Prev. Nutr. Food Sci. 2015;20(3):162-168
http://dx.doi.org/10.3746/pnf.2015.20.3.162
pISSN 2287-1098 · eISSN 2287-8602

Photoprotective Effect of Lotus (*Nelumbo nucifera* Gaertn.) Seed Tea against UVB Irradiation

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ABSTRACT: Lotus (*Nelumbo nucifera* Gaertn.) seed is widely used as a traditional medicine in countries of Asia. Among many functions of the lotus seed, one interesting activity is its skin protection from the sunlight and scar. In this study, we focused on the skin protective property of lotus seed tea against ultraviolet B (UVB) irradiation. Two groups of a hairless mouse model, water as control (water group) and lotus seed tea (LST group), were administrated a fluid drink water for six months. After 6 month of administration, UVB exposure was carried out to both groups for another 3 months. During and after the administration, the skin moisture content and the morphological and histopathological analyses through biopsy were carried out. Prior to UVB irradiation, no significant difference was discovered in the skin moisture content for the water group and LST group ($P<0.05$). However, drastic changes were observed after the UVB treatment. The LST group showed a clear evidence of skin protection compared to the control group ($P<0.05$). The moisture content, epidermal and horny layer thickness, and protein carbonyl values all revealed that the intake of the lotus seed tea enhanced protection against UVB exposure. As a result, the long-term intake of the lotus seed tea showed the effect of preventing loss of skin moisture, mitigating the formation of abnormal keratinocytes, and contributing to protein oxidation inhibition.

Keywords: lotus (*nelumbo nucifera*) seed tea, photoprotective, skin moisture content, horny layer, protein oxidation

INTRODUCTION

The process of skin aging can be categorized into aging due to internal factors such as age and photoaging due to external factors such as sunlight. Aging due to external factors, especially due to ultraviolet light (UV), is called photoaging (1).

UV radiation represents one of the most important environmental factors affecting human health. UV radiation is divided into three sections as UVA (320∼400 nm), UVB (280∼320 nm), and UVC (200∼280 nm) by wavelength. Among these, the toxic effects of UVB are a major concern for human health (2). UVB initiates a photo-oxidative reaction that changes anti-ROS-sensitive signaling pathways, which increases the cellular level of ROS (3). Toxic products mainly derived from lipid peroxidation and protein carbonylation under UVB exposure accelerate further oxidative damage. The major acute effects of UV irradiation in normal human skin include erythema, edema, sunburns, hyperpigmentation, and hyperplastic responses, whereas the chronic effects include photoaging, immunosuppression, and photocarcinogenesis. UV irradiation especially damages keratinocytes of the epidermis to cause dyskeratosis where the keratin layer thickens and dehydrates, leading to the skin losing its elasticity, becoming rough, and making wrinkles (4). People live in an environment full of exposure to UV radiation. Due to the increase in life span and outdoor activities, it has become easier to be exposed to UV radiation. Increased skin damage may be due to UV exposure because the ozone layer is continuously being destroyed by environmental pollution, which increases the absolute amount of UV (5). Currently, the typical methods used to stop skin damage due to UV include using sunblock or using cosmetics that contain substances for preventing wrinkles, or directly applying medicine on the skin. However, the drawbacks of these methods are that they are restricted to the areas of the skin where medicine or cosmetics are applied and that they are only temporary. Recently, there has been an increasing number of studies that have focused on the UV protective effects of food on skin to maintain healthy skin through diet. The food items that have been researched include green tea (6), red ginseng (7), mug-

Received 17 August 2015; Accepted 17 September 2015; Published online 30 September 2015

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wort (8), and sesame oil (9).

