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Original article

An open-label clinical trial of *Geranium dielsianum* extract administered for 12 weeks: Anti-glycative actions, skin quality, and intestinal environment

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Abstract

***Objectives*:** Numerous *in vitro* and *in vivo* studies of the functionality of *Geranium dielsianum* extract (GDE: MISKAMISKA™) have found many novel functions, including improvements in the intestinal environment. The present study was conducted to evaluate the effects of GDE in preventing glycation, maintaining beautiful skin, and improving the intestinal environment in healthy women, and to verify its safety in an uncontrolled open-label clinical trial using a test material based on GDE.

***Methods*:** Thirty-three women, mainly those aged 30 to <50 years who had a constipation tendency and rough skin problems, and preferred eating sweets and carbohydrates in their daily diet, were recruited. From among these 33, the 13 women having the highest values of the glycative stress index skin AGE deposition (Auto Fluorescence: AF) as determined using the AGE Reader® (DiagnOptics), were selected. One woman dropped out of the study; the remaining 12 women (mean age: 41.5 ± 5.0 years) received the test material for 12 weeks. The daily consumption of the primary ingredients of the test material was 1,200 mg of GDE and 800 mg of each crystalline cellulose and calcium stearate. Before taking the test material (Week 0), and at Weeks 8 and 12 of its ingestion, blood chemistry, a lifestyle survey using the Anti-Aging QOL Common Questionnaire (AAQOL), a questionnaire-based survey on the skin, and a questionnaire-based survey on bowel movement were performed. Skin AF was determined as a glycative stress index. As dermatological endpoints, skin elasticity (R2, R7), water content of the stratum corneum, and transepidermal water loss were measured using the Cutometer (Courage & Khazaka), Corneometer, and Tewameter, respectively. Blood oxidative stress (bOS) and blood antioxidant power (bAP) in plasma samples were measured as oxidative stress indexes using Spotchem (Arklay).

***Results*:** The skin questionnaire survey revealed that the subjective symptom items 'Dry skin', 'Make-up becomes runny', 'Dull skin', and 'Skin smoothness' improved significantly ($p<0.05$) at Week 12 of ingestion of the test material. 'Skin moisture' improved significantly ($p<0.05$) at Weeks 8 and 12 of ingestion. The AAQOL survey showed that 'Constipation' symptoms were significantly relieved, and the defecation frequency began to increase significantly at Week 1 of ingestion, and continued to increase up to Week 12 ($p<0.001$). Blood chemistry showed that HDL cholesterol increased significantly after ingestion of the test material (baseline value, 66.3 ± 14.7 mg/dL; 7.8%, $p=0.034$), and that blood oxidative stress (bOS), an oxidative stress index, did not change, but another index, blood antioxidant power (bAP), increased significantly (14.5%, $p=0.013$). Skin moisture retention test revealed a significant improvement (14.0%, $p=0.040$) in the water content of the stratum corneum in the right upper arm at Week 12 of ingestion, and a significant reduction in transepidermal water loss in the right upper arm at Week 8 of ingestion (−18.9%, $p=0.020$) and at Week 12 (−25.3%, $p=0.002$).

Subclass analysis was performed in two groups according to pre-ingestion skin AF: those with a relatively high value of ≥2.19 (median) and those with a relatively low value of <2.19. In the subjects with higher skin AF values, the AF value and fasting blood glucose did not change, but HbA1c decreased significantly at Week 12 (baseline value, 5.45 ± 0.22; −2.2%, $p=0.034$). Supplementary analysis was performed to determine the relationship between skin AF value and actual age. The AF value from actual age was calculated. In 3 of the 4 subjects with a skin AF value of ≥25% higher than the baseline actual age, the AF value decreased at Week 12. No serious adverse events were found during or after the end of the study period.

***Conclusion*:** The 12-week uncontrolled open-label clinical trial with the GDE-based test material demonstrated bowel movement improvement and skin moisture-retaining effects in support of the previously reported *in vitro* and *in vivo* studies. No definite data demonstrating the anti-glycation action of GDE were obtained in the present study. However, since *in vitro* studies revealed potent anti-glycation action and carbohydrate absorption suppressing action, and since supplementary analysis of selected subjects with high age-corrected AF values revealed AF value reductions, the anti-glycation action of GDE is expected to be demonstrated through a double-blind study with limited subject conditions in the future. The safety of ingested GDE in humans was established.

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KEY WORDS: *Geranium dielsianum*, oxidative stress, glycative stress, constipation, moisturizing action

Introduction

A number of plants belonging to the family *Geraniaceae*, such as *Geranium dielsianum*, *Geranium lecheri* Knuya, and *Geranium ayabacense* Wild ex HBK, are native to the highlands in the Central Andean Mountains in Peru. In particular, *Geranium dielsianum* (GD) is reportedly indigenous exclusively to the Peruvian Andes as its only habitat in the world, and has traditionally been recognized as being highly effective against diabetes mellitus [1,2]. In Peru, GD is infused to make a popular herbal tea.

To elucidate the useful bioactivities of GD, we have conducted chemical and physiological investigations using MISKAMISKA™, a *Geranium dielsianum* extract powder (GDE). To date the functionality of GDE has been studied with a focus on *in vitro* or *in vivo* experimentation [3,4], with few human studies reported; therefore data to clarify the bioactivities of *Geranium dielsianum* is lacking.

