ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Anti carcinogenic activity of Methanolic Extract of Fennel Seeds (*Foeniculum vulgare*) against breast, colon, and liver cancer cells.

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Manuscript Info

Manuscript History:

Received: 15 March 2015
Final Accepted: 19 April 2015
Published Online: May 2015

Key words:

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Abstract

Cancer is a disease of gene disorder that occurs in the normal processes of cell division. Undesirable side effects of chemotherapy and surgery have triggered the search of new compounds from plant to fight cancer because they are relatively safer than synthetic drugs. Apoptosis is a process of internally programmed cell death. It plays important roles in cancer development and treatment, thus the ability of bioactive compounds to increase the sensitivity of cancerous cells towards cellular damage and activate the apoptotic response is the most desirable. *Foeniculum vulgare* has been used as folk medicine for various ailments. Foeniculum vulgare, one of the most common use in Egyptian kitchen as a spice and beverage as well as in traditional medicine for its estrogenic, lactagouge, diuretic, antioxidant, immune booster and its usefulness in dyspepsia. That makes studies of their anticarcinogenic very essential. It 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 *Foeniculum vulgare* are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C. Therefore, cancer prevention effects using anticarcinogenicity, showed clear variation between different treated and non treated cancer cells. *Foeniculum vulgare* has various biological activities such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective and antimelanogenesis effects. To study the anticarcinogenic activity of *Foeniculum vulgare* in MCF-7 (Human breast cancer cell line), HePG-2 (human hepatocellular carcinoma cell line), and HCT 116(colon carcinoma cell line). *Foeniculum vulgare* extracts were prepared by Soxhlet extraction method using methanol as a solvent. The cytotoxicity activity of the plant extracts was determined using sulphodiamine-B assay (SRB assay). . Detremenation of proteins in cancer cell lines by SDS polyacylamide gel electrophoresis. DNA fragmentation induced by the plant extracts was evaluated through DNA extraction using agarose gel electrophoresis. RNA extraction to evaluate the expression of genes P53 and Bax genes using Semi-quantitave RT-PCR analysis. **Results & Discussion:** SRB assay results showed that MCF7 cells were inhibited by all the extracts with IC50 24.5±.08 while IC50 of HEPG-2 is 28.7±.04 and IC50 of HCT 116 is 59.8±.09µg/ml. SDS polyacylamide gel electrophoresis showed clear variation between different classes of treated and nontreated cancer cells. DNA extracted from treated cells showed fragmentation of the DNA was observed between treated and non treated (control) cancer cells

suggesting the occurrence of apoptosis. RT-PCR analysis showed increase in expression of genes P53 and Bax genes.

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INTRODUCTION

Cancer is one of the most life threatening diseases in which deregulating proliferation of abnormal cells invades and disrupts surrounding tissues**. Gennari *et al*., 2007.** There is evidence of cancer found in ancient human remains and in the medical literature since antiquity, dating back to the times of the Pharaohs in ancient Egypt and the classical world. Natural products play a relevant role in cancer therapy today with substantial numbers of anticancer agents used in the clinic being either natural or derived from natural products from various sources such as plants, animals and microorganisms (also of marine origin).

During the last few years, natural-product based drug discovery is increasing based on new technologies, such as combinatorial synthesis and high-throughput screening, and their associated approaches. **Stefania, et al., 2009.** Cancer occurs due to excessive free radical damage which ultimately causes damage to the genetic material DNA, protein and lipids. **Roszkowski et al., 2014**. Apoptosis is the major form of programmed cell death which takes place in all the cells to maintain homeostasis and cellular integrity. Cancer treatment therapies target this apoptotic pathway by increasing apoptosis in cells and thus preventing cancer**. Zhao et al., 2014.** It constitutes serious public health problems in both developed and developing countries. The limited success of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in treatment of cancer, indicates that there is an imperative need of alternative strategies in cancer management. **Dai et al., 2010.** The use of traditional medicine is widespread and plants provide a large source of natural antioxidants that might serve as leads for the development of novel drugs. Therefore, investigations of natural antioxidants and bioactive compounds for preservation of traditional medicines and use in treating certain human diseases have received much attention. **Lin YW, et al., 2010.**

Fennel (*Foeniculum vulgare*) is a small green brown seed belonging to the family Umbelliferae. It grows in the Mediterranean region and western Asia. In folk medicine, fennel seeds alone and in preparations are used to cure various disorders, acting as a carminative, lactagogue. and diuretic agent and used to treat respiratory and gastrointestinal problems. **Krizman et al., 2007**.

