Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : September 2, 2016
Accepted : November 21, 2016
Published online : December 31, 2016

Original Article

Anti-glycation and skin beautification properties from ingestion of mixed herb extract: A placebo-controlled, double-blind, randomized, parallel-group study.

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Abstract

***Purpose*:** The anti-glycation properties of mixed herb extract (MHE) composed of *Chamaemelum nobile* (roman chamomile), *Crataegus laevigata* (hawthorne berries), *Houttuynia cordata* (dokudami) and *Vitis vinifera* (grape leaves) have been reported in various clinical trials. However, these studies used high dosages and composite products that combine other functional ingredients, so this new study evaluated the anti-glycation and skin beautification properties in low doses of a single product.
***Methods*:** The subjects were 24 Japanese women (12 in the MHE group, 12 in the placebo group) 40 to 64 years of age who had high amounts of advanced glycation end products (AGEs) deposits on the skin as measured by an AGE Reader™. A placebo-controlled, double-blind, randomized, parallel-group comparative study was conducted using MHE as the test food, which was administered in capsules of 100 mg per day for 12 weeks. The subjects underwent blood tests, skin-related measurements and medical interviews before administration as well as after 8 and 12 weeks of administration. Note that this study has been conducted upon approval by an ethics review committee (Japan Clinical Trial Registry ID: UMIN000015242).
***Results*:** In all cases, significant reductions were observed in the MHE group compared to the placebo group for both Melanin Index (MHE group: -0.04, placebo group: +0.01, $p = 0.009$) and color difference b* (yellow) (MHE group: -0.66, placebo group: +0.16, $p = 0.025$) for the skin of the upper arm. Also, a significant reduction in *Brown Spots* on the face was observed for the MHE group after 12 weeks' administration compared to before administration (48.39 → 46.49, $p = 0.005$). For the subgroup of healthy subjects that excluded 2 subjects suspected of having diabetes, (10 in the MHE group, 12 in the placebo group), significant reductions in 3-deoxyglucosone (3DG) were observed in the MHE group when 3DG levels after 12 weeks were compared to pre-administration levels (16.96 → 13.73 ng/mL, $p = 0.043$). Likewise for the hemoglobin oxygen saturation index (Hb SO_2 Index) (an index for complexion) in the cheek skin, a significant increase was observed in the MHE group compared to the placebo group (MHE group: +7.38, placebo group: +4.36, $p = 0.020$).
***Conclusion*:** MHE suppressed the yellowing and browning of skin color. This is possibly caused by a reduction in carbonylation due to suppression of 3DG generation. Furthermore, the improvement in complexion could possibly be attributed to the herb constituents.

***KEY WORDS*:** Mixed herb extract, anti-glycation, skin beautification, advanced glycation end products (AGEs), 3-deoxyglucosone (3DG)

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Glycative Stress Research 2016; 3 (4): 236-245

Introduction

It is reported that the carbonylation of skin proteins is involved in yellowing and reduced clarity of the skin [1]. Also, the mixed herb extract composed of *Chamaemelum nobile* (roman chamomile), *Crataegus laevigata* (hawthorne berries), *Houttuynia cordata* (dokudami) and *Vitis vinifera* (grape leaves) in *in vitro* experiments suppresses the generation of 3-deoxyglucosone (3DG) [2], glyoxal, methylglyoxal and other compounds that cause carbonylation.

MHE has been reported to have various anti-glycation properties in clinical trials, but these evaluations involved high dosages or composite products that contain other functional ingredients [3-6]. Therefore, we evaluated anti-glycation and skin beautification properties of a single product at low doses.

Method

Subjects

The subjects for this study were Japanese women 40 to 64 years of age, and to achieve a sample size of 10 or more women in a group, the target size was set to 24 people to account for possible drop-outs (12 in the MHE group, 12 in the placebo group). A screening test was applied to a pool of applicants who had passed the selection criteria and exclusion criteria, to select subjects with relatively higher levels of skin AGEs deposits as measured by an AGE Reader™ (Diagnoptics Technologies B.V., Groningen, Netherlands). The group allocator used random numbers to create the allocation table. In the allocation, highest priority was given to matching the skin AGEs deposit levels, and then to ensure an even distribution of the average and standard deviation of age and BMI (***Table 1***).

Trial Design

The clinical trial design was a placebo-controlled, double-blind, randomized, parallel-group comparative study.

The test food was MHE supplied in capsules, 100 mg per day. Over 12 weeks, the trial subjects generally ingested a placebo or MHE once per day on an empty stomach. Blood tests, physical tests and medical interviews were conducted prior to administration, and after 8 and 12 weeks of administration for a total of 3 times. Furthermore, in addition to the above, the questionnaire portion only was conducted after the fourth week by mail.

The trial subjects fasted on the day prior to testing, ingesting only water from 10 pm until the end of testing on the next day. On the day of testing, blood and stratum corneum samples were collected from the subjects, who also completed all other tests from 9 am to 2 pm.

The primary endpoints were related to the anti-glycation properties: skin AGEs deposit levels, skin elasticity, stratum corneum carboxymethyl lysine (CML) and blood test results (CML, 3DG).

Secondary endpoints were skin color differences, imaging diagnosis of skin using VISIA™, blood test results (glucose, hemoglobin [Hb] A1c and insulin), and medical interviews (Anti-Aging QOL Common Questionnaire and a skin questionnaire).

Other blood tests were conducted as safety endpoints: total bilirubin, aspartate aminotransferase (AST) (glutamic oxaloacetic transaminase [GOT]), alanine aminotransferase (ALT) (Glutamic Pyruvic Transaminase [GPT]), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyltransferase (γ-GTP), creatine phosphokinase (CPK), total protein, albumin, albumin/globulin (A/G) ratio, creatinine, urea nitrogen, uric acid, triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-

Table 1. Baseline demographic and clinical characteristics.

