Effects of cosmetics therapy using isoflavone and pine bark extract on the skin and QOL: A double-blind placebo-controlled trial

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Abstract

Objective: The efficacy and safety of anti-aging cosmetics that use isoflavone and pine bark extract (Flavangenol(r)) as the primary ingredients were studied in a double-blind, non-crossover manner on 21 female volunteers in the control group (age: 46.6 ± 6.7 years) and 20 female volunteers in the cosmetics-treated group (age: 45.8 ± 4.9 years).
Methods: The subjects used a sample product that was indistinguishable between the groups, and conducted a self facial skincare twice a day, in the morning and at night, for 4 weeks. Physical examinations were conducted and a questionnaire was administered before and after this skincare regimen.
Results: The cosmetics-treated group showed significant improvements in parameters related to physical symptoms such as "tendency to gain weight" and "excessive sensitivity to cold," and mental symptoms such as "irritability," "loss of motivation," "reluctance to talk with others," "pessimism," and "lapse of memory." In the control group, diastolic blood pressure dropped significantly (121.3/77.0 mmHg to 121.4/73.2 mmHg), while both diastolic and systolic blood pressures lowered significantly (127.7/79.0 mmHg to 119.6/73.9 mmHg) in the cosmetics-treated group. In a study of oxidative stress markers, the cosmetics-treated group showed significant improvements in serum lipid peroxide and 8-isoprostane production rate. In a study on skin measurements, the amount of melanin on the forehead had markedly increased in the control group, but this was suppressed in the cosmetics-treated group. No adverse events were seen throughout the study period.
Conclusion: These findings confirm the efficacy and safety of this product.

Key words: Anti-aging medicine, cosmetics, stress, pine bark extract, estrogen, quality of life

Introduction

The objective of anti-aging medicine is to help people live a long, healthy life. This is a medical treatment aimed not simply at prolonging life but at preventing physical and mental deterioration caused by aging, and also maintaining a high quality of life (QOL).[1] Anti-aging drugs are classified as preventive medicine. After undergoing a medical evaluation or screening to assess the degree of aging, one may choose from a range of anti-aging treatment options including diet, exercise, and other lifestyle therapies, drug therapies including nutritional supplement therapies, and hormone replacement therapies. Lifestyles have been shown to have a major influence on health and longevity.1 However, among the different lifestyle activities, very few studies have thus far been conducted on the use of cosmetic creams, especially on whether such use would favorably influence QOL, health, and longevity, besides direct effects on the skin. Estrogen, a female hormone whose secretion declines with age, is closely

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related not only to health and longevity but also to the aging of the skin.[2-4] Recent reports that isoflavone contained in soybean extract possesses an estrogen-like activity are drawing strong interest from the perspective of estrogen secretion and the aging of the female skin.[5]

In our previous study, via an open, non-blinded test, we investigated the safety and efficacy of cosmetics (skincare products) that contained soybean extract (eterrite,® manufactured by Charle Corporation, Kobe, Japan), as well as their possible relation to QOL and longevity.[6] We found the cosmetic product improved several subjective symptoms related to QOL, reduced the level of cortisol, a stress hormone, and occasionally elevated the estradiol level. However, we were unable to identify if these effects were attributable to the application of the makeup, the activity of common components of the makeup, or to the characteristic ingredients contained in eterrite, principally isoflavone and pine bark extract (Flavangenol).®

In this study, via a controlled, double-blind, non-crossover test, we investigated the efficacy and safety of an anti-aging skin cream (eterrite Brightening Force; hereafter referred to as "the Product"). Double-blind means that neither the subjects nor the clinicians conducting the study are aware of the particular treatment each subject enrolled in the study is receiving.

Materials and Methods

The subjects in our study were women aged 40 to 59 years, who had used other companies' cosmetics products (skincare) 3 or more times a week, and who gave their informed consent to participate in this study. Patients with mental disorders, severe hepatic, renal, cardiac and/or hematological diseases, and personnel related to the cosmetics business were excluded from the study. This was in accordance with ethical guidelines set forth by the Japan Anti-Aging foundation.

Forty-one female subjects were randomly divided into 2 groups: a control group A (21 subjects; 46.6 ± 6.7 years) and an active test group B (20 subjects; 45.8 ± 4.9 years). The subjects used samples that were indistinguishable in appearance between the 2 groups to carry out a skincare program that included a 15- to 30-minute self facial skincare twice a day, in the morning and at night, for 4 weeks.

The ingredients of each sample type are as follows: control cream-water, glycerin, BG (butylene glycol), DPG (propylene glycol), sorbitol, licorice extract, PEG-40 hydrogenated castor oil (Polyethylene glycol 2000), methylpolysiloxane, polyglycerol stearate-10, xanthan gum, carbomer, octyl dodecanol, sodium polyacrylate, sodium hydroxide, titanium oxide, silica, alumina, mica, ethanol, phenoxyethanol, methylparaben, and propylparaben; active test cream-the same ingredients as the control cream, plus soybean extract (isoflavone), extract of the bark of a pine tree found on the French sea coast (Flavangenol),® tocopherol, ascorbyl phosphate Mg, *Saxifraga stolonifera* extract, peony extract, kudzu root extract, chlorella extract, hydrolase extract, grapefruit extract, and eglantine extract (these are all considered to be active ingredients).

