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Cytotoxic and Apoptotic Effects of *Scrophularia Umbrosa* Dumort Extract on MCF-7 Breast Cancer and 3T3 Cells

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Abstract

Background: Breast cancer is the most prevalent cancer in women worldwide, especially in developing countries. *Scrophularia umbrosa* Dumort, a medicinal plant, has been used to treat various diseases in traditional medicine. In this study, we investigated the anti-cancer and cytotoxic effects of *S. umbrosa* Dumort extracts on a human breast cancer cell line.

Methods: The methanol and other *S. umbrosa* Dumort factions, including those from dichloromethane, water, n-butanol, ethyl acetate, and petroleum ether, were examined. The cytotoxic effects of the fractions on MCF-7 human breast cancer adenocarcinoma and 3T3 mouse embryonic fibroblast cells were evaluated by MTT assays. In addition, apoptotic induction was determined by propidium iodide flow cytometry.

Results: The water, n-butanol. petroleum ether, and ethyl acetate fractions had no cytotoxic effects. The methanol and dichloromethane fractions showed significant cytotoxic affects in a dose-dependent manner on the malignant cells while causing no damage to non-malignant cells. In addition, the cell death assay indicated that the *S. umbrosa* dichloromethane fraction triggered apoptosis in the MCF-7 cells.

Conclusions: *S. umbrosa* induced apoptosis in MCF-7 cells. The *S. umbrosa* dichloromethane fraction exhibited the greatest cytotoxic effect on these cells. This work presents a first evaluation of the cytotoxic effects of *S. umbrosa* and further studies are needed to determine the cytotoxic mechanism.

Keywords: Apoptosis, Breast cancer, Cytotoxicity, MCF-7 cell line, *Scrophularia umbrosa* Dumort.

Introduction

Breast cancer is the most common cancer and leading cause of cancer mortality among women worldwide, accounting for 25% of all cancers and 15% of cancer deaths in females (1). The main treatment methods for breast cancer include radiation, hormone treatments, targeted and chemotherapy, and surgery. Although chemotherapy is the most common cancer treatment (2), it causes various undesirable side effects (3). Currently, the use of medicinal plants for cancer therapy has increased due to their low side effects and high efficiency. Several anti-cancer drugs including Taxol, vinca alkaloids, camptothecin, and podophyllotoxin are derived from bioactive plant compounds (4, 5). The genus Scrophularia of the *Scrophulariaceae* family comprises about 300 species, which are is widely distributed in temperate regions of the northern hemisphere. Some species have been used since ancient times in folk medicines to treat inflammation, itching, wounds, pain, dermatitis, fever, constipation, and tumors (6, 7) Several bioactive compounds have been isolated from these species, including iridoids, iridoid glycosides, phenylpropanoid glycosides,

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phenylethanoid glycosides, flavonoids, terpenoids, and saponins (8-10). These compounds have shown antitumor, anti-oxidative, anticoagulant, antimicrobial, antipyretic, antiprotozoal, antifungal, antidiabetic, anti-inflammatory, hemodynamic, choleretic, immunomodulatory, hepatoprotective, and neuroprotective effects (10-12). Previous findings demonstrated that anticancer properties of *Scrophularia* species can be mediated through the induction of apoptosis (13).

In present study we evaluated cytotoxic effects and apoptosis induction of *Scrophularia (S.) umbrosa* Dumort extracts on MCF-7 cells, a human breast cancer adenocarcinoma cell line, and investigated possible adverse effects of these fractions on 3T3 cells, a mouse fibroblast cell line obtained from Swiss albino mouse embryo tissue.

Materials and methods

Ethical considerations

The present study was approved by the Medical Research Committee of Mashhad University of Medical Sciences, Mashhad, Iran (approval no 941647).

Materials

The cell culture media (DMEM and RPMI1640), fetal bovine serum (FBS), penicillin-streptomycin, and trypsin/EDTA solution were obtained from Gibco BRL (Life Technologies, Paisley, Scotland). Cell lines were obtained from the cell bank (Pasteur Institute, Tehran, Iran). 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), and dimethyl sulfoxide (DMSO) were acquired from Sigma-Aldrich (Germany).

