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Original article

An exploratory clinical trial on the effects of mixed herb extract on the inhibition of glycative stress

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Abstract

***Objective*:** Accumulation of advanced glycation end products (AGEs) produced by glycative stress promotes the progression of age-related diseases. We conducted an exploratory study in humans using mixed herb extracts that have both enhancing action of oxidized protein hydrolase (OPH) activity in the body and AGEs crosslink cleaving activity.

***Method*:** The subjects were 24 women 40 years or older and less than 65 years of age with high levels of skin AGEs deposits. Three kinds of the following herbs were extracted in hot water: fenugreek (*Trigonella foenum-graecum*) seeds, fennel (*Foeniculum vulgare*) seeds, and hibiscus (*Hibiscus sabdariffa*) calyxes and bracts. Solid contents of the extracts and dextrin were mixed in a ratio of 6 : 4 to produce mixed herb extract (MHE) powder (brand name: Satonaceil). The test food was a food product in a capsule form. The daily intake of the test food was 100 mg/day for the L group (n = 12) and 300 mg/day for the H group (n = 12) as MHE. The ingestion period was 12 weeks. Before ingestion, and at 6 and 12 weeks post-ingestion, physical examination, blood test, urine test, skin-related tests, and a questionnaire were performed. Note that this study was conducted upon approval by an ethics review committee. (UMIN Clinical Trial ID: UMIN000025986).

***Results*:** Because two subjects violated the exclusion criteria after the start of the study, 11 subjects each were to be analyzed in the L group and the H group. Significant reductions were observed in the levels of pentosidine and fasting blood glucose in both groups at 12 weeks post-ingestion compared to those pre-ingestion. Significant reductions were also observed in the score of wrinkle (on the left and right) measured with VISIA in the L group and HOMA-IR in the H group. In addition, there was a tendency for 3-deoxyglucosone (3DG) to decrease in the L group and for carboxymethyl lysine (N^{ε}-(carboxymethyl)lysine: CML) to decrease in the H group. Further, significant reductions in several liver-function-related indices were observed. In the safety evaluation, no adverse events or abnormal variations in laboratory parameters attributable to the test food were observed.

***Conclusion*:** Effects of MHE ingestion on the reduction of AGEs seen in basic studies can be expected in humans, as demonstrated by the significant decrease or tendency to decrease of glycative-stress-related parameters. Furthermore, ingestion of this MHE was confirmed to be safe.

***KEY WORDS*:** mixed herb extract, anti-glycation, skin beautification, advanced glycation end products (AGEs), pentosidine

Introduction

Advanced glycation end products (AGEs) are involved in aging and age-related diseases such as skin aging, Alzheimer's disease, hypertension, arteriosclerosis, and osteoporosis [1)]. Potential methods of reducing AGEs that have already accumulated in the body include 1) improving the metabolism of the body, that is, enhancing oxidized protein hydrolase (OPH) in the body to promote the decomposition of AGE-modified protein, and 2) directly cleaving the AGE crosslink in the body [2)].

In a previous study, our group reported that hot water extracts of *Trigonella foenum-graecum* (fenugreek) seeds, *Foeniculum vulgare* (fennel) seeds, and *Hibiscus sabdariffa* (hibiscus) calyxes and bracts have both OPH activity enhancing action and AGEs crosslink cleaving activity [3)].

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Currently, we prepared mixed herb extracts using hot water extracts of fenugreek, fennel, and hibiscus (brand name: Satonaceil), and conducted an exploratory clinical trial.

Method

Subjects

The subjects for this study were Japanese women 40 years or older and less than 65 years of age publicly recruited as paid volunteers by TTC Co., Ltd. (Shinjuku-ku, Tokyo, Japan). Given that this was an exploratory study in humans, a target sample size of 10 in each group was deemed appropriate for statistical analysis. However, the sample size was set at 12 in the low-dose (L) group and 12 in the high-dose (H) group to account for possible drop-outs.

Screening tests were performed on the applicants. Among the applicants who passed the exclusion and the inclusion criteria, those with relatively higher levels of skin AGEs deposits (measured by AGE Reader) were selected. The group allocator created an allocation table using random numbers, and randomly allocated the selected 24 people into two groups of 12 each. The exclusion criteria were 31 items including 1) people with smoking habit, 2) those with average sleep time of less than 5 hours per night, 3) those with regular use of anti-glycation foods and anti-glycation cosmetics, and 4) those with regular use of medications, health foods, foods for specified health uses, and foods with function claims that may affect skin beautification. In addition, during the trial period, 11 restrictions that the subjects had to comply with were established, including restrictions on excessive exercise, dieting or overeating that greatly deviates from their daily life, keeping lifestyle habits such as sleeping and bathing consistent as much as possible, and maintaining their regular daily life.

Test Food

Fenugreek seeds, fennel seeds, and hibiscus calyxes and bracts were extracted in hot water to prepare MHE by mixing solid contents of the extracts and dextrin in a ratio of 6 : 4. The test food was a food product in a capsule form, containing MHE (100 mg/capsule). The test food composition and nutritional constituents are listed in ***Table 1*** and ***Table 2***, respectively.

Table 1. Test food composition (Compounded amount: mg/capsule).

Dextrin	143
Mixed herb extract (as Satonaceil)	100
Total	243

Table 2. Test food nutritional constituents (Administration amount per day).

		L group	H group
Energy	(kcal)	0.86	2.57
Protein	(g)	0.06	0.18
Lipid	(g)	0.00	0.00
Carbohydrate	(g)	0.15	0.46
Sodium	(mg)	0.44	1.31

Trial Design

The food product is in capsule form and is used as test food. The subjects in the L group ingested the test food 100 mg/day (1 capsule) and those in the H group 300 mg/day (3 capsules). The subjects, as a general rule, ingested the test food once a day on an empty stomach over 12 weeks. Physical examination, blood tests, urine tests, skin-related tests, body measurements, a questionnaire, and medical interviews were conducted prior to ingestion, and after 6 and 12 weeks of ingestion for a total of 3 times.

