAE per 100 g of fresh fruit pulp) than in Namwa (30.05 ± 0.50 mg GAE per 100 g of fresh fruit pulp), according to the Folin-Ciocalteu method. The level of β-carotene in Khai was 79.20 ± 4.76 μg per 100 g of fresh fruit pulp and was undetectable in the fresh fruit pulp of Namwa when measured using our modified method. The content of phenolic and β-carotene was in accordance with previous studies [14,19,25].

To eliminate the impact of the different water contents in the two varieties of fresh fruit, we also compared total phenolic and β-carotene in the fruit pulps dried by lyophilization. Similar to the results obtained from the fresh fruit pulps, the total phenolic content of the dried fruit pulp of Khai (137.11 ± 4.96 mg GAE per 100 g) was higher than that of Namwa (89.43 ± 1.79 mg GAE per 100 g). When the β-carotene content in the dried fruit pulp was measured, 282.85 ± 16.95 μg GAE per 100 g was found in Khai, but not detected in Namwa.

The skin is a particularly vulnerable organ because of its potential exposure to solar radiation, especially UVB. UVB causes skin disorders through the generation of free radicals [26,27] and cyclobutane pyrimidine dimers in DNA [28]. It has been reported that phytochemicals, including carotenoids and polyphenols, are natural antioxidants which help to prevent skin damage from free radicals [14,15] and cyclobutane pyrimidine dimers [28], and also help to promote the endogenous cellular defenses [15,16]. Therefore, the continuous administration of phytochemicals has been suggested as a possible way to enhance antioxidant capacity and improve defenses against UVB [19]. Owing to the higher phenolic content of Khai, we hypothesized that Khai might show a greater preventive effect than Namwa on the UVB-induced accumulation of damaged molecules. Moreover, as lipids are the main structures within the cell and serves as a major target of free radical attack [6], and β-carotene is a carotenoid that prevents free radical attack in the lipid part of the cellular membrane [29], consumption of Khai with a high β-carotene content might also lead to the reduced accumulation of oxidized lipid molecules in the skin.

3.2. Oral Consumption of Banana Prevents Epidermal Thickening and Dermal Connective Tissue Damage after UVB

Chronic UVB exposure causes skin damage and dermal connective tissue disarrangement; this is known as photodamage. After repeated exposure to UVB, free radicals such as ROS are generated. Afterward, skin cells, particularly keratinocytes, respond to ROS by the secretion of pro-inflammatory mediators, which lead to skin inflammation, epidermal hyperplasia, and dermal connective tissue damage [30,31]. Epidermal hyperplasia or thickening, and connective tissue damage are clear signs of skin disorder. Here, epidermal thickening (20.17 ± 1.94 μm for the non-UVB (control) group vs 27.95 ± 3.85 μm for the UVB + water group), coupled with histological changes in dermal tissue in UVB-irradiated mouse skin was observed (Fig. 1A and B). The epidermal thickening was completely prevented by L-ascorbic acid (19.01 ± 1.66 μm) and substantially reduced by consumption of Khai (21.95 ± 2.04 μm). The damage to the dermal connective tissues was also prevented in these two groups. The epidermal hyperplasia (26.37 ± 1.94 μm) and damage in dermal connective tissues were markedly found in the UVB + Namwa group.

The beneficial effects of Khai may result from its constituent natural antioxidants, although these phenolic compounds have lower antioxidant activity than L-ascorbic acid [32]. Nevertheless, some studies have reported the prevention of premature aging or UV-induced skin damage by the continuous consumption of natural antioxidants such as phenolic compounds [20,33,34]. Moreover, β-carotene and phenolic compounds have the ability to absorb UVB photons [35]. The scavenging of ROS and photoprotection by natural antioxidants would not only prevent ROS-related cellular reactions, but also decrease the production of inflammatory cytokines in UVB-irradiated skin, which would consequently prevent UVB-induced epidermal and dermal alterations.

3.3. Effect of Banana Consumption on Lipid Peroxidation in UVB-irradiated Mouse Skin

Polyunsaturated fatty acids are excellent substrates for lipid peroxidation. Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are lipid peroxidation end products that can diffuse to distant organs. They play a vital role in the adducting of cellular components such as proteins, which will lead to their subsequently malfunction and the development of various diseases [6].

