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Fermented soybean product (Cheonggukjang) improved some attributes of protein and growth hormone measurements in Sprague-Dawley rats

In Sik Hwang[a,1], *Ji Eun Kim*[a,1], *Young Ju Lee*[a], *Moon Hwa Kwak*[a], *Jun Go*[a], *Hong Joo Son*[b], *Dong Sup Kim*[c], *Dae Youn Hwang*[a,*]

[a] *Department of Biomaterials Science, College of Natural Resources & Life Science/Life and Industry Convergence Research Institute, Pusan National University, Miryang, Republic of Korea*

[b] *Department of Life Science & Environment Biochemistry, College of Natural Resources & Life Science, Pusan National University, Miryang, Republic of Korea*

[c] *Department of Food Science & Technology, College of Natural Resources & Life Science, Pusan National University, Miryang, Republic of Korea*

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ABSTRACT

We hypothesized that the administration of Cheonggukjang (CKJ) would exert positive effects on factors implicated with growth in Sprague-Dawley (SD) rats. To test this hypothesis, we measured specific aspects of bone and organ growth in male SD rats that were treated for 6 weeks with 3 concentrations of CKJ. Although the CKJ extract contained high concentrations of flavonoids and phenolic compounds, no significant differences in body length, organ weights, or femur weight were detected between the CKJ- and vehicle-treated groups. However, thicknesses of the epiphyseal growth plate in the proximal femoral epiphysis and the compact bone in the linea aspera were broadest in the femur of the 2 CKJ-treated groups when compared with the vehicle-treated groups. Furthermore, the levels of growth hormone (GH) and calcium ion were higher in the sera of the high-concentration CKJ–treated groups, whereas the expression level of GH receptor was higher in muscle tissue of all CKJ-treated groups and in the liver tissue of the high-concentration CKJ–treated group. In the GH receptor downstream signaling pathway, the phosphorylation levels of Akt and Erk were expressed differently between liver and muscle tissues upon CKJ treatment. However, the phosphorylation level of STAT5 was very similar to the expression level of the GH receptor in all CKJ-treated groups. These results indicate that CKJ extract may increase the thickness of the epiphyseal growth plate and the compact bone of the femur, elevate GH secretion, and stimulate regulation of the GH receptor downstream signaling pathway in the liver and muscle tissues of SD rats.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; CKJ, Cheonggukjang; GH, growth hormone; H-CKJ, high-concentration CKJ; H&E, hematoxylin and eosin; L-CKJ, low-concentration CKJ; LDH, lactate dehydrogenase; M-CKJ, medium-concentration CKJ; NGF, nerve growth factor.

* *Corresponding author.* Department of Biomaterials Science, College of Natural Resources & Life Science, Pusan National University, 50 Cheonghak-ri, Samnangjin-eup Miryang-si, Gyeongsangnam-do 627-706, Republic of Korea. Tel.: +82 55 350 5388; fax: +82 55 350 5389.
E-mail address: dyhwang@pusan.ac.kr (D.Y. Hwang).

[1] These authors contributed equally to this study.

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1. Introduction

Cheonggukjang (CKJ) is a fermented product that is primarily manufactured by short-term fermentation of soybeans using *Bacillus subtilis*, which contains many enzymes, microorganisms, and bioactive compounds that are absent from unfermented soybeans [1,2]. During CKJ fermentation, flavonoid glycosides are converted into aglycones by hydrolysis, and many of the proteins are degraded into small peptides and amino acids [3,4].

CKJ displays diverse biological and pharmacological activities, including antiobesity, antidiabetic, anti-inflammatory, and neuroprotective effects against human chronic diseases [5-8]. CKJ supplementation has been shown to significantly reduce visceral fat mass and apolipoprotein B100/apolipoprotein A1 levels in human subjects [9] as well as improve body weight, epididymal fat accumulation, serum total cholesterol, and low-density lipoprotein cholesterol in diet-induced obesity animals [5]. Furthermore, CKJ supplementation has been shown to significantly reduce blood glucose and glycosylated hemoglobin levels as well as improve insulin tolerance in C57BL/Ksj-*db/db* mice [6,10]. In addition, CKJ treatment is found to decrease passive cutaneous anaphylaxis in rat models of type I hypersensitivity and arachidonic acid–induced ear edema [7]. Moreover, CKJ treatment for 8 weeks recovered the concentration of nerve growth factor (NGF) in serum and the phosphorylation level of TrkA and Erk in the NGF receptor TrkA signaling pathway in the brain of Tg2576 mice [8]. Although large controlled intervention trials have indicated that CKJ has beneficial effects on various chronic diseases, its effects on body growth sensitivity have yet to be investigated.

