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Gluconacetobacter hansenii GK-1 Ingestion for 8 Weeks Improves Skin Discomfort in Healthy Adults: A Randomized, Double-Blind, Placebo-Controlled Study

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Abstract

Gluconacetobacter hansenii GK-1 is an acid-resistant gram-negative bacterium used in vinegar brewing. Oral ingestion of GK-1 was previously reported to help maintain immunity and reduce nasal discomfort. Considering the suggested mechanism of action of activation of regulatory T cells via TLR4 to control Th1/Th2 balancing, GK-1 is also assumed to reduce skin discomfort secondary to immune reactions; however, this has not been validated in humans. Thus, we conducted a randomized, double-blind, placebo-controlled, parallel-group study on 100 healthy Japanese men and women (mean age, 47.6 ± 1.01 years) aged 20–64 years who consumed GK-1 (9×10^9 cells) daily for 8 weeks. Visual analog scale for overall, facial, arm, and leg skin discomfort was assessed before and after ingestion. The cumulative days of skin discomfort during the ingestion period were assessed. Compared with the placebo group, the *G. hansenii* GK-1 group had a significantly lower visual analog scale for overall and facial skin discomfort after 8 weeks and cumulative days of skin discomfort. Moreover, there were no adverse events attributable to *G. hansenii* GK-1. This study confirmed that oral ingestion of *G. hansenii* GK-1 contributed to skin integrity. The study protocol was preregistered at the Clinical Trials Registry System (registration no. UMIN000053005, December 7, 2023).

Keywords

Acetic Acid Bacteria, Ingestion, Skin Itch, Paraprobiotics, Functional Food

1. Introduction

Acetic acid bacteria are aerobic gram-negative bacteria that are widely present in nature, such as in flowers and fruits, and are also used in traditional fermented food, such as vinegar, kefir, and kombucha [1]-[3]. Among the 19 acetic acid bacteria that have been reported to date [4], heat-killed *Gluconacetobacter hansenii* GK-1 (GK-1) has been found to have various functions in healthy adult men and women [5]-[8]. Yoshioka *et al*. [5] reported that ingestion of 4×10^{10} GK-1 cells per day for 8 weeks reduced nasal discomfort secondary to pollen and house dust [5]. In addition, Yamashita *et al*. reported that ingestion of 1.5×10^{10} GK-1 cells per day for 12 weeks increased sIgA and decreased cumulative days of runny nose, nasal congestion, sneezing, sore throat, cough, hoarseness, malaise, arthralgia, phlegm production, chills, fever, and fatigue) [6]. Tanaka *et al*. reported that ingestion of 9×10^9 GK-1 cells per day for 12 weeks increased thepDC activity markers and reduced the cumulative days of cold-like symptoms, such as feeling ill, runny nose, stuffy nose, sneezing, coughing, head and face discomfort due to stuffy nose, dull headache, phlegm production or chest discomfort, and malaise [7]. Moreover, Oe M *et al*. reported that ingestion of 9×10^9 GK-1 cells per day for 8 weeks suppressed IFNα reduction and cumulative days of cold-like symptoms, such as runny nose, throat discomfort, cough, and fever [8]. These reports suggested that GK-1 ingestion contributes to immune-related healthy functions.

A previous study reported that oral ingestion of lipopolysaccharide (LPS) extracted from acetic acid bacteria reduced the number of scratches among Japanese cedar pollinosis model mice sensitized with Japanese cedar pollen [9]. However, there had been no human studies evaluating the effect of GK-1 ingestion on alleviating skin discomfort. Therefore, we conducted a randomized, double-blind, placebo-controlled, parallel-group study to investigate and verify the function of GK-1 ingestion for 8 weeks in relieving skin discomfort among healthy adults.

2. Materials and Methods

2.1. Study Samples

The test foods were 9×10^9 cells/ day of GK-1 (Kewpie Corporation, Tokyo, Japan) and dextrin (Matsutani Chemical Industry Co., Ltd., Hyogo, Japan) as placebo. Both were made as capsule supplements (Aliment Industry Co., Ltd., Yamanashi, Japan) with uniform taste, appearance, and nutritional composition (**Table 1**).

Table 1. Nutrition facts of each test sample.

		Placebo	GK-1
Energy	(kcal)	1.1	1.1
Protein	(g)	<0.1	<0.1
Fat	(g)	<0.1	<0.1
Carbohydrates	(g)	0.2	0.2
Salt equivalent	(g)	0.0	0.0

2.2. Study Design

The study was conducted at Miura Medical Clinic, Japan between January and March 2024 under the supervision of a physician. A total of 100 patients were allocated into two groups using the block randomization method, to prevent pre-test bias in sex and age. Allocation information was strictly sealed from the completion of allocation to the end of the study, and the patients, researchers, test food providers, and outcome assessors were blinded. Both the placebo and GK-1 groups received one capsule/day for 8 weeks. Using a visual analog scale (VAS), skin discomfort was assessed before and after 8 weeks of ingestion. Skin symptom questionnaires and diaries were recorded daily during the ingestion period.

