Antioxidant Activity of Flavonoids-rich Fractions of Jakwangchalbyeo, Chalbyeo and Ilpumbyeo in H4ⅡE cells

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Objectives

Experiments were performed to investigate antioxidant activity of flavonoid-rich fractions of rice (Jagkwangchalbyeo, Chalbyeo and Ilpumbyeo) extracts in H4ⅡE cells.

Materials and Methods

- **Materials** : Flavonoid-rich fractions of Jagkwangchalbyeo, Chalbyeo and Ilpumbyeo that were cultivated in Konkuk University experimental farm in year of 2002.
- **Cell viability Assay (MTT assay)** : H4ⅡE cells were plated at a density of 4×10^4 cells/well in a 96-well plate. Cells were exposed to 1 mM hydrogen peroxide (H_2O_2) containing medium for 1 hr under 5% CO_2 at 37℃. Then, various concentration of each extracts (10 *u*g/mL, 25 *u*g/mL, 50 *u*g/mL, and 100 *u*g/mL, respectively) were added into the well and incubated for 1, 3, 5, 24 hours. The cell viability was assessed by measuring the degree of formazan crystal formation by MTT assay method.
- **Measurement DCFH-DA oxidation by FACS** : H4ⅡE cells (1×10^6 cells/mL) were washed with PBS. Then, 2', 7'-dichlorofluorescein diacetate (DCFH-DA) was added to 1 mL of the cell suspension, and incubated in 37℃ water-bath for 10 min. Then, the cells were treated with 100 *u*g/mL rice extracts dissolved in DMSO, in the presence of or in the absence of 250 *u*M H_2O_2. Anti-oxidant activity of rice extracts were evaluated by measuring the generation of DCF fluorescence in each samples through FACS analysis.

Results and Discussion

- MTT assay was performed to choose a optimal concentration of H_2O_2 and a proper exposer time for challenging to cells. Results showed that approximately 57 % of total cell population was viable when exposed to 1 mM H_2O_2 for one hour (Fig. 1).
- Chalbyeo extract and Ilpumbyeo extract did not cause any anti-oxidant effect on the H_2O_2-induced oxidative stress (Fig. 3, 4). However, It was shown that Jakwangchalbyeo extract recovered the cell viability upto 74% at concentration of 100 ug/mL for 24 h treatment, suggesting that Jakwangchalbyeo extract was able to suppress the H_2O_2-induced cell death (Fig. 2).
- Generation of DCF fluorescence increased significantly in the cells treated with H_2O_2 or in the cells treated with DMSO plus H_2O_2 (Fig. 5). Whereas, generation of DCF fluorescence decreased drastically by the treatment of rice extracts dissolved in DMSO in the presence of H_2O_2 (Fig. 6). These result indicated that rice extracts itself was able to inhibit the generation of DCF fluorescence, implying its anti-oxidant activity

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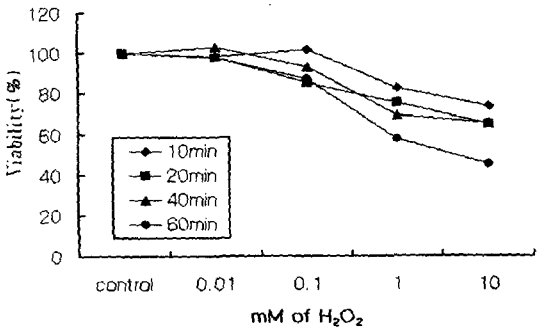

Figure 1. Effect of hydrogen peroxide (H_2O_2) on H4ⅡE cell viability

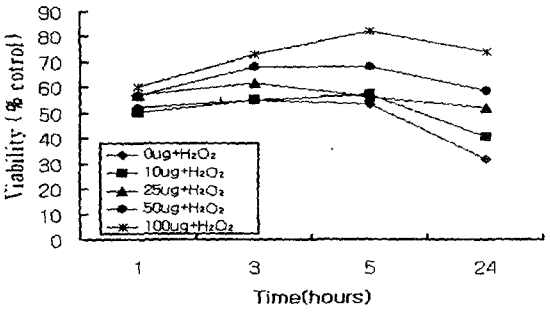

Figure 2. Effect of Jakwangchlbyeo extract in the presence of H_2O_2 on the viability of H4ⅡE cell

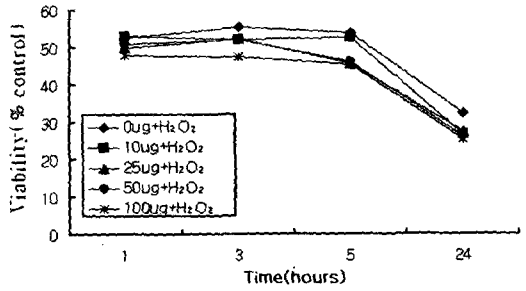

Figure 3. Effect of Chalbyeo extract in the presence of H_2O_2 on the viability of H4ⅡE cell.

