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RESEARCH NOTE

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Dandelion (*Taraxacum officinale*) Flower Ethanol Extract Inhibits Cell Proliferation and Induces Apoptosis in Human Ovarian Cancer SK-OV-3 Cells

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Abstract This study investigated the proapoptotic effect of ethanol extracts obtained from dandelion (*Taraxacum officinale*) flower on human ovarian cancer SK-OV-3 cells. Cells were treated with dandelion flowers ethanol extract (DFE) ranging from 1.5625 to 100 μg/mL for 24 hr. Significant antiproliferative effects of DFE were first observed from at 6.25 μg/mL ($p<0.05$), and this inhibition showed in a dose-dependent manner. When cells were treated with more than 6.25 μg/mL DFE, cell-cycle analysis showed that DFE caused an increase in the percentage of sub-G0/G1 cells and arrested at the S and G2/M phase in a dose-dependent manner. Moreover, apoptosis induction by DFE involved p53 activation and bax upregulation as well as downregulation of bcl-2. Our findings indicate that DFE resulted in apoptotic cell death, suggesting that DFE possesses potential anticancer properties.

Keywords: dandelion, *Taraxacum officinale,* apoptosis, cell proliferation

Introduction

In recent, natural phytochemicals therapies are accepted as common form of medicine, and epidemiological and experimental studies have demonstrated that traditional herbs have decreased the incidence of certain forms of cancer (1,2). Herbal remedies are now becoming widely accepted that such botanical components can make an important contribution to human health.

Dandelion (*Taraxacum officinale*) is well-known as a folk medicine and home remedy with various reported health benefits. In Korea, dandelion has been used to treat women's diseases such as breast and uterine cancer (3,4). It has been reported that dandelion juice contains more than 30 phenolic compounds (5). Previous studies have also demonstrated that some dandelion have physiological effects that benefit human health (6,7). Furthermore, dandelion has been found to possess marked antioxidant activity in both biological and chemical models (8,9) and to inhibit the proliferation of several types of cancer cells (10,11). These studies suggest that the effects of dandelion seem to be associated with its phenolic acids and flavonoids.

Such phenolic acids and flavonoids, which are considered a new category of chemotherapeutic agents, have demonstrated anticancer activity through the induction of apoptosis (12-14). Apoptosis is an important series of events that leads to programmed cell death and is essential for the development and homeostasis of tissues. The potential mechanisms for the apoptotic process involve the balance between factors of apoptosis induction and apoptosis inhibition. Recently, regulation of apoptosis has been proposed as a promising target for cancer chemotherapy (15,16). The present study was designed to investigate the proapoptotic effects of dandelion flowers ethanol extract (DFE) on human ovarian cancer SK-OV-3 cells.

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Materials and Methods

Preparation of ethanol extract from dandelion flowers (DEF) Dandelion flowers were purchased from a Kyungdong drugstore in Seoul, Korea. The shade-dried powder of flowers was extracted in a Soxhlet extracter with 70% ethanol. The collected ethanol supernatant was evaporated in a rotary evaporator (NYC-2000; Eyela, Tokyo, Japan) under reduced pressure. In addition, the remaining ethanol was dried in desiccators with a high vacuum pump (W2v40; Woosung Automa Co., LTD., Gyeonggi-do, Korea). This dehydrated powder was diluted in dimethyl sulfoxide (DMSO) to 10 mg/mL just before use.

Cells culture Human ovarian cancer SK-OV-3 cells were purchased from the KCLB (Korean Cell Line Bank, Korea). Cells was routinely maintained in RPMI 1640 (Invitrogen [Molecular Probes], Gibco, Carlsbad, CA, USA), supplemented with 10% fatal bovine serum (FBS) and antibiotics such as 50 U/mL of penicillin and 50 mg/mL streptomycin (Gibco) at 37°C in a humidified atmosphere containing 5% CO_2. In cell proliferation experiments, cells were treated with DFE at various concentrations (1.5625, 3.125, 6.25, 12.5, 25, 50, and 100 μg/mL) or vehicle alone for 24 hr. In apoptosis assay, cells were treated with DFE at 6.25, 12.5, 25, 50, and 100 μg/mL or vehicle alone for 24 hr.

Determination of cell proliferation The inhibitory effect of the DFE on cell proliferation was determined by the methyl thiazolyl tetrazolium (MTT) assay. The cells exposed to DFE were added to MTT. Four hr later, DMSO was added to each well to dissolve the resulting formazan

crystals and then absorbance was recorded at 490 nm in a microplate reader (SpectraMax Plus; Molecular Devices, Sunnyvale, CA, USA).

Cell cycle distribution Cells were harvested, washed with cold phosphate buffered saline (PBS), and processed for cell cycle analysis. Briefly, the cells were fixed in absolute ethanol and stored at –20°C for later analysis. The fixed cells were centrifuged at 800×g and washed twice with cold PBS. RNase A (20 μg/mL final concentration) and propidium iodide staining solution (50 μg/mL final concentration) were added to the cells and incubated for 30 min at 37°C in the dark. The cells were analyzed on a FACS Calibur instrument (BD Biosciences, San Jose, CA, USA) equipped with CellQuest 3.3 software. ModFit LT 3.1 trial cell cycle analysis software was used to determine the percentage of cells in the different phases of the cell cycle.