Immunofluorescent staining of skin tissues for the examination of MDA and 4-HNE levels are shown in Fig. 2A, and the relative percentage fluorescence intensity of the peroxidation end products contained in the skin tissues is shown in Fig. 2B. The intensity in the non-UVB (control) group was adjusted to 100%. After exposure to UVB radiation for 12 weeks, the intensity of the staining of both end products was significantly higher ($p < 0.05$) in the UVB + water group (213.10 ± 24.32% for intensity of MDA, 199.09 ± 13.42% for intensity of 4-HNE) compared with the control group. The localization of the end products was most noticeable in the epidermal region, where lipids are abundant in the outermost layer. On the other hand, daily oral administration of Khai or L-ascorbic acid significantly inhibited the increases in MDA (130.71 ± 7.55% for the UVB + Khai group, 59.97 ± 17.51% for the

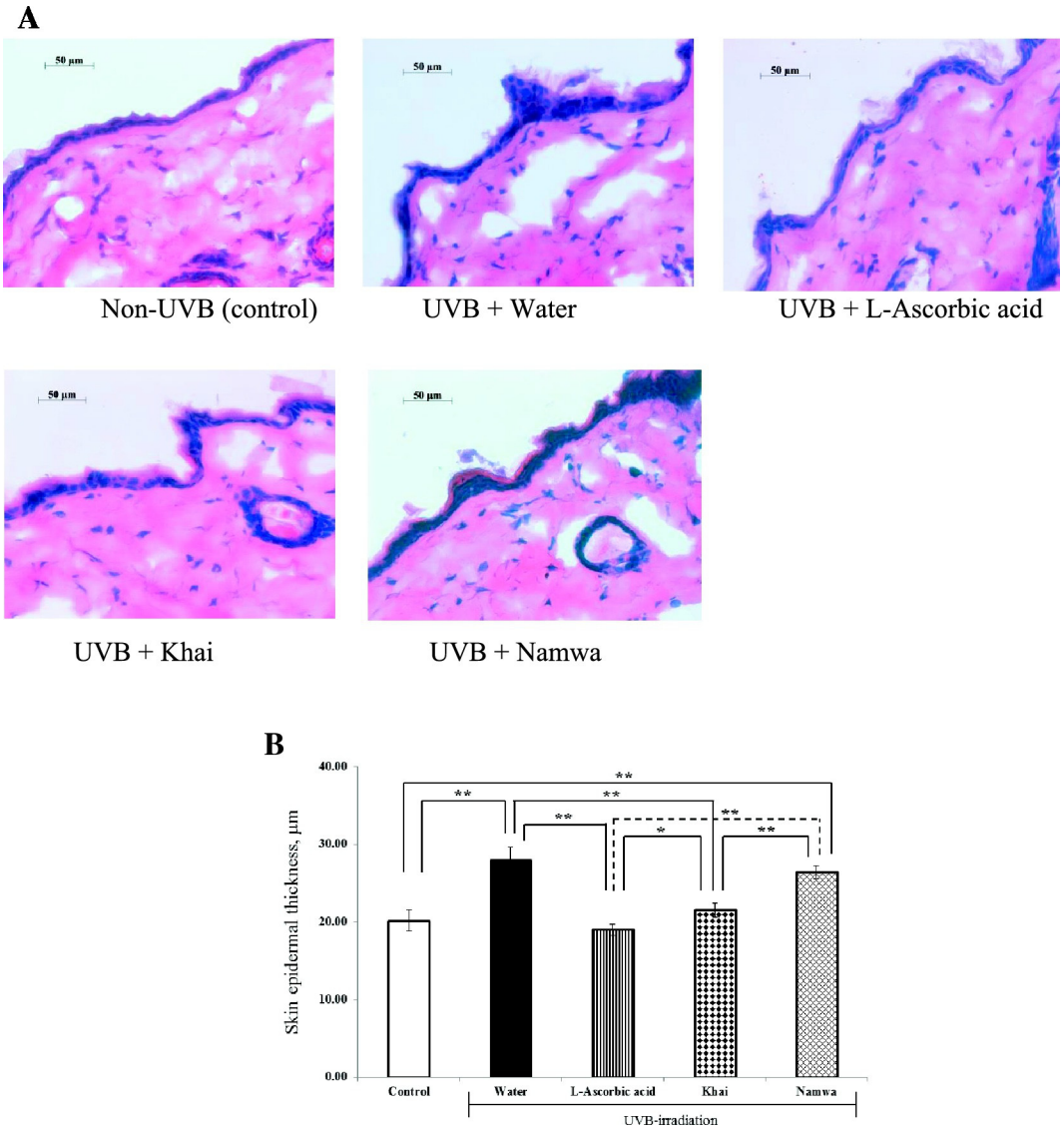

Fig. 1. A H & E skin sections showing skin thickening after 12-week course of UVB radiation counteracted by oral water, 50 mg/day L-ascorbic acid, 1 mg/g body weight/day of Khai or Namwa. B Corresponding measurements of skin thicknesses (panel A). Bars are mean ± s.e.m.; n = 5 mice. Control is non UVB-irradiated mice. Student's *t*-test indicates statistically significant differences between groups. $^{*}p < 0.05$, $^{**}p < 0.01$.

UVB + L-ascorbic acid group) and 4-HNE (133.38 ± 22.31% for the UVB + Khai group, 95.60 ± 13.06% for the UVB + L-ascorbic acid group) in UVB-irradiated mouse skin. In a comparison between the two varieties of bananas, Khai exhibited much greater inhibition of MDA and 4-HNE than Namwa. The high content of β-carotene, a lipid soluble antioxidant, in Khai might be involved in radical scavenging in the lipidic parts of skin and prevention of the generation of lipid peroxidation end products. The rapid antioxidant activity of L-ascorbic acid might minimize the occurrence of further reactions, or a chain reaction, between