Fennel essential oil is used in cosmetics, pharmaceuticals, and perfumery and as a food additive. **Flav Frag J. 1999**. Fennel seeds are a potential source of powerful natural antioxidants, such as vitamins E and C, phenolic compounds, and oleoresins, and they display antioxidant properties in foods and biological systems. **Krizman et al., 2006.** Fennel essential oil has a potent hepatic-protective action against carbon tetrachloride–induced hepatic damage in rats. **O¨ zbek et al., 2003**. Various bark extracts of Fennel have also been reported to possess antifungal activity against Candida albicans **Pai et al., 2010.** The essential oil of Fennel has been reported to show hypoglycaemic activity in Streptozotocin induced diabetic rats.This makes the possibility of its inclusion in antidiabetic drug industry **El-Soud et al., 2011**. Oral administration (200 mg/kg) of *Foeniculum vulgare* fruit methanolic extract has been reported to show inhibitory effects against acute and subacute inflammatory diseases. **Choi and Hwang, 2004.** In recent years, estragole has become the subject of several research due to its being the major compound in fennel this volatile compound could possess potential carcinogenic properties. **Raffo et al.,2011.** *Foeniculum vulgare* has antimicrobial properties of the essential oil have been recognized. **Kawther FA. 2007.** Fennel has been reported to show radical scavenging and antioxidant activity. **Oktay M, et al., 2003**. Analgesic and anti inflammmatory property have also been reported. **Rosemayre et al., 2005.** It has also bronchodilatory effects and a depressive action on arterial blood pressure **Boskabady et al., 2004,** that may be due to fennel extract which acts mainly as a diuretic and a natriuretic **El Bardai et al., 2001.** It is widely used for dyspeptic complaints such as mild, spasmodic gastric-intestinal complaints, bloating and flatulence. **Alexandrovich et al., 2003.** Different studies had shown that the extract of *Foeniculum vulgare* is effective in the treatment of colic in breastfed infant. **Savino et al., 2005.**

Fennel contains minor amounts of polyacetylenes in nonpolar extracts, which shows cytotoxicity against five different lymphoblastic cell lines **Zidorn et al., 2005** .Fennel seeds are eaten raw, sometimes with some sweetener

to improve eyesight. Extracts of fennel seeds have been shown in animal studies to have a potential use in the treatment of glaucoma, as a diuretic and a potential drug for the treatment of hypertension. It has been used as a galactagogue improving the milk supply of a breast feeding mother. This is suggested to be due to the presence of phytoestrogens present in fennel which promote growth of breast tissue **Agarwal et al., 2008**.

Fennel 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. **Parejo, et al., 2004**.

The present study evaluated the efficacy of fennel methanolic extract as an agent with an antitumor activity in cancer cells.

2. Materials and methods:

2.1. Preparation of methanolic fennel extract.

Extracts were prepared by soxhlet apparatus for 10 hours according to the Association of Offecial Analytical Chemists **(AOAC, 1970)** procedure using methanol as a solvent fifty grams of dried sample were poured into a 200 ml of methanol . The heat given to the solvent was just to evaporate it slowly. Then crude extract was left at room temperature to dry.

2.2. Cell lines and culture maintenance.

Human breast cancer cell line (MCF-7), human hepatocellular carcinoma cell line (HePG-2), and colon carcinoma cell line (HCT116) were obtained from VACSERA - Cell Culture Unit, Cairo, Egypt. This cell line originally obtained from the American Type Culture Collection (ATCC). The cell count was done and the cell viability was tested by trypan blue using haemocytometer. Cells were cultured in RPMI medium (Gibco, Glasgow, UK) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, 1% non-essential amino acids in tissue culture flasks and incubated in a CO2 incubator in a 5% CO2 and 95% humidity atmosphere. Once the cells attained confluent growth, the cells were trypsinized using Trypsin-EDTA (PAA) for carrying out various assays. **Alan, 1998; Jennie, 1998**

2.3. Measurements cytotoxicity by sulphodiamine-B assay(SRB)

The cytotoxic assay was performed at the Center of Genetic Engineering at Al-Azhar university , using the sulforhodamine B assay. **Vichai, et al., 2006.**The stock cells maintained in 75 cm2 polystyrene flasks (Falcon) with minimal essential medium containing 10% fetal bovine serum, penicillin 100 IU/mL and streptomycin 100 lg/mL. in a humidified atmosphere of 5% CO2 at 37 ^{O}C. The cells were dissociated with 0.2% trypsin (Sigma) in phosphate-buffered saline (PBS) solution (Sigma). Cell lines in exponential growth phase were washed with PBS solution and trypsinized and resuspended in complete culture media. Cells were equal to 20.000 cells /well and were plated in 96-well plates for 24 hours before treatment to allow attachment of the cells to the wall of the plate. After incubation, the cells were exposed to various concentrations. The plant extracts were added to the cells at serial concentrations of 100,10,1,0.1 and 0.01 µg /ml. The control well received only maintenance of medium. Triplicate wells were prepared for each individual dose. The monolayer cells in the plates were incubated at 37^{o}C in a humidified incubator with 5% CO2 for 48 hours, the cells were fixed using 10% trichloroacetic acid washed, and stained with sulforhodamine B stain. Excess stain was washed off with acetic acid, and the attached stain was recovered with 50 µl/well of 10 mM tris base (PH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well will be measured spectrophotometrically at 570 nm with an ELISA microplate reader. The relationship between the surviving fraction and the drug concentration was plotted to determine The 50% inibitory concentrations (IC50) and calculated using sigmoidal concentration response curve fitting models (Sigmaplot software).

2.4. SDS polyacylamide Gel Electrophoresis. Laemmli, 1970; Stegemann et al., 1988.

The cells were precipitated and lysed directly utilizing 150 µl of lysis buffer and heated for 10 min at 100oC. Each sample was separated in 12% SDS polyacylamide gel.The separating gel was inserting between the glass plates and allows polymerizing for 15- 30 min at room temperature.

The stacking gel was inserting on the top of separating gel and comb was inserted to form wells. After polymerization, the comb was removed wisely to avoid tearing the edges of the wells before polymerization. The slab gel apparatus chamber was filled with running buffer.15µl of the prepared samples were loaded into each well. The power supply was connected to the gel electrophoresis tