Effects of Dietary Paprika Xanthophylls on Ultraviolet Light-Induced Skin Damage: A Double-Blind Placebo-Controlled Study

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Abstract: Generation of singlet oxygen by solar ultraviolet (UV) radiation causes acute inflammatory responses in the skin. Accumulation of singlet-oxygen-quenching antioxidants in the skin can suppress this photo-oxidative stress. This study evaluated the effect of dietary xanthophylls from red paprika fruit extract on UV-induced skin damage. A randomised double-blind placebo-controlled parallel group comparison study involving 46 healthy volunteers was performed. The minimal erythema dose (MED) of each individual was determined prior to the study. A capsule containing paprika xanthophylls (9 mg) or a placebo was administered daily for 5 weeks. The MED, minimal tanning dose (MTD), skin physiology parameters (skin color, hydration, and barrier function), and facial skin physiology parameters were evaluated at weeks 0, 2, and 4. The MED of the verum group at 2 and 4 weeks after administration was significantly higher than that of the placebo group. At 4 weeks, the suppression of UV-induced skin darkening by the verum diet was significantly greater than that of the placebo. There were no significant differences in facial skin parameters between the verum and placebo groups. Our results indicate the efficacy of dietary paprika xanthophylls in suppression of UV-induced skin damage.

Key words: red paprika, xanthophyll, photodamage, skin, erythema, oral administration

1 INTRODUCTION
The effect of oral nutritional supplements on human skin is emerging as a topic of interest in public health. The ability of natural antioxidants such as polyphenols and carotenoids to protect against the harmful effects of ultraviolet (UV) radiation on human skin has been investigated[1][2]. Skin is continuously exposed to UV radiation. Overexposure to UV is a major cause of skin disorders, including sunburn, which is mediated by UV-induced oxidative stress; e.g., production of reactive oxygen species. Among the reactive oxygen species, singlet oxygen is a major factor in photo-induced skin damage[3][5]. UV-induced singlet oxygen induces lipid peroxidation and prostaglan- din E2 release, which are related to skin inflammation and melanin synthesis[7]. Hence, natural antioxidants with high radical-scavenging and singlet-oxygen-quenching properties have potential for ameliorating skin photodamage. Carotenoids are a group of naturally occurring pigments that are widely distributed in plants; they play important roles in the protection of photosynthetic machinery and organs from reactive oxygen species generated by sunlight. Carotenoids can be divided into two classes, carotenes and xanthophylls. Xanthophylls are oxygenated carotenoids that contain hydroxy, keto, epoxy, methoxy, or carboxylic acid groups. Recently, we reported that red paprika fruit is a valuable source of carotenoids. Red paprika differs from other carotenoid-rich vegetables such as carrots, tomatoes, and spinach, not only in its high xanthophyll content but also in its unusual xanthophyll composition[6][9]. We recently found that two characteristic xanthophylls of red paprika, capsanthin and capsorubin, show strong singlet-oxygen-quenching activity[10]. Furthermore, in human studies, oral administration of paprika carotenoid beverage[11] and paprika juice[12] increased plasma carotenoid concentrations. Thus, dietary paprika xanthophylls may reduce UV-induced photodamage by quenching singlet oxygen. In this study, we evaluated the effect of oral administration of red paprika fruit extract containing xanthophylls with high singlet-oxygen-quenching activity on UV-induced skin photodamage and facial skin parameters.

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863
2 EXPERIMENTAL PROCEDURES

