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Cytotoxic effects of hydroalcoholic extract of Iranian cranberry (*Vaccinium arctostaphylos*) on breast cancer cell lines MCF-7 and MDA-MB2321

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ABSTRACT

Background & Aim: Cancer is currently recognized as the second leading cause of death worldwide, following cardiovascular diseases, with breast cancer being the most common malignant tumor among women. Despite ongoing efforts, there is still no successful treatment for this disease, and chemotherapy, while widely used, not only fails to guarantee a cure but also carries significant side effects. This study aimed to evaluate the cytotoxic effects of Iranian cranberry (*Vaccinium arctostaphylos*) extract on human breast cancer cell lines.

Experimental: The MCF-7 and MDA-MB231 cell lines were obtained from the Geniran Institute, and after cell culture and extract preparation, concentrations of 25 µg/mL, 50 µg/mL, 75 µg/mL, 100 µg/mL, 125 µg/mL, 150 µg/mL, 175 µg/mL, and 200 µg/mL were tested on 96-well plates for 24, 48, and 72 h. Cell viability was assessed using the MTT assay.

Results: The results indicated that the concentration of 200 µg/mL after 72 h had the greatest impact on reducing the survival of cancer cells, eliminating over 70% of cancer cells in both lines during this period. Given the significant efficacy of the Iranian cranberry extract in eliminating cancer cells in vitro, further clinical studies on this plant are recommended.

Recommended applications/industries: According to the results obtained, the extract of Iranian cranberry has the potential to be used in pharmaceutical industries and research centers as a supplement for the treatment of breast cancer. Of course, further clinical studies on this extract are recommended.

1. Introduction

Breast cancer is the most common malignant tumor in women and the leading cause of cancer-related mortality, with its incidence rising steadily. Currently, 36% of cancer patients are diagnosed with breast tumors (Nardin *et al.*, 2020). A large proportion of patients in industrialized countries and more than half in developing countries are affected, with the highest mortality rates reported in developing and underdeveloped nations (Bellanger *et al.*, 2018).

Predisposing factors and etiologies include gender, age, economic development, hormonal conditions, genetics, radiation exposure, alcohol consumption, diet, obesity, and smoking (Smołarz, 2022). Women account for 99% of cases, while only 1% occurs in men (Religioni, 2020). Women under 50 years old are at a higher risk (Lima *et al.*, 2021). The highest rates of incidence are linked to limited access to healthcare, poor nutrition, and stressful living conditions in developing countries (Ghoncheh *et al.*, 2016). Hormonal factors, such as the timing of estrogen exposure, early menstruation, late menopause, age at first childbirth, and number of children, also influence breast cancer susceptibility (Lima *et al.*, 2021; Torre *et al.*, 2017; Ghoncheh *et al.*, 2016). About 5–10% of

breast cancer cases are genetically linked, often due to mutations in the BRCA1 and BRCA2 genes (Mehrgou *et al.*, 2016). Alcohol consumption increases the risk of breast cancer by up to 9% with a daily intake of 10 grams through liver damage and indirect effects on estrogen metabolism (Nardin *et al.*, 2020). Obesity promotes cancer cell survival by releasing cytokines stored in fat, inhibiting apoptosis, and promoting cell proliferation, invasion, angiogenesis, and metastasis (Tornatore *et al.*, 2012; Prasad *et al.*, 2010). Moreover, obesity reduces treatment effectiveness and increases recurrence risk (Lee *et al.*, 2019). Nicotine in tobacco stimulates neutrophil N2 formation and creates pre-metastatic niches in the lung, contributing to breast cancer development (Tyagi *et al.*, 2021).

Breast cancer diagnosis and prognosis rely on mammography, biomarker assays, and biopsy, with biomarker assays providing more precise information on cancer progression compared to mammography. Key diagnostic biomarkers include TNM, HER-2, Ki67 protein, hormonal factors, and the pathological complete response (pCR) (Smołarz *et al.*, 2022). Treatment options encompass surgery, breast-conserving therapy (BCT), chemotherapy, herbal medicines, and the role of non-coding RNA in gene therapy (Smołarz *et al.*, 2022).

Major surgical treatments include nine procedures (Towpik *et al.*, 2022; Samulak *et al.*, 2008), while breast-conserving therapies include three surgeries, with cosmetic outcomes also being considered (Burstein *et al.*, 2019).