	MHE group	Placebo group	p-value
Target size	12	12	(t-test)
Age (years of age)	51.5 ± 6.3	51.4 ± 5.5	p = 0.973
Height (cm)	155.40 ± 5.15	158.38 ± 4.68	p = 0.153
Weight (kg)	55.00 ± 6.81	56.76 ± 4.99	p = 0.478
Body fat (%)	29.38 ± 3.51	28.60 ± 3.62	p = 0.596
BMI (kg/m^2)	22.77 ± 2.46	22.64 ± 2.07	p = 0.894
Skin AGEs deposit level	2.43 ± 0.17	2.41 ± 0.18	p = 0.791
Glucose (mg/dL)	88.8 ± 11.0	83.7 ± 6.3	p = 0.173
HbA1c [NGSP] (%)	5.71 ± 0.59	5.58 ± 0.24	p = 0.473
Insulin (μU/mL)	5.77 ± 1.65	4.83 ± 1.86	p = 0.203

Measured value: Average ± standard deviation. MHE, mixed herb extract; BMI, body mass index; AGEs, advanced glycation end products; NGSP, National Glycohemoglobin Standardization Program.

density lipoprotein (LDL) cholesterol, Na, K, Cl, Ca and Fe, as well as hematology tests (white blood cell counts, red blood cell counts, hemoglobin levels, hematocrit, platelet counts and hemograms).

For basic measurements, height (only on first time), body weight, body fat ratio, fat mass, lean body mass, BMI, basal metabolic rate, blood pressure and pulse rate. The examinations were conducted by physicians.

Trial subjects recorded ingestion status of the test food, any harmful events, and any changes to lifestyle (diet, exercise, alcohol ingestion, etc.) in their diaries.

The test period was from September 25 to December 19, 2014. Physician interviews, blood pressure measurement and blood collection was administered at the Senrigaoka Kyoritsu Clinic, and skin-related tests were conducted at the A-Kit, Inc. Life and Medical Sciences Inspection Center directly afterwards.

Test Food

The test food was a food product in capsule form containing the MHE (product name: AG Herb MIX) (100 mg/capsule) or a placebo capsule. To the extent possible, the placebo was made to be indiscernible from the MHE food product in appearance, taste and odor. The test food composition and nutritional constituents are listed in ***Table 2*** and ***Table 3***.

Testing Methods

Subjective Symptoms (Questionnaire Items)

The evaluation of subjective symptoms was mainly divided into *physical symptoms* and *mental symptoms*. The Anti-Aging QOL Common Questionnaire (AAQol) was used, and evaluations were made on a five-point scale similar to previous reports[3]. Also, a Skin Questionnaire was administered at the same time.

Body Measurements and Physical Testing

Body fat ratio, lean body mass, and other items in addition to height, body weight, blood pressure and heart rate were measured by bioelectrical impedance analysis, using a Well-SCAN500 (Canon Lifecare Solutions, Inc.: Bunkyo-ku, Tokyo, Japan) body composition analyzer.

Blood Testing

For the blood tests, 3DG was tested at SRL, Inc. (Tachikawa, Tokyo, Japan), and the rest were done at LSI Medience Corporation (Chiyoda-ku, Tokyo, Japan).

Skin-Related Testing

The skin-related tests below were started after washing the face and cleansing the measurement area, then acclimatizing to an environment with constant temperature and humidity (21 ± 1°C, 50 ± 5%) for 20 minutes.

1) Skin AGEs deposit levels

The skin AGEs deposit levels were measured using an AGE Reader™. Following a previous report[7], the measured area for the determination was the inner side of the upper right arm (about 10 cm from the olecranon toward the shoulder).

2) Skin elasticity

Skin elasticity was measured using a Cutometer MPA580 Dual (Courage + Khazaka electronic GmbH, Cologne, Germany) skin elasticity measurement device. The measurement area for the determination was the left cheek (the median position between the bottom of the ear lobe and the corner of the mouth) as well as the inner part of the upper right arm.

Table 2. Test food composition (Compounded amount: mg/capsule).

	MHE food	Placebo
Dextrin	117.5	217.5
MHE (as AG Herb MIX)	100.0	0.0
Starch	25.0	25.0
Calcium stearate	7.5	7.5
Total	250.0	250.0

MHE, mixed herb extract.

Table 3. Test food nutritional constituents (Administration amount per day: per capsule).

	MHE food	Placebo
Energy (kcal)	1.2	1.2
Protein (g)	0.003	0
Lipid (g)	0.001	0
Carbohydrate (g)	0.276	0.287
Sodium (mg)	0.243	0.139

MHE, mixed herb extract.

3) Stratum corneum CML

Following a previous report [8], the stratum corneum CML was measured. An adhesive film was applied to the skin to collect the stratum corneum (tape stripping method). This test was conducted three times in the same location, and the samples were stored frozen for measuring CML levels in the stratum corneum. The collection area was the inner part of the upper right arm. Measurements were conducted at A-Kit, Inc. (Ogaki, Gifu, Japan).

4) Skin color difference

Skin color difference was measured using a CM-600d spectrophotometer and the CM-SA (Konica Minolta, Inc., Chiyoda-ku, Tokyo, Japan) skin analysis software package. The measurement area was the left cheek and the inner part of the upper right arm.

5) Imaging analysis of the facial skin

Imaging analysis of the facial skin was conducted by using the VISIA™ Evolution (Canfield Imaging Systems, Inc., NJ, USA) system.

Statistical Analysis

Analysis was done using the IBM SPSS Statistics 22 (Japan IBM Corporation, Chuo-ku, Tokyo, Japan) package. Dunnett's multiple comparison test was used as the assay method for before and after comparisons of the measured items prior to administration, and after the 8 weeks and 12 weeks of administration. For inter-group comparisons, at-test (two-group comparison) was used. Friedman's multiple comparison test was used as the assay method for before and after comparisons of questionnaire items prior to administration, and after 4 weeks, 8 weeks and 12 weeks of administration. The Mann-Whitney test was used for inter-group comparisons. The significance level was a relative risk below 5% for all two-tailed tests.