This study was conducted following the intent of the Declaration of Helsinki and the Ethical Guidelines for Biological Sciences and Medical Research for Human Patients [Ethical Guidelines for Medical and Health Research Involving Human Patients (MEXT MHLW METI, Japan). The study was approved by the ethics committee of Miura Clinic, Medical Corporation Kanonkai (approval number: R2307, approval date: November 30, 2023). The study protocol was preregistered at the Clinical Trials Registry System (UMIN-CTR) (UMIN000053005).

The study population included 37 men and 63 women, and the mean age was 47.6 ± 1.01 years. The study enrolled 100 Japanese adult men and women who were aware of dry skin and judged to be non-ill by the investigator's internal medicine specialist and the subinvestigator's dermatologist. The inclusion criteria were 1) Healthy and aged 20 - 64 years; 2) Aware of skin discomfort and dryness; 3) Voluntarily agreed to participate in the study after receiving full explanation of the purpose and content of the study. The main exclusion criteria were 1) Presence of skin diseases, such as atopic dermatitis; 2) Presence of serious diseases, such as diabetes mellitus; 3) Individuals who have undergone gastrointestinal surgery; 4) People with diseases during treatment; 5) Allergy to food and drugs; 6) Women who wished to become pregnant or were breastfeeding during the study period; 7) Habitual engagement in strenuous sports and were on a diet; 8) Extreme irregularities in diet; 9) Possible excessive exposure to ultraviolet light and daily sunlight; 10) Use of skin care products or visited cosmetic salons during the study; 11) Unable to discontinue consumption of health food or designated quasi-drugs; 12) Continuous receipt of skin medical treatment that can affect immunity or cause pruritus (e.g., laser); 13) Considered by the investigator to be inappropriate for the study.

The main patient compliance items during the study period were as follows: 1) No significant change in prior lifestyle habits, such as diet, drinking, exercise, bedtime, smoking, and use of moisturizer-related cosmetics; 2) Avoidance of exercise and eating that were excessive for the range of daily life; 3) Prohibition of intake of pharmaceutical products, including topical preparations, and health foods; 4) Prohibition of changing the cosmetics used; 5) Prohibition of visits to a cosmetic salon for skin care purposes; 6) Prohibition of alcohol drinking and excessive exercise until the examination on the day before the examination was completed.

The study was conducted on patients who gave written informed consent after thorough explanation of the study details.

Sample sizes were estimated from relevant prior studies [10]. When α was 0.05 and power $(1 - \beta)$ was 70%, the required sample size was 76. The sample size calculation software was Cancer Research and Biostatistics Statistical Tools (https://stattools.crab.org/).

2.3. VAS

Based on symptoms on the face to upper back, arms, and legs (lower than bold), VAS was used to assess skin discomfort as the primary endpoint and dryness as the secondary endpoint. The average value of three items for each site was recorded as overall. A 100-mm line was drawn, with the left end as the best condition without any symptoms and the right end as the worst condition experienced before and after 8 weeks of ingestion.

2.4. Skin Symptom Questionnaire

Degree of skin discomfort was assessed daily using a questionnaire from the first day of ingestion until the day after the 8th week (total, 57 days). Questionnaire responses were recorded using an eight-point Likert scale (zero for no symptoms, one for light symptoms, and seven for very severe symptoms).

2.5. Statistical Analysis

The data were presented as mean ± SE, and a hazard ratio of <5% was judged to be significant. Computer software IBM SPSS Statistics 29.0 (IBM Corp., NY, USA) was used for statistical analyses. Changes in the VAS were compared between groups using Shapiro-Wilk test to confirm normality followed by analysis of covariance using the initial value as a covariate. Paired t-test was performed for comparisons before and after ingestion. For the questionnaire for skin manifestations, cumulative days was categorized into two as no symptoms (normal: 0) and with symptoms (light, mild, moderate, or severe: 1 - 7) and compared between groups using chi-square test.

3. Results

The patient characteristics are shown in **Table 2**. There were no significant differences between the placebo and GK-1 groups by sex and age. There was no dropout during the study period. Of 100 patients in the intent-to-treat set, 13 in the placebo group and 8 in the GK-1 group were excluded because of continuous intake of pharmaceutical products, use of topical drugs, excessive ultraviolet light exposure, lifestyle changes, and errors in the description. Finally, 79 patients were included in the analysis as the per protocol set (**Figure 1**). The ingestion rate of the tested foods was 98.9%. The patients included in the analysis had the same dietary and lifestyle habits as before the target during the study period.