The remarkable effects of herbal medicines, along with their lower side effects and accessibility, have led to a third of available drugs originating from plants (Scartezzini *et al.*, 2000). The anticancer effects of bioactive plant compounds such as curcumin, quercetin, berberine, and allicin—as well as various flavonoids and saponins—have been well documented (Wani *et al.*, 2014; Sabale *et al.*, 2013). However, many of these studies were conducted on cell lines and have not been tested in vivo. Moreover, among these bioactive compounds, the effects of quercetin and allicin on breast cancer have not been specifically investigated (Siddiqui *et al.*, 2022).

Cranberry (*Vaccinium Arctostaphylos*) of the Ericaceae family is rich in phenolic andanthocyanin compounds, contributing to its potent antioxidant properties (Ayaz *et al.*, 2005). This plant also contains significant anticancer compounds such as quercetin (Sepehri Far *et al.*, 2009; Hasanaloo *et al.*, 2013) and has been shown to have potent effects against prostate cancer (Ghorbanzadeh *et al.*, 2018) and colon cancer (Karami *et al.*, 2022). The American species of this plant has also demonstrated significant cytotoxic effects on metastatic breast cancer cell lines (MDA-MB231) (Boivin *et al.*, 2008).

Given the antioxidant and anticancer properties of cranberry, as well as its documented effects on prostate and colon cancer, this study aimed to evaluate the in vitro cytotoxic effects of Iranian cranberry extract on breast cancer cell line.

2. Materials and Methods

2.1. Preparation of the plant extract

In this study, dried Iranian *Vaccinium arctostaphylos* (Qareqat) fruits were purchased from reputable herbal stores in Isfahan province, Iran, and the herbarium code (4302) was confirmed by the Agricultural Research Center of Chaharmahal and Bakhtiari province.

The hydroalcoholic extract was prepared at the Medicinal Plants Research Center, Islamic Azad University, Shahrekord, Using the percolation method with 70% ethanol at a temperature of 45-55 for 24-48 hours, then drying the extract at a temperature of 40 for 48-72 hours and finally dissolving it in normal saline.

2.2. Cell line culture

MCF-7 and MDA-MB231 breast cancer cell lines (representing non-metastatic and metastatic breast cancer, respectively) were used. The cells were cultured in RPMI medium supplemented with 10% FBS and 1% penicillin and streptomycin. Cultured cells were maintained in T25 flasks inside a 37°C incubator with 5% CO_2 and 95% humidity. Once cell confluence reached 80%, the cells were detached from the flask using trypsin and counted with a Neubauer hemocytometer (sharifzadeh *et al.*, 2021).

2.3. MTT cell viability assay

For this assay, 20 µL of counted cells were added to each well of 96-well plates containing 180 µL of culture medium, ensuring approximately 10,000 cells per well. Eight different extract concentrations (25, 50, 75, 100, 125, 150, 175, 200 µg/mL) were tested in eight rows with four replications. Control samples for each extract concentration were tested by adding only

PBS. After 24, 48, and 72 h of exposure to the extract, the culture medium in each well was removed, and 20 µL of MTT solution was added. Plates were incubated for 4 h, allowing mitochondrial succinate dehydrogenase in viable cells to reduce the yellow MTT to insoluble purple formazan crystals. The medium was then removed, and 200 µL of DMSO was added to each well to solubilize the formazan. Absorbance was measured at 570 nm, and cell viability was calculated by dividing the absorbance of treated cells by that of control cells and multiplying by 100 (Sharifzadeh *et al.*, 2021).

2.4. Ethical approval

This study was approved by the Ethics Committee of Islamic Azad University, Shahrekord Branch, under the ethics code IR.IAU.SHK.REC.1403.369. It was conducted in a laboratory setting as an interventional study with a control group.

2.5. Statistical analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Tukey tests at a probability level of 5% ($P<0.05$). Data were expressed as the mean ± SEM. REST 2009 software was employed for data analysis.

3. Results and discussion

The results showed that the viability of metastatic and non-metastatic breast cancer cell lines decreased upon treatment with various concentrations of Qareqat extract, particularly at 48 and 72 h of incubation at higher concentrations compared to the control group.

As can be seen, only concentrations of 175µg/mL and 200µg/mL were able to kill about 10% of cancer cells (MCF-7) in 24 h (Figure 1).

It is observed that in 24 h, none of the cranberry extract affects the survival of cancer cells (MDA-MB231) (Figure 2).