Preparation of extracts

Scrophularia umbrosa Dumort plant was collected from Razavi Khorasan Province, Iran, in May 2015. The plant specimen (no.13145) is stored in the herbarium of the Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. To prepare extracts, roots were rinsed, dried, powdered (150 g), and macerated in 80% methanol solution within 48 hours. The water, n-butanol, petroleum ether, ethyl acetate, and dichloromethane extracts were obtained from the methanol extract using a separatory funnel. Each extract was condensed on a rotary evaporator (Heildolph, Germany) at 45 ºC, freeze dried, (Martin Christ, Germany), and stored at –20 ºC until the experiments. Dimethyl sulfoxide was used to dissolve the dichloromethane and petroleum ether fractions. Other fractions were dissolved in culture media.

Cell culture

The 3T3 and MCF-7 cells were cultured in DMEM and RPMI1640 media, respectively. The cell culture media was supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cells were incubated at 37 ºC in a humidified atmosphere with 5% CO2. To detect viability and apoptosis, cells were seeded in 96- or 6-well plates. All experiments were performed in triplicate.

MTT assay

Cytotoxic effects of the different *S. umbrosa* extracts were evaluated in MCF-7 and 3T3 cells using the MTT assay. The method is based on the ability of live cells to metabolize the yellow tetrazolium bromide to purple formazan products. The MCF-7 and 3T3 cells were exposed to 6.25-1000 µg/ml methanol extract concentrations in 96-well plates for 24 hr. The other fractions were evaluated at concentrations of 6.25-300 µg/ml. Dimethyl sulfoxide at concentration of 0.5% (v/v) was included as solvent control. At the end of treatment, 100 µL of MTT solution was added to each well. After 4 hr incubation, the insoluble formazan crystals were solubilized with DMSO and the optical density was measured at 570 nm using an ELISA reader.

Flow cytometry analysis

MCF-7 cell lines were harvested into 6-well plates and exposed to the dichloromethane fraction for 24 hr. After 24 hr the cells were washed, incubated with RNase A for 15 minutes at room temperature, and then with 100 µg/mL PI, 0.1 % sodium citrate, and 0.1% Triton X-100 for 3 hr at 4 °C. The flow cytometry was performed on a flow cytometer (Bio-Rad, USA) and data was analyzed with CellQuest (USA) software.

Statistical analysis

Results were expressed as means ± SDs. Statistical differences between treated and control groups were compared using one-way analysis of variance (ANOVA) followed by Tukey post-test. P values less than 0.05 were considered significant.

Results

Cytotoxic effects of Scrophularia umbrosa extracts on MCF-7 and 3T3 cells

Viability of MCF-7 cells incubated with *S. umbrosa* methanolic extracts at concentrations from 6.25 to 1000 µg/ml was significantly less than that of the controls ($p<0.001$) (Fig. 1). The concentrations inducing 50% cell growth inhibition (IC50) against MCF-7 and 3T3 cells were calculated to be 530 and 1550 µg/ml, respectively. The *S. umbrosa* methanolic extract exhibited no significant cytotoxic activity against 3T3 cells ($p>0.05$) (Fig. 1). The cytotoxic effects of the other solvent fractions were analyzed at concentrations of 6.25-300 µg/ml. The water, n-butanol, petroleum ether, and ethyl acetate extracts had no detectable effect on cell growth. We conclude from these results that these extracts all have IC50s greater than 300 µg/ml. The dichloromethane fraction, however, showed a prominent cytotoxic effect on MCF-7 cells in a dose-dependent manner with an IC50 of 159 ± 2.7 µg/ml (Table 1 and Fig. 2). Similar to the methanolic fraction, it also significantly reduced cell viability at the lowest concentration measured. No significant differences in cell viability were observed between the dichloromethane fraction-treated and control-treated 3T3 cells (Fig. 2). Based on these results, the dichloromethane fraction was further used to characterize apoptosis.