ROS and cellular components, which would consequently lower the accumulation of peroxidation end products. Moreover, it has been reported that L-ascorbic administration inhibited lipid peroxidation in the skin of mice exposed to γ-radiation, probably via enhancement of glutathione peroxidase activity to detoxify lipid peroxides in the skin tissue [27].

3.4. Effect of Banana Consumption on Protein Oxidation in UVB-irradiated Mouse Skin

Proteins are known to be potential targets for oxidative modification. The oxidative reaction or oxidative cleavage of the amino acid residues of lysine, arginine, proline, and threonine leads to protein carbonyl formation [36]. Additionally, during lipid peroxidation or reducing sugar reaction, proteins may produce a carbonyl group through reactions with aldehydes (4-HNE and MDA) [6,37].

The immunohistochemical staining of the skin tissues for the examination of carbonyls is shown in Fig. 3A, and the percentage intensity of carbonyls contained in the skin tissues is shown in Fig. 3B. The intensity of the control group was adjusted to 100%. After chronic exposure to UVB for 12 weeks, carbonyl content was found to be significantly increased in the UVB + water group (141.28 ± 12.66%) compared with the control group. In contrast, oral consumption of Khai or L-ascorbic acid significantly inhibited the UVB radiation-induced increase in carbonyl content (102.80 ± 7.91% for the UVB + Khai group, 102.44 ± 10.75% for the UVB + L-ascorbic group). The inhibition was not significant in the UVB + Namwa group (119.33 ± 16.54%). These results were consistent with the study of the lipid peroxidation end products. Again, the consumption of Khai (high phenolic content) and L-ascorbic acid (high antioxidant activity) might prevent accumulation of oxidation end products in UVB-irradiated skin. For the UVB + Namwa group, the increased accumulation of carbonyl products in comparison with the UVB + Khai group might occur because of the lower content of

