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Original Research Article

Apoptotic induction of crude extract of *Foeniculum vulgare* extracts on cervical cancer cell lines

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A B S T R A C T

Regarding the presence of many active biological constituents in *foeniculum vulgare* the present investigation was carried out to study apoptotic activity of crude methanolic leave extract in cervical cancer cell lines (HeLa). The induction of apoptosis was determined by analyzing DNA fragmentation in cervical cancer cells treated with active fraction of crude methanol extract using agarose gel electrophoresis. Fragmentation of the DNA was observed at different plant sample concentrations. Morphological observations were carried out and apoptosis body was observed at 125µg/ml of the extract. The results suggested that the medicinal plant *foeniculum vulgare* could probably induce apoptosis on cervical cancer cell line and inhibit cell proliferation through DNA fragmentation.

Introduction

Cancer is still a serious health problem and has amajor social and economic impact worldwide. Despite recent advances in diagnosis, prevention, and therapy, cancer still affects quality of life in patients due to some limitations of these current medical practices. Consequently, more and more people resort to alternative medicine, which is defined as health care practices used instead of standard ones. Herbal medicine, one type of the alternative medicine, is based on the use of plants or plant extracts to treat diseases and promote health and has been offered especially for cancer treatment over the last century. This alternative treatment is more widely accepted at the present time. Therefore, medicinal plants have become important and reliable sources for anticancer agents and worldwide efforts are ongoing to find new plants with biological activity (Newman *et al.*, 2002; Newman *et al.*, 2003; Schwartsmann *et al.*, 2002).

A very limited scientific data can be accessed regarding the beneficial effect of herbal medicine, especially herbal plants, the effect of *Foeniculum vulgare* extract

on human cervical carcinoma cells was studied as a preliminary exploration. *Foeniculum vulgare*- Fennel is a plant belonging to the Umbelliferae (Apiaceae) family, known and used by humans since antiquity. Because of its flavor, it was cultivated in every country surrounding the Mediterranean sea. Its therapeutic (Puelo, 1980) and culinary utilization was so large that fennel was exported from country to country for centuries. Today, all these cultures have given rise to a great complexity, and simple observation of the plant's morphological characteristics is not sufficient to classify the different fennel species and subspecies (Jansen, 1981).

In the present study, cell cytotoxicity was determined after treating with plant extract. The cytotoxicity effect of plant extract was evaluated at gene level based on genomic assay such as DNA fragmentation from gel electrophoresis.

Materials and Methods

Collection of plant materials

The plant sample was collected from the local areas of Coimbatore. The plant sample has been authenticated by TNAU, Cbe.

Preparation of plant extract

The stems, shoots, leaves of *Foeniculum vulgare* were washed thoroughly and shade dried at room temperature. The dried leaves were subjected to size reduction to fine power by using dry grinder and passed through a sieve. 20gm plant powder was dissolved in 200ml of distilled water and methanol in a different conical flask and mixed well and the extract was filtered through Whatmann no.1 filter paper. This extract was used for the present study.

Cell culture

HeLa cell lines was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a humidified atmosphere of 50 μg/ml CO_2 at 37 °C.

Analysis of dna fragmentation

Dispense 0.5 ml of cell suspension.

Centrifuge cells at 200xg at 4^0C for 10 min.

Add to the pellet 0.5 ml of TTE Solution and Vortex Vigorously. This procedure allows the release of fragmented chromatin from nuclei, after cell lysis (due to the presence of Triton X- 100 in the TTE solution) and disruption of the nuclear structure (following Mg^{++} chelation by EDTA in the TTE Solution).

To separate fragmented DNA from intact chromatin, centrifuge tubes at 20,000xg for 10 min at 4^0C.

Carefully transfer supernatants in new tubes labeled T (Top).

Add to the small pellet in tubes 0.5 ml of TTE solution.

Add 0.5 ml volume of Ice-cold %M NaCl and vortex vigorously. The addition of the Salt should be able to remove histones from DNA.
Add 0.7 ml of ice-cold isopropanol and vortex vigorously.

