Original Article

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The anti-proliferative effects of Ferulago angulata on human promyelocytic leukemia cell line (HL-60)

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Abstract

Ferulago angulata is a medicinal plant with bioactive compounds that have antioxidant activities. *F. angulata* extract can be a good candidate as chemo- preventive agent against cancer cells. In this study, the pro-apoptotic activities of *F. angulata* were investigated on human promyelocytic leukemia cell line (HL-60). HL-60 cell line was cultured in RPMI 1640 medium and treated with different concentrations of F. angulata extracts. The effect of extract on cell viability was measured by MTS assay. The apoptosis was evaluated using flowcytometry. IC_{50} was calculated by probit analysis and the groups were compared using Kruscal wallis test. Our results showed that F.angulata decreased the cell viability in a concentration of more than 500 µg/ml with an IC_5 of about 1000 µg/ml. Apoptosis was observed in all concentrations, but only was remarkable in more than 500 µg/ml. F.angulata can induce anti-proliferative activities against HL-60 cells. A complete understanding of molecular events and pharmacokinetics of the elements and clinical trials in animal models are required for dose determination and its interaction with other components of combination chemotherapy.

Keywords: Apoptosis, *Ferulago angulata*, HL-60 cell line, MTS.

1. Introduction

Leukemia is defined by the autonomous proliferation of immature hematopoietic cells in the bone marrow. This condition is classified into chronic and acute types. Acute leukemia is an aggressive disorder, generated by malignant changes in hematopoietic precursor cells. Acute myeloid leukemia (AML) is a heterogeneous disorder of myeloid precursors with atypical differentiation (1). AML is the most prevalent type of acute leukemia throughout adolescence and the first months after birth (2, 3). The progress of, HL-60 cell line therapeutic approaches for AML are limited, and despite primary responses to chemotherapy, prognosis of AML is generally poor for the majority of patients due to initial resistance and recurrent relapse (4, 5).

The development of novel therapeutic agents and protocols is in a crucial demand for improving outcomes in patients with AML. A favorable strategy for cancer prevention is introducing compounds that block cancer development. Natural compounds and their derivatives have been considered for the treatment of various diseases including cancer (6-9). Plant products stimulate cell death through diverse mechanisms. The most prevalent mechanisms are programmed cell death (PCD)-type I (apoptosis), PCD-type II autophagic cell death, and necrosis (10).

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Apoptosis is a way of self-cannibalism, which includes exclusive cells and does not cause inflammation to the close cells (11, 12). Therefore, apoptosis, which is described by a series of biochemical and morphological alterations, including deoxyribonucleic acid (DNA) fragmentation, nuclear condensation, externalization of phosphatidylserine (PS), membrane blebbing, and damage of mitochondrial membrane potential (MMP), has become a mechanism of interest in drug study (13, 14). Mitochondria are established to be an essential factor in the regulation of apoptosis (14). An omission in MMP leads to the translocation of proapoptotic Bax to mitochondria, which results in the release of cytochrome c and the motivation of caspase cascades (15). Moreover, extreme production of reactive oxygen species leads to oxidative stress and the depletion of the glutathione level has been described to trigger apoptotic signaling (16, 17).

Ferulago angulata, recognized as Chavir in Iran, is a common plant distributed in Iran, Turkey, Iraq, Greece, Serbia, and Macedonia (18, 19). *F. angulata* has been traditionally used as a treatment for dyspepsia. In addition, it has been reported to have anti-microbial activity (20). The leaves and flowers of the plant have demonstrated angiogenesis and migration suppressive effect versus the human umbilical vein endothelial cells by down-regulating the expression of vascular endothelial growth factor (VEGF)-A and VEGF receptor-2 mRNA (21). Furthermore, the cytotoxic effect of *F. angulata* against some cell lines has also been reported (22, 23). The present study was designed to investigate the anti-proliferative activity of *F. angulata* leaves on the HL-60 leukemia cell line.

2. Materials and methods

2.1. Cell culture

Human promyelocytic leukemia Cell Line (HL-60) (Pasteur Institute, Iran) was grown in RPMI1640 culture medium supplemented with 10% fetal calf serum, 0.3 mg/ml glutamine, 100 IU penicillin, and 100 µg/ml streptomycin, and was kept in a standard condition (humidified atmosphere containing 5% CO_2 at 37 °C). Cells in the exponential phase were applied for all experiments. Cell count was done by hemocytometer and tripane blue dye.

