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ORIGINAL ARTICLE

Study of Cytotoxic Effect of Methanolic Extract of Ferula Assa-Foetida Resin of Mashhad and Yazd on MDA-MB-231 Cell Line

Leila Vahabi*[1], Kahin Shahanipour[2], Ramesh Monajemi[3], Fatemeh Mortazavifar[4]
[1]Young Researchers and Elite Club, Isfahan (Khorasgan) Branch, Islamicn Azad University, Isfahan, Iran
[2]Department of Biochemistry, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
[3]Department of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
[4]Department of educational management, Isfahan (Khorasgan) Branch, Islamicn Azad University, Isfahan, Iran
E-mail:leilastudent1404@yahoo.com

ABSTRACT
New cell-culture biotechnology enables us to culture cancer cells in vitro and to find, design, and manufacture new drugs. Active ingredients in herbal medicines enjoy biological balance because other materials accompany them and, hence, these medicines do not cause side effects for the human body and, accordingly, have substantial superiority and advantage over chemical drugs. Assa-foetida gum was extensively used in traditional Iranian medicine. Plants of the same species that grow in different habitats have different percentages of active ingredients depending on the water, air, climatic conditions, and even angle of incidence in their habitats. Therefore, this study was conducted to evaluate and compare cytotoxic effects of methanol extracts prepared from assa-foetida gum obtained from South Khorasan and Yazd Provinces on the breast cancer cell line MDA-MB-231. Methods and Materials: Assa-foetida gum was collected from South Khorasan and Yazd and its methanol extract was prepared. The cancer cell line MDA-MB-231 was cultured on RPMI-1640 culture medium containing 10% fetal bovine serum and 5% CO2 and was incubated in the presence of various methanol extract concentrations for 24, 48, and 72 hours. The MTT method was employed to calculate the survival rate of cells. Discussion and Conclusions: Results indicated that, of the three exposure intervals of 24, 48, and 72 hours, methanol extracts of assa-foetida gum from South Khorasan and Yazd had their maximum cytotoxic effect on the cancer cell line MDA-MB-231 at the 48-hour exposure interval.
Keywords:MDA-MB-231, Assa-foetida, Gum, Cytotoxic, Methanol extract

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INTRODUCTION

Cancer is unnatural growth of cells in the body that can result in death (M10).Cancer disrupts the harmony that exists between cells and tissues and, as a result, not only do cancerous cells meet difficulties in carrying out their functions but they damage other cells in the body too [2]. Breast cancer is the most common cancer in women throughout the world and constitutes one third of all cancer cases in women [14], and many women and men die of it every year. Despite the many advances made in the early diagnosis and suitable treatment of breast cancer, it is still the most important fatal cancer in women [6]. So far, many herbal plants with various therapeutic characteristics have been identified [8]. Medicinal plants can play an important part in maintaining human health, and in defending people against various kinds of diseases including cancer, without causing any toxic side effects [10].

The herbal plant assa-foetida (*Ferula assa-foetida L.*) grows in non-arable regions, in dry and sandy lands containing lime compounds situated in warm regions with altitudes of 190-2400meters, and in very rolling terrain with slopes of 15-70 percent and low rainfall of about 250-350 millimeters. Moreover, this plant grows in shallow, steeply sloped, and eroded. Assa-foetida tolerates low temperatures and salinity, is a member of the Umbelliferae Family, has an oleo-gum-resin that is very rich in chemical compounds, and was of great interest in traditional medicine (16). Iranian Steppes are the main habitats of assa-foetida [13]. In Iran, the Central Iranian Plateau, the desert regions reaching the Zagros Mountains in the Provinces of Fars, Kerman, Yazd, Hormuzgan, Sistan and Baluchistan, Isfahan, Lorestan, Kohgiluyehand

Boyer- Ahmad, and Bushehr [7].The growing season lasts until early spring or up to the middle of the first month of summer [12].
Unfortunately, in recent years, uncontrolled, unscientific, and traditional harvesting of this plant has reduced its growth leading to very low plant densities in the rangeland, and has put this useful plant under the danger of extinction [16]. Assa-foetida gum is rich in chemical compounds that can be used for treating diseases including cancer. The purpose of this study was to determine cytotoxic effects of methanol extracts prepared from assa-foetida gum collected in two cities of South Khorasan and Yazd Provinces on the breast cancer cell line MDA-MB-231 at the three exposure intervals of 24, 48, and 72 hours.