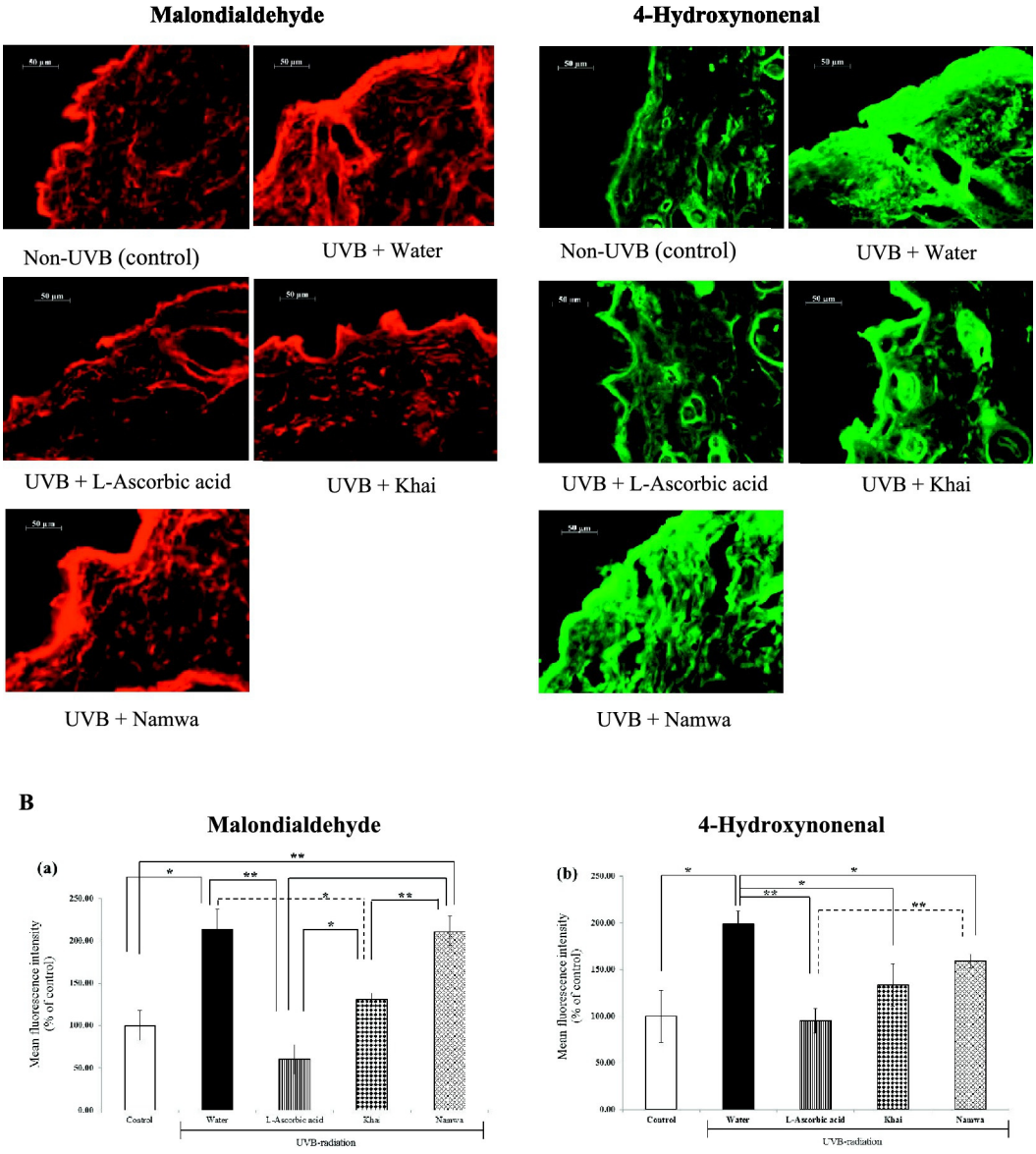

Fig. 2. Immunofluorescence staining in the same mice as in Fig. 1 showing lipid peroxidation end products (MDA and 4-HNE). A Frozen sections were treated with anti-MDA antibody or anti-4-HNE antibody revealed by Cy5 (red) or FITC (green) conjugated goat anti rabbit IgG antibody. Fluorescence was averaged from region of interest in each photograph and quantitated using Image J software. B Corresponding quantitated fluorescence intensity, each bar graphs show quantification of fluorescence intensity for the MDA level (a) and 4-HNE level (b) normalized to control ± s.e.m.; n = 5. Control is non UVB-irradiated mice. Significantly different between groups, $^{*}p < 0.05$, $^{**}p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

natural antioxidants. Moreover, as Namwa fruit pulps have a higher content of sugar than Khai fruit pulps [38], Namwa consumption might increase blood glucose level, which would result in carbonyl accumulation in the skin [39].

