Introduction

Cancer is believed to be one of the most considerable causes of mortality all around the world\(^1\). Numerous studies have shown the preventive effects of Ferula assa-foetida, as a medicinal herb, on cancer development. Cytotoxic effects of this plant are linked to the components in its gum. Several lab trial studies have also proven these characteristics\(^2\).

Gastrointestinal malignancies have always been one of the prevalent and mortal cancers in the world and in Iran\(^3\). Pancreatic cancer, with a high mortality rate and 5-year survival rate of 4%, leads to approximately 250,000 deaths per year worldwide\(^4\).

Ovarian cancer, as the second most common malignancy of women, is the first cause of cancer-related deaths among women. It is estimated that more than 140,000 deaths in the world occur annually due to this cancer. Unfortunately, sixty percent of all patients with ovarian cancer are diagnosed with metastatic stage of the tumor, reducing 5-year survival rate to less than 50\%\(^5\).

Cancer is characterized by uncontrolled growth of abnormal cells resulted from genetic transformations\(^6\). So far, many medicinal herbs are proved to have different therapeutic properties. Medicinal herbs, with the least side effects, can play an important role in maintaining human health and defending against variety of diseases including cancer\(^7\). Being rich in components such as phytosterols, flavonoids, carotenoids and terpenoids, these plants act as antioxidants and eliminate free radicals. On the other hand, they disrupt DNA formation of cancer cells and metabolic pathways associated with metastasis, through stimulating the immune system\(^8\). Different types of Ferula assa-foetida are endemic in central Asia and are used as food or medicinal herb. The anti-progressive effect of this plant on cancer cells are shown in previous studies\(^9\). Furthermore, having anti-inflammatory properties, Ferula assa-foetida can be used in other medical conditions\(^10\).

Cytotoxic Effects of Methanolic Extract of Ferula assa-foetida on SKOV-3 and MIA PaCa-2 Cells

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Abstract

Introduction: Previous studies have demonstrated cytotoxic effects of Ferula assa-foetida. In the present study, the anti-proliferative effect of this plant on two species of cancer cells related to pancreatic cancer (MIA PaCa-2) and ovarian cancer (SKOV-3) was investigated.

Materials and Methods: 100 grams of powdered herb dissolved in 500 milliliter of methanol was placed in Soxhlet extractor for 72 hours. After adding trypsin to the medium, cells were cultured in serum containing medium. A serial dilution of extract was created with 25, 50, 100, 200 and 400 microgram per milliliter concentrations. Plates were fed with 200 microliters of new mediums at the end of their growth and 50 microliters of MTT were added to all wells of 1 to 11 columns. After incubation, mediums and MTT were removed from the wells and remaining crystals were resolved by adding DMSO. After adding glycine buffer (25 μl per well), we immediately read the results at wavelength of 570 nanometer using an ELISA reader.

Results: Concentrations of 25, 50, 100, 200 and 400 micrograms per milliliter of methanolic extract of Ferula asa-foetida had significant cytotoxic effect on SKOV-3 and MIA PaCa-2 cancer cells with a P-Value of <0.05. These changes were time-dependent.

Discussion and Conclusion: Besides their several medical uses, medicinal herbs have recently turned out to have antineoplastic effects. One of these herbs is Ferula assa-foetida. In the present study, we evaluated the anti-proliferative effects of this plant on ovarian and pancreatic cancer cells.

Keywords: Cancer, Medicinal herbs, Ferula assa-foetida, Cytotoxicity

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In the present study, considering the increasing incidence rate of malignancies, we tried to evaluate the possible effects of an accessible medicinal herb on cancer cells growth. We assessed the anti-proliferative effect of Ferula assa-foetida on two species of cancer cells related to pancreatic cancer (MIA PaCa-2) and ovarian cancer (SKOV-3).

Materials and Methods

Extraction

Soxhlet extraction method was carried out. The herbs were dried and then powdered. 100 grams of powdered herb dissolved in 500 milliliter (ml) of methanol was placed in Soxhlet extractor for 72 hours, at a lower temperature than the boiling degree of the solvent. The liquid extract was filtered using Whatman filter paper, and then was concentrated in a rotary evaporator inside a vacuum at temperature of 40℃.

MTT Method

Cells were placed in serum containing medium to which trypsin was added. 200 grams of the cell suspension was centrifuged for 5 minutes. The cells were dissolved again in the culture medium and were counted. Depending on the cell growth rate (doubling time), 2.5-50×10³ cells per ml were diluted in 20 ml volume. The obtained suspension was transferred to a 9 centimeters petri dish. Then we transferred 200 microliter (μl) of it, using a micro-multicell holder, to each of the wells of the 96-house plate, from the second to the eleventh column (0.5-10×10³ cells per well). A serial dilution of extract including concentrations of 25, 50, 100, 200 and 400 micrograms per ml was obtained. Four plates were used for each concentration. There were four duplicate of control sample, receiving no medication, which other concentrations were compared with them. At the end of effecting time of Ferula assa-foetida, the medium was removed from all the containing cell wells and 200 μl of new mediums were added. The plates were fed daily, up to two or three cell divisions. We fed plates with 200 μl of new mediums at the end of their growth and added 50 μl of MTT to all wells of 1 to 11 columns. Plates were then put in the foil and incubated for 4 hours at a 37℃ temperature. Mediums and MTT were removed from the wells and the remaining crystals were resolved by adding 200 μl of Dimethyl Sulfoxide (DMSO) to all columns of 1 to 11. Then we added glycine buffer (25 μl per well) to all wells containing DMSO. According to instability of the product, we immediately read the absorption at the wavelength of 570 nanometers (nm). Column 1 wells, containing the medium and MTT without any cell, were used as the blank mode of the plate reader. This method is simpler compared to other cell proliferation methods, making it applicable in most laboratories. All steps were performed on 96-well cell culture plates, and the results were read using an ELISA reader at 570 nm wavelength.