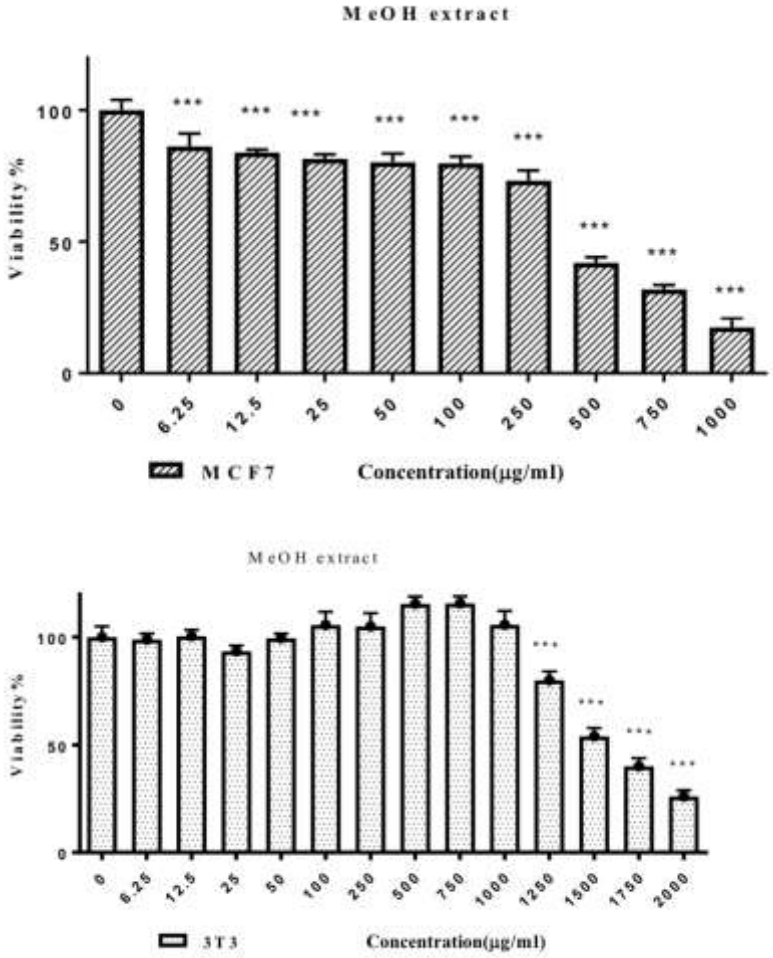

Fig.1. Cytotoxic effect of *S. umbrosa* methanolic extracts on MCF-7 and 3T3 cells (6.25-1000 µg/ml) after 24 hrs. Data are presented as means ± S.Ds. ***$p<0.001$ compared with control.

Table 1. Doses inducing IC50 of solvent fractions of *S. umbrosa* against MCF-7 and 3T3 cells. Cells were incubated with various extract concentrations for 24 hrs. IC50 values were expressed as the mean s± SDs (n = 6).

	Cell line	Fraction			
	Petroleum ether	**Dichloromethane**	**Ethyl acetate**	**n-Butanol**	**H_2O**
MCF-7	>300	159±2.7	>300	>300	>300
3T3	>300	>300	>300	>300	>300