Ethical Standards

The study complied with the ethical principles set forth in the Helsinki Declaration and the Japan Personal Information Protection Act, and was conducted in accordance with the Japan Ministerial Ordinance on Good Clinical Practice for Drugs (GCP) (Ordinance of the Ministry of Health and Welfare No. 28 of March 27, 1997). Review documents were submitted to the Kenshokai Ethics Committee (Fukushima-ku, Osaka, Japan), and were reviewed and approved on August 27, 2014. Informed consent was obtained from trial subjects prior to screening tests, and the subjects sufficiently understood the content of the testing plan and voluntarily expressed their willingness to participate in the test by submitting the consent form. The Clinical Trial Registry ID was UMIN000015242 for this study.

Results

Target Group for Analysis

Fig. 1 shows the tracking flow diagram for trial subjects. The solicitation for trial subjects was conducted between August 28 to September 7, 2014, and screening tests were conducted on the 9th and 10th of September. 24 people participated in the trial with twelve people in the MHE group and twelve people in the placebo group allocated in a random manner. All subjects completed the trial, and none dropped out. Also, no subjects were deemed unsuited for analysis, and all were included in the analysis. In addition, the changes in physical testing are shown in ***Table 4***.

Baseline Data

Table 1 shows baseline demographic and clinical characteristics for each group. Furthermore, participants were limited to the Asian race (Japanese) and female sex.

Primary Endpoints

For the primary endpoints of skin AGEs deposit levels, skin elasticity, stratum corneum CML and blood test results (CML, 3DG), significant differences were not observed for both between the MHE group and the placebo group and within the MHE group in changes from before administration to the 12th week of administration (***Table 5*** and ***Table 6***). However, parameters identified to have undergone changes opposite to improvement were listed under the safety endpoints described later on in the paper.

Furthermore, changes in the blood 3DG levels for the subgroup of healthy subjects (excluding 2 persons who showed HbA1c values higher than the standard range) are shown in ***Fig. 2***. There was a significant reduction in the MHE group (before administration 16.96 ± 2.92 → 12th week 13.73 ± 4.00 ng/mL [p = 0.043]), whereas the placebo group did not show any significant change (before administration 15.09 ± 3.59 → 12th week 13.15 ± 3.63 ng/mL [p = 0.191]). In an inter-group comparison, no significant inter-group differences were noted (p = 0.456).

Secondary Endpoints

For the secondary endpoints of skin color differences, imaging diagnosis of the skin using VISIA™ (***Table 6***), blood testing (glucose, HbA1c and insulin) (***Table 5***) and medical interview (Anti-Aging QOL Common Questionnaire, skin questionnaire) (***data not shown***), improvements were demonstrated in the three items 1) to 3) below. However, items showing changes opposite to improvement were listed under the safety endpoints described later in the paper. Furthermore, the subgroup analysis is shown in 4).

1) Skin (upper arm) Melanin Index

Changes in the Melanin Index in the skin of the upper arm are shown in ***Fig. 3***.

There were significant reductions in the MHE group (before administration 0.70 ± 0.09 → 12th week 0.66 ± 0.10 ng/mL [p = 0.001]), whereas the placebo group did not show any significant change (before administration 0.70 ± 0.06 → 12th week 0.70 ± 0.09 ng/mL [p = 0.727]). In an inter-group comparison, the MHE group showed significant reductions in contrast to the placebo group (p = 0.009).

2) Skin (upper arm) color difference b* (yellow)

Fig. 4 shows the color difference b* (yellow) for the skin of the upper arm. There were significant reductions in the MHE group (before administration 14.84 ± 1.31 → 12th week 14.18 ± 1.72 ng/mL [p = 0.005]), whereas the placebo group did not show significant change (before administration 14.78 ± 1.31 → 12th week 14.93 ± 1.42 ng/mL [p = 0.819]). In an inter-group comparison, the MHE group showed significant reductions in contrast to the placebo group (p = 0.025).

