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Original article

Effect of *Chongkukjang* on histamine-induced skin wheal response: A randomized, double-blind, placebo-controlled trial

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ABSTRACT

Background: Studies in animals have demonstrated the antiallergenic properties of *Chongkukjang* (CKJ), a traditional Korean food made by fermentation of soybean with *Bacillus subtilis*. CKJ might therefore be used as an ingredient in a functional food designed to suppress allergies. The purpose of this study was to investigate the effect of CKJ on histamine-induced skin wheal response in healthy participants.
Methods: A randomized, double-blind, placebo-controlled trial was conducted. Sixty participants (48 women and 12 men) were randomly assigned to one of two groups: One group received 35 g CKJ daily for 12 weeks, and the other received a placebo at the same dosing frequency. A skin prick test with histamine (10 mg/mL) was conducted on the ventral forearm 10 cm from the elbow, and assessed 15 minutes later. Outcomes included measurement of efficacy [skin wheal response, immunoglobulin E (IgE), histamine, interferon-gamma, interleukin-4, eosinophil, and eosinophil cationic protein (ECP)], and safety (adverse events, laboratory test results, electrocardiogram, anthropometric values, and vital signs).
Results: Fifty-five participants (28 in the CKJ group and 27 in the placebo group) completed the study. After 12 weeks of supplementation, participants in the CKJ group showed a significant reduction in histamine-induced skin wheal areas compared with placebo group ($p < 0.05$). At 12 weeks, the CKJ group showed a significant improvement in percentage change from baseline in histamine-induced wheal area, compared with the placebo group ($p < 0.05$). CKJ did not influence blood levels of IgE, histamine, interferon-gamma, interleukin-4, eosinophil, or ECP.
Conclusion: Oral administration of CKJ for 12 weeks resulted in a reduction of the skin wheal response to histamine, with no apparent adverse effects. Trial registration: ClinicalTrials.gov: NCT01402141.

1. Introduction

Allergic disease is a consequence of exposure to normally innocuous substances that elicit the activation of mast cells, which mediate tissue swelling, redness, pain, and respiratory symptoms [1–6]. A variety of pharmaceutical agents may be used to suppress the inflammation of an allergic response. Since the notion that a

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daily intake of certain foods having antiallergic activity may potentially reduce or eliminate the need for drugs is highly attractive, the anti-inflammatory and antiallergic activities of *Chongkukjang* (CKJ) have been investigated [7].

The traditional Korean food CKJ has the shortest fermentation period (2–4 days) and is fermented at a high temperature (40–43°C) with *Bacillus subtilis*. CKJ has its soybean protein degraded from the protein degradation enzyme, and free amino acid is produced along with related peptides afterwards. Because of this, CKJ has its own special characteristics and aroma. It also serves as a great source of nutrients that provide adequate amounts of amino acids in Korean peoples' diets, where rice constitutes a common and substantial part of most meals [8].

In its long history, CKJ has sometimes been used to treat superficial inflammatory skin disorders. The bioactive compounds in CKJ include the isoflavonoids genistein and daidzein [9]. During the fermentation period, the flavonoid aglycones accumulate through hydrolysis of the flavonoid glycosides [10]. CKJ also contains poly-gamma-glutamic acid (gamma-PGA), an anionic polymer composed of D- and L-glutamic acid units linked through the alpha-amino and gamma-carboxylic acid groups [11]. Gamma-PGA is produced by *Bacillus subtilis* during the fermentation of soybeans and is not present in humans [12]. Gamma-PGA is water soluble, biodegradable, edible, and nontoxic, and is therefore compatible with use in food and cosmetic products [13]. Recent reports also attribute antiallergic and immunomodulatory activities to gamma-PGA [7,14–17], although these activities are not confirmed in humans consuming CKJ.

Histamine is a mediator of the wheal and flare response, one of the highly irritating symptoms of allergy that impairs quality of life and prompts sufferers to seek relief in the form of medications. In the development and comparison of prospective antihistamine medications, the histamine-induced wheal and flare skin test has been used most frequently. The histamine-induced wheal and flare test is based on the planimetric assessment of local swelling caused by plasma extravasation (wheal) and reflex vasodilatation (flare) after a histamine challenge [18].