Clinical test parameters were examined twice, once before the skincare creams were used and again after completing the period of using the creams. Subjective symptoms were divided into physical and mental symptoms. They were investigated by using an Anti-Aging QOL Common Questionnaire (AAQOL).[7] The numerical scale is a subjective one for each physical symptom queried; however, the degree is related. For example, for the assignment of value 3, the subject might begin to consider a visit to a doctor, or self medicate with over the counter remedies. Values of 1 and 2 might be perceived as within a normal range over the population. In addition to the general blood and biochemical tests, the values of estradiol, progesterone, insulin-like growth factor-I (IGF-I), dehydroepiandrosterone sulfate (DHEA-s), and cortisol were measured.

Lipid peroxide, 8-hydroxy-2'deoxyguanosine (8-OHdG), and 8-isoprostane F-2α (isoprostane) were used as indicators of the degree of oxidative stress[8-10] These indicators were measured at the Japan Institute for Control of Aging. The level of lipid peroxide in the blood was measured. In addition, early-morning urine (initial urine for the day) was sampled and 8-OHdG, isoprostane, and creatinine levels were measured. The rate of 8-OHdG production, the rate of isoprostane production, and the creatinine correction amount were calculated based on the urine amount and urine-collection period (the time from the final urination the previous evening to the time of the first urination in the morning).

Facial skin was evaluated at 2 sites-the forehead and the left cheek-to investigate the blood flow and the volume of moisture, sebum, melanin, and erythema. We used changes in the electrical capacitance of the skin to estimate the amount of water in the keratin layer, and adapted a corneometer (CM825SPANC; Courage and Khazaka Electric GmbH, Germany) for this purpose.[11,12] To measure the volume of sebum on the skin's surface, we used the changes in the penetration of exclusive film, and adapted a sebumeter (SM810SPANC; Courage and Khazaka Electric

GmbH, Germany) for this purpose.[13,14] To measure the skin's melanin volume and the degree of erythema, we used a mexameter (MX18; Courage and Khazaka Electric GmbH, Germany). With this device, light of a specific wavelength was irradiated onto the skin, the reflected light was detected and measured by using a light diode, and the melanin and erythema indices were calculated.[11,15,16] SKInfo® (Integral Co., Ltd.), which integrates various measurement devices, was used to record these parameters. The skin's blood flow was measured 5 times using PeriScan PIMII (PERIMED, Sweden), a laser Doppler blood flow measurement device, and the mean values were used as the measurement.[17,18]

The test was to be promptly discontinued if severe adverse reactions or events occurred, and appropriate treatment, based on the judgment of the physician in charge of the test, would be provided.

The t-test, paired t-test, or rank sum test, where appropriate, were performed to statistically analyze the survey results. The results were shown in terms of mean ± standard deviation, and a probability value of less than .05 was considered to be significant. The Wilcoxon Matched-Pairs Signed-Ranks Test was not applied in the statistical analysis.

Results

Tables 1 to 4 show the results of the investigation on subjective symptoms, based on the QOL Common Questionnaire. The physical symptoms that improved significantly among the group A subjects were "blurry eyes," for which the score changed significantly from 2.4 ± 0.9 to 2.0 ± 0.8 (P = 0.001), "skin problems," for which the score changed significantly from 2.6 ± 1.0 to 2.2 ± 0.8 (P = 0.014), and "constipation," for which the score changed significantly from 3.0 ± 1.3 to 2.6 ± 1.2 (P = 0.008) (Table 1). Among scores for the group B subjects, "tendency to gain weight" changed significantly from 3.4 ± 1.1 to 3.1 ± 1.2 (P = 0.015), "skin problems" changed significantly from 3.2 ± 0.8 to 2.4 ± 0.8 (P = 0.004), and "excessive sensitivity to cold" changed significantly from 2.7 ± 1.3 to 2.3 ± 1.0 (v.029) (Table 3). The parameters that worsened were scores for "headache" in group A, which changed significantly from 2.4 ± 1.0 to 2.7 ± 1.1 (P = 0.048), and "tinnitus" in group B, which changed significantly from 1.7 ± 1.1 to 1.9 ± 1.1 (P = 0.048).

Table 1. Physical symptoms-group A

Variable	Before	4 weeks later	P – level
Tired eyes	2.9 ± 1.1	2.5 ± 1.0	0.083
Blurry eyes	2.4 ± 0.9	2.0 ± 0.8	0.001‡
Eye pain	2.0 ± 0.7	1.8 ± 0.7	0.164
Stiff shoulders	3.3 ± 1.3	3.4 ± 1.1	0.288
Muscular pain/stiffness	2.7 ± 1.0	2.9 ± 1.0	0.226
Palpitations	1.9 ± 0.9	2.1 ± 0.8	0.102
Dyspnea	1.8 ± 0.8	1.8 ± 0.7	0.374
Tendency to gain weight	3.0 ± 0.9	3.0 ± 1.0	0.288
Weight loss; thin	1.3 ± 0.7	1.4 ± 0.6	0.214
Lethargy	2.6 ± 1.0	2.5 ± 1.1	0.417
No feeling of good health	2.5 ± 1.0	2.3 ± 1.0	0.206
Thirst	1.9 ± 1.0	1.9 ± 0.8	1.00
Skin problems	2.6 ± 1.0	2.2 ± 0.8	0.014†
Anorexia	1.8 ± 0.8	1.9 ± 0.7	0.358
Heavy stomach	2.0 ± 0.7	2.0 ± 0.8	1.00
Gastric pain	2.0 ± 0.7	1.9 ± 0.8	0.165
Liable to catch a cold	2.1 ± 0.8	2.1 ± 0.8	0.288
Coughing and sputum	2.0 ± 0.9	2.1 ± 1.2	0.226
Diarrhea	2.0 ± 0.9	1.9 ± 0.8	0.189
Constipation	3.0 ± 1.3	2.6 ± 1.2	0.008‡
Headache	2.4 ± 1.0	2.7 ± 1.1	0.048†
Dizziness	2.0 ± 1.0	2.2 ± 0.9	0.068
Tinnitus	1.8 ± 1.0	2.0 ± 0.9	0.129

