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Effects of fennel, asafetida and ginseng ethanolic extracts on growth and proliferation of mouse breast cancer $4T_1$ cell lines

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Original article

ABSTRACT

Background and aims: The $4T_1$ cells tumor growth and metastatic pattern in BALB/c mice very closely mimic human breast cancer. These herbal remedies used in traditional folk medicine have been the source of many medically beneficial drugs. The aim of the present study was to evaluate the anti-proliferative activity of the asafetida, ginseng and fennel ethanolic extracts on mouse breast cancer $4T_1$ cell line in vitro.

Methods: In this experimental study, asafetida, gensing and fennel were extracted; $4T_1$ mouse mammary tumor cell line were cultured in 48-well flat bottom plate at density of 50x103 per well in 100µl RPMI-1640 medium. Then, different dilutions of each extract (25, 50, 100, 200, 500, and 2000µg/ml) were added to cell culture. Cells then were incubated at 37°C for 24 hours. After 24 h, cell proliferation was determined by the BrdU assay.

Results: Mouse breast cancer $4T_1$ cell line was incubated with different doses of extracts. After 24h, cell proliferation was determined by the BrdU assay and the dose of 50µg/ml of fennel showed the best inhibitory effect.

Conclusion: Anti-tumor activities of fennel, asafetida and ginseng ethanolic extracts may decrease proliferation activity of $4T_1$ cell line in vitro. The results suggested that fennel, asafetida and ginseng ethanolic extracts induce apoptosis and inhibit cell proliferation in vitro and fennel had the best anti proliferation effect.

Keywords: Fennel, Asafetida, Ginseng, $4T_1$ Cell line, Breast cancer.

INTRODUCTION

Plant extracts have been used for centuries as a popular remedy against several health disorders.[1] Natural products and herbal remedies used in traditional folk medicine have been the source of many medically beneficial drugs.[2] Many of medicinal plants have been shown to present interesting biological and pharmacological activities and are used as therapeutic agents.[3] To date, very little research has been done to investigate these traditionally used medicinal plants. Foeniculumvulgare (FVE) is a well-known umbelliferous plant.[1] FVE fruits have been used as traditional herbal medicine around the world for centuries.[4] The seeds of this plant have been known to be able to increase milk secretion, promote menstruation, facilitate birth, and alleviate the symptoms of dysmenorrhea, alleviate the symptoms of female climacteric syndrome, and increase libido.[5,6] It also possesses emnenagague and galactagogue

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properties.[7] A study conducted by Dukic, et al. showed an antifungal activity of fennel essential oil. Moreover, different doses of fennel essential oil (25 and 50µg/ml) significantly decreased the level of oxytocin and prostaglandin E and induced uterine contractions in primary dysmenorrhea.[8] Fennel seed extract has been shown to have estrogenic, antioxidant, and anti hirsutism activities.[9] FVE is natively found in North and North West regions of Iran.[10] Ginseng is one of the commonly used herbal medicines whose underlying mechanism is not clear.[11] Recent investigations have shown that ginseng extract and its components could suppress tumor promoting activity[12] and induce apoptosis in cancer cells.[13,14] Breast cancer cell lines have been the most widely used models to investigate how proliferation, apoptosis and migration become deregulated during the progression of breast cancer.[15] According to its beneficial activity, the present study was conducted to evaluate the potential activity of ethanolic extract of fennel seeds in proliferation activity in mouse mammary tumor cell line. All procedures of the present study were performed at the Department of Immunology of Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

METHODS

In this experimental study, fennel seeds and asafetida plants were collected from desert areas in Yazd, Iran. Roots of ginseng were bought from herbal medicine shop. Following identification, drying, and powdering seeds of fennel, roots of ginseng and pedicle of asafetida were soaked in 80% methanol for 48 hours at room temperature. After filtration the residue was drained into the same flask. The solvent was dried by rotary evaporation under reduced pressure and at a temperature of maximally 45°C. Before running the test, the extracts were dissolved in dimethylsulfoxide (DMSO), and then, diluted in RPMI-1640. The extracts were filtered and stored at 4°C.

$4T_1$ mouse mammary tumor cell line (Pasteur Institute, Tehran, Iran)[16] maintained in RPMI-1640 (Sigma-Aldrich, MO, USA)[17] was supplemented with 10% fetal bovine serum and 100 units/ml penicillin-streptomycin (Gibco, BRL, Grand Island, NY, USA).[18] 72 hours before giving effect of extract, cells were seeded in multi-well dishes such that they were confluent at the time of the experiment.