3.5. Effect of Banana Consumption on MMP-1 Expression in UVB-irradiated Mouse Skin

MMPs are group of zinc-dependent proteolytic enzymes that can be divided into different subgroups, such as collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9) and stromelysins (MMP-3 and -11). MMP-1, which is predominantly produced by dermal fibroblasts, is responsible for the first degradation step of the collagen deposited in the dermal layer. The active form of MMP-1 is a secreted form [40]. Increased secretion of MMP-1 results in a fragmented collagen fibril, which results in the loss of biomechanical properties in the skin [41]. A major stimulator of MMP-1 expression and secretion is UV radiation [42], and some studies have revealed that phenolics and L-ascorbic acid can suppress the production and secretion of MMP-1 in UV-irradiated fibroblasts through the blocking of the ROS-receptor and/or cytokine-receptor downstream pathways, such as the mitogen-activated protein kinase (MAPK) pathway [43,44].

The immunofluorescence staining of MMP-1 in skin tissue is shown in Fig. 4A, and the fluorescence intensity is presented as a percentage of the control fluorescence intensity in Fig. 4B. The intensity of the control group was adjusted to 100%. In comparison to the control group, repeated exposure to UVB radiation dramatically increased MMP-1 (163.91 ± 15.55% for the UVB + water group), along with dermal tissue damage (Fig. 1A) in the mouse skin. The consumption of banana (129.15 ± 12.40% for the UVB + Khai group, 136.70 ± 10.22% for the UVB + Namwa group) or L-ascorbic (100.78 ± 20.77%) inhibited the overexpression of MMP-1 induced by chronic UVB exposure but significant inhibition was found only in group of mice receiving L-ascorbic acid. In our previous study, we reported that oral administration of Khai or L-ascorbic acid prevented dermal tissue damage by the maintenance of the type I collagen level in UVB-irradiated mouse skin [19]. The

