

RESEARCH ARTICLE

Anticancer Activity of *Petroselinum sativum* Seed Extracts on MCF-7 Human Breast Cancer Cells

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Abstract

Pharmacological and preventive properties of *Petroselinum sativum* seed extracts are well known, but the anticancer activity of alcoholic extracts and oil of *Petroselinum sativum* seeds on human breast cancer cells have not been explored so far. Therefore, the present study was designed to investigate the cytotoxic activities of these extracts against MCF-7 cells. Cells were exposed to 10 to 1000 µg/ml of alcoholic seed extract (PSA) and seed oil (PSO) of *Petroselinum sativum* for 24 h. Post-treatment, percent cell viability was studied by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-biphenyl tetrazolium bromide (MTT) and neutral red uptake (NRU) assays, and cellular morphology by phase contrast inverted microscopy. The results showed that PSA and PSO significantly reduced cell viability, and altered the cellular morphology of MCF-7 cells in a concentration dependent manner. Concentrations of 50 µg/ml and above of PSA and 100 µg/ml and above of PSO were found to be cytotoxic in MCF-7 cells. Cell viability at 50, 100, 250, 500 and 1000 µg/ml of PSA was recorded as 81%, 57%, 33%, 8% and 5%, respectively, whereas at 100, 250, 500, and 1000 µg/ml of PSO values were 90%, 78%, 62%, and 8%, respectively by MTT assay. MCF-7 cells exposed to 250, 500 and 1000 µg/ml of PSA and PSO lost their typical morphology and appeared smaller in size. The data revealed that the treatment with PSA and PSO of *Petroselinum sativum* induced cell death in MCF-7 cells.

Keywords: MCF-7 cells - *Petroselinum sativum* - cellular morphology - cytotoxicity

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Introduction

Breast cancer is one of the most common causes of the cancer in females in whole world (WCR, 2008). It has been observed that breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represents 13.7% of all cancer deaths due to the breast cancer in male and female (Ferlay et al., 2000). It is the most frequent cancer in both developed and developing regions, but the rate of human breast cancer is higher in developing countries in compared to developed nations (Ferlay et al., 2000). Alternative and complementary medicines are now considered to play an emerging role in the cancer prevention. Natural products are an extremely promising strategy for chemoprevention to block the development of cancer in human. Many natural plants have furnished modern medicine with the drugs that are used in cancer therapy as cytotoxic agents. Investigations from basic research are confirming that many chemopreventive dietary compounds are active at molecular levels (Russo et al., 2005; Pan et al., 2008). Since many years, plant

extracts have been used as traditional remedies to treat a various diseases including cancer (Salem, 2005; Padhye et al., 2008; Gaidhani et al., 2009; Svejda et al., 2010; Khan et al., 2011; Randhawa and Alghamdi, 2011; Sharma et al., 2011). Experimental studies also revealed that seed oil (Al-Oqail et al., 2013) and extracts of the various plants can also protect against human breast cancer (Pratumvinit et al., 2009; Abu-Dahab et al., 2012; Abdelhamed et al., 2013). The *Petroselinum sativum* (*P. sativum*) or parsley, a member of the family of Umbelliferae, has been reported to have antioxidant (Kreydiyyeh et al., 2001; Ahmed et al., 2010), antidiabetic (Yanardag et al., 2003), anti-inflammatory, antiedema antihypertensive, antimicrobial (Wahba et al., 2010) activities. *P. sativum* has also been found to reconstruct kidney tissue after nephrotoxicity (Kohjimoto et al., 2004; Saeidi et al., 2011). Due to the diverse pharmacological and preventive properties of *P. sativum* plant extracts, present investigation was carried out to investigate the cytotoxic activities of alcoholic extract and seed oil of *P. sativum* against human breast adenocarcinoma cells (MCF-7).

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Materials and Methods

Chemicals and consumables

MEM culture medium, antibiotics-antimycotic solution, fetal bovine serum (FBS) and trypsin were purchased from Invitrogen, Life Sciences, USA. Consumables and culture wares used in the study were procured from Nunc, Denmark. Ethanol and all other specified reagents and solvents were purchased from Sigma Chemical Company Pvt. Ltd. St. Louis, MO, USA.

Plant material and extractions

The *Petroselinum sativum* seeds used in this study were obtained from the local market of Riyadh, Saudi Arabia. The seeds were screened manually to remove bad ones. The oil from *P. sativum* seeds was extracted by continuous extraction in Soxhlet apparatus for 12 h using petroleum ether (60-80°C boiling range) as a solvent according to the method described by AOCS (Horwitz, 1980). At the end of the extraction the solvent was evaporated. The oil thus obtained was dried over anhydrous sodium sulphate and stored -4°C for further analysis. For the preparation of alcoholic extract, the seeds were macerated in alcohol and then filtered. The procedure was repeated several times. The solvent was then evaporated using a rotary evaporator and the residue so obtained was called as the alcoholic extract.

