

Research Article

Methanolic Fractions of *Ornithogalum cuspidatum* Induce Apoptosis in PC-3 Prostate Cancer Cell Line and WEHI-164 Fibrosarcoma Cancer Cell Line

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Abstract

Purpose: The present study, was aimed to assess the cytotoxic effects of *Ornithogalum cuspidatum* methanolic fractions on PC-3, prostate cancer cells and WEHI-164, Fibrosarcoma cells.

Methods: Methanolic fractions of *O. cuspidatum* were prepared using solid phase extraction and the cells were treated with different concentrations for 12 and 24 hours. Cytotoxicity and cell viability were measured by MTT assay. ELISA was also employed to assess the histone-associated DNA fragments and the involvement of apoptotic mechanisms.

Results: 10 and 20% fractions had not significant cytotoxic effects ($p > 0.05$) but other fractions exerted growth inhibition on both cancer cell lines ($p < 0.05$). After 24h of incubation with 40, 60, 80 and 100% fractions, the IC₅₀ values were: 165, 85, 65 and 45 µg/ml on PC-3 cells and 200, 96, 76 and 73 µg/ml against WEHI-164 cell line, respectively. ELISA results also revealed that, both cell lines had undergone apoptosis.

Conclusion: It is deduced that, 80% and 100% methanolic fractions had significant anti-proliferative and apoptotic impacts on PC-3 and WEHI-164 cells in vitro and could be considered for developing chemo-preventive substances.

Introduction

Prostate cancer is one of the most common malignancies worldwide and the second common cause of cancer-related mortality in men.¹ It has been estimated that, 15–80% of males with over the age of 50 have undiagnosed microscopic prostate cancer.² Fibrosarcoma is also a malignant mesenchymal tumour derived from fibrous connective tissue.³ Recently radiotherapy and immunotherapy included in the treatment of cancer beside the surgery and chemotherapy.⁴ Many patients are resistant to these therapies and some of these treatments have a wide range of side effects. Medicinal plants have been used for prevention, therapy and remission of human diseases during thousands of years.⁵ To the extent that, more than 60% of the anti-cancer drugs are derived from natural resources and herbal origins. The search for plant-derived anti-cancer drugs was started in the 1950s by discovery of the vinca alkaloids, vincristine and cytotoxic podophyllo toxins.^{6,7} These plants derived chemotherapy drugs including etoposide, vincristine, Cisplatin and Taxol exert their anti-cancer effects via inducing programmed cell death in cancer cells.^{7,8}

Ornithogalum cuspidatum is an Iranian species of Liliaceae family. Diverse species of this family is distributed in Europe, Asia and Africa with different climatic conditions. recently some studies suggested

Ornithogalum species contain compounds that exhibit cytotoxic properties in a variety of malignant tumor cells.⁹⁻¹² Apoptotic effect of *O. cuspidatum* methanolic extract on Fibrosarcoma cells has been previously studied.¹³ The aim of the present study was to find the appropriate methanolic fractions with optimum concentration and exposure time for inhibition of growth in PC-3 and WEHI-164 cells and induction of apoptosis.

Materials and Methods

Preparation of *O. cuspidatum* extract

O. cuspidatum was collected from Maraghe city in East Azarbaijan of Iran and was identified by Dr. Abbas Delazar (Department of Pharmacognosy). Plant material organized by 100 g of ground bulbs. Extraction was performed in a Soxhlet apparatus with 1 liter of methanol. Extraction process carried out in 6 hours and then methanol was evaporated with a rotary evaporator (Heildolph-Germany).^{13,14}

Preparations of *O. cuspidatum* methanolic extract fractions

SPE (Solid Phase Extraction) was used for preparation of methanolic fractions. 2 gr of *O. cuspidatum* methanolic extract was solved in methanol and water, in a ratio of 10:90. Then the solvent was applied to Sep-Pak ODS (Octa Decyl Silica) column (Waters, USA) and eluted

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with 200 ml of 10%, 20%, 40%, 60%, 80% and 100% methanol and 200 ml of Dichloromethane (DCM, Merck-Germany). Finally, the six fractions were filtered and evaporated at 50°C under negative pressure with rotary evaporator.¹⁵

Cell culture

Human prostate cancer, PC-3 and mouse fibrosarcoma, WEHI-164 cell lines were purchased from the Cell Bank of Pasteur Institute - Tehran-IRAN (NCBI CODE=C200&C427). The cell lines were cultured in RPMI-1640 medium (Sigma-Germany) supplemented with 100U/mL penicillin (Gibco-UK), 100 µg/mL streptomycin(Gibco-UK) and 10% FBS(Gibco-UK) . The cells were grown and maintained at 37°C in a humidified atmosphere of 5% CO₂.

