

Effects of Oral Administration of Ellagic Acid-Rich Pomegranate Extract on Ultraviolet-Induced Pigmentation in the Human Skin

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Summary We performed a double-blind, placebo-controlled trial to clinically evaluate the protective and ameliorative effects of ellagic acid-rich pomegranate extract on pigmentation in the skin after ultraviolet ray (UV) irradiation, using female subjects in their 20s to 40s. Thirteen healthy volunteers per group were randomly assigned to three groups; namely, high dose (200 mg/d ellagic acid), low dose (100 mg/d ellagic acid) and control (0 mg/d ellagic acid: placebo). Each group received the respective test foods for 4 wk. Each subject received a 1.5 MED (minimum erythema dose) of UV irradiation on an inside region of the right upper arm, based on the MED value measured on the previous day. Luminance (L), melanin and erythema values were measured before the start of the test food intake, and after 1, 2, 3 and 4 wk following the start of the test food intake. Further, questionnaires were conducted regarding the condition of the skin before the start of the test food intake and at the termination of the test food intake. As a result, decreasing rates of L values from the baseline in the low- and high-dose groups were inhibited by 1.35% and 1.73% respectively, as compared to the control group. Further, a stratified analysis using subjects with a slight sunburn revealed an inhibited decrease of L values compared with the control group at 1, 2 ($p < 0.01$, respectively) and 4 wk ($p < 0.05$) after the start of the test food intake in the low-dose group, and at 2 and 3 wk ($p < 0.05$) in the high-dose group. Furthermore, the results of questionnaires showed ameliorating tendencies due to the test food, in some items such as “brightness of the face” and “stains and freckles.” Based on the above-mentioned results, it is suggested that ellagic acid-rich pomegranate extract, ingested orally, has an inhibitory effect on a slight pigmentation in the human skin caused by UV irradiation.

Key Words ellagic acid, pomegranate extract, skin pigmentation, UV irradiation, double-blind controlled trial

The pomegranate (*Punica granatum* L.) has been used extensively in traditional medicines in many countries. The Chinese, for example, have used the pomegranate as a traditional product in anti-bacterial, anti-inflammatory and homeostasis applications. Extracts from different parts of this plant, such as the juice (1, 2), seed oil (3) and peel (4), have been reported to exhibit strong anti-oxidant activity. Pomegranate juice has potent anti-atherogenic effects in humans and in atherosclerotic mice, which may be attributable to its anti-oxidative properties (2, 5). Dry pomegranate seed contains the steroid estrogen estron, the isoflavone phytoestrogens genistein and daidzein, the phytoestrogen coumestrol (6), as well as others. Pomegranates are also rich in phenolic compounds. Pomegranate juice is an important source of anthocyanins, 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin and pelargonidin (7). In addition, pomegranate bark (8) is very rich in ellagitannins and gallotannins.

Ellagic acid is a naturally occurring polyphenol found

in many natural sources such as fruits, vegetables and nuts. Ellagic acid has been found to have anti-carcinogenic (9, 10), anti-fibrosis (11) and anti-oxidative (12) properties. It has been reported that ellagic acid has a high affinity for copper at the active site of tyrosinase, and inhibits its activity by binding to the copper (13). Further, ellagic acid topically applied on the skin of brownish guinea pigs was reported to have an inhibitory effect on pigmentation in the skin, when irradiated with ultraviolet rays (UV) (13). In a topical application study on the human skin, the inhibitory effect of ellagic acid on UV-induced pigmentation in the skin was observed (14).

In a study previously performed, pomegranate extract containing ellagic acid at a concentration of 90% inhibited tyrosinase activity in mushrooms, and orally administered pomegranate extract inhibited pigmentation, in a dose-dependent manner, in the skin of brownish guinea pigs receiving UV irradiation, where the number of melanocytes in the epidermis was decreased in a dose-dependent manner (15).

In the present study, we evaluated the protective and

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ameliorative effects of a pomegranate extract rich in ellagic acid, administered orally to humans, on pigmentation in the skin caused by UV irradiation.

Materials and Methods

1. Test food and control food

1) Pomegranate extract: Pomegranate (*Punica granatum* L.) fruit rinds were subjected to extraction three times, with 50% aqueous ethyl alcohol, at 60 to 70°C for 2 h. The ethyl alcohol was removed under vacuum. The resulting aqueous solution was acidified with hydrochloric acid and then refluxed at 70°C for 6 h. Upon dilution with water, ellagic acid was precipitated. The precipitate was collected by filtration and dried in vacuum tray driers. This pomegranate extract contained ellagic acid at a concentration of 89.5% (confirmed by HPLC analysis). This extract was provided by Sabinsa Japan Corporation (Tokyo, Japan).