2.2. Analysis of cell viability

Cells at 10×10^3 cells/ml were cultured in a 96-well tissue culture plate for 24 h. The cells then were treated with different concentrations of *F. angulata* (0-100 µM) for 24, 48, and 72 h in the above-mentioned culture media. MTS was added to the cells and were incubated for 3 h more at 37 °C in a dark position. The optical density (OD) against the background (culture medium with DMSO) at 490 nm was evaluated using ELISA reader (stat fax-2100 awareness). Every independent test was repeated at least three times and the concentration required for a 50% inhibition of growth (IC_{50}) was determined. The OD is comparative to the total number of living cells.

2.3. Apoptosis assay

Apoptosis was detected by flow cytometry method with a FITC Annexin V Apoptosis Detection Kit according to the manufacture instruction. HL-60 cells were seeded in a six well plate in complete media for 24 h. The cells then were treated with different concentrations of *F. angulata* for 48 h, afterwards were harvested and washed in PBS twice. 10^6 cells were re-suspended in 1 ml 1×binding buffer. 10^5 cells were incubated with FITC-labeled annexin V and propidium iodide (PI) at room temperature in dark place for 20 min. The tube was mixed gently, and kept on ice and examined by flow cytometry (PARTEC, Germany). Apoptotic cells are FITC positive and could be at first or in the final stage of apoptosis. Both populations were documented as apoptotic cells. Untreated cells were considered as the negative control for comparison.

2.4. Statistical analysis

All data were presented as mean±SD and analyzed by SPSS version 18 using Kruskal-Wallis and Dunn's multiple comparison test. The difference between the control and each concentration considered significant if the *P* value <0.05. All tests were repeated at least 3 independent times. Probit analysis was exert for IC_{50} calculation and GraphPad prizm for Graphs.

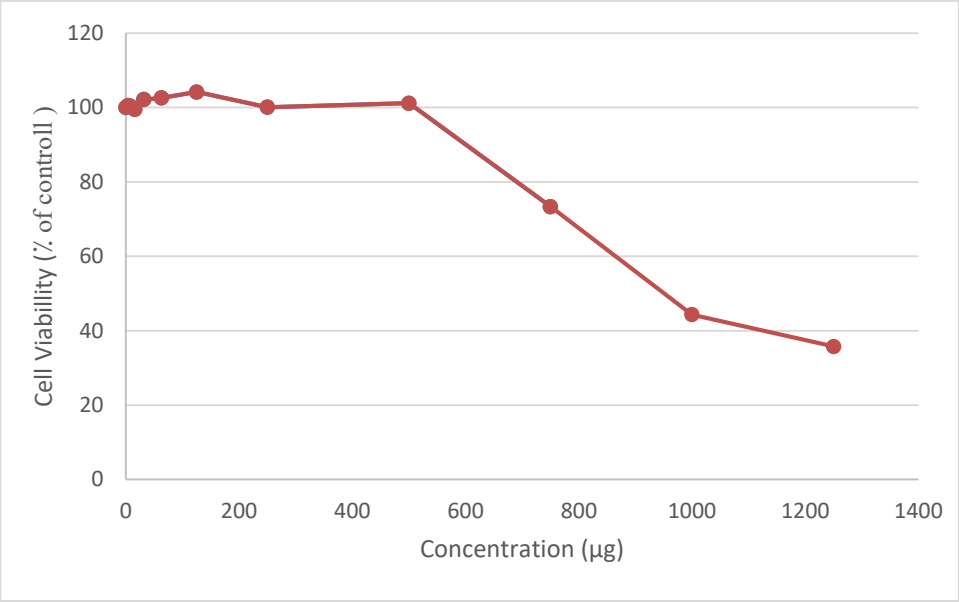

Figure 1. The effect of *F. angulata* on HL-60 cell line viability by MTS assay. Cells were incubated with different concentrations of *F. angulata* for 48 h and cell viability was determined compared with the untreated cells.

3. Results

3.1. F. angulata decreased cell viability in a dose-dependent behavior

HL-60 cells were incubated with various concentrations (0-1000 µM) of *F. angulata* for 48h. Reduction of cell viability was observed after incubation time in a 500 µg dose of *F. angulata*. The inhibition concentration (IC_{50}) was 1000 µg/ml in 48 h (Figure. 1).

