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The Effect of Hydroalcoholic Extract of Cuscuta Europaea on Breast Cancer Cells (Mcf7) in Comparison with Kidney Epithelial Cells (Vero cell line)

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ABSTRACT

BACKGROUND AND OBJECTIVE: Studies have shown that plants of Family Cuscutaceae can affect the growth and development of cancer cells. It is important that the herbs used for non-cancerous cells do not have cytotoxic effects. The present study was conducted to evaluate the effects of hydroalcoholic extracts of Cuscuta europaea on the viability of breast cancer cells (MCF7) compared to non-cancerous kidney epithelial cells (Vero cell line).

METHODS: In this experimental study, Vero cells and MCF7 cells were prepared from Pasteur Institute Cell Bank and divided into the control group and the groups treated with doses of 0.01, 0.1, 1 and 10 mg/ml. 48 hours after exposure, the cytotoxic effect of the extract was measured by MTT.

FINDINGS: Compared with the control group, exposure to dose of 0.01, 0.1, 1 and 10 mg/ml of hydroalcoholic extract of Cuscuta europaea reduced the viability of MCF7 cells (54.47±0.35, 55±0.42, 44.62±0.38, and 4.64±0.73%, respectively) and Vero cells (58.42±0.45, 48.61±0.55, 43.14±0.23, and 5.35±0.27%, respectively) ($p<0.001$).

CONCLUSION: The results of this study showed that the hydroalcoholic extract of Cuscuta europaea can simultaneously cause toxicity in non-cancerous cells, although it has toxic effects on breast cancer cells, and in this regard, the use of this extract in the treatment of breast cancer does not seem favorable due to its toxic effects on non-cancerous cells.

KEY WORDS: *Cuscuta Europaea, MCF7, Vero, Viability.*

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Introduction

Breast cancer has a high prevalence worldwide (1). Among all types of cancers, breast cancer has high mortality rates. Common treatments for this disease include hormone therapy, surgery, chemotherapy, and radiotherapy, but due to their significant side effects, they have their own deficiencies and disabilities. The use of alternative therapies, especially the use of plant-derived anticancer compounds, can be one of the most important solutions to the treatment of breast cancer (2). Several studies have shown that flavonoids in plant extracts can be effective in the treatment of cancer, especially breast cancer (3, 4).

Cuscuta europaea is one of the most famous parasitic plants of the Bindweed family and has been proved to be an appropriate drug for neurological diseases, insomnia, melancholia, headache, joint pain and cancer (5). Studies have shown that medicinal herbs are effective in the treatment of various cancers such as gastrointestinal cancer (6), skin cancer (7), oral cancer (8), reproductive cancer and breast cancer (9). Among medicinal herbs, parasitic plants are effective in the treatment of gastrointestinal cancers, skin cancer, oral cancer, reproductive cancer, and breast cancer (10). Research has shown that the extract of Cuscuta europaea, which is of parasitic plant family, has anticancer effects (5). Moreover, studies have shown that parasitic plants can affect cancer of the reproductive system (11).

Medicinal herbs, especially parasitic plants, are effective in the treatment of breast cancer (12 – 14). Research results have shown that the extract of Cuscuta europaea can be effective in inhibition and prevention of breast cancer (15). In contrast, some studies have shown that the extract of this plant stimulates the proliferation of breast cancer cells and thus contributes to the growth and development of this cancer (16). On the other hand, studies show that medicinal herbs, including parasitic plants, have no cytotoxic effects on non-cancerous cells in most cases, especially non-cancerous kidney epithelial cells (15,16). Considering the high prevalence of cancers, particularly the prevalence of breast cancer in the world (19), and Iran (20), as well as the widespread complications of this cancer in affected people and the imposition of significant social and financial costs on the family and community, and considering the side effects of conventional treatments based on radiotherapy and chemotherapy, and given that previous studies on the subject of this study have contradictory results in many cases (15–18), the present study was conducted to investigate the cytotoxic effects of hydroalcoholic extract of Cuscuta europaea on the viability of breast cancer cells compared to non-cancerous kidney epithelial cells, so that the results of this study can be used to decide whether or not to use Cuscuta europaea for the treatment of breast cancer.

Methods

In this experimental study, after approval by the Ethics Committee of Islamic Azad University of Hamedan with the code 7529/d/1397, plant samples were collected from the pastures of Guilan province at 2050 m heights of Ashkoor valley route in Rudsar County. There is a sample of the plant with the herbarium code of 2314 at the Agriculture Department of Islamic Azad University, Karaj Branch. The aerial parts of the plant contain stems and flowers, and the plant has no leaves. The aerial parts of the plant were used in this study. Hydroalcoholic extract of the plant was prepared based on previous studies (21–23).

