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The chemosensitizing effect of aqueous extract of sweet fennel on cisplatin treated HeLa cells

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Summary

Background: Cisplatin is an important chemotherapeutic agent that is widely used in treatment of several malignancies, but its side effects on normal tissues and organs limit its use. The aim of this study was to evaluate the effect of aqueous extract of sweet fennel alone and in combination with cisplatin on human cervical cancer adenocarcinoma cell line (HeLa cells) searching for an effective, inexpensive therapy with minimal side effects. *Materials and Methods:* HeLa cell line was used to study the cytotoxic effect of different concentrations of the aqueous extract of sweet fennel alone and in combination with 50 μg/ml cisplatin. Quantitative measure of drug interaction was quantified by the combination index. Gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) were used to analyze the sweet fennel decoction. MTT assay was used to examine cell viability percentage. Electron microscopy was applied to study the ultrastructure of the cells. *Results:* The phenyl propanoids (23%) and phenols (12%) constituted the highest percentage of the aqueous extract. Increasing the concentration of sweet fennel from 50 μg/ml to 80 μg/ml, decreased the percentage of the cell viability of HeLa cells from 86.74% to 78.28%, respectively. Further decrease to 11.31% was demonstrated when 50 μg/ml of fennel was combined with 50 μg/ml cisplatin (additive effect). In addition to the signs of apoptosis observed in HeLa cells at 50 μg/ml of fennel, disruption of both nuclear and cytoplasmic membranes and presence of autophagolysosomes were noticed at a dose of 80 μg/ml. Combination of 50 μg/ml of cisplatin with 60, 70, and 80 μg/ml of sweet fennel revealed no significant difference in comparison to cisplatin alone. The combination with 50 μg/ml of sweet fennel revealed marked vacuolization of the cytoplasm, fragmentation of the nucleus, and complete disruption of nuclear membrane. *Conclusion:* Combination of cisplatin and the 50 μg/ml of the fennel could enhance cervical cancer growth inhibition. This combination could be effective in lowering the dose of single or repeated cumulative courses of cisplatin and hence decreases its hazardous side effects. In vivo studies and the evaluation of different combination doses of cisplatin and sweet fennel are recommended.

Key words: HeLa cell line; Sweet fennel; Cisplatin; Ultrastructure; Cytotoxicity.

Introduction

Cervical carcinoma, although largely preventable, is one of the most prevalent cancers worldwide [1]. Several chemotherapeutic, cytotoxic, and immunomodulating agents are available to treat cancer. Besides being enormously expensive, these drugs are associated with serious side effects and morbidity [2].

Cisplatin (CP, cis-dichlorodiammine-platinum) is an important chemotherapeutic agent widely used in clinical treatment against several malignancies like ovarian, cervical, testicular, bladder, lung, gastric, and head and neck cancers. At the molecular level, it interacts with cellular proteins and lipids and forms DNA adducts causing cell cycle arrest in G2/M phase [3].

Ongoing search continues for an ideal treatment that has minimal side effects and is cost-effective. The present interest and widespread use of herbal remedies has created the possibility of interaction between them and pharmaceutical drugs if they are used simultaneously [2].

Foeniculum vulgare Mill. (F.vulgare) sweet fennel; a plant that belongs to the family Apiaceae (Umbelliferaceae) has been reported to contain 6.3% of moisture, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fibre, and 42.3% carbohydrates. The minerals and vitamins present in F. vulgare are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin, and vitamin C. The relative concentration of essential oil compounds varies considerably depending on the phonological state and origin of the fennel. The essential oil composition of F. vulgare exhibits considerable chemodiversity depending upon the method of extraction [4]

Sweet fennel (F. vulgare.) contains volatile oils (trans-anethole, thymol, fenchone, carvacrol, terpinene, P-thymene, and thymol methyl ether), phenolic glycosides, flavonoids, phytosterols, triterpines, and saponins [5, 6]. Fennel also contains minor amounts of polyacetylenes in non-polar extracts, which shows cytotoxicity against five different lymphoblastic cell lines [7].

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It is known that natural bioactive compounds may act in synergy with chemotherapeutics used in clinical treatment in programmed cell death induction. They also diminish the side effects of cytostatic drugs [8]. In order to find a natural and cost effective cytotoxic medication that may replace the hazardous chemotherapeutics or decrease their dose by combination, the aim of the present work was to evaluate the effect of the inexpensive medicinal plant, sweet fennel, either alone or in combination with cisplatin on HeLa cell line. This was applied by studying the cytotoxic effect using MTT assay and morphological pattern of the cells using electron microscope.