The subjects took a bath the day before the tests and went to bed by 12 : 00 am. Also, taking a shower or bath was prohibited on the day of testing. In addition, the subjects fasted, except for water, on the day prior to testing, from 10 : 00 p.m. until the end of testing on the following day. The time of their visit was set to the same time for every visit so that the time of the test would be the same every time (within ± 2 hours).

The study period was from January 2017 to May 2017. Esthetic evaluations and physical measurements were performed at the evaluation center of DRC Co. (Kita-ku, Osaka, Japan), Ltd. and blood draw at Ikiiki Clinic (Kita-ku, Osaka).

Endpoints

No primary outcomes or secondary outcomes were established as this was an exploratory effectiveness study.

For glycation-related endpoints, blood tests (pentosidine, 3-deoxyglucosone (3DG), and carboxymethyl lysine (N^{ε}-(carboxymethyl)lysine: CML) and skin-related tests (skin AGEs deposit levels, AGEs levels in the body, and stratum corneum CML) were performed.

For esthetic-related endpoints, skin-related tests (skin viscoelasticity, skin color difference, and imaging analysis of the skin) were performed.

For other efficacy endpoints, the following tests were performed: blood tests (total cholesterol, LDL cholesterol, HDL cholesterol, non-HDL cholesterol, triglyceride, free fatty acid, atherogenic index, glucose (GLU), HbA1c, insulin, HOMA-IR, AST (GOT), ALT (GPT), γ-GTP, ALP, LDH, total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, A/G ratio, creatinine, urea nitrogen, uric acid, Na, K, Cl, Ca, Fe), and a questionnaire (Anti-Aging QOL Common Questionnaire).

For safety endpoints, hematology tests (white blood cell counts, red blood cell counts, hemoglobin level, hematocrit level, platelet counts, and hemogram) and urine tests were performed.

For the body measurements, body weight, body mass index (BMI), body fat percentage, abdominal circumference, hip circumference, waist-hip ratio, systolic blood pressure, and diastolic blood pressure were measured.

The subjects recorded the ingestion status of the test

food, any adverse events, and any changes to lifestyle (diet, exercise, alcohol intake, etc.) in their journals.

Testing Method

Subjective Symptoms (Questionnaire Items)

The Anti-Aging QOL Common Questionnaire (AAQol)[4] was used for an evaluation of subjective symptoms. They were divided into 33 items for "physical symptoms" and 21 items for "mental symptoms." This evaluation was made on a five-point scale with the best condition being rated as 1 point and the worst condition being rated as 5 points.

Body Measurements and Physical Testing

In addition to height, body weight, blood pressure, and pulse rate, body fat percentage was measured via the bioelectrical impedance analysis, using Inbody 720 (Inbody Japan Inc., Koto-ku, Tokyo, Japan), a body composition analyzer.

Blood Testing

Among blood test parameters, glycation-related parameters such as pentosidine were measured at the Glycative Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University (Kyotanabe, Kyoto, Japan), and 3DG was measured at BML, Inc. (Shibuya-ku, Tokyo), while other parameters were measured at LSI Medience Corporation (Chiyoda-ku, Tokyo).

Skin-related Testing

The skin-related tests for measurements of AGEs in the body and imaging with VISIA were started after washing the measurement areas, and acclimatizing at room temperature for 10 minutes. Then, subjects were kept standby in a resting sitting position in an environmental room with constant temperature and humidity for 20 minutes (20°C to 22°C, 50 ± 5%). After acclimatization, skin color difference and skin viscoelasticity were measured in the environmental room, and measurement of skin AGEs deposit level and tape stripping to collect samples to measure stratum corneum CML were performed outside the environmental room.

1) Skin AGEs deposit levels

The skin AGEs deposit levels were measured using an AGE Reader (Diagnoptics Technologies B.V., Groningen, Netherlands). Following a previous report[5], the measurement area was the inner side of the upper arm (about 10 cm from the olecranon toward the upper arm).

2) AGEs in the body

An AGEs sensor (Air Water Biodesign Inc., Kobe, Hyogo, Japan) was used to measure AGEs in the body. Following the manual, the area for measurement was the middle finger of the left hand.

3) Skin viscoelasticity

Skin viscoelasticity was measured using a Cutometer MPA580 Dual (Courage + Khazaka electronic GmbH, Cologne, Germany), a skin viscoelasticity measurement device. The area for measurement was the inner side of the upper arm.

4) Stratum corneum CML

Following a previous report[6], the stratum corneum CML level was measured. An adhesive film was applied to the skin to collect the stratum corneum (tape stripping method). This test was conducted three times at the same site, and the samples were stored frozen for measuring CML level in the stratum corneum. The collection area was the inner side of the upper arm. The measurements were conducted at A-Kit, Inc. (Ogaki, Gifu, Japan).

5) Skin color difference

The skin color difference was measured using a CM-2600d, a spectrophotometer, and a CM-SA (Konica Minolta, Inc., Minato-ku, Tokyo), a skin analysis software package. The areas for measurement were the cheek and the inner side of the upper arm.

6) 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).

Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics V23 (IBM Japan, Ltd., Chuo-ku, Tokyo), a statistics software. At each time point post-ingestion, changes in test values from pre-ingestion in each group were analyzed using one-sample t-tests. For changes in test values from pre-ingestion to post-ingestion, the difference between the L group and the H group was analyzed using two-sample t-test. For the questionnaire, scores at each time point post-ingestion in each group were compared to those pre-ingestion using Wilcoxon signed-rank test. Also, at each time point post-ingestion, for changes in scores from pre-ingestion, the difference between the L group and the H group was analyzed using Mann-Whitney U test. For comparisons of values of the endpoints between pre-ingestion and post-ingestion in each group, Bonferroni method was used to adjust for multiplicity. The significance levels were considered to be significant at a risk rate of less than 5% and to have a tendency to decrease at less than 10% for all two-tailed tests.

