Original Article

A Comparison of the Effects of the Hydro-Alcoholic and Aqueous Extracts of Aloe Vera on the Proliferation of MCF7

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Abstract

Background and Aim: Apart from being the most common type of cancer, breast cancer is the second leading cause of death induced by cancer among women. Chemotherapy drugs are often associated with adverse effects on patients. Common medicinal therapies for breast cancer have often short-term impacts, and the consumption of some of them leads to drug resistance. The target of this study was to determine the effects of aqueous and hydro-alcoholic extract of Aloe Vera on MCF7 cells (human breast adenocarcinoma cell line). This cell line is a largely investigated epithelial cancer cell line, which is derived from breast adenocarcinoma that has the characteristics of differentiated mammary epithelium.

Materials and Methods: After culturing MCF7 cancer cells in the PRMI 1460 medium, both the aqueous and hydro-alcoholic extracts of Aloe Vera were prepared in concentrations of 20, 40, 60, 80 and 100 μg/mL from bulk solution. Subsequently, MCF7 cells were contacted with different concentrations of the prepared extract. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) test and Acridine Orange / Propidium Iodide (AO/PI) stained were used to determine the survival rate of cells. Then, the researchers applied Doxorubicin (0.1 Mm) as positive control. The T-Student test was utilized in order to analyze the data using SPSS 21. p< 0.05 was considered as a significant level.

Results: The results of MTT assay and viability showed that different concentrations of the ethanol extracts of Aloe Vera plant remarkably and dose-dependently reduced the cell proliferation of MCF7 gets (p<0.05).

Conclusion: Hydro alcoholic extract could be utilized as a natural compound in the treatment of breast cancer.

Keywords: Hydroalcoholic, Aloe Vera, Breast cancer, MCF7

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Introduction

As the most common malignancy among women all around the world, breast cancer is widely known as the second major cause of death among women. Breast cancer comprises 21.4 percent of all types of reported
cases of cancer in Iran. This type of cancer develops in the lobules of lactiferous ducts walls which supply milk. The signs of breast cancer include touchability of the mass in the breasts, deformation of the breasts, appearance of hollow on the skin of the breast and also secretion of liquid from the nipples (1). Special risk factors, which include obesity, lack of movement, having the first period at a very young age, and having mutated genes, are highly influential in the development of breast cancer. Survival in breast cancer depends on the type of the cancer, the severity of the disease, and the age of the patient (2). Common medicinal therapies for breast cancer are usually characterized by short-term treatment impacts that also induce resistance to drugs in some cases (3). Meanwhile, the use of natural compounds extracted from herbs and plants has been frequently addressed by researchers, because on the one hand, they have useful treatment effects, and on the other hand, they do not have adverse side-effects. The cancerous cell MCF7 was first removed from a 69-year-old woman suffering from breast cancer in a double mastectomy operation in 1970. This cell contains estrogen and cytokeratin receptors, and is sensitive towards them. Moreover, it originates from the first invasive breast ductal carcinoma (4). Among the most significant features of this cell line in comparison to other cell lines is its long durability in laboratory conditions. Furthermore, contrary to previously separated cell lines, this cell line shows a suitable passage. Fortunately, this cell line is accessible from Iran’s cell bank (5). MCF7 stands for “Michigan Cancer Foundation-7”, a region where it was first recognized (6).

Aloe Vera is the name of a plant belonging to Aloe genus, Asparaguses order, Asphodelaceae family. It is native to North Africa, and is stemless. Ninety-six percent of Aloe Vera gel is formed by water. The remaining 4 percent includes several materials 75 percent of which has been recognized so far. This plant has hydroxy-anthracene derivatives including 25 to 40 percent Aloe A2 and B out of the whole chromone derivatives, including Aloe resin A2, B and C. Other significant ingredients of Aloe include sugars such as glucose, mannose, enzymes such as oxidase, amylase, catalase, and also vitamins such as B1, B2, B6, C and E as well as folic acid and minerals such as calcium, sodium, magnesium, zinc, copper and chrome. Aloe Vera contains materials such as aloein famodin, anthracene, isobar balsein and also 12 percent resin (7). This plant has important therapeutic specifications, including improving and balancing body immune system in inflammatory and viral diseases (such as Herpes simplex), curing cancers, dressing wounds, and treating damages caused by burns. Moreover, Aloe Vera is an anti-inflammatory plant, and contributes to the anti-apoptosis activity of liver cells and tubular cells (8). Aloe Vera gel has several uses, including being utilized in esophagus reflux. New research shows the increasing use of this plant by patients, especially those suffering from cancer who refer to different branches of alternative medicine for receiving a treatment (9). Normally, the method of extracting any material from the plant can induce changes to the active ingredients. Nevertheless, no scientific investigation has been carried out on the distinction in the performance in different extracts.

