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Investigations of antioxidative activity against oxidative stress induced by H_2O_2 in PC-12 neuronal cells from enzymatic hydrolysates of *Phellinus linteus*

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상황버섯 효소 가수분해 추출물의 항산화성 및 신경세포 보호효과에 관한 연구

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Objectives

Phellinus linteus has been used as a traditional medicine in Korea, China, Japan and other Asian countries for the treatment of various diseases, including oral ulcer, gastroenteric disorder, lymphatic disease and other cancers. It has been reported that *Phellinus linteus* showed anti-inflammation, and anti-angiogenesis effects, stimulating humoral and cell-mediated immunity, as well as inhibiting tumor growth and metastasis. In this study suggest that enzymatic extracts of *Phellinus linteus* possess antioxidative activity.

Materials and Methods

○ Materials

Phellinus linteus was sourced from a local market (Chungju, Korea). In addition PC-12 Cells was obtained from Pukyong National University.

○ Methods

Free radical scavenging DPPH radical - A sample solution of 30 μL of each enzymatic extracts, was added to 30 μL of DPPH (30 μM) in methanol solution. After mixing vigorously for 10 sec, the solution was then transferred into a 100 μL Teflon capillary tube, and the scavenging activity of each enzymatic extract on DPPH radical was measured using a JES-FA ESR spectrometer (Jeol Ltd., Tokyo, Japan).

Alkyl radical - Alkyl radicals were generated by AAPH. The phosphate-buffered saline (PBS, pH 7.4) reaction mixtures containing 10 mM AAPH, 10 mM 4-POBN, and indicated concentrations of tested samples were incubated at 37℃ in a water bath for 30 min and then transferred to a 100 μL teflon capillary tube. The spin adduct was recorded on an ESR spectrometer.

Flow cytometer For sub-G1 and cell cycle analysis, PC-12 cells were suspended in ethanol with 0.5% Tween-20 and left for 24 hr at 4℃. The cells were harvested by centrifugation and resuspended in 1.0 mL of PBS with 0.05 mg/mL of propidium

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iodide and 10 μg/mL of RNase A, and incubated at 37℃ for 30 min. The analysis of apoptotic cell

death was performed by measuring the hypodiploid DNA contents using a flow cytometer (FACS-caliber; Becton Dickinson, NJ, USA). The cells in sub-G1 population was considered as apoptotic cells and percentage of each phase of cell cycle was determined.

Results

The *Phellinus linteus* were enzymatically hydrolyzed by 7 carbohydrases (Dextrozyme, AMG, Promozyme, Maltogenase, Termamyl, Viscozyme and Celluclast). The DPPH radical scavenging activity of Celluclast extracts was the highest, and the IC_{50} values was 823 μg/mL. The Dextrozyme extracts showed the highest alkyl radical scavenging activity, and the IC_{50} values was 713 μg/mL. In addition, the Dextrozyme extracts decreased cell death in PC-12 cells against H_2O_2-induced oxidative damage.

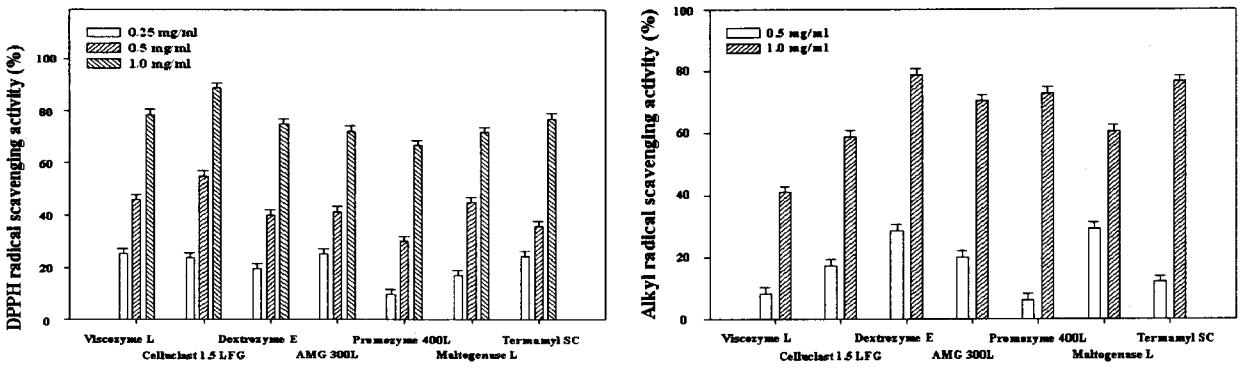

Fig 1. DPPH (left) and alkyl (right) radical scavenging activity of various enzymatic extracts by carbohydratic hydrolysis from *Phellinus linteus*

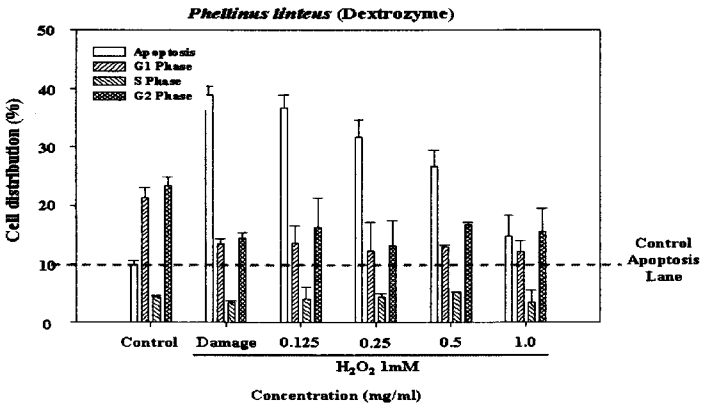

Fig 2. Cell death and cell cycle of PC-12 after treating the Dextrozyme extracts from *Phellinus linteus* prior H_2O_2 treatment.