Nelumbo nucifera commonly known as lotus or sacred lotus is an aquatic perennial plant belonging to the *Nelumbonaceae* family (10). Lotus has been used as a food for about 7,000 years in Asia and is cultivated for its edible roots, seeds, and leaves. Various lotus plant parts like buds, flowers, fruits, leaves, stalks, and rhizomes have been used as herbal medicines for the treatment of many diseases including cancer, depression, diarrhea, heart problems, hypertension, and insomnia (10). Lotus seeds have also been widely used as food or medicine in Asia because they have high nutritional values and much functionality. One particular effect of lotus seeds is their protective effect on skin. Liu et al. (11) reported that ethanol extracts of *Nelumbo nucifera* seeds are effective in the treatment of tissue inflammation and cancer, and Park et al. (12) reported that *Nelumbo nucifera* seeds can inhibit oxidizing damages to DNA. Huang et al. (13) reported that lotus leaves extracts have protective effects on skin against UVB irradiation, and Kim et al. (14) reported that lotus leaves extracts have anti-wrinkle effects. These studies are evidence that lotus is beneficial to the skin. There has been active research on utilizing these properties to develop cosmetics for whitening and wrinkle care using lotus extracts (15-17). Some cosmetic products on the market actually contain the extracts. The present study was performed to examine the possibilities of lotus seed tea as a functional beverage by creating the tea using *Nelumbo nucifera* seeds and investigating its protective effects on skin.

MATERIALS AND METHODS

Production of the lotus seed tea

Lotus seed tea was prepared as in a previous experiment. Local farmers kindly provided the lotus seeds harvested in Haman, Korea. The coats of lotus seeds were removed. The uncoated seeds were roasted until browned and then extracted with hot water to produce the lotus seed tea. The optimal conditions for the concentration and taste of the lotus seed tea were determined by its color and sensory.

Animal experiments

For the experiments on the photoaging of the skin, 5-week old male SKH-1 hairless mice were purchased from Central Lab Animal, Inc. (Seoul, Korea). All animals had free access to water and market available chows for 1 week for adjustment. Animals were housed at a temperature of 22±2°C and relative humidity of 50±10%. A 12-h light/dark cycle was followed, and the intensity of illumination was 200~300 Lux. All the experimental procedures and protocols used in this study were reviewed and approved by the Inje University Animal Care and Use Committee, Inje University, Gimhae, Korea. After 1 week of adjustment, the mice were randomly divided into two groups containing 10 mice each: the test group was given the lotus seed tea (LST group), and control group was given drinking water (water group) as beverages. The diet was given with the chow for both groups. After six months, the mice in each group were again divided into two groups each (n=5), one with UVB irradiation and another without UVB irradiation (water group, water-UV group, LST group, and LST-UV group). The UVB irradiation was performed for three months.

UVB irradiation

UVB lamp FS 40 (UV Lighting International, Brook Park, OH, USA) was used as the light source, and the irradiation dose was measured by Solarmeter® (Solartech, Inc., Glenside, PA, USA). Mice were put inside an acryl case designed for this study, and the back of the mouse was irradiated with UVB at the strength of 1.8 mW/s and 50 mJ/cm^2 three times per week. The dose of irradiation was increased by 20% every week for 15 weeks.

Measuring the moisture content of the skin of hairless mouse

To measure the moisture content of the epidermis of hairless mice, a Corneometer (CM820®, Courage+Khazaka Electronic GmbH, Cologne, Germany) was used. The measurement was conducted in a room where there is no air movement or direct sunlight. The temperature was 23±2°C and the relative humidity was 43±4%.

Histopathological studies

To assess the histopathological damage on the animals' skin, biopsies were performed on hairless mice by collecting skin (1 cm × 1 cm) from the center of the dorsal side. To obtain an accurate cross-section when preparing a microscope sample, the skin tissues were fixed in 4% formalin diluted in phosphate buffered saline (PBS) for 24 h, embedded in paraffin, and cut into 5 μm thick sections in a rotary microtome. The sections were stained with haematoxylin-eosin (H&E) dye and observed under a microscope (IX51, Olympus, Tokyo, Japan) for histopathological changes in the skin. The epidermis and keratin layers were observed using a Nano Zoomer Digital Pathology instrument (microDimensions, München, Germany). Five random locations on a skin tissue were selected, and their average values were used.