Growth hormone (GH), a hormone secreted from the anterior pituitary gland, is considered an important regulator of body growth and composition in mammals [11]. GH secretion can be promoted by injection or administration of various compounds, such as acipimox [12], estradiol [13], ghrelin [14], rivastigmine [15], astragalus [16], glucocorticoid [17], glycine [18], creatine [19], L-dihydroxyphenylalanine [20], and α-glycerylphosphorylcholine [21]. However, a few studies have shown the correlation between soy-related products and GH-stimulating capacity. The concentration of GH in the blood of healthy women increased to 5.0 ± 0.8 μg/L after ingestion of soy proteins, relative to placebo ingestion [22]. Therefore, we hypothesized that CKJ would exert beneficial effects on growth-related factors, including body growth, bone growth, GH secretion, and GH sensitivity in Sprague-Dawley (SD) rats. Specifically, we investigated whether oral administration of CKJ extract for 6 weeks improves body growth via GH secretion in male SD rats.

2. Methods and materials

2.1. *Preparation and component analysis of CKJ extract*

Pungwon soybean strain was supplied by the National Institute of Crop Science in Miryang, Korea. The *B. subtilis* used for the preparation of CKJ was obtained from the Applied and Environmental Microbiology Laboratory at Pusan National University. Whole soybeans (100 g) were washed and soaked with 3 volumes of tap water at room temperature for 12 hours. Afterwards, they were steamed for 30 minutes at 121°C and then allowed to cool to 37°C. The steamed soybeans were subsequently inoculated with 5% (wt/wt) *B. subtilis* (1×10^9 cells/mL) and fermented for 48 hours at 37°C. CKJ extract was freeze dried and then homogenized, then the CKJ powder was stored at –75°C until use [8,23,24].

The chemical compositions of CKJ were analyzed in triplicate using the official method suggested by the Association of Official Analytical Chemists [25]. Moisture content was measured by the weight method at 105°C, and crude protein was detected using a Kjeldahl nitrogen/protein analyzer (FOSS Tecator SE/Auto 2399, Hoganas, Sweden) after complete degradation of CKJ (1 g) in K_2SO_4 and H_2SO_4 buffer (10 mL). Crude fat was detected using a Soxtec system (FOSS Quality Assurance; Soxtec TM 2045), and crude ash was measured by weighing the sample before and after heating the CKJ sample at 550°C for 4 hours to remove all organic compounds from the sample.

For bioactive component analysis, CKJ extract (1 g) was subjected to extraction with 10 mL of dH_2O at 70°C for 2 hours. This mixture was then centrifuged (10 minutes, 1000 rpm), and then the supernatant was collected for analysis. The amount of total phenolics in CKJ extract was determined according to the Folin-Ciocalteu method [26]. Briefly, the collected sample (20 μL) was mixed with 100 μL of 0.2 N Folin-Ciocalteu reagent for 5 minutes, after which 300 μL of 20% sodium carbonate was added. Following incubation at room temperature for 2 hours, the absorbance of the reaction mixture was measured at 765 nm. Gallic acid was used as a standard to produce the calibration curve. Total phenolic content was expressed in milligrams of gallic acid equivalents per gram of CKJ extract. The amount of total flavonoids in CKJ extract was determined according to the method described by Meda et al [27]. Briefly, CKJ extract (200 μL) was added to test tubes containing 60 μL of 5% potassium nitrite, 600 μL of distilled water, and 60 μL of 10% aluminum chloride. After incubation at 25°C for 5 minutes, the absorbance of the reaction mixture was measured at 510 nm. Total flavonoids content was determined using a standard curve with quercetin as a standard and expressed as milligrams of quercetin equivalents per gram of CKJ powder.

The concentrations of daidzein and genistein in the CKJ extract were analyzed according to the method suggested by Zheng et al [28]. Standard samples of 98% daidzein and 99% genistein were purchased from Sigma-Aldrich (St Louis, MO, USA). Briefly, aqueous CKJ extracts were mixed with 50% methanol, after which the resulting mixture was filtered through a 0.2 μm membrane filter (Waters Co, Millfod, MA) before high performance liquid chromatography (HPLC) injection. Concentrations of daidzein and genistein were analyzed using an iLC 3000 HPLC system (Interface Engineering, Seoul, Korea) that was equipped with a Corona CAD Detector (ESA Bioscience, Inc, Chelmsford, MA). Chromatographic separation was conducted using a CAPCELL PAK MG C18 column (4.6 × 250 mm; particle size, 5 μm; Shiseido Co Ltd, Tokyo, Japan). The mobile phase consisted of solvent A (deionized water) and solvent B (acetonitrile) applied in the following gradient elution program: 0 to 25 minutes, 30% to