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Research Note

Cytotoxic Effects of Strawberry, Korean Raspberry, and Mulberry Extracts on Human Ovarian Cancer A2780 Cells

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ABSTRACT: Reactive oxygen species are tumorigenic by their ability to increase cell proliferation, survival, and cellular migration. The purpose of the present study was to compare the antioxidant activity and cytotoxic effects of 3 berry extracts (strawberry, Korean raspberry, and mulberry) in A2780 human ovarian carcinoma cells. Except for raspberry, the ethyl acetate or methylene chloride fractions of berries containing phenolic compounds exerted dose dependent free radical scavenging activities. In the raspberry fractions, the hexane fraction also exhibited potent antioxidant activity. The cytotoxic effects of berries extracts in A2780 human ovarian carcinoma cells were measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Surprisingly, co-treatment with *n*-butanol (BuOH) fractions of berries showed stronger cytotoxic effects compared to the other fractions. These findings suggest that potent anticancer molecules are found in the BuOH fractions of berries that have stronger cytotoxic activity than antioxidants.

Keywords: antioxidant, berry, ovarian cancer, cytotoxic effect

INTRODUCTION

Oxidative stress is defined as an imbalance between production of free radicals and antioxidants, which are substances that eliminate oxidative stress by protective mechanisms (1). One of the pivotal characteristics of tumor cells is their increased ability to survive compared with normal cells (2). Reactive oxygen species (ROS) are reported to be tumorigenic by their ability to increase cell proliferation, survival, and cellular migration (3). ROS can induce DNA damage, leading to genetic lesions that initiate tumorigenicity, and subsequent tumor progression (4).

Ovarian cancer is one of the most common types of cancer in females. Prevention of ovarian cancer is important because females with ovarian cancer may have not only concerns about their sexual health but also fertility problems (5). Earlier findings have shown that antioxidants such as vitamins C, E, β-carotene, selenium, lutein, and lycopene significantly reduced the risk of ovarian cancer (6-8). Antioxidants have the potential to suppress cancer and to reduce the risk of cancer development by scavenging reactive oxygen species. In line with this notion, berries are rich sources of natural chemopreventive agents including vitamins A, C, and E, selenium, carotenoids, anthocyanins, flavonols, flavanols, proanthocyanidins, ellagitannins, and phenolic acids that have anticancer effects (9).

Strawberry, Korean raspberry, and mulberry extracts are known to have anticancer effects on cervical and breast cancer cell lines (10). Strawberry phenolic compounds inhibited the growth of human oral (CAL-27 and KB), colon (HT29 and HCT-116), and prostate (LNCaP and DU145) cancer cells (11). Strawberry extracts inhibited the growth of human colon (HCT-116), lung (A549), stomach (SNU-638), and fibrosarcoma (HT-1080) cancer cells (12). In addition, previous studies reported that dietary freeze-dried strawberries and Korean raspberries inhibited N-nitrosomethylbenzylamine (NMBA)-induced tumorigenesis in the rat esophagus (13,14). Mulberry inhibited Ehrlich ascites tumor in mice (15). Korean raspberry inhibited the growth and induced apoptosis of human cervical (HeLa, SiHa, and C-33A) cancer cells.

Berries including strawberry, Korean raspberry, and mulberry may have beneficial effects against oxidative stress mediated diseases such as cancer. The purpose of

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the present study was to compare the antioxidant activity and cytotoxic effects of berry extracts in A2780 human ovarian carcinoma cells.

MATERIALS AND METHODS

Preparation of extract and fractions

The dried powders (each 10 g) of strawberry, Korean respberry, and mulberry were extracted three times with methanol under reflux. The resultant extracts were combined and suspended in water, and then fractionated successively with hexane, methylene chloride (MC), ethyl acetate (EtOAc), and *n*-butanol (BuOH), leaving residual aqueous fraction. All extract and fractions were stored at refrigerator until use.