Materials and Methods

The study employed the use of the following: HeLa cell line (CCL-81), Dulbecco's modified Eagle's medium (DMEM), cisplatin 50 mg/100 ml) was prepared as a 3.3 mM stock solution in 0.9% saline daily as done previously [9], plant extract: sweet fennel was purchased from local market, cleaned and grinded, and MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

The following procedures were accomplished in the tissue culture unit at King Fahd Medical Research Center (KFMRC).

Preparation of aqueous extract of fennel

One hundred grams of clean, dried, and grinded seeds of sweet fennel were mixed with 1,000 ml of distilled water and boiled at 100°C for 30 minutes. The decoction was centrifuged, filtered, frozen, and then lyophilized [10]. Twenty µg/ml of the powder was dissolved in DMEM and supplemented with 10% fetal calf serum (FCS) before its addition to mono cell layer of HeLa cells for 24 hours.

Gas chromatography–mass spectrometry (GC-MS):

Analysis was performed using a GCMS-QP2010 plus and a DB-5 column (30 m × 0.25 mm i.d.; film thickness 1.00 µm) was included in the gas chromatograph system. The GC column temperature was programmed to rise from 100°C to 320°C at a rate of 4°C per minute and the total GC run time was 60 minutes. The inlet temperature was kept at 280°C and helium was used as a carrier gas at a constant flow rate of 39.0 cm per second. A sample of 1.0 µL was injected using the splitless mode and the mass conditions were as follows: ionization voltage: 70 eV; ion source temperature: 200°C; full scan mode in the m/z range 35–600 with a 0.20 s/scan velocity.

High-performance liquid chromatography (HPLC)

The combined diethyl ether layers were evaporated and the resultant residue was dissolved in HPLC grade MeOH to give 1.000 ppm. Twenty µl was injected into the HPLC YL 9100 system. Identification of the phenolic compounds in the sample was determined by comparing the retention times of known peaks. Column C/8, ultraviolet, (UV) 220 nm, Refractive Index (RF) = 1.

Culture of HeLa cells

HeLa cells were cultured in DMEM supplemented with 10% FCS, 100 µg ml^{-1} penicillin/ streptomycin. Cells were maintained in 5% CO2- 95% air at 37°C. HeLa cells were seeded in 96-well plates at 5 x 10^4 cells/well. Triplicate wells were prepared.

Experimental design:

The following groups were incubated for 24 hours: Group 1: cultured HeLa cells used as negative control; Group 2:cultured HeLa cells treated with cisplatin (50 µg/ml); Group 3: cultured HeLa cells treated with aqueous extract of sweet fennel (50, 60, 70 and 80 µg/ml); Group 4: cultured HeLa cells treated with Cisplatin and aqueous extract of fennel simultaneously.

Cytotoxicty Test (MTT assay)

The assay detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product, which reflects the normal function of mitochondria and cell viability [11]. HeLa cells were seeded in 96-well plates at 5 x 10^4 cells/well. The media on the cells was changed to the test solution of cisplatin 50 µg/ml. As renal toxicity is enhanced with repeated and cumulative doses of the drug and is decreased on using single course [12] so a single high dose of cisplatin was chosen for this trial. Based on previous literature, the IC50 of the methanolic extract of fennel seed on the MCF7 cancer breast cell line was 50 µg/ml [13]. Accordingly ascending doses of the sweet fennel were used.

Viable cells percentage was counted as: [Mean O.D of treated cells/Mean O.D of control cells] ×100% [13]. Monolayer cells were incubated either with cisplatin only or in combination with different doses of sweet fennel (50, 60, 70 ,and 80 µg/ml) for 24 hours in 37°C in 5% CO_2-95% air. Triplicate wells were prepared. Cells grown under the same conditions as non-treated were considered as control cells.

To assess cell viability, the absorbance at 570 nm was determined after 24-hour incubation. This was done using an ELISA reader and was compared with the control cultures.