The present study seeks to investigate the effects of the aqueous and hydro-alcoholic extracts of Aloe Vera on the of MCF7 cells proliferation.

Materials and Methods

This study follows an experimental design. Aloe Vera was purchased from a botanical institute in Urmia. After preparing the Aloe Vera plant, its type and species were specified by a herbarium expert from the biology department in the Faculty of Science of Urmia University. Subsequently, the whole plant was powdered by a home mixer. To prepare the hydro-alcoholic extract, 0.1 gram of the powder was weighed that was followed by 20 milliliters of 80 percent methanol poured on it. To prepare the aqueous extract, the same amount was added to water, and then the mixture was shaken well and put in water bath (Bain-marie) in 50 °C for 2 hours. After 2 hours, the extracts were filtered by a filter paper, and the volume of the hydro-alcoholic extract was 20 milliliters that was poured into a volumetric flask containing 80 percent methanol solvent (10). Subsequently, the cancerous MCF7 cells were prepared from the Cell Bank of Pasteur Institute of Tehran, which were then cultured in a RPMI1460 medium. Then, the MCF7 cells were treated by aqueous and hydro-alcoholic extracts in
concentrations of 20, 40, 60, 80 and 100 micrograms in millilitre. Doxorubicin was used as positive control with concentration of 0.1 mM. The cytotoxicity effects of the extracts were evaluated by MTT method. To this end, MCF7 cells with the accumulation of 10000 cells were transferred to 96 well plates, and were exposed with the above-mentioned densities for 24 hours. At the end of 20 µL incubation time of the solvent, 5 milligrams in MMT millilitre was added to all wells, and the plate was incubated for 4 hours. Finally, formazan color crystals sediments in the cells cytoplasm were solved by adding 100 micro-liters DMSO to each well, and then the intensity of the color was recorded in wave length of 492 nanometers by means of Elisa method (11). Fluorescence coloring and cell count under microscope are the simplest and fastest means to recognize the living and dead cells. In this method, the fluorescence colors of Acridine Orange / Ethidium Bromide were used to determine the survival rate of cells (12).

Eventually, through the use of Ethidium Bromide /Acridine Orange solvent, the alive cells were seen as having green color, and the dead cells were seen as having orange to brown color (13). To this end, 100 micrograms in millilitre of acridine orange and 100 micrograms in millilitre Ethidium Bromide are solved in equal amounts in 1 millilitre PBS solvent, and then 10 microliters of the above solvent is mixed with 250 microliters of cellular suspension. Then, 10 microliters of the mixture is placed on a clean slide which is covered by another slide. Subsequently, by using X 40 lens of fluorescence microscope, at least 100 cells are counted and investigated to inspect whether they are alive or dead. The number of green cells (alive) divided by the total number of green (alive) and orange (dead) cells signifies the percentage of the living cells (14). T-Student test was used in order to analyze the data using SPSS 21. p<0.05 was considered as the significant level. All the data were reported in the form of Mean ± SD.

**Results and Discussion**

By comparing the data derived from MTT, and evaluating the percentage of the survival rate of treated cancerous cells with aqueous and hydro-alcoholic extracts, it turned out that the difference between the means of percentage of cells survival rates in both of the treatment groups indicated a significant distinction with the control group at p<0.05 level of significance. The percentage of the survival rate of the cells was significantly lower in the treatment group compared to the aqueous extract group. The survival means of cancerous cells in the group treated by Doxorubicin was lower compared to the group treated by aqueous and hydro-alcoholic extracts (p<0.05). One hundred cells were counted, and the proportion of living cells to apoptotic cells was determined. The results of Acridine Orange / Propidium Iodide (AO/PI) coloring indicated that the rate of apoptosis in treated cells with aqueous and hydro-alcoholic extracts was greater compared to the negative control group that exhibited a significant increase in apoptosis rate compared to the aqueous group (p<0.05), but it was lower compared to Doxorubicin group. The increase in the concentration of aqueous extract of Aloe Vera has led to the decline of the numbers estimated for MTT test and the rise of the percentage of the apoptotic MCF7 cells. This relationship is linear but with the changes in concentration, no significant difference is made in the totality of the relationship. The minimum concentration of the aqueous extract of Aloe Vera in this test, which decreased the significance of the MCF7 cells growth, was 60 microgram/ml. According to MTT test results, hydro-alcoholic extracts apoptosis on the growth of MCF7 cells is linear and concentration-dependent. The minimum and maximum hydro-alcoholic extracts concentrations in this test, which increased the significance of MCF7 cells, were 40 and 100 microgram/ml respectively. Considering the results, hydro-alcoholic extracts suppresses cancerous MCF7 cells more efficiently compared to the aqueous extract. The results associated with the investigation on aqueous and hydro-alcoholic extracts as well as the comparison between the two groups have been shown in figures 1 and 2.