The present study was conducted as an uncontrolled open-label clinical trial in women who had a constipation tendency and rough skin problems, and preferred eating sweets, to evaluate the anti-glycation effect, skin-conditioning effect, and intestinal environment-improving effect of GDE taken for 12 consecutive weeks.

Methods

Subjects

Thirty-three Japanese healthy women aged 30 to < 50 years meeting all the following three criteria were recruited: i) preferring eating sweets and carbohydrates, ii) suffering constipation, and iii) having rough skin problems. From among them, 13 women with large amounts of advanced glycation end products (AGEs) deposited in the skin [5] as determined using the AGE Reader® (DiagnOptics, Groningen, The Netherlands), were enrolled in the study. One subject experienced cystitis during the study period, and was excluded from the analysis set at the investigator's discretion, leaving 12 subjects for data analysis [age, 41.50 ± 5.02 years; height, 158.38 ± 2.92 cm; body weight, 54.94 ± 10.32 kg; body mass index (BMI), 21.95 ± 4.34].

Study design

The present study was conducted as an uncontrolled open-label clinical trial. The test material was a tablet formulation containing 150 mg of *Geranium dielsianum* extract powder (GDE). Each subject took 8 tablets every day (GDE dose of 1,200 mg/day) in two divided portions (4 tablets taken after breakfast and 4 tablets after dinner) for 12 weeks. Even when no food was taken, each subject took the test material alone. The test material was supplied by Towa Corporation (Tokyo, Japan).

Subjective symptoms were checked, and somatometry, blood chemistry, and oxidative stress tests were performed before and at 8 and 12 weeks after the start of the study. Each subject recorded in the life diary the presence/absence and severity of adverse events, test material ingestion status, lifestyle habits, and dietary and exercise habits during the study period from January 8, 2015 to April 27, 2015.

Properties of the test material

The nutritive components and composition of the test material are shown in ***Tables 1*** and ***2***.

Table 1. Nutrition information of the test material per 8 tablets.

Item	Amount
Calorie	7.96 Kcal
Protein	14.0 mg
Lipid	56.0 mg
Carbohydrate	1.85 mg
Sodium	0.06 mg

Table 2. The composition of the test materials per 8 tablets.

Use		Amount
Active ingredient	*Geranium dielsianum* extract (GDE)	1,200 mg
Excipient	Crystalline cellulose, calcium stearate	800 mg
Total		2,000 mg

Endpoints

(1) Subjective symptoms

Subjective symptoms were evaluated in two categories: physical symptoms and mental symptoms. Their scores were rated according to five grades (points 1 to 5) as previously reported [6-9] using the Anti-Aging QOL Common Questionnaire (AAQOL). A questionnaire-based survey on skin symptoms was performed using a previously reported questionnaire form (24 items) [8]. The number of defecations per day was calculated from the records in their diary.

(2) Somatometry

Somatometry was performed for height (cm), body weight (kg), body fat ratio (%), body mass index (BMI), basal metabolic rate (kcal), systolic and diastolic blood pressure (mmHg), and pulse rate (/min). The body composition was determined using a body composition analyzer (WELL-SCAN500; Canon Lifecare Solutions Inc., Tokyo, Japan).

(3) Blood chemistry

The following hematology parameters were measured: white blood cell count (WBC) (/μL), red blood cell count (RBC) (x 10^4/μL), hemoglobin (Hb) (g/dL), hematocrit (Hct) (%), platelet count (Plt) (x 10^4/μL), MCV (fL), MCH (pg), MCHC (%), and differential WBC counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils: %). Blood chemistry parameters consisted of total protein (g/dL), albumin (g/dL), A/G ratio, CPK (IU/L), AST (GOT, IU/L), ALT (GPT, IU/L), LDH (IU/L), ALP (IU/L), γ-GTP (IU/L), creatinine (mg/dL), uric acid (mg/dL), blood urea nitrogen BUN (mg/dL), fasting blood glucose (mg/dL), HbA1c [NGSP] (%), total cholesterol (TC) (mg/dL), HDL cholesterol (HDL-C) (mg/dL), LDL cholesterol (LDL-C) (mg/dL), triglycerides (TG) (mg/dL), serum electrolytes (Na, K, Cl (mEq/L), Ca (mg/dL), Fe (μg/dL)), and total bilirubin (mg/dL). Blood chemistry testing was performed by LSI Medience Corporation (Tokyo, Japan).

(4) Evaluation of glycative stress and skin functions

The measurements shown below were started after cleaning the measuring sites of the body following 20-minute acclimation in a constant-temperature constant-humidity room (21 ± 1°C, 50 ± 5%). Tests were performed at A-KIT Corporation (Ibaraki, Osaka, Japan).

a) Amount of AGEs deposited on the skin (glycative stress index)

As a glycative stress index, AGE-derived fluorescence intensity was measured three times at one site of the body using the AGE Reader® [5] as previously reported [8,9]. The mean skin auto fluorescence (AF) value was adopted as the test value. The measuring site of the body was the medial part of the right upper arm.

b) Water content of the stratum corneum

The water content of the stratum corneum was measured five times at the center of each measuring site of the body using the Corneometer (CM825; Courage+khazaka, Cologne, Germany) [11,12] as previously reported [9,10]. The mean of three measurements (excluding the data of the maximum and minimum values, was adopted as the test value. The measuring sites of the body were the left cheek (midpoint between the earlobe and lip margin) and the medial part of the right upper arm (approx. 10 cm from the olecranon toward the shoulder).

