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Original Article

Evaluation of Cytotoxicity Effects of Oleo-Gum-Resin and Its Essential Oil of *Ferula assa-foetida* and Ferulic Acid on 4T1 Breast Cancer Cells

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Abstract

Background: Cancer causes significant morbidity and mortality and is a major public health problem worldwide. Breast cancer is a leading cause of cancer-associated mortality in women, and the incidence is also on the rise in the entire world. Medicinal plants have been an important source of several clinically useful anticancer agents. **Aim:** In this study, we studied the growth inhibitory effect of asafoetida and its essential oil and ferulic acid on antitumor activity using mouse breast cancer cell line. **Materials and Methods:** For this aim, cells were exposed to these components at different concentrations and for different time durations. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to characterize the cytotoxicity of the constituents used. **Results:** Our results showed that all three constituents could inhibit 4T1 cell proliferation. Our MTT assay results showed a significant cytotoxicity effect in a time- and concentration-dependent manner. It also demonstrated that essential oil of asafoetida has a stronger effect in decreasing viability breast cancer cells. Ferulic acid showed a significant effect only at a dose of 500 µg/ml. **Conclusions:** Based on the results of cellular carried out in this study, we could demonstrate that asafoetida and its essential oil and ferulic acid have inhibitory effect on the growth of breast cancer cell line. As evidenced from these preliminary results, asafoetida and its derivative constituents may be considered as attractive alternatives to serve as lead compounds in drug development for breast cancer as an adjuvant therapy. However, much remains to be done before such agent could be introduced to the clinic.

Keywords: *4T1 cell line, asafoetida, breast cancer, essential oil, ferulic acid*

Introduction

Cancer is a major public health problem in many parts of the world. It is currently the second leading cause of death and is expected to surpass heart diseases as the leading cause of death in the next few years.[1] Breast cancer is the most common type of cancer among women, especially in industrialized countries.[2] Despite the advance of cancer prevention including better diagnostic for early detection and used of antiestrogenic drugs such as tamoxifen and raloxifene, which resulted in the reduction of breast cancer incident and mortality, breast cancer is still the most commonly diagnosed cancer that is associated with mortality in women.[3] The conventional therapies for cancer include chemo- and radiotherapies mediated by inducing apoptosis or inhibiting proliferation in neoplastic cells.[4] These therapies cause damage to healthy tissues around the tumors[5] and also develop resistance by numerous tumors.[6] In the recent years, researchers have been studying alternatives of cancer therapy by applying potential biological molecules to target neoplastic tumors.[7] Herbs have been identified as an important source of novel bioactive compounds for medicine development including cancer chemotherapeutic drugs.[8] *Ferula assa-foetida* L. which belongs to *Apiaceae* family is an herbaceous perennial with an unpleasant odor that grows wildly in central area of Iran.[9] The part used of this plant and several other species of *Ferula* is an oleo-gum-resin [asafoetida] that obtained by incision of stem and root.[10] In Iranian folk medicine, asafoetida is considered as an anticonvulsant, diuretic, antispasmodic, antihelminthic, and carminative agent.[11] Recent pharmacological and biological studies have also shown several pharmacological activities such as antioxidant, anticonvulsant, antidiabetic,

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antispasmodic, and hypotensive.[11] It also demonstrated that this oleo-gum-resin has antileishmanial,[12] diuretic,[13] antinociceptive,[14] and aphrodisiac effects.[15] Asafoetida also has been reported to act as anticarcinogen in many traditional systems[16] which confirmed by a number of new studies.[11] A meta-analysis study showed that in countries such as Japan, Russia, China, and Indonesia, the rate of cancer is higher in the comparison of countries that usage of asafoetida is common.[17] These evidences provided reliable reasons for investigation of anticancer effect of asafoetida on viability 4T1 cells *in vitro*.

