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In Vitro Cytotoxic Activity of Matricaria Chamomilla Root Extract in Human Breast Cancer Cell Line MCF-7

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Abstract: Breast cancer is among the most common causes of cancer deaths in women. Recently, phytochemicals and their effects on the prevention and treatment of cancer have drawn a lot of attention. According to previous research, *Matricaria chamomilla* is a source of phytochemicals that have anticancer effects. In this research, the cytotoxic effects of the hydroalcoholic extract of *Matricaria chamomilla* root on the MCF-7 breast cancer cell line were investigated. **Materials and Methods:** The MCF-7 cells were treated for 24, 48 and 72 hours with different concentrations (200 $\mu\text{g/ml}$ -2000 $\mu\text{g/ml}$) of *Matricaria chamomilla* root extract in DMEM (Dulbecco's Modified Eagle's Medium) culture medium containing of Fetal Bovine Serum 10% (FBS). The half maximal inhibitory concentration (IC_{50}) was assayed by MTT method. Cell colony formation was also determined in percentage through a clonogenic assay. In this assay, a total of 1000 cells of the MCF-7 cell line were put in the wells of a 6-well plate and after 24 hours every well was treated with dosages of extract varying from 100 to 800 ($\mu\text{g/ml}$). Nine days following the treatment, the percentage of colony formation was measured. **Results:** The MCF-7 cells exposed to different dosages of *Matricaria chamomilla* root extract demonstrated 50% cell death after 24, 48 and 72 hours with 1954 ± 4.2 ($\mu\text{g/ml}$), 1700 ± 5.1 ($\mu\text{g/ml}$), and 1560 ± 5.3 ($\mu\text{g/ml}$) dosages, respectively. In addition, a drop in cell colony formation was observed. **Conclusion:** The results indicated that *Matricaria chamomilla* root extract is cytotoxic to the MCF-7 breast cancer cell line. In order to study the effects of *Matricaria chamomilla* root extract on cell colony formation a clonogenic assay was performed. The assay revealed a decline in colony formation. Seemingly, *Matricaria chamomilla* root extract can contribute to the prevention or treatment of breast cancer. Hence, more studies can be conducted to use proper dosages of active ingredients of this substance for prevention or treatment of breast cancer.

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Keywords: MTT, *Matricaria chamomilla*, Breast Cancer, Cytotoxic activity, Clonogenic Assay

1. Introduction

Breast Cancer is the most chronic non-contagious common diseases in women. Breast cancer is the first common cancer and one of the most important causes of death in women (Jemal et al., 2011). Over centuries, herbs had been the only accessible source of treatment of pains and diseases. Today, in spite of the considerable progress and development of synthetic medicine, herbs and different forms of drugs obtained from them are still widely used (Hoffman, 2010). The number of studies on phytochemicals capable of reducing the outbreak of a number of tumors is increasing and special attention has been paid to food derivatives which are almost nonpoisonous (Liu, 2012). Human has long recognized the medicinal properties of many plants and has used them to treat many human diseases. It has been indicated that many plants contain chemo-protective materials (Gullett et al., 2010). Examples of such plants are members of the Asteraceae family

such as *Matricaria chamomilla* and *Artemisia*. These plants are rich sources of chemo-protective phytochemicals such as phytosterols, flavonoids, carotenoids, and terpenoids. These phytochemicals stimulate the immune system and inhibit advancement of metabolic pathways associated with development of cancer (Craig, 1999). Plant members of the Compositae or Astraceae family are among herbaceous plants. One of the valuable members of this family is German Chamomile which is scientifically known as *Matricaria chamomilla* L (Lim, 2014). Chamomile (*Matricaria chamomilla*) is an aromatic and medicinal plant with antioxidant, anticancer, antigen toxic, anti-inflammatory, antimicrobial and neuroprotective activities (Lim, 2014).