By principle, in traditional medicine, the extracts prepared from all parts of plants are used for therapeutic purposes. Naturally, selecting the type of solvent is effective in the level of active ingredient and also their combination (15). The results of the present study signify the cytotoxic effects of both aqueous and hydro-alcoholic extracts. However, these results
clearly indicate that hydro-alcoholic extract has cytotoxic effects even in low doses at which aqueous extract does not show any performance. Clearly, using a medicinal compound in lower doses with suitable efficiency decreases the possible side effects of the drug compound (16). Hence, the hydro-alcoholic extract seems to be a better candidate for being used as a cytotoxic drug at least for MCF7 cells compared to the aqueous extract.

In spite of using different therapeutic courses of actions, including surgery, chemotherapy, and radiotherapy, the fatality rate of patients suffering from cancer is high, which is indicative of the inefficiency of these remedies. Furthermore, the harmful effect of chemotherapy and radiotherapy on normally dividing cells is another disadvantage of such remedial processes (17). It has been proven that consumption of foods that have antioxidant characteristics is highly effective in the prevention and reduction of suffering induced by cancer (18). Aloe Vera extract is among the plant compounds that have been dealt with in this respect. In the present study, the effects of different concentrations of Aloe Vera extract on MCF7 cells were evaluated through Trypan blue

### Table 1: The results of MCF7 cells proliferation and apoptosis after treatment by microgram/milliliter of Aloe Vera extract and concentration of µm Doxorubicin (* means the significant difference of p<0.05 in treatment group compared to control group).

<table>
<thead>
<tr>
<th>Group under study</th>
<th>Proliferation rate (MTT)</th>
<th>Apoptosis percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>0.0±716.028</td>
<td>14.00±2.00</td>
</tr>
<tr>
<td>20µg/ml</td>
<td>0.0±617.012*</td>
<td>24.00±1.00*</td>
</tr>
<tr>
<td>40µg/ml</td>
<td>0.0±527.027*</td>
<td>35.00±3.6*</td>
</tr>
<tr>
<td>60µg/ml</td>
<td>0.0±489.011*</td>
<td>41.66±6.02*</td>
</tr>
<tr>
<td>80µg/ml</td>
<td>0.0±475.013*</td>
<td>52.33±3.51*</td>
</tr>
<tr>
<td>100µg/ml</td>
<td>0.0±407.016*</td>
<td>55.33±4.04*</td>
</tr>
<tr>
<td>Doxorubicin 0.1 mM</td>
<td>0.0±324.012*</td>
<td>71.33±2.51*</td>
</tr>
<tr>
<td>Probable Amount (P)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

### Table 2: The results of proliferation and apoptosis rate of MCF7 cells after treatment by microgram/milliliter of Aloe Vera extract and concentration of µm Doxorubicin.

<table>
<thead>
<tr>
<th>Group under study</th>
<th>Proliferation rate (MTT)</th>
<th>Apoptosis percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>0.0±716.028</td>
<td>14.00±2.00</td>
</tr>
<tr>
<td>20µg/ml</td>
<td>0.0±662.030*</td>
<td>22.00±2.03*</td>
</tr>
<tr>
<td>40µg/ml</td>
<td>0.0±601.023*</td>
<td>28.00±1.08*</td>
</tr>
<tr>
<td>60µg/ml</td>
<td>0.0±473.022*</td>
<td>35.11±4.06*</td>
</tr>
<tr>
<td>80µg/ml</td>
<td>0.0±531.010*</td>
<td>40.01±2.11*</td>
</tr>
<tr>
<td>100µg/ml</td>
<td>0.0±488.012*</td>
<td>46.08±2.08*</td>
</tr>
<tr>
<td>Doxorubicin 0.1 mM</td>
<td>0.0±324.017*</td>
<td>71.33±2.51*</td>
</tr>
<tr>
<td>Probable Amount (P)</td>
<td>0.05</td>
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</tbody>
</table>
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and MTT methods. The results showed that the minimum and maximum concentrations of the hydro-alcoholic extract of *Aloe Vera*, which increased the significance of MCF7 cells, were 40 and 100 microgram/ml respectively. *Aloe Vera* contains different components each of which shows special effectiveness. Amodim and Aloe-edomin are among these materials which have synergetic effects on each other (19). Recently, some research studies have been carried out on the anticancer effects of these herbal products. The cytotoxic effect of Aloe-edomin on cancerous cells in stomach cancer has been investigated through MTT test. It has been found that the survival rate of cancerous cells in stomach cancer decreases dependent on the dose and time duration of Aloe-edomin treatment (20). The results of this research confirm the findings of the present study in that it approves the effectiveness of *Aloe Vera*
derivative products against breast cancer cells. Cancerous cells (23). A research that investigated

