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Original article

Apoptotic Potential of Two Caryophyllaceae Species in MCF-7 and MDA-MB-468 Cell Lines

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Abstract

Background and objectives: Plants have been used to treat diseases like cancer for many years and today the trend towards their use is increasing. One of the most effective mechanisms of plants against cancer is inducing apoptosis. Apoptosis is a programmed cell death which acts opposite to cell division. It starts in response to some stimuli. Despite the effectiveness of apoptosis inducing agents, their use has been limited due to side effects and resistance to these treatments; so, applying medicinal herbs due to their lower cost and toxicity has drawn attentions. Recent research at the Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences on two medicinal plants *Acanthophyllum bracteatum* and *A. microcephalum* has shown cytotoxic effects of these two species, but the mechanism of their toxicity has remained unknown; thus, the present study was designed to evaluate the apoptotic potential of *Acanthophyllum bracteatum* and *A. microcephalum*. **Methods:** In the present study, the cytotoxic effects of the methanol extract of *Acanthophyllum bracteatum* and *A. microcephalum* was evaluated against MCF-7 and MDA-MB-468 cells by MTT assay; furthermore, their apoptosis potential has been evaluated by annexin-V/propidium iodide assay and Hoechst 33258 staining in the same cell lines. **Results:** The methanol extract of *A. microcephalum* and *A. bracteatum* showed cytotoxic effects against MCF-7 and MDA-MB-468 cell lines with IC_{50} values of 64, 159 and 102, 250 µg/mL, respectively. The results of the apoptosis assays confirmed the potential of the two plants extracts to induce apoptosis in both cell lines while *A. microcephalum* demonstrated more considerable results. **Conclusion:** *A. microcephalum* could be a suitable choice for further breast cancer studies.

Keywords: *Acanthophyllum bracteatum*; *Acanthophyllum microcephalum*; Annexin-V/propidium iodide; apoptosis; MTT assay

Introduction

Caryophyllaceae is a wide-spread family with about 86 genera and 2200 species [1]; the genus *Acantophyllum* from this family has 32 species in Iran growing in central deserts to mountainous

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areas in center and south regions of Iran [2,3]. Caryophyyllaceae plants are known to possess foaming agents called saponins which are plant metabolites with 600 to 2000 Da weight. Saponins appear as glycosides, the aglycones have found to be mostly gypsogenin, gypsogenic acid or quillaic acid in Caryophyllaceae family [4]. Saponins have been reported to have haemolytic, molluscicidal, anti-inflammatory, antifungal, antibacterial, antiparasitic, cytotoxicity, anti-tumor and antiviral activities [5]. There has been records about the biological properties such as antioxidant [6,7], immunomodulatory [8] and cytotoxic [9] properties of different species of the genus *Acanthophyllum*. One report is about the cytotoxicity of *Acanthophyllum bracteatum* Boiss. and *A. microcephalum* Boiss. methanol extracts in human adenocarcinoma cells (MCF-7) in previous studies [9]; thus, in the present work, the apoptotic potential of these extracts in two human breast adenoma carcinoma cells has been evaluated.

Material and Methods

Plant material

The aerial parts of *Acanthophyllum bracteatum* and *A. microcephalum* were collected in 2010 from Kohgiluyeh-va-Boyer Ahmad and Ardebil Provinces, Iran, respectively and their identity was confirmed by botanists at the Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. The aerial parts were shade dried and ground.

Extraction and preparation of samples

Ten g of each species was macerated with methanol at room temperature with continuous shaking overnight. The process was repeated for thrice. The concentrated and dried extract was dissolved in DMSO and serial dilutions were made with cell culture medium to prepare the final concentrations (3.125-400 µg/mL). The final concentration of DMSO was 1%.