2.1 Subjects

Inclusion criteria were Japanese male or female aged 30–50 years who had skin phototype II according to the Fitzpatrick classification. The following were excluded: subjects who had been routinely taking supplements and cosmetics that contain carotenoids or that have an anti-inflammatory or skin-lightening effect; subjects with a diagnosis of photosensitive disorder or who were taking drugs that affect skin photosensitivity; subjects diagnosed with diseases in the region of measurement by a dermatologist; subjects who had undergone beauty or special skincare treatment in the region of measurement within the past 6 weeks or who planned to have such treatment; subjects exposed to excessive UV-light radiation in daily life due to, for example, long hours of outside work, sports, sea bathing, or leisure activities, or who expected to be exposed to excessive UV-light radiation during the study period; subjects who had serious disorders of carbohydrate metabolism, lipid metabolism, liver function, or kidney function, or of the cardiovascular, respiratory, endocrine, or nervous system, or with a history of psychiatric disorders; subjects who had participated in other similar clinical studies within the previous 2 months; subjects who had participated in another clinical trial within the previous 4 weeks, or who planned to participate in such a trial; and subjects deemed inappropriate for inclusion by a physician. During the study period, participants were requested to maintain their dietary habits and to avoid supplements, cosmetics, or quasi-drugs with anti-inflammatory or skin-lightening activity.

2.2 Intervention

A commercial paprika-xanthophyll preparation (PapriX-oil; Glico Nutrition Co., Ltd., Osaka, Japan) was used as the source of red paprika xanthophylls. Gelatin capsules containing 333 mg of PapriX-oil (9 mg total xanthophylls, 5 mg capsanthin, and 0.5 mg β-cryptoxanthin) were used as the verum diet. Gelatin capsules containing vegetable oil that were indistinguishable from the verum diet were used as a placebo. The subjects ingested one capsule orally with a meal every evening for 5 weeks.

2.3 Apparatus

Minimal erythema UV dose (MED) was evaluated using a xenon arc solar simulator (LPF302 (1-mm-thick) and luv360 (1-mm-thick)) filters. The simulator complies with the international sun protection factor (SPF) test method (2006) and mimics a tropical sun spectrum. Measurements of skin color (redness and lightness; using the CIE L*a*b* color system), transdermal water loss (TEWL) and stratum corneum hydration were performed using a CM-2600d Spectrophotometer (Konica Minolta, Japan), Vaposcan (Asahi Biomed, Yokohama, Japan) and Corneometer (Courage & Khazaka, Germany), respectively. Corneometer data are shown as skin capacitance arbitrary units (au). MED and minimal tanning dose (MTD) determinations were performed at 22 ± 2°C and 50 ± 10% relative humidity. All other measurements were performed at 21 ± 1°C and 50 ± 5% relative humidity.

2.4 Experimental design

This randomized, placebo-controlled, parallel-group comparative, clinical study was performed to evaluate the effect of paprika xanthophylls on UV-induced skin damage. Group allocation was carried out by an independent person. Subjects and investigators were blinded to the group assignment. The allocation was concealed in a sealed, opaque envelope until the final analysis. The experimental design is shown in Fig. 1.

First, the minimal erythema UV dose (MEDi) on the skin of the back was determined prior to the initiation of this study by irradiating the subjects with solar-simulated UV. Skin color, TEWL, and stratum corneum hydration were also measured before UV irradiation. Before administration (day 1 [D1]), small areas of skin at six locations on the back were exposed to solar-simulated UV at six consecutive doses with a common ratio of 1.2 centered around 1.0 (i.e., 0.69, 0.83, 1.0, 1.2, 1.4, and 1.7) MEDi. At 16–24 h after UV irradiation (D2), skin color was evaluated, MED was determined, and the change in the redness parameter (Δa*) compared before irradiation with 1.4 MEDi was measured. The MTD was determined at 7 days after UV irradiation (D8). At the same time, the skin color, TEWL, and stratum corneum hydration were measured, and changes in the lightness parameter (ΔL*), TEWL (ΔT), and stratum corneum hydration (ΔH) at the location irradiated with 1.4 MEDi were assessed. After completion of the pre-administration measurements, subjects started intake of verum or placebo capsules. After 2 and 4 weeks (D22 and D36), skin
color, TEWL, and stratum corneum hydration were measured, and UV irradiation of the back skin was carried out. The MED and skin redness values were evaluated at 2 weeks (D23) and 4 weeks (D37) after initiation of capsule administration. MTD, skin lightness, TEWL, and stratum corneum hydration were evaluated on D29 and D43.

