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Original Article

The brand-new compound CPS219 exhibits anti-oxidant and estrogen-like actions

Kyung-Min Jeong, Yu-Jin Choi, Seung-Hwan Kim, Hyun-Ja Jeong*

Division of Food and Pharmaceutical Engineering, Hoseo University, Asan 31499, Republic of Korea

ABSTRACT

CPS219, a new concept compound composed of coffee (CO), *Pueraria Tomsonii* flower (PF), and Sambou bamboo salt™ (BS), was developed as a coffee beverage to alleviate menopausal symptoms. The purpose of this study is to evaluate the anti-oxidant and menopausal alleviating effects of CPS219 combined as the optimal ratio of each component selected through sensory evaluation and blind consumer test. For CPS219, the optimal ratio of CO, PF, and BS was determined to be 1:0.1:0.017 through various sensory evaluations and blind consumer tests. CPS219 significantly enhanced the superoxide dismutase-like activity compared to the CO or CO plus PF (CP). The proliferation of MCF-7 cells was considerably increased after 24 hours by treatment with CO, CP, or CPS219, but only CPS219 significantly boosted the proliferation of MCF-7 cells after 48 hours. Moreover, CPS219 had an estrogen-like effect by dramatically increasing the expression of estrogen receptor-β mRNA in MCF-7 cells but not CO and CP. Treatment of MCF-7 cells with CO, CP, or CPS219 did not cause any cytotoxicity. In conclusion, these findings imply that anti-oxidant and estrogen-like properties of CPS219 can be used to prevent and cure postmenopausal symptoms.

Keywords CPS219, menopause, superoxide dismutase, estrogen receptor-β

INTRODUCTION

Every woman experiences the natural biological period of menopause at some point in her life.[1] Significant hormonal changes, such as estrogen insufficiency in women, cause menopause.[2] Menopausal women commonly experience abnormal vaginal bleeding, hot flashes, bone loss, migraines, excess weight, forgetfulness, vaginal and urinary problems, anxiety, and mood swings.[3] Such symptoms have a detrimental effect on life in general.[4] As a result, several researchers are developing risk-free and efficient substitutes for controlling menopausal biomarkers.[5,6]

These years, there has been an increase in interest in the pharmacologic function of bioactive chemicals found in plants.[7] This is due to the fact that the medical phytoestrogen derived from herbs has been utilized to treat menopause on a large scale.[8] Plant-derived chemicals called phytoestrogens act as estrogen receptors (ERs) modulators by attaching to ERs in place of estrogen.[7] *Pueraria Tomsonii* flower (PF), which mostly consists of isoflavones, has anti-oxidant, anti-inflammatory, and anti-cancer properties.[9-11] PF specifically stimulates estrogen-like action.[12] Therefore, menopausal women may utilize PF as a replacement to treatment for menopause based on estrogen.[13] Additionally, coffee (CO) contains a variety of bioactive substances.[14] Through its anti-oxidant and anti-inflammatory properties, CO has significant pharmacological and biological effects

*Correspondence: Hyun-Ja Jeong
E-mail: hjjeong@hoseo.edu
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on human health.[14,15] Bamboo salt is produced using techniques that produce less toxicity and convert acidity to strong alkalinity compared to sun-dried salts, and it is mostly taken for health reasons.[16] Bamboo salt has anti-aging, anti-allergic, immune-enhancing, anti-oxidant, and anti-inflammatory properties since it includes a lot of minerals and an important microelement for keeping the human body in working order.[17,18] The new concept natural compound CPS219, composed of CO, PF, and Sambou bamboo salt™ (BS) was developed to alleviate menopausal symptoms. Based on the above reports, we hypothesized that CPS219 could regulate various menopausal symptoms. Thus, our present study thus aimed to evaluate the anti-oxidant and menopausal alleviating effects of CPS219 in ER-positive human breast cancer cell line, MCF-7 cells.