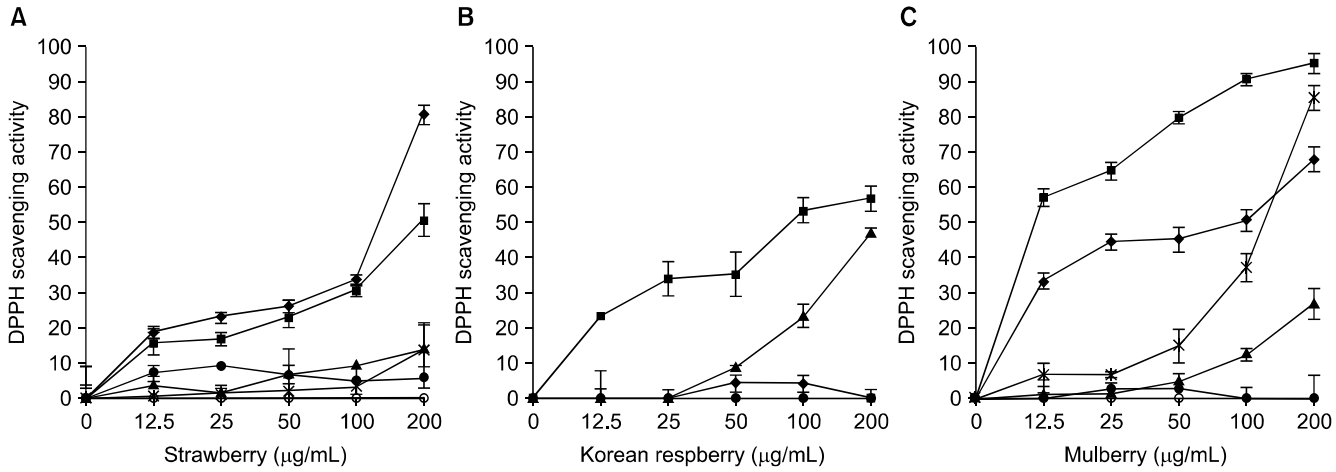

Fig. 1. DPPH radical scavenging activity of strawberry, Korean raspberry, and mulberry extracts. Berry extracts were mixed with DPPH (0.1 mM) in ethanol. After 20 min incubation at room temperature, the absorbance was read at 517 nm using a microplate reader. ▲, methanol extract; ×, hexane fraction; ●, aqueous fraction; ■, ethyl acetate fraction; ○, *n*-butanol fraction; ◆, methylene chloride fraction.

