Protective role of soybean extract from H_2O_2-induced oxidative stress and cell death in human keratinocyte HaCaT Cells

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Abstract

Oxidative stress is closely related to incidence of various skin diseases. The soybean has been known as beneficial food that is demonstrated to possess a variety of antioxidants. Here, we examined whether soybean extract had a protective role against oxidative stress-induced cell death in human keratinocyte HaCaT cells. Firstly, we determined bioactive components in the soybean extract (Cheongja-3 black soybeans) using ultra performance liquid chromatography (UPLC) analysis. We found that 1.38 μg of γ-tocopherol and 9.738 ng of lutein existed in 100 mg of soybean extract. Soybean-treatment markedly decreased hydrogen peroxide-generated intracellular reactive oxygen species (ROS) levels at 100 μg/ml without no cytotoxicity. Furthermore, soybean extract protected cells from H_2O_2-induced cell death by assessment of MTT assay and caspase activities. From these results, antioxidant function of soybean extract could be potential for the protection of human keratinocytes HaCaT cells from oxidative stress and triggered cell death.

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Nutritional contents in three varieties of soybean extracts

	Newly developed Korean soybeans	α-T3 (mg/100g)	γ-T (mg/100g)	Lutein (mg/100g)
	Saedanbaek	1.073	2.592	0.001
	Daechan	ND	ND	ND
	Cheongja-3	ND	1.382	0.010

* α-T3: alpha-tocotrienol, γ-T: gamma-tocopherol

Methods

Cell Culture
HaCaT cells were cultured in Dulbecco modified eagle medium (DMEM) containing 10% Fetal Bovine Serum, and kept in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

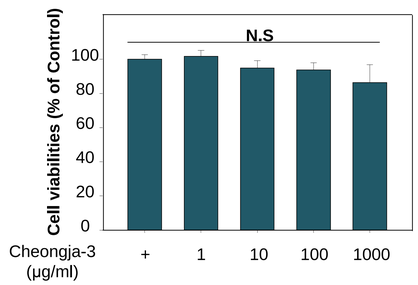
Preparation of soybean extract
Each soybean was extracted in 40% ethanol, and evaporated and dried for use.

Intracellular reactive oxygen species (ROS) levels
Cells were stained with 10 μM DCF-DA for 30 min, and treated with indicated concentration of soybean extract in the presence or absence of hydrogen peroxide (H_2O_2) for further 3 hr. Then, the fluorescence were detected by fluorometric microplate reader at ex/em=485/535.

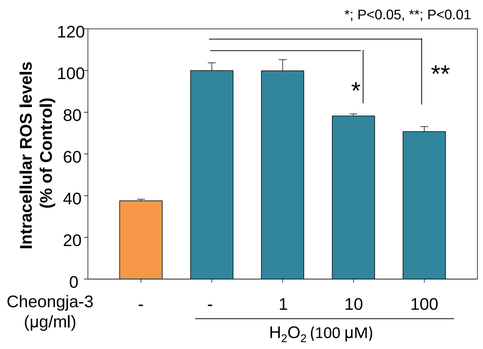
Western blot
Protein samples were loaded on SDS-PAGE, and transferred to PVDF membrane. Then, the membranes were blocked with 5% skim milk, and incubated with primary antibodies (1:1000). The next day, the membranes were incubated with secondary antibody conjugated with horse radish peroxidase (HRP). The results were visualized using enhanced chemiluminescence .

Results

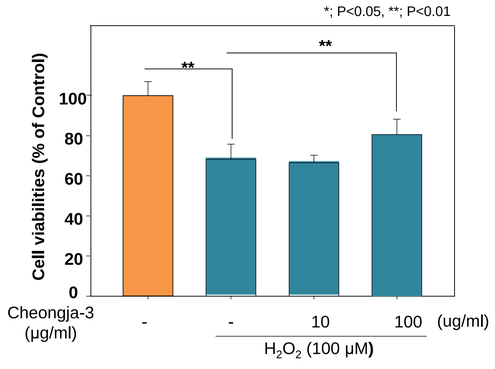
Effects of soybean extract on HaCaT cell viabilities



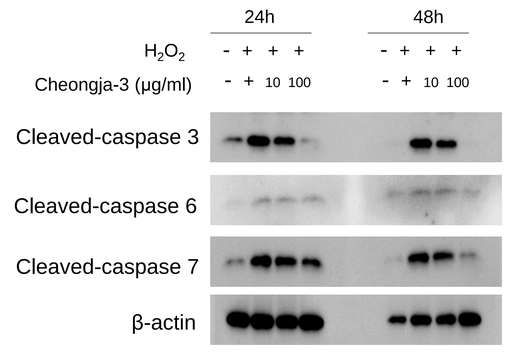
Reduced ROS levels by Cheongja-3



Protective effects of Cheongja-3 from oxidative stress-induced cell death



Protective effects of Cheongja-3 from oxidative stress-induced cell death



Summary & Conclusions

- Cheongja-3 soybean extract which is containing high amounts of antioxidants had protective effects from oxidative stress-induced cell death in HaCaT human keratinocytes.
- **Supported by Rural Development Administration (PJ011885), Republic of Korea**