The present study, therefore, was carried out to investigate the antiallergic effects of CKJ, administered orally to human participants, and assessed using the histamine skin prick test. Findings from the present study shed light on the efficacy, safety, and underlying mechanisms of CKJ, which are relevant to the development of a dietary supplement to treat symptoms of allergy in the skin.

2. Materials and methods

2.1. Participants

The study participants were recruited from the Clinical Trial Center for Functional Foods (CTCF2) in Chonbuk National University Hospital (Jeonju, Republic of Korea) between January 2011 and September 2011. A total of 60 healthy volunteers (12 male, 48 female; 34.9 ± 12.3 years) participated in this study. The volunteers had not taken any drugs that might affect histamine response, such as antihistamines, mast cell stabilizers, or antidepressants for at least 7 days prior to enrolling in this study. Inclusion criteria were: (1) healthy volunteers aged 20–80 years and (2) positive response to the histamine skin prick test (a wheal size > 3 mm). Exclusion criteria were: (1) a severe generalized skin condition such as eczema, psoriasis, or atopic dermatitis; (2) a history of severe allergic reaction; (3) use of oral antihistamines or topical corticosteroids in the preceding 3 months; (4) any acute or chronic illness; (5) cardiovascular disease, liver, or kidney disease; (6) allergies to soy-containing foods; (7) use of any prescribed or investigative medication during the 2 months preceding enrollment; (8) excessive use of a drug or alcohol in the preceding 2 months; and (9) laboratory tests results as well as medical or psychological conditions that could interfere with successful participation in the study as judged by the investigators.

The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from each participant before the study began. The protocol was approved by the Functional Foods Institutional Review Board (FFIRB) of Chonbuk National University Hospital (FFIRB number: 2010-02-007). The protocol was registered with www.clinicaltrials.gov (NCT01402141).

2.2. Study design

This current study was conducted under a 12-week, randomized, double-blind, placebo-controlled trial to evaluate the safety and efficacy of CKJ in healthy individuals in Korea. Participants were recruited through advertisements in local newspapers and the hospital website and bulletin boards. Candidates were interviewed and evaluated to determine eligibility. After completing a screening test, eligible participants were randomized to either the CKJ group or the placebo group. The treatment period consisted of 1 week of baseline assessments, 12 weeks of treatment, and 3 weeks of follow-up, for a total study period of 16 weeks. All participants and investigators were blinded to the type of treatment received until completion of the study.

To avoid allocation bias, concealed allocation using a sealed envelope was employed in this study. A statistician randomized participants using a computer-generated random table in a 1:1 ratio with block size 2, and clinical research coordinators (CRC) used the random table to assign the CKJ and placebo treatments. CKJ or placebo pills were prescribed to the participants every 4 weeks. The CKJ group ($n = 30$) took 35 g (11.7 g pills per pack, 3 times per day) of freeze-dried CKJ daily for 12 weeks, which was equivalent to 70 g of fresh CKJ; the placebo group ($n = 30$) took the same amount. CKJ and the placebo were provided by the Institute of Sunchang Fermented Soybean Products (Sunchang, Republic of Korea). Briefly, white soybeans (Sunchang, Republic of Korea) were sorted, washed, and soaked in water for 12 hours at 15°C and boiled for 0.17 hours at 121°C. The cooked soybeans were cooled to 40°C and fermented with *Bacillus licheniformis* SRCM 100027 at 37°C for 24 hours. CKJ was freeze-dried using a freeze-dryer (model PVTFD 100R, Ilsinlab, Yangju, Republic of Korea), and then made into pills (Imshil Herbal Medicine Co., Imshil, Republic of Korea). The placebo had the same taste and appearance but did not have the principal ingredient that was present in CKJ. The placebo supplements were composed primarily of rice and wheat flours.