Lower back pain	2.4 ± 0.9	2.5 ± 0.9	0.270
Joint pain	2.2 ± 1.2	2.5 ± 0.9	0.134
Swelling	2.0 ± 0.8	2.0 ± 0.7	0.374
Easy to perspire	2.3 ± 1.0	2.4 ± 0.7	0.189
Polakisuria	1.8 ± 0.7	2.0 ± 0.7	0.068
Hot flash	2.0 ± 0.9	2.1 ± 0.8	0.189
Excessive sensitivity to cold	2.7 ± 1.2	2.8 ± 1.2	0.314

Table values are mean ± SD score from Anti-Aging QOL Common Questionnaire,[7] range of values, 1 to 5; 1 = none, 2 = almost none, 3 = a little, 4 = moderate, 5 = severe. n = 21, paired *t*-test.

Table 2. Mental symptoms-group A

Variable	Before	4 weeks later	P – level
Irritability	2.8 ± 0.9	2.7 ± 0.8	0.247
Easily angered	2.6 ± 0.9	2.5 ± 0.8	0.093
Loss of motivation	2.4 ± 0.9	2.4 ± 0.4	0.374
No feeling of happiness	2.3 ± 1.0	2.3 ± 0.8	1.00
Nothing to look forward to in life	2.1 ± 1.1	2.0 ± 0.7	0.324
Daily life is not enjoyable	2.3 ± 0.9	2.2 ± 0.7	0.270
Loss of confidence	2.1 ± 0.8	2.2 ± 0.8	0.252
Reluctance to talk with others	2.0 ± 0.9	2.0 ± 0.7	0.417
Depressed	2.2 ± 0.8	2.0 ± 0.7	0.240
A sense of uselessness	1.9 ± 0.7	1.9 ± 0.6	1.00
Shallow sleep	2.5 ± 0.9	2.8 ± 1.0	0.081
Difficulty falling asleep	2.0 ± 1.0	2.1 ± 0.8	0.413
Pessimism	2.8 ± 0.9	2.7 ± 0.9	0.247
Lapse of memory	2.9 ± 1.2	3.0 ± 1.0	0.386
Inability to concentrate	2.1 ± 1.0	2.2 ± 0.8	0.165
Inability to solve problems	2.1 ± 0.8	2.1 ± 0.6	1.00
Inability to make judgments readily	2.0 ± 0.7	2.1 ± 0.6	0.041†
Inability to sleep because of worries	2.2 ± 1.0	2.3 ± 0.8	0.374
A sense of tension	2.2 ± 1.0	2.2 ± 0.8	1.00
Feeling of anxiety for no special reason	2.2 ± 1.2	2.1 ± 0.9	0.362
Feeling of vague fear	2.0 ± 1.1	1.7 ± 0.7	0.142

Table values are mean ± SD score from Anti-Aging QOL Common Questionnaire,[7] range of values, 1 to 5; 1 = none, 2 = almost none, 3 = a little, 4 = moderate, 5 = severe. n = 21, paired *t*-test.

Table 3. Physical symptoms-group B

Symptom	Before	4 weeks later	P – level
Tired eyes	2.9 ± 0.7	2.8 ± 1.1	0.395
Blurry eyes	2.3 ± 0.9	2.6 ± 0.9	0.109
Eye pain	1.8 ± 0.8	2.1 ± 1.1	0.134
Stiff shoulders	3.3 ± 1.2	3.3 ± 1.1	1.00
Muscular pain/stiffness	2.5 ± 0.9	2.8 ± 1.1	0.165
Palpitations	1.8 ± 0.6	1.9 ± 0.7	0.270
Dyspnea	1.8 ± 0.6	1.8 ± 0.6	1.00
Tendency to gain weight	3.4 ± 1.1	3.1 ± 1.2	0.015†
Weight loss; thin	1.3 ± 0.6	1.6 ± 0.7	0.011†

Lethargy	2.4 ± 0.9	2.4 ± 0.9	0.417
No feeling of good health	2.2 ± 0.8	2.1 ± 0.8	0.303
Thirst	2.0 ± 0.9	1.9 ± 1.0	0.214
Skin problems	3.2 ± 0.8	2.4 ± 0.8	0.004‡
Anorexia	1.6 ± 0.8	1.7 ± 0.6	0.374
Heavy stomach	1.8 ± 0.7	2.0 ± 0.9	0.129
Gastric pain	1.8 ± 0.7	2.0 ± 0.9	0.068
Liable to catch a cold	2.0 ± 0.6	1.9 ± 0.8	0.134
Coughing and sputum	1.8 ± 0.7	1.8 ± 0.9	0.386
Diarrhea	1.9 ± 0.7	2.0 ± 0.8	0.214
Constipation	2.7 ± 1.1	2.8 ± 1.0	0.247
Headache	2.2 ± 0.7	2.2 ± 0.7	1.00
Dizziness	2.2 ± 1.1	2.1 ± 1.1	0.358
Tinnitus	1.7 ± 1.1	1.9 ± 1.1	0.048†
Lower back pain	2.9 ± 1.3	2.7 ± 1.1	0.224
Joint pain	2.0 ± 1.3	2.0 ± 0.9	1.00
Swelling	2.3 ± 1.2	2.2 ± 1.0	0.363
Easy to perspire	2.9 ± 1.0	2.7 ± 1.2	0.107
Polakisuria	2.1 ± 0.7	2.2 ± 0.7	0.214
Hot flash	2.2 ± 1.3	2.3 ± 1.1	0.226
Excessive sensitivity to cold	2.7 ± 1.3	2.3 ± 1.0	0.029†

Table values are mean ± SD score from Anti-Aging QOL Common Questionnaire,[7] range of values, 1 to 5; 1 = none, 2 = almost none, 3 = a little, 4 = moderate, 5 = severe. n = 20, paired *t*-test.