Combination index

Determination of drug synergy or antagonism was quantified by the combination index (CI) [14]. CI provides a quantitative measure of the extent of drug interaction and the numerical value was calculated as described in the following equation:

$$CI = \frac{\text{Cisplatin \% + sweet fennel \%}}{\text{Cisplatin/sweet fennel \%}}$$

Cisplatin % and sweet fennel % = the mean percentage of dead cells after treatment with separate drugs, and cisplatin/fennel % after treatment with both drugs. CI = 1 indicates an additive effect, < 1 — synergy, while > 1 — antagonism.

Electron microscopic studies

Upon convergence of the cultured HeLa cells, cells were accessed with trypsin diluted in phosphate buffer saline (PBS) at a density of 200,000 cells per ml, and fixed in 2.5% glutaraldehyde at RT for at least two hours at 4ºC. Cells were centrifuged three times (1,500 grams, ten minutes) with 0.1 M fresh phosphate buffer and post-fixed in 1% osmium tetroxide in phosphate buffer for 30 minutes. Finally, cells were washed again and dehydrated in acetone and embedded in Epon. After sectioning, cells were contrasted with uranyl acetate for 30 minutes and lead citrate for five min [15]. The stained grids were examined and photographed with transmission electron microscope at 60 kV. Processing was done according to electron microscopic unit protocol in KFMRC at KAU.

Statistical analysis

Statistical analysis was performed by using SPSS program version 16 (IBM-USA). The one-way analysis of variance (ANOVA) test was used. When equal variance could be as-

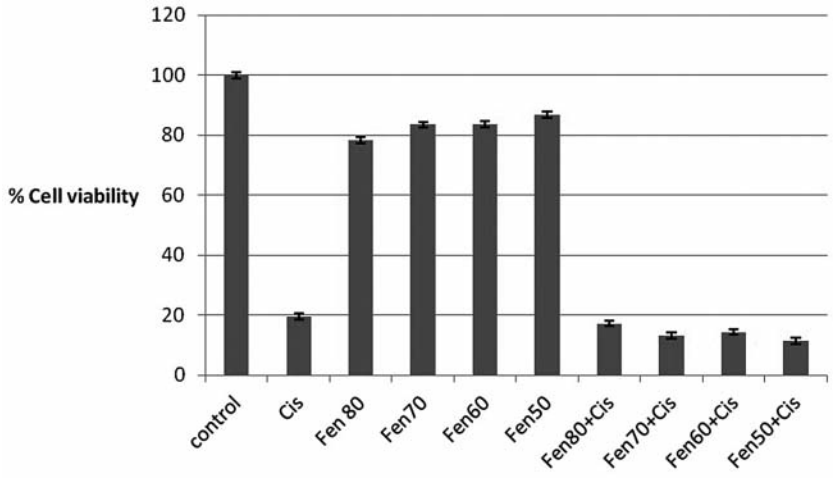

Figure 1. — Effect of cisplatin (cis) (50 ug/ml) and different concentrations of fennel extract (80, 70, 60, and 50 µg/ml) alone or in combination with cis on the cell viability of HeLa cells.

sumed, the LSD *t*-test was applied. Data was presented as mean ± standard deviation (SD). A $p < 0.05$ was considered statistically significant.

Results

Analysis of decoction of fennel

According to GC-MS spectroscopy and HPLC analysis, phenyl propanoids constituted the highest component. Other components are revealed in Table 1.

Table 1. — *Composition percentage of different chemical categories of the sweet fennel decoction.*

Chemical category	% composition
A-pinene	1.5
B-myrcene	0.6
Limonene	0.8
Phenyl propanoids	**23**
Hydrocarbons	0.1
Phenols	12
Remaining are impurities	

Cytotoxicty test

The results of MTT revealed that aqueous extract of sweet fennel, to a certain extent, was cytotoxic to HeLa cells compared to cisplatin. As the concentration of sweet fennel increased from 50 µg/ml to 80 µg/ml, the percentage of the cell viability of HeLa cells decreased from 86.74% to 78.28%, respectively. On the other hand, 50 µg/ml cisplatin resulted in only 19.59% cell viability of HeLa cells. There was a highly significant difference between the cytotoxic effect of cisplatin compared to the cytotoxic effect of sweet fennel in its different concentrations (Figure 1). Further decrease in cell viability of HeLa cells to 11.31% was demonstrated when 50 µg/ml of fennel was used in combination with 50 µg/ml cisplatin (Figure 1). This additive effect was proved by the combination index (CI) (Table 2). On the other hand combination of 80, 70, and 60 µg/ml of sweet fennel with cisplatin did not show any significant difference as compared to cisplatin alone.