c) Transepidermal water loss

Transepidermal water loss was measured five times at the center of each measuring site of the body using the Tewameter (TM300; Courage+khazaka) [13] as previously reported [9,10]. The mean of three measurements (excluding the data of the maximum and minimum values) was adopted as the test value. The measuring sites of the body were the same as stated above in b), the left cheek and the right upper arm.

d) Measurement of color differences

Color differences were measured five times at each measuring site of the body using a spectrophotometer (CM-600d; Konica Minolta, Inc., Osaka, Japan) as previously reported [8,10]. The mean of three measurements (excluding the data of the maximum and minimum L^* values) was adopted as the test value of each parameter. The evaluation parameters consisted of Melanin Index, Hb Index, $HbSO_2$ Index, and L^*, a^*, and b^* values. The measuring sites of the body were the same as stated above, the left cheek and the right upper arm.

e) Measurement of skin viscoelasticity

Skin viscoelasticity was measured five times around each measuring site of the body using a single suction method with a Cutometer (CT580; Courage+khazaka) [14] as previously reported [8-10]. The mean of three measurements (excluding the data of the maximum and minimum R2 values) was adopted as the test value of each parameter. The evaluation parameters consisted of R2 and R7. The measuring sites of the body were the same as stated above, the left cheek and the right upper arm.

f) Diagnostic imaging of the skin

Image analysis was performed using VISIA (VISIA® Evolution; Canfield Scientific, Fairfield, NJ, USA), as previously reported [10] to obtain data on pores, freckles, and other findings. Data for the left face were recorded.

(5) Evaluation of oxidative stress

Physical oxidative stress was evaluated on the basis of measurements of blood oxidative stress (bOS) and blood antioxidant power (bAP) using the i-Pack Oxystress Test (Spotchem; Arkray, Kyoto, Japan) [15]. The correlation between bOS and derivatives of reactive oxygen metabolite (d-ROM) was determined to be r=0.973, and the correlation between bAP and biological antioxidant potential (BAP) to be r=0.975 [16].

• Statistical analyses

Data were statistically analyzed by performing paired-t test, two-way repeated measure ANOVA, or Dunnett's test using the Dr.SPSSII statistical analysis software (IBM SPSS Japan, Tokyo, Japan). A significance level of ≤5% was considered to indicate a significant difference.

• Ethical review

After the ethics and rationality of the study were reviewed and approved at a human study ethics committee meeting by the Ethics Committee of Tokyo Synergy Clinic (Chuo-ku, Tokyo, Japan), the present study was started and implemented in accordance with the study protocol approved thereby.

Results

Subjective symptoms

The score improved significantly for 3 of the 32 items of physical symptoms in the Anti-Aging QOL Common Questionnaire: 'Lethargy', 'Skin problems', and 'Constipation' (***Table 3***). The score did not change significantly in any of the 21 items of mental symptoms.

The Week-12 score improved significantly for 5 of the 24 items of skin symptoms: 'Dry skin', 'Make-up becomes runny, 'Dull skin', 'Skin smoothness', and 'Skin moisture' ($p<0.05$) (***Table 4***). The score tended to improve for the items 'Concerned about pores', 'Rough skin', 'Corners of eyes sagging', and 'Make-up does not stay on' ($p<0.01$).

Defecation frequency analysis results are shown in ***Table 5***. The defecation frequency began to increase significantly at Week 1 of GDE ingestion, and this increase continued up to Week 12 ($p<0.001$).

• Somatometry and physical test results

Results of somatometry and physical test (body composition) are shown in ***Table 6***. Blood pressure rose significantly after ingestion of the test material. All of the changes in systolic (6.2%, $p<0.001$) and diastolic blood pressure (9.1%, $p<0.001$) fell within the respective ranges of reference values. No significant change was found in any other parameter.

• Blood chemistry

Data analysis results for blood chemistry are shown in ***Table 7***. HDL cholesterol increased significantly at Week 12 of ingestion (7.8%, $p=0.034$). Although significant changes were also found in white blood cell count (8.8%, $p=0.022$), monocyte count (−18.1%, $p=0.019$), and fasting blood glucose (5.3%, $p=0.040$), all these changes fell within the respective ranges of reference values.

• Evaluation of skin functions (*Table 8*)

The color difference test revealed significant changes in L^* (baseline value, 70.22 ± 3.55; 1.3%, $p=0.014$) and a^* (baseline value, 5.88 ± 1.52; −8.8%, $p<0.050$) at Week 12, with no changes found in any other parameters.

Skin elasticity test revealed no significant change in the upper arm. In the cheek, however, the R2 (baseline value, 0.80 ± 0.04; −5.0%, $p<0.001$) and R7 (baseline value, 0.45 ± 0.06; −6.7%, $p=0.027$) were significantly decreased at Week 12.

Table 3. Physical symptoms by AAQOL.

	0W	8W	12W	p values	
				8W	12W
Lethargy	2.67 ± 1.15	2.08 ± 0.90	1.50 ± 0.52	0.581	**0.021**
Skin problems	2.83 ± 0.72	2.25 ± 0.62	1.83 ± 0.72	0.798	**0.043**
Constipation	3.50 ± 0.80	2.50 ± 0.80	2.25 ± 0.97	0.239	**0.034**

Anti-Aging QOL Common Questionnaire: (AAQOL) [6-9] is used and results are expressed as mean ± standard deviation, Dunnett's test vs. 0W, n = 12.