During a 12-week intervention period, participants were asked to continue their usual diets and activities and were asked not to take any other functional foods or dietary supplements. The skin prick test was performed, and immunoglobulin E (IgE), histamine, interferon-gamma, interleukin-4, eosinophil, and eosinophil cationic protein (ECP) were measured before and after the intervention period for both groups. Every 4th week the participants were asked to report for assessment of any adverse events or any changes in training, lifestyle, or eating patterns, and to assess pill compliance.

2.3. Assessments

The skin prick tests with histamine solution, CKJ, and saline were performed on the forearms using prick lancets. Histamine was dissolved in distilled water at 10 mg/mL. A droplet of 10 mg/mL histamine solution was introduced into the skin by piercing with a

sterile lancet. The droplet was gently wiped off after 1 minute, and the wheal and flare reaction was evaluated under a bright lamp after 15 minutes. The size of the wheal and flare were outlined with a thin-tipped marker pen. All tests were carried out by the same investigator to minimize variability in the application technique. The area of the wheal and flare was measured with planimetry, by a person blinded to the study protocol, and expressed as square millimeters.

Our primary outcome was the difference in wheal response induced by the histamine challenge at baseline and at 12 weeks after randomization. The wheal response was assessed as the mean diameter or with planimetry at 15 minutes after the challenge. The secondary outcomes were the mean changes in IgE, histamine, interferon-gamma, interleukin-4, eosinophil, and ECP. These biomarkers were checked baseline and 12 weeks.

2.4. Safety measures

Participants reported all unexpected adverse events related to CKJ intake to the investigator, who then recorded the event on the individual case report form. Safety assessment was based on clinical laboratory test results, vital signs, anthropometric values, electrocardiogram, and adverse events. Clinical laboratory tests, including aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), glucose, total protein, albumin, blood urea nitrogen (BUN), creatinine, red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin, hematocrit, number of platelets, and number of differentiated cells were performed at baseline and 12 weeks. Vital signs of each participant were checked while monitoring adverse events (nausea/vomiting, fatigue, allergic reaction, and any adverse events related to CKJ) after every visit.

2.5. Statistical analysis

A sample size calculation was performed, using as primary outcome measure the histamine-induced wheal size, whereby 5.0 square millimeters with a standard deviation of 6.85 was assumed to represent a clinically relevant difference between the two groups for alpha set at 5% and power at 80%. This resulted in a required number of 24 participants in each group. Assuming a dropout rate of 20%, a total of 60 participants were enrolled in this trial.

Statistical analysis was performed using SAS version 9.0 for Windows (SAS Institute, Cary, NC, USA). Data were presented as mean ± SD values. General characteristics were analyzed by the independent t test or Fisher's exact test. Student paired t test was used for continuous measures to assess differences before and after the 12-week intervention period. A linear mixed-effects model was applied to repeated measures data for each continuous outcome variable. Fixed effects included treatment group, treatment visit, and interaction between the treatment group and visits. A value of $p < 0.05$ was considered statistically significant.