Ethical Standards

The study complied with the ethical principles set forth in the Helsinki Declaration and Japan's Personal Information Protection Act, and was conducted in accordance with the Ethical Guidelines for Medical Research Involving Human Subjects (public notice of Ministry of Education, Culture, Sports, Science and Technology/Ministry of Health, Labour and Welfare). The documents for this study were submitted to the Aisei Hospital Ueno Clinic Research Ethics Committee (Taito-ku, Tokyo) in advance, and were examined and approved on January 12, 2017. 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 outline of this study was registered in the UMIN Clinical Trials Registry system (UMIN-CTR) (UMIN Clinical Trial ID: UMIN 000025986).

Results

Target Group for Analysis

Figure 1 shows the tracking flow diagram for trial subjects. Subject recruitment began in January 2017. Twenty-four subjects, who had been selected from the screening tests held on February 7, were randomly allocated to the L group of 12 subjects and to the H group of 12 subjects and participated in this study. Thereafter, one subject received a diagnosis of anemia, and could not undergo laboratory tests at 12-week testing. One subject developed pollinosis and used a rhinitis medication for a long time. The two subjects above were excluded from effectiveness analysis because they violated the exclusion criteria. Therefore, a total of 22 subjects, 11 in the L group and 11 in the H group, were included in the analysis. The characteristics of these subjects are shown in ***Table 3***.

Glycation-related indices

Results of glycation-related endpoints are shown in ***Table 4***. There were significant reductions in pentosidine levels in both groups at 12 weeks post-ingestion ($p = 0.022$ for the L group and $p = 0.011$ for the H group, ***Fig. 2***). A tendency to decrease was observed in 3DG level in the L group at 12 weeks post-ingestion ($p = 0.052$), and a significant reduction was observed in 3DG level in subgroup analysis of subjects with values outside the reference range (7 subjects with 3DG > 18.14 ng/mL) in the L group at 12 weeks post-ingestion ($p = 0.042$). There was a tendency for the CML level to decrease in the H group at 12 weeks post-ingestion ($p = 0.086$).

Esthetic-related indices

Results of esthetic-related endpoints are shown in ***Table 5***. Imaging diagnosis of the skin showed a significant improvement in wrinkles in the L group at 12 weeks post-ingestion ($p = 0.011$ on the left and $p = 0.011$ on the right, ***Fig. 3***). There was a tendency for the skin color difference (upper arm) b* (yellow) to decrease in the L group at 12 weeks post-ingestion ($p = 0.078$). There were significant reductions in color difference (cheek) a* (red) in both groups at 12 weeks post-ingestion ($p = 0.036$ for the L group and $p = 0.004$ for the H group).

Other Effectiveness Indices

Results of other effectiveness indices are shown in ***Table 6***.

1) Glycometabolism-related indices

There was a significant reduction in fasting blood glucose level in the L group at 12 weeks post-ingestion ($p = 0.024$), and in the H group at 12 weeks post-ingestion ($p = 0.016$, ***Fig. 4***). There was a significant decrease in HOMA-IR levels in the H group at 12 weeks post-ingestion ($p = 0.038$) and a tendency for the HOMA-IR levels to decrease in the L group at 12 weeks post-ingestion ($p = 0.080$, ***Fig. 5***). A tendency to decrease was shown in insulin levels in the H Group at 12 weeks post-ingestion ($p = 0.092$).

2) Liver-function-related indices

There was a significant reduction in AST level in the H group at 12 weeks post-ingestion ($p = 0.004$, ***Fig. 6***). There was a significant reduction in ALT level in the H group at 12 weeks post-ingestion ($p = 0.006$, ***Fig. 7***). A significant reduction was observed in γ-GTP level in a subgroup analysis of the H group excluding one subject with a value outside the reference range (γ-GTP > 100 U/L) at 12 weeks post-ingestion ($p = 0.011$). A significant reduction in ALP level was observed in the H group at 12 weeks post-ingestion ($p < 0.001$), and significant reductions were also seen in LDH level in the L group and the H group at 12 weeks post-ingestion ($p = 0.014$ for the L group and $p < 0.001$ for the H group).

Safety Endpoints

Among adverse events that occurred during the study period, no serious adverse events were reported.

All adverse events were "mild" except that the severity of one case of "iron deficiency anemia" in the L group was "moderate". In addition, the principal investigator judged that any of these adverse events were "unrelated" or "probably unrelated" to the test food. Thus, there were no adverse events attributable to the test food.

Discussion

1. Safety

Absence of serious adverse events attributable to the ingestion of MHE might be because each herb has been used in spices, Chinese medicines, and herbal teas from ancient times[7-9], as a food material with a long history of dietary use. Therefore, the MHE was considered to be completely safe as a food material.

2. Inhibitory action of glycative stress

By ingesting the test food, significant reductions were seen in pentosidine levels in the L group and the H group at 12 weeks post-dose compared to the level pre-ingestion. Also, there was a tendency for the 3DG levels to decrease in the L group at 12 weeks post-ingestion compared to the level pre-ingestion, and there was a tendency for the CML levels to decrease in the H group at 12 weeks post-dose compared to the level pre-ingestion.

It was suggested that these actions to reduce blood AGEs and glycation intermediates might be due to the effects of fenugreek, fennel, and hibiscus demonstrated in a previous study[3], that is, 1) enhancing OPH to promote the decomposition of AGE-modified protein, and 2) directly cleaving the AGE crosslink.