Cell culture

Human breast adenocarcinoma cells (MCF-7) was cultured in MEM, supplemented with 10% FBS, 0.2% sodium bicarbonate and antibiotic/antimycotic solution (100x, 1/100 ml of medium). Cells were grown in 5% CO₂ at 37°C in high humid atmosphere. Before the experiments, viability of cells was assessed following the protocol of (Siddiqui et al., 2008). MCF-7 cells showing more than 95% cell viability and passage number between 16 and 18 were used in the present study.

Experimental design

MCF-7 cells were exposed to various concentrations of alcoholic extract (PSA) and seed oil (PSO) of *P. sativum* (10 to 1000 µg/ml) for a period of 24 h. Following the exposures of PSA and PSO, cells were subjected to assess the cytotoxic responses using MTT, and NRU assays and morphological alterations using phase contrast inverted microscope.

Drug solutions

The seed extracts of *Petroselinum sativum* were not completely soluble in aqueous medium solution, therefore the stock solutions of all the extracts were prepared in dimethylsulphoxide (DMSO) and diluted in culture medium to reach the desired concentrations. The concentration of DMSO in culture medium was not more than 0.1% and this medium was used as control.

Cytotoxicity Screening

MTT assay: Percent cell viability was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium

bromide (MTT) assay as described (Siddiqui et al., 2008). Briefly, cells (1×10⁴) were allowed to adhere for 24 h CO₂ incubator at 37°C in 96 well culture plates. After the respective exposure, MTT (5 mg/ml of stock in PBS) was added (10 µl/well in 100 µl of cell suspension), and plates were incubated for 4 h. Then, supernatants were discarded and 200 µl of DMSO were added to each well and mixed gently. The developed color was read at 550 nm using Multiwell Microplate Reader (Thermo Scientific, USA). Untreated sets were also run under identical conditions and served as control.

Neutral red uptake (NRU) assay: Neutral red uptake (NRU) assay was carried out following the protocol described by (Siddiqui et al., 2010). Briefly, after the respective exposure, the medium was aspirated and cells were washed twice with PBS, and incubated for 3 h in a medium supplemented with neutral red (50 µg/ml). Medium was washed off rapidly with a solution containing 0.5% formaldehyde and 1% calcium chloride. Cells were subjected to further incubation of 20 min at 37°C in a mixture of acetic acid (1%) and ethanol (50%) to extract the dye. The plates were read at 540 nm using multiwell microplate reader (Thermo Scientific, USA). The values were compared with the control sets run under identical conditions.

Morphological analysis

Morphological changes in MCF-7 cells exposed to increasing concentrations (10-1000 µg/ml) of PSA and LSO were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20× magnification.

Statistical analysis

The results were expressed as mean and standard error of means (SEM). One way ANOVA was employed to detect differences between the groups of treated and control. The values showing p<0.05 were considered as statistically significant.

Results

MTT and NRU assays

Cytotoxicity of alcoholic seed extract (PSA) and seed oil (PSO) of *P. sativum* was assessed using MTT and NRU assays, after exposing the MCF-7 cells at 10-1000 µg/ml

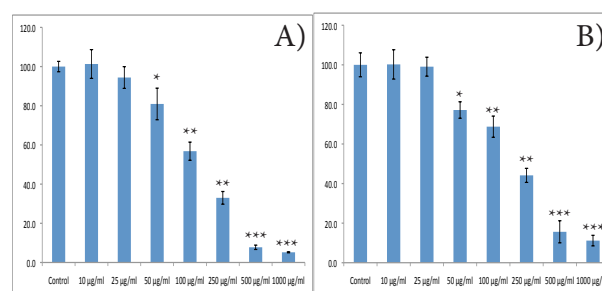


Figure 1. Cytotoxicity Assessments by A) MTT and B) NRU Assay in MCF-7 Cells Following the Exposure of Various Concentrations of Alcoholic Seed Extract of *Petroselinum Sativum* (PSA) for 24 h. Values are mean±SE of three independent experiments (*p<0.05, **p<0.01, ***p<0.001 Vs Control)