Table 2. — *Combination index (CI) analysis of combined treatment of HeLa with sweet fennel and cisplatin for 24 hours. CI > 1 antagonism; CI < 1 synergism; CI = 1 additive effect.*

Conc. of cisplatin	Conc. of Fennel	CI
50 µg/ml	50 µg/ml	1.00
	60 µg/ml	1.24
	70 µg/ml	1.06
	80 µg/ml	1.21

Electron microscopic study

Control HeLa cells revealed intact plasma membrane and numerous microvilli on the cell surface. The nucleus had intact nuclear membrane and prominent nucleolus. Multiple mitochondria with evident cristae were also observed in the cytoplasm (Figure 2).

In group 2, the HeLa cells treated with 50 µg/ml cisplatin revealed disrupted nuclear membrane and karyorrhexed nucleus. Numerous autophagolysosomes were observed, some of which opened to the outside of the cell through disrupted cellular plasma membrane. Loss of microvilli on the cell surface was evident (Figure 3).

HeLa cells treated with 50 µg/ml of aqueous extract sweet fennel (group 3) revealed shrunken condensed cytoplasm. The nucleus had condensed chromatin and partly fragmented into smaller micronucleus, but with intact nuclear membrane. Multiple vacuoles and vesicles were noted. The intact cytoplasmic membrane revealed focal areas of blebbing (Figure 4). HeLa cells treated with 80

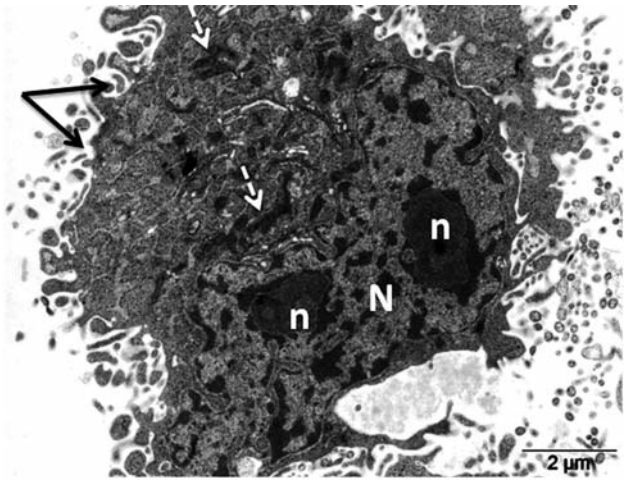

Figure 2. — An electron micrograph of HeLa cells from the control group showing intact plasma membrane and numerous microvilli (thin arrows) on the cell surface.The nucleus (N) has intact nuclear membrane and prominent nucleoli (n). The cytoplasm shows multiple mitochondria with evident cristae (dashed arrows).

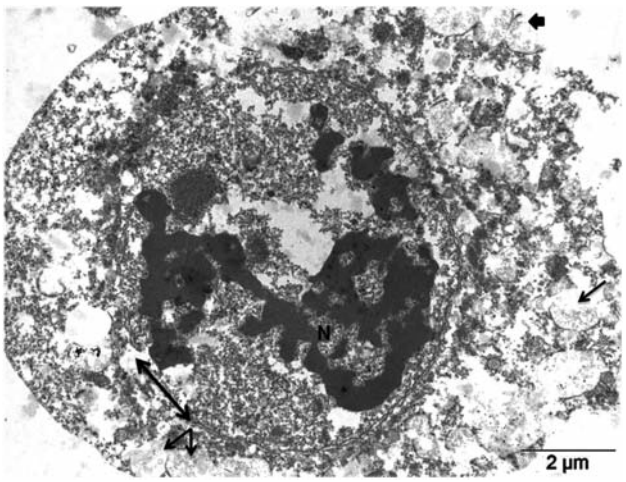

Figure 3. — An electron micrograph of HeLa cells treated with 50 µg/ml cisplatin showing disrupted nuclear membrane (double arrow), karyorrhexis nucleus (N), and numerous autophagolysosomes (thin arrows). Some opened to the outside of the cell through disrupted cellular plasma membrane (thick arrow). Loss of microvilli on the cell surface is noticed.