Table 4. Skin symptoms.

	0W	8W	12W	p values	
				8W	12W
Concerned about pores	3.25 ± 1.06	2.50 ± 0.90	2.42 ± 0.90	0.106	0.055
Dry skin	3.25 ± 1.14	2.50 ± 1.09	2.08 ± 0.90	0.197	**0.005**
Make-up becomes runny	3.17 ± 1.11	2.50 ± 1.00	2.17 ± 0.83	0.683	**0.027**
Rough skin	3.08 ± 0.90	2.25 ± 0.75	2.00 ± 0.74	0.239	0.055
Corners of eyes sagging	2.67 ± 1.07	1.92 ± 0.67	1.75 ± 0.75	0.239	0.086
Dull skin	3.08 ± 1.08	2.33 ± 0.78	2.00 ± 0.85	0.347	**0.012**
Make-up does not stay on	2.67 ± 1.07	2.08 ± 0.90	1.75 ± 0.75	1.000	0.086
Skin smoothness	3.58 ± 1.16	2.75 ± 1.06	2.33 ± 0.89	0.161	**0.007**
Skin moisture	3.42 ± 1.08	2.33 ± 0.65	1.92 ± 0.51	**0.027**	**0.001**

Skin Symptom Questionnaire [8] is used and results are expressed as mean ± standard deviation, Dunnett's test vs. 0W, n = 12.

Table 5. Change of bowel movement.

Time course	Stool frequency per day	p value
0W	0.40 ± 0.11	
1W	0.74 ± 0.32	**<0.001**
2W	0.82 ± 0.40	**<0.001**
3W	0.85 ± 0.28	**<0.001**
4W	0.90 ± 0.37	**<0.001**
5W	0.80 ± 0.28	**<0.001**
6W	0.85 ± 0.28	**<0.001**
7W	0.82 ± 0.28	**<0.001**
8W	0.77 ± 0.25	**<0.001**
9W	0.83 ± 0.28	**<0.001**
10W	0.87 ± 0.38	**<0.001**
11W	0.85 ± 0.38	**<0.001**
12W	0.80 ± 0.35	**<0.001**

Stool frequency per day is calculated by using the data of test diary. Results are expressed as mean ± standard deviation, Dunnett's test vs. 0W, n = 12.

Table 6. Physical information.

		0W	8W	12W	p values		
					Two-way ANOVA	8W	12W
Height	cm	158.38 ± 2.92					
Weight	kg	54.94 ± 10.32	54.88 ± 10.76	55.12 ± 10.43	0.659	0.978	0.826
Body fat	kg	24.78 ± 6.55	24.43 ± 6.87	24.38 ± 5.97	0.569	0.601	0.521
BMI.	–	21.95 ± 4.34	21.92 ± 4.52	22.02 ± 4.41	0.623	0.953	0.827
BMR	kcal/kg	1144.75 ± 47.36	1144.67 ± 52.08	1147.17 ± 53.37	0.570	0.999	0.568
Systolic BP	mmHg	102.25 ± 13.75	106.33 ± 13.57	108.58 ± 11.46	**0.019**	0.112	**0.011**
Diastolic BP	mmHg	69.42 ± 9.63	73.83 ± 9.18	75.71 ± 6.46	**0.001**	**0.013**	**0.001**
Puls	/min	69.75 ± 7.40	71.29 ± 6.89	71.96 ± 7.24	0.413	0.562	0.330

Data are expressed as mean ± standard deviation, two-way repeated measure ANOVA and Dunnett's test vs. 0W, n = 12. BMI, body mass index; BMR, basal metabolic rate; BP, blood pressure.

