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ORIGINAL ARTICLE

The effect of Cytotoxicity aqueous and alcoholic extract of shallot (*Allium ascalonicum*) on cancer cells derived from the SD rat on induced-DMBA breast tumor

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ABSTRACT
Allium plants are an important part of the diet of many populations and there is a long-held belief in their health-enhancing properties such as cancer prevention. This study aims to investigate the effect of aqueous-alcoholic shallot extract on breast cancer cells in rats. The aqueous and alcoholic extract of shallot is prepared. Then, using Trypan blue and MTT, in different concentrations and times their cytotoxicity on breast cancer cells are examined. By dying fluorescent hoechst and propidium iodide (PI), morphological and apoptogenic changes are examined. Statistical differences between treatments are analyzed using one-way ANOVA, SPSS statistical software and Tukey test and p 0.05 are considered statistically significant.The results of this study reveal that shallot extract at concentration range of (0.01 to 0.05 µg/ml) has controlling effects on cancer cells and the effect of alcoholic extract is more than aqueous extract. ***Conclusion****: This study shows that aqueous and alcoholic extract of shallots has cytotoxicity effects on cancer cells, and this plant as a medicinal plant extract against breast cancer may be the subject of further researches.*
Keywords*: Shallot extract; breast cancer; DMBA*

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INTRODUCTION

Breast cancer is the most common cancer among women worldwide, especially in western countries where there have been a lot of advances in medical science, still breast cancer remains as a danger factor and cause of death. Based on researches in recent years, breast cancer is the most frequent type of cancer among women [1]. Comparing the new data with the previous reports, the incidence of this disease increases rapidly in our country [2]. The high numbers of this type of cancer's patients necessitate further research to understand the mechanisms of cancer development and more effective therapeutic methods [3].

Chemotherapy is one of the therapeutic approaches to reduce the symptoms of cancer. One of the most difficulties in chemotherapy is the lack of an active and effective drugs and drug resistance to the existing medicine. Therefore, research on the development of strategies that can overcome these problems and prevent the appearance of the disease is one of the essential major subjects [4]. it is Found that many common plants have chemoprotective properties (cancer prevention) such as, Allium species (garlic, onions and shallots), members of the Labiatae family (basil, mint, thyme and rosemary), family members of zingiberaceae (ginger and turmeric) [5].

Shallot (*Allium ascalonicum*) is the Iranian native plant. Allium plants such as garlic and onion have been used as medicine and flavor and their usage dated back to many years ago [6]. Allium plants have been prevalent as a traditional medicine for many centuries. Their usage is because of their values and medicinal properties. These plants are rich in flavonols and organosulfur compounds showed anti-cancer properties in laboratory conditions [7, 8].

MATERIALS AND METHODS

This is an experimental study in which rats treated with DMBA with required size of tumor, then animals were anesthetized by Dietyleter, Their hair around the tumor was shaved and the dissection area was sterilized using 70% alcohol and diluted Savln. After splitting, the entire tumor was removed and

transferred to the laminar hood. The most internal parts of the tumor were cut and transferred to Falcons containing 10 ml of sterile HBSS. Then the samples were removed from sterile HBSS were removed and in fresh medium. After separating the blood and the additional tissues, add the remaining part of tumor was chopped in to very fine particles and the obtained suspension was passed several times through the needle 5ml in order to separate the cell from tissues completely. The resulting mixture was transferred to Falcon 15ml and centrifuged with cycle of 1500rpm for 5 min, then the solution was discarded, and added to the sediment of Falcon 5 ml medium DMEM containing FBS 10% . After peptizing, 1/5 to 2 ml of the homogenized mixture was transferred to T25 flasks and the volume of flask internal medium was reached to 5 ml. Flask was placed inside a CO2 incubator. After 24 hours, the surface medium of flask evacuated and 5 ml of fresh medium was added to the flask

After shallot preparation and drying, it grinds into the powder. It is extracted three times with ethanol 95% (proportion of 1 to 5) at room temperature for 24 h. Then it is filtered and the solvent removed and extract is concentrated [9]. To prepare the aqueous extract of shallot, about 100 grams of healthy bulbil of shallot was selected and outer peel was removed, then the bulbil was changed to the soft substance in an electric machine and shallot tissue was completely crushed ,was mixed with deionized water proportion of 1 to 1 and was gently stirred. After complete extraction, the extract was centrifuged and filtered under the cycle of 14,000 rpm for 5mil. The surface liquid was separated and powdered by the freeze dryer. The powder is kept in the freezer, for each use; the required quantity is weighed and dissolved in medium [10].