First, the plant samples were dried in shade, and the aerial parts of the plant were grinded. Then, 100 g of powder was soaked in 300 cc of hydroalcoholic solvent 50% (500 cc of ethanol 96% with 500 cc distilled water) for 24 hours and extracted by Soxhlet extractor (Behr, Germany) and the resulting extract was dried in the oven at 40 to 50 °C within two to three days. Finally, the extracts were placed in an incubator at 37 °C for 24 hours and the water was completely evaporated and dried. In the next step, the extract was weighed and dissolved in PBS solvent, and a solution of 100 mg/ml was prepared as reservoir.

Breast cancer cells (MCF7 cell line) and kidney epithelial cells (Vero cell line) were also prepared from Pasteur Institute Cell Bank. These cells were kept in a complete culture medium with 10% fetal bovine serum

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(FBS) and 1% antibiotics (penicillin/streptomycin). Cells (106 cells/ml) were cultured in T-25 flasks containing 5 ml of complete culture medium under atmospheric air at 95 percent relative humidity and 5% carbon dioxide at 37 °C. The culture medium was replaced every 48 hours. As soon as the cells reached 95% confluency, the culture medium was aspirated and the cell layer was washed three times with phosphate buffer. The cell layer was treated with 1 ml 0.25% Trypsin-EDTA solution and incubated at 37 °C. The cells were evaluated by the inverted microscope (XDS-2 model manufactured by OPTIKA, Italy) in terms of separation of cells from each other.

Based on previous studies (5, 11-14), the MCF7 cell line and Vero cell line were randomly divided into control group and groups exposed to doses of 0.01, 0.1, 1, and 10 mg/ml of the extract. The control group was not under any treatment. Considering sufficient culture medium for cells and at least six repetitions, the extracts were added to the wells and the plates were stored in the incubator for 48 hours. In this regard, 96 – well plate was used, and cells with a cell density of 104 cells per well were placed in 96 – well plate. After the desired time, the liquid was evacuated from the plate and the MTT color was added. Four to six hours after the addition of the paint, the MTT solution was evacuated and the DMSO solvent was added. After complete dissolution, the absorbance of the solutions was read at wavelengths of 570 nm and 630 nm using Elisa Plate Reader. Finally, for statistical analysis, the normal distribution of data was first evaluated using Kolmogorov – Smirnov test, and after confirming the normal distribution of data, the data were analyzed using SPSS software, one-way ANOVA and the Bonferroni post hoc test, and $p<0.05$ was considered significant.

Results

According to the results of this study, the viability of breast cancer cells in groups exposed to extracts of Cuscuta europaea at doses of 0.01, 0.1, 1 and 10 mg/ml was significantly lower than that of the control group ($P < 0.001$). On the other hand, the viability of breast cancer cells in the group exposed to the extract at a doses of 10 mg/ml was significantly different from all other groups ($P<0.001$), while the viability of breast cancer cells in groups exposed to 0.1 mg/ml was not significantly different from 0.01 mg/ml.

In addition, the viability of breast cancer cells in groups exposed to the extract at doses of 1 and 10 mg/ml had a significant difference compared to the group exposed to the extract at 0.1 mg/ml ($P<0.05$, and $P<0.001$, respectively) (Fig 1).

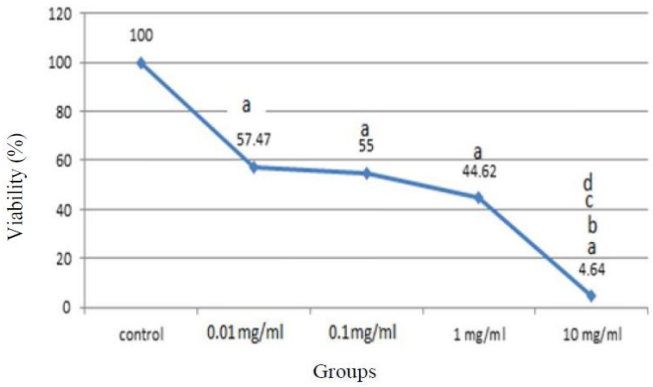

Figure 1. MCF7 cell viability in exposure to different doses of extracts of Cuscuta europaea in cell culture medium.