MATERIALS & METHODS

Materials

CO was purchased from Dongsuh Foods Co. (Seoul, Republic of Korea). PF was purchased from Dongui Herb Co. (Seoul, Republic of Korea). BS was provided by Sambou bamboo salt Inc. (Jeonbuk, Republic of Korea). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and 17β estradiol (E2) were purchased from Sigma chemical Co. (St. Louis, MO, USA). RPMI 1640 and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). PF was boiled at 100℃ for 2 h and then lyophilized. The yield of PF was about 18% (54 g). E2 was dissolved in DMSO.

Sensory Evaluation

Each sample was prepared according to the various proportions and placed in refrigerated conditions until testing. About 20 ml of each sample was provided in a white 50 ml paper cup with a random 4-digit number written on it. All samples were evaluated for overall quality (color, smell, taste, bitterness, acidity, and preference) by 15 trained sensory evaluation personnel from the Department of Food Science and Technology, Hoseo University. Sensory evaluation results were used in the 7-point hedonic scale (low, 1 indicates a strong hate, while high, 7 indicates a strong preference).

Blind customer test

Blind consumer test was conducted with the finally determined experimental groups and control groups. Similarly, about 20 ml of each sample was provided in a white 50 ml paper cup with a random 4-digit number written on it. The preference of the samples was evaluated by 34 people. Blind consumer test results used a ranking method.

Cell culture

MCF-7 cells were bought from the Korean Cell Line Bank (KCLB®, Seoul, Republic of Korea), and they were grown in RPMI 1640 containing 10% FBS and 1% penicillin/streptomycin at 37°C with 5% CO2 and 95% humidity. MCF-7 cells, which are ER-positive, are typically utilized to examine estrogen activity.

Superoxide dismutase (SOD) assay

An anti-oxidant effect was analyzed using EZ-SOD assay kit (DoGenBio Co., Ltd, Republic of Korea). In summary, the samples diluted with various concentrations were put into the well, the

WST working solution and the enzyme working solution were added and then mixed carefully. Plates were incubated at 37ºC for 20 min and then absorbance was analyzed at 450 nm with an ELISA reader.

Bromodeoxyuridine (BrdU) assay

The growth of MCF-7 cells was measured using a colorimetric immunoassay based on the measurement of bromodeoxyuridine (BrdU) incorporated by DNA synthesis according to the manufacturer's protocol (Roche Diagnostics GmbH, Mannheim, Germany).

Quantitative real-time PCR analysis

Using an easy-BLUETM RNA extraction kit, total RNA was extracted from MCF-7 cells in accordance with the manufacturer's instructions (iNtRON Biotech, Republic of Korea). The commercial cDNA synthesis kit (Bioneer Corporation, Daejeon, Republic of Korea) was used to create the cDNA. SYBR Green master mix was used for quantitative real-time PCR, and an ABI StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was used for mRNA detection analysis. Data were normalized to housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Primers were as follows: GAPDH (5′ TCG ACA GTC AGC CGC ATC TTC TTT 3′; 5′ACC AAA TCC GTT GAC TCC GAC CTT 3′), and ER-β (5′ TTC CCA GCA ATG TCA CTA ACT T3′; 5′TTG AGG TTC CGC ATA CAG A 3′). With a melting curve analysis, the typical profile timings were 95°C for 10 min in the first phase, then 15 s at 95°C in the second step, and 30 s at 60°C for 40 cycles. The ΔΔCT method was used to examine the data.

MTT assay

MTT assay was used to measure cell viability. Briefly, 100 μl of cell suspension (1×10^4 cells/well) was cultured in 96-well plates after treatment with CPS219. After adding the MTT solution (5 mg/ml), the cells were incubated at 37°C for 4 h. The DMSO was used to dissolve the insoluble formazan product. The optical density was then assessed at 590 nm with an ELISA reader.

Statistical analysis

GraphPad Prism software (GraphPad Software Inc., CA, USA) was used to conduct all statistical analyses and to create figure graphs. Data from at least three independent experiments that were carried out in duplicates or triplicates are presented as the mean ± standard error mean (SEM). Treatment effects were analyzed using one-way ANOVA or the independent *t*-test. A value of $p < 0.05$ was regarded to be statistically significant.