Immunoblotting assay Cells were lysed in radio-immunoprecipitation assay buffer [1% nonidet P-40, 150 mM NaCl, 0.05% 4-chloro-2,5-dimethoxyamphetamine (DOC), 1% sodium dodecyl sulfate (SDS), 50 mM Tris, pH 7.5] containing protease inhibitor for 1 hr at 4°C. The supernatant was separated by centrifugation, and protein concentration was determined by Bradford protein assay kit II (Bio-Rad Laboratories, Hercules, CA, USA). Proteins (25 μg/well) denatured with sample buffer were separated by 10% SDS-polyacrylamide gel. Proteins were transferred onto nitrocellulose membranes (0.45-μm). The membranes were blocked with a 1% BSA solution for 3 hr and washed twice with PBS containing 0.2% Tween-20, and incubated with the primary antibody overnight at 4°C. Antibodies against $p21^{Cip1}$, p53, bax, bcl-2, and β-actin were purchased from Santa Cruz (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and used to probe the separate membranes. The next day, the immunoreaction was continued with the secondary goat anti-rabbit horseradish-peroxidase-conjugated antibody after washing for 2 hr at room temperature. The specific protein bands were detected by Opti-4CN Substrate kit (Bio-Rad).

Statistical analyses Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test (Sigma Stat; Jandel, San Rafael, CA, USA). For all comparisons, differences were considered statistically significant at $p<0.05$.

Results and Discussion

It has been reported that rich flavons such as luteolin and luteolin 7-glucoside exist in the dandelion (5,8,9), which contributed to noted *in vitro* antioxidant and anticancer activity (14,17). These flavones are also showed the biological activity without introducing cytotoxicity (8).

In the present study, the antiproliferative effects of DFE were investigated on SK-OV-3 cells. Human ovarian cancer SK-OV-3 cells were used because we wanted to investigate the estrogen-independent effects of DFE. SK-OV-3 cells are reported to be estrogen-unresponsive because they express a truncated, dysfunctional ERa, and were described to express very low levels of ERb (18,19).

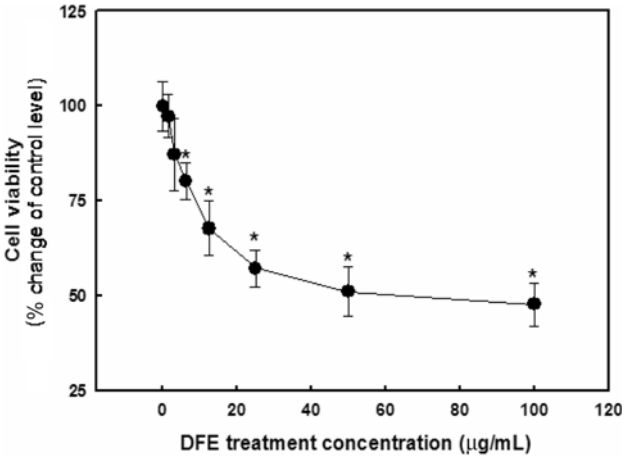

Fig. 1. Effect of DFE on cell proliferation of human ovarian cancer SK-OV-3 cells. Cells were exposed to DFE at various concentrations (1.5625, 3.125, 6.25, 12.5, 25, 50, and 100 μg/mL) and incubated for 24 hr. All data are reported as the % change in comparison with the vehicle-only group, which were arbitrarily assigned 100% viability. *$p<0.05$, significantly different from the vehicle-only group (only solvent without DFE).

When cells were exposed to various concentrations of DFE ranging from 1.5625 to 100 μg/mL for 24 hr, DFE showed antiproliferative activity in a dose-dependent manner (Fig. 1). This finding is consistent with previous reports indicating that dandelion may be an effective agent against cell growth in some types of cancer cells (10,11). Thus DFE apparently has antiproliferative effects and exhibits anticancer activity.

Then after, to investigate the apoptosis induced by DFE, cell-cycle was analyzed in SK-OV-3 cells exposed to DFE at over 6.25 μg/mL, the concentration that showed the first significant effect. In the present study, DFE caused G1/S transition together with S and G2/M phase progression (Fig. 2A). After exposure to 25, 50, and 100 mg/mL DFE for 24 hr, we observed that the proportion of SK-OV-3 cells in the G1 phase decreased by 42.3, 37.5, and 35.2% compared with control value 61.3% and the proportion of S-phase cells increased by 28.5, 30.1, and 29.5% compared with control value 13.3%, respectively. This decrease was accompanied by an increase in the number of G2/M phase cells.

In the present study, the DFE-induced accumulation of the cell population at either the S or G2/M phase resulted in apoptosis induction. DFE led to DNA accumulation in the sub-G0/G1 phase. A significant increase in the cell population of sub G0/G1 phase was seen with DFE treatment at concentrations of 25, 50, and 100 μg/mL (15.2, 26.4, and 41.0% of the cell population compared with control value 1.2±0.2%, $p<0.05$).