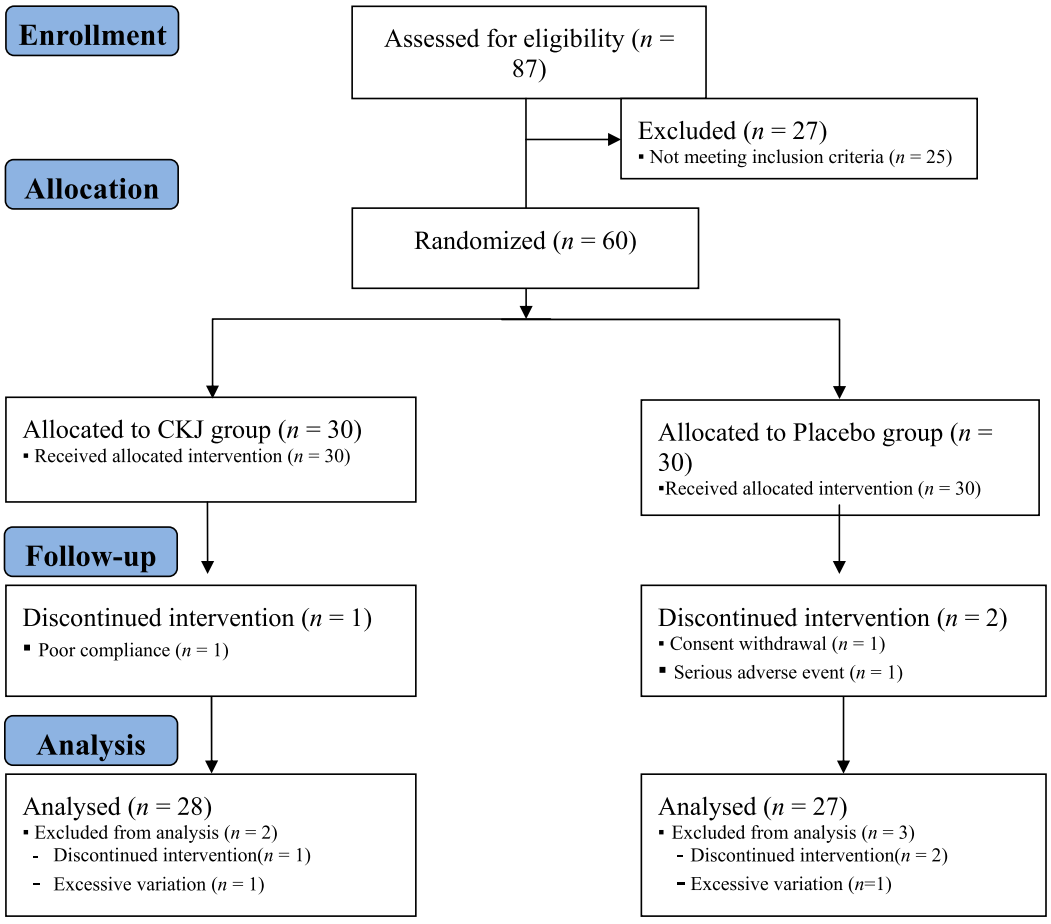

Fig. 1. Flow chart used for the study participants. Number of study participants enrolled, allocated, followed, and analyzed shown using the CONSORT 2010 Flow Diagram.

Table 1
Demographic characteristics of the study participants.

	CKJ group ($n = 30$)	Placebo group ($n = 30$)	Total ($n = 60$)	p^*
Sex (M/F)†	10/20	2/28	12/48	0.021
Age (y)	35.5 ± 11.9	34.2 ± 12.9	34.9 ± 12.3	0.679
Weight (kg)	61.9 ± 11.4	58.7 ± 8.7	60.3 ± 10.2	0.147
Height (cm)	164.2 ± 9.3	161.1 ± 7.3	162.6 ± 8.4	0.209

Values are presented as mean ± standard deviation.
CKJ, *Chongkukjang;* F, female; M, male.
* Analyzed with independent *t* test.
† Analyzed with Fisher's exact-test.

3. Results

3.1. Participants

The flow chart is summarized in Fig. 1 for the number of participants who completed the study. From 87 participants screened initially, 27 participants were excluded because they failed the inclusion criteria by the skin prick test and/or laboratory tests. The remaining 60 participants were randomly distributed between the CKJ and placebo groups. Five participants were excluded from the statistical analysis. One of these participants failed in compliance; one participant voluntarily withdrew a written informed consent for personal reasons; one participant experienced a serious adverse event (SAE); and two participants were excluded from analysis because of excessive variation, which likely reflected an error on statistical analysis. As a result, 55 participants (CKJ = 28 and placebo = 27) remained for analysis.

3.2. General characteristics of the participants

Baseline characteristics of participants in the CKJ and placebo groups are summarized in Table 1. The mean ages of participants in the CKJ and placebo groups were 35.5 ± 11.9 years and 34.2 ± 12.9 years, respectively. The 60 participants were 34.9 ± 12.3 years old, overall. There were no significant differences in baseline characteristics such as age, weight, and height between the CKJ and placebo groups.