RESULT

CPS219 was developed through sensory evaluations and blind customer test

Sensory evaluation was conducted on three types of freeze-dried CO (Sumatra, Colombia, and Maxim) and their characteristics were compared. In the evaluation, CO with a good balance of color, taste, and smell and relatively low bitterness and acidity produced good results. As shown in Fig. 1A, Maxim CO was rated highest on a 7-point hedonic scale.

Next, to determine the CO concentration, the preference according to the coffee concentrations (1%, 2%, 3%, and 4%) was investigated. Maxim CO 3% was harmonious in

color, taste, and smell. In addition, it was found that bitterness and acidity had a significant effect on the preference results (Fig. 1B). Finally, in order to determine the optimal ratio of the three compounds, CO (3%), PF (0.075, 0.15, and 0.3), and BS (0.025, 0.05, and 0.1%) were variously combined and the preference was investigated through sensory evaluation and blind consumer test. The strongest preference in sensory evaluation was seen in samples 9292, 5223, and 5319 (Fig. 1C, $p < 0.05$). The most preferred number in the blind consumer test, 5229 (CPS219), consisted of 3% CO, 0.3% PF, and 0.05% BS. According to all of the findings, the optimal ratio of CO, PF, and BS was determined as 1:0.1:0.017.

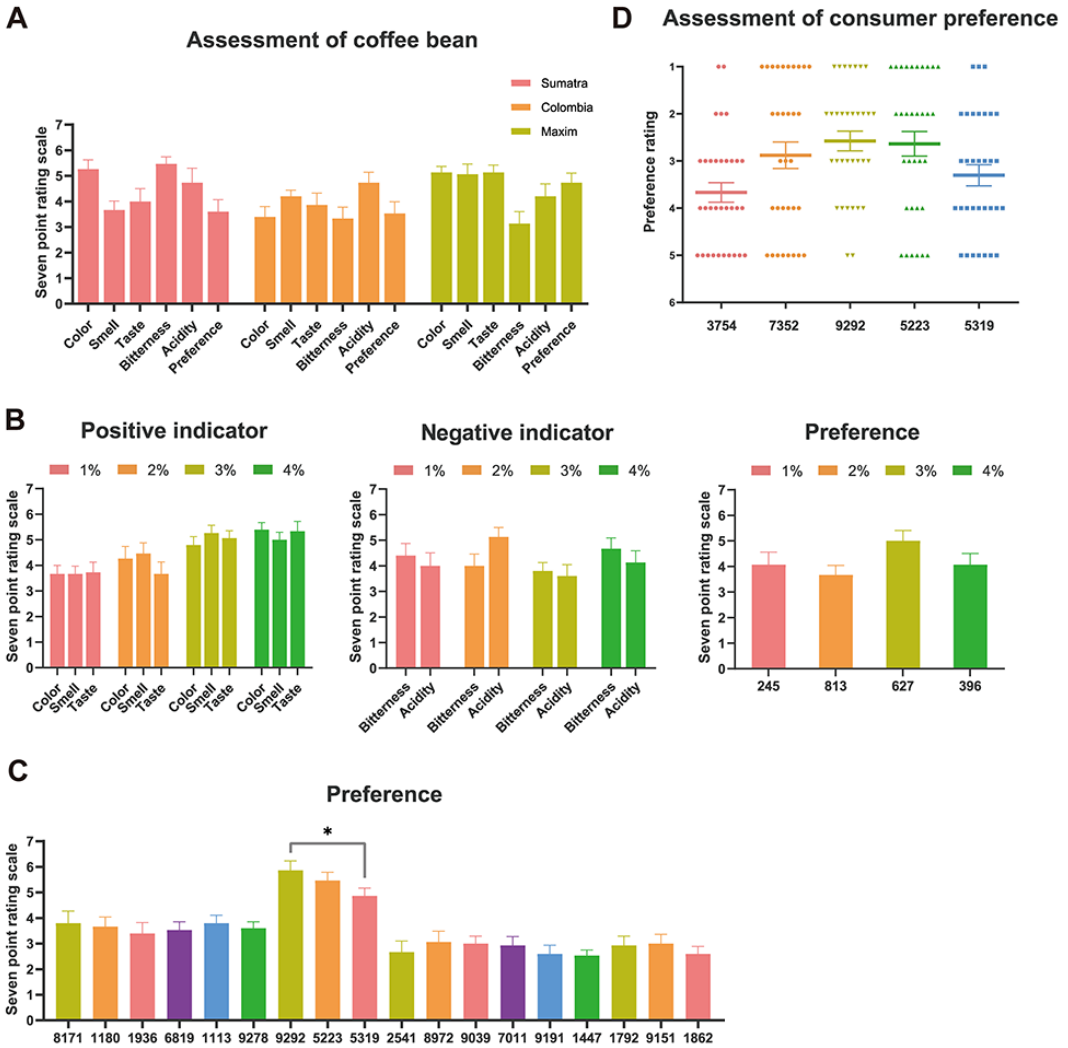

Fig. 1. Sensory evaluation of CPS219. (A-C) Sensory evaluation of CO beans with different characteristics, CO content, and preference of CPS concentration were used in the 7-point hedonic scale where dislike extremely = 1 and like extremely = 7. (D) Blind consumer test (N = 34). No.3754, CO; No.7352, CP; No.9292, CPS0.025; No.5223, CPS0.05 (CPS219); 5319, CPS0.1. *$p < 0.05$; significantly different from No.1862.