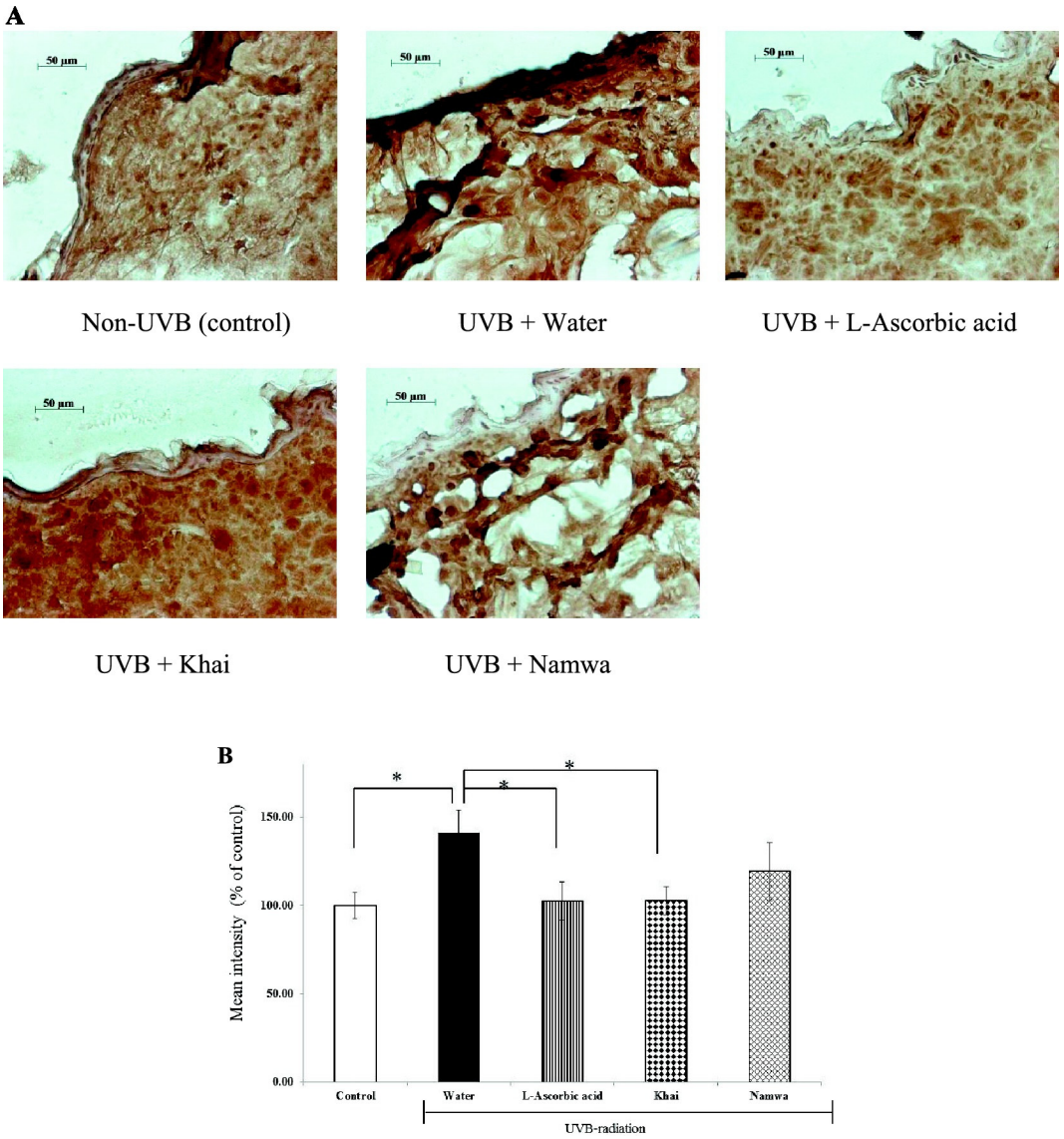

Fig. 3. Immunohistochemistry in the same mice as in Fig. 1 showing carbonyls adducted protein in mice tissues. A Frozen sections were treated with anti-DNP antibody to observe carbonyls adducted protein in mice tissues. Brown color was represented carbonyls level. The brown spots were averaged from region of interest in each photograph and quantitated using Image J software. B Bar graphs illustrate the level of carbonyls in mice skin tissues by immunohistochemistry. Brown spots were averaged from region of interest in each photograph and quantitated normalized to control ± s.e.m.; n = 5. Control is non UVB-irradiated mice. Significantly different between groups, $^{*}p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

results from our previous study, coupled with the results from our present study, indicate that the prevention of UVB-induced dermal connective tissue damage might, at least partially, result from the inhibition of the overexpression of MMP-1. However, further study should be conducted to identify the mechanism(s) underpinning the prevention of MMP-1 overexpression in the UVB-irradiated mouse skin by banana or L-ascorbic consumption.

3.6. Effect of Banana Consumption on γ-Glutamylcysteine Synthetase Enzyme Expression in UVB-irradiated Mouse Skin

γ-Glutamylcysteine synthetase (γ-GCS) is a rate-limiting enzyme for glutathione synthesis. Glutathione exists intracellularly in either an oxidized (GSSG) or reduced (GSH) state. GSH, coupled with glutathione peroxidase, detoxifies peroxides by functioning as an electron donor in the reduction, which produces GSSG as an end product. The reduction of GSSG is catalyzed by glutathione reductase in a process that requires NADPH. The maintenance of glutathione level is critical for the survival and function of cell, and a correlation between reduced GSH level and degenerative diseases has been reported [45]. Moreover, it has also been indicated that the induction of MMP-1 production by fibroblasts was correlated to a low glutathione level in the fibroblasts [46].

The beneficial effects of phytochemicals such as β-carotene and polyphenols have been reported on the glutathione synthesis system [15,16]. Our previous study reported that the level of total glutathione level in mouse skin was significantly decreased following repeated exposed to UVB in comparison with non-UVB-irradiated skin. The glutathione level in UVB-irradiated mouse skin was restored in Khai or L-ascorbic acid administration groups [19], but this restoration was not observed in the group that was administered Namwa. In the present study, we investigated γ-GCS expression in UVB-irradiated mouse skin. As shown in Fig. 5, a small but significant increase in the expression of γ-GCS was found in the skin after repeated UVB exposure in comparison with the control group. This might result from the skin defense systems promoting biosynthesis of the enzyme to producing more GSH in order to counteract ROS production from the chronic UVB exposure. This hypothesis is based on a previous report that indicated the increased expression of Nrf-2, an antioxidant response element gene