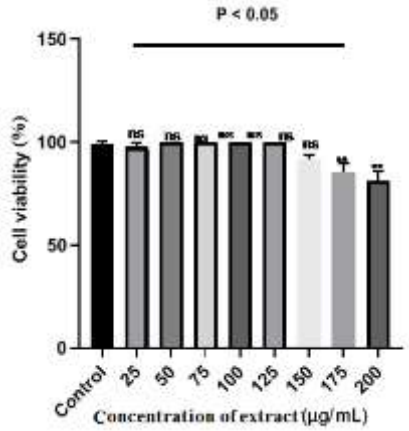

Figure 1. Effect of different concentrations of Iranian cranberry extract on the survival of breast cancer cell lines (MCF7) in 24 h (ns: none significant,**:P<0.01).

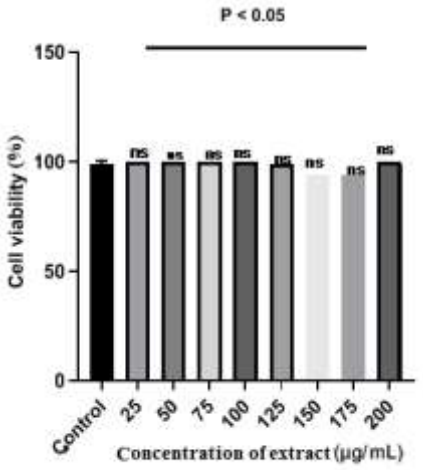

Figure 2. Effect of different concentrations of Iranian cranberry extract on metastatic breast cancer cell lines (MDA-MB231) in 24 h (ns: none significant).

As can be seen, out of 150, 175, and 200µg/mL, more than 50% of breast cancer cells (MCF-7) were destroyed in 48 h (Figure 3). The concentration of 150, 175, and 200µg/mL were observed to kill more than 30% of the cancer cells (MDA-MB231) (Figure 4).

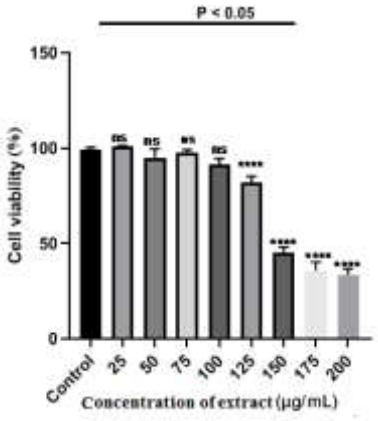

Figure 3. Effects of different concentrations of Iranian cranberry extract on breast cancer cell (MCF-7) survival in 48 h (ns: none significant,****:P<0.0001).

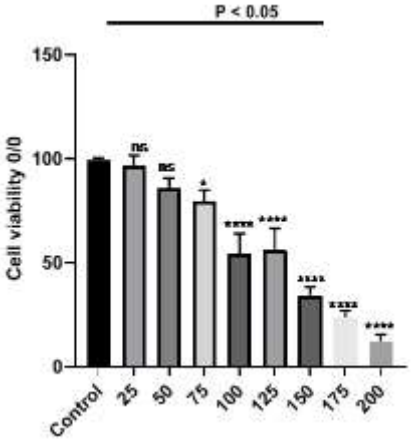

Figure 5. Effect of different concentrations of Iranian cranberry extract on breast cancer cell line (MCF-7) survival in 72 hours (ns: none significant, *:P<0.05,****:P<0.0001).

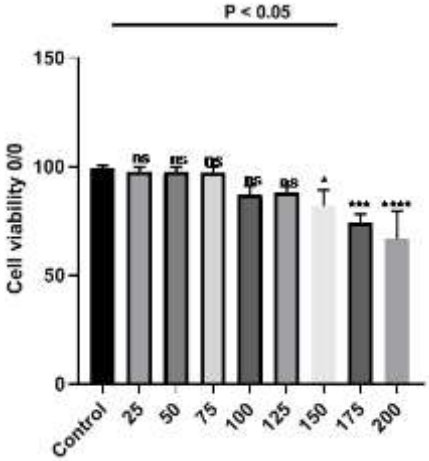

Figure 4. Effect of different concentrations of Iranian cranberry extract on metastatic breast cancer cells (MDA-MB231) in 48 hours (ns: none significant, *:P<0.05,***:P<0.001, ****:P<0.0001).