CPS219 improved SOD-like activity

To evaluate the SOD-like activity of CPS219, we performed the SOD assay. As a result, CP had lower SOD-like activity than control. CO or CPS219 significantly increased the SOD-like activity compared to the control (Fig. 2, $p < 0.05$). In addition, CPS219 significantly increased the SOD-like activity compared to the CO (Fig. 2, $p < 0.05$).

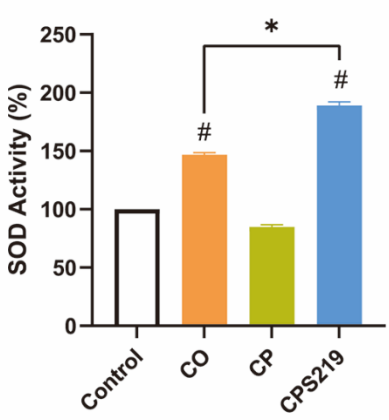

Fig. 2. CPS219 improved SOD-like activity. SOD-like activity of CO, CP, or CPS219 was measured using SOD assay kit. Each datum represents the mean ± SEM of three independent experiments. #$p < 0.05$; significantly different from the Control. *$p < 0.05$; significantly different from the CO.

CPS219 increased proliferation of MCF-7 cells

We investigated the effect of CPS219 on proliferation of MCF-7 cells (an estrogen-dependent cell line). For this purpose, MCF-7 cells were treated with CO, CP, CPS219, or E2 for 24 h or 48 h. Proliferation of MCF-7 cells was measured using a BrdU incorporation assay. Proliferation of MCF-7 cells was considerably increased by treatment with CO, CP, or CPS219 after 24 h, but only CPS219 significantly boosted the proliferation of MCF-7 cells after 48 h (Fig. 3A-B, $p < 0.05$). E2 showed a both significantly increased for 24 h and 48 h (Fig. 3A-B, $p < 0.05$). Microscopic observations showed that cells treated with CPS219 showed a higher proliferation rate compared to cells cultured in blank conditions (Fig. 3C).