MTT assay

Two human breast adenocarcinoma cell lines (MCF-7 and MDA-MB-468) were obtained from Pasture Institute, Tehran, Iran. The cells were seeded in 96-well plates (13500 and 8000 cells/well for MCF-7 and MDA-MB-468 cells, respectively). Exposure of the cells to the extract, 5-fluorouracil as the positive control and DMSO 1% as the negative control took place the next day and continued for 48 h. On the final day, the medium containing the samples was replaced with medium containing MTT (prepared in PBS) with the final concentration of 0.5 mg/mL. The plates were incubated for another 4 h; thereafter, the MTT containing medium was removed and the formed formazan crystals were dissolved in DMSO. The absorbance was then recorded using a micro plate reader at 570 nm. The viability of the cells in regard to the control (cells receiving DMSO 1%) was calculated and the IC_{50} was obtained using Microsoft Excel program [10,11].

Hoechst 33258 assay

MCF-7 and MDA-MB-468 cells were seeded in 96-well plates and exposed to samples with the same method as mentioned for MTT assay at IC_{50} value of the extracts. Completing the exposure time (48 h) the cells were fixed with paraformaldehyde 4% for 30 min at room temperature. Then the fixative solution was removed and the cells were washed with PBS. Hoechst 33258 (1 µg/mL) was added to the wells. The plates were kept in darkness (15 min, 37 ºC), the dye was removed afterwards and the cells were washed again. They were then examined afterwards using a florescent microscope for glowing condensations of chromatin [10].

Apoptosis assay

Considering the IC_{50} values of the extracts in MCF-7 and MDA-MB-468 cells, the cells were exposed to extracts for 24, 48 and 72 h; afterwards, they were collected and evaluated for apoptosis induction using Invitrogen FITC Annexin V/Dead Cell Apoptosis Kit following

the instructions. Briefly, the cells were incubated for 15 min in darkness with 5 µL of annexin-V solution, then PI was added and the incubation continued for another 15 min. The cell suspension was moved to flow cytometry tubes and analyzed by flow cytometer and the percentage of live, early apoptotic, late apoptotic and the cells undergoing necrosis was presented [12].

Results and Discussion

Regarding MTT assay, a colorimetric cytotoxicity method for evaluating the toxicity of materials to the cells [13], *Acanthophyllum microcephalum* and *A. bracteatum* showed cytotoxicity to MCF-7 and MDA-MB-468 cells with IC_{50} values of 64, 159 and 102, 250 µg/mL in the above cell lines, respectively. IC_{50} values of 5-fluorouracil as the positive control were 0.83 and 1.04 µg/mL, respectively. The results of Hoechst staining have been demonstrated in figures 1 and 2 for MCF-7 and MDA-MB-468 cells, respectively. Brilliant condensed chromatin is an indication of apoptosis in the cells.

For quantifying the apoptotic potential of the extracts, annexin V-FITC and propidium iodide fluorescence staining was used. This method evaluates dislocation of phosphatidylserine from the inner to the outer layer of the plasma membrane. Control cells were negative for both annexin V-FITC and PI. The cells at early apoptotic stage were annexin V-FITC-positive, PI-negative cells while annexin V-FITC-positive, PI-positive cells were considered to be late apoptotic and PI-positive cells were considered as necrotic. Flow cytometery analyses have been presented in figures 3-6. As could be observed in the figures, by passing exposure time of the cells to the extracts from 24 h to 72 h, the apoptosis rate (sum of early and late apoptotic cells) has increased slightly and the percentage of live cells has decreased while the early apoptotic cells were converted to late apoptotic ones compared to the control (cells treated with DMSO 1%). This finding was more considerable for *Acantophyllum microcephalum* which had also shown lower IC_{50} in the examined cell lines. There has not been much research about the cytotoxicity of *Acanthophyllum* species.