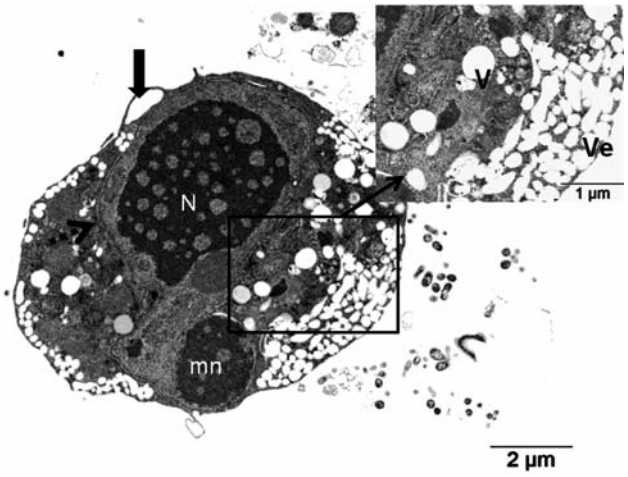

Figure 4. — An electron micrograph of HeLa cells treated with fennel (50 µg/ml) only showing shrunken condensed cytoplasm. The nucleus (N) shows condensed chromatin and partly fragmented into smaller micronucleus (mn) but with intact nuclear membrane (dashed arrow). Multiple vacuoles (V) and vesicles (Ve) are noted in the higher magnification. The intact cytoplasmic membrane shows focal areas of blebbing (thick arrow).

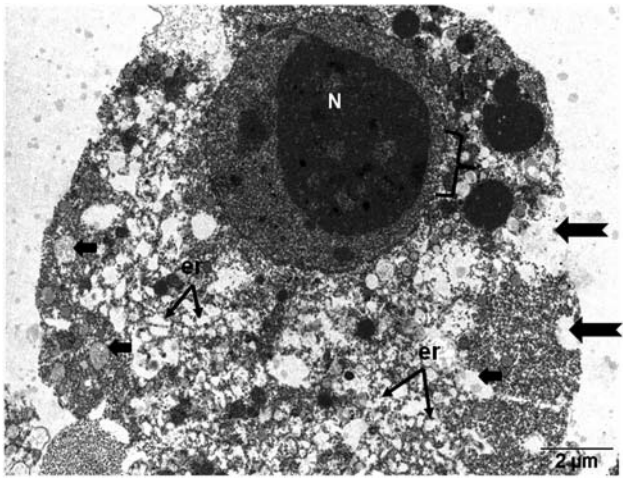

Figure 5. — An electron micrograph of HeLa cells treated with 80 µg/ml fennel showing condensed nuclear chromatin and focal areas of disintegrated nuclear envelope (brace). The cytoplasm reveals multiple autophagolysosomes (thick arrows) some of which opened to the outside of the cell via the disrupted cell membrane (notched arrows). Dilated cisternae of rough endoplasmic reticulum (er) occupy the cytoplasm.

µg/ml aqueous extract of sweet fennel showed condensed nuclear chromatin and focal areas of disintegrated nuclear envelope. Multiple autophagolysosomes were noted in the cytoplasm some of which opened to the outside of the cell via the disrupted cell membrane. Dilated cisternae of rough endoplasmic reticulum occupied the cytoplasm (Figure 5).

In group 4, HeLa cells treated with combination of 50 µg/ml cisplatin and 50 µg/ml of fennel revealed nuclear fragmentation with complete disruption of the nuclear envelope and presence of apoptotic bodies. Extensive vacuolation of the cytoplasm was manifested. Loss of villi was also noted (Figure 6).

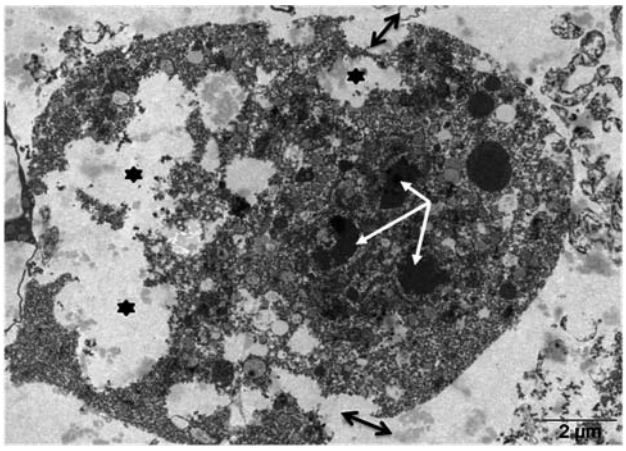

Figure 6. — An electron micrograph of HeLa cells treated with both 50 µg/ml cisplatin and 50 µg/ml of fennel showing nuclear fragmentation with complete disruption of the nuclear envelope and apoptotic bodies (white arrows), extensive vacuolation of the cytoplasm (✱), some of which open outside the cell (double-headed arrow).