Regarding the mental symptoms in group A, scores for "inability to make judgments easily" changed significantly for the worse, from 2.0 ± 0.7 to 2.1 ± 0.6 (P = 0.041) (Table 2). In group B, "irritability" score changed significantly from 2.4 ± 0.8 to 2.0 ± 0.8 (P = 0.004), "loss of motivation" score changed significantly from 2.6 ± 1.0 to 2.0 ± 0.7 (P = 0.012), "reluctance to talk with others" score changed significantly from 2.3 ± 1.1 to 1.7 ± 0.7 (P = 0.018), "pessimism" score changed significantly from 2.4 ± 0.7 to 2.0 ± 0.8 (P = 0.008), and "lapse of memory" score changed significantly from 3.0 ± 0.7 to 2.7 ± 0.7 (P = 0.028), all in the direction of improvement (Table 4). In other words, although group A showed no improvement in any of the scores for mental symptoms, group B showed significant improvements in 5 parameters.

Table 4. Mental symptoms-group B

Symptom	Before	4 weeks later	P – level
Irritability	2.4 ± 0.8	2.0 ± 0.8	0.004‡
Easily angered	2.3 ± 0.7	2.2 ± 0.9	0.190
Loss of motivation	2.6 ± 1.0	2.0 ± 0.7	0.012†
No feeling of happiness	2.3 ± 1.1	2.0 ± 0.9	0.074
Nothing to look forward to in life	2.3 ± 1.2	1.9 ± 0.9	0.084
Daily life is not enjoyable	2.3 ± 1.1	1.9 ± 0.9	0.080
Loss of confidence	2.3 ± 1.0	2.1 ± 0.8	0.117
Reluctance to talk with others	2.3 ± 1.1	1.7 ± 0.7	0.018†
Depressed	2.1 ± 0.9	2.0 ± 0.8	0.134
A sense of uselessness	2.0 ± 0.8	1.9 ± 0.6	0.165
Shallow sleep	2.3 ± 1.1	2.2 ± 1.0	0.270
Difficulty falling asleep	2.2 ± 1.1	2.0 ± 1.0	0.093

Pessimism	2.4 ± 0.7	2.0 ± 0.8	0.008‡
Lapse of memory	3.0 ± 0.7	2.7 ± 0.7	0.028†
Inability to concentrate	2.6 ± 0.6	2.4 ± 0.7	0.107
Inability to solve problems	2.2 ± 0.8	2.3 ± 0.8	0.303
Inability to make judgments readily	2.3 ± 0.7	2.2 ± 0.7	0.165
Inability to sleep because of worries	2.0 ± 0.6	2.0 ± 0.7	1.00
A sense of tension	2.5 ± 0.5	2.3 ± 0.8	0.165
Feeling of anxiety for no reason	2.0 ± 0.6	1.9 ± 0.6	0.214
Feeling of vague fear	1.6 ± 0.7	1.7 ± 0.5	0.374

Table values are mean ± SD score from Anti-Aging QOL Common Questionnaire,[7] range of values, 1 to 5; 1 = none, 2 = almost none, 3 = a little, 4 = moderate, 5 = severe. n = 20, paired *t*-test.

Results of the physical and blood examinations are shown in Tables 5 and 6. Diastolic blood pressure changed significantly from 121.3/77.0 mmHg to 121.4/73.2 mmHg in group A (systolic/diastolic; $P = 0.012$), while both diastolic and systolic blood pressures changed significantly from 127.7/79.0 mmHg to 119.6/73.9 mmHg in group B ($P = 0.002$, $P = 0.004$). While significant differences were seen in terms of changes in the erythrocyte count, hemoglobin amount, platelet count, gamma-glutamyltranspeptidase (γ-GTP), uric acid, and triglycerides in group A, as well as in the platelet count and urea nitrogen in group B, they were all within the range of physiological variations, and thus, were assessed as having no clinical significance.

Table 5. Clinical test results-group A

Variable	Unit	Before	4 weeks later	P – level
Weight	kg	59.4 ± 11.3	59.6 ± 11.0	0.282
Body mass index		23.8 ± 3.9	23.9 ± 3.7	0.292
Percentage body fat	%	29.5 ± 5.7	29.9 ± 6.4	0.303
Systolic BP	mmHg	121.3 ± 14.9	121.4 ± 17.9	0.474
Diastolic BP	mmHg	77.0 ± 13.4	73.2 ± 11.3	0.012†
Leukocyte count	/mm^3	5805 ± 1912	5565 ± 1575	0.178
Erythrocyte count	10^{4}x/mm^3	431.5 ± 25.5	421.0 ± 25.5	0.004‡
Hemoglobin	g/dl	12.4 ± 1.6	12.1 ± 1.5	0.009‡
Platelet count	10^{4}x/mm^3	25.1 ± 4.4	26.6 ± 6.3	0.027†
GOT	U/l	20.1 ± 3.5	19.8 ± 4.0	0.278
GPT	U/l	18.1 ± 5.6	16.8 ± 7.7	0.082
ALP	U/l	200.5 ± 51.2	197.8 ± 50.7	0.330
γ-GTP	U/l	28.4 ± 26.2	23.4 ± 15.6	0.032†
Creatinine	mg/dl	0.602 ± 0.085	0.602 ± 0.106	1.00
Urea nitrogen	mg/dl	13.7 ± 3.2	13.2 ± 2.5	0.099
Uric acid	mg/dl	4.63 ± 1.05	4.32 ± 0.93	0.018†
Total cholesterol	mg/dl	205.5 ± 34.7	201.2 ± 34.7	0.106
Triglyceride	mg/dl	85.0 ± 44.3	73.4 ± 37.1	0.020†
Sodium	mEq/l	141.1 ± 1.7	141.0 ± 1.9	0.362
Potassium	mEq/l	4.1 ± 0.4	4.2 ± 0.3	0.126
Chlorine	mEq/l	103.3 ± 1.5	103.3 ± 1.5	0.118
Lipid peroxide	nmol/ml	0.37 ± 0.09	0.35 ± 0.08	0.165
Fasting blood sugar	mg/dl	94.7 ± 10.8	93.5 ± 11.6	0.186
IGF-I	ng/ml	224.0 ± 63.0	185.1 ± 63.7	0.001‡
Cortisol	μg/dl	9.3 ± 6.2	9.5 ± 4.9	0.432

DHEA-s	ng/ml	1326.9 ± 777.1	1273.4 ± 629.3	0.234
Estradiol	pg/ml	80.6 ± 59.5	63.0 ± 67.4	0.196
Progesterone	ng/ml	3.3 ± 6.7	2.8 ± 4.7	0.221

ALP, alkaline phosphatase; BP, blood pressure; IGF-I, insulin-like growth factor-I; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; γ-GTP, gamma-glutamyltranspeptidase; DHEA-s, dehydroepiandrosterone sulfate. n = 21, mean ± SD, paired *t*-test.

Table 6. Clinical test results-group B

Variable	Unit	Before	4 weeks later	P – level
Weight	kg	56.6 ± 6.8	56.3 ± 6.4	0.124
Body mass index		22.9 ± 2.9	22.7 ± 2.7	0.118
Percentage body fat	%	28.0 ± 5.2	27.8 ± 4.0	0.389
Systolic BP	mmHg	127.7 ± 18.1	119.6 ± 18.9	0.002‡
Diastolic BP	mmHg	79.0 ± 11.4	73.9 ± 10.5	0.004‡
Leukocyte count	/mm^3	5064 ± 1186	5300 ± 1452	0.236
Erythrocyte count	10^{4}x/mm^3	430.0 ± 30.3	428.6 ± 29.2	0.379
Hemoglobin	g/dl	12.6 ± 1.5	12.5 ± 1.6	0.283
Platelet count	10^{4}x/mm^3	24.8 ± 6.4	26.0 ± 7.4	0.049†
GOT	U/l	20.0 ± 4.0	19.7 ± 4.8	0.332
GPT	U/l	16.2 ± 6.1	15.8 ± 5.6	0.315
ALP	U/l	182.5 ± 61.7	179.5 ± 56.0	0.253
γ-GTP	U/l	17.6 ± 12.2	17.1 ± 8.1	0.307
Creatinine	mg/dl	0.589 ± 0.065	0.594 ± 0.058	0.324
Urea nitrogen	mg/dl	11.8 ± 2.2	12.7 ± 2.5	0.018†
Uric acid	mg/dl	4.60 ± 0.78	4.43 ± 0.89	0.092
Total cholesterol	mg/dl	197.7 ± 27.4	200.2 ± 29.8	0.138
Triglyceride	mg/dl	76.7 ± 46.8	66.4 ± 30.3	0.093
Sodium	mEq/l	141.3 ± 2.0	141.5 ± 1.9	0.180
Potassium	mEq/l	4.0 ± 0.3	4.1 ± 0.3	0.080
Chlorine	mEq/l	103.4 ± 2.1	103.4 ± 2.0	0.449
Lipid peroxide	nmol/ml	0.44 ± 0.11	0.39 ± 0.08	0.006‡
Fasting blood sugar	mg/dl	92.8 ± 6.0	91.6 ± 5.9	0.182
IGF-I	ng/ml	201.9 ± 49.5	176.0 ± 43.8	0.001‡
Cortisol	μg/dl	10.1 ± 6.2	9.5 ± 5.3	0.327
DHEA-s	ng/ml	1179.1 ± 560.5	1140.7 ± 572.7	0.245
Estradiol	pg/ml	71.7 ± 57.8	70.0 ± 50.6	0.438
Progesterone	ng/ml	3.9 ± 6.7	4.4 ± 6.2	0.267

ALP, alkaline phosphatase; BP, blood pressure; IGF-I, insulin-like growth factor-I; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; γ - GTP, gamma-glutamyltranspeptidase; DHEA-s, dehydroepiandrosterone sulfate. n = 20, mean ± SD, paired *t*-test.

In endocrine examinations associated with anti-aging medicine, IGF-I fell significantly in both group A and group B, from 224.0 ± 63.0 ng/ml to 185.1 ± 63.7 ng/ml (P = 0.001), and from 201.9 ± 49.5 ng/ml to 176.0 ± 43.8 ng/ml (P = 0.001), respectively. No significant changes were seen in cortisol, DHEA-s, estradiol, or progesterone.