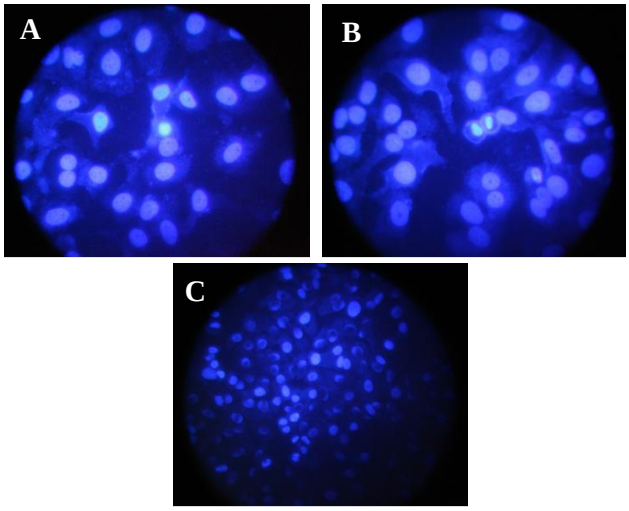

Figure 1. Results of Hoechst 33258 staining in MCF-7 cell line. The cells were treated with 100 µg/mL *Acanthophyllum bracteatum* extract (A); 50 µg/mL *Acanthophyllum microcephalum* extract (B) and DMSO 1% (C)

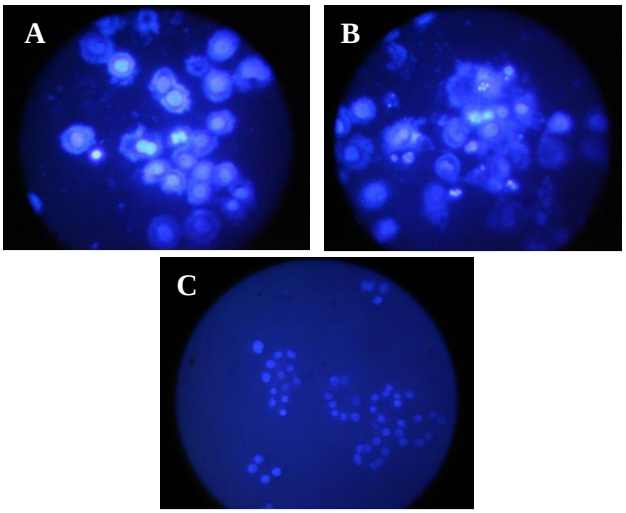

Figure 2. Results of Hoechst 33258 staining in MDA-MB-468 cell line. The cells were treated with 200 µg/mL *Acanthophyllum bracteatum* extract (A); 75 µg/mL *Acanthophyllum microcephalum* extract (B) and DMSO 1% (C)