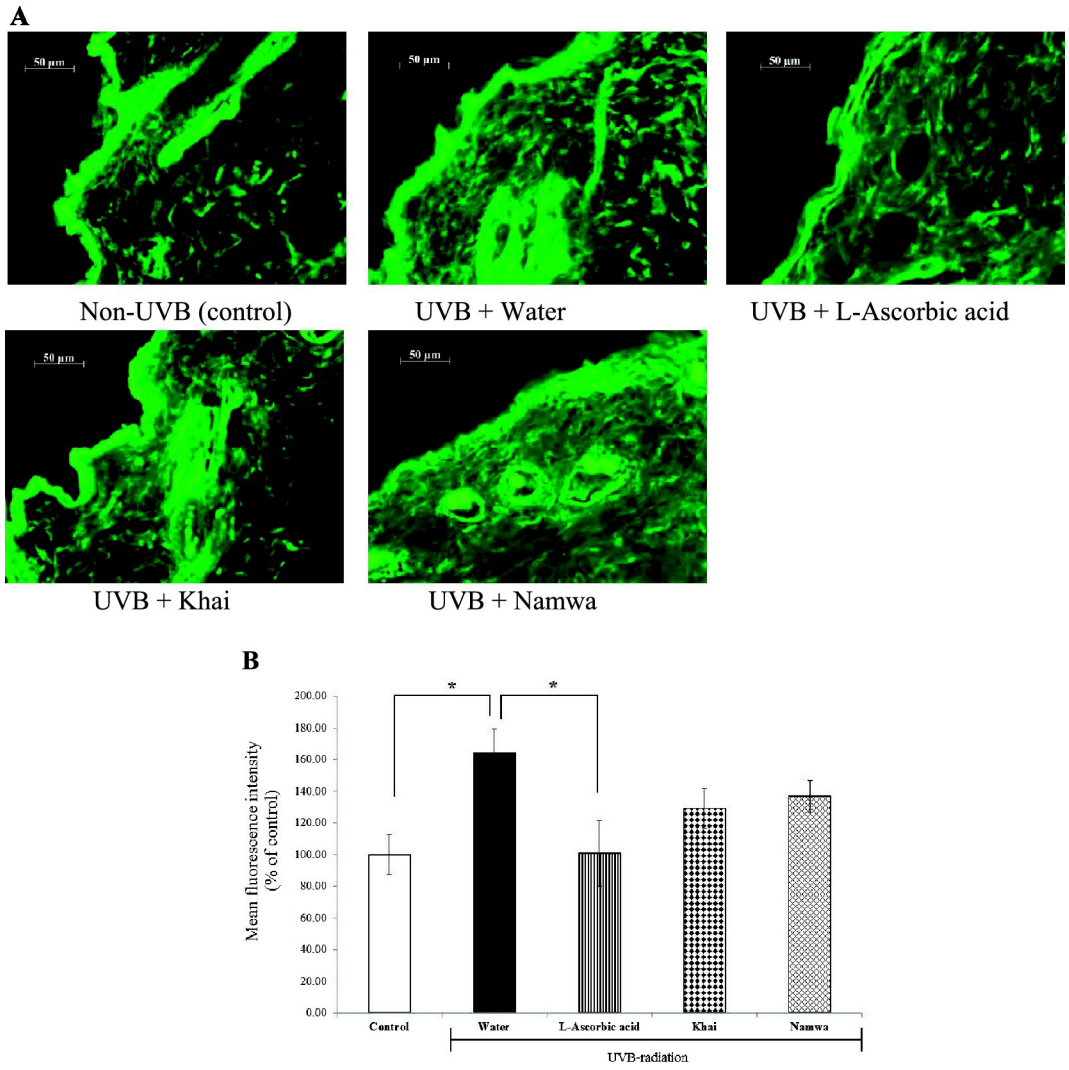

Fig. 4. Immunofluorescence in the same mice as in Fig. 1 showing MMP-1 expression in mice tissues. A Frozen sections were treated with anti-MMP-1 antibody revealed with FITC conjugated goat anti rabbit IgG antibody. Fluorescence was averaged from region of interest in each photograph and quantitated using Image J software. B Corresponding quantitated fluorescence intensity, each bar graphs showing semi-quantification of mean fluorescence intensity of MMP-1 normalized to control ± s.e.m.; n = 5. Control is non UVB-irradiated mice. Significantly different between groups, $^{*}p < 0.05$.

that controls γ-GCS gene expression, was caused by accumulated ROS [47]. However, overproduction of ROS in UVB-irradiated skin may have potentially overwhelmed such a defensive system, which consequently resulted in a decrease in the level of glutathione in skin and skin disorders. Nevertheless, a large increase in the level of γ-GCS expression was observed in UVB-irradiated skin of mice receiving Khai in comparison with the γ-GCS level found in the control group. We hypothesized that the phenolics and β-carotene in Khai could scavenge free radicals and also activate Nrf-2 [16,17] to induce γ-GCS expression and subsequent glutathione synthesis. Thus, the long-term consumption of Khai could prevent an accumulation of oxidized lipids and protein end products. This prevention would not occur in the group administered Namwa.