Materials and Methods

Essential oil and oleo-gum-resin preparation

F. assa-foetida oleo-gum-resin was collected from Tabas region (Yazd Province, Iran), and the plant species was botanically identified by Dr. Abbas Zarezadeh in Yazd Agricultural Research Center. The dried powder of asafoetida was soaked in distilled water overnight at room temperature and the yielded suspension was used.[18] Concentrations and dosages of the extract were expressed as crude amount of the dried oleo-gum-resin used in preparing the stock solution. For oil isolation of asafoetida, oleo-gum-resin (100 g) was dissolved in 1 L of distilled water and the oils were isolated by hydrodistillation using a Clevenger-type apparatus for 3 h. The distillated oils were dried over anhydrous sodium sulfate and stored at 4°C until used.

Cells and reagents

The 4T1 cells, which were purchased from the Pasteur Institute of Iran, were maintained in RPMI 1640 (Sigma-Aldrich) medium containing 10% (v/v) fetal bovine serum (Gibco Laboratories), 300 μg/ml glutamine, 100 μg/ml streptomycin, and 100 units/ml penicillin. The cell cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma, USA, and ferulic acid was purchased from Sigma, USA. Transwell plates for transwell migration assay were purchased from SPL Life Sciences Co. Ltd., Korea.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution

The MTTs were dissolved in sterile phosphate-buffered saline at 5 mg/ml and stored in dark condition at 4°C for a period lasting <3 weeks. After the final dilution with prewarmed sterile unsupplemented culture medium, the MTT solution was filtered through a 0.22 μm filter.

Cytotoxicity assay

Tumor cells in the exponential growth phase were harvested from culture flasks using 0.05% ethylenediaminetetraacetic acid (Gibco Laboratories) for 3 min. The cells were washed in standard growth medium and counted using a hemocytometer. A number of 3×10^3 cells per well were plated on 96-well flat-bottomed plates. One day after the seeding of the cells at 37°C, cells were treated for 24, 48, and 72 h with various concentrations of oleo-gum-resin and its essential oil and ferulic acid. Cells treated with serum-free medium for the same periods of time were used as a control. After that, the MTT solution (5 mg/ml in phosphate-buffered solution) was added to each well. After 3.5 h of incubation, purple crystals were formed by mitochondrial dehydrogenase enzyme of living cells. Then, the medium was discarded and 150 μl of dimethyl sulfoxide was added to dissolve the formazan crystals. The absorbance of each sample was read at 540 nm using a microplate reader (BioTek Instrument, Box 998). Results were expressed as percentage of cell viability with respect to untreated control cells (as 100%). The percent viability of each well was calculated from the following:

$$\text{Percent viability} = \frac{\text{Absorbance of test} - \text{Absorbance of blank}}{\text{Absorbance of control} - \text{Absorbance of blank}} \times 100$$

Statistical analyses

All data were expressed as means ± standard deviation. Graph Pad Prism version 5 (San Diego, California, USA) was used for data analysis. Statistically significant differences were determined using one-way ANOVA with Tukey–Kramer posttest for multiple comparisons. $P < 0.05$ was considered statistically significant.

Results

Cytotoxicity effect of oleo-gum-resin on 4T1 cells

The oleo-gum-resin at 1–1000 μg/ml did not show any obvious cytotoxicity on breast cancer 4T1 cells *in vitro* after incubated for 24 h [Figure 1]. The inhibition effect of oleo-gum-resin on 4T1 cells was enhanced after incubated for 48 and 72 h. Cell viability was about 10% even at the highest concentration of 1000 μg/ml after 72 h.