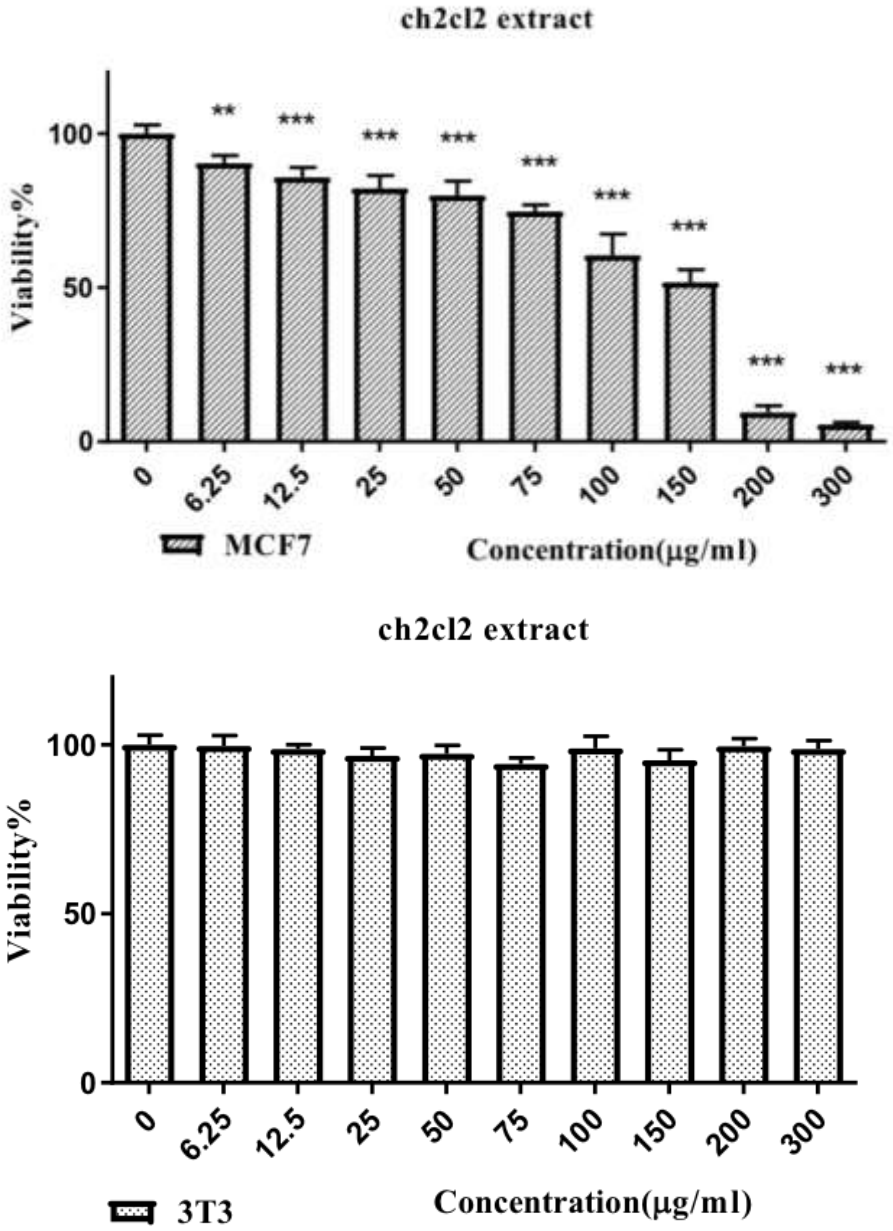

Fig. 2. Cytotoxic effect of *S. umbrosa* CH2CL2 extracts on MCF-7 and 3T3 cells (6.25-300 µg/ml) after 24 hrs. Data are presented as means ± S.Ds. **P < .01 and ***p<0.001 compared with control.

Apoptotic effects of dichloromethane fractions of *Scrophularia umbrosa* on MCF-7 cells

The apoptotic effect of the *S. umbrosa* dichloromethane fractions was determined by PI staining and flow cytometry. The dichloromethane fraction was examined at the IC50 concentration of 150 µg/ml obtained from the MCF-7 cell MTT assays. Incubation of the MCF-7 cells with the dichloromethane fraction resulted in a sub-G1 peak in the histogram, indicating DNA fragmentation and apoptotic processes in induced cell toxicity (Fig. 3).