Allow precipitation to proceed overnight at -20^0C. This step can be shortened by putting samples in a bath of ethanol/dry ice for 1 hr.
After, precipitation, recover DNA by pelleting for 10 min at 20,000x g at 4^0C.

Discard supernatants by aspiration or by rapidly inverting tubes and carefully remove any drops or fluid remaining adherent to the wall of the tubes with a paper towel corner.

This can be critical step because the pellet could be loosen and transparent, hard to be seen.

Rinse the pellets by adding 0.5-0.7 ml ice-cold 70% ethanol.

Centrifuge tubes at 20,000x g for 10 min at 40C.

Discard supernatants by aspiration or by rapidly inverting tubes. Carefully remove any drops or fluid remaining adherent to the wall of the tubes by inverting tubes over an absorbent paper towel for 30 min. Let air dry the tubes in upright position for at least 3 hr before proceeding.

Dissolve DNA by adding to each tube 20-50 µl of TE solution and place the tubes at 37^0C. Mix the samples of DNA with loading buffer by adding 10x loading buffer to a final concentration of 1x. The addition of loading buffer to samples allows to load gel wells more easily and to monitor the run of samples. Run the electrophoresis in standard TBE buffer after setting the voltage to the desired level. During electrophoresis it is possible to monitor the migration of samples by following the migration of bromophenol blue dye contained in the loading dye.

Stop the electrophoresis when the dye reaches about 3 cm from the end of the gel.

To visualize DNA, place the gel on a UV Transilluminator and take photos of the gel. Wear eye and skin protection when UV is on.