3.3. Skin prick tests

Results of the skin prick tests are summarized in Table 2. Histamine challenge caused a marked wheal response accompanied by itching in all participants. The baseline wheal area (prior to administration of CKJ or placebo) did not differ significantly between groups (14.88 ± 7.44 mm^2 vs. 14.16 ± 5.88 mm^2, respectively). After 12 weeks of supplementation, participants in the CKJ group produced a significant reduction of histamine-induced wheals than the placebo group did ($p = 0.023$).

The percentage change from baseline in histamine-induced wheal area for the CKJ group and placebo group demonstrated that they were comparable (Fig. 2). At 12 weeks, the percentage change from baseline in histamine-induced wheal area showed a decrease in the CKJ group (−2.58 ± 41.78 %), whereas the placebo group showed an increase (30.17 ± 61.99 %). The CKJ group showed a significant improvement compared with the placebo group ($p = 0.027$).

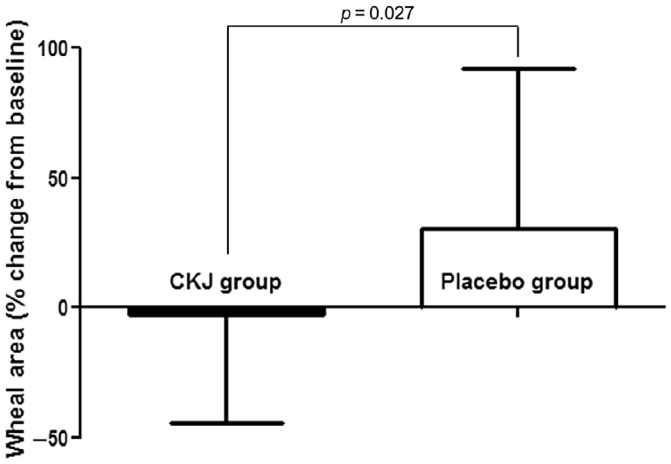

Fig. 2. Percentage change from baseline in histamine-induced wheal area. Values are presented as mean ± standard deviation. Analyzed with independent *t* test and *p*-values compared to placebo.

3.4. Blood profiles

Changes in allergenic mediators in blood before and after the 12-week intervention period are shown in Table 3. No significant changes were observed in blood IgE, histamine, interferon-gamma, interleukin-4, eosinophil, and ECP in either the CKJ or placebo group at 12 weeks.

3.5. Safety

At every visit, reports of symptoms and adverse effects from each participant were recorded. One participant in the placebo group experienced a SAE (a herniated lumbar disc at L5-S1) during the study, which led them to withdraw from the study. This one serious adverse effect, as judged by the principal investigator, was unlikely to be related to the study protocol. No significant differences were found between the two groups. The evaluation was expanded to include clinical laboratory tests, vital signs, anthropometric vales, and electrocardiogram findings during the participants' visits. All test results were within the normal range, and no significant changes were observed between the CKJ and placebo groups (data not shown).

4. Discussion

This randomized, double-blind, placebo-controlled trial was conducted to determine whether taking CKJ could reduce the

Table 2
Histamine-induced wheal area.

	CKJ group ($n = 28$)			Placebo group ($n = 27$)			p for treatment effect†
	Baseline	12 weeks	p^*	Baseline	12 weeks	p^*	
Wheal area (mm^2)	14.88 ± 7.44	13.1 ± 5.69	0.121	14.16 ± 5.88	16.54 ± 5.89	0.101	0.023

Values are presented as mean ± SD.
CKJ, Chongkukjang.
* Analyzed with paired *t* test between baseline and 12 weeks in each group.
† Analyzed with linear mixed-effect model.

Table 3
Blood profiles.