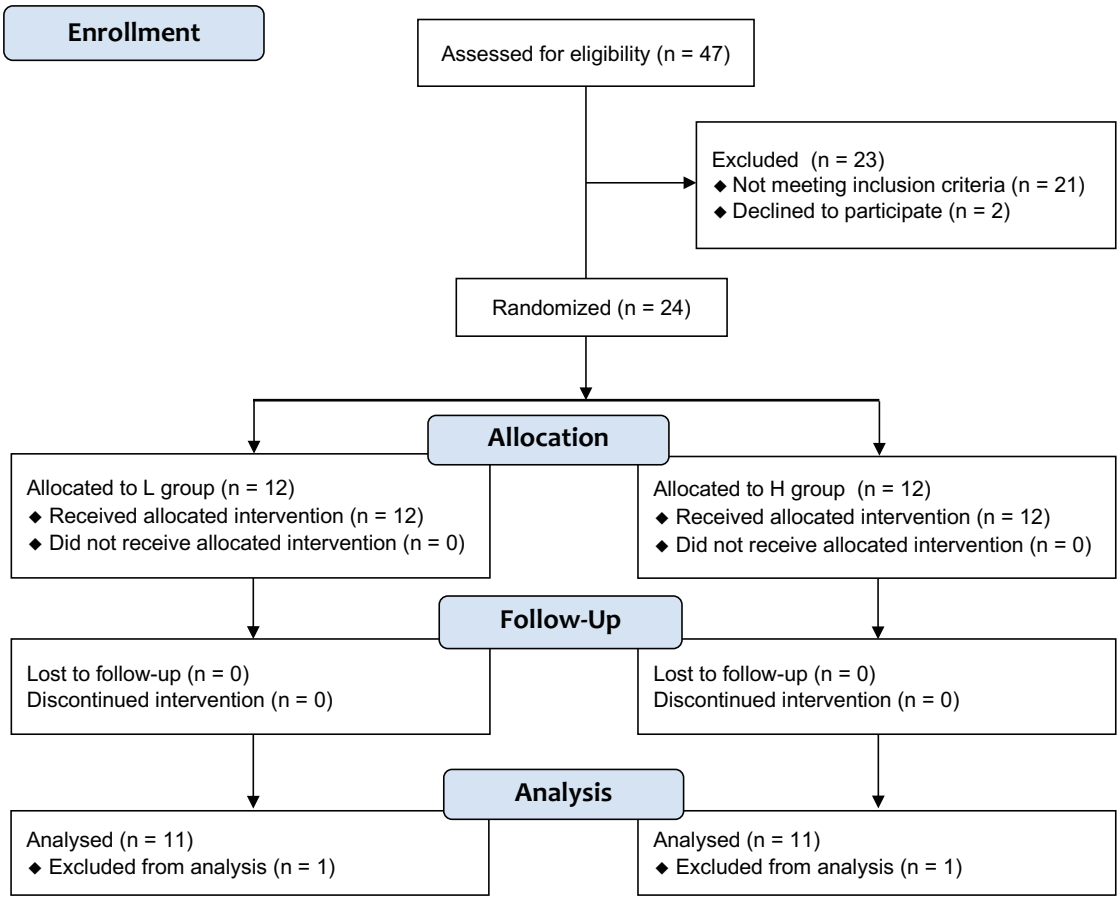

Fig. 1. **Tracking flow diagram for trial subjects.**

Table 3. Baseline demographic and clinical characteristics (Target group for analysis).

	Unit	Total	L group	H group	p-value
Target size		22	11	11	(t-test)
Age	years of age	47.5 ± 7.4	49.2 ± 8.6	45.8 ± 5.9	p = 0.300
Height	cm	156.3 ± 4.8	156.8 ± 5.3	155.8 ± 4.4	p = 0.629
Weight	kg	51.08 ± 7.77	50.91 ± 7.14	51.25 ± 8.70	p = 0.922
BMI	kg/m^2	20.84 ± 2.43	20.65 ± 2.22	21.03 ± 2.72	p = 0.719
Body fat	%	29.95 ± 3.49	30.88 ± 3.33	29.01 ± 3.55	p = 0.217
Systolic blood pressure	mmHg	116.1 ± 17.3	113.8 ± 13.3	118.4 ± 20.9	p = 0.550
Diastolic blood pressure	mmHg	70.6 ± 12.5	68.1 ± 8.5	73.2 ± 15.6	p = 0.353
Pulse rate	bpm	69.7 ± 11.8	69.6 ± 7.6	69.8 ± 15.3	p = 0.972
Skin AGEs deposit level		2.189 ± 0.191	2.206 ± 0.180	2.171 ± 0.209	p = 0.680

Measured values: Average ± standard deviation. BMI, body mass index; AGEs, advanced glycation end products.

Table 4. Glycation-related testing.

Parameter		Unit	Reference range	Group	0W	6W	12W
AGEs in blood	Pentosidine	ng/mL	9.15 - 4.31	L	3.75 ± 2.26	–	3.30 ± 2.39 #
				H	2.64 ± 0.24	–	2.17 ± 0.27 #
	3DG	ng/mL	3.76 - 18.14	L	19.47 ± 2.96	–	16.82 ± 2.43
				H	16.20 ± 4.15	–	15.42 ± 2.68
	CML	μg/mL	–	L	1.94 ± 0.26	–	1.85 ± 0.33
				H	2.67 ± 1.52	–	2.25 ± 1.12
Skin AGEs accumulation level	AF		–	L	2.21 ± 0.18	2.20 ± 0.21	2.18 ± 0.20
				H	2.17 ± 0.21	2.25 ± 0.24 ##	2.21 ± 0.21
	CML in stratum corneum	μg/mg protein	–	L	96.1 ± 31.4	–	81.6 ± 23.1
				H	93.3 ± 26.7	–	95.4 ± 38.3
Internal body AGEs level	AF		–	L	0.532 ± 0.067	0.559 ± 0.080	0.525 ± 0.073
				H	0.513 ± 0.072	0.554 ± 0.064 #	0.514 ± 0.048

Subjects: n = 11 (L group), n = 11 (H group). Values: Average ± standard deviation. * $p < 0.05$, ** $p < 0.01$: Comparison of two groups (t-test), # $p < 0.05$, ## $p < 0.01$: Comparison with before administration (Bonferroni (3 times), paired t-test (twice)). AGEs, advanced glycation end products; 3DG, 3-deoxyglucosone; CML, N^{ε}-(carboxymethyl)lysine; AF, skin autofluorescence.