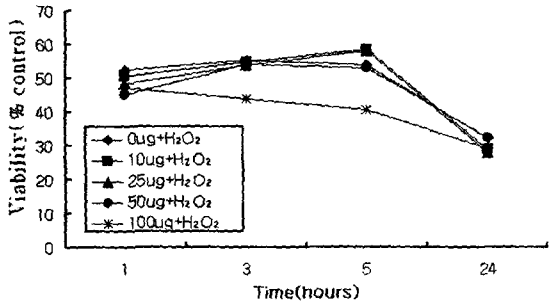

Figure 4. Effect of Ilpumbyeo extract in the presence of H_2O_2 on the viability of H4ⅡE cell

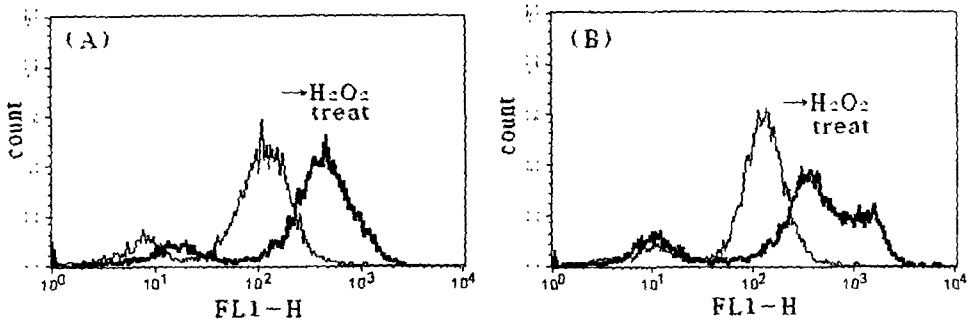

Figure 5. FACS analysis of DMSO-mediated anti-oxidant activity in H4ⅡE cell after DCFH-DA treatment. (A) H4ⅡE cells with no treatment and H4ⅡE cells treated with H_2O_2. treatment. (B) H4ⅡE cells treated with DMSO and H4ⅡE cells treated with DMSO plus H_2O_2.

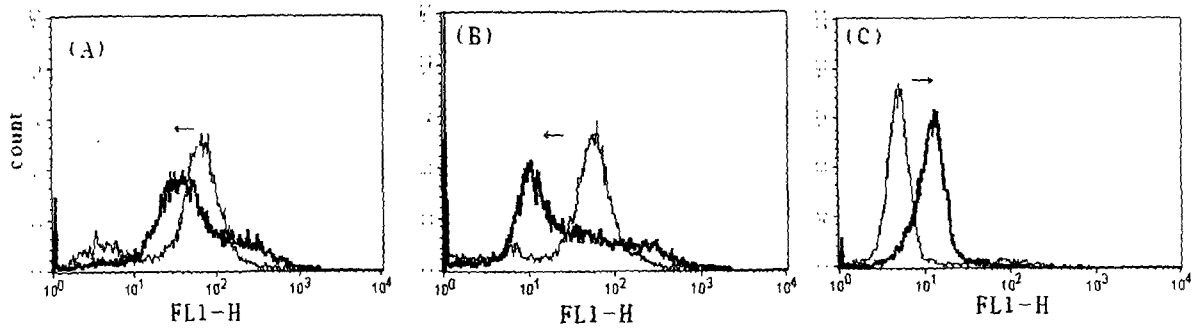

Figure 6. FACS analysis of rice extracts-mediated anti-oxidant activity in H4ⅡE cell after DCFH-DA treatment. (A) H4ⅡE cells with Jakwangchalbyeo extract treatment and H4ⅡE cells treated with Jakwangchalbyeo extract plus H_2O_2. (B) H4ⅡE cells with Chalbyeo extract treatment and H4ⅡE cells treated with Chalbyeo extract plus H_2O_2. (C) H4ⅡE cells with Ilpumbyeo extract treatment and H4ⅡE cells treated with Ilpumbyeo extract plus H_2O_2.