Tables 7 and 8 show the results of the measurements of the skin. No significant changes were seen in the sebum levels in either group A or group B. Moisture had increased significantly on the forehead and the left cheek for both groups A and B. Blood flow had decreased significantly,

from 0.630 ± 0.256 U to 0.481 ± 0.179 U in group A (P = 0.001), and from 0.621 ± 0.203 U to 0.471 ± 0.168 U in group B (P = 0.001). A significant difference was seen between the groups in terms of the volume of melanin in the forehead. The volume increased significantly in group A, from 160.3 ± 33.1 U to 171.7 ± 36.4 U (P = 0.003) and did not show any significant differences in group B, between 156.6 ± 35.3 U and 159.2 ± 31.1 U (P = 0.308).

Table 7. Measurements of the skin-group A

Variable	Unit	Before	4 weeks later	P – level
Forehead: sebum	U	0.865 ± 1.592	1.170 ± 1.830	0.230
Forehead: moisture	U	49.86 ± 10.70	58.41 ± 10.80	0.008‡
Forehead: melanin	U	160.3 ± 33.1	171.7 ± 36.4	0.003‡
Forehead: erythema	U	310.1 ± 60.7	281.5 ± 49.0	0.002‡
Left cheek: sebum	U	0.325 ± 0.862	0.430 ± 0.542	0.303
Left cheek: moisture	U	52.8 ± 11.0	62.5 ± 8.0	0.003‡
Left cheek: melanin	U	149.5 ± 35.0	151.0 ± 46.4	0.392
Left cheek: erythema	U	301.6 ± 57.7	283.9 ± 45.2	0.026†
Blood flow	U	0.630 ± 0.256	0.481 ± 0.179	0.001‡

n = 21, mean ± SD, paired *t*-test. U; units are proper and depend on the measurement apparatus

Table 8. Measurements of the skin-group B

Variable	Unit	Before	4 weeks later	P – level
Forehead: sebum	U	0.535 ± 0.707	0.965 ± 2.55	0.211
Forehead: moisture	U	51.34 ± 11.12	56.77 ± 11.92	0.049‡
Forehead: melanin	U	156.6 ± 35.3	159.2 ± 31.1	0.308
Forehead: erythema	U	302.8 ± 65.1	273.8 ± 61.7	0.002‡
Left cheek: sebum	U	0.321 ± 1.116	0.347± 0.313	0.461
Left cheek: moisture	U	55.2 ± 10.6	61.6 ± 8.8	0.010‡
Left cheek: melanin	U	151.8 ± 33.1	155.8 ± 35.1	0.278
Left cheek: erythema	U	292.6 ± 47.4	277.8 ± 53.5	0.114
Blood flow	U	0.621 ± 0.203	0.471 ± 0.168	0.001‡

n = 20, mean ± SD, paired *t*-test. U; units are proper and depend on the measurement apparatus.

Regarding the investigation into the degree of oxidative stress(Tables 9 and 10), no significant differences were seen in blood lipid peroxide in group A, from 0.37 ± 0.09 nmol/ml to 0.35 ± 0.08 nmol/ml (P = 0.165); however, a significant decrease was seen in group B, from 0.44 ± 0.11 nmol/ml to 0.39 ± 0.08 nmol/ml (P = 0.006). Moreover, no significant differences were seen in terms of the rate of isoprostane production (in nanograms per kilogram per hour) in group A, between 1.80 ± 1.16 and 1.39 ± 0.72 (P = 0.072); however, a significant decrease was seen in group B, from 2.83 ± 2.46 to 2.30 ± 1.95 (P = 0.044). No significant differences were seen in either group A or group B in terms of the rate of urinary 8-OHdG production, which is an indicator for oxidative DNA damage.

No adverse reactions or events were seen in either group A or B throughout the course of treatment.

Table 9. Degree of oxidative stress-group A

Variable	Unit	Before	4 weeks later	P – level
Urinary 8-OHdG	ng/ml	5.08 ± 2.28	5.07 ± 3.05	0.494
Urinary creatinine	mg/dl	68.7 ± 37.5	66.5 ± 28.6	0.378

Urinary isoprostane	ng/ml	2.06 ± 1.62	1.53 ± 0.88	0.075
Urine volumeml		341.0 ± 114.7	359.5 ± 128.0	0.289
Urine-collection period	hour	5.99 ± 1.48	6.29 ± 1.19	0.266
8-OHdG production rate	ng/kg/h	4.80 ± 2.03	4.65 ± 2.33	0.363
Isoprostane production rate	ng/kg/h	1.80 ± 1.16	1.39 ± 0.72	0.072
8-OHdG/CRE	ng/mg CRE	8.69 ± 3.82	7.80 ± 2.79	0.078
Isoprostane/CRE	ng/mg CRE	2.97 ± 1.16	2.37 ± 1.08	0.023†
Blood lipid peroxide	nmol/ml	0.37 ± 0.09	0.35 ± 0.08	0.165

8-OHdG, 8-hydroxy-2'deoxyguanosine; CRE, creatinine. n = 21, mean ± SD, paired *t*-test.