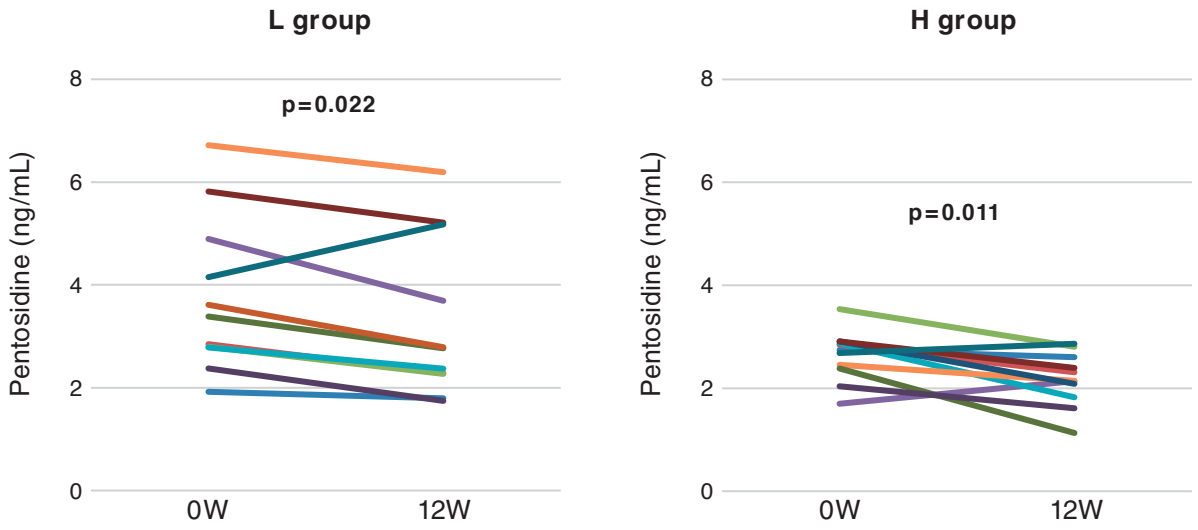

Fig. 2. **Changes in the blood pentsidine levels.**

a) L group, **b)** H group. Analysis: Paired t-test (twice).

Table 5. Beauty-related testing.

Parameter		Unit	Group	0W	6W	12W
Imaging analysis by VISIA	Skin wrinkles (left cheek)		L	0.134 ± 0.128	–	0.105 ± 0.113 #
			H	0.118 ± 0.147	–	0.085 ± 0.079
	Skin wrinkles (right cheek)		L	0.144 ± 0.106	–	0.112 ± 0.072 #
			H	0.136 ± 0.148	–	0.110 ± 0.106
Skin viscoelasticity (Cheek)	R2		L	0.815 ± 0.055	0.823 ± 0.046	0.826 ± 0.062
			H	0.835 ± 0.037	0.830 ± 0.043	0.829 ± 0.038
	R7		L	0.533 ± 0.070	0.527 ± 0.059	0.538 ± 0.064
			H	0.557 ± 0.050	0.547 ± 0.059	0.552 ± 0.049
Color difference (Cheek)	L*		L	66.46 ± 2.71	66.55 ± 2.81	66.41 ± 2.66
			H	65.01 ± 2.72	65.85 ± 2.07	66.15 ± 1.65
	a*		L	10.91 ± 2.29	10.32 ± 1.89	9.76 ± 1.56 #
			H	12.72 ± 3.19	11.14 ± 2.66 #	10.56 ± 2.67 ##
	b*		L	17.33 ± 2.06	17.06 ± 2.14	17.47 ± 2.06
			H	15.62 ± 3.28	16.58 ± 3.05	16.75 ± 2.71
	Melanin Index		L	1.052 ± 0.164	1.035 ± 0.202	1.055 ± 0.203
			H	1.054 ± 0.104	1.050 ± 0.136	1.049 ± 0.117
	Hb SO_2 Index	%	L	62.11 ± 8.38	59.72 ± 8.69	57.90 ± 7.50 #
			H	56.65 ± 7.63	57.97 ± 8.40	55.25 ± 5.67
Color difference (Upper arm)	L*		L	69.90 ± 1.78	69.77 ± 1.96	69.99 ± 1.93
			H	70.36 ± 1.62	70.35 ± 1.36	70.57 ± 1.53
	a*		L	5.61 ± 1.00	5.96 ± 1.34	5.78 ± 1.28
			H	5.75 ± 1.34	5.85 ± 0.97	5.76 ± 1.22
	b*		L	16.08 ± 1.79	15.73 ± 1.37	15.56 ± 1.65
			H	15.99 ± 1.67	15.58 ± 1.76	15.67 ± 1.58
	Melanin Index		L	0.802 ± 0.156	0.802 ± 0.146	0.792 ± 0.159
			H	0.794 ± 0.121	0.787 ± 0.125	0.774 ± 0.127
	Hb SO_2 Index	%	L	62.95 ± 7.12	57.77 ± 9.01 #	62.98 ± 10.94
			H	59.86 ± 7.20	57.55 ± 5.79	57.42 ± 8.35

Subjects: n = 11 (L group), n = 11 (H group). Values: Average ± standard deviation. * $p < 0.05$, ** $p < 0.01$: Comparison of two groups (t-test). # $p < 0.05$, ## $p < 0.01$: Comparison with before administration (Bonferroni (3 times), paired t-test (twice)). Hb, hemoglobin; SO_2, oxygen saturation.