	CKJ group ($n = 28$)			Placebo group ($n = 27$)			p for treatment effect[†]
	Baseline	12 weeks	p[*]	Baseline	12 weeks	p[*]	
IgE (IU/mL)	76.54 ± 93.05	69.64 ± 82.26	0.161	70.03 ± 112.46	63.39 ± 98.45	0.084	0.648
Histamine (μg/g creatinine)	0.56 ± 0.55	0.76 ± 0.58	0.144	0.64 ± 0.52	0.81 ± 0.74	0.280	0.901
Interferon-γ (IU/mL)	14.4 ± 3.01	17.57 ± 1.9	<.0001	15.79 ± 5.5	18.11 ± 2.24	0.030	0.481
Interleukin-4 (pg/mL)	0.17 ± 0.14	0.27 ± 0.2	0.015	0.57 ± 1.58	0.36 ± 0.79	0.506	0.322
Eosinophil (%)	2.7 ± 2.18	2.58 ± 2.26	0.601	3.33 ± 2.93	2.63 ± 2.59	0.094	0.207
ECP (μg/L)	17.86 ± 17.52	18.77 ± 17.73	0.679	23.21 ± 21.34	21.86 ± 26.59	0.615	0.511

Values are presented as mean ± SD.
CKJ, *Chongkukjang;* ECP, eosinophil cationic protein; IgE, immunoglobulin E.
[*] Analyzed with paired *t* test between baseline and 12 weeks in each group.
[†] Analyzed with linear mixed-effect model.

histamine-induced skin wheal response in healthy Korean volunteers. We found that consuming CKJ for 3 months resulted in significant attenuation of the skin wheal reaction and that the CKJ supplement was well tolerated. CKJ supplementation showed a significant improvement in percentage change from baseline in histamine-induced wheal area compared with the placebo group.

The CKJ is a Korean fermented soybean containing microorganisms, proteinase, and diverse bioactive compounds, including isoflavones, peptides, and gamma-PGA. Preparations of CKJ are reported to exert antihypertensive, fibrinolytic, antidiabetic and anti-inflammatory activities. These activities may be attributable to fermentation products of the *Bacillus* bacteria through growth on soy.

The anti-inflammatory action of CKJ in the histamine-induced skin wheal response [7,14–17] may in part represent inhibition of 5-lipoxygenase (5-LOX). The enzyme 5-LOX presents an important target for anti-inflammatory drug development through its role in leukotriene biosynthesis. Inhibitors of 5-LOX may also suppress allergy. In a rat model of passive cutaneous anaphylaxis (PCA), CKJ showed significant inhibition. The anti-inflammatory activity of CKJ was evaluated using an arachidonic acid-induced model of edema in the mouse ear, in which cysteinyl-leukotrienes are major mediators. In this model, the CKJ dose-dependently inhibited edema in the ear. The gamma-PGA component of CKJ, in turn, may significantly reduce expression of transient receptor potential A1 (TRPA1) receptors in suburothelial afferents. Histamine-induced excitation of sensory neurons and PAR-2 activation may activate or sensitize the TRPV1 receptors. This same pathway may be a target of the CKJ in attenuation of the histamine-induced skin wheal response.

Findings in the present study may be limited in that we did not control for variations in diet or activity levels of the participants; this uncertainty may be resolved in a future study that includes a diet and exercise component. Also, we did not evaluate the placebo effect.

Nevertheless, the significant inhibitory effect of CKJ, by oral administration, on the histamine-induced skin wheal response may have clinical relevance. Many studies have used this test to assess the relative potency of antihistamines. To the best of our knowledge, no previous study has evaluated the antiallergenic activity of CKJ in human participants. Therefore, our findings may contribute to the development of new cost-effective remedies for mild allergic diseases.

This study demonstrated the safety and tolerability of CKJ administered orally in 35-g doses in healthy volunteers. Administration of the CKJ in a 12-week regimen resulted in attenuation of the histamine-induced skin wheal response, suggesting that CKJ may be used to prepare a safe, well-tolerated, and effective functional food for the treatment of allergic conditions.

Conflicts of interest

All authors have no conflicts of interest to declare.

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