Measurement of protein carbonyl content in skin tissues

To evaluate the extent of oxidization of skin tissues, the carbonyl content was measured according to the methods described by Oliver et al. (18), which use 2,4-dini-

trophenylhydrazine (DNPH) on skin tissues. The skin homogenate was divided into 2 equal aliquots containing approximately 0.7~1.0 mg of protein each. Both aliquots were precipitated with 10% trichloroacetic acid. One sample was treated with 2 N HCl, and the other sample was treated with an equal volume of 0.2% (w/v) DNPH in 2 N HCl. Both samples were incubated at 25°C and stirred at 5-min intervals. The samples then reprecipitated with 10% trichloroacetic acid. The pellets were carefully drained and dissolved in 6 M guanidine with 20 mM sodium phosphate buffer, pH 6.5. Insoluble debris was removed by centrifugation at 6,000 *g* at 4°C. The absorbance was measured at 370 nm. The difference in the spectrum of the DNPH-treated sample versus the HCl control was determined, and the carbonyl content was calculated using the molar absorption coefficient ($22\times 10^6\ M^{-1}\cdot cm^{-1}$).

Statistical analysis

Statistical analysis was performed with SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). Results were expressed as mean±SD. One-way ANOVA, and Duncan's multiple range test were performed to test the significance at $P<0.05$ across different groups.

RESULTS

Food efficiency ratio and beverage consumption

The first animal experiment was performed for six months feeding chow to hairless mice. For the LST group (n=10), the lotus seed tea was provided as a drinking fluid, whereas tap water was provided for the control group (water group). The results show that the final weight averaged was 36.9±1.5 g for the control group and 37.2±1.7 g for the case group, with the differences not being significant. There was no significant difference in food consumption between the control group consuming 7.6±0.4 g/d and the case group consuming 8.0±0.5 g/d. In the consumption of beverage, the control group consumed 20.9±4.6 mL, whereas the case group consumed more, 21.8±5.5 mL. The increase in weight for both groups was 0.6±0.1 g/d. Food efficiency ratio was 7.8±1.7% for the control group and 7.6±1.5% for the case group (Table 1).

After feeding the lotus seed tea and tap water for 6 months, the mice in each group were separated into two groups (n=5), one with UVB irradiation and the other one without UVB irradiation. After 3 months, the final weight, the amount of food consumption, the amount of beverage consumption, the increase in weight, and food efficiency ratio were not significantly different across the different groups ($P<0.05$) (Table 2).

Differences in the hairless mouse skin's ability to contain moisture

To investigate the effects of long-term consumption of lotus seed tea on the animal skin's moisture content, the moisture content of the skin was measured using a Corneometer CM-825. This instrument is affected by the changes in the dielectric constant of the contact region and shows the relative amount of moisture content

Table 1. Changes in final weight, food intake, water intake, body weight gain, and food efficiency in hairless mice administrated lotus seed tea or tap water for 6 months

Groups	Final body weight (g)	Food intake (g/d)	Water intake (mL/d)	Body weight gain (g/d)	Food efficiency (%)
Water	36.9±1.5	7.6±0.4	20.9±4.6	0.6±0.1	7.8±1.7
LST	37.2±1.7	8.0±0.5	21.8±5.5	0.6±0.1	7.6±1.5

All values are mean±SD (n=10 animals in each group).
Water group, administrated water only; LST group, administrated lotus seed tea instead of water.
There was no significant difference between water group and LST group in final body weight, food intake, water intake, body weight gain, and food efficiency.

Table 2. Changes in final weight, food intake, water intake, body weight gain, and food efficiency in hairless mice irradiated UVB

Groups	Final body weight (g)	Food intake (g/d)	Water intake (mL/d)	Body weight gain (g/d)	Food efficiency (%)
Water	37.0±1.4	7.2±0.6	20.6±2.6	0.6±0.1	7.6±1.2
LST	37.2±1.1	7.6±0.3	21.2±2.3	0.6±0.1	7.8±1.1
Water-UV	37.0±0.8	7.3±0.4	20.8±1.8	0.5±0.1	7.4±0.8
LST-UV	36.1±1.2	7.2±0.4	20.4±2.3	0.6±0.1	7.9±1.2

All values are mean±SD (n=5 animals in each group).
Water group, administrated water only; LST group, administrated lotus seed tea instead of water; Water-UV group, UVB irradiated to water group; LST-UV group, UVB irradiated to LST group.
There was no significant difference between the four groups in final body weight, food intake, water intake, body weight gain, and food efficiency.

by measuring the electrostatic capacity corresponding to the moisture content with the highest dielectric constant in the skin. This instrument is widely used for measuring the extent of moisturizing in the skin since it can measure deep inside the tissue and also shows a constant sensitivity even at low levels of moisture content (19,20).