In the UVB + L-ascorbic acid group, we identified low expression of γ-GCS in UVB-irradiated mouse skin. In general, L-ascorbic acid is a potent antioxidant, which rapidly eliminates free radicals and promotes glutathione recycling in cells, but does not induce the expression of γ-GCS [48,49]. L-ascorbic acid can reduce oxidized glutathione by the rapid activation of glutathione recycling activity. The synergistic activity in the antioxidant and glutathione recycling of L-ascorbic acid was therefore responsible for the prevention of skin damage from chronic UVB exposure observed in the present study. From the obtained results, we concluded that Khai and L-ascorbic acid functioned through different mechanisms to prevent skin damage from chronic UVB exposure.

4. Conclusion

Mouse skin exposed to chronic UVB radiation resulted in epidermal thickening, dermal connective tissue damage, and high accumulation of lipid peroxidation and protein oxidation end products in skin. Moreover, higher expression of an enzyme involved collagen degradation, MMP-1, was observed. The diminished accumulation of protein and lipid peroxidation end products was observed in the skin of UVB-irradiated mice receiving Khai fruit pulp, along with reduced MMP-1 expression, prevention of epidermal thickening and dermal connective tissue damage, and increased γ-GCS expression. These effects were attributed to the antioxidant action and/or the ability to activate the cellular signaling pathway of phenolics and β-carotene that are abundant in the fruit pulp of Khai. The prevention of dermal skin damage, epidermal thickening, MMP-1 overexpression, and oxidation product accumulation was

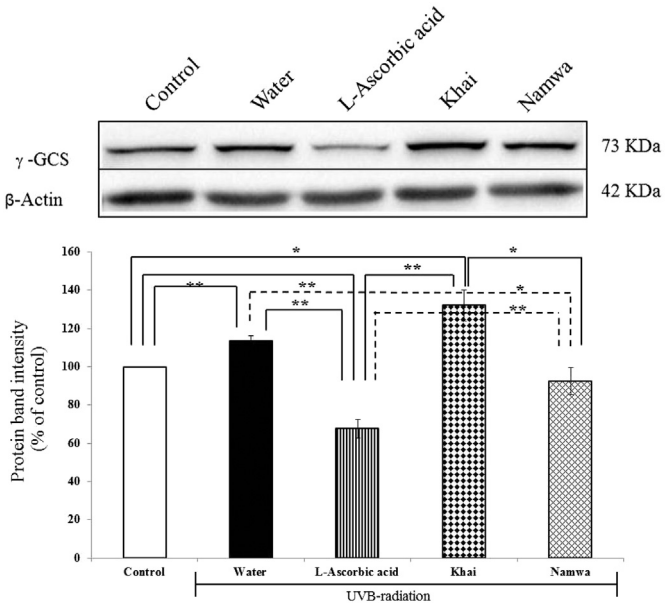

Fig. 5. The effect of oral administration of Khai, Namwa and L-ascorbic acid on γ-glutamylcysteine synthetase (γ-GCS) level in chronic UVB-irradiated mice. Intensity of γ-GCS protein level was normalized to control (non UVB-irradiated mice) from the protein bands. Each bar represents mean ± s.e.m.; n = 5 mice. Significantly different between groups, $^{*}p < 0.05$, $^{**}p < 0.01$.

also found in the skin of UVB-irradiated mice that were administered L-ascorbic acid. However, the preventive mechanisms of Khai and L-ascorbic acid were different. The preventive effect of Khai occurred through the induction of γ-GCS, whereas that of L-ascorbic acid arose from its glutathione recycling ability.

Conflict of Interest

On behalf of all authors, the authors have declared no conflicts of interest.

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