Table 10. Degree of oxidative stress-group B

Variable	Unit	Before	4 weeks later	P – level
Urinary 8-OHdG	ng/ml	7.52 ± 5.57	6.43 ± 4.34	0.150
Urinary creatinine	mg/dl	87.9 ± 56.1	93.9 ± 66.0	0.344
Urinary isoprostane	ng/ml	3.08 ± 2.20	2.33 ± 1.89	0.072
Urine volume	ml	288.5 ± 114.6	311.3 ± 105.7	0.148
Urine-collection period	hour	5.53 ± 1.92	5.23 ± 1.22	0.238
8-OHdG production rate	ng/kg/h	6.50 ± 4.23	6.24 ± 4.20	0.280
Isoprostane production rate	ng/kg/h	2.83 ± 2.46	2.30 ± 1.95	0.044†
8-OHdG/CRE	ng/mg CRE	8.46 ± 2.14	7.48 ± 3.47	0.078
Isoprostane/CRE	ng/mg CRE	3.70 ± 1.97	2.51 ± 0.81	0.002‡
Blood lipid peroxide	nmol/ml	0.44 ± 0.11	0.39 ± 0.08	0.006†

8-OHdG, 8-hydroxy-2'deoxyguanosine; CRE, creatinine. n = 20, mean ± SD, paired *t*-test.

Discussion

No appropriate assessments have thus far been made on the efficacy of cosmetic products containing ingredients that have estrogen-like activities such as isoflavone, or those containing antioxidative substances such as pine bark extract. In our present study, to clear up these and other doubts, we conducted 2 double-blind tests in a clinical setting. The results showed that, compared with a control group, women given the active Product saw improvements in both mental and physical symptoms. Favorable effects were also seen in the latter group in the oxidative stress tests and in their skin data. Whether these test results can be explained by the presence of isoflavone and pine bark extract will be examined hereunder.

Pine bark extract is a polyphenol, which is extracted from the bark of a pine tree *(Pinus pinaster Ait.)* originating in France. Its primary ingredient, oligomeric proanthocyanidin, or OPC, is believed to have antioxidative and vascular endothelial protecting activities.[19,20] It is also said to suppress proteases such as collagenase, hyaluronidase, and elastase. Some of its characteristic ingredients are other antioxidative substances such as tocopherol (vitamin E) and ascorbyl phosphate Mg (stabilized vitamin C derivative), which are believed to help the activity of pine bark extract.

Isoflavone is believed to have estrogen-like activity. Estrogen maintains femininity,[2-4] as well as youthful and healthy skin. In addition, it is said to prevent bone density from declining, prevent arteriosclerosis from advancing, and, in the long-term, it reportedly works to prevent the advancement of senility.[3,4] There were 5 mental symptom parameters in which group B saw favorable effects: "irritability," "loss of motivation," "reluctance to talk with others," "pessimism," and "lapse of memory." Estrogen is known to have neuropsychiatric effects such as boosting of sexual feelings, enhancement of cognitive ability, and improvement of depressive states.[3-4] It is true that replenishing estrogen to treat menopausal disorders can improve the subjects' mental status after a relatively short period. Therefore, improvement of mental symptoms may be explained as the effects of estrogen. However, some

facts should also be taken into account, that is, the effects of isoflavone are only about several tenths those of estrogen, and that it is unclear if isoflavone is absorbed by the skin. It is believed that pine bark extract is not involved in this process.

Regarding oxidative stress, neither group saw improvements in 8-OHdG, although lipid peroxide in the blood and the rate of isoprostane production improved in group B. The substances contained in the Product, which group B had used, had little or no effect on DNA oxidative damage, but were shown to significantly suppress the hyperoxidation of lipids. This, however, is largely due to the involvement of pine bark extract; it is believed that isoflavone is not involved in this process.

As for the skin measurement test, favorable effects were seen on the volume of melanin on the forehead. The question here is whether the interpretation, "Group A had increased volumes of melanin, while such increases were suppressed in group B" is reasonable. If it is true that estrogen works to maintain a youthful and healthy skin, it could also have a favorable effect on the melanin deposits in the skin.[3] Because isoflavone is administered directly on the skin along with the cosmetic base, it is definitely possible that effective doses may have worked on the skin.

Then, what about indirect actions? These are derived from the fact that the Product used by group B improved the mental symptoms (which indicates feeling pleasant) when actually used. This was determined by survery question. If people actually use the Product and see their skin conditions improve in addition to experiencing a pleasant feeling while using it, they may end up happier. This may be the comprehensive effect of isoflavone and pine bark extract contained in the Product. However, it does not matter too much if there are aspects that cannot be fully explained by the pharmacological activity of isoflavone and pine bark extract. It is common to see favorable effects on a person's mental state, which in turn induce favorable effects on their physical state.

Let us examine the consistency between the previous test and the present one. Results of the previous clinical test, conducted as an open, non-blinded study, showed that the action of applying a skin cream [1] improved a number of subjective symptoms related to QOL,[2] reduced the level of cortisol (a stress hormone) in subjects over the age of 40 years, and [3] elevated the level of estradiol.6 Meanwhile, results of the present study showed improvements in QOL items that were different from those of the previous study. Further, no significant differences were seen in the cortisol or estradiol levels in both groups A and B. No significant differences were seen in the value of estradiol itself between the 2 groups.

In the previous study, improvements were seen with respect to the following 4 mental symptom parameters: "no feeling of happiness," "reluctance to talk with others," "pessimism," and "inability to sleep because of worries." In the present study, improvements were seen with respect to the following 5 mental symptom parameters: "Irritability," "loss of motivation," "reluctance to talk with others," "pessimism," and "lapse of memory." As seen in both tests, "reluctance to talk with others" and "pessimism" were improved. We all know that it is not easy to adequately identify the changes in mental symptoms and as a result of comparing the 2 tests, we concluded that their reproducibility was adequate.