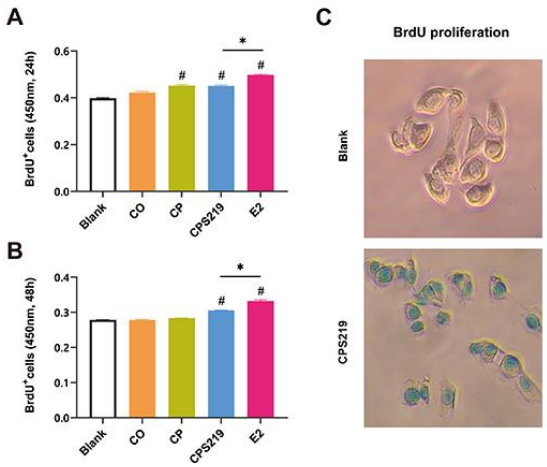

Fig. 3. CPS219 increased proliferation of MCF-7 cells. MCF-7 cells (1×10^4) were treated with CO, CP, CPS219, or E2 for (A) 24 h or (B) 48 h. Proliferation of MCF-7 cells was measured using a BrdU incorporation assay kit. (C) Optical microscopy photographs of BrdU-labeled MCF-7 cells. Each datum represents the mean ± SEM of three independent experiments. #$p < 0.05$; significantly different from the Blank. *$p < 0.05$; significantly different from the CO.

CPS219 increased ER-β mRNA expression in MCF-7 cells

Estrogen binds to ER-β to have a biological effect.[8] We examined the mRNA expression of ER-β in MCF-7 cells to see if the cell proliferation induced by CPS219 was connected to the function of ER-β. When compared to the blank, CPS219 dramatically boosted the mRNA levels of ER-β (Fig. 4, $p < 0.05$). Additionally, the mRNA levels of ER-β significantly increased in E2-treated cells (Fig. 4, $p < 0.05$).

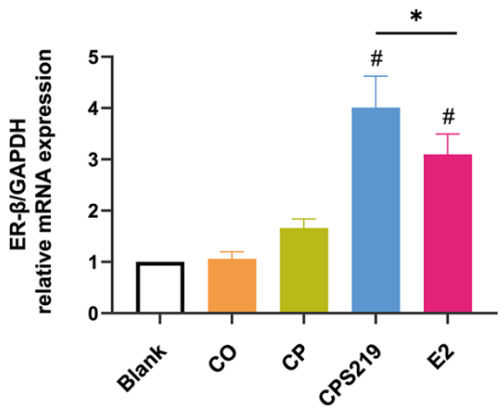

Fig. 4. CPS219 increased mRNA expression of ER-β in MCF-7 cells. MCF-7 cells (1 × 10^6) were treated with CO, CP, CPS219, or E2 for 10 h. The ER-β mRNA expression was analyzed with quantitative real-time PCR analysis. Each datum represents the mean ± SEM of three independent experiments. #p < 0.05; significantly different from the Blank. *p < 0.05; significantly different from the CO.

Effect of CPS219 on MCF-7 cell viability

Finally, we investigated the effect of CPS219 on the viability of MCF-7 cells. MCF-7 cells were treated with CO, CP, CPS219, or E2 for 24 h. Cell viability was determined using an MTT assay. The viability of cells treated with CO, CP, CPS219, or E2 was not significantly different from blank (Fig. 5).

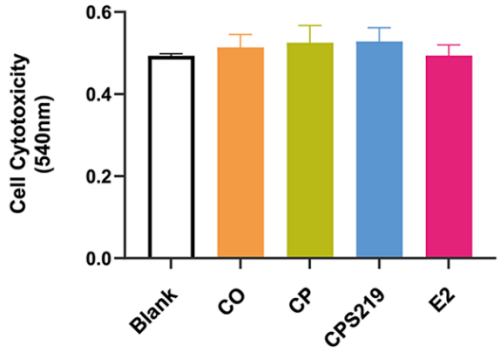

Fig. 5. Effect of CPS219 on cell viability. MCF-7 cells (1 × 10^4) were pretreated with CO, CP, CPS219, or E2 for 24 h. Cell viability was determined using an MTT assay. Each datum represents the mean ± SEM of three independent experiments.