Table 7. Blood chemistry test

			0W	12W	p values
WBC	/μL	3300 – 9000	4558.33 ± 1427.30	4958.33 ± 1465.02	**0.022**
RBC	x10^4/μL	380 – 500	431.00 ± 39.44	433.92 ± 34.73	0.525
Hb	g/dL	11.5 – 15.0	11.67 ± 2.27	11.73 ± 2.21	0.610
Hct	%	34.8 – 45.0	37.06 ± 5.35	37.22 ± 4.91	0.699
Plt	x10^4/μL	14.0 – 34.0	31.86 ± 5.41	31.01 ± 7.00	0.317
MCV	fL	85 – 102	85.67 ± 9.74	85.83 ± 9.93	0.689
MCH	Pg	28.0 – 34.0	27.03 ± 4.51	27.04 ± 4.73	0.929
MCHC	%	30.2 – 35.1	31.25 ± 2.06	31.30 ± 2.20	0.751
Neutrophil	%	40.0 – 75.0	61.66 ± 8.34	61.58 ± 8.33	0.970
Lymphocyte	%	18.0 – 49.0	28.46 ± 7.62	29.58 ± 7.23	0.477
Monocytes	%	2.0 – 10.0	6.37 ± 1.07	5.22 ± 1.12	**0.019**
Eosinophil	%	0.0 – 8.0	2.95 ± 1.37	2.97 ± 2.02	0.973
Basophil	%	0.0 – 2.0	0.57 ± 0.27	0.65 ± 0.46	0.628
Total protein	g/dL	6.7 – 8.3	6.93 ± 0.34	6.95 ± 0.25	0.870
Albumin	g/dL	3.8 – 5.2	4.18 ± 0.27	4.18 ± 0.21	1.000
A/G ratio		1.1 – 2.0	1.54 ± 0.20	1.54 ± 0.18	1.000
CPK	U/L	40 – 150	81.00 ± 30.47	74.58 ± 21.67	0.495
AST (GOT)	U/L	10 – 40	17.75 ± 5.12	16.42 ± 3.15	0.223
ALT (GPT)	U/L	5 – 45	13.50 ± 6.29	12.08 ± 5.32	0.328
LDH	U/L	120 – 240	170.33 ± 24.55	161.92 ± 17.27	0.123
ALP	U/L	100 – 325	156.58 ± 54.78	157.08 ± 43.77	0.957
γ-GTP	U/L	30 以下	17.92 ± 8.93	17.33 ± 9.62	0.510
Creatinine	mg/dL	0.47 – 0.79	0.66 ± 0.08	0.63 ± 0.07	0.093
Uric acid	mg/dL	2.5 – 7.0	4.41 ± 1.25	4.33 ± 1.21	0.658
BUN	mg/dL	8.0 – 20.0	13.49 ± 3.77	12.06 ± 3.99	0.067
FPG	mg/dL	70 – 109	82.92 ± 8.86	87.33 ± 9.87	**0.040**
HbA1c [NGSP]	%	4.6 – 6.2	5.47 ± 0.23	5.41 ± 0.30	0.206
TG	mg/dL	30 – 149	76.25 ± 54.87	61.58 ± 26.05	0.157
TC	mg/dL	120 – 219	190.33 ± 32.40	192.17 ± 27.86	0.622
HDL-C	mg/dL	40 – 95	66.33 ± 14.66	71.50 ± 12.72	**0.034**
LDL-C	mg/dL	65 – 139	108.25 ± 21.71	106.42 ± 18.98	0.602
Na	mEq/L	137 – 147	139.33 ± 1.50	139.58 ± 1.24	0.600
K	mEq/L	3.5 – 5.0	4.14 ± 0.28	4.28 ± 0.28	0.281
Cl	mEq/L	98 – 108	104.33 ± 1.56	104.50 ± 1.31	0.674
Ca	mg/dL	8.4 – 10.4	9.24 ± 0.36	9.25 ± 0.28	0.889
Fe	μg/dL	40 – 180	67.83 ± 40.68	73.58 ± 46.81	0.594
Total bilirubin	mg/dL	0.2 – 1.2	0.68 ± 0.23	0.69 ± 0.34	0.836

Data are expressed as mean ± standard deviation, paired-t test, n = 12.

Table 8. Skin test.

			0W	8W	12W	p values		
						Two-way ANOVA	8W	12W
Color difference (Upper arm)	Melanin Index		0.70 ± 0.20	0.70 ± 0.18	0.70 ± 0.19	0.869	0.972	0.924
	Hb Index		1.01 ± 0.32	0.87 ± 0.30	0.88 ± 0.20	0.051	0.054	0.071
	Hb SO_2 Index (%)		51.18 ± 12.07	53.48 ± 10.15	52.96 ± 8.85	0.567	0.494	0.646
	L*		70.22 ± 3.55	70.98 ± 3.30	71.14 ± 2.94	**0.018**	**0.046**	**0.014**
	a*		5.88 ± 1.52	5.23 ± 1.53	5.36 ± 1.15	**0.017**	**0.013**	**0.050**
	b*		14.74 ± 1.99	15.05 ± 1.74	15.18 ± 1.80	0.294	0.463	0.222
(Cheek)	Melanin Index		0.99 ± 0.16	0.96 ± 0.14	1.00 ± 0.14	**0.013**	0.058	0.593
	Hb Index		1.14 ± 0.32	1.06 ± 0.30	1.13 ± 0.32	0.249	0.209	0.947
	Hb SO_2 Index (%)		55.18 ± 5.28	56.61 ± 7.58	55.47 ± 5.70	0.269	0.219	0.927
	L*		67.90 ± 2.63	68.57 ± 2.58	67.86 ± 2.53	**0.045**	0.063	0.987
	a*		8.62 ± 1.94	8.06 ± 1.54	8.60 ± 1.76	0.131	0.135	0.998
	b*		17.65 ± 1.91	17.65 ± 1.62	17.84 ± 1.94	0.711	1.000	0.690
Elasticity (Upper arm)	R2		0.90 ± 0.02	0.89 ± 0.02	0.88 ± 0.02	0.147	0.670	0.098
	R7		0.69 ± 0.04	0.69 ± 0.04	0.67 ± 0.03	0.129	0.958	0.114
Elasticity (Cheek)	R2		0.80 ± 0.04	0.79 ± 0.05	0.76 ± 0.04	**＜0.001**	0.515	**＜0.001**
	R7		0.45 ± 0.06	0.45 ± 0.06	0.42 ± 0.05	**0.022**	0.996	**0.027**
Stratum corneum water content	Right upper arm		21.73 ± 6.18	24.11 ± 8.19	24.78 ± 6.38	0.053	0.117	**0.040**
	Left cheek		33.94 ± 12.08	36.47 ± 9.30	36.38 ± 11.27	0.307	0.298	0.319
TEWL	Right upper arm		8.42 ± 2.05	6.83 ± 1.44	6.29 ± 1.40	**0.003**	**0.020**	**0.002**
	Left cheek		13.61 ± 2.72	12.66 ± 3.23	13.44 ± 4.33	0.282	0.236	0.945
Image analysis by VISIA	Brown Spots	Score (%)	43.83 ± 8.35	44.40 ± 8.26	44.69 ± 8.02	0.297	0.491	0.229
	Pores	Score (%)	22.09 ± 11.52	21.82 ± 11.11	22.59 ± 12.26	0.610	0.942	0.819
	Porphyrin	Score (%)	9.50 ± 10.03	10.67 ± 13.83	11.05 ± 15.39	0.550	0.697	0.541
	Red	Score (%)	22.37 ± 4.32	22.75 ± 3.32	24.00 ± 4.90	0.198	0.883	0.154
	Spots	Score (%)	34.55 ± 6.81	35.05 ± 7.09	34.74 ± 6.14	0.875	0.828	0.972
	Texture	Score (%)	9.84 ± 2.70	10.16 ± 3.26	10.82 ± 3.73	0.270	0.819	0.200
	UV Spots	Score (%)	33.45 ± 10.57	33.65 ± 10.92	31.72 ± 9.80	0.266	0.982	0.308
	Wrinkles	Score (%)	7.20 ± 6.50	9.53 ± 10.34	7.30 ± 5.86	0.242	0.229	0.996