The viability of kidney epithelial cells in groups exposed to Cuscuta europaea extract at doses of 0.01, 0.1, 1 and 10 mg/ml was significantly lower than the control group ($P<0.001$). In addition, the viability of kidney epithelial cells in group exposed to 10 mg/ml dose was significantly different from that of all other groups ($P<0.001$), while the viability of kidney epithelial cells in groups exposed to Cuscuta europaea extract at 0.1 and 1 mg/ml doses was not significantly different from 0.01 mg/ml group. On the other hand, viability of kidney epithelial cells in groups exposed to Cuscuta europaea extract at doses of 1 and 10 mg/ml showed a significant difference compared with the group exposed to 0.1 mg/ml dose ($p<0.05$, and $p<0.001$, respectively), while the viability of kidney epithelial cells in group exposed to 0.01 mg/ml Cuscuta europaea extract did not show a significant difference compared to the group exposed to the extract at 0.1 mg/ml dose. The viability of kidney epithelial cells in groups exposed to Cuscuta europaea extract at doses of 0.1 and 10 mg/ml was significantly different from the group

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exposed to the extract at a dose of 1 mg/ml (P<0.05, P<0.001, respectively) (Fig. 2).

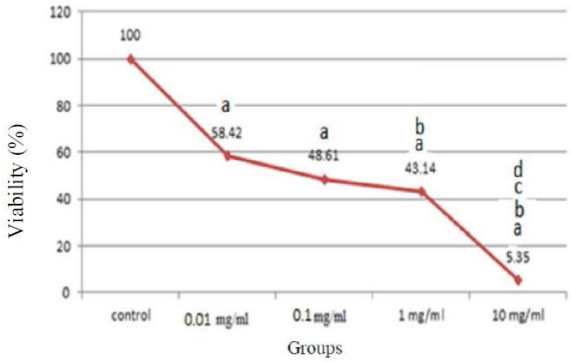

Figure 2. Vero cell line viability in exposure to different doses of extracts of Cuscuta europaea in cell culture medium

Discussion

The results of this study showed that the hydroalcoholic extract of Cuscuta europaea reduces the proliferation of breast cancer cells as well as non-cancerous kidney epithelial cells. This clearly showed that the hydroalcoholic extract of Cuscuta europaea has cytotoxic effects on non-cancerous cells and can also harm normal cells of the body if used as an anticancer extract. In this regard, studies have shown that various species of Cuscuta have anticancer effects. Research results indicate that various species of this genus are effective in the treatment of gastrointestinal cancers, skin, oral, reproductive system, and breast cancers (10, 14). Consistent with the findings of this study, studies suggest that the extract of Cuscuta europaea has anticancer effects on bladder (24) and gastrointestinal cancer (25).

Moreover, according to the results of previous studies, the extract of Cuscuta europaea in traditional treatments was found to be effective in preventing and treating breast cancer in humans (5, 15). Some studies, however, have shown that the extract of Cuscuta europaea stimulates the proliferation of breast cancer cells (16). The results of the present study, on the other hand, showed that the extract of Cuscuta europaea also has cytotoxic effects on non-cancerous cells, suggesting that the use of the extract as anticancer agent against breast cancer cells may have cytotoxic side effects on non-cancerous cells. Therefore, the extract of Cuscuta europaea should be cautiously applied as anticancer agent regarding its side effects on normal body cells. However, contrary to the findings of this research, some research results have shown that the extracts of some Cuscuta europaea family members have no cytotoxic effects on non-cancerous cells, including epithelial cells (17, 18).

In terms of the possible mechanism of action of the Cuscuta europaea extract on breast cancer cells and non-cancerous kidney cells, the cytotoxic effect of this extract may be attributed to the chemical compounds present in the extract. The results of studies have shown that the extracts of Cuscuta family contain compounds such as trisaccharide, a glycosidic acid called caustic acid, and various amino acids along with organic acids (26). Studies also have shown that glucoside containing compounds have anticancer effects (27). In addition, glucoside compounds inhibit the heparinase, which causes anti-proliferative effects on cancer cells (28). Amino acid compounds can also prevent cancer (29). Therefore, considering the compounds found in the extract of Cuscuta europaea, which often have anti-cancerous properties, this extract can be introduced as an herbal extract with potent cytotoxic anticancer effects.

The results of this study showed that although hydroalcoholic extract of Cuscuta europaea has toxic effects on breast cancer cells, it can simultaneously cause cytotoxicity in non-cancerous cells, and in this regard, the use of this extract does not seem desirable due to the adverse effects on non-cancerous cells.

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