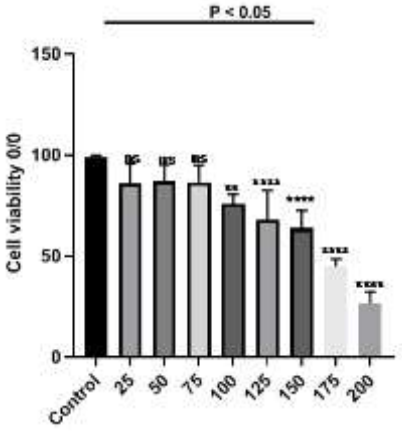

Figure 6. Effect of different concentrations of Iranian cranberry extract on metastatic breast cancer cell (MDA-MB231) survival in 72 hours (ns: none significant,**:P<0.01,****:P<0.0001).

It can be seen that concentrations of 100 and 125µg/mL were successful in killing more than 40%, 150 and 175µg/mL were effective in killing more than 70%, and 200µg/mL was effective in killing more than 80% of MCF-7 cancer cells (Figure 5) while the concentrations of 175µg/mL and 200µg/mL were effective in killing 60% and 70% of the MDA-MB231 cancer cells, respectively (Figure 6).

Statistical analysis using the t-test revealed significant differences at 48 h for MDA-MB231 cells treated with 175, and 200 µg/mL concentrations (P<0.001), and for MCF-7 cells treated with 125 µg/mL (P<0.0001) (Figures 3 and 4). At 72 h, significant differences were also observed at 100 µg/mL for MDA-MB231 (P<0.05) and at 75 µg/mL for MCF-7 cells (P<0.01) (Figures 5 and 6).

Currently, chemotherapy remains the most commonly recommended treatment for breast cancer, either as monotherapy or in combination with other drugs. However, chemotherapy drugs have narrow therapeutic indices, leading to non-selective toxicity to healthy tissues and an increased risk of infections. Despite their effectiveness, chemotherapy and even radiotherapy are associated with several side effects, including nausea and vomiting (in 75% of patients), post-mastectomy edema (in 30–60% of patients), and joint pain (in more than 40% of patients). Neutropenia, cachexia, fatigue, pain, hair loss, hot flashes, and stress are also frequently seen, hindering treatment effectiveness. Thus, various options for reducing breast cancer risk have been proposed, including modifying non-genetic risk factors such as obesity, alcohol consumption, and physical inactivity (Liao *et al.*, 2013). The use of medicinal plants is also considered important for cancer prevention. One key factor in breast cancer development is oxidative stress. Reactive oxygen species (ROS) and oxidative damage are central to human disease research. Antioxidants are compounds that neutralize ROS and protect against oxidative damage. Therefore, the consumption of medicinal plants rich in antioxidants is especially recommended for high-risk groups (Ali Mohammadi *et al.*, 2021).

Our findings demonstrated that Qareqat extract significantly reduced the viability and proliferation of both non-metastatic and metastatic breast cancer cells, particularly at 200 µg/mL after 72 h, killing over 80% of non-metastatic and over 70% of metastatic breast cancer cells.

Ghorbanzadeh *et al*. (2018) found that Qareqat extract reduced viability in human prostate cancer cells (PC-3) and increased the expression of glutathione S-transferase, possibly via CPG demethylation affecting cancer pathways.

Karami *et al*. (2022) also reported cytotoxic effects of Qareqat extract on colorectal cancer cells (HCT-116) using the MTT assay. Antioxidants are widely used as dietary supplements to prevent diseases like cancer. Anthocyanins and flavonoids are the primary bioactive compounds in Qareqat, acting as natural antioxidants and playing roles in preventing oxidative DNA damage by ROS and inducing apoptosis.

Sun *et al*. (2006) demonstrated that concentrations of 10–50 µg/mL of Qareqat extract increased the expression of pro-apoptotic genes such as caspase-3, cdk-3, and cdk-4, and reduced the expression of inhibitory genes such as caspase-8 and flip, arresting A549 lung cancer cells in the G1 phase and preventing their proliferation. Similarly, Boivin *et al*. (2007) reported that 10–25 µl/mL of the *American Vaccinium* extract showed a stronger effect on the expression of tumor suppressor genes and apoptotic genes compared to other berry family members, significantly reducing viability in breast, colorectal, gastric, and prostate cancer cells.

4. Conclusion

This study showed that Iranian cranberry extract at concentrations of 200 µg/ml and above can kill over 50% of breast cancer cells in 72 hours. Overall, Qareqat extract, due to its high content of antioxidant and anticancer compounds, appears not only effective on cancer cells but also potentially associated with fewer side effects, raising hopes for a more accessible and affordable therapeutic supplement for patients. Given its cytotoxic effects on human breast cancer cell lines, further studies could pave the way for using this plant as a potential adjunct therapy in breast cancer prevention and treatment.

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