Regarding the cortisol value, our previous study found that the mean for subjects aged 40 years and older was relatively high, at 13.7 $\pm$ 6.0 g/dl, and that, when the numerical values were examined for each patient, those with high cortisol levels saw a reduction, while those whose levels were not high initially had no changes. In other words, the activity of putting on makeup could be regarded as working to maintain the body's homeostasis in an auxiliary manner, rather than to reduce the cortisol level. In the present study, the value of cortisol was not too high in the beginning, at 10.1 $\pm$ 6.2 g/dl, and ultimately became 9.5 $\pm$ 5.3 g/dl, which did not constitute a significant change. However, the figures are consistent in terms of maintaining homeostasis or constancy.

Although the same view could be also applied to estradiol, we determined the trial period to be 4 weeks, so as to eliminate as much as possible the influence of the subjects' menstrual cycles.

IGF-I and the amount of blood flow in the facial skin decreased in both group A and group B. Although there is a possibility that IGF-I may have decreased because of the activity of applying makeup, compared with ordinary biochemical tests, there still are many measurement errors, so the possibility of the effects of variations/discrepancies cannot be ruled out. Similar measurement errors must be taken into account for the amount of blood flow in the facial skin as well.

In this paper, we have described the results of our present test and the interpretation thereof. In conclusion, the efficacy of anti-aging cosmetic products that contain characteristic ingredients (used by group B) was shown by a double-blind test method.

References

1. Yonei Y. Mechanism of Aging and Life Span. Tokyo: Nippon Jitsugyou Shuppan 2003 (In Chinese and Korean: in press).
2. Aso T. Management of health in middle-aged and older females; the role of gynecologists. Tochigi Prefectural Obstetrics/Gynecology Medical Report 1999; 26: 73-75.
3. Ota H, Makita K. Manual for hormone therapy conducted at the obstetrics/gynecology department, IV. Menopause and other menopausal syndromes. Obstet Gynecol 2001;68(Extra Number):200-206.
4. Ouchi M. Manual for medical care in the elderly, Volume 2. Hormone replacement therapy that protects the health of elderly women: Current status and outlook. Treatment 2001;83:2723-2728.
5. Iwami K. Clinical nutritional keywords for 2002: nutritional ingredients, soybean isoflavone and its physiological activity. Clin Nutrition 2002;100:779-783.
6. Yonei Y, Mizuno Y. Effect of an isoflavone-containing cosmetics, eterrite*P*“, on the mental and physical parameters associated with QOL and aging. Anti-Aging Med 2004; 2:19-26 .
7. Yonei Y, Mizuno Y, Orimo H, Mizushima Y. Effect of Treatment with a high-electropotential apparatuses (Serente) therapy on symptoms associated with QOL and aging. Anti-Aging Med 2003;2:16-23.
8. Thompson HJ, Heimendinger J, Haegele A, Sedlacek SM, Gillette C, O'Neill C, Wolfe P, Conry C: Effect of increased vegetable and fruit consumption on markers of oxidative cellular damage. Carcinogenesis 1999; 20: 2261-2266
9. Urso ML, Clarkson PM: Oxidative stress, exercise, and antioxidant supplementation. Toxicology 2003 189: 41-54
10. Piconi L, Quagliaro L, Ceriello A: Oxidative stress in diabetes. Clin Chem Lab Med 2003; 41: 1144-1149,
11. Fluhr JW, Kuss O, Diepgen T, Lazzerini S, Pelosi A, Gloor M, Berardesca E: Testing for irritation with a multifactorial approach: comparison of eight non-invasive measuring techniques on five different irritation types. *Br J Dermatol* 2001; 145: 696-703
12. Annen M, Yamamoto K. Our experience of using a lotion containing bioceramide LS and its usefulness for pediatric atopic dermatitis. *J Japan Soc Pediatr Dermatol* 1998; 17:45-50.
13. Sator PG, Schmidt JB, Honigsmann H: Comparison of epidermal hydration and skin surface lipids in healthy individuals and in patients with atopic dermatitis. *J Am Acad Dermatol* 2003; 48: 352-358
14. Rode B, Ivens U, Serup J: Degreasing method for the seborrheic areas with respect to regaining sebum excretion rate to casual level. Skin Res Technol 2000; 6: 92-97
15. Clarys P, Alewaeters K, Lambrecht R, Barel AO:Skin color measurements: comparison between three instruments: the Chromameter(R), the Derma Spectrometer(R) and the Mexameter(R). Skin Res Technol 2000; 6: 230-238
16. Manuskiatti W, Sivayathorn A, Leelaudomlipi P, Fitzpatrick RE:Treatment of acquired bilateral nevus of Ota-like macules (Hori's nevus) using a combination of scanned carbon dioxide laser followed by Q-switched ruby laser. *J Am Acad Dermatol* 2003; 48: 584-591
17. Vertuani S, Ziosi P, Solaroli N, Buzzoni V, Carli M, Lucchi E, Valgimigli L, Baratto G, Manfredini S: Determination of antioxidant efficacy of cosmetic formulations by non-invasive measurements. Skin Res Technol 2003; 9: 245-253
18. Wilhelm KP, Kaspar K, Funkel O: Comparison of three techniques for evaluating skin erythemal response for determination of sun protection factors of sunscreens: high resolution laser Doppler imaging, colorimetry and visual scoring. Photodermatol Photoimmunol Photomed 2001; 17: 60-65
19. Masquelier J: Oligom_res procyanidoliques. Cosm_tologie 1990; 95:89-97
20. Blazs_G, G_bor M, Rohdewald P: Antiinflammatory activities of procyanidin-containing extracts from Pinus pinaster Ait. after oral and cutaneous application. Pharmazie 1997; 52: 380-382