Discussion

The clinical outcomes of cervical cancer patients treated with weekly cisplatin chemo-radiation therapy were based on pretreatment cisplatin in vitro chemo response testing. It was found that cisplatin chemosensitivity testing of pretreatment cervical biopsies correlated with clinical response and disease-free outcomes for patients with advanced stage cervical cancer treated with weekly cisplatin chemo-radiation therapy [16].

In the present study, the major constituents of the F. vulgaire (sweet fennel) were 23% phenyl propanoids and 12% phenols. The phenolic compounds present in F. vulgare were reported to be associated with the prevention of diseases thought to be induced by oxidative stress such as cardio vascular diseases, cancer, and inflammation [17]. Taking into account the numerous defensive roles of phenyl propanoids and their derivatives, these compounds are of great interest, especially for medicinal use as antioxidant, UV screens, anticancer, and anti-viral [18]. Phenyl propanoids also blocked the cell cycle and induced apoptosis in MCF-7 breast tumor cells [19].

In the present work the use of 50 µg/ml of aqueous extract of sweet fennel resulted in 86.74% HeLa cells viability. Whereas combining the same dose with 50 µg/ml of cisplatin resulted in 11.31% cell viability, which is lower than cisplatin alone 19.59%. On the other hand, combination of 80, 70, and 60 µg/ml of sweet fennel with cisplatin did not show any significant difference as compared to cisplatin alone.

As previously reported, the effect of herbs can be assessed by the inhibited cell growth, the loss of cell viability percentage, and inhibited biomarkers. The major subphysiological changes in the treatment of cancer cells with herbs are metabolic integrity loss, hypoxia, and oxygen starvation of tumor cells with more burden of rapid growth (proliferation). The herbal therapeutic effect presumably is competitive inhibition of available energy molecules to fast growing tumor cells. Moreover, the biochemical concept is mainly based on the blocking of the anaerobic glucose utility sites in glucose oxidation and tricarboxylic acid cycle (TCA cycle) of cancer cell. This augments the aerobic energy metabolism which leads to lactic dehydrogenase (LDH) inhibition and lactate accumulation. LDH plays an active role in development of malignancy as the enzyme LDH generates product NAD+ and two pyruvate molecules from one glucose [20].

The morphological study of HeLa cells revealed apoptotic changes on using 50 µg/ml of sweet fennel in addition to multiple autophagolysosomes on using 80 µg/ml. This was in accordance to previous researchers [21] who stated that compounds that block or suppress the proliferation of tumor cells by inducing apoptosis are considered potential antitumor agents.

Apoptosis can be induced by stresses acting upon specific organelles including nuclei, mitochondria, the endoplasmic reticulum, or lysosomes. In spite of the heterogeneity of potential cell death inducers, apoptosis is characterized by common morphological and biochemical alterations. This suggests the existence of interorganellar crosstalk [22].

In this study the autophagic vacuoles observed on using high doses of sweet fennel alone and in cisplatin treated HeLa cells were in association with disrupted nuclear membrane; this implies cell death. In fact, autophagy has been reported to initiate cell death in response to intracellular damage caused by hypoxia, chemotherapeutic agents, virus infection, or toxins [23].

Autophagy is a type of programmed cancer cell death as observed by other researchers [24]. They reported that it is caspase-independent cell death characterized by the accumulation of autophagic vacuoles in the cytoplasm accompanied by degradation of the Golgi apparatus and endoplasmic reticulum, preceding destruction of the nucleus. Therapeutically increased autophagy could represent an alternative way to destroy the cancer. It was reported that if autophagic activity is insufficient, long-lived proteins and defective organelles accumulate and apoptotic cell death occurs. In contrast, if autophagy destroys the cytosol and organelles beyond a certain threshold, autophagic cell death will occur, especially in apoptosis-insufficient cells [25].