Anti proliferative activity assay

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) is a yellow component that is reduced by mitochondrial dehydrogenase of viable cells to blue formazan which reflects the normal function of mitochondria and cell viability. PC-3 and WEHI-164 cells (1×10⁴ per well) were seeded into 96-well culture plates and allowed to be attached. After reaching 70% confluency, the cells were treated with different concentrations of fractions (10, 20, 40, 50, 100, 150, 200 and 400 µg/ml) for 12 h and 24h. Then, the supernatants were removed and fresh media with 50 µl of the MTT solution (2mg/ml, Sigma-Germany) were added to each well. The plates were incubated at 37°C for 4 h and at the end of incubation, media were discarded. 25 µl of the Sorensen's Buffer and 200 µl of DMSO (Merck-Germany) were added and the plates were incubated for 30 min to solubilize the formazan crystals. The absorbance was quantified with a spectrophotometer at 570 nm wavelength. All experiments were performed in triplicates. Control groups received the same amount of DMSO without fractions of plant extract (5, 15, and 18).

Assessment of necrosis and apoptosis

PC-3 and WEHI 164 cells (10⁴) were treated with 50, 100, 150 and 200 µg/ml fractions and 0.2 % (v/v) DMSO at 37°C for 24 hrs. The optimum concentrations were determined by MTT assay. Then, Cell Death Detection ELISA^{plus} kit (Roche Diagnostics GmbH- Germany) was utilized for the assessment of cell apoptosis and necrosis via quantification of histone associated DNA in mono and oligonucleosome forms. According to the manufacturer's instructions, the culture supernatants (for detection of necrosis) and cell lysates (for detection of apoptosis) were prepared and incubated in the streptavidin coated microtiter plate. The simultaneous binding of nucleosomes to both "biotin labeled anti histone" and "anti- DNA- POD" antibodies facilitated their fixation on wells and substrate mediated color development. Eventually, the results were analyzed spectrophotometrically using an ELISA plate reader at 405 nm.^{13,14,16,17}

Statistical analyses

All experiments were performed in triplicates. Statistical differences of mean values among fractions were determined by one-way ANOVA using SPSS 16 software. Differences were considered significant at p< 0.05.

Results

MTT cell viability assay

Cytotoxicity of the fractions were evaluated by MTT colorimetric assay after 12 and 24h of incubation. According to the results, 10% and 20% fractions did not exert any cytotoxic effect on PC3 and WEHI-164 cells in 12 and 24h. Concentrations of 200 and 400 µg/ml of 40% fraction exhibited moderate cytotoxicity and decreased the viability to 40% in 24h. However, lower concentrations of this fraction did not show an acceptable cytotoxic activity in 12 and 24 h. The 60%, 80% and 100% fractions demonstrated significant cytotoxic effects (p<0.05) on both cell lines and in all concentrations in 12 and 24 h (Figure 1). IC50 value for 60%, 80% and 100% fractions (the dose required for 50% inhibition) were 165, 105 and 100 µg/ml in WEHI-164 cells and 145, 115 and 170 µg/ml in PC3 cell lines in 12h, respectively. After 24h of incubation, IC50 values corresponding to 40%, 60%, 80% and 100% fractions were 200, 96, 76 and 73 µg/ml against WEHI-164 cell lines and 165, 85, 65 and 45 µg/ml for PC3 cells. The order of the cytotoxicity was: F100%>F80%>F60%>F40% in both cell lines.

Cell Death Detection ELISA assay

According to MTT results, the 40%, 60%, 80% and 100% fractions at concentrations of 50, 100, 150 and 200 µg/ml and incubation time of 24h were chosen for the assessment of apoptosis by cell death detection ELISA assay. Cells treated with Camptothecin were used as positive control and result of control group was normalized to 100%. By shifting the fraction class from 40% to 100%, the spectrophotometric results were found in an ascending order. Figure 2 interprets the results of this experiment in PC-3 and WEHI-164 cells.