Table 2. Background characteristics of the patients.

	Placebo Mean ± SE	GK-1 Mean ± SE	*P* value
Number of participants	Male: 12 Female: 25	Male: 15 Female: 27	0.759
Age (y)	47.5 ± 1.67	48.3 ± 1.53	0.735

P value for comparison was calculated by Chi-square test for number participants and unpaired t-test for age.

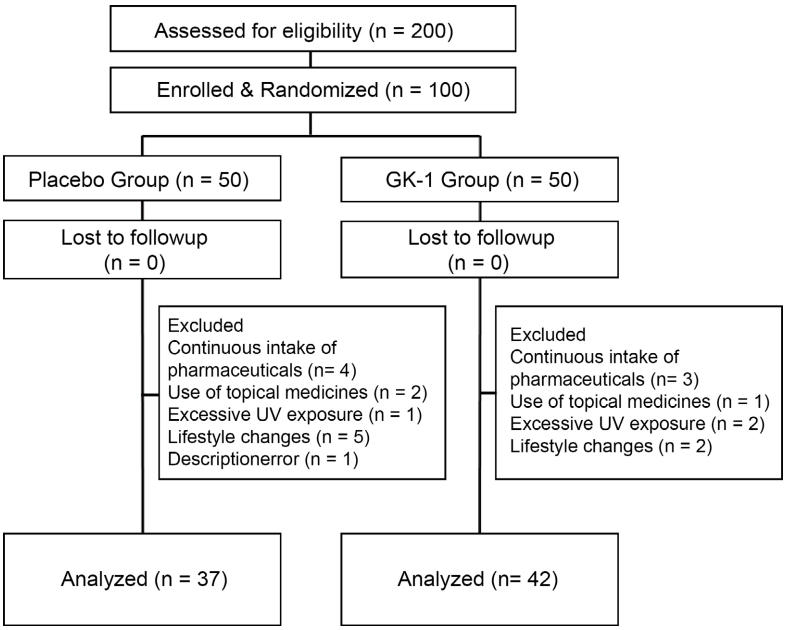

Figure 1. Flowchart of patient recruitment and assignment to the study groups.

3.1. VAS

As shown in Table 3, compared with the placebo group, the GK-1 group had significantly lower value in the changes of VAS for overall skin discomfort (P = 0.02572) and facial skin discomfort (P = 0.04995) but had similar changes in the VAS for skin discomfort on the arms and legs. Table 4 shows that although the changes in the VAS for overall, facial, arm, and leg skin dryness were similar between the placebo and GK-1 groups, those for skin dryness and discomfort at all sites significantly decreased in both groups at 8 weeks after ingestion (P < 0.001).

The cumulative days of skin discomfort were significantly lower in the GK-1 group than in the placebo group (P < 0.001) (Table 5).

The cumulative days of symptoms are 2108 in the placebo group (36 patients × 57 days and 1 patient × 56 days because of missing values) and 2394 in the GK-1 group (42 patients × 57 days).

3.2. Safety

No adverse events related to ingestion of GK-1 or placebo were reported during the study.

Table 3. Comparison of the change in the VAS for skin discomfort after 8 weeks of ingestion between placebo and GK-1.

		Week 8 Mean ± SE	*P* value a	*P* value b
Overall	Placebo (n = 37)	−34.9 ± 2.67	0.02572*	<0.001†††
	GK-1 (n = 42)	−42.9 ± 2.43		<0.001†††
Face	Placebo (n = 37)	−30.6 ± 4.59	0.04995*	<0.001†††
	GK-1 (n = 42)	−43.8 ± 4.29		<0.001†††
Arm	Placebo (n = 37)	−35.4 ± 4.84	0.15489	<0.001†††
	GK-1 (n = 42)	−43.4 ± 4.25		<0.001†††
Leg	Placebo (n = 37)	−38.7 ± 4.44	0.67527	<0.001†††
	GK-1 (n = 42)	−41.4 ± 4.17		<0.001†††

a: P value represents comparison between GK-1 and placebo using ANCOVA; *P < 0.05 vs. placebo group; b: P value represents comparison from baseline using paired t-test; †††P < 0.001 vs. baseline.

Table 4. Comparison of the change in the VAS for skin dryness after 8 weeks of ingestion between placebo and GK-1.