To evaluate the effect of paprika xanthophylls on non-involved skin, the L* value, a* value, TEWL, and stratum corneum hydration on the left cheek were measured on D2 (before administration) and D23 and D37 (after 2 and 4 weeks after administration, respectively).

2.5 Ethics
The study was conducted according to the Declaration of Helsinki, and the protocol was reviewed and approved by the Kenshokai Ethical Review Board. All subjects provided informed consent. This study was registered in the UMIN Clinical Trials5 Registry (UMIN-CTR) under the trial number UMIN000024014.

2.6 Statistical analysis
For skin color, TEWL, and stratum corneum hydration data, Welch’s and paired t-tests were used for between-group (placebo vs. paprika) and within-group comparisons, respectively. For MED and MTD, between-group comparisons were performed using the Mann–Whitney U-test and Wilcoxon signed-rank test, respectively. Holm’s method was used to adjust the significance level for multiple comparisons in the Wilcoxon signed-rank test. A p-value < 0.05 was considered indicative of statistical significance.

3 RESULTS
3.1 Effect of paprika xanthophylls on UV-induced erythema formation
Forty-six healthy adults (9 males and 37 females, age 30–49 years) were randomly allocated to the placebo or verum group using a random number table. However, two subjects dropped out for personal reasons and one finished the protocol but was excluded due to lack of administrative compliance. Finally, the verum group was composed of 3 males and 19 females with a mean age of 40.7 years, and the placebo group was composed of 4 males and 17 females with a mean age of 41.4 years. No undesirable effect due to the treatment was reported. The baseline characteristics of the subjects are shown in Table 1.

The effect of paprika xanthophylls on UV-induced erythema formation was evaluated by determining the MEDs, which are expressed as multiples of MEDi. Paprika xanthophylls administration protected against erythema formation (Fig. 2). In the paprika xanthophyll group, the mean MED pre-administration (1.08 MEDi) significantly increased to 1.17 and 1.30 MEDi after 2 and 4 weeks, respectively. In contrast, the placebo group showed no significant change in mean MED between before (1.07 MEDi) and after (1.08 and 1.14 MEDi at 2 and 4 weeks, respectively) administration. The MEDs of the verum group at 2 and 4 weeks were significantly higher than those of the placebo group.

3.2 Effect of paprika xanthophylls on UV-induced skin tanning
The effect of paprika xanthophylls on UV-induced skin tanning was evaluated by determining the MTDs, which are expressed as multiples of MEDi (Fig. 3). In the paprika xanthophyll group, the mean MTD pre-administration (1.26 MEDi) significantly increased to 1.35 and 1.43 MEDi at 2 and 4 weeks, respectively. The mean MTD of the placebo group showed no significant change between before (1.22 MEDi) and after (1.29 and 1.31 MEDi at 2 and 4 weeks, respectively) administration. The mean MTD of the paprika xanthophyll group at week 4 tended to be higher than that of the placebo group (p = 0.057).

Table 1 Baseline characteristics of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>Paprika</th>
<th>Placebo</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (female/male)</td>
<td>22 (19/3)</td>
<td>21 (17/4)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>40.7 ± 4.3</td>
<td>41.4 ± 4.7</td>
<td>0.62</td>
</tr>
<tr>
<td>MEDi (mJ/cm²)</td>
<td>4391 ±1067</td>
<td>4299 ±838</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation.

Fig. 2 Changes in minimal erythema dose (MED) before and after ingestion of red paprika extract. Values are means ± standard error (SE). a: p < 0.05 vs. pre-administration, b: p < 0.05 vs. week 2, *: p < 0.05 vs. placebo group. Within-group comparisons were performed by Wilcoxon signed-rank test with Holm’s method, and between-group comparisons were conducted by Mann–Whitney U test.
than in the placebo group, and the amelioration of the increase in skin redness caused by UV irradiation \( \Delta a^* \) was significantly larger in the verum group than in the placebo group. The increase in skin redness caused by UV irradiation \( \Delta a^*_w \) tended to be larger in the verum group than in the placebo group (Table 2). Briefly, the increase in skin color after UV irradiation was suppressed by administration of red paprika xanthophylls. Disruption of the skin barrier function by UV irradiation was insufficient, as indicated by the fact that all \( \Delta T \) values were within 0.5 g/h・m\(^2\). There were no significant differences in the decrease in \( \Delta T \) values between the verum and placebo groups (Table 2), while in the within-group comparison at week 4, the \( \Delta T \) value of the verum group was significantly lower than that pre-administration (data not shown). UV exposure caused a significant decrease (\( \sim 4 \) au) in stratum corneum hydration (data not shown). Administration of paprika xanthophylls showed no significant effect on the UV-induced decrease in skin hydration in both the within- and between-group comparisons.