To further examine DFE-induced apoptosis, p53 activation was investigated in SK-OV-3 cells, which demonstrated significant cell-cycle arrest and sub-G0 DNA fragmentation. The tumor suppressor gene p53 is regarded as a key factor in a balance between cell survival and cell death via regulation of both the G_1 and G_2/M portions of the cell cycle (20). Activation of p53 in response to DNA damage led to cell cycle arrest and inhibition of cell proliferation

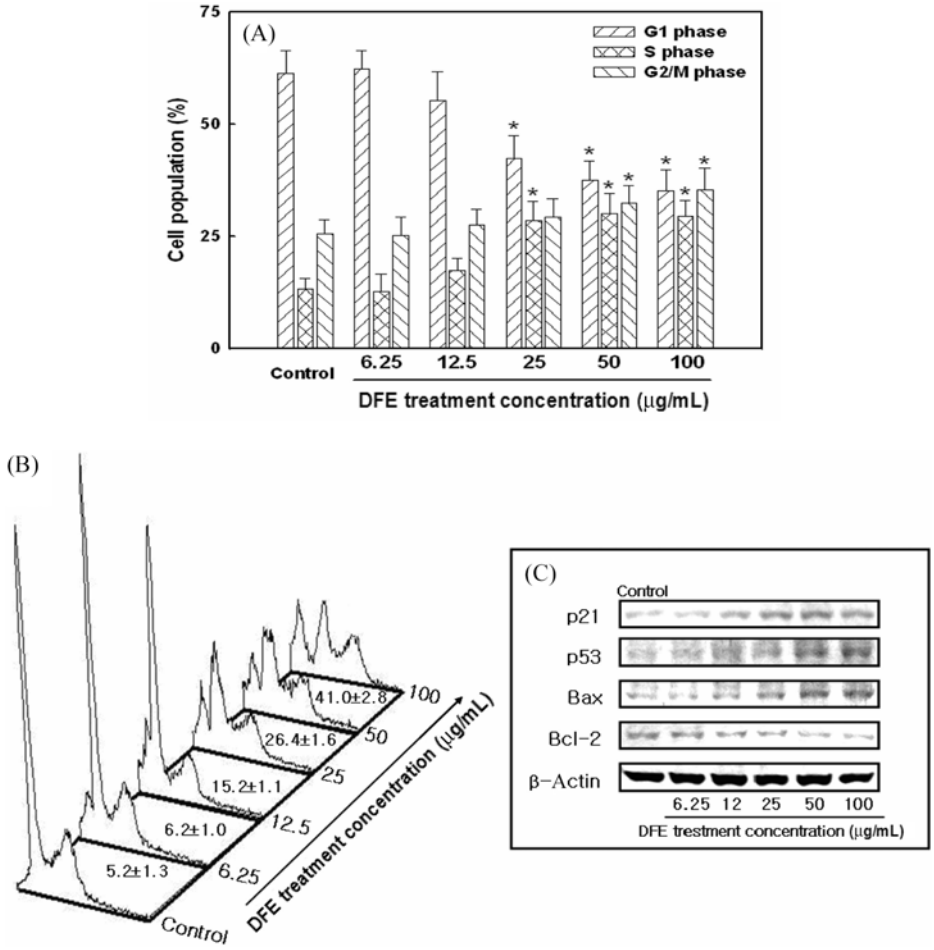

Fig. 2. Effect of DFE on cell cycle arrest (A), sub-G1 cell population (B), and apoptosis-related gene expression (C) of human ovarian cancer SK-OV-3 cells. Cells were exposed to DFE at various concentrations (6.25, 12.5, 25, 50, and 100 μg/mL) and incubated for 24 hr. Values are expressed as percentage of the cell population in the G1, S, and G1/M phase of cell cycle. *$p<0.05$, significantly different from the vehicle-only group (only solvent without DFE).

(21-23). In the present study, DFE exposure increased p53 expression, suggesting that activation of p53 plays a role in the DFE-induced cell-cycle arrest at S and G2/M phase and apoptosis of SK-OV-3 cells (Fig. 2C). Moreover, DFE treatment resulted in an increase in $p21^{Cip1}$ expression as a dose-dependent manner. The $p21^{Cip1}$, a down-stream target of p53, could inhibit the G1-S phase transition and result in G1 phase cell cycle arrest (24-26).

In the present study, modulation of BCl-2 and Bax was observed after exposure to DFE in dose-dependent manner (Fig. 2C). Members of the Bcl-2 family of proteins are critical regulators of the apoptotic pathway, controlling mitochondrial permeability and cytochrome c expression (27,28). These proteins consist of the major antiapoptotic family members, Bcl-x(L) and Bcl-2, and the proapoptotic proteins, Bax and Bak.

Based on our results, we suggest that p53-dependent pathways activated by DFE result in apoptotic cell death of human ovarian cancer SK-OV-3 cells. The apparent anticancer activities of DFE might contribute to cancer chemotherapy. However, further studies are needed to isolate the active components and to elucidate the exact mechanism of action *in vitro* and *in vivo*.

Acknowledgments

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