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Measurement of free radical scavenging activity and neuroprotective effect by oxidative damage from various enzymatic extracts of *Inonotus obliquus*

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Objectives

Most mushrooms are rich in vitamins, fiber, and amino acids and low in fat, cholesterol, and calories. These mushrooms contain a large variety of biologically active polysaccharides with immunostimulatory properties, which contribute to their anticancer effects. *Inonotus obliquus* used as a folk medicine in Asia, Europe and most of the another countries. In this study suggest that enzymatic extracts of *Inonotus obliquus* possess antioxidative activity.

Materials and Methods

○ Materials

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

○ Methods

Free radical scavenging activity 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 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

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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 *Inonotus obliquus* 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} value was 52 μg/mL. The Maltogenase extracts showed the highest alkyl radical scavenging activity, and the IC_{50} value was 79 μg/mL. In addition, the Maltogenase extracts decreased cell death in PC-12 cells against H_2O_2-induced oxidative damage.

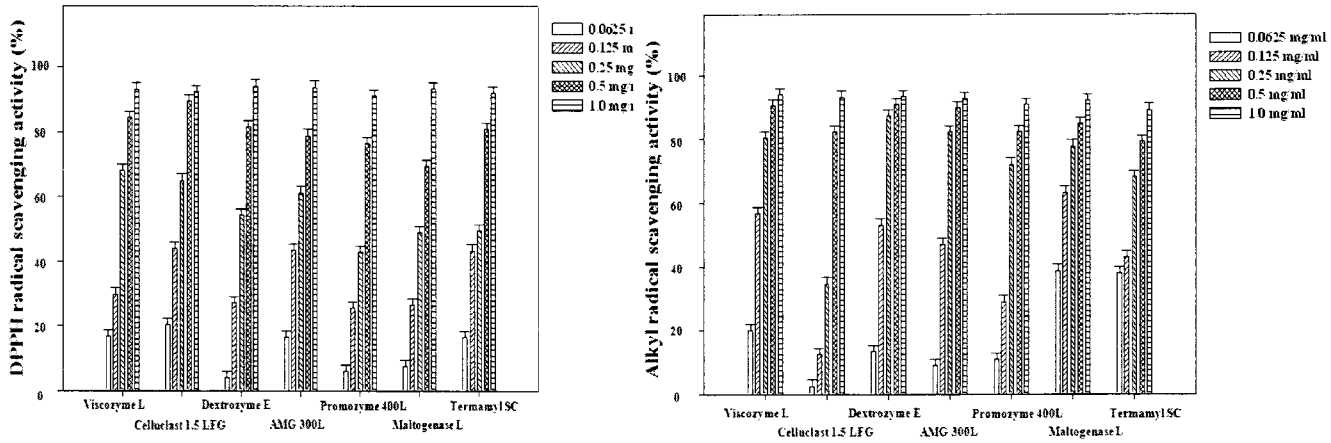

Fig 1. DPPH (left) and alkyl (right) radical scavenging activity of various enzymatic extracts by carbohydratic hydrolysis from *Inonotus obliquus*

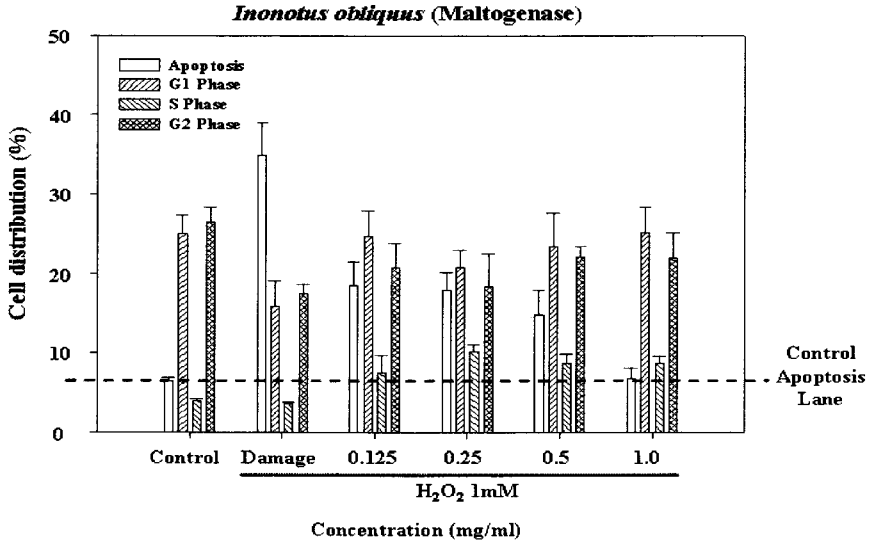

Fig 2. Cell death and cell cycle of PC-12 after treating the Maltogenase extracts from *Inonotus obliquus* prior H_2O_2 treatment.