It thus appears that aqueous extract of sweet fennel was cytotoxic to the HeLa cells alone. but its combination in high doses with cisplatin called for its antioxidant signaling. Accordingly, the cytotoxic effect of the combined therapy was almost similar to that of cisplatin alone. This was confirmed by the combination index results. This ex-

planation is in accordance with other studies that revealed that plant-derived phenyl propanoids as potent radiotherapeutic agents due to their relatively safe level of cytotoxicity [26]. It was also reported that these compounds render antioxidative activities involved in the oxidation process. They can play a role as reducing agents, proton donors, and metal chelating agents due to their high redox potential, thus providing antioxidant properties [27].

It was reported that the water and ethanol extract of fennel exhibited strong antioxidant activity; 100 grams of water and ethanol extracts exhibited 99.1% and 77.5% inhibition of peroxidation in linoleic acid system, respectively [28]. On the other hand, it was declared that radiosensitizing effects of these phytochemicals are thought to interact with several intracellular signaling molecules which then mediate signaling cascades including cell cycle arrest and cell death, while the radioprotective effect of those has been highly dependent on their antioxidant activities for protection against radiation-induced damage [29].

Conclusion

The cytotoxicity to HeLa cells was tested for various doses of aqueous extract of sweet fennel. It exhibited cytotoxic effect on HeLa cells as revealed by the apoptotic changes at low dose and signs of cell death at higher doses. However the cell viability of highest dose used was 78.28%. It has been observed that combination of cisplatin and the 50 µg/ml of the fennel could enhance cervical cancer growth inhibition where the cell viability was only 11.31%. This combination can be useful in lowering the dose of cisplatin and hence decreases the hazards to its side effects. Experimental study in vivo and the evaluation of different combination doses of cisplatin and sweet fennel are recommended.

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References

[1] DuPont, N.C., Monk B.J.: "Chemotherapy in the management of cervical carcinoma". *Clin. Adv. Hematol. Oncol.*, 2006, *4*, 279.

[2] Aggarwal B.B., Ichikawa H., Garodia P., Weerasinghe P., Sethi G., Bhatt I.D., *et al.*: "From traditional Ayurvedic medicine to modern medicine: identification of therapeutic targets for suppression of inflammation and cancer". *Expert Opin. Ther. Targets*, 2006, *10*, 87.

[3] Pabla N., Dong Z.: "Curtailing side effects in chemotherapy: a tale of PKCdelta in cisplatin treatment. *Oncotarget*, 2012, *3*, 107.

[4] Díaz-Maroto I.J., Vila-Lameiro P., Guchu E., Díaz-Maroto M.C.: "A comparison of the autecology of Quercus robur L.Q. pyrenaica Wild.: present habitat in Galicia, NW Spain". *Forestry*, 2007, *80*, 223.

[5] Park I.K., Shin S.C.: "Fumigant activity of plant essential oils and components from garlic (Allium sativum) and clove bud (Eugenia caryophyllata) oils against the Japanese termite (Reticulitermes speratus Kolbe)". *J. Agric. Food Chem.*, 2005, *53*, 4388.

[6] Council of Europe: "European Pharmacopoeia", 5th edition. EDQM, 2, 2005.

[7] Zidorn C., Jöhrer K., Ganzera M., Schubert B., Sigmund E.M., Mader J., *et al.*: "Polyacetylenes from the Apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities". *J. Agric. Food Chem.*, 2005, *53*, 2518.

[8] Jakubowicz-Gil J., Paduch R., Ulz Z., Badziul D., Głowniak K., Gawron A.: "Cell death in HeLa cells upon imperatorin and cisplatin treatment". *Folia Histochem. Cytobiol.*, 2012, *50*, 381.

[9] Hanigan M.H., Deng M., Zhang L., Taylor P.T. Jr., Lapus M.G.: "Stress response inhibits the nephrotoxicity of cisplatin". *Am. J. Physiol. Renal. Physiol.*, 2005, *288*, F125.

[10] Mohamed A.M., Metwally N.S.: "Antiaflatoxicogenic activities of some aqueous plant extracts against AFB1 induced renal and cardiac damage". *Journal of Pharmacology and Toxicology*, 2009, *4*, 1.