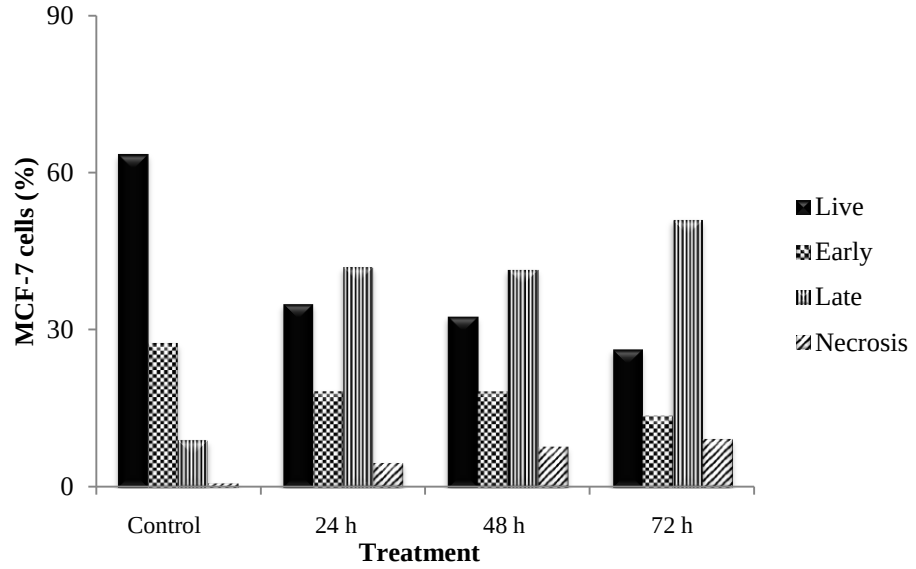

Figure 3. Percentage of live, early apoptotic, late apoptotic and necrotic MCF-7 cells after 24,48 and 72 h treatment with *Acanthophyllum bracteatum*

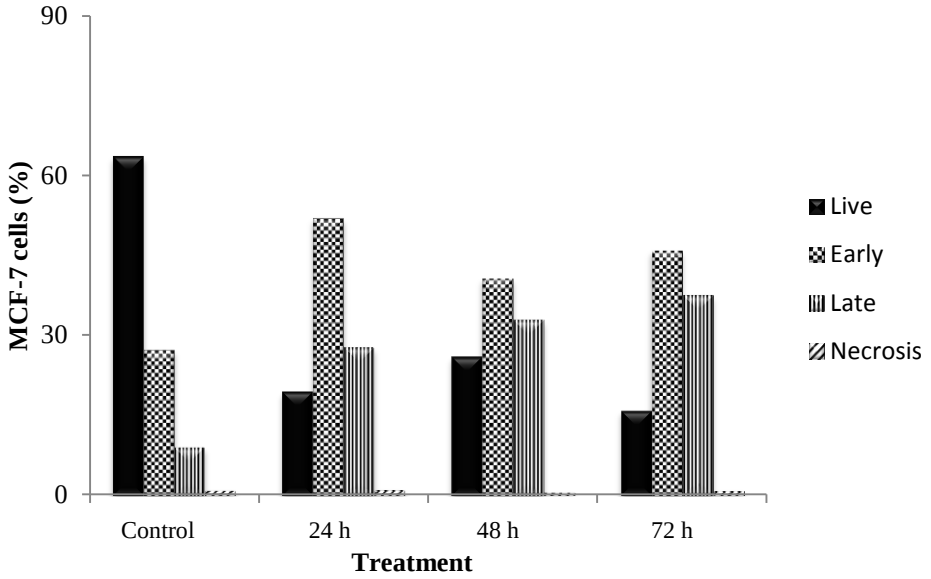

Figure 4. Percentage of live, early apoptotic, late apoptotic and necrotic MCF-7 cells after 24,48 and 72 h treatment with *Acanthophyllum microcephalum*