Discussion

Cancer is a growing health concern worldwide, which associates with substantial mortality and morbidity. Many patients are resistant to conventional treatment strategies. Subsequently, new studies aim to improve the survival rate and life quality of cancer individuals.¹⁸ Natural compounds seem to be appropriate choices for prevention and treatment of various diseases such as cancer. Among herbal medicines, *Ornithogalum* genus is shown to contain cytotoxic and antimicrobial compounds.⁹ In instance, the anti-proliferative effect of *Ornithogalum saundersiae* OSW-1 on malignant tumor cell lines including: Leukemia, Lymphoma, ovarian (SKOV3 cell line), brain (U87-MG cell line) and pancreas tumors (AsPC-1 cell line) has been identified.^{10,11,19,20} Other study by Kuroda et al.

demonstrated the cytotoxic effects of steroidal glycosides of *Ornithogalum thyrsoides* on leukemic cancer cells.¹² In the present study, the cytotoxic and apoptotic properties of methanolic fractions of *O. cuspidatum* extract on PC-3, prostate cancer cell line and WEHI-164, Fibrosarcoma cancer cell line were investigated. MTT results showed that during 12 and 24 hours, different

concentrations of 10% and 20% fractions did not exert any notable anti-proliferative effects. The cytotoxicity was evident in 100%, 80%, 60%, 40% fractions respectively, which indicates the accumulation of cytotoxic compounds in more polar fractions. The 60%, 80% and 100% fractions had significant differences with the control group at all concentrations in 12 and 24h.