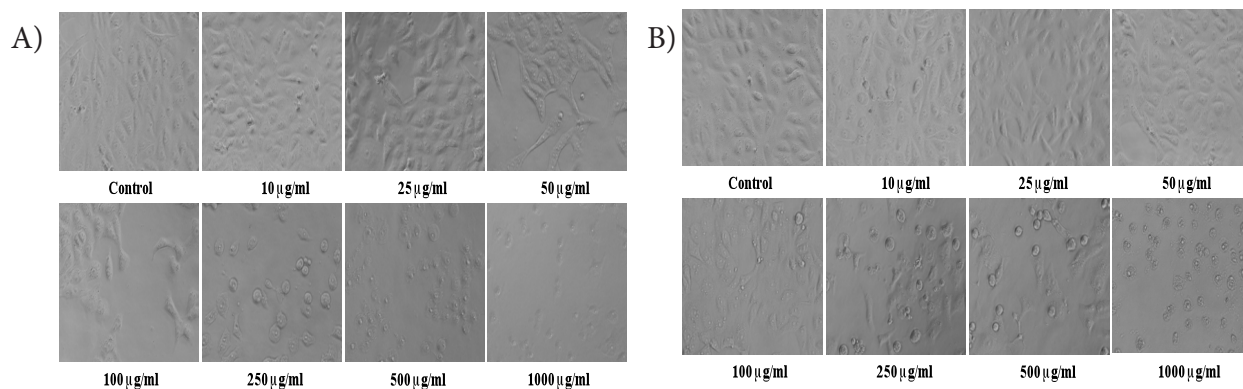


Figure 2. Morphological Changes in MCF-7 Cells Exposed to Various Concentrations of A) Alcoholic Seed Extract and B) Seed Oil of *Petroselinum Sativum* (PSA) for 24 h. Images were taken using an inverted phase contrast microscope (OLYMPUS CKX 41) at 20X magnification

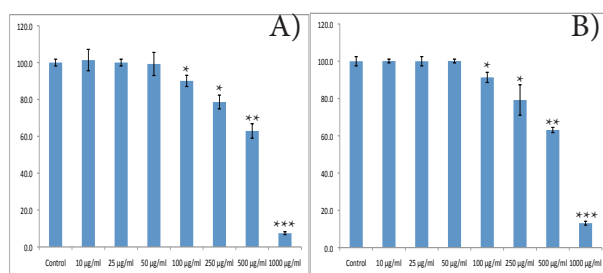


Figure 3. Cytotoxicity assessments by A) MTT and B) NRU assay in MCF-7 cells following the exposure of various concentrations of seed oil of *Petroselinum sativum* (PSO) for 24 h. Values are mean ± SE of three independent experiments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ Vs Control)

concentrations for 24 h. The percent cell viability in MCF-7 cells as observed by MTT and NRU assays PSA are presented in Figures 1. Figures 1 shows that PSA induced statistically significant ($p < 0.001$) decrease in cell viability of MCF-7 cells in a concentration dependent manner. MCF-7 cells exposed with 50 µg/ml concentration and above concentrations of PSA for 24 h were found to be cytotoxic, and cell viability was found 81%, 57%, 33%, 8% and 5% at 50, 100, 250, 500 and 1000 µg/ml of PSA respectively by MTT (Figure 1A) and 77%, 69%, 56%, 16%, and 11% at 50, 100, 250, 500 and 1000 µg/ml of PSA respectively by NRU assay (Figure 1B). Concentrations 25 µg/ml and lower of PSA did not cause any effect on the viability of MCF-7 cells.

A concentration dependent reduction in cell viability of MCF-7 cells was also observed in case of PSO, but magnitude of reduction was lower than PSA. Figure 3 shows that PSO induced statistically significant ($p < 0.001$) decrease in cell viability of MCF-7 cells exposed for 24 h. MCF-7 cells exposed to 100 µg/ml and above concentrations of PSO for 24 h were found to be cytotoxic. Cell viability at 100 µg/ml, was found to be 90% by MTT (Figure 3A) and 91% by NRU (Figure 3B) assays, whereas, reduction in cell viability at 250, 500, and 1000 µg/ml of PSO was found to be 78%, 62%, and 8% by MTT (Figure 3A) and 79%, 63% 13% by NRU assays (Figure 3B), respectively. The concentrations of PSO at 50 µg/ml and lower did not cause any adverse effect on the viability of MCF-7 cells as shown by MTT and NRU assays (Figure 3). Although both PSA and PSO were

found to reduce significant cell viability in a concentration dependent manner, but the MCF-7 cells were found to be more sensitive towards PSA extract.