Data are expressed as mean ± standard deviation, two-way repeated measure ANOVA and Dunnett's test vs. 0W, n = 12.

Skin moisture retention test revealed a significant increase in the water content of the stratum corneum (14.0%, p=0.040) in the upper arm at Week 12 of ingestion, and significant decreases in transepidermal water loss in the upper arm at Week 8 (–18.9%, p=0.020) and Week 12 (–25.3%, p=0.002) of GDE ingestion. In the cheek, no significant change was found. Diagnostic imaging with VISIA revealed that no parameters that changed significantly.

• Glycative stress index (*Table 9*)

The amount of AGEs deposited on the skin, a glycative stress index, increased significantly from 2.14 ± 0.30 at baseline to 2.26 ± 0.22 at Week 12 (5.6%, p=0.028).

• Oxidative stress index (*Table 10*)

Blood antioxidant power (bAP), an oxidative stress index, increased significantly from 5,166 ± 835 μmol/L at baseline to 5,917±527 μmol/L at Week 12 (14.5%, p=0.013). Another oxidative stress index, blood oxidative stress (bOS), did not change significantly during the study period.

• Subclass analysis

Subclass analysis on the 12 subjects was performed in two groups divided by the amount of AGE deposited on the skin (AF value): those with higher values (6 subjects) and those with lower values (6 subjects) (*Table 11*). In the subjects with an AF value of ≥2.19 (median of the measurements), fasting blood glucose did not change significantly, but HbA1c decreased significantly from 5.45 ± 0.22% at baseline to 5.33 ± 0.23% at Week 12 (–2.2%, p=0.034). In the subjects with an AF value of <2.19, neither fasting blood glucose nor HbA1c changed significantly.

In the subjects with higher AF values, the AF value did not change. In the subjects with lower AF values, the AF value increased significantly from 1.88 ± 0.13 at baseline to 2.09 ± 0.12 at Week 12 (11.2%, p=0.013), whereas the transepidermal water loss (right upper arm) was markedly decreased at Week 12 (–35.4%, p=0.001).

Safety evaluation

No serious adverse events were found during or after the end of the study period. Hematology and blood chemistry revealed significant elevations of fasting blood glucose and HDL cholesterol within the respective ranges of physiological changes, but no clinically problematic variation was found; there was no problematic finding concerning the safety of the test material.

Discussion

GDE, the primary component of the test material, is an extract from *Geranium dielsianum* (GD), a plant of the family *Geraniaceae* reportedly indigenous exclusively to the Peruvian Andes. GD, traditionally used as a herb tea, has been said to be pharmacologically effective against diabetes mellitus and other conditions. Recent studies have focused on the effects of GDE for preventing inflammation, adjusting intestinal function (mitigation of diarrhea symptoms)[2], controlling diabetes mellitus[1,3], and improving lipid metabolism[16-18], with anti-glycation action to suppress AGE production, as demonstrated in an *in vitro* experimental system[19]. In a human serum albumin (HSA)/glucose reaction system, GDE more potently suppresses the production of fluorescent AGEs than aminoguanidine. In a collagen/glucose reaction system as well, GDE suppresse the production of fluorescent AGEs and carboxymethyllysine (CML), and these activities are more marked than those of aminoguanidine.

As the functionality of GDE has been mainly investigated *in vitro* or *in vivo*, but not in humans, we conducted a preliminary study to elucidate the likely useful bioactivities of GDE in humans. We evaluated the effects of a GDE-containing test material in preventing glycation, maintaining beautiful skin, and improving the intestinal environment in women, and verifying its safety. Thirty-three women aged 30

Table 9. Skin AF

	0W	12W	p value
Skin AF	2.14 ± 0.30	2.26 ± 0.22	**0.028**

Skin AGE deposit is evaluated as AF measured by AGE Reader®. Data are expressed as mean ± standard deviation, paired-t test vs. 0W, n = 12. AGEs, advanced glycation end products; AF, skin auto fluorescence.

Table 10. Oxidative stress.

		0W	12W	p value
bOS	μmol/L	13.43 ± 2.69	12.64 ± 2.66	0.136
bAP	μmol/L	5.166 ± 835	5.917 ± 527	**0.013**

bOS and bAP values are measured by i-Pack Oxystress Test (Spotchem). Data are expressed as mean ± standard deviation, paired-t test vs. 0W, n = 12. bOS, blood oxidative stress; bAP, blood antioxidant power.