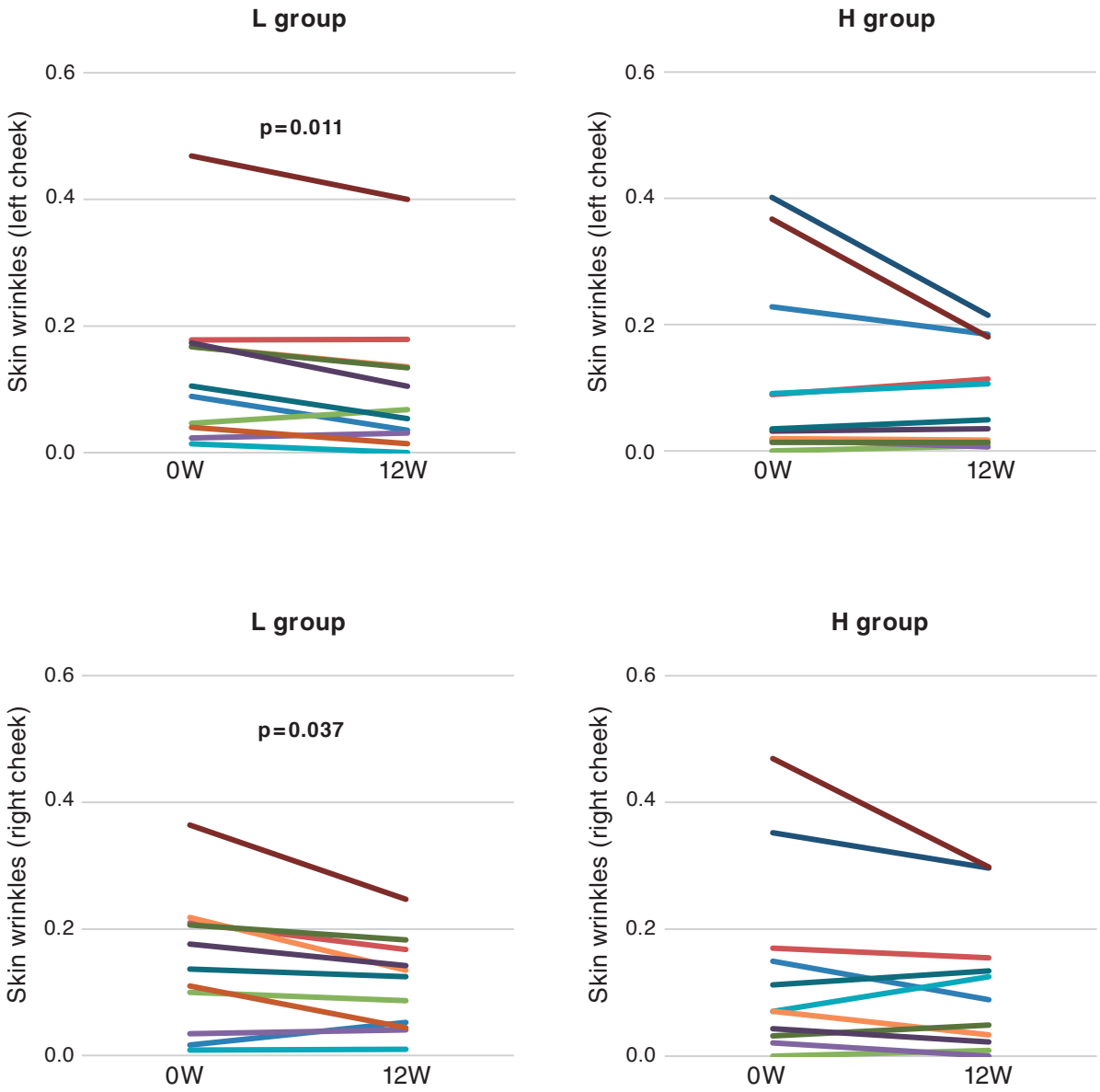

Fig. 3. **Changes in the skin wrinkles.**

a) L group, left cheek, **b)** H group, right cheek, **c)** L group, right cheek, **d)** H group, right cheek. Analysis: Paired t-test (twice).

Table 6. Other outcomes.

Parameter		Unit	Reference range	Group	0W	6W	12W
Glucose metabolism	Glucose	mg/dL	70 - 109	L	83.0 ± 5.9	79.0 ± 5.1	76.4 ± 5.0 #
				H	78.9 ± 8.0	77.7 ± 5.9	74.3 ± 4.6 #
	HOMA-IR		1.6 or below	L	0.920 ± 0.575	0.793 ± 0.230	0.589 ± 0.213
				H	0.793 ± 0.457	0.730 ± 0.385	0.557 ± 0.351 #
	Insulin	μU/mL	1.7 - 10.4	L	4.42 ± 2.63	4.06 ± 1.16	3.11 ± 1.11
				H	4.08 ± 2.27	3.72 ± 1.76	3.00 ± 1.76
Liver function	AST (GOT)	U/L	10 - 40	L	20.3 ± 3.4	21.7 ± 4.4	18.9 ± 3.2
				H	19.6 ± 5.3	18.5 ± 3.9	16.7 ± 4.2 ##
	ALT (GPT)	U/L	5 - 45	L	15.8 ± 3.5	18.4 ± 9.8	15.6 ± 3.9
				H	14.9 ± 6.4	11.4 ± 4.8	10.8 ± 3.3 ##
	γ-GTP	U/L	30 or below	L	18.5 ± 5.4	25.1 ± 26.8	17.5 ± 8.1
				H	24.5 ± 35.2	15.5 ± 10.0	14.1 ± 8.0
	ALP	U/L	100 - 325	L	181 ± 28	179 ± 38	173 ± 33
				H	202 ± 65	194 ± 50	181 ± 57 ##
	LDH	U/L	120 - 240	L	189 ± 24	181 ± 21	176 ± 24 #
				H	164 ± 18	157 ± 16	153 ± 17 ##