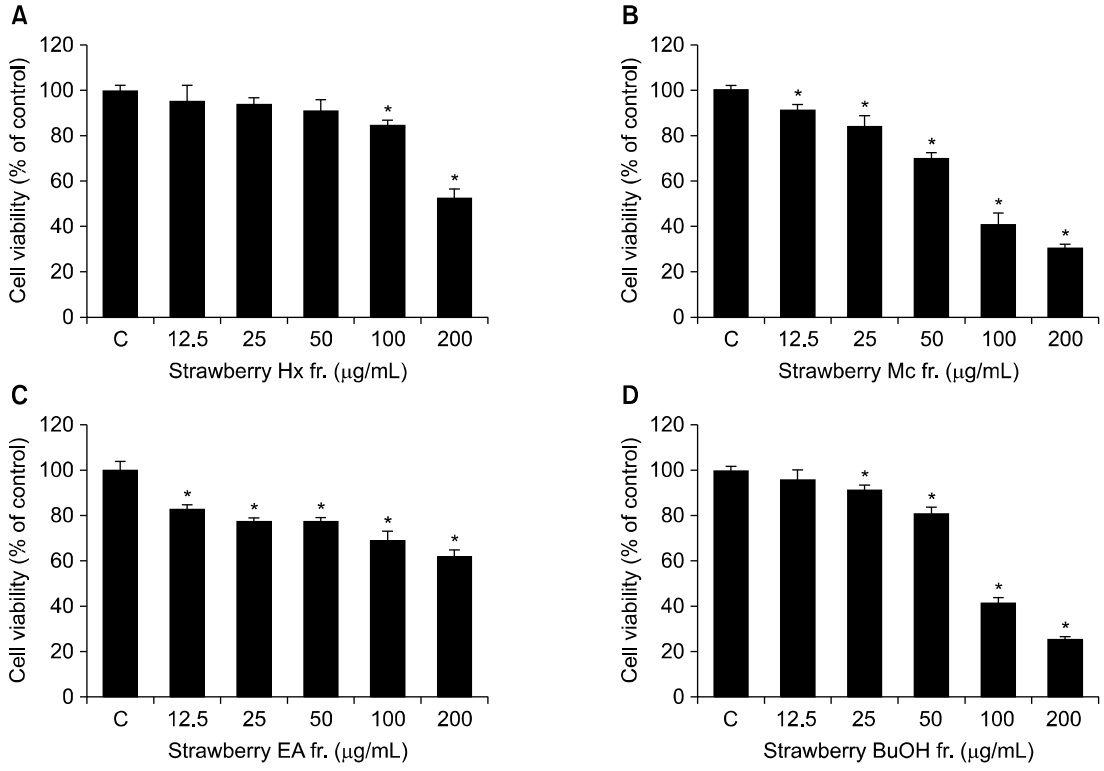

Fig. 2. Effects of strawberry extracts on cytotoxicity in A2780 human ovarian carcinoma cells. When the cells were approximately 80% confluent, they were seeded in 96-well culture plates at 1×10^4 cells per well and incubated for 24 h for adhesion. Then, the cells were treated with the control (0.5% DMSO), or indicated concentrations of berry extracts for 24 h. *Significantly different compared to the control at $P<0.05$. Hx, hexane; Mc, methylene chloride; EA, ethyl acetate; BuOH, *n*-butanol.

Chemicals

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) reagent was purchased from Sigma Aldrich Co. (St. Louis, MO, USA). The Ez-Cytox cell viability assay kit was purchased from Dail Lab Service Co. (Seoul, Korea). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen (Grand Island, NY, USA).

DPPH scavenging activity assay

DPPH scavenging activity was measured by the DPPH assay. In brief, berry extracts were mixed with DPPH (0.1 mM) in an ethanol solution. After 20 min incubation at room temperature, the absorbance was read at 517 nm using a microplate reader (PowerWave XS; BioTek Instruments, Winooski, VT, USA). Then, the DPPH scavenging activity (%) or percent inhibition was calculated using following equation:

$$\text{DPPH scavenging activity (\%) or percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 was the absorbance of the control reaction and A_1 was the absorbance in presence of the test or standard sample.

Cell culture and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay

A2780 human ovarian carcinoma cells were used to evaluate the anti-proliferative effects of berries extracts. A2780 human ovarian carcinoma cells were purchased from the American Type Culture Collection (Rockville, MD, USA), and cultured in RPMI1640 medium (Cellgro, Manassas, VA, USA), supplemented with 10% FBS, 1% penicillin/streptomycin (Invitrogen), and 4 mM L-glutamine in an atmosphere of 5% CO_2 at 37°C. Viability of the cells was determined by the Ez-Cytox cell viability detection kit. When the cells were approximately 80% confluent, they were seeded in 96-well culture plates at 1×10^4 cells per well and incubated for 24 h for adhesion. Then, the cells were treated with the control [0.5% dimethyl sulfoxide (DMSO)], or indicated concentrations of berry extracts for 24 h. After incubation, 10 μL of Ez-Cytox reagent was added to each well and incubated for 2 h. Cell viability was measured by reading the absorbance at 450 nm using a microplate reader (PowerWave XS; BioTek Instruments).

Statistical analysis

Statistical significance was determined through analysis of variance (ANOVA) followed by a multiple comparison test with a Bonferroni adjustment. *P*-values of less than 0.05 were considered statistically significant. The