2) Test food: The test food was a round tablet (300 mg in weight and 9 mm in diameter) containing 112 mg (equivalent to 100 mg ellagic acid) or 56 mg (equivalent to 50 mg ellagic acid) of the pomegranate extract and other components such as dextrin, cellulose, maltitol and sucrose esters of fatty acids. The control food was a placebo tablet containing dextrin, cellulose, maltitol, sucrose esters of fatty acids and caramel colour.

2. Subjects. Thirty-nine female volunteers in their 20s to 40s were enrolled in the study. The exclusion criteria were as follows.

- 1) Those who routinely used medical products and/or cosmetics with whitening effects, such as ellagic acid and vitamin C.
- 2) Those who had atopic dermatitis or other skin disorders.
- 3) Those who had severe diseases such as diabetes mellitus, liver disorders, renal disorders or cardiovascular diseases.
- 4) Those who had a food allergy.
- 5) Those who were pregnant, breast-feeding or expected to become pregnant during the study period.
- 6) Those who had a menstrual disorder or whose skin condition was substantially changed by menstruation.
- 7) Those who were already enrolled in another study at the start of this study.
- 8) Those who were found to be unsuitable for this study by the investigator.

The dropout and discontinuation criteria for test subjects were as follows.

- 1) Those with a low intake rate of the test food (less than 80%).
- 2) Those detracting from the reliability of study results by acts such as missing recording in the diary and others.
- 3) Those who became unable to continue the study due to personal reasons.

No new volunteers were recruited to replace dropout subjects.

Informed consent for the study was obtained from all

subjects in accordance with the objectives of the Helsinki Declaration.

Subjects were instructed to wear long sleeves or outerwear whenever leaving home in order to avoid sunbeams reaching the region irradiated with UV rays. Further, they were instructed to maintain a moderate lifestyle, avoiding excessive eating and drinking, as well as excessive exercise.

3. Study design. Thirteen subjects per group were randomly assigned to the high-dose pomegranate extract (200 mg/d ellagic acid: 2 high-dose tablets/d), low-dose pomegranate extract (100 mg/d ellagic acid: 2 low-dose tablets/d) and placebo (control: 2 placebo tablets/d) groups. The study was a double-blind, placebo control trial.

4. Study procedure

1) Intake of the test food and control food: Each subject took orally the two tablets prescribed together with cold or warm water, taking care not to masticate the tablets, after breakfast for 4 wk. When they did not eat breakfast or missed taking the tablets, they took the tablets within that day.

2) Measurement of whitening effects (protective or ameliorative effects on UV-induced pigmentation): On the day before the start of ingesting the test food, each subject received UV irradiation on the inside of the upper left arm, and on the next day (at the start of the test food intake), a minimum erythema dose (MED) was measured. Before ingesting the test food, each subject received 1.5 MED of UV irradiation on the inside of the upper right arm. Luminance (L), melanin and erythema values were determined before the start of the test food intake and at 1, 2, 3 and 4 wk after the start of the test food intake. A spectro-colorimeter (Minolta Co., Ltd.) was used for measuring L values, and a Mexameter for measuring melanin and erythema values. The skin color around the region irradiated with UV rays was also measured, for correcting the values, because the trial was performed in the summer.

3) Questionnaire: Questionnaires consisting of 20 items were conducted regarding the state of the skin before and after the test food intake period. Subjects checked "yes" or "no" for questionnaire items before the test food intake, and evaluated the state of the skin in five grades (improved, slightly improved, no change, slightly worsened, worsened) after the end of the test food intake period.

4) Diary: Subjects entered the state of taking the test food, physical condition and other information every day in their diary.

5) Evaluation of whitening effects: The inhibitory and ameliorative effects of the test food on UV-induced pigmentation in the skin were evaluated by intergroup comparison of the values before and at 1, 2, 3 and 4 wk after the start of test food intake, as well as the data of questionnaires before and after the test food intake period, between the test food groups and control group.

6) Statistical analysis: A paired *t*-test was used to assess intergroup differences of the changing rate of values determined before and at 1, 2, 3 and 4 wk after

Table 1. Age of subjects and intake rate of respective test foods in the low-dose, high-dose and control groups.

	Low-dose group	High-dose group	Control group
Number of subjects	12	13	12
Age (y)			
Range	34–47	30–43	33–49
Mean±SE	37.8±0.29	36.3±0.28	40.0±0.45
Intake rate of respective test foods (%)			
Range	85.7–100	89.3–100	85.7–100
Mean±SE	98.5±0.35	97.8±0.31	97.3±0.40

Table 2. Change in L value in the UV-irradiated region on the inside of the upper arm after the start of test food intake in the placebo (control) group and test food (low- and high-dose) groups.