MATERIALS AND METHODS

Assa-foetida gum was collected from Khorasan and Yazd Provinces. To prepare the methanol extract, five grams of gum powder was dissolved in 20 milliliters of methanol and, while being continuously shaken, was kept in the dark for 48 hours. The solution was then filtered and dried at a suitable temperature (1). Depending on the rate of growth, the cells in the culture medium containing RPMI and FBS and 10% antibiotic covered the surface of the flask after a few days and turned yellow after consuming the nutrients. To change the culture medium of sticky cells in the culture medium, 25% trypsin was used. After cells proliferated and were in the ascending growth phase, they were counted using a hemocytometer by transferring them to 96-well plates. The cells were exposed to the various concentrations of the extracts for the three exposure intervals of 24, 48, or 72 hours, and the MTT method was then used to study the cytotoxic effects of the extracts.
Pasteur Institute of Iran provided the MDA-MB-231 cell line in a flask to be used in this research. The code of the cell line was C578 based on the NCBI Cell Catalog used in Pasteur Institute of Iran. Growth inhibition of the breast cancer cell line MDA-MB-231 by the five concentrations of methanol extracts prepared from gum collected in Khorasan and Yazd was determined by considering the extent of growth of these cells in the negative control samples to be 100 percent. Doxorubicin, which was used as the positive control, substantially reduced growth of the cells.Each extract concentration was used in three separate experiments and each experiment was conducted in four replications. Extracts that inhibited cell growth by the equivalent of at least 50 percent were considered cytotoxic. Results were analyzed using the SPSS19 software.

RESULTS

Diagrams 1-6 show the cytotoxic effects of methanol extracts prepared from assa-foetida gum in the three exposure intervals of 24, 48, and 72 hours. The gum had been collected from two cities in South Khorasan and Yazd Provinces.

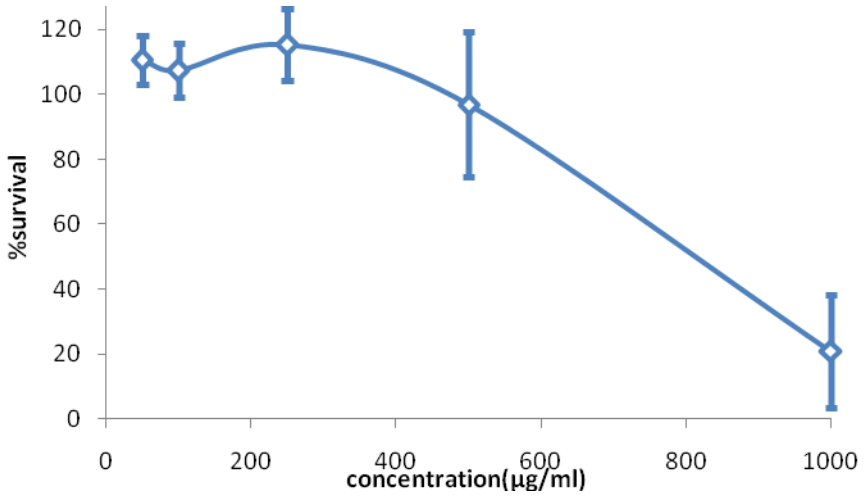

Diagram 1: The cytotoxic effects of methanol extracts prepared from assa-foetida gum collected in South Khorasan on the cell line MDA-MD-231 during a 24- hour exposure interval