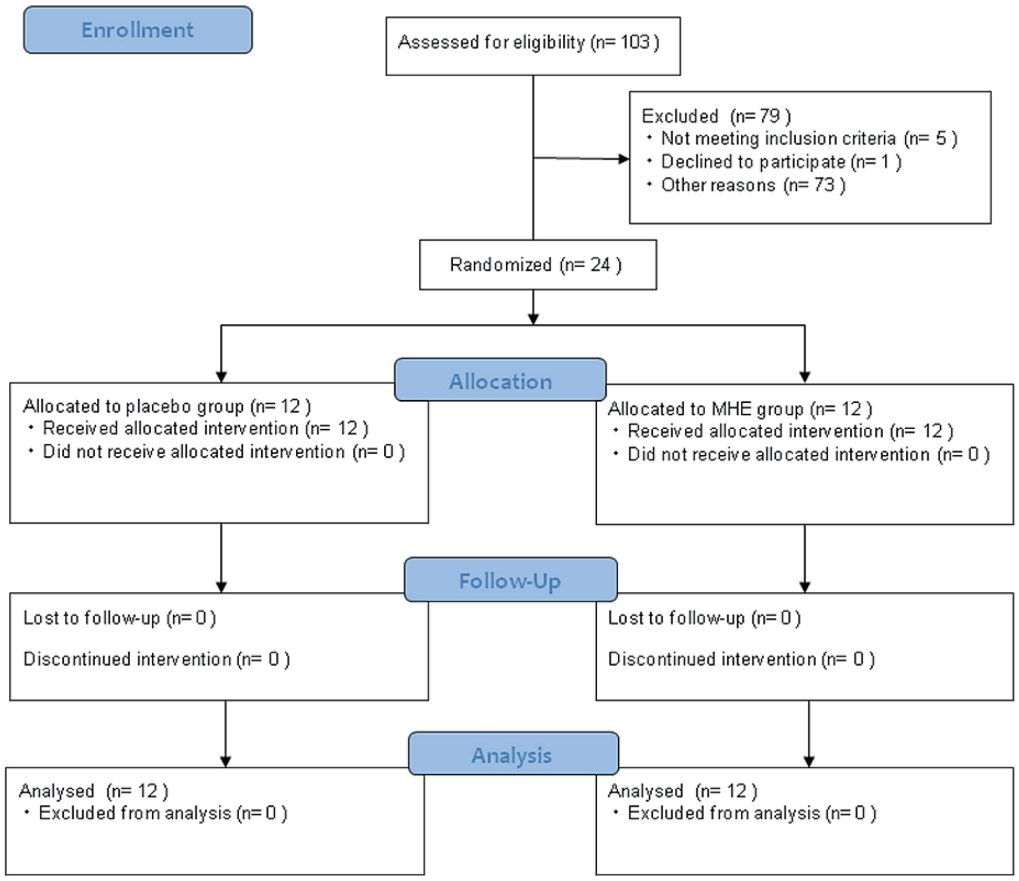

Fig. 1. **Tracking flow diagram for trial subjects.**
MHE, mixed herb extract.

Table 4. Physical testing.

Parameter		Unit	Group	0W	8W	12W
Body composition	Weight	kg	MHE	55.0 ± 6.7	55.4 ± 6.7	55.1 ± 6.9
			Placebo	57.0 ± 5.1	57.0 ± 5.2	57.2 ± 5.2
	Body fat	%	MHE	29.9 ± 3.0	29.8 ± 3.9	28.9 ± 4.3
			Placebo	28.7 ± 3.6	29.1 ± 3.3	28.2 ± 3.3
	Fat mass	kg	MHE	16.5 ± 3.3	16.7 ± 3.9	16.0 ± 3.7
			Placebo	16.4 ± 3.1	16.7 ± 3.0	16.2 ± 2.8
	Lean body mass	kg	MHE	38.5 ± 3.9	38.8 ± 3.7	39.1 ± 4.5
			Placebo	40.5 ± 3.3	40.4 ± 3.1	41.0 ± 3.6
	BMI	kg/m^2	MHE	22.8 ± 2.4	22.9 ± 2.4	22.8 ± 2.5
			Placebo	22.7 ± 2.1	22.7 ± 2.1	22.8 ± 2.0
	Basal metabolic rate	kcal/day	MHE	1072 ± 64	1074 ± 62	1076 ± 68
			Placebo	1097 ± 52	1095 ± 49	1101 ± 50
Blood pressure	systolic	mmHg	MHE	121 ± 14	121 ± 17	122 ± 12
			Placebo	124 ± 17	128 ± 16	125 ± 18
	diastolic	mmHg	MHE	81 ± 10	79 ± 10	79 ± 11
			Placebo	76 ± 6	80 ± 8 #	80 ± 8 #
Pulse rate		bpm	MHE	78 ± 11	74 ± 8	74 ± 11
			Placebo	70 ± 11	67 ± 8	68 ± 12

Subjects: n = 12 (MHE group), n = 12 (Placebo group). Values: means ± standard deviation. * $p < 0.05$, ** $p < 0.01$: Comparison with the Placebo group (t-test). # $p < 0.05$, ## $p < 0.01$: Comparison with before administration (Dunnett). MHE, mixed herb extract; BMI, body mass index.

Table 5. Clinical chemistry testing.

Parameter	Unit	Reference range	Group	0W	8W	12W
CML in stratum corneum	μg/mg protein	–	MHE	36.7 ± 4.9	–	37.8 ± 14.9
			Placebo	39.0 ± 16.0	–	42.7 ± 11.0
CML	μg/mL	–	MHE	0.65 ± 0.05	–	0.88 ± 0.16 ##
			Placebo	0.71 ± 0.23	–	0.96 ± 0.32 ##
3-deoxyglucosone	ng/mL	3.76 - 18.14	MHE	18.0 ± 3.9	14.6 ± 3.8 #	15.1 ± 5.1
			Placebo	15.1 ± 3.6	15.0 ± 3.0	13.1 ± 3.6
Glucose	mg/dL	70 - 109	MHE	88.8 ± 11.0	84.6 ± 10.3	88.3 ± 13.0
			Placebo	83.7 ± 6.3	80.0 ± 5.7 ##	81.0 ± 6.1 #
HbA1c/NGSP	%	4.6 - 6.2	MHE	5.71 ± 0.59	5.60 ± 0.44	5.64 ± 0.46
			Placebo	5.58 ± 0.24	5.52 ± 0.22	5.57 ± 0.17
Insulin	μU/mL	1.7 - 10.4	MHE	5.77 ± 1.65	5.27 ± 1.48	5.63 ± 1.58
			Placebo	4.83 ± 1.86	4.84 ± 1.56	3.59 ± 1.05 #
Total protein	g/dL	6.7 - 8.3	MHE	7.3 ± 0.4	7.2 ± 0.3	7.3 ± 0.2
			Placebo	7.3 ± 0.3	7.3 ± 0.3	7.2 ± 0.3
Albumin	g/dL	3.8 - 5.2	MHE	4.3 ± 0.3	4.3 ± 0.3	4.3 ± 0.2
			Placebo	4.4 ± 0.3	4.4 ± 0.3	4.4 ± 0.3
A/G ratio		1.1 - 2.0	MHE	1.48 ± 0.23	1.50 ± 0.21	1.47 ± 0.21
			Placebo	1.57 ± 0.27	1.55 ± 0.19	1.57 ± 0.27
CPK	U/L	40 - 150	MHE	67.3 ± 42.0	82.1 ± 64.2	64.2 ± 23.7
			Placebo	100.2 ± 33.4	121.1 ± 54.3 #	112.2 ± 57.1
AST (GOT)	U/L	10 - 40	MHE	18.0 ± 3.7	21.5 ± 4.4 ##	19.6 ± 3.9
			Placebo	19.2 ± 3.8	20.5 ± 3.8	20.5 ± 3.7
ALT (GPT)	U/L	5 - 45	MHE	15.3 ± 7.9	19.3 ± 10.4	15.9 ± 7.0
			Placebo	15.9 ± 5.3	16.0 ± 3.9	15.4 ± 3.7
LDH	U/L	120 - 240	MHE	175.0 ± 24.7	176.4 ± 27.3	190.3 ± 28.8 #
			Placebo	181.1 ± 35.4	186.2 ± 32.3	196.3 ± 29.9 ##
ALP	U/L	100 - 325	MHE	207.3 ± 69.9	212.7 ± 70.4	223.8 ± 69.7 #
			Placebo	182.8 ± 59.0	187.0 ± 58.2	184.2 ± 58.0
γ-GTP	U/L	30 以下	MHE	26.3 ± 15.0	27.7 ± 17.7	26.7 ± 18.7
			Placebo	20.8 ± 10.5	20.3 ± 8.7	22.5 ± 17.0
Creatinine	mg/dL	0.47 - 0.79	MHE	0.65 ± 0.08	0.63 ± 0.10	0.63 ± 0.10
			Placebo	0.69 ± 0.07	0.66 ± 0.07 #	0.65 ± 0.09 ##
Uric acid	mg/dL	2.5 - 7.0	MHE	4.66 ± 1.26	4.49 ± 1.19	4.60 ± 1.15
			Placebo	4.98 ± 0.95	4.68 ± 0.68	4.74 ± 0.72
Urea nitrogen	mg/dL	8.0 - 20.0	MHE	11.8 ± 2.5	10.9 ± 2.4	12.7 ± 2.8
			Placebo	10.9 ± 3.1	11.5 ± 3.1	11.6 ± 3.1
TG (Triglyceride)	mg/dL	30 - 149	MHE	124.1 ± 50.1	108.7 ± 44.4	114.2 ± 52.6
			Placebo	96.8 ± 43.3	93.0 ± 41.3	86.0 ± 36.5
Total cholesterol	mg/dL	120 - 219	MHE	248.3 ± 47.3	235.7 ± 36.9	243.2 ± 43.4
			Placebo	218.9 ± 29.3	222.3 ± 28.8	216.4 ± 28.7
HDL cholesterol	mg/dL	40 - 95	MHE	61.3 ± 12.8	61.6 ± 12.3	63.4 ± 11.5
			Placebo	64.1 ± 8.6	68.5 ± 11.3	68.5 ± 11.1
LDL cholesterol	mg/dL	65 - 139	MHE	159.3 ± 45.0	149.0 ± 38.4	154.2 ± 41.6
			Placebo	133.4 ± 27.3	134.7 ± 26.3	127.0 ± 26.2
Sodium	mEq/L	137 - 147	MHE	141.3 ± 1.4	140.7 ± 1.6	140.8 ± 2.0 *
			Placebo	142.2 ± 0.8	141.3 ± 1.2 #	140.5 ± 1.5 ##
Potassium	mEq/L	3.5 - 5.0	MHE	4.25 ± 0.39	4.23 ± 0.36	4.50 ± 0.49 ##
			Placebo	4.12 ± 0.20	4.28 ± 0.16	4.48 ± 0.26 ##
Chloride	mEq/L	98 - 108	MHE	104.7 ± 1.5	104.1 ± 1.6	104.5 ± 1.6
			Placebo	105.0 ± 1.7	104.6 ± 1.6	104.1 ± 1.6
Calcium	mg/dL	8.4 - 10.4	MHE	9.53 ± 0.39	9.33 ± 0.31	9.47 ± 0.38
			Placebo	9.47 ± 0.39	9.53 ± 0.32	9.38 ± 0.24
Serum iron	μg/dL	40 - 180	MHE	93.9 ± 42.0	83.2 ± 46.5	73.8 ± 39.2 #*
			Placebo	83.1 ± 44.4	94.7 ± 49.3	88.9 ± 38.9
Total bilirubin	mg/dL	0.2 - 1.2	MHE	0.79 ± 0.24	0.78 ± 0.34	0.68 ± 0.16 *
			Placebo	0.69 ± 0.17	0.68 ± 0.12	0.71 ± 0.17

Subjects: n = 12 (MHE group), n = 12 (Placebo group). Values: means ± standard deviation. * $p < 0.05$, ** $p < 0.01$: Comparison with the Placebo group (t-test). # $p < 0.05$, ## $p < 0.01$: Comparison with before administration (Dunnett [3 times], paired t-test [twice]). MHE, mixed herb extract; CML, carboxymethyl lysine; NGSP, National Glycohemoglobin Standardization Program; A/G, albumin/globulin; CPK, creatine phosphokinase; AST (GOT), aspartate aminotransferase (glutamic oxaloacetic transaminase); ALT (GPT), alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 6. Skin-related testing.