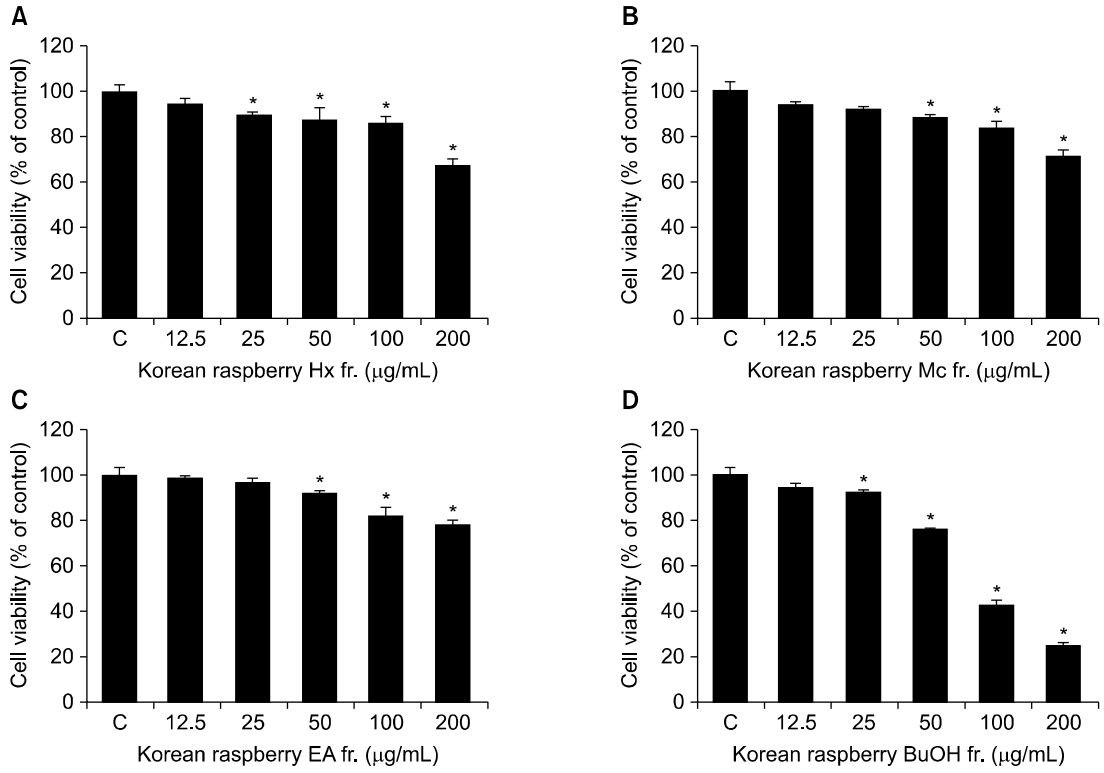

Fig. 3. Effects of Korean raspberry extracts on cytotoxic effects in A2780 human ovarian carcinoma cells. When the cells were approximately 80% confluent, they were seeded in 96-well culture plates at 1×10^4 cells per well and incubated for 24 h for adhesion. Then, the cells were treated with the control (0.5% DMSO), or indicated concentrations of berry extracts for 24 h. *Significantly different compared to the control at $P<0.05$. Hx, hexane; Mc, methylene chloride; EA, ethyl acetate; BuOH, *n*-butanol.

analysis was performed using SPSS ver. 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Berries are known as a powerful source of natural antioxidants that are widely used in traditional medicine due to their health benefits. Several berries contain phenolic compounds, including hydroxybenzoic and hydroxycinnamic acid derivatives, anthocyanins, flavonols, flavanols, condensed tannins (proanthocyanidins), hydrolyzable tannins (16), ellagitannins, proanthocyanidins, and anthocyanins (17). In general, most of the polyphenolic substances being detected in the EtOAc fraction have antioxidant and anti-inflammatory activities (18).

Among different berries, strawberries contain many antioxidant phenolic compounds. Cyanidin-3-glucoside, pelargonidin, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, kaempferol, quercetin, kaempferol-3-(6'-coumaroyl)glucoside, 3,4,5-trihydroxyphenyl-acrylic acid, glucose ester of (E)-*p*-coumaric acid, and ellagic acid are known to inhibit the growth of human oral (CAL-27 and KB), colon (HT29 and HCT-116), and prostate (LNCaP and DU145) cancer cells (14). Korean raspberry and mulberry contain the antioxidant vitamins A, B, and C (19), flavonoids including quercetin 3-(6-malonylglucoside), rutin, isoquercitin, cyanidin 3 rutinoside, and cyanidin 3-glucoside (20), and phenolic compounds, including flavonoids, anthocyanins, and carotenoids (21). These are known to have antioxidant, anticancer, anti-inflammatory and anti-neurodegenerative biological properties (19,22).

In the present study, the antioxidant activities of berry extracts and their fractions were determined using the DPPH radical scavenging activity test. All the berry extracts were divided into five fractions: hexane, EtOAc, MC, BuOH, and residual aqueous fractions. DPPH assay is rapid and widely used method to measure antioxidant capacity by using its decolorization in the presence of antioxidants (7,12). The scavenging ability of berry extracts was represented in Fig. 1. The MC fraction showed the highest DPPH radical scavenging activity compared to the other fractions in the strawberry extract (Fig. 1A). The EtOAc fraction showed the highest DPPH radical scavenging activity compared to the other fractions in the Korean raspberry extract (Fig. 1B). Similarly, the EtOAc fraction showed the highest DPPH radical scavenging activity compared to the other fractions in the mulberry extract (Fig. 1C). Except for raspberry, the EtOAc or MC fractions of berries containing phenolic compounds exerted dose dependent free radical scavenging activities. In the raspberry fractions, the hexane fraction also exhibited potent antioxidant activity (Fig. 1C). Therefore,