To assess the biological strength of cells were treated with aqueous and alcoholic extracts of Shallot, the cells isolated with trypsin and after centrifuge, cell sediment in the fresh medium reached to a volume of 1ml. 300 microliters of the resulted cell suspension in the same volume of trypan Blue was mixed and placed in incubated for 2 min. The numbers of live and dead cells were examined by hemocytometer slide and reported in percent. In this method, due to the permeability of dead cells to trypan blue, the blue color was observed. In addition to blue trypan technique, MTT was used to study the biological strength of the treated cells. In this method succinate deaqueousgenase enzyme was able to restore the yellow dimethyl diphenyl tetrazolium to insoluble purple formazan crystals .Cancer cells were cultured for 24 h in 96- cell plate and adhered to plate with the above concentration of the aqueous and alcoholic extracts shallots treatment, after 48 and 72 h, 10 ml MTT per 100 ml of medium was added to the sinks and incubated for 4 h, then the resulted formazan crystals dissolved in DMSO and its absorbance by ELISA reader (SCO diagnostic, Germany) at a wavelength of 505 nm was measured. Stuck cells in 24-cell plates at a dose of 0.04 and 0.03g for aqueous and alcoholic extracts were treated for 48 hours (according to the statistical results of the Trypan blue and MTT test ,48 hours were required to study the morphological investigation).

Chromatin dying with Hoechst to study nucleus morphology and PI to distinguish between dead and live cells was performed by fluorescence microscopy (Olympus IX 70).

Statistical tests

The statistical software SPSS ANOVA, Tukey test was used and performed on the data, and $p < 0.05$ was considered statistically significant.

RESULTS

In this study, we evaluated the effects of the aqueous and alcoholic extract of *A. ascalonicum* on viability of the breast cancer cells and normal cells using Trypan blue and MTT assays. On the basis of obtained results, after 48 and 72 h of incubation, Results Indicates that aqueous and alcoholic shallot extract in a 48 hour treatment dose-dependently reduced viability of cancer cells. The effect of shallot extract on controlling the breast cancer cells is greater than aqueous one (Figure1).

Figure 2 indicates the effect of aqueous and alcoholic extracts of shallot cancer on cells treated with 72-hours.

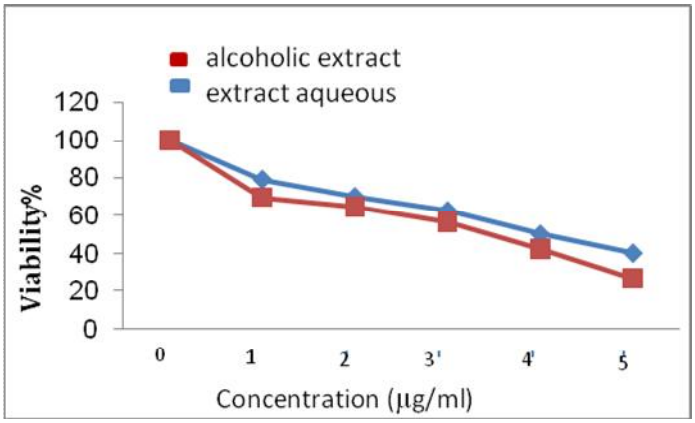

Figure1. graphical viability cells percentage comparison after 48 hours treatment under the influence of hydro-alcoholic shallot extract.