Parameter		Unit	Group	0W	8W	12W
Skin AGEs deposit level	AF		MHE	2.36 ± 0.21	2.29 ± 0.18	2.23 ± 0.26 #
			Placebo	2.45 ± 0.21	2.37 ± 0.23	2.27 ± 0.23 ##
Skin viscoelasticity (Upper arm)	R2		MHE	0.90 ± 0.02	0.88 ± 0.03 #	0.88 ± 0.03 *
			Placebo	0.90 ± 0.02	0.88 ± 0.03 #	0.90 ± 0.02
	R7		MHE	0.66 ± 0.04	0.65 ± 0.06	0.66 ± 0.05
			Placebo	0.68 ± 0.05	0.66 ± 0.05 #	0.69 ± 0.05
Skin viscoelasticity (Cheek)	R2		MHE	0.72 ± 0.07	0.73 ± 0.05	0.75 ± 0.06
			Placebo	0.75 ± 0.08	0.73 ± 0.07	0.77 ± 0.06
	R7		MHE	0.37 ± 0.07	0.40 ± 0.05	0.39 ± 0.06
			Placebo	0.41 ± 0.08	0.39 ± 0.07	0.43 ± 0.06
Color difference (Upper arm)	Melanin Index	%	MHE	0.70 ± 0.09	0.68 ± 0.09	0.66 ± 0.10 ###**
			Placebo	0.70 ± 0.06	0.69 ± 0.09	0.70 ± 0.09
	Hb Index		MHE	0.77 ± 0.13	0.84 ± 0.19	0.89 ± 0.21
			Placebo	0.79 ± 0.17	0.92 ± 0.27	0.87 ± 0.25
	Hb SO_2 Index		MHE	56.4 ± 9.1	52.7 ± 7.4	53.2 ± 5.7
			Placebo	57.8 ± 7.2	53.9 ± 10.6	57.5 ± 8.4
	L*		MHE	70.0 ± 1.4	70.1 ± 1.5	69.9 ± 1.6
			Placebo	70.1 ± 1.5	69.6 ± 1.8	70.1 ± 1.9
	a*		MHE	4.82 ± 0.61	4.95 ± 0.87	5.21 ± 1.19
			Placebo	5.04 ± 0.90	5.43 ± 0.91	5.45 ± 1.36
	b*		MHE	14.8 ± 1.3	14.7 ± 1.4	14.2 ± 1.7 ##*
			Placebo	14.8 ± 1.3	14.7 ± 1.6	14.9 ± 1.4
Color difference (Cheek)	Melanin Index	%	MHE	1.11 ± 0.12	1.06 ± 0.13 ##	1.04 ± 0.12 ##
			Placebo	1.15 ± 0.13	1.11 ± 0.14 #	1.10 ± 0.10 ##
	Hb Index		MHE	1.08 ± 0.22	1.06 ± 0.27	1.14 ± 0.22
			Placebo	1.01 ± 0.15	1.06 ± 0.23	1.11 ± 0.23
	Hb SO_2 Index		MHE	51.7 ± 7.0	56.2 ± 7.1 ##	58.5 ± 7.1 ##
			Placebo	54.5 ± 4.5	59.1 ± 5.0 ##	58.8 ± 4.2 ##
	L*		MHE	64.6 ± 2.6	65.8 ± 2.4 ##	65.8 ± 2.4 ##
			Placebo	65.1 ± 2.0	65.6 ± 2.1	65.8 ± 2.0 #
	a*		MHE	8.44 ± 1.07	8.40 ± 1.55	8.83 ± 1.31
			Placebo	8.53 ± 0.85	8.80 ± 0.99	9.09 ± 1.22
	b*		MHE	18.5 ± 1.5	18.2 ± 1.6	17.8 ± 1.5 ##
			Placebo	19.2 ± 1.6	18.8 ± 1.9	18.5 ± 1.6 #
Imaging analysis by VISIA	Brown Spots	Score × 100	MHE	48.4 ± 4.9	47.2 ± 4.8	46.5 ± 5.3 ##
			Placebo	48.1 ± 6.6	47.4 ± 8.2	47.3 ± 7.4
	Pores	Score × 100	MHE	18.4 ± 9.9	20.0 ± 10.8	20.6 ± 11.9
			Placebo	17.7 ± 9.0	18.1 ± 8.5	18.2 ± 9.5
	Porphyrin	Score × 100	MHE	10.7 ± 8.5	8.9 ± 7.6	8.0 ± 6.7
			Placebo	8.5 ± 9.2	6.2 ± 7.4	6.6 ± 9.2
	Red	Score × 100	MHE	25.9 ± 5.1	26.4 ± 6.0	28.2 ± 7.1
			Placebo	26.8 ± 5.1	29.1 ± 6.7	31.7 ± 10.7
	Spots	Score × 100	MHE	38.9 ± 6.3	39.4 ± 7.2	37.7 ± 8.1
			Placebo	39.2 ± 8.8	38.8 ± 10.0	38.2 ± 9.8
	Texture	Score × 100	MHE	12.2 ± 5.8	12.1 ± 5.5	12.7 ± 7.0
			Placebo	13.6 ± 5.3	12.5 ± 6.0	12.5 ± 6.1
	UV Spots	Score × 100	MHE	30.4 ± 9.2	32.0 ± 9.4	31.0 ± 8.2
			Placebo	32.3 ± 3.7	33.1 ± 2.8	33.9 ± 5.9
	Wrinkles	Score × 100	MHE	11.6 ± 8.8	11.7 ± 7.2	11.0 ± 7.8
			Placebo	16.8 ± 10.2	13.8 ± 16.4	15.3 ± 14.1

Subjects: n = 12 (MHE group), n = 12 (Placebo group). Values: means ± standard deviation. * $p < 0.05$, ** $p < 0.01$: Comparison with the Placebo group (t-test). # $p < 0.05$, ## $p < 0.01$: Comparison with before administration (Dunnett). MHE, mixed herb extract; AGEs, advanced glycation end products; AF, auto fluorescence; Hb, hemoglobin; SO_2, oxygen saturation; UV, ultraviolet.

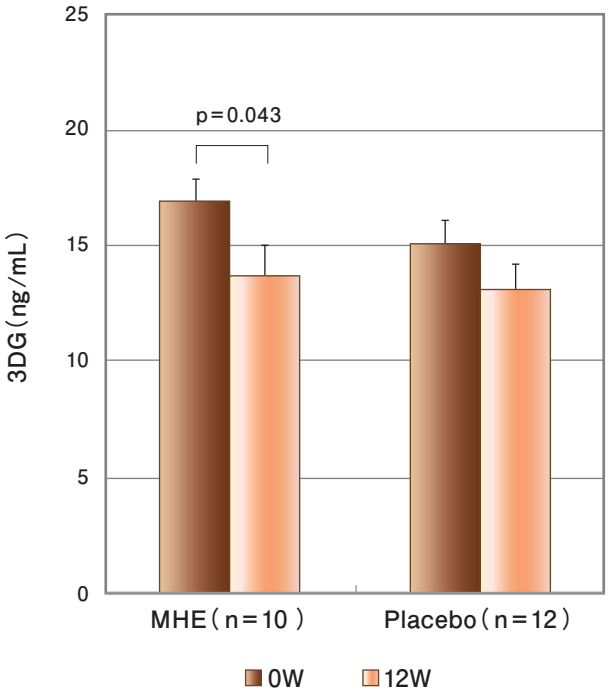

Fig. 2. **Changes in the blood 3DG levels (Healthy subjects).**
Measured value: Average ± standard error mean (SEM). Analysis: Dunnett (intra-group) and t-test (inter-group). 3DG, 3-deoxyglucosone; MHE, mixed herb extract.

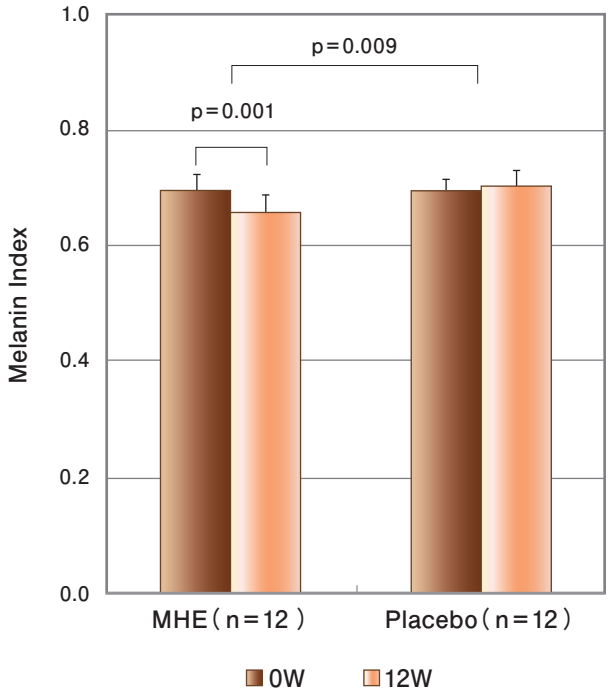

Fig. 3. **Changes in the skin (upper arm) Melanin Index.**
Measured value: Average ± standard error mean (SEM). Analysis: Dunnett (intra-group) and t-test (inter-group). MHE, mixed herb extract.

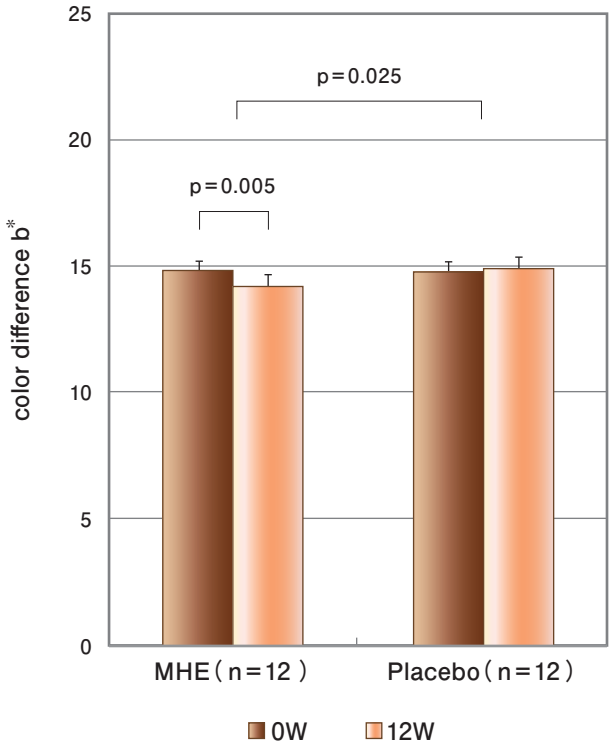

Fig. 4. **Changes in the skin (upper arm) color difference b* (yellow).**
Measured value: Average ± standard error mean (SEM). Analysis: Dunnett (intra-group) and t-test (inter-group). MHE, mixed herb extract.

3) *Brown spots* on skin (face)

Fig. 5 shows the changes in *brown spots* on the skin (face). There were significant reductions in the MHE group (before administration 48.39 ± 4.95 → 12th week 46.49 ± 5.31 ng/mL [$p = 0.005$]), whereas the placebo group did not show significant change (before administration 48.08 ± 6.62 → 12th week 47.35 ± 7.41 ng/mL [$p = 0.396$]). In an inter-group comparison, a significant difference in the change in amount between the two groups was not recognized ($p = 0.171$).

4) Hb SO_2 Index (complexion) in the skin (cheek)

Changes in Hb SO_2 Index in the subgroup of healthy subjects (excluding the 2 persons who showed HbA1c values higher than the standard range) are shown in ***Fig. 6***.

There were significant increases in the MHE group (before administration 52.70 ± 7.34 → 12th week 60.08 ± 6.68 ng/mL [$p < 0.001$]) as well as in the placebo group (before administration 54.45 ± 4.48 → 12th week 58.81 ± 4.15 ng/mL [$p < 0.001$]). In an inter-group comparison, the MHE group showed significantly higher changes in contrast to the placebo group ($p = 0.020$).

Hb SO_2 Index is one of the indices used to judge complexion.

Safety Endpoints

Harmful events that occurred during the trial period included *cold, fever, headache, sinusitis, (suspected) cystitis, vomiting, diarrhea, soft stool, stomachache, constipation and menstrual pain*. However, all events became mild or recovered during the administration period and therefore the physicians judged that there was no causal relationship with MHE.

Test parameters were included in the scope if there were changes opposite to improvement, regardless of whether they were efficacy endpoints or not.

For changes in individual subjects, parameter values that were not abnormal prior to administration but shifted into the deviation range after 12 weeks were white blood cell count, MCHC (mean corpuscular hemoglobin concentration), neutrophils, lymphocytes, LDH, ALP, γ-GTP, creatinine, triglyceride, total cholesterol, LDL cholesterol, K and Fe. However, these might have fortuitously crossed the borderline, or might have been just below the borderline within the standard range and had crossed over the threshold to fall into the deviation range due to a slight fluctuation. All were judged to be within the range of physical change.