Groups	L values after UV irradiation (mean±SE)				
	0 wk (before irradiation)	1 wk	2 wk	3 wk	4 wk
Low-dose	65.19±0.775	58.65±0.770 (90.09±1.58%)	59.39±0.568 (91.21±1.17%)	59.33±0.534 (91.11±1.12%)	59.77±0.437 (92.04±1.18%)
High-dose	64.52±0.741	58.36±0.825 (90.47±0.90%)	59.19±0.916 (91.73±0.88%)	59.89±0.961 (92.80±0.79%)	59.90±0.949 (92.83±1.01%)
Control	65.19±0.536	57.83±0.763 (88.74±1.11%)	58.72±0.600 (90.09±0.70%)	59.10±0.620 (90.71±1.05%)	59.64±0.516 (91.54±0.91%)

Each value (%) in parentheses is a rate versus baseline.

the start of test food intake. Questionnaire data was assessed by intergroup comparison of the scores at 4 wk after the start of the test food intake between the test food groups and control group using Wilcoxon's paired test (ystat2004). A *p* value less than 0.05 was considered to indicate statistical significance.

RESULTS

Hereinafter, the low-dose pomegranate extract group, high-dose pomegranate extract group and control group are referred to as groups L, H and P, respectively.

1. Age of subjects and intake rate of the test food

One subject in Group L and one subject in Group P dropped out from the study due to voluntary withdrawal and a lower rate for the test food intake (71%) than the dropout criterion, respectively. Therefore, 12 subjects in Group L, 13 subjects in Group H and 12 subjects in Group P were included in the analysis. The mean age and mean intake rate of the test food for subjects of each group are shown in Table 1. Mean ages (mean±SE) were 37.8±0.29, 36.3±0.28 and 40.0±0.45 for groups L, H and P, respectively.

2. Measurements with a spectrophotometer (L values)

Changes in L values and rates of change in L value at each measuring time point versus baseline values before the test food intake, which were considered as 100%, are shown in Table 2. Rates of change in L value for groups L and H did not show statistically significant differences compared to Group P. However, a decrease in L value at 1 wk after the start of the test food intake was inhibited by 1.35% and 1.73% in groups L and H, respectively, as compared to Group P. Results of stratified analysis using subjects with slight sunburn (1 to 10

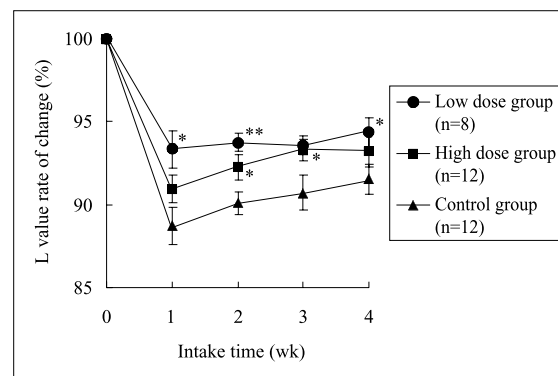


Fig. 1. Change in L values in the UV-irradiated region on the inside of the upper arm after the start of test food intake in low-dose (100 mg/d ellagic acid), high-dose (200 mg/d ellagic acid) and control groups by stratified analysis using subjects with slight sunburn (1 to 10 of rate of change in L value at 1 wk after receiving UV irradiation). Rate of change in L value at each measuring time point versus the baseline values considered as 100% are shown. Data are means±SE. **p*<0.05 versus control group, ***p*<0.01 versus control group.

for rate of change in L value at 1 wk after receiving UV irradiation, where the maximum value of pigmentation is generally observed) are shown in Fig. 1, indicating statistically significant inhibition against decreasing L values at 1, 2 (*p*<0.01) and 4 wk (*p*<0.05) after the start of test food intake in Group L, and at 2 and 3 wk (*p*<0.05) after the start of test food intake in Group H. The rate of change from 1 wk after the UV irradiation generally showing maximum pigmentation (i.e.,

Table 3. Change in melanin value in the UV-irradiated region on the inside of the upper arm after the start of test food intake in the placebo (control) group and test food (low- and high-dose) groups.