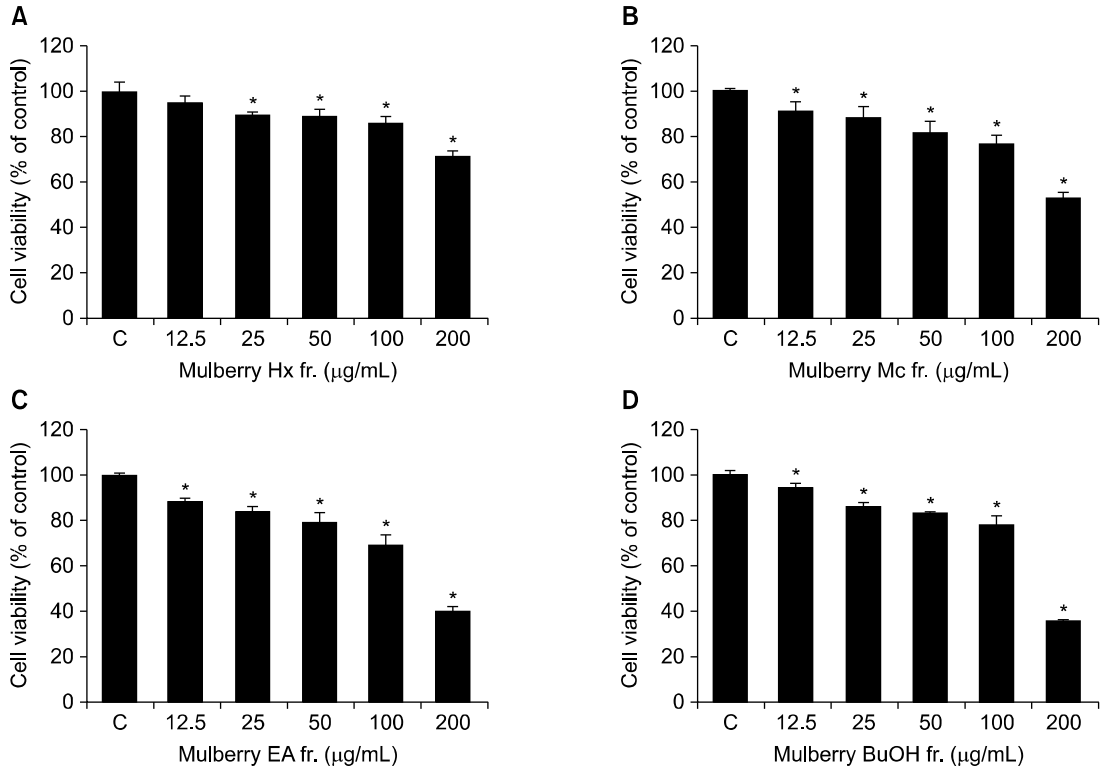

Fig. 4. Effects of mulberry extracts on the cytotoxic effects in A2780 human ovarian carcinoma cells. When the cells were approximately 80% confluent, they were seeded in 96-well culture plates at 1×10^4 cells per well and incubated for 24 h for adhesion. Then, the cells were treated with the control (0.5% DMSO), or indicated concentrations of berry extracts for 24 h. *Significantly different compared to the control at $P<0.05$. Hx, hexane; Mc, methylene chloride; EA, ethyl acetate; BuOH, *n*-butanol.

the free radical scavenging compounds of berries are mainly contained in the EtOAc fraction. These findings are in accordance with the earlier reports on the detection of most of the polyphenolic substances in the EtOAc fraction that exhibits antioxidant and anti-inflammatory activities (18).

The cytotoxic effect of berry extracts in A2780 human ovarian carcinoma cells was measured using the MTT assay. A2780 cells were treated with various concentrations of berries and their fractions. The BuOH fraction showed the highest cytotoxic effects compared to the other fractions in the strawberry extract (Fig. 2). Similarly, the BuOH fraction showed the highest cytotoxic effects compared to the other fractions in the raspberry extract (Fig. 3). Also, the BuOH fraction showed the highest cytotoxic effects compared to the other fractions in the mulberry extract, and the ethyl acetate fraction showed the same tendency (Fig. 4). Surprisingly, co-treatment with BuOH fractions of berries showed stronger cytotoxic effects compared to the other fractions. These findings suggest that potent anticancer molecules for human ovarian carcinoma A2780 cells are found in the BuOH fractions of berries that have stronger cytotoxic activity than EtOAc fraction containing antioxidants.

Berries including strawberries, Korean raspberry, and mulberry are effective antioxidants and exerted cytotoxic effects in A2780 human ovarian carcinoma cells. Further chemical identification of the BuOH fractions may aid in the development of cancer prevention diets for ovarian cancer patients.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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