The inductive apoptosis effects of Aloe-emodin on cells have been stated in a number of research studies (21). Most probably, the Aloe-emodin in the Aloe Vera extract influences MCF7 cell membrane, and stimulates the death receptors especially Fas Protein. Moreover, it activates caspasas, and eventually contributes to the programmed death of the cell or its apoptosis from outside the cell. A study conducted on the anti-tumoral effect of the natural extract of Aloe Vera leaves revealed that in addition to anti-tumoral impacts, the extract has a protective effect on healthy cells of the lung in human embryonic stem cells, while the commercial extract of this plant lacks the above binary effects. In another study, the anticancer effect of Aloe-emodin as a compartment of Aloe Vera extract on the survival rate of some cell lines, and apoptosis induction with the rise in Fas (CD95) have been stated (22). This research indicates that Aloe-emodin shows a dual behavior in cell growth. For example, this material stimulates the growth of primary liver cells, and at one due to apoptosis induction in hepatocellular carcinoma (HCC) and neuroectodermal tumors brings about the death of mitochondria, and subsequently the death of different types of the cell lines of neuroectodermal tumor such as IMR-32 and IMR-5 revealed that the Aloe-emodin (hydroxyanthraquinone) in the Aloe Vera leaf contained an anti-tumor material with selective activity against neuroectodermal cancer cells in laboratory and in vivo situation (24). I another study conducted on bladder cancerous cells, it was observed that Aloe-emodin could prevent the proliferation of cancerous cells and call a halt to cells cycle 2G/1 M phase. Furthermore, it was indicated that Aloe-emodin could induce apoptosis by activating Caspase 3, P53, P21, Bax and Fas (25). An understanding of the important mechanisms involved in cancer is crucial for the advancement and progress of useful therapeutic methods for such diseases (26). A series of mutations in cells cause resistance against apoptosis and cells death. Therefore, apoptosis induction is one of the main objectives in curing the disease (27). It has been indicated that a series of natural compounds, including plants, play a key role in apoptosis induction that has been controlled in cancerous cells. These compounds could induce apoptosis routes in cancerous cells and stop their proliferation through different mechanisms. Hence, apoptosis routes induction could be attributed
to anticancer characteristics of *Aloe Vera* extract and the presence of its material in the effective compounds of the extract (28). Atul et al. (2012) examined the anticancer properties of *Aloe Vera* extract on melanoma cells. The results of their investigation indicated that 50 milligram/milliliter concentration of *Aloe Vera* extract could reduce cancer cells proliferation for more than 50 percent. These results confirm the anticancer effects of this extract (28). The difference in the concentration used in their study with that of the present study could be attributed to the difference in the susceptibility between the melanoma cells line and MCF7 cancerous cells and also differences in susceptibility of cells lines in response to different extract concentration.

Kaiyuan et al. (2010) investigated the effect of Aloe-emodin on cells proliferation in colon cancer, and indicated that 0.37 mM of Aloe-emodin could inhibit the growth of cancer cells (30). Pankaj et al. (2013) showed that disaccharides of *Aloe Vera* extract could prevent the proliferation of cancerous cells due to the inhibition of the link between benzopyrene and liver cells in rats and by means of this mechanism (31). It could be stated the anticancer properties of *Aloe Vera* stem from the presence of effective compounds within it (27). In line with the investigations carried out in the aforementioned research, the results of the present study revealed that hydro alcoholic and aqueous extracts of *Aloe Vera* could have anti-proliferating properties in MCF7 cancerous cells. Investigations of the proliferation rate and apoptosis showed that with increasing the concentration, the anti-proliferating effects and survival rate decrease, which in turn show that it is concentration-dependent. A comparison of the results between the two groups indicated that the anti-proliferating effect of the hydro alcoholic extract was greater than that of the aqueous extract. Most probably, the differences between the degree of anti-proliferating properties in hydro alcoholic and aqueous extracts could be due to the quality of the extraction process and the characteristics of the solvent used for that purpose. It is possible to achieve antimicrobial compounds through recognizing and extracting these materials.

**Conclusion**

To sum up, the results of the present study confirm the anticancer properties of *Aloe Vera*, particularly the impact of its hydro alcoholic extract on MCF7 breast cancerous cells. The naturalness, low cost and accessibility of this product for the public are among its considerable advantages. The results of the present study confirm that *Aloe Vera* could be used as an anticancer drug for curing breast cancer or for being used as a herbal supplement in diets along with other routine medicines used for curing breast cancer. Nevertheless, further in-situ research is required in this respect.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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