Measured items identified to have significant changes from pre-administration to the 12th week within the MHE group or inter-group were the blood CML, LDH, ALP, and K with significant increases, and upper arm skin elasticity (R2) and Fe with significant decreases (***Table 5*** and ***Table 6***). However, either all were relatively small changes that fell within the standard range, or the change was smaller than that in the placebo group, or there was no significant difference within the subgroup of healthy subjects. Thus, these were not deemed to be problematic from the clinical perspective. For questionnaire items, *pessimism, easily breaking into a sweat,* and *make-up comes off more readily* showed significant improvement in the placebo group in contrast to the MHE group, but there was no deterioration in the MHE group. Therefore, this was deemed not to be problematic.

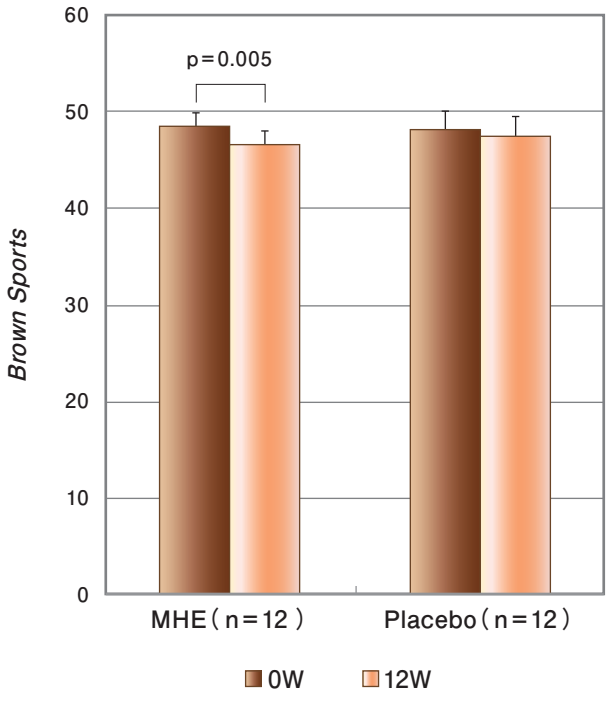

Fig. 5. **Changes in *Brown Spots* on the skin (face).**

Measured value: Average ± standard error mean (SEM). Analysis: Dunnett (intra-group) and t-test (inter-group). MHE, mixed herb extract.

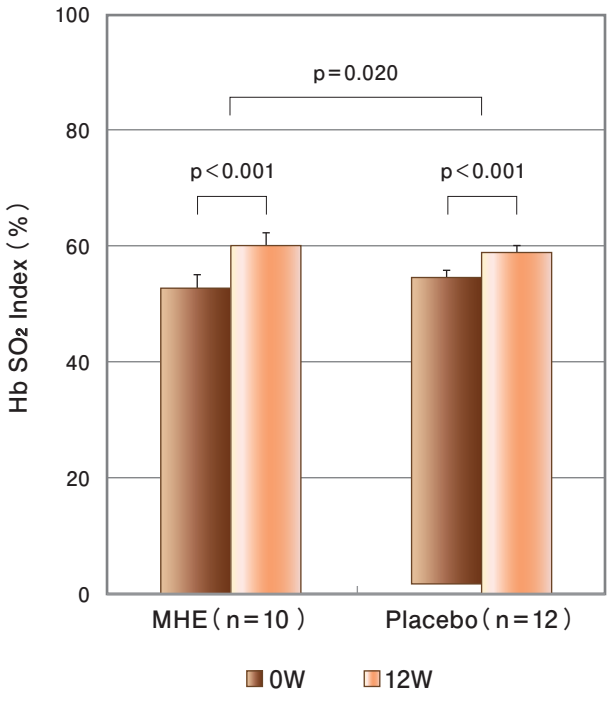

Fig. 6. **Changes in Hb SO_2 Index in the skin (cheek) (Healthy subjects).**

Measured value: Average ± standard error mean (SEM). Analysis: Dunnett (intra-group) and t-test (inter-group). MHE, mixed herb extract.

Discussion

The ingestion of food products containing MHE resulted in all cases in the suppression of yellowing or browning of the skin. Also, for the subgroup of healthy subjects (excluding the subjects suspected of having diabetes), there were significant reductions in blood 3DG after 12 weeks in contrast to pre-administration levels.

Prior research reported 3DG generation inhibition by MHE *in vitro* for MHE either as each individual herbs or a mixture of the four types [2]. Also, in an open study targeting type 2 diabetes at a dosage of 600 mg/day (six times the dosage in this study), significant reductions in blood 3DG were noted after 8 weeks and after 12 weeks in comparison to pre-administration levels [4]. Thus, it is likely that the dosage of 100 mg/day was not sufficient for subjects who have high blood glucose levels.

Significant differences were noted in the skin Melanin Index before and after administration and in contrast to the placebo group. The area measured is the inner part of the upper arm, which is normally not exposed and unlikely to be impacted by solar radiation, so according to the measurement principle, the value is deemed to include glycation end products. Therefore, it is possible that a reduction in glycation end products was expressed in the Melanin Index. Previous studies have reported that the presence of carbonyl-modified compounds due to UV radiation and glycation in the skin is related to the progressive yellowing of the skin [1,9].

On the other hand, a comparison of the subgroup of healthy subjects shows that complexion was significantly improved compared to the placebo group. The flavonoids and polyphenols in dokudami [10], hawthorne berries [11] and grape leaves [12] in the MHE have vasodilator and antioxidant properties, and thus these constituents were deemed to have acted to improve blood flow. Also, the result was the same as that for the previously reported MHE-containing vinegar beverage [6] and we surmise that the same action has occurred again in this study.

Conclusion

MHE suppressed yellowing and browning of the skin. This is possibly caused by a reduction in carbonylation due to suppression of 3DG generation. The improvement in complexion could be possibly attributed to the herb constituents. Additionally, a high degree of safety was confirmed, even with continuous ingestion.

Conflict of Interest Declaration

This study was funded by ARKRAY, Inc. and A-Kit, Inc. was consigned with the implementation.

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