Groups	Melanin values after UV irradiation (mean±SE)				
	0 wk (before irradiation)	1 wk	2 wk	3 wk	4 wk
Low-dose	487.75±3.030	511.11±2.686 (104.82±0.65%)	509.69±3.520 (104.51±0.51%)	510.17±2.769 (104.61±0.48%)	509.08±2.933 (104.10±0.46%)
High-dose	491.38±3.649	516.92±5.887 (105.17±0.65%)	515.92±5.411 (104.98±0.58%)	510.44±4.266 (103.89±0.61%)	506.59±3.953 (103.11±0.51%)
Control	487.47±2.434	512.06±2.846 (105.05±0.51%)	512.08±3.763 (105.06±0.70%)	507.83±2.755 (104.19±0.55%)	505.92±2.366 (103.80±0.49%)

Each value (%) in parentheses is a rate verses baseline.

Table 4. Change in erythema value in the UV-irradiated region on the inside of the upper arm after the start of test food intake in the placebo (control) group and test food (low- and high-dose) groups.

Groups	Erythema values after UV irradiation (mean±SE)				
	0 wk (before irradiation)	1 wk	2 wk	3 wk	4 wk
Low-dose	575.19±4.197	614.53±6.072 (106.88±1.16%)	605.97±5.884 (105.37±0.95%)	603.86±4.630 (105.02±0.88%)	598.31±4.120 (103.92±0.73%)
High-dose	575.10±3.432	616.33±4.729 (107.18±0.74%)	608.18±5.245 (105.76±0.73%)	605.03±4.603 (105.20±0.53%)	600.36±5.443 (104.39±0.64%)
Control	570.78±4.772	612.03±6.508 (107.25±0.95%)	606.33±5.963 (106.25±0.89%)	607.58±5.888 (106.50±1.16%)	597.11±4.993 (104.63±0.60%)

Each value (%) in parentheses is a rate verses baseline.

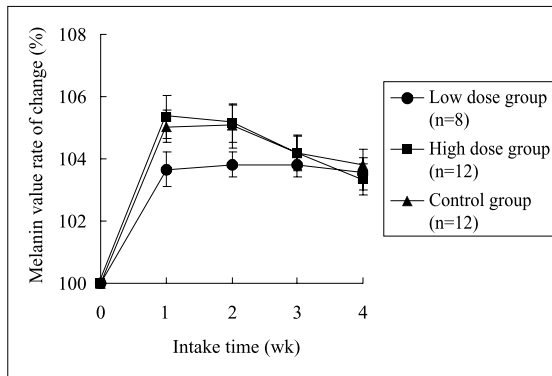


Fig. 2. Change in melanin values in the UV-irradiated region on the inside of the upper arm after the start of test food intake in low-dose (100 mg/d ellagic acid), high-dose (200 mg/d ellagic acid) and control groups by stratified analysis using subjects with slight sunburn. Rate of change in melanin value at each measuring time point versus the baseline values considered as 100% are shown. Data are means±SE.

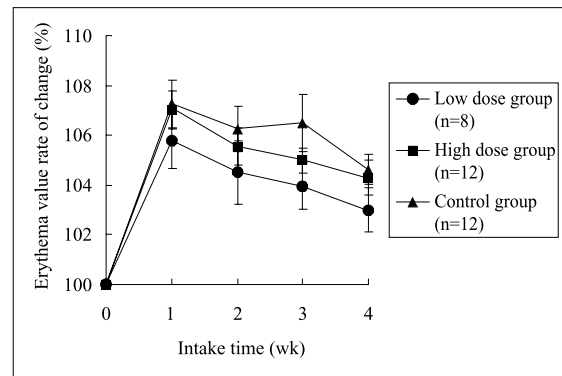


Fig. 3. Change in erythema values in the UV-irradiated region on the inside of the upper arm after the start of test food intake in low-dose (100 mg/d ellagic acid), high-dose (200 mg/d ellagic acid) and control groups by stratified analysis using subjects with slight sunburn. Rate of change in erythema value at each measuring time point versus the baseline values considered as 100% are shown. Data are means±SE.

recovery rates of pigmentation) was not significant in the total data or stratified data in groups L and H, as compared to Group P.

3. Measurements with Mexameter (melanin and erythema values)

Changes in melanin value and rate of change in melanin value at each measuring time point versus baseline values considered as 100% are shown in Table 3. The rate of change in melanin values in groups L and H was not statistically significant compared with Group P.

Results of the stratified analysis conducted in the same manner as that for L values are shown in Fig. 2. A tendency to inhibit the increase in melanin values was observed in Group L at 1 wk after the start of test food intake.

Changes in erythema values and rate of change in erythema values at each measuring time point with the baseline values considered as 100% are shown in Table 4. Rate of change in erythema values in groups L and H was not statistically significant as compared to Group P.

Table 5. Number of subjects who reported ameliorative effects for each questionnaire item.