		Week 8 Mean ± SE	*P* value a	*P* value b
Overall	Placebo (n = 37)	−35.02 ± 2.44	0.50903	<0.001†††
	GK-1 (n = 42)	−37.64 ± 2.76		<0.001†††
Face	Placebo (n = 37)	−32.2 ± 3.94	0.42295	<0.001†††
	GK-1 (n = 42)	−38.1 ± 4.79		<0.001†††
Arm	Placebo (n = 37)	−35.9 ± 4.40	0.81450	<0.001†††
	GK-1 (n = 42)	−39.0 ± 4.72		<0.001†††
Leg	Placebo (n = 37)	−37.0 ± 4.39	0.90795	<0.001†††
	GK-1 (n = 42)	−35.9 ± 4.94		<0.001†††

a: P value represents comparison between GK-1 and placebo using ANCOVA; b: P value represents comparison from baseline using paired t-test; †††P < 0.001 vs. baseline.

Table 5. Cumulative days of symptoms.

	No symptoms	Symptom(+)	*P* value
Placebo (n = 37)	78	2,030	0.001***
GK-1 (n = 42)	297	2,097	

P value represents comparison between GK-1 and placebo using Chi-square test; ***P < 0.001 vs. placebo group.

4. Discussion

In this randomized, double-blind, placebo-controlled, parallel-group study, ingestion of GK-1 at a daily dose of 9×10^9 cells for 8 weeks was confirmed to improve the primary endpoint of changes in the VAS for overall and facial skin discomfort.

VAS is an assessment method published as a subjective evaluation indicator in the atopic dermatitis guideline in the U.S.A. and Japan, and this evaluation method hold academic validity as a discomfort evaluation of the skin [11] [12]. To date, no human studies have reported the function of orally ingested GK-1 for skin discomfort, although there had been reports that administration of LPS extract from acetic acid bacteria to animals inhibited skin scratching [9] and topical application of creams containing LPS improved mild atopic dermatitis [13]. LPS is presumed to act particularly on the GK-1 structure to inhibit allergy and inflammation through TLR4 and Treg. Interleukin (IL) 33 has been implicated in the mechanism that leads to skin discomfort and itch in dry skin by stimulating sensory neurons via receptors [14] [15]. Acetic acid bacteria bind to TLR2 and TLR4 in the intestinal epithelium and inhibit Th2 through Tregs [15]-[19]. Therefore, the Th2-type cytokine IL33 may also be involved in the acetic acid bacteria mechanism that leads to reduced skin discomfort.

In this study, GK-1 ingestion significantly improved skin discomfort on the overall and facial sites but not on the arms and legs. Considering the aforementioned mechanisms of action, improvement of skin discomfort may not be site-specific. Nevertheless, the improvements on the overall and facial sites were confirmed to significantly positively correlate with those on the face, arms, and legs (data not shown). Therefore, GK-1 ingestion may improve the overall immune function of the body and reduce skin discomfort. These results might have been influenced by the anatomical difference in the superficial skin layer between the face and upper extremities and the distribution of sebaceous glands and resident bacteria on the skin [20]. In addition, the face may have been more sensitive to the perception of itch, based on a previous report that compared reproducible itch induced by external factors on the face and arms [21].

Skin discomfort, such as itching, may be caused by external factors, such as contact and sweat, and diverse factors, such as psychological stress and allergy [22]. A limitation of this study was that the cause of skin discomfort could not be identified, because none of these factors were specified. In the future, further studies on more patients will be required to identify the kind of skin discomfort that is remarkably alleviated by GK-1 in relation to these factors. Moreover, the extent of the association between GK-1 effects and confounders, such as various elements (e.g., diet, sleep, fatigue, hormonal balance, sun exposure, air dryness, and epidermal microbiome) that are intricately associated with skin conditions [23]-[26], were not fully considered. Further research on the relationships among the effects of GK-1 ingestion on the skin and the different skin properties, lifestyle, and immune parameters of patients is required in the future.

Oral ingestion of GK-1 for 8 weeks alleviated skin discomfort in healthy Japanese men and women and is expected to be used as a functional food that contributes to the maintenance of healthy skin.

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Authors' Contributions

Conceptualization: J.M. and R.M.; Methodology, software, validation, formal analysis, and investigation: M.O. and M.Y.; Resources: M.R., Y.T., and K.K.; Data curation; M.O. and M.Y.; Writing—original draft preparation: M.O.; Writing—review and editing: M.R. and Y.T.; Visualization: M.O.; Supervision: J.M., N.M., and M.K.; Project administration and Funding acquisition: R.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

M.O., M.Y., K.K., Y.T., M.K., and R.M., are employees of Kewpie Corporation. J.M. received consulting fees from Kewpie Corporation. The remaining authors have no other conflicts of interest to declare.

Data Availability

The data underlying this article cannot be shared publicly due to ethical restrictions. The data will be shared on reasonable request to the corresponding author.

Institutional Review Board Approval

The study was conducted according to the Declaration of Helsinki guidelines and approved by the ethics committee of Miura Clinic, Medical Corporation Kanonkai (protocol code R2307; date of approval November 30, 2023).

Informed Consent

Informed consent was obtained from all patients involved in the study.

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