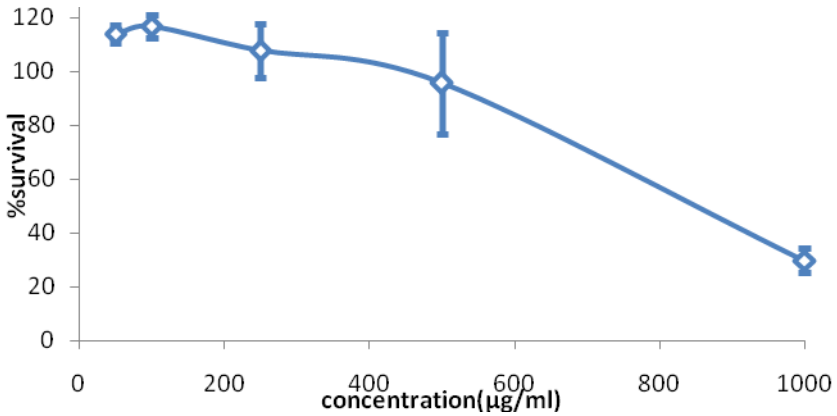

Diagram 2: The cytotoxic effects of methanol extracts prepared from assa-foetida gum collected in Yazd on the cell line MDA-MD-231 during a 24- hour exposure interval

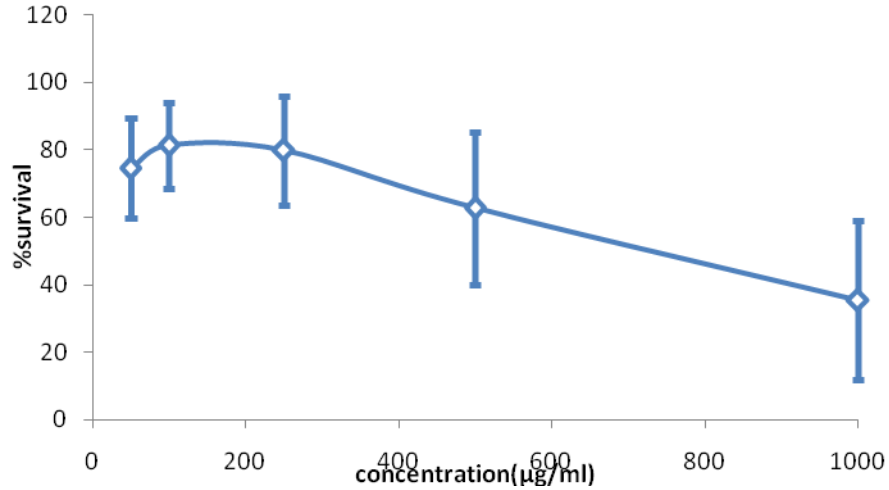

Diagram 3: The cytotoxic effects of methanol extracts prepared from assa-foetida gum collected in South Khorasan on the cell line MDA-MD-231 during a 48- hour exposure interval