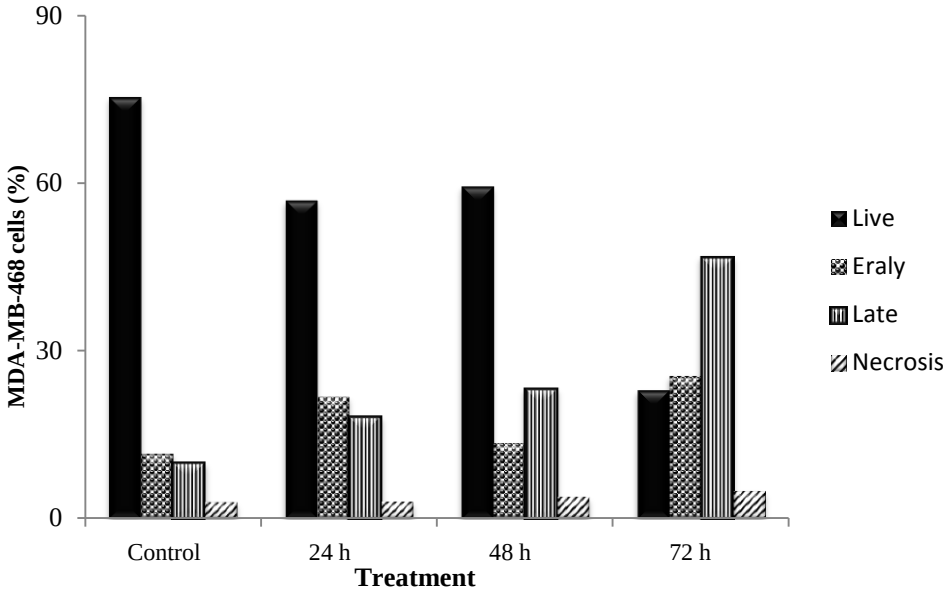

Figure 5. Percentage of live, early apoptotic, late apoptotic and necrotic MDA-MB-468 cells after 24,48 and 72 h treatment with *Acanthophyllum bracteatum*

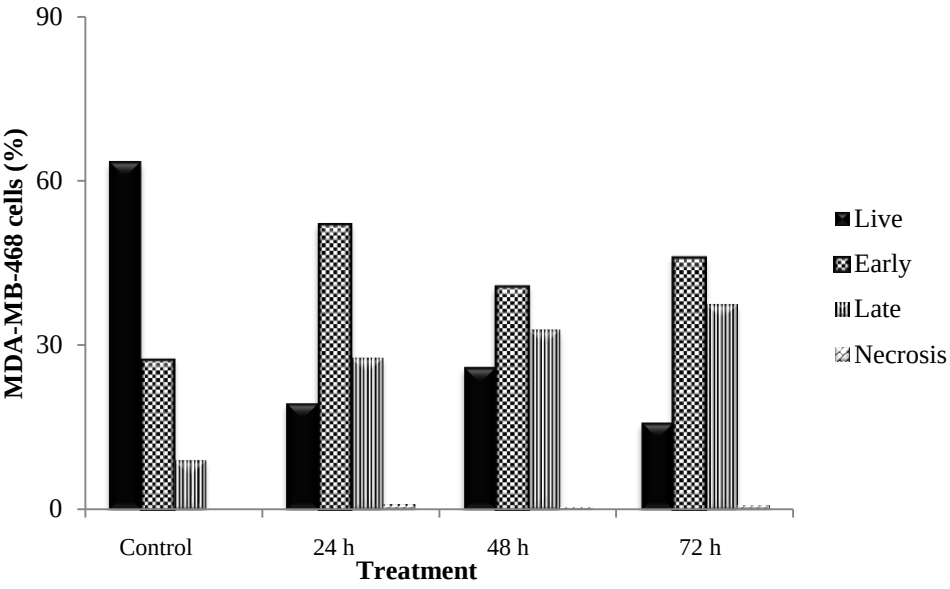

Figure 6. Percentage of live, early apoptotic, late apoptotic and necrotic MDA-MB-468 cells after 24,48 and 72 h treatment with *Acanthophyllum microcephalum*

Few reports regarding the cytotoxic activity of the genus have shown their effectiveness against various cell lines; these include the glycosides isolated from three species of the genus which

have shown cytotoxicity to HCT-116 and HT-29 cells [14] and the triterpenoid saponins from the roots of *A. squarrosum* which demonstrated anti-proliferative activity to lymphocyte [15]. In a study for evaluating the cytotoxicity of 17 species of Caryophyllaceae family, *Acanthophyllum bracteatum* and *A. microcephalum* were evaluated against A-549, HepG-2, HT-29, MCF-7 and MDBK cells and showed cytotoxic results. The reported IC_{50} in MCF-7 were lower compared to our study. Regarding that the plants evaluated in our study were collected in 2010 it could be suggested that the difference could be due to the time gap between plant collection and fulfilling the experiments which might have resulted in decomposition of the cytotoxic compounds.

Coming to a conclusion, the results of the present study suggest *A. bracteatum* and *A. microcephalum* as promising sources for future molecular investigations especially regarding breast cancer studies.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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