DISCUSSION

Food qualities including color, smell, and taste may all be accurately measured using sensory evaluation in a controlled setting.[19] These data are critical to demonstrating that sensory evaluation ensures product success by understanding and connecting consumers with the product.[20] This sensory evaluation was used to find the optimal ratio of CPS219. In the present study, we showed that preference was highest when the contents of CO, PF, and BS were 3%, 0.3%, and 0.05%, respectively. In addition, we found that while external factors (color and smell) and rich flavor (taste) are important in determining CO beans and their content, bitterness and acidity have a strong and direct effect. Therefore, we propose that 1:0.1:0.017 was the ideal CO, PF, and BS ratio.

The SOD enzyme regulates the amounts of different reactive oxygen species, limiting their potential toxicity and regulating significant parts of cell longevity governed by their signaling roles.[21] Reactive oxygen species (ROS) are unstable and highly reactive factors that contain free radicals generated by lack of electrons, and affect various physiological processes ranging from egg maturation, fertilization, embryonic development, and pregnancy.[22,23] Increased ROS in the body cause oxidative stress (OS), which triggers apoptotic pathways in the ovaries and causes diseases that adversely affect the female reproductive system, such as menopause.[23,24] DNA damage in the ovarian epithelium caused by this OS can be prevented with anti-oxidant.[23] Isoflavones and triterpenoid saponins fractionated from PF have been reported to exert various pharmacological actions, including

protection against the effects of alcohol metabolism,[25] and BS has been reported to be a unique anti-oxidant that protects cells from the damaging effects of reactive oxygen species and toxic metabolites.[26] Our results showed that CPS219 significantly increased the SOD-like activity, suggesting that CPS219 has an anti-oxidant effect in menopause.

In terms of chemical composition, phytoestrogens and mammalian estrogen, E2, are quite comparable.[27] E2 is a key regulator of differentiation, growth, and function in target tissues, including mammary glands, skeletal systems, and reproductive tracts.[28] The main biological actions of E2 are mediated via intracellular receptors, ER-α and ER-β.[29] Under physiological circumstances, the amounts of ER-β expression and estrogen play a role in how estrogen behaves.[30-32] In this study, we showed that CPS219 significantly promotes the proliferation of MCF-7 cells. Moreover, CPS219 increased the ER-β mRNA expression. These results indicate that CPS219 acts as a functional estrogen ligand and promotes the proliferation of MCF-7 cells. Therefore, we suggested that CPS219 has a potent estrogenic effect through the ER-mediated signaling pathways.

In conclusion, our findings revealed that CPS219 has anti-oxidant and estrogen-like effects (Fig. 6). Therefore, we suggest that CPS219 may be a potent functional food that can be utilized to treat menopausal disorders.

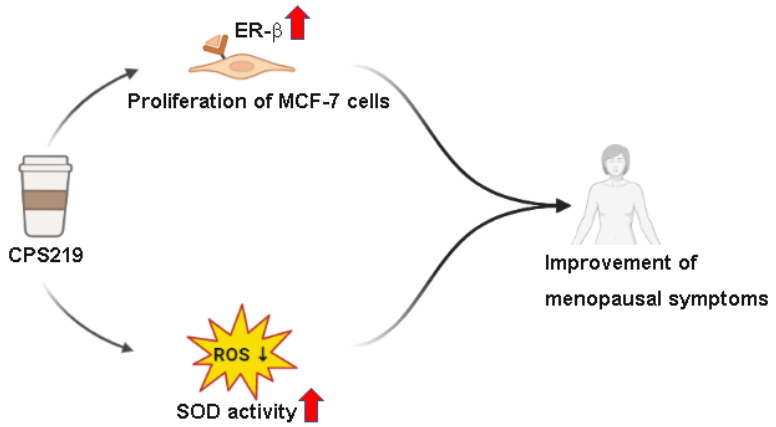

Fig. 6. Schematic diagram of CPS219. CPS219 will improve menopausal symptom through the increasing the anti-oxidant and estrogen-like properties.

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None

CONFLICT OF INTEREST

The authors have no conflicting financial interests.

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