Data analysis

MTT results were analyzed using SPSS 20, with a significance level of P <0.05.

Results

Tables 1 and 2 demonstrate anti-proliferative effects of the methanolic extract of Ferula assa-foetida in different concentrations on MIA PaCa-2 and SKOV-3 cells after 3 time periods of 24, 48 and 72 hours. Concentrations of 25, 50, 100, 200 and 400 μg per ml showed anti-proliferative effects on SKOV-3 and MIA PaCa-2 cells. These changes were also dependent on time. The results of MTT test indicated a remarkable reduction in survival of SKOV-3 and MIA PACA-2 cells at concentrations of 200 and 400 μg per ml.

Table 1 shows that the cells viability rate of MIA PaCa-2 cells after 24 hours exposure to concentrations of 25, 50, 100, 200 and 400 μg per ml of methanolic extract of Ferula assa-foetida were 99.87%, 96.26%, 93.34%, 86.07% and 49.06% respectively, with a p value of <0.05. By reaching time period to 48 hours, in exposure to concentrations of 25, 50, 100, 200 and 400 μg per ml of methanolic extract of Ferula assa-foetida, MIA PaCa-2 cells viability rate were 96.26%, 94.15%, 93.22%, 84.57% and 47.99% respectively, with a p value of <0.05.

After 72 hours, MIA PaCa-2 cells viability rate in exposure to concentrations of 25, 50, 100, 200 and 400 μg per ml of methanolic extract of Ferula assa-foetida, MIA PaCa-2 cells viability rate were 96.26%, 94.15%, 93.22%, 84.57% and 47.99% respectively, with a p value of <0.05.

In the table 2, it is shown that the SKOV-3 cells vi-
ability rate after 24 hours exposure to concentrations of 25, 50, 100, 200 and 400 μg per ml of methanolic extract of Ferula assa-foetida were 91.28%, 84.55%, 79.41%, 72.79% and 59.55% respectively, with a p value of <0.05.

The SKOV-3 cells viability rate after 48 hours exposure to concentrations of 25, 50, 100, 200 and 400 μg per ml of methanolic extract of Ferula assa-foetida were 90.44%, 76.42%, 73.10%, 56.81% and 26.51% respectively, with a p value of <0.05.

It is shown that viability rate of SKOV-3 cells after 72 hours exposure to concentrations of 25, 50, 100, 200 and 400 μg per ml of methanolic extract of Ferula assa-foetida were 89.73%, 71.48%, 68.93%, 40.68% and 15.58% respectively, with a p value of <0.05.

Discussion

Medicinal herbs have an undeniable role in protecting human against variety of diseases. They have recently turned out to have some antineoplastic effects. One of these plants is Ferula assa-foetida. Some previous studies have emphasized the antineoplastic effects of the compounds in the gum of this herb.

In the present study, the effect of methanolic extract of Ferula assa-foetida on SKOV-3 and MIA PaCa-2 cancer cells, which are pancreatic and ovarian cancer cells, was evaluated. The cytotoxic changes were observed 24, 48 and 72 hours after the addition of the extract at concentrations of 25, 50, 100, 200 and 400 μg per ml. It was found that concentrations of 25, 50, 100, 200 and 400 μg per ml had anti-proliferative effects on SKOV-3 and MIA PaCa-2 cells. These changes were also time-dependent.

A similar study has found that in the exposure of Ferula assa-foetida extract, the size of tumors containing 4T1 breast cancer cells, were significantly reduced. Necrosis was also observed in neoplastic regions. These results confirm the results of the present study on the cytotoxic effects of Ferula assa-foetida. However, it seems to be necessary to use a certain and accurate concentration to eliminate cancer cells, observing any damage to normal cells of the body.

In another study, the significant cytotoxic effects of this plant on MCF-7 and PC-3 cells, as prostate cancer cells, were observed. Also by evaluation NIH cells, as normal and non-cancerous cells, no damages were detected. We also observed cytotoxic effects of Ferula assa-foetida on two other species of cancer cells.

Another study, suggested that at low concentrations, Ferula assa-foetida has a rejuvenation effect in fibroblasts, while in higher concentrations, it facilitates cell apoptosis. These findings indicate the dose-dependent effect of this medicinal herb on cellular functioning. When using this medicinal herb, we should consider its dose-dependent effects.

In a study, the widespread therapeutic effects of this plant, including antioxidant and anti-inflammatory effects are underlined. It is demonstrated in another study that extract of this plant can reduce the duration and extent of damage to the nervous cells caused by seizure.

So far, few studies on therapeutic effects of Ferula assa-foetida have been conducted. Regarding remarkable results of previous studies and the present study, further studies on this field considering the exact mechanism of functioning and any possible adverse effects are needed to be carried out.

Conclusion

Considering the increasing incidence of different types of cancer and the lack of effective treatments, as well as the limitations of screening methods in identifying some of the aggressive types, curative effects of an accessible treatment can be hopeful. In this study, we observed the anti-proliferative effects of a medicinal herb, named Ferula assa-foetida, on ovarian and pancreatic cancer cells. In recent years, despite the cytotoxic and antioxidant effects of this plant, little efforts have been made to obtain more information of its characteristics. We suggest further studies to be carried out on this field.

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