3.2. F. angulata increased apoptosis of promyelocytic leukemia cells

The apoptosis was evaluated by a double staining method with Annexin V-FITC/PI. HL-60 cells were incubated with various concentrations (0, 500, 750, 1000 µM) of *F. angulata* for 48 h. Apoptosis was increased after treatment with different concentrations of the drug parallel to the control group (Figure 2). Figure 3 (A and B) show

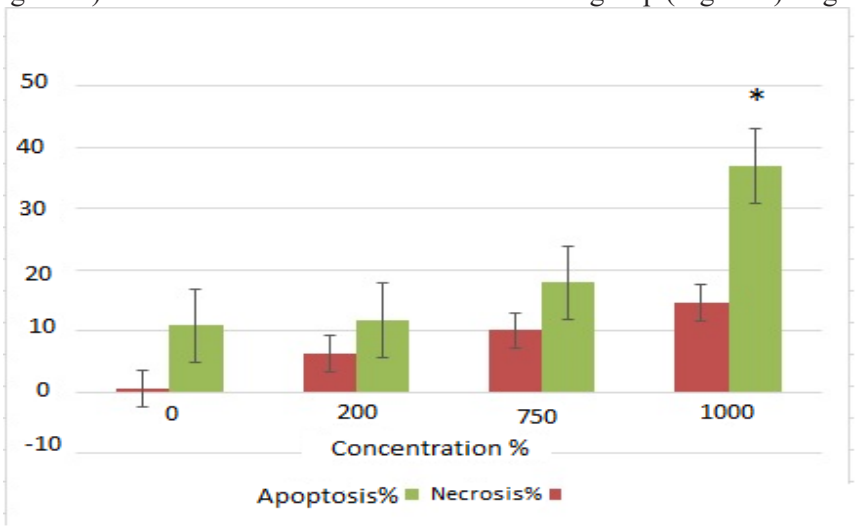

Figure 2. The effect of different concentrations of *F. angulata* on HL-60 cells apoptosis after 48 h incubation compared with the control group.
(* shows a significant difference compared with the control group).