2. Materials and Methods

2.1. Obtaining *Matricaria chamomilla* Extract

For the purpose of this research, bushes were scooped out of the soil in the reproductive stage of the life of *Matricaria chamomilla*. The bushes were washed and dried through shading in room temperature. In order to extract, bushes were drenched. The dried roots were mildly grinded using an electric mill and then 50 grams of the powder was mixed with 500 ml of 70% ethyl alcohol. The mixture was shaken for 10 hours. The solution was filtered and dried in room temperature. Desired concentrations (50 to 2000 $\mu\text{g} / \text{ml}$) of the solution were diluted in DMEM culture medium. The solution was passed through a 0.2 μm filter for sterilization (Srivastava and Gupta, 2007).

2.2. Cell Culture

MCF-7 cell lines was obtained from the Pasteur Institute of Iran, and then were cultured in DMEM, with 10% FBS, 100 u/ml penicillin, and 100 $\mu\text{g} / \text{ml}$ streptomycin. The culture was maintained in a 37°C incubator with 95% moisture and 5% of CO₂ (Han et al., 2012).

2.3. MTT Assay

The cells was treated by different concentrations of the extract (200 to 2000 $\mu\text{g} / \text{ml}$) for 24, 48 and 72 hours, triplicate for each concentration. The sample control contained cells and a culture medium without extract. Inhibition of cells viability was assayed using the MTT method. Then, 1×10^4 cells were cultured in every well of a 96-well plate and treated 24, 48 and 72 hours by the extract and then, added MTT solution (5 $\mu\text{g} / \text{ml}$) into each wells for 4 hours. Following the formation of formazan crystals, the crystals were solved in 100 $\mu\text{g} / \text{ml}$ of DMSO (Dimethylsulfoxid) and the absorbance at 570 nm was read by ELISA reader (BioTek ELx800). The data was analyzed by SPSS software and one way of variance (ANOVA) test (Tang et al., 2014).

2.4. Clonogenic Assay

In this assay, 1000 cells were seeded in the wells of a 6-well plate and after 24 hours incubation every well was treated with different dosages of extract concentration from 100 to 600 $\mu\text{g} / \text{ml}$. At the end of nine days of treatment, the total culture medium was removed from the wells and cells were washed with PBS (phosphate buffer saline). The cells fixed by methanol and acetic acid solution (3:1 ratio) and incubated for 5 minutes. Afterwards, the fixative solution was removed from the wells and 0.5% crystal violet (in methanol) stained for 15 minutes.

Finally, the stain was rinse with tap water. In every well, the number of colonies with cells more than 50 was counted by Olympus SZX16 stereo microscope and the number of colony treated to control were calculated (Samoszuk et al., 2005).

3. Results

3.1. Cells Viability Percentage

The results showed decrease of viability MCF-7 cells is depended on time duration and concentration of *Matricaria chamomilla* root extract in the MTT assay after 24, 48 and 72 hours (Figure 1). The IC₅₀ of *Matricaria chamomilla* extract obtained after 24, 48 and 72 hours for the MCF-7 cell line is shown in Table 1.

Table 1: Cytotoxic activity of *Matricaria chamomilla* (M \pm SEM)

Cell line	IC50($\mu\text{g}/\text{mL}$)		
	24h	48h	72h
MCF-7	1954 \pm 4.2	1700 \pm 5.1	1560 \pm 5.3

Cells were treated with different concentration of *Matricaria chamomilla* root extract for 24, 48, and 72 hours. Viability was quantitated by MTT assay.

3.2. Colony Formation Percentage

In order to this study the effects of *Matricaria chamomilla* root extract on colony formation evaluated by Clonogenic Assay. The results show that decrease of colony formation of MCF-7 cell line dependent on time and concentration in all groups (Table 2). Colony formation show in affected the extract groups and control group (Figure 2).