Table 11. Subclass analysys divided into 2 groups with high AF and low AF.

Subclass group	Item		0W	12W	p values
AF ≧ 2.19 (n = 6)	FPG	mg/dL	84.17 ± 6.74	87.17 ± 6.74	0.312
	HbA1c/NGSP	%	5.45 ± 0.22	5.33 ± 0.23	**0.034**
	AF		2.43 ± 0.16	2.43 ± 0.15	0.888
	Stratum corneum water content	Right upper arm	20.93 ± 8.11	25.68 ± 8.67	0.070
		Left cheek	31.02 ± 13.74	32.55 ± 10.64	0.715
	TEWL	Right upper arm	7.45 ± 2.02	6.51 ± 1.69	0.445
		Left cheek	13.04 ± 2.00	13.47 ± 3.42	0.824
AF < 2.19 (n = 6)	FPG	mg/dL	81.67 ± 11.11	87.50 ± 12.99	0.091
	HbA1c/NGSP	%	5.48 ± 0.26	5.48 ± 0.37	1.000
	AF		1.88 ± 0.13	2.09 ± 0.12	**0.013**
	Stratum corneum water content	Right upper arm	22.52 ± 4.11	23.87 ± 3.53	0.070
		Left cheek	36.86 ± 10.58	40.22 ± 11.44	0.462
	TEWL	Right upper arm	9.39 ± 1.71	6.07 ± 1.15	**0.001**
		Left cheek	14.19 ± 4.02	13.41 ± 5.44	0.636

Data are expressed as mean ± standard deviation, paired-t test vs. 0W. AF, autofluorescence measured by AGE Reader®. FPG, fasting plasma glucose; NGSP, National Glycohemoglobin Standardization Program; TEWL, transepidermal water loss.

to <50 years who had a constipation tendency and rough skin problems, and preferred eating sweets and carbohydrates in their daily diet, were recruited. From these 33, 13 women with the highest values of AGE deposited on the skin were selected via screening. One dropout was removed; the remaining 12 women took 1,200 mg/day of GDE for 12 weeks in an uncontrolled open-label clinical trial. To obtain more definite evidence for the characteristic anti-glycation action of GDE, we attempted to identify 'subjects with great glycative stress' via screening; however, the number of subjects identified with a high glycative stress index value was insufficient because the mean AF value was 2.14 for the mean age of 41.9 years.

Administration of GDE for 12 weeks relieved the subjective symptom 'Constipation', and improved the skin symptom items 'Dry skin', 'Make-up becomes runny', 'Skin darkening', 'Skin smoothness', and 'Skin moisture'. Blood testing revealed an increased blood antioxidant power (14.5%), an increased water content of the stratum corneum (upper arm) (14.0%), and a decreased transepidermal water loss (upper arm) (–25.3%); a marked skin moisturizing effect was observed. Skin findings included reductions in elasticity indexes R2 (–5.0%) and R7 (–6.7%).

However, no definite results were obtained for glycolipid metabolism and glycative stress. In the present study, body weight and body fat did not change significantly, and HDL cholesterol increased (7.8%); however, skin AF increased (5.6%) as a result of an increase in fasting blood glucose level (5.3%).

Taking into consideration the results of the present study, the digestive tract functions of the subjects and changes in their dietary habits must be discussed. GDE was shown to act on the intestinal flora in laboratory animals [2]. In rats, orally administered GDE favorably influenced the intestinal flora and increased *Bifidobacteria* and *Lactobacilli* and decreased the *Clostridium leptum* subgroup and *Bacteroides* group [2]. With its essential effect of improving the intestinal flora, GDE improves not only bowel movement, but also various digestive tract functions. When analyzing the diary data, the subjects seemed to have felt generally healthier; it is likely that increased physical activity and appetite might have increased their food consumption. The increase in food consumption seems to have been such that fasting blood glucose was increased by 5.3% with no significant changes induced in

body weight, LDL cholesterol, or triglyceride levels. The skin AF exhibited a similar increase (5.6%). The increased HDL cholesterol (by 7.8%) seems to suggest increased physical activity.

Oxidative stress determinations revealed a 14.5% increase in blood antioxidant power (bAP), although there was no change in blood oxidative stress (bOS). Various methods are available for evaluating the blood antioxidant powers of foods, with oxygen radical absorbance capacity (ORAC) commonly used as an index of blood antioxidant power in the US and Europe. GDE has a potent anti-oxidative potential with an ORAC value of 5,100 μmol TE/g,[4] which is considered to have increased the blood antioxidant power (bAP).

Subclass analysis was performed in two groups: those with higher skin AF values and those with lower values. In the subjects with higher AF values, the AF value and fasting blood glucose level did not change significantly, however the first-stage glycation product HbA1c decreased slightly but significantly (−2.2%) (***Table11***). GDE inhibits α glucosidase [3], an enzyme that decomposes oligosaccharides, such as maltose and sucrose. Inhibition of α glucosidase activity inhibits the digestion of oligosaccharides in the small intestine, and retards their absorption, which in turn relieves postprandial hyperglycemia. The α glucosidase inhibitory activity of GDE may suppress postprandial hyperglycemia and reduce HbA1c.