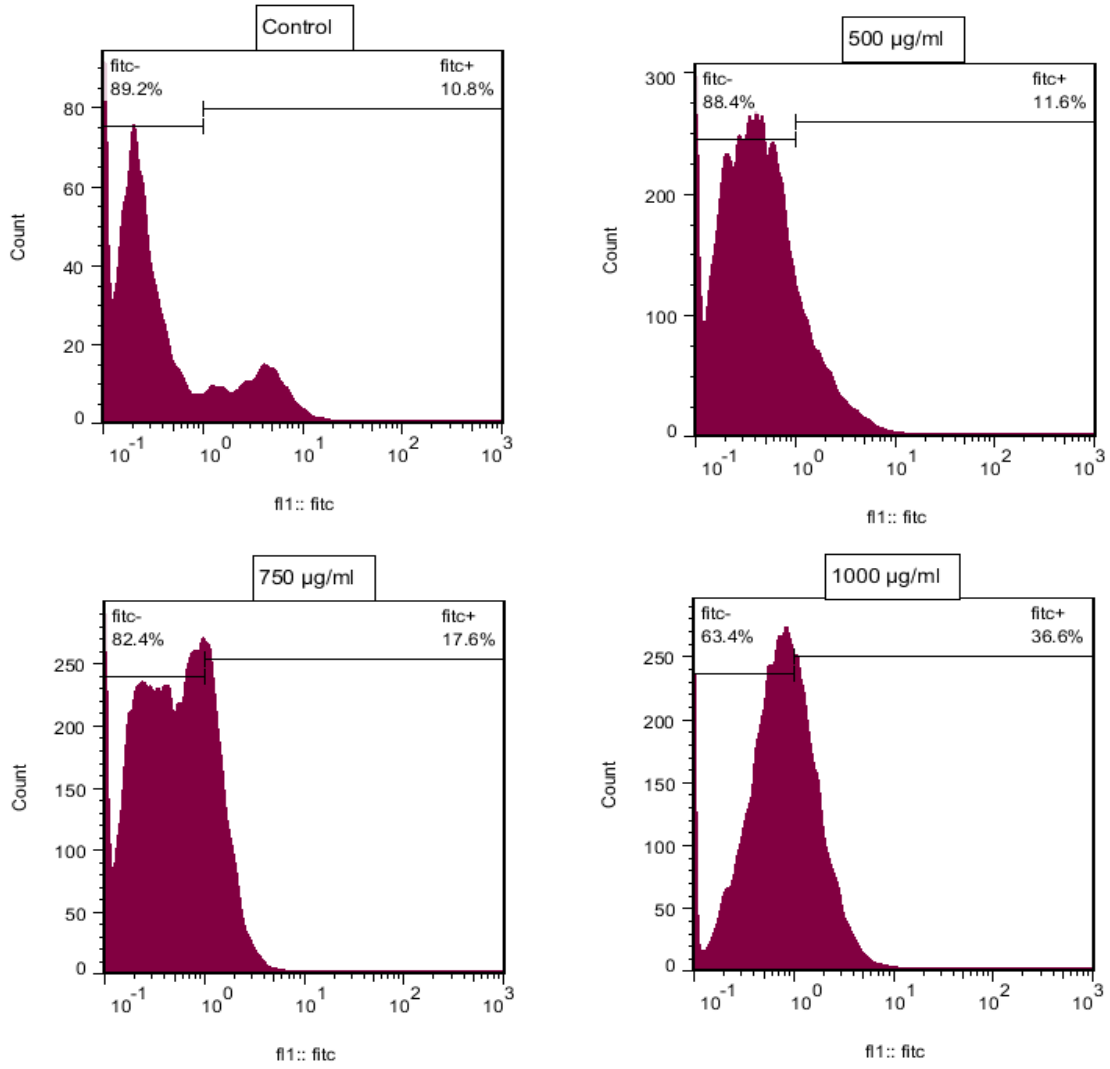

Figure 3-A. Concentration-dependent apoptosis induction by *F. angulata* in HL-60 cells. Cells were exposed to different concentrations of *F. angulata* (500, 750, and 1000 µM) for 48 h. Apoptosis induction was assessed by flow cytometry after annexin V-FITC stating.

flow cytometric analysis using Annexin V staining for apoptosis in HL-60 cells. Data demonstrates the values of three independent experiments. *F. angulata* at the concentrations of 500, 750, and 1000 µM induced apoptosis in about 11.7, 17.9, and 37% of the cells, respectively.

4. Discussion

The usage of natural products and plant extracts has been increasingly rised for their potential anti-cancer activity over the last decades. In fact, so many plant-derived natural compounds, or their derivatives, have been confirmed for the treatment of a wide range of solid tumors and hematological malignancies, such as vinblastine for bladder cancer, breast cancer, and Hodgkin's lymphoma, vincristine for lymphoid leukemia and lymphomas, and etoposide for lung and testicular cancers, and certain types of leukemia and lymphoma (24-26). Furthermore, several novel plant-