[11] Lau C.B., Ho C.Y., Kim C.F., Leung K.N., Fung K.P., Tse T.F., Chan H.H., *et al.*: "Cytotoxic activities of Coriolus versicolor (Yunzhi) extract on human leukemia and lymphoma cells by induction of apoptosis". *Life Sci.*, 2004, *75*, 797.

[12] Piccart M.J., Lamb H. and Vermorken J.B.: Current and future potential roles of the platinum drugs in the treatment of ovarian cancer. Annals of Oncology, 2001. 12: p. 1195-1203.

[13] Sul'ain M.D., Zazali K.E. and Ahmad N.: "Screening on anti-proliferative activity of psidium guajava leaves extract towards selected cancer cell lines". *Journal of US-China Medical Science*, 2012, *9*, 30.

[14] Bradford, M.M.: "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding". *Ana. Biochem.*, 1976, *72*, 248.

[15] De Castro Pazos M., Pacheco-Soares C., Soares da Silva N., DaMatta R.A., Pacheco M.T.: "Ultrastructural effects of two phthalocyanines in CHO-K1 and HeLa cells after laser irradiation". *Biocell.*, 2003, *27*, 301.

[16] Grigsby P.W., Zighelboim I., Powell M.A., Mutch D.G., Schwarz J.K.: "In vitro chemoresponse to cisplatin and outcomes in cervical cancer". *Gynecol Oncol.*, 2013, *130*, 188.

[17] De Marino S., Gala F., Borbone N., Zollo F., Vitalini S., Visioli F. *et al.*: "Phenolic glycosides from Foeniculum vulgare fruit and evaluation of antioxidative activity". *Phytochemistry*, 2007, *68*, 1805.

[18] Bermejo P., Abad M.J., Díaz A.M., Fernández L., De Santos J., Sanchez S., *et al.*: "Antiviral activity of seven iridoids, three saikosaponins and one phenylpropanoid glycoside extracted from Bupleurum rigidum and Scrophularia scorodonia". *Planta Med.*, 2002, *68*, 106.

[19] Pozo-Guisado E., Merino J.M., Mulero-Navarro S., Lorenzo-Benayas M.J., Centeno F., Alvarez-Barrientos A., *et al.*: "Resveratrol-induced apoptosis in MCF-7 human breast cancer cells involves a caspase-independent mechanism with downregulation of Bcl-2 and NF-kappaB". *Int. J. Cancer*, 2005, *115*, 74.

[20] Sharma R.: "Recommendations on herbs and herbal formula in cancer prevention". *The Open Nutraceutica*, 2010, *3*, 129.

[21] Frankfurt O.S. Krishan A.: "Apoptosis-based drug screening and detection of selective toxicity to cancer cells". *Anticancer Drugs*, 2003, *14*, 555.

[22] Ferri, K.F., Kroemer G.: "Organelle-specific initiation of cell death pathways". *Nat. Cell. Biol.*, 2001, *3*, E255.

[23] Kondo Y., Kanzawa T., Sawaya R., Kondo S.: "The role of autophagy in cancer development and response to therapy". *Nat. Rev. Cancer*, 2005, *5*, 726.

[24] Lefranc, F., Facchini V., Kiss R.: "Proautophagic drugs: a novel means to combat apoptosis-resistant cancers, with a special emphasis on glioblastomas". *Oncologist*, 2007, *12*, 1395.

[25] Nishida, K., Yamaguchi O., Otsu K.: "Crosstalk between autophagy and apoptosis in heart disease". *Circ. Res.*, 2008, *103*, 343.
[26] Kim W., Seong K.M., Youn B.: "Phenylpropanoids in radioregulation: double edged sword". *Exp. Mol. Med.*, 2011, *43*, 323.
[27] Javvadi P., Segan A.T., Tuttle S.W., Koumenis C.: "The chemopreventive agent curcumin is a potent radiosensitizer of human cervical tumor cells via increased reactive oxygen species production and overactivation of the mitogen-activated protein kinase pathway". *Mol. Pharmacol.*, 2008, *73*, 1491.
[28] Oktay, M., Gülçin İ., Küfrevioğlu Ö.İ.: "Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts". *LWT - Food Science and Technology*, 2003, *36*, 263.
[29] Tsao, R., Deng Z.: "Separation procedures for naturally occurring antioxidant phytochemicals". *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, 2004, *812*, 85.

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