In the subjects with lower skin AF values, the AF value increased significantly by 11.2%, whereas the transepidermal water loss (right upper arm) improved markedly (−35.4%) (***Table 11***). The water content of the stratum corneum tended to be lower in the subjects with higher AF values, and higher in the subjects with lower AF values, whereas the transepidermal water loss tended to be smaller in the subjects with higher AF values, and larger in the subjects with lower AF values. The smaller transepidermal water loss observed in the subjects with higher AF values is attributable to lower water contents, and thus the lack of transpiring water. In the subjects with higher AF values as well, both the water content of the stratum corneum and the transepidermal water loss tended to improve; however, the differences were statistically insignificant. The action mechanism for the skin moisturizing effect of GDE remained unclear in this study; further research is needed.

Next, AF values from actual ages were calculated using the formula for the relationship between AF value and actual age [5]. After the correction for age, the calculated values were compared with actual measured values, and the subjects with high AF values for their age were selected (***Table 12***). Results showed that the subject's baseline value was worse than the actual age value by ≥25% in 4 subjects, of whom 3 (ID; 1501001, 1501022, and 1501032) had markedly decreased AF change ratios of 94.1% to 99.0% at Week 12 of ingestion of the test material.

No serious adverse events due to GDE were found during or after the end of the study period. However, the critical findings that blood pressure rose significantly within the range of physiological changes, and that skin elasticity indexes R2 and R7 decreased need to be discussed.

Systolic blood pressure rose by 6.2% from 102 ± 14 mmHg at baseline, and diastolic blood pressure rose by 9.1% from 69 ± 10 mmHg at baseline. The study subjects included a number of hypotensive subjects, 4 with a systolic blood pressure of <95 mmHg and 4 with a diastolic blood pressure of <61 mmHg. Among them, some had their blood pressures elevated by GDE into the normal range of blood pressure. When analyzing the data exclusively for normotensive subjects (8 subjects with a systolic blood pressure of 100 to 140 mmHg and a diastolic blood pressure of ≥60 mmHg), no significant rise in systolic blood pressure was found (***Table 13***). The elevations of diastolic blood pressure also fell within the range of physiological changes, and the change ratio was low (4.1%, $p=0.033$). Therefore, the elevations of blood pressure found in the present study were not considered to be adverse events.

Generally, skin elasticity indexes R2 and R7 are considered to indicate higher elasticity and hence more favorable as they approach 1.0, *i.e.*, have higher values. However, this observation is only applicable to cases where the skin water content is normal or constant. At low skin water contents, skin elasticity indexes R2 and R7 sometimes seem apparently high; an improvement of skin water content could reduce the apparently high R2 and R7 to their true levels. Therefore, the changes in skin elasticity indexes R2 and R7 found in the present study were not considered to be adverse events.

Based on the lack of adverse effects, the safety of GDE in chronic administration has been established in rats [20]. No safety issues were found in the present study as well.

Conclusion

In the present uncontrolled open-label clinical trial, the GDE-based test material taken for 12 weeks relieved subjective skin symptoms, improved bowel movement, had a skin moisture retention effect, and improved the anti-oxidative potential. Safety of GDE in humans was established. Although no definite data were obtained, the anti-glycation action of GDE is expected to be demonstrated by conducting a double-blind study with limited subject conditions in the future.

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Conflict of interest statement

A part of this work was supported by Towa Corporation.

Table 12. Profile of skin AF

ID	Age	AF 0W	AF 8W	AF 12W	AF predicted*	AF (0W) ／AF (predicted*)	% change of AF 8W	% change of AF 12W
1501001	41	2.48	2.50	2.33	1.83	1.3555	101.0%	94.1%
1501002	33	1.76	1.68	1.97	1.69	1.0414	95.2%	112.0%
1501004	44	1.85	2.18	2.23	1.88	0.9844	117.7%	120.6%
1501006	34	1.89	2.01	2.04	1.71	1.1054	106.3%	108.0%
1501008	42	1.78	2.06	2.16	1.85	0.9657	115.6%	121.2%
1501009	38	2.32	2.52	2.42	1.78	1.3079	108.2%	104.3%
1501015	48	2.13	2.06	2.19	1.95	1.0917	96.9%	102.6%
1501016	41	1.86	2.09	1.95	1.83	1.0183	112.1%	104.6%
1501020	45	2.37	2.23	2.39	1.90	1.2499	93.7%	100.8%
1501022	48	2.62	2.51	2.59	1.95	1.3437	95.8%	98.6%
1501028	46	2.39	2.59	2.60	1.92	1.2451	108.4%	109.1%
1501032	38	2.24	2.22	2.22	1.78	1.2615	99.0%	99.0%

Skin AGE deposit is evaluated as AF measured by AGE Reader®. *Predicted AF value (y) is calculated, using chronological age (x), by the equation; y=0.0175x+1.1121, as previously reported in reference 5). AGEs, advanced glycation end products; AF, skin auto fluorescence.

Table 13. Profile of blood pressure in the subjects with normal blood pressure.

			0W	8W	12W	p values	P value by ett's	
						Two-way ANOVA	8W	12W
Age		years	39.75 ± 4.40					
Blood pressure	Systolic	mmHg	109.44 ± 10.57	113.00 ± 10.92	112.88 ± 10.90	0.357	0.344	0.368
	Diastolic	mmHg	75.06 ± 5.87	78.75 ± 6.46	78.31 ± 6.35	**0.033**	**0.034**	**0.049**

Data are expressed as mean ± standard deviation, two-way repeated measure ANOVA and Dunnett's test, n = 8.

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