Results and Discussion

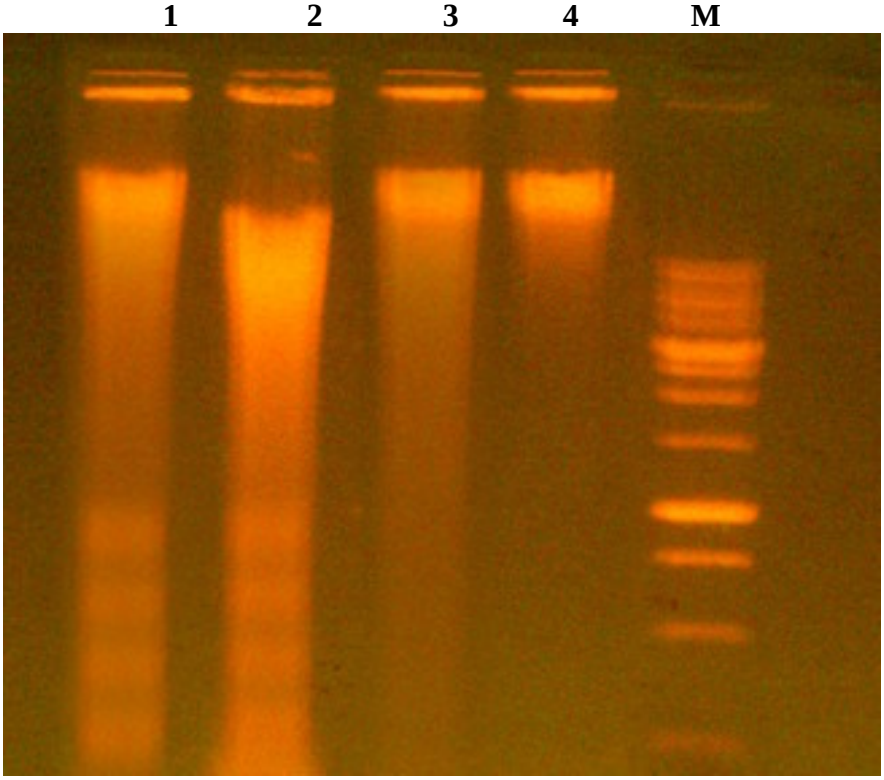
DNA Fragmentation of treated cells

The conventional agarose gel electrophoresis was performed on the cells treated with different concentration of plant extract for 48 hours. The result showed that inter nucleosomal DNA cleavage produced ladder pattern for the methanolic extract treated cells (Fig. 1). DNA fragmentation was detected. According to Walker (1998), cells and untreated cells could produce a discrete band from 700 to 1000 kbp, which was unrelated to apoptotic DNA cleavage, but attributed to the migration of any DNA fragment larger than 700 kbp (Walker, 1998). This indicates that the DNA might be cleaved after treatment but in a large number of base pair. The explanation also describes the presence of apoptotic bodies in the cell morphological study. This observation was also happened to the positive control cells treated with cisplatin. An extensive DNA fragmentation might be occurred which could not be detected in this study.

It was found that the methanolic extract could cause DNA degradation at 125 µg/ml. The fragmented DNA was observed around 5 to 8 kbp, which was smaller than the typical fragmentation of DNA at 20 to 300 kbp when entering early stage of apoptosis (Cohen,1992). According to Wyllie (1980), the biochemical hallmark of apoptosis is cleavage of the nuclear DNA into ~200 base pair multiples. This specific DNA cleavage is due to the activation of endogenous endonuclease that cleaves at the exposed linker regions between nucleosomes. It is worthy to highlight that necrosis was not happened in this study because it associates with the random form of DNA cleavage (Darling, 2000).

Foeniculum vulgare is well known for its medicinal therapeutic value, Therefore, the effect of *Foeniculum vulgare* extracts on cervical carcinoma cell line was studied. Methanolic extracts of the plant have different effects on DNA fragmentation.DNA fragmentation was found in the cells treated with the methanolic extract at around 200 Kbp.

Figure.1 DNA Fragmentation after treatment with *Foeniculum vulgare* extracts compared to the marker(m) values



Lane 1: 125µg/ml; Lane 2: 62.5µg/ml; Lane 3: 31.2µg/ml; Lane 4: Control
Lane M: Marker

In summary, our data provide experimental evidence for the first time that the methanolic extract of *Foeniculum vulgare* showed selective cytotoxic effects on cervical cancer cells, Furthermore, these extracts exerted their cytotoxic effects to eliminate cancerous cells via specific apoptotic cell death.

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References

Cohen, G.M., Sun, X.M., Snowden, R.T., Dinsdale, D., Skilleter, D.N. 1992. Key morphological features of apoptosis may occur in the absence of inter nucleosomal DNA fragmentation. *Biochemical J*., 286, 331-334.

Darling, J.L. 2000. Neuronal and glial tumours in vitro: An overview, in: Doyle, A., Griffiths,J.B. Eds., *Cell and tissue culture for medical research*. John Wiley and Sons Ltd, London, pp. 306 – 320.

Jansen, P. C. M. 1981 Spices, condiments and medicinal plants in Ethiopia, their taxonomy and agricultural significance. *Belmontia* 12, 20-29.

Newman, D. J., Cragg, G. M., Holbeck, S. and Sausville, E.A. 2002. Natural products and derivatives as leads to

cell cycle pathway targets in cancer chemotherapy.*Current Cancer Drug Targets.* 2, 279-308.

Puelo, M. A. 1980 Fennel and anise as estrogenic agent. *J. EthnopharmacoL* 2, 337-344.

Schwartsmann, G., Ratain, M. J., Cragg, G. M., Wong, J. E.,Saijo, N., Parkinson, D. R., Fujiwara, Y., Pazdur, R.,Newman, D. J., Dagher, R. and Di Leone, L. 2002.Anticancer drug discovery and development throughout the world. *Journal of Clinical Oncology*. 20, 47S-59S.

Walker, B.K., Lei, H., Kragg, S.S. 1998. A functional link between N-linked glycosilation and apoptosis in Chines Hamster Ovary cells. *Biochem. Biophys. Res. Comm.*, 250,264-270.

Wyllie, A.H. 1980. Glucocorticoid induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature,* 284, 555-556.