After one month of drinking the lotus seed tea, the moisture content of the animals' skin were 73.41±9.8% for the LST group and 71.65±8.32% for the water group. Although the value for the LST group was higher than the control group, there were no significant differences ($P<0.05$) (Fig. 1A). After 4 months of fluids administration, the water content of the skin in the LST group was 57.0±7.4%, and the water group showed 50.1±5.6%. The value for the LST group was higher than the control group, and there was no significant difference ($P<0.05$) (Fig. 1B). The moisture contents determined after 4

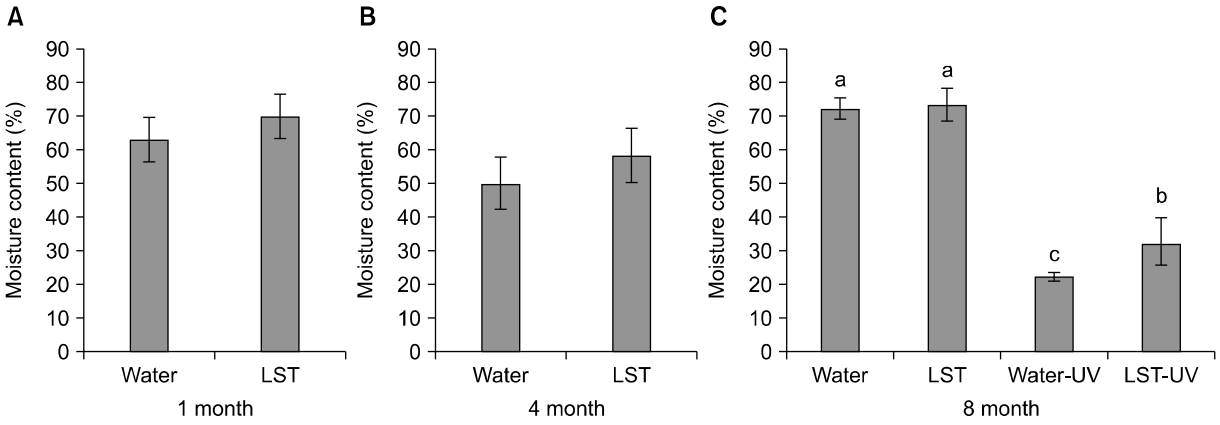

Fig. 1. Moisture content on hairless mice skin. Figures show the moisture content after 1 month (A), 4 months (B), and 8 months (C) of water (water group) and lotus seed tea (LST group) administration. In this time, two month of UVB irradiation was treated to water-UV and LST-UV group. Different letters (a-c) on the bars indicate significant difference ($P<0.05$) by the Duncan's multiple range test. Water group, administrated water only; LST group, administrated lotus seed tea instead of water; Water-UV group, UVB irradiated to water group; LST-UV group, UVB irradiated to LST group.