Subjects: n = 11 (L group), n = 11 (H group). Values: Average ± standard deviation. * $p < 0.05$, ** $p < 0.01$: Comparison of two groups (t-test). # $p < 0.05$, ## $p < 0.01$: Comparison with before administration (Bonferroni (3 times), paired t-test (twice). HOMA-IR, homeostasis model assessment for insulin resistance; AST (GOT), aspartate aminotransferase (glutamic oxaloacetic transaminase); ALT (GPT), alanine aminotransferase (glutamic pyruvic transaminase); γ-GTP, γ-glutamyltransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

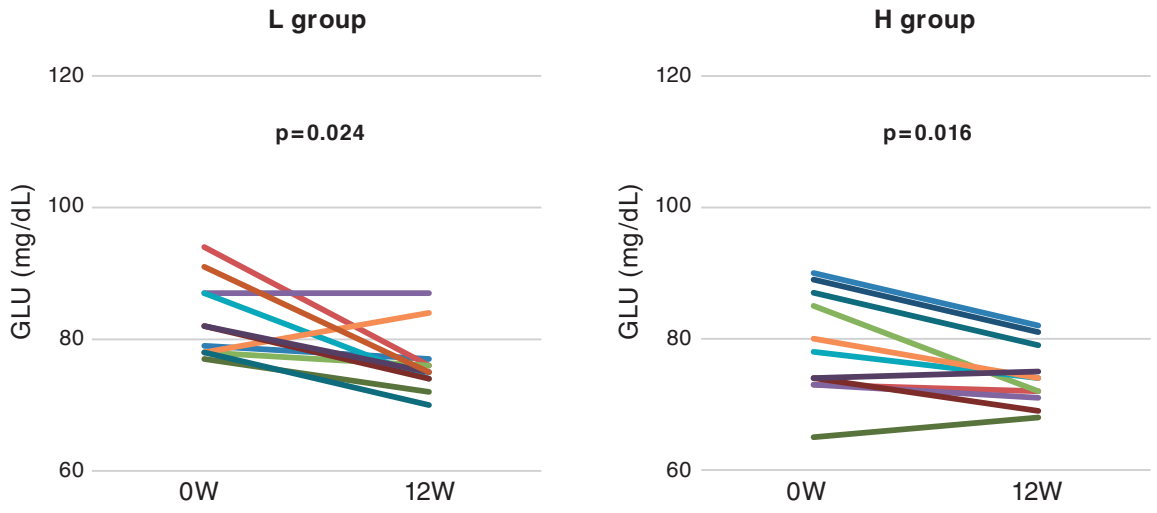

Fig. 4. **Changes in the blood glucose levels.**

a) L group, **b)** H group. Analysis: Bonferroni (3 times). GLU, glucose.

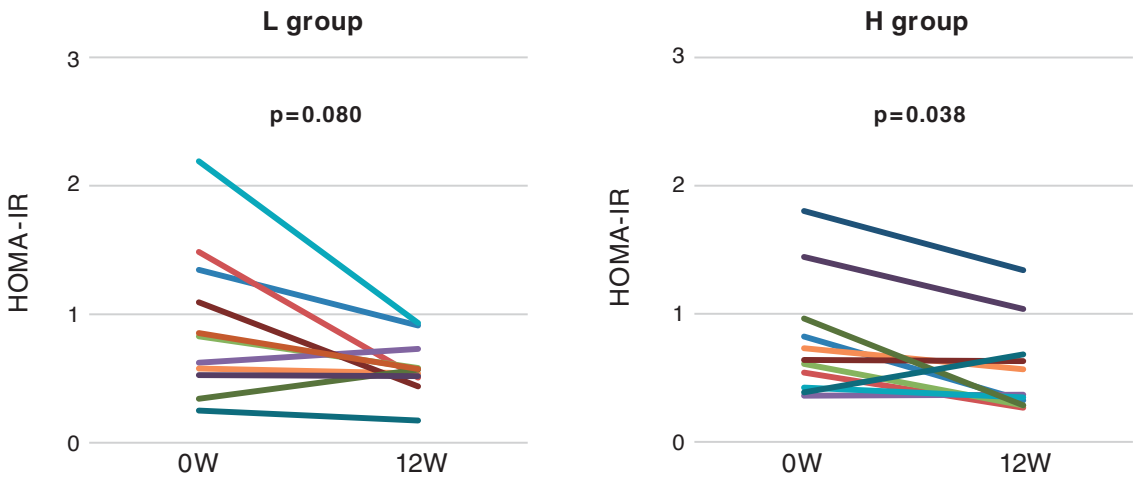

Fig. 5. **Changes in HOMA-IR levels.**

a) L group, **b)** H group. Analysis: Bonferroni (3 times). HOMA-IR, homeostasis model assessment for insulin resistance.