Items	Low-dose group	High-dose group	Control group
Brightness of the face	5	6	2
Stains and freckles	4	6	2
Hydrated skin condition	4	5	3
Moisturated skin condition	5	6	2
Turgor and elasticity of the skin	3	3	2
Softness of the skin	5	3	2
Glossy skin condition	3	3	2
Pimples/eczemae	3	2	1

Results of the stratified analysis conducted in the same manner as that for L values are shown in Fig. 3. A tendency to inhibit the increase in erythema values was observed in Group L at 1 wk after the start of test food intake.

4. Results of questionnaire

Data obtained from the questionnaire on 20 items concerning the state of the skin, conducted at the end of the test food intake period, were analyzed using Willcoxon's paired test, indicating no statistically significant difference in Group L or H as compared to Group P. However, the number of subjects reporting improvements in some items, including "brightness of the face" and "stains and freckles," was larger in groups L and H than that in Group P, as shown in Table 5, suggesting an ameliorating tendency.

DISCUSSION

Ellagic acid is a naturally occurring polyphenol found in many natural sources. It is known that ellagic acid inhibits melanin formation by acting on tyrosinase, which is the main enzyme-producing melanin (5, 7).

In the present study, a supplement containing ellagic acid extracted from the pomegranate was orally administered to women in their 20s to 40s over 4 wk to evaluate the protective and ameliorative effects on UV-induced pigmentation. Healthy subjects confirmed to have no skin disorder were allocated to a high-dose group (Group H: 200 mg/d ellagic acid), low-dose group (Group L: 100 mg/d ellagic acid) and control group (Group P: 0 mg/d ellagic acid [placebo]). MEDs were measured for all subjects before starting test food intake, and each subject received a 1.5 MED of UV irradiation on an inside region of the upper right arm just before starting test food intake. L, melanin and erythema values were determined as parameters of whitening effects before and at 1, 2, 3 and 4 wk after the start of test food intake. Although the rate of change in these values versus the baseline values considered as 100% did not show statistically significant differences in Group L or H as compared to Group P, decreasing rates in the L value at 1 wk after the start of test food intake were inhibited by 1.35% and 1.73% in groups L and H, respectively. Further, a stratified analysis using subjects with slight sunburn indicating L value changes of 1 to 10 at 1 wk after the start of test food intake revealed an inhibited decrease in L values having statis-

tical significance, as compared to the control group at 1, 2 and 4 wk after the start of test food intake in Group L, and at 2 and 4 wk after the start of test food intake in Group H. Melanin and erythema values were also analyzed in the same manner as that for L values, where the increase in values had a tendency to be inhibited at 1 wk after the start of test food intake in Group L. Furthermore, the results of questionnaires showed an ameliorating tendency in certain items, such as "brightness of the face" and "stains and freckles." Based on the above-mentioned results, it is suggested that ellagic acid ingested orally has whitening effects on slight sunburn even at the low doses tested in this study. Kamide et al. (14) reported that 6-wk repeated application (three times per day) of cosmetic cream containing ellagic acid, beginning just after the first UV irradiation out of three times (once per day for 3 d), inhibited pigmentation from 1 wk after the start of the topical application, indicating a significant protective effect for pigmentation. In our present study, a tendency toward inhibiting pigmentation at an early stage (at 1 wk after the start of test food intake) was also observed. In a previous study where pomegranate extracts containing ellagic acid at a concentration of 90% were orally administered to brownish guinea pigs (receiving UV irradiation on 7, 9 and 11 d after the start of administration) for 7 wk at dose levels of 100 and 1,000 mg/kg/d, UV-induced pigmentation in the skin was inhibited in a dose-dependent manner. In that study, it was demonstrated that the number of melanocytes in the epidermis was decreased in a dose-dependent manner. These results suggest that ellagic acid orally administered is absorbed into the body and the ellagic acid and/or its metabolites inhibit proliferation of melanocytes in the skin, resulting in inhibited synthesis of melanin by tyrosinase in melanocytes.

In our present study, it is suggested that ellagic acid-rich pomegranate extract orally administered to humans has a protective effect on slight sunburn caused by UV irradiation even at such low doses as were used in the study. The safety of orally administered ellagic acid has been made clear in many previous studies. It can be said that the results obtained in this study suggest a possibility for ellagic acid-rich pomegranate extract to be developed as a whitening health food.

The dose-effect relationship was not observed in this study. Further study may be needed to clarify the whit-

ening effects of ellagic acid-rich pomegranate taking into consideration dose levels and the sample size of subjects, upon confirming the absorbability of the extract into the body.

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