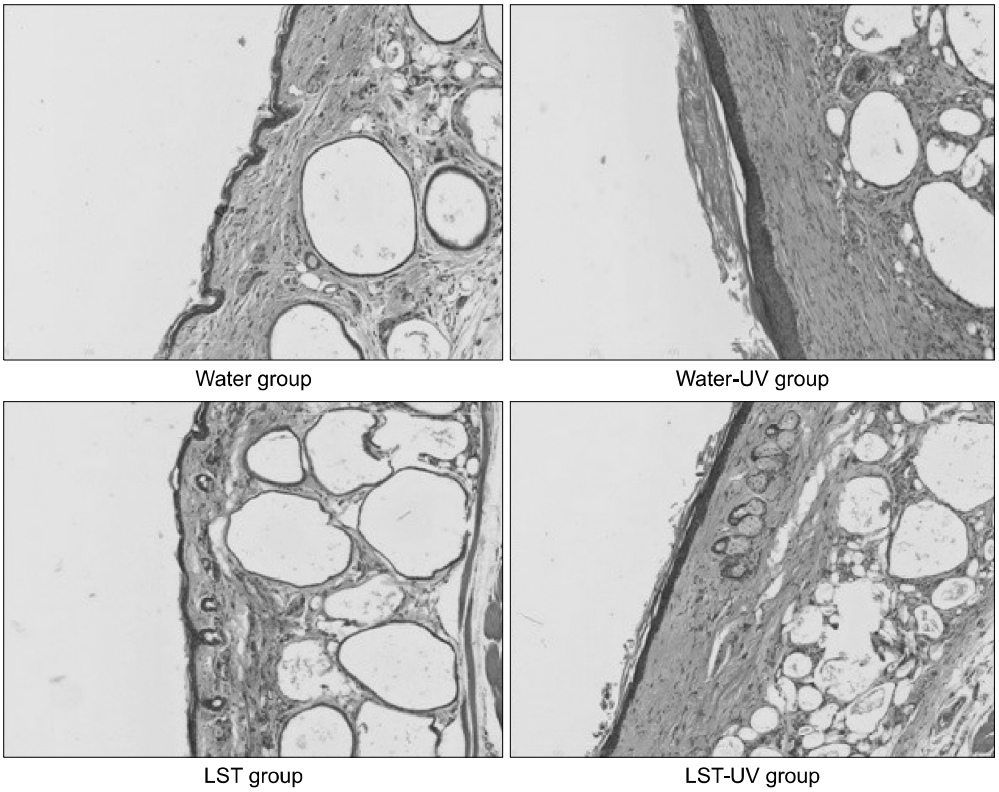

Fig. 2. Sections of the dorsal skin tissues of UVB irradiated mice showing the epidermis and horny layers (H&E staining, 100×). Water group, administrated water only; LST group, administrated lotus seed tea instead of water; Water-UV group, UVB irradiated to water group; LST-UV group, UVB irradiated to LST group.

months of administration were substantially lower than the moisture contents after 1 month in both groups. Although the animals stayed in the humidity controlled condition, it seems the animals were affected by the weather because the measurement of 4 months took place in the winter. The amount of moisture in the skin was measured 2 months after UVB irradiation (8 months after the start of rearing the mice). The results show that the moisture content of the skin was 73.53±4.68% for the LST group and 72.55±3.45% for the water group. The difference was not significant ($P<0.05$). However, with UVB irradiation, the moisture content decreased substantially, with different results depending on the type of beverage. The moisture content of the skin for the LST-UV group was 32.60±6.95%, but it was 22.67±1.25% for the water-UV group, showing that the LST-UV group had higher moisture content than the water-UV group ($P<0.05$) (Fig. 1C).

Histopathological observations of skin tissues

Histopathological observations of skin tissues show that compared to the group with UVB irradiation, the group without UVB irradiation had thinner epidermis and horny layers with virtually no loss of the horny layers. On the other hand, the group with UVB irradiation had an abnormally enlarged epidermis and horny layers, but the LST-UV group that consumed the lotus seed tea had thinner horny layers than the water-UV group (Fig. 2). When the thickness of epidermis was measured, the thickness was 13.13±1.55 μm and 13.14±1.12 μm for the water group and the LST group, respectively, not showing much difference. However, the thickness of the epidermis was 24.11±4.33 μm and 24.13±4.60 μm for the water-UV group and the LST-UV group, respectively, which was much thicker than the groups with UVB irradiation. There were no significant differences between the two groups at $P<0.05$ (Fig. 3). However, the water group had 6.55±0.82 μm of the horny layers and the LST group had 5.40±0.44 μm of the horny layers, showing that the LST group had a relatively thinner horny layer. In the water-UV group, the horny layer thickness was 16.58±5.41 μm, and it was 10.55±3.58 μm for the LST-UV group, showing that the horny layers for the LST group was thinner (Fig. 4).