$4T_1$ mouse mammary tumor cell line were cultured in 48-well flat bottom plate in density of 50x103 per well in 100µl medium. After 48h of incubation, cells were exposed to fennel, asafetida and ginseng extracts (50, 100, 200, 500, and 2000µg/ml). Cells then were incubated at 37oC for 24 hours.

The anti-proliferative activity was analyzed by BrdU assay.[19] BrdU can be incorporated into the newly synthesized DNA of replicating cells (during the S phase of the cell cycle) and substituting for thymidine during DNA replication. Antibodies specific for BrdU can then be used to detect the incorporated chemical and indicating cells that were actively replicating their DNA. Binding of the antibody requires denaturation of the DNA, usually by exposing the cells to acid or heat.[20] Because it is neither radioactive nor myelotoxic at labeling concentrations, it is widely preferred for in vivo studies of cancer cell proliferation.[21] The resulting DNA-bound bromouracil moiety was subsequently detected by commercial anti-BrdU mAb without the need for a denaturation step.[22] The end product color was then analyzed by measuring absorbance at 450nm with a

reference wavelength at 630nm.[16,23] Data are expressed as percent of cell viability compared with that of control culture, defined as 100%.

The results obtained from the samples were compared with each other and with the control group. The data of experiments were collected and analyzed by SPSS software 19. Significant level of data was examined using one-way ANOVA test and $P<0.05$ was considered as the level of significance.

RESULTS

To evaluate the anti-proliferative activity of fennel, asafetida and ginseng ethanolic extracts, mouse breast cancer $4T_1$ cell line were incubated with different doses of plant extracts. After 24h, cell proliferation was determined by the BrdU assay. The results of optical absorption measurements based on different concentrations of extracts compared to cell proliferation are shown in Table 1.

Table 1: Absorbance of the test indifferent concentrations of ginseng, fennel and asafetida

Extracts / Concentration	Asafetida		Ginseng		Fennel	
	OD	SD	OD	SD	OD	SD
0 µg/ml	2.43	0.40	2.43	0.40	2.43	0.04
25 µg/ml	0.71	0.46	0.97	0.22	0.63	0.19
50 µg/ml	0.59	0.01	1.03	0.01	0.41	0.01
100 µg/ml	0.74	0.00	1.10	0.04	0.61	0.03
200 µg/ml	1.20	0.00	1.47	0.01	0.77	0.02
500 µg/ml	1.63	0.02	1.82	0.05	1.20	0.00
2000 µg/ml	0.82	0.00	1.24	0.04	1.24	0.04

OD: Optical density; SD: Standard Deviation.

Comparison of the maximum absorbance which was obtained for the concentration of 500 µg/ml ginseng and the minimum absorbance which was obtained for the concentration of 50 µg/ml asafetida.

DISCUSSION

Today, Medicinal plants have a good place in the diet of people in the world.[24] Herbal therapy has thus been introduced partly because herbs consist of constituents with multiple purposes, and partly, because there is a long tradition of using herbs in Asian and European countries.[11] The traditional folklore and ethno pharmacological knowledge are helpful to identify plants with presumably health-beneficial effects or potential anti-inflammatory and/or antitumor activities.[25] The treatment of cancer is still a big challenge and development of new drugs is urgent.[26] Soltanzad et al. demonstrated that methanol extracts of Ferula inhibited A549 cell proliferation in a concentration-dependent manner.[27] Lee et al. discussed the anti-inflammatory effects of ginseng extracts and ginsenosides on cellular responses triggered by different inducers including endotoxin, tumor necrosis factor-alpha, interferon-gamma and other stimuli.[28] Also Mohammad et al. demonstrated that volatile oil of fennel seeds (Foeniculumvulgare) may have remarkable anticancer potential against a breast cancer cell line (MCF7) and liver cancer cell line.[29] In the present study, the effect of fennel, asafetida and ginseng on $4T_1$ breast cancer cell line was assessed with BrdU method. All of them showed a potent anti-proliferation activity. This study provides the evidence that the anti-tumor activities of fennel, asafetida and ginseng

ethanolic extracts may decrease proliferation activity of $4T_1$ cell line in vitro. In conclusion, our screening shows significant cytotoxic activity of all three types of extract, but out of these three extracts, fennel at dose of 50µg/ml showed the best inhibitory effect. Further research especially in vivo is needed to identify the major anti proliferative activity of these extracts.

CONCLUSION

This study provides the evidence that the anti-tumor activities of fennel, asafetida and ginseng ethanolic extracts may decrease proliferation activity of $4T_1$ cell line in vitro. The results suggested that fennel, asafetida and ginseng ethanolic extract induced apoptosis and inhibited cell proliferation in vitro and fennel had the best anti proliferation effect at 50µg/ml dose of extract.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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