Effect of ferulic acid on 4T1 cells viability

Ferulic acid at 0.5–50 μg/ml did not show any obvious cytotoxicity on breast cancer 4T1 cells after incubated for

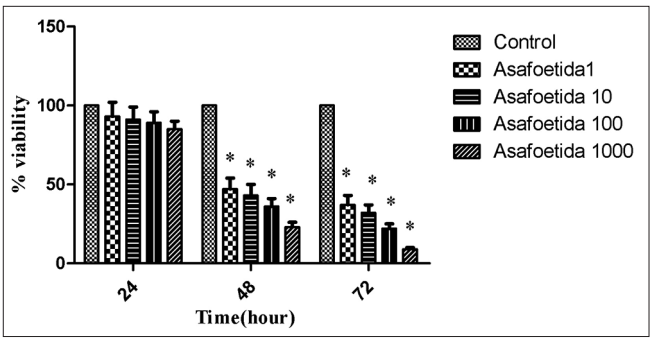

Figure 1: Cytotoxicity effect of asafoetida on 4T1 cells after 24, 48, or 72 h incubation. Data were expressed as mean ± standard deviation

24, 48, or 72 h [Figure 2]. Although the inhibition effect of ferulic acid on 4T1 cells was enhanced after incubated for 72 h only at 500 µg/ml, the cell viability was about 20% even at the highest concentration of 500 µg/ml.

Effect of essential oil of asafoetida on 4T1 cells

Essential oil of asafoetida at 0.01 and 0.1 µl/ml did not show any obvious cytotoxicity on breast cancer cells after incubated for 24 h [Figure 3]. At doses of 1 and 10 µl/ml, a significant difference in viability percentage was observed in all the three times. The lowest dose of essential oil (0.01 µl/ml) could decrease significantly viability percentage only after 72 h. Furthermore, a significant difference was observed after 48 and 72 h at 0.1 µl/ml dose of essential oil.

Discussion

Throughout the world, Iranian medicinal *F. assa-foetida* has been widely consumed as health promoting, food supplements, and medicine. Given the increasing popularity promotion of *F. assa-foetida* for adjuvant cancer treatment, there is an interest to study the potential protection of *F. assa-foetida* against cancer, especially breast cancer cells. In the present study, we aimed to evaluate the antitumor effect of *F. assa-foetida* oleo-gum-resin, its essential oil, and ferulic acid, one of the main components of *F. assa-foetida* oleo-gum-resin, using the highly invasive mouse mammary carcinoma 4T1 cells. Several investigations demonstrated the cytotoxic activity of *Ferula* species. Bagheri *et al*.[10] determined the cytotoxicity of some *Ferula* species on *Artemia salina* as a model for evaluating general cytotoxicity. Hence, the species of *Ferula* could be as a good source of cancer chemopreventive agents. Asafoetida is dried latex exuded from the living rhizome, rootstock, or taproot of an umbelliferous plant of varied species.[11] Our results revealed that 48 and 72 h after incubation with asafoetida, cell viability was significantly decreased compared to control group that was dose- and time-dependent manner [Figure 1]. Our findings are consistent with the results of other researchers. In a study, it has been shown that asafoetida inhibits microsomal activation-dependent mutagenicity of 2-acetamidoflourene.[19] In spite of some old evidence about genotoxicity and mutagenicity of asafoetida, recent studies have revealed its potential antioxidant, antimutagenicity, and cancer chemopreventive activities.[11] In an *in vivo* study, Saleem *et al*. showed that pretreatment of animals with acetone extract of asafoetida could cause the reversal of early events of carcinogenesis.[16] Another study showed that asafoetida reduced the multiplicity and size of palpable mammary tumors in Sprague–Dawley rats.[11] Different mechanisms seem to impact on this activity such as radical scavenging activity and lipoxygenase inhibitory activity.[20] In the previous study, we showed that asafoetida has remarkable antioxidant and lipoxygenase inhibitory activity.[21] In addition, there are a number of biological activities in asafoetida that have been reported for this compound including cancer chemoprevention and apoptosis induction in cancer cells. Phytochemical analysis showed that asafoetida contains about 40%–64% resin, 25% endogenous gum, 10%–17% volatile oil, and 1.5%–10% ash. The resin portion is known to contain farnesiferol, asareninotannols, ferulic acid, umbelliferone, the gum includes glucose, galactose, l-arabinose, rhamnose, glucuronic acid, polysaccharides, and glycoproteins, and the volatile fraction contains sulfur-containing compounds, monoterpenes, and other volatile terpenoids.[11] Umbelliprenin is one of these components that has been shown to have remarkable cancer chemoprevention *in vitro* and *in vivo*.[22] Moreover, it was reported recently that farnesiferol C[23] from *F. assa-foetida* may be a potential candidate for the treatment of cancer. Phenolic compounds present in asafoetida have been reported to have strong antioxidants and antitumor properties.[24] In this study, we also investigated anticancer effect of ferulic acid which is one of the main phenolic constituents in asafoetida. Results showed that ferulic acid has anticancer at a dose of 500 µl [Figure 2]. The previous study showed that it has anticancer effect by increasing the effect of radiation treatment on human cervical carcinoma cells[25] and also has a synergistic role to inhibit cancer cell proliferation.[23]