The protein carbonyl values of skin tissues

Fig. 5 shows the effects of providing either water or lotus seed tea to mice on the oxidation of skin proteins. The carbonyl content which shows the protein oxidation were 12.39±3.15 nmole for the water group, 14.51±1.69 nmole for the LST group, 21.81±2.55 nmole for the water-UV group, and 17.18±0.74 nmole for LST-UV group per mg of protein. There were no differences between the water group and the LST group, but the values for the water-UV group were higher than those of the LST group, with no significance ($P<0.05$).

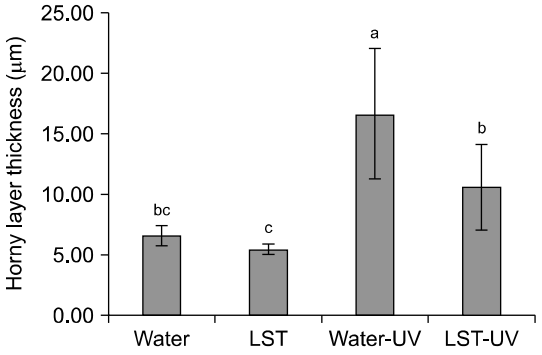

Fig. 4. Effects of lotus seed tea (LST) against the horny layer thickness on hairless mice skin by UVB irradiation. Different letters (a-c) on the bars indicate significant difference ($P<0.05$) by the Duncan's multiple range test. Water group, administrated water only; LST group, administrated lotus seed tea instead of water; Water-UV group, UVB irradiated to water group; LST-UV group, UVB irradiated to LST group.

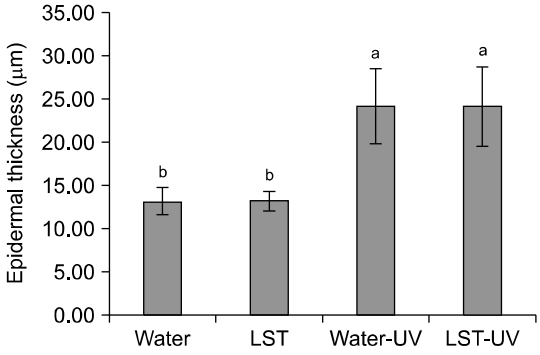

Fig. 3. Effects of lotus seed tea (LST) against the epidermal thickness on hairless mice skin by UVB irradiation. Different letters (a, b) on the bars indicate significant difference ($P<0.05$) by the Duncan's multiple range test. Water group, administrated water only; LST group, administrated lotus seed tea instead of water; Water-UV group, UVB irradiated to water group; LST-UV group, UVB irradiated to LST group.

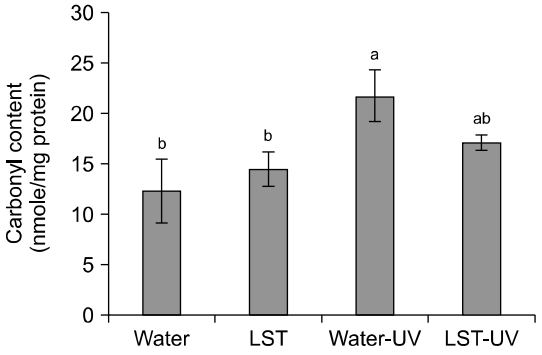

Fig. 5. Effects of lotus seed tea (LST) against on skin tissue of protein carbonylation. Different letters (a, b) on the bars indicate significant difference ($P<0.05$) by the Duncan's multiple range test. Water group, administrated water only; LST group, administrated lotus seed tea instead of water; Water-UV group, UVB irradiated to water group; LST-UV group, UVB irradiated to LST group.