4. Discussion

In this study, the effects of cytotoxicity of *Matricaria chamomilla* root extract on the MCF-7 breast cancer cell line were analyzed after 24, 48 and 72 hours of treatment with 1954 \pm 4.2 $\mu\text{g}/\text{ml}$, 1700 \pm 5.1 $\mu\text{g}/\text{ml}$, and 1560 \pm 5.3 $\mu\text{g}/\text{ml}$ dosages of the extract. Other studies performed on the effect of *Matricaria chamomilla* flower extract on other cancer cell lines more than effect of *Matricaria chamomilla* root extract (Srivastava et al., 2009). In another study it was shown that chamomilla has a high rate is flavonoid aglycone and apigenin and anti-cancer properties and prevent cell growth selectively and apoptosis in cancer cells, which in the present study also ranked in the category of chamomilla MCF-7 showed (Wang et al., 2010). Currently available information suggests that some extracts are more effective than their raw materials (Jo et al., 2005).

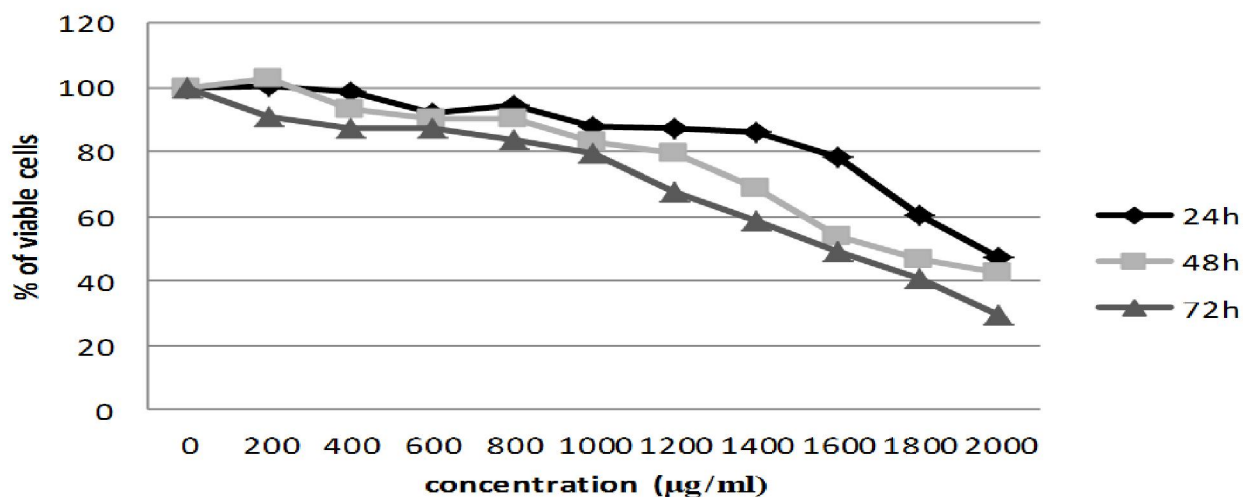


Figure 1. The cytotoxic effect of hydroalcoholic extract of *Matricaria chamomilla* Root on MCF-7 cells, 24, 48 and 72 h after exposure. The effect was measured by MTT cell viability assay. The data are mean \pm SD of three independent experiments



Figure 2: Colony formation in the MCF-7 cell line.

Cell lines	Control	100 μ g/ml	200 μ g/ml	400 μ g/ml	600 μ g/ml	800 μ g/ml
MCF7	100	86.8 \pm 2.6*	61.3 \pm 2.0*	40.6 \pm 1.4*	23.3 \pm 1.0*	11 \pm 1.7

Cells were treated with different concentration of *Matricaria chamomilla* root extract for 24, 48, and 72 hours. Viability was quantitated by MTT assay.

Moreover, the combination of some of the materials in herbal extracts may play a significant role in their final biological activity (Sebastian and Thampan, 2007). Extracts of other plants such as the liquorice root extract, soy extract, and green tea extract have also demonstrated anticancer properties. This is a finding that complies with results of the present research (Roomi et al., 2005, Hakimuddin et al., 2004). In this study it was indicated that application of *Matricaria chamomilla* hydroalcoholic

extract has a cytotoxic effect on breast cancer cells. This finding also proves that *Matricaria chamomilla* root can have a cytotoxic effect on the MCF-7 cell line. According to the results of this study and previous research, our long-term goal is to characterize antiproliferative and apoptotic effects of *Matricaria chamomilla* flower extract on breast cancer cell lines.

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