Morphological changes

The morphological alterations observed in MCF-7 cells are shown in Figures 2. Changes in morphology were found to be concentration dependent manner. Cells exposed to 50 µg/ml and above concentrations of PSA for 24 h reduced the normal morphology of MCF-7 cells, and cell adhesion capacity as compared to control (Figure 2A). In case of PSO, the morphology of MCF-7 cells at 100 µg/ml have shown to reduce the normal morphology and cell adhesion capacity in compared to control (Figure 2B). Most of the cells exposed to 250, 500 and 1000 µg/ml of PSA and PSO lost their typical morphology and appeared smaller in size, but the lower concentrations did cause any effect on cellular morphology of MCF-7 cells (Figures 2).

Discussion

World Health Organization investigation shows that 80% of world populations relies on traditional medicines (WHO, 1993). From these, at least 30% utilized medicinal plants from clinical indication (Martins et al., 1992). Available literatures on medicinal plants indicate that promising photochemicals can be developed for many health problems (Gupta, 1994; Rodeiro et al., 2008; Farshori et al., 2013). Plant extracts as a traditional remedies are already being used to treat a variety of diseases including cancer (Zheng et al., 1992; Svejda et al., 2010; Khan et al., 2011; Randhawa and Alghamdi, 2011; Sharma et al., 2011). The utilization of medicinal plants is more common in underdeveloped countries (Heck et al., 2000) and experimental studies showed that the extracts of the various plants can also protect against breast cancer cells (Pratumvinit et al., 2009; Abu-Dahab et al., 2012; Abdelhamed et al., 2013). In the view to the above mentioned facts, the present study was designed to evaluate the potential therapeutic capabilities of anticancer property of alcoholic extract and oil of *Petroselinum sativum* (*P. sativum*) seeds in MCF-7 cells, a human breast adenocarcinoma cells. The cytotoxic responses of the extracts were determined by the 3-(4,5-dimethylthiazol-2-yl)-2, 5-biphenyl tetrazolium bromide (MTT) and

neutral red uptake (NRU) assays. The results indicate that both *P. sativum* extracts have significant in vitro inhibitory effect on the growth rate of MCF-7 cells. The results also demonstrate that both alcoholic seed extract (PSA) and seed oil (PSO) of *P. sativum* reduced the cell viability of MCF-7 cells in a concentration-dependent manner. The *P. sativum* plant extracts have been reported to have antioxidant (Kreydiyyeh et al., 2001; Ahmed et al., 2010), antidiabetic (Yanardag et al., 2003), anti-inflammatory, antiedema antihypertensive, antimicrobial (Wahba et al., 2010) activities. *P. sativum* has also been found to reconstruct kidney tissue after nephrotoxicity (Kohjimoto et al., 2004; Saeidi et al., 2011). Our results are in well correlation with the previous findings in which the plant extracts were found to induce cytotoxicity on human breast cancer T47D cells (Abdolmohammadi et al., 2008) due to the sensitivity of cancerous cells towards the death flavanoids. Other studies also showed that certain constituents of plant also inhibit the growth of human gastric adenocarcinoma (MK-1), human uterus carcinoma (HeLa), and murine melanoma (B16F10) cells (Fujika et al., 1999). The growth inhibitory effects of other extracts on cancerous cells have also been observed by different investigators on other human cancer cell lines (Li et al., 1995; Kim et al., 2002; Kumi-Diaka and Butler, 2000). Recently, we have also showed that fenugreek seed oil decreased the cell viability of various cancer cells including human breast cancer cells (Al-Oqail et al., 2013). The alcoholic extract of seed showed a significantly higher cytotoxic potential than the seed oil of *Petroselinum sativum*. This kind of difference in the cytotoxicity of different extracts have also been observed by Samarakoon et al. (2010), explaining that this cytotoxicity is possibly due to the active components and herbal preparation (Samarakoon et al., 2010). Alcoholic seed extract (PSA) and seed oil (PSO) of *P. sativum* were also further investigated to determine their mechanism of cell death by observing them under phase contrast inverted light microscope. From the microscopic images it was observed that MCF-7 cells lose their typical morphology when they were exposed to various concentrations of the PSA and PSO for 24 h. These morphological changes in MCF-7 cells were in well agreement with the previous studies showing this kind of alteration in the cells could be a possible mechanism of cell death (Berrington and Lall, 2012).

In conclusion, our results show that PSA and PSO significantly reduced the cell viability, and altered the cellular morphology of MCF-7 cells in a concentration dependent manner. The data revealed that the exposure of alcoholic seed extract (PSA) was more effective than the seed oil (PSO) of *Petroselinum sativum* in MCF-7 cells. Further molecular studies are undergoing to elucidate the mechanism(s) of action of these extracts on human breast cancer cells.

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