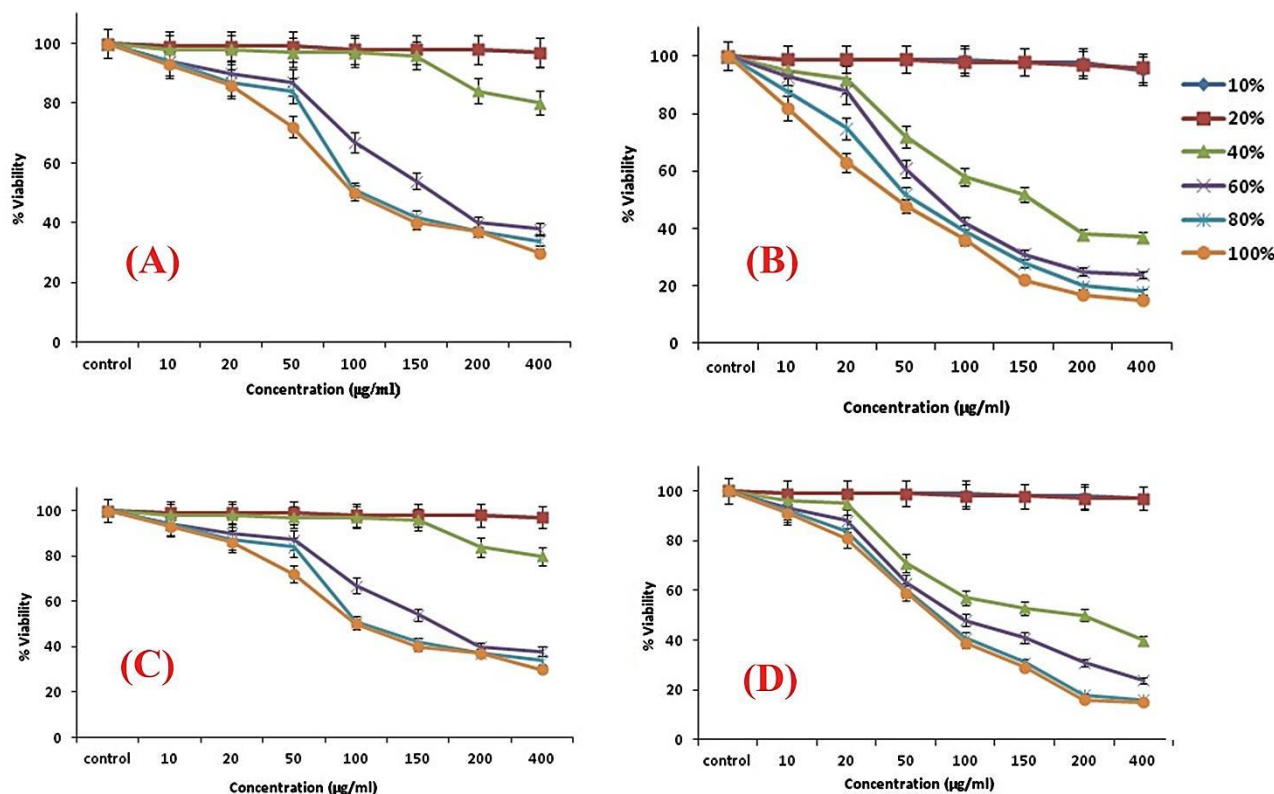


Figure 1. Cytotoxicity of *O. cuspidatum* methanolic fractions (10%, 20%, 40%, 60%, 80% and 100%) on cancerous cell lines: PC-3 in 12 hours (A), PC-3 in 24 hours (B), WEHI-164 in 12 hours (C) and WEHI-164 in 24 hours (D). Cell viability was measured by MTT assay. The data represent the mean±SE of three independent experiments each in triplicate format ($p < 0.05$).

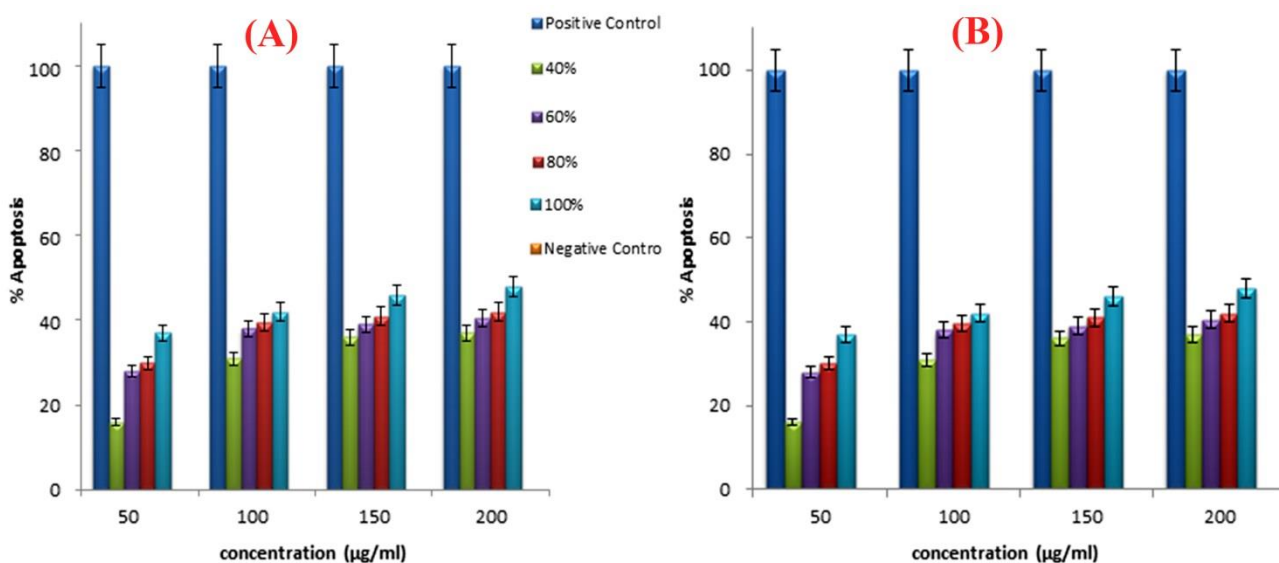


Figure 2. Apoptotic effect of *O. cuspidatum* methanolic fractions (40%, 60%, 80% and 100%) on PC-3 (A) and WEHI- 164 cells (B) in 24 hours. The data represent the mean±SE of three independent experiments each in triplicate format ($p < 0.05$).

It should be noticed that, a desired anticancer agent must affect cancerous cells without influencing normal cells and necrosis bourn inflammatory responses. Hence, the identification of more efficient compounds with apoptotic features and minimal immune response has been intended in pharmacological studies. In previous study Samavati et al., confirmed that the cytotoxic effects of *O. cuspidatum* methanolic extracts on WEHI-164 cells is through apoptotic pathways.¹³ According to the results of cell death detection ELISA, the apoptosis rate was raised in a dose and time dependent manner.

Conclusion

The present study was performed to assess the anticancer and apoptotic properties of *O. cuspidatum* methanolic fractions. Our results showed the cytotoxic effects in 40%, 60%, 80% and 100% fractions. Furthermore, apoptosis in a dose and time dependent manner was observable. Briefly, methanolic fractions of *O. cuspidatum*, could be considered in development of chemo-preventive substances.

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Conflict of Interest

The authors report no conflicts of interest.

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