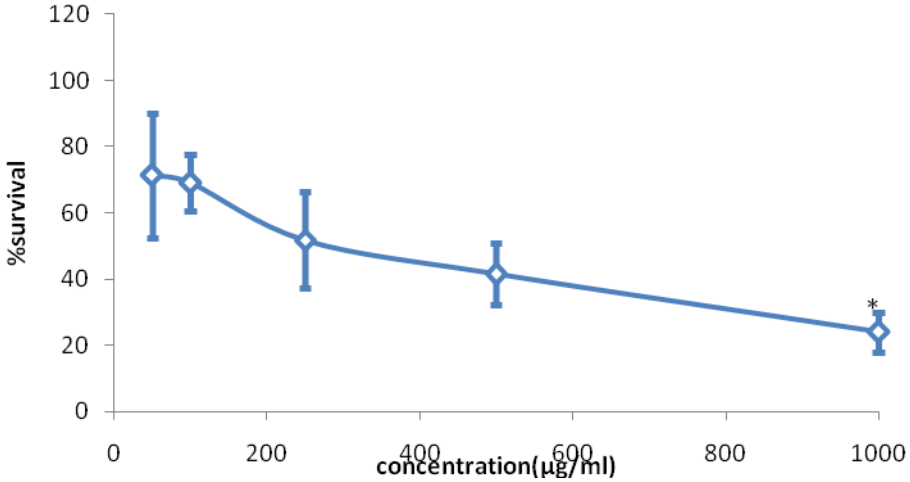

Diagram 4: The cytotoxic effects of methanol extracts prepared from assa-foetida gum collected in Yazd on the cell line MDA-MD-231 during a 48- hour exposure interval

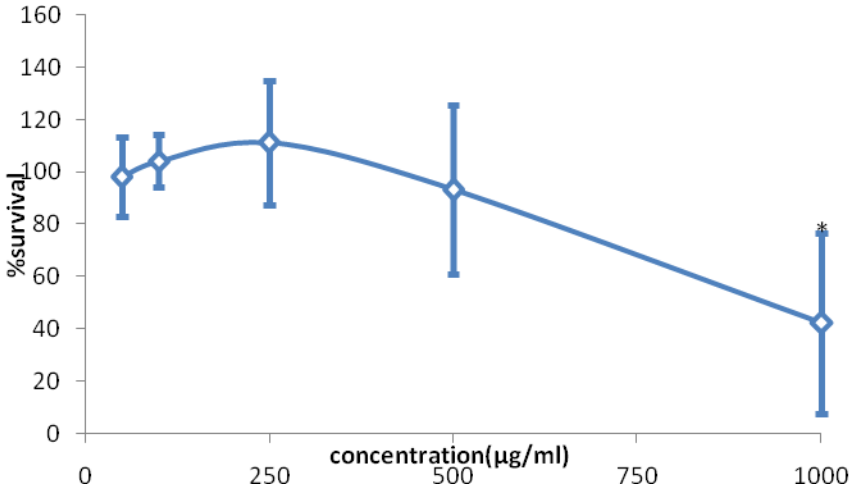

Diagram 5: The cytotoxic effects of methanol extracts prepared from assa-foetida gum collected in South Khorasan on the cell line MDA-MD-231 during a 72- hour exposure interval

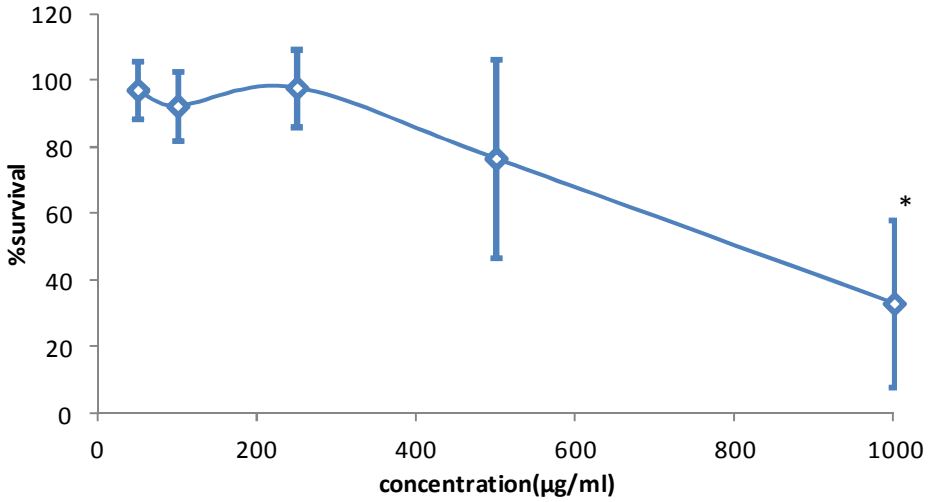

Diagram 6: The cytotoxic effects of methanol extracts prepared from assa-foetida gum collected in Yazd on the cell line MDA-MD-231 during a 72- hour exposure interval

So far, many common plants have been shown to have characteristics that protect people from cancer including species of garlic, onions and Persian shallot, members of the mint family such as basil, peppermint, thyme, and rosemary, and members of the ginger and turmeric family. These plants are rich in phytosterols, flavonoids, carotenoids, and terpenoids, act as antioxidants, sweep up free radicals, stimulate the immune system, and suppress the formation of DNA and carcinogen complexes and the metabolic pathways associated with metastasis [4]. Since very little research has been conducted so far in relation to the cytotoxic effects of Ferula assa-foetida gum in vitro, and considering compounds extracted from its gum have anticancer effects in other plants, this study was carried out to open the way for further research on the effects of assa-foetida gum. Researchers have found that the use of assa-foetida resin prevents the growth of breast cancer cells due to the action of nitrosourea and prolongs tumor latency period [11]. Moreover, it has been discovered that assa-foetida gum administration inhibits metalloproteinase activity and thus suppresses invasion of cancer cells [15].Research has shown administration of farnesiferol can be effective in suppressing vascular endothelial growth factor. Suppressing this growth factor suppresses proliferation, migration, invasion, angiogenesis, and connective tissue production processes in cancer cells. Oral administration of assa-foetida sap inhibits breast cancer growth due to the action of nitrosourea. Moreover, assa-foetida gum reconstructs antioxidant systems that are damaged by nitrosourea administration [9]. As farnesiferol is one of the main constituents of assa-foetida resin, and is a terpene, pure methanol extracts can extract the farnesiferol and thus provide cytotoxic effects. Methanol extracts at concentrations of over 800

microliters per millilitre were very effective and this shows that increasing the concentrations of cytotoxic active ingredients enhances their cellular toxicity and leads to cell death.

Comparison of cytotoxic effects at various durations of exposure to methanol extracts prepared from assa-foetida gum collected in its habitats situated in South Khorasan and Yazd showed that sensitivity of the breast cancer line MDA-MB-231 to the methanol extracts of assa-foetida gum collected from Yazd at exposure duration of 48 hours was more compared to other extracts. However, after the 48 hours the cells started to grow again. Moreover, methanol extracts prepared from gum collected in Yazd and South Khorasan Provinces had cytotoxic effects at exposure duration of 48 hours.Assa-foetida gum has volatile compounds such as sesquiterpene alcohol that is known as farnesiferol [3]. Therefore, the reduction in the cytotoxic effects of methanol extracts after 48 hours can be explained by the presence of volatile compounds in these extracts.

Plants of the same species that grow in various habitats have different percentages of active ingredients depending on the water, air, climatic conditions, and even angle of incidence in their habitats. Moreover, the quantities of phenols and other products obtained from plants depend on environmental and genetic factors, and on postharvest conditions too [5].In general, considering the obtained statistical results, it can be concluded that methanol extracts of gum collected at both habitats had, in general, toxic effects on the breast cancer cell line MDA-MB-231. However, the maximum toxic effects belonged to the methanol extracts of Yazd and South Khorasan, respectively, at exposure duration of 48 hours. Low cytotoxic effect during the first 24 hours can be due to insufficient exposure duration, and at the 72-hour exposure time due to the presence of volatile compounds in the gum.Results of this research show the relatively strong cytotoxic effects of methanol extracts of assa-foetida gum and the high content of phenols in these extracts. Based on these results, assa-foetida gum can be a suitable choice for designing and manufacturing anticancer drugs. Of course, more extensive should be conducted to prove this claim. Moreover, assa-foetida can be considered a strategic plant because, despite containing valuable compounds [16], its medicinal-industrial potential is incorrectly and insufficiently utilized, it is exported without any control (which will lead to its loss, despite the history it has in Iranian traditional medicine), and because it is under the danger of extinction and of being forgotten. Engaging in, and paying attention to, research such as this can prove useful and effective in the preservation and survival of valuable plant species that are under the danger of extinction in Iran.

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