DISCUSSION

To investigate one of the most important physiological functions of the lotus seed, which is the protective effects for skin, we fed the tea to hairless mice for a long time to observe the protective effects on skin against photoaging due to UVB irradiation. When the moisturizing status of the skin was measured with a Corneometer, the moisture content of the skin was slightly higher in the LST group without UV irradiation, but there was no significance ($P<0.05$). Similar results were shown when measured after 1 month or 4 months. When the moisture content of the skin was measured after 8 months with UV irradiation, the LST-UV group showed significantly higher moisture content with 32.6±6.95%, compared to 22.67± 4.68% of the water-UV group. Moisture content is crucial in maintaining the beauty and health of the skin. With enough amount of moisture, the skin is smooth and glossy, but when the moisture content falls below a threshold, the skin loses its elasticity and glossiness. Small wrinkles also form, leading to aging of the skin. The moisturizing capabilities of the elderly falls below those of the young (21), but the present study showed that the moisture content of the skin fell substantially with photoaging due to UVB irradiation, compared to the non-photoaging groups. However, since it was also confirmed that the LST group had higher moisture content than the water group, lotus seed tea intake has been shown to inhibit drying of the skin due to UV radiation to a certain degree.

In the present study, when the thicknesses of the epidermis and the horny layers were measured, the thickness of the epidermis was substantially thicker in the group with UV irradiation (water-UV and LST-UV) than in the group without UV irradiation (water and LST), but there was not much difference between the LST-UV group and the water-UV group, both of which went through UV irradiation ($P<0.05$). However, in terms of the horny layer, the thickness of the horny layer was substantially thicker in the group with UV irradiation (water-UV and LST-UV) than the group without UV irradiation (water and LST) ($P<0.05$). Throughout the experiment, we could understand the protective effects of the lotus seed tea on the skin against UVB. The horny layer is the outermost layer, contacting with the outside directly. Although the keratin layer is composed of dead cells, it has an important role of being a skin barrier. In other words, the horny layer inhibits the loss of moisture and electrolytes to offer an environment where the skin can maintain normal biological functions, and it also acts as the primary defense that blocks harmful factors, such as toxic material, microbes, mechanical stimulation, and UV, from entering the skin. However, when the horny layer becomes abnormally thick due to photoaging, the skin roughens and loses its elasticity. The horny layer being thin means that the skin is relatively soft. By comparing the thickness of keratin layers, we can differentiate the extent of protective effects of the skin against photoaging.

The protein carbonyl values used to measure the extent of protein oxidation of the skin tissue was lower in the LST-UV group (17.18±0.74 nmole/mg protein) than in the water-UV group (21.81±2.55 nmole/mg protein), but the differences were not significant ($P<0.05$). In the skin dermis, reactive oxygen species (ROS) are produced in large amounts when UVB irradiation causes photoaging. These ROS cause lipid peroxidation, DNA damage, and oxidation of protein tissues (9). The peroxidation of lipids or the carbonylation of proteins would cause the skin to lose its resistance to photoaging and would accelerate the oxidative damaging. Since the values of protein carbonyl of skin are lower in the LST group, it suggests that the lotus seed tea has an antioxidative effect against tissue oxidation. In fact, lotus leaf (14,22) or lotus seed extract (23) shows high antioxidative effects *in vitro* and *in vivo*. Major antioxidative compounds in lotus seeds include alkaloids, saponins, and phenolics (23). Major antioxidative compounds in lotus leaf include hyperin, isoquercetin, astragalin, kaempferol, myricetin, and catechin (13). Most of the compounds that protect the skin from the toxicity of UVB come from plant extracts with high antioxidative effects (24,25). The antioxidative effects of lotus tea seems to come from the synergic effects of antioxidative compounds from the lotus seed and the products made from the Maillard reactions that occurs during the roasting process of the lotus seed. Throughout this study, we argued that a continuous consumption of lotus seed tea would have protective effects against photoaging and regular aging. In addition, there is a needs for further research on the extent of whitening, wrinkle formation, and collagen formation for using lotus seed tea as a functional beverage with protective effects on skin against photoaging.

ACKNOWLEDGEMENTS

This study was supported by the research grant from the Inje Research Foundation of Inje University.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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