3.4 Effect of paprika xanthophylls on facial skin conditions

To evaluate the effect of paprika xanthophylls on non-UV-irradiated skin, facial skin colour \( L^*, a^* \), TEWL, and stratum corneum hydration values were determined. Oral intake of paprika xanthophylls resulted in no significant improvement in any of these parameters in comparison to placebo (Table 3). Although the TEWL values increased in both groups during the study period, the increase in the paprika xanthophyll group at 4 weeks tended to be less than that in the placebo group \( p = 0.098 \).

### Table 2: Changes in various parameters of UV-irradiated skin.

<table>
<thead>
<tr>
<th></th>
<th>Week 2 –Pre-administration</th>
<th>Week 4 –Pre-administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paprika</td>
<td>Placebo</td>
</tr>
<tr>
<td>( \Delta a^* )</td>
<td>(-1.4 \pm 0.4^c)</td>
<td>(-0.7 \pm 0.4^c)</td>
</tr>
<tr>
<td>( \Delta L^* )</td>
<td>(2.4 \pm 0.4^d)</td>
<td>(2.4 \pm 0.5^c)</td>
</tr>
<tr>
<td>( \Delta T )</td>
<td>(-0.3 \pm 0.1^c)</td>
<td>(-0.4 \pm 0.2^d)</td>
</tr>
<tr>
<td>( \Delta H )</td>
<td>(1.6 \pm 0.8^d)</td>
<td>(1.1 \pm 0.7^c)</td>
</tr>
</tbody>
</table>

\(^a\) (D23-D22)–(D2-D1).
\(^b\) (D37-D36)–(D2-D1).
\(^c\) (D29-D22)–(D8-D1).
\(^d\) (D43-D36)–(D8-D1).

Values are means ± standard error (SE).

*Statistical analysis by Welch’s \( t \)-test.
Effects of Dietary Paprika Xanthophylls on Skin Photodamage

Table 3 Changes in facial skin parameters.

<table>
<thead>
<tr>
<th></th>
<th>Week 2 -Pre-administration</th>
<th>Week 4 -Pre-administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paprika</td>
<td>Placebo</td>
</tr>
<tr>
<td>a*</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>L*</td>
<td>0.0 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>TEWL</td>
<td>0.9 ± 0.6</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>SC hydration</td>
<td>−0.5 ± 1.6</td>
<td>−3.2 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE.

*Statistical analysis by Welch’s t-test.

Administration of paprika xanthophylls tended to suppress the reduction of skin barrier function compared with placebo, although the TEWL and hydration values of both groups worsened during the test period. Seasonal environmental changes can affect facial skin (e.g., increased dryness) because the temperature and humidity decrease from October to November (when this study was conducted) in Japan. Oral intake of other xanthophylls such as lutein and astaxanthin reportedly exerts a protective effect on skin barrier function and elasticity. It is required for further investigation of the efficacy of dietary paprika xanthophylls in suppression of UV-induced skin damage.
5 CONCLUSION

The present study indicates that administration of red paprika xanthophylls suppresses UV-induced erythema and skin darkening. Lipophilic xanthophylls remain in the skin for several weeks after discontinuance of treatment. Furthermore, efficacy may be enhanced by combination oral and topical xanthophyll treatment. Factors other than UV radiation are risk factors for oxidative stress and skin aging. Daily intake of red paprika extract would reduce skin oxidative stress and maintain skin health.

References


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Effects of Dietary Paprika Xanthophylls on Skin Photodamage