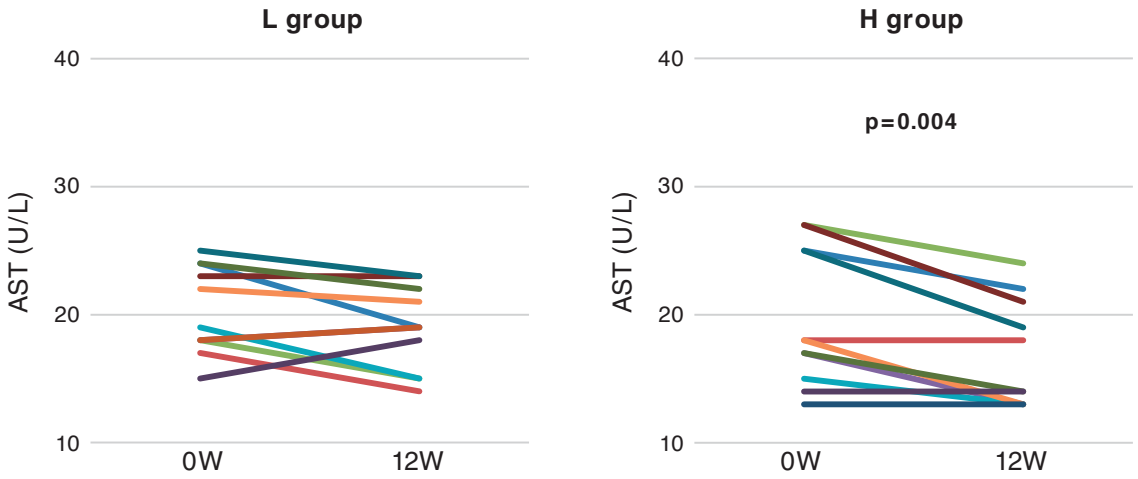

Fig. 6. **Changes in AST levels.**

a) L group, **b)** H group. Analysis: Bonferroni (3 times). AST, aspartate aminotransferase.

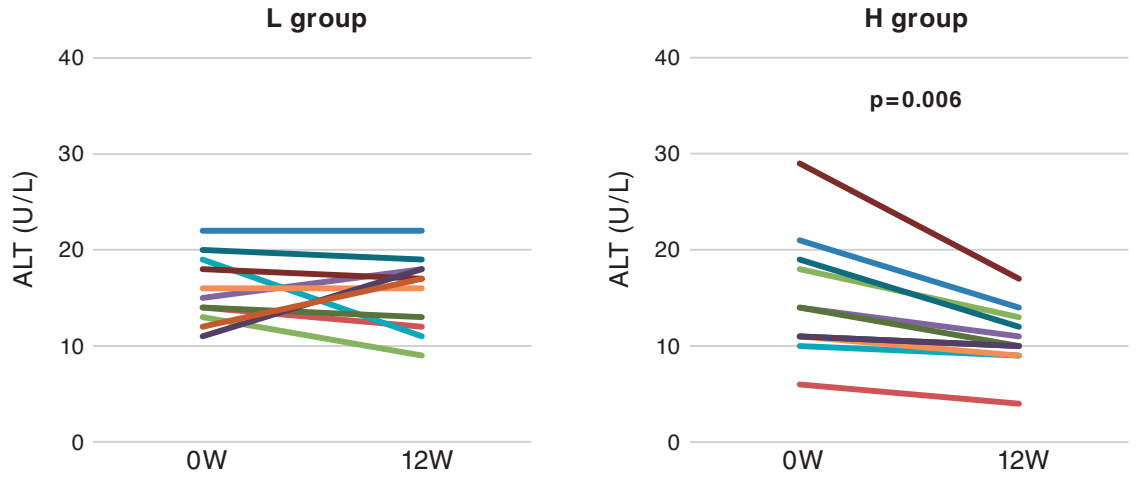

Fig. 7. **Changes in ALT levels.**

a) L group, **b)** H group. Analysis: Bonferroni (3 times). ALT, alanine aminotransferase.

In addition, there were significant reductions in the scores of wrinkles on the left and right measured via imaging diagnosis of the skin in the L group at 12 weeks post-ingestion compared to the score pre-ingestion in this study. There was a tendency for the skin color difference (upper arm) b* (yellow) to decrease in the L group. The effects of glycation on the skin has been reported; for example, glycation of collagen and other proteins in the skin are involved in reduction in skin elasticity and formation of wrinkles [10], and carbonylation of skin proteins can cause yellowing of the skin [1]. Therefore, it is suggested that improvement in wrinkles and reduction of skin yellowing by ingesting MHE may be due to its contribution of inhibitory actions on AGEs and glycation intermediates in the body. As for color difference (cheek) a* (red), further evaluation is warranted because the possibility of seasonal changes cannot be excluded.

On the other hand, changes by MHE ingestion were seen in glycometabolism-related indices (fasting blood glucose and HOMA-IR) and in liver-function-related indices (AST, ALT, and others). The effect of fenugreek seeds contained in the MHE to decrease blood glucose level has been reported in a clinical study in humans [11], and the effect to improve liver function has been reported in a study in rats [12], and the effectiveness in humans has also been suggested. These ingredients may have acted to cause changes in glycometabolism and liver function. Improvement in glycometabolism, in particular, is expected to reduce the risk of glycation in the body.

Conclusion

Because significant reductions or a tendency to decrease were achieved in glycative-stress-related indices (pentosidine, 3DG, and CML) by MHE ingestion, its action to reduce AGEs, as demonstrated in basic studies using fennel, fenugreek, and hibiscus, may be exerted in humans. Additionally, improvements in esthetic-related parameters in this study may be due to the contribution of these actions. Thus, the findings of this study suggest that ingestion of MHE may reduce AGEs in humans who have already accumulated AGEs in the body due to glycative stress, and have potential application in the esthetic field. Furthermore, ingestion of this MHE was confirmed to be safe.

Conflict of Interest Declaration

This study was funded by Arkray, Inc. and properly conducted under the management of TTC Co., Ltd. Dr. Sumio Kondo, who is affiliated with Medical Corporation Kenshokai Fukushima Healthcare center, is the principal investigator for this study and performed the contracted work, and has no conflicts of interest to disclose.

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