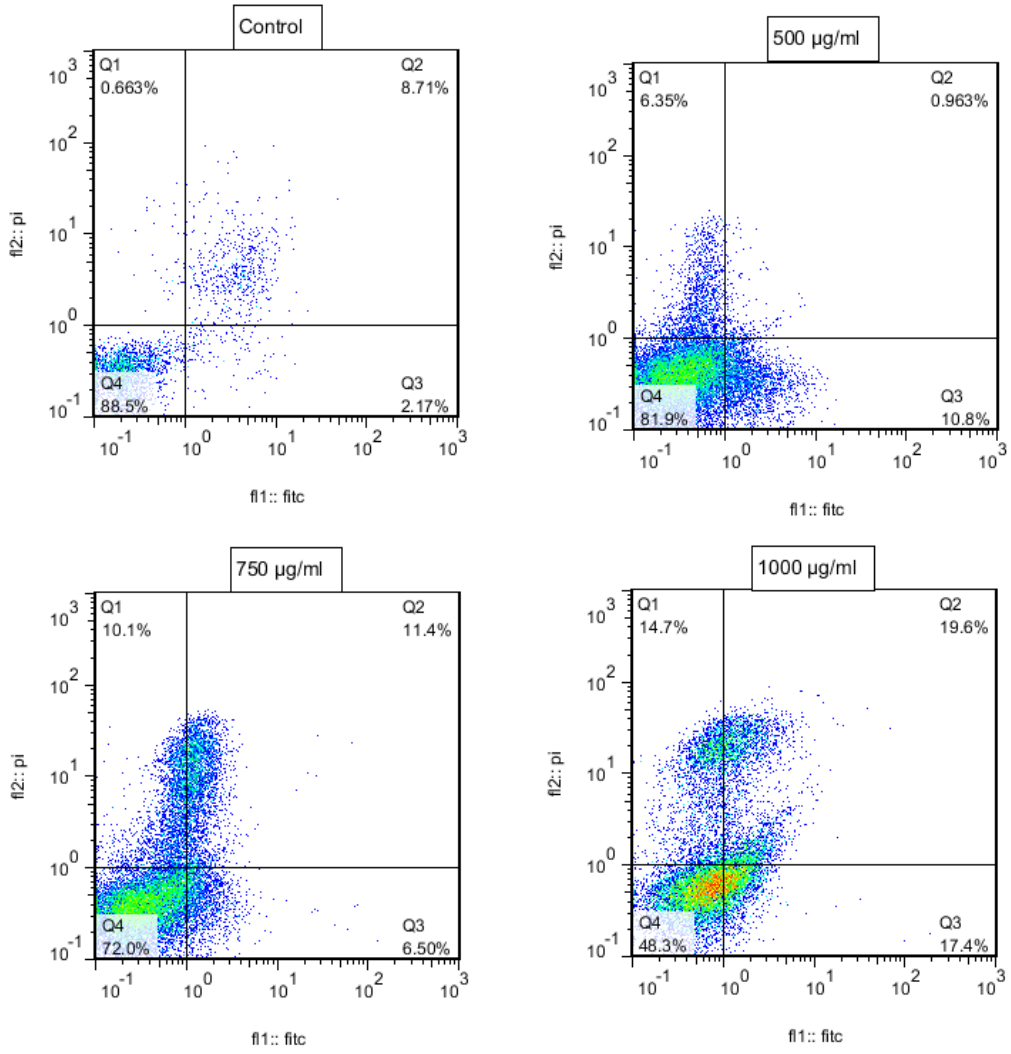

Figure 3-B. Concentration-dependent apoptosis induction by *F. angulata* in HL-60 cells. Cells were exposed to different concentrations of *F. angulata* (500, 750, and 1000 µM) for 48 h. Dot plots shows the results of a representative apoptosis/necrosis assay.

derived natural products with substantial anti-cancer activity against a wide array of tumor types have been recently discovered such as noscapine, bruceantin, and silvestrol (27-29). Some studies indicated that plant and natural products are effective on the prevention of malignant cell proliferation and are strong apoptotic inducers in hematological and non-hematological cancers. Additionally, they have synergistic effect in combination with some chemical drugs (30-31).

AML is one of the hematological malignancies for which novel and effective treatments are essentially needed, and in which the investigation of plant-derived compounds has been comparatively limited so far. It is an aggressive leukemia and one of the most common infantile malignancies, which also occurs with high incidence in the aging. Unfortunately, these group of patient do not respond well to current chemotherapy treatments (4, 5).

In this study, we have shown that *F. angulata* induces apoptosis activity in HL-60 cell lines.

The antitumor and cytotoxic effects of *F. angulata*-plant have also been investigated is other studies (32-36). Kiziltas *et al*. reported that *F. angulata* has a protective effect on the liver tissue of rat by changing the antioxidant enzymes (32). In another study, which was conducted by Heidari *et al*., the apoptotic and antioxidant properties of F. angulata were approved on the stomach cell line (33). Similarly, Karimian *et al*. reported apoptotic activities of *F. angulata*, along with a decline in the production of BCL2 and stopping the cells in step G1 cell cycle. In this study, IC-50 was 5.3±0.82 µg/ml, which was deferent from our results(34).

A significant reduction in the expression level of BCL2 and HSP70 in MCF-7 cancer cell line was also reported (36).

The cytotoxic effects of *F. angulata* have also been studied on some other cell lines. The results showed that the methanolic extract of *F. angulata* at concentrations of 50-800 µg/ml demonstrated more than 50% proliferation inhibition (35). In our study, IC_{50} was higher than most of the previous studies. In a study conducted by Amirghofran *et al*. on the Jurkat T-cell leukemia, the effect of inhibiting proliferation was observed on a concentration less than 8 µg/ml. However, they used MTT method, which is slightly different from ours (22). The different IC_{50} of the extract in different studies is somehow related to the cells behavior. The purity of the extract and other environmental factors can also impact the output.

5. Conclusion

TThis study demonstrated that *F. angulata* can induce apoptosis in human promyelocytic leukemia cell line (HL-60), which can show its potential as an anti-cancer therapy. The study of molecular events will provide further insights into the development of new therapeutic agents.

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Conflict of Interest

None declared.

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