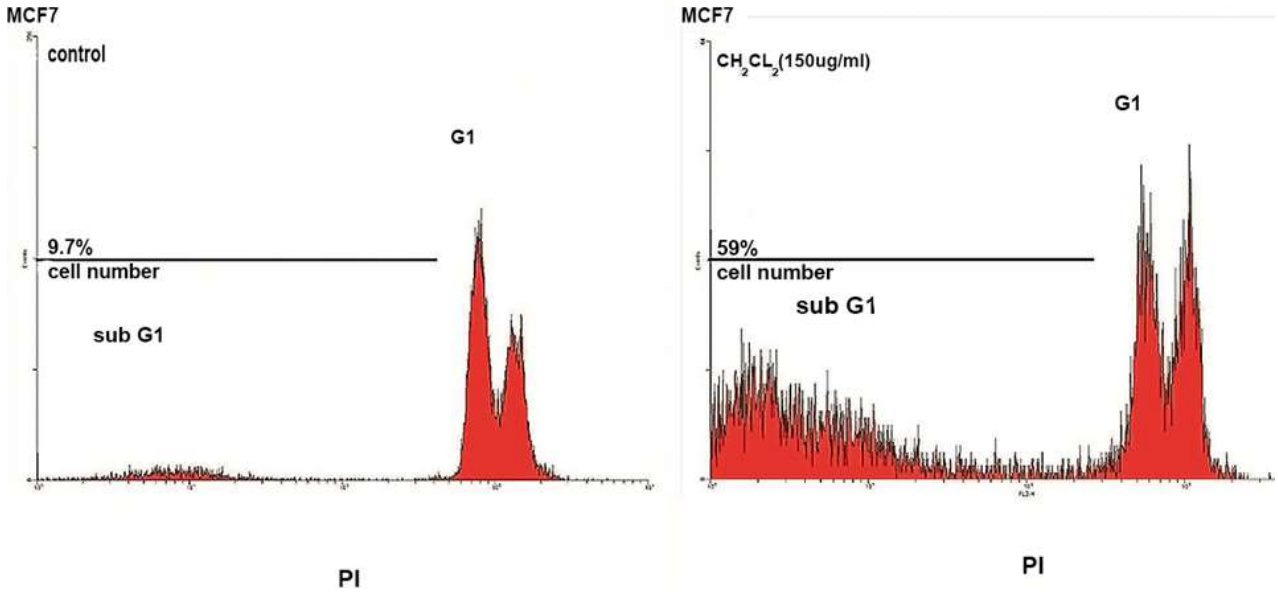

Fig. 3. Flow cytometry histograms of MCF-7 cell apoptosis. MCF-7 cells were treated with 150 µg/ml of the *S. umbrosa* CH2Cl2 fraction for 24 h. Apoptosis was analyzed with propidium iodide and flow cytometry. The sub-G1 peak indicates an apoptotic process in the *S. umbrosa* CH2Cl2 fraction induced cell death.

Discussion

Medicinal plants are important sources of bioactive compounds in cancer prevention and treatment (5). Plants of the *Scrophularia* genus were used to treat cancer in traditional medicines (14).

Many studies have demonstrated that these plants have anticancer effects. In one study, *S. oxysepala* dichloromethane and methanol extracts significantly inhibited cell growth (IC50=22.7 µg/ml) and viability, and induced apoptosis in MCF-7 cells in a dose-dependent manner while having no deleterious effects on non-cancerous HUVAC cells (2). In another study, an *S. variegata* ethanolic extract was cytotoxic to MCF-7 cells (IC50=200 µg/ml) and induced programmed cell death via a mitochondrial intrinsic pathway by cell cycle arrest in G2/M phase, and increases in caspase 3 and caspase 9 levels (13). The ethanolic extract of *S. megalantha* also showed cytotoxic activity on human Jurkat cells, a lymphoblast-like cell line (IC50>200 µg/ml) (15), while the total water extract of *S. striata* had a strong anticancer effect via an apoptotic mechanism on 1321 cells, an astrocyte cancer cell line (IC50= 6 µg/ml) (16).

In the present study, the anti-cancer effects of methanolic extract and water, n-butanol, petroleum ether, ethyl acetate dichloromethane fractions were investigated on normal and breast cancer cell lines. The methanolic extract had cytotoxic activity with an IC50 of 530 µg/ml on the cancer cell line without affecting normal cells. This result indicates that the *S. umbrosa* methanolic extract contains effective compound(s) for the treatment breast cancer.

In the next stage, the water, n-butanol petroleum ether, ethyl acetate, and dichloromethane fractions were isolated from the methanol extract based on polarity. In accordance with the results of other studies, the dichloromethane fraction exhibited the highest cytotoxic activity against breast cancer cells without affecting the normal cells.

Programmed cell death, or apoptosis, is a key mechanism of cancer treatment drugs (14). In this study, the PI assay was performed to examine cell

apoptosis. The flow cytometry results suggest that apoptosis is, at least in part, one mechanism of cell death in the dichloromethane fraction-treated MCF-7 cells.

In this study *S. umbrosa* demonstrated an anti-cancer effect on MCF-7 cells by inducing apoptosis. The cytotoxic effect was most pronounced in the *S. umbrosa* dichloromethane fraction. This work presents a first evaluation of cytotoxic effects of *S. umbrosa* and further studies are needed to determine the cytotoxic mechanism.

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The authors declare no conflict of interest.

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