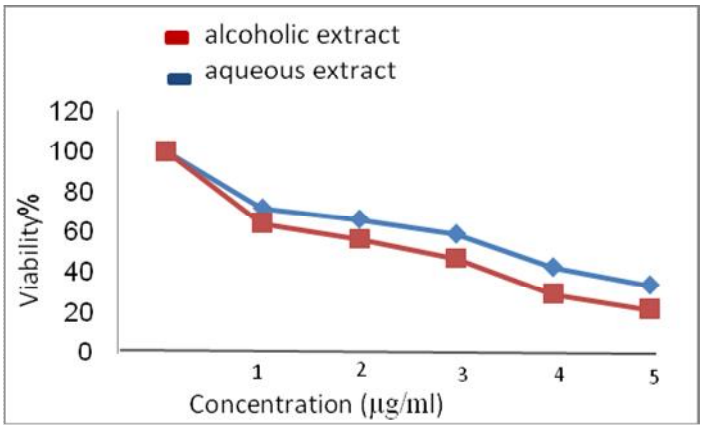

Figure2: graphical viability cells percentage comparison after 72 hours treatment under the influence of hydro-alcoholic shallot extract.

As well as Results of the morphological changes indicates cells after treatment with the extract in 48 hours by fluorescent dying Hoechest included nucleus deformation compared with the control group (Figure 3).

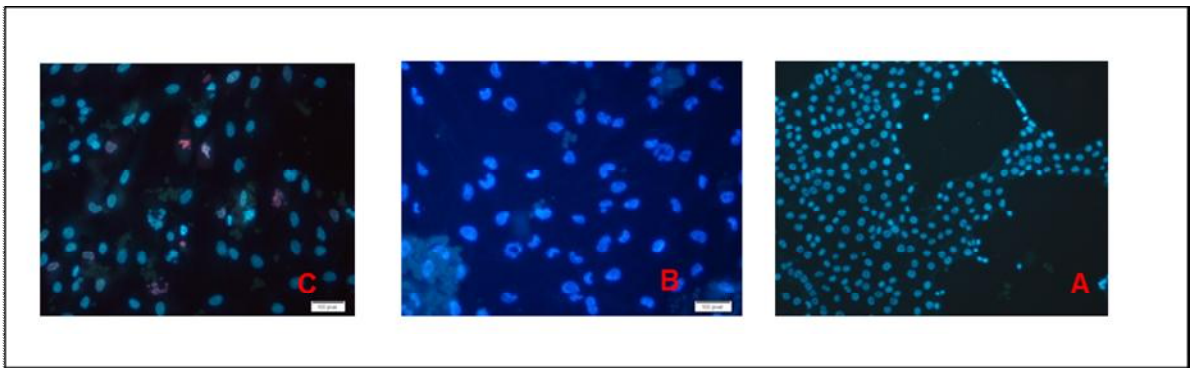

Figure 3. Florescence dying of cancer cells for 48 hours
A: control sample
B: treatment cell with dose of .03mlg/l alcoholic extract
C: treatment cell with dose of .04mlg/l alcoholic extract

DISCUSSION

Diet plays a major role in cancer etiology and prevention. Although inconsistencies exist across studies that have investigated the relationship between diet and cancer, dietary factors undoubtedly influence

cancer risk. There is increasing evidence that fruits and vegetables have chemopreventive properties because of then supplement and synergistic effects of various phytochemicals present in these nourishments [11].

Based on the results of trypan blue staining and MTT, reduction in the existence of cancer cells in rats treated with aqueous and alcoholic extracts shallots was related to the doze and time. Shallot extract on different cell classes depended on the cell type, and had different cytotoxic effects and IC50 shallot extract for each cell class was different [12]. Their results support the results of the present study.

Although the control mechanism of shallot is not clear, but the results of various studies have shown that Quercetin in shallot had anti propagation effects through increased induction of p53 and reduction of anti-apoptotic proteins Survivin and Bcl-2 [13], also allicin stimulated apoptotic formation, stop cell cycle, nucleus condensation and DNA laddering and activate caspase 3, 8, and 9 [14]. Further studies will be required to analyze the chemical component in each extract and their influence on cancer cells and their reaction on drugs.

However, following treatment of cancer cells with shallot extract within 48 hours, morphological changes such as changing the shape of core and also increase the number of dead cells, was observed In considering the process of caspase enzymes activation in treated cells and the role of this enzyme in broken cell genome, this enzymatic activity may cause cell deformation.

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