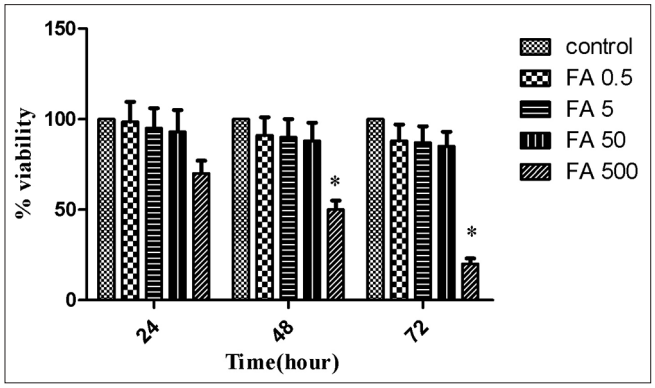

Figure 2: Cytotoxicity effect of ferulic acid on 4T1 cells after 24, 48, or 72 h incubation. Data were expressed as mean ± standard deviation

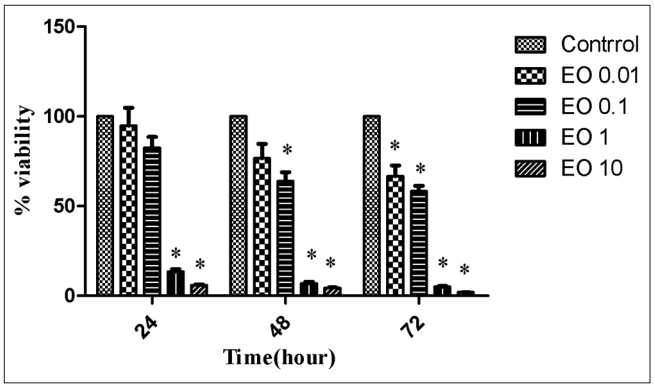

Figure 3: Effect of asafoetida essential oil on 4T1 cells after 24, 48, or 72 h incubation. Data were expressed as mean ± standard deviation

Among the components were tested, the essential oil has strongest cytotoxicity effect on 4T1 cells at the higher concentrations of 1 and 10 μg/ml [Figure 3]. Previously, researchers showed that this essential oil contains sulfur components and it is observed that E-1-propenyl sec-butyl disulfide (40.15%) and Z-1-propenyl sec-butyl disulfide (23.93%) were the major constituents of the oil.[26]

However, we observed that sulfur compounds of asafoetida have stronger anticancer effects, but we do not know the mechanism of these effects. However, some evidence suggests that organosulfur compounds modulate the activity of several metabolizing enzymes that activate (cytochrome P450s) or detoxify (glutathione S-transferases) carcinogens and inhibit the formation of DNA adducts in several target tissues.[27]

Conclusions

Our results demonstrated that asafoetida has a cytotoxic activity against mouse breast 4T1 cells. It seems that asafoetida has a set of natural compounds that have anticancer effects. In this study, it was found that the asafoetida oil has more potential to suppress cancer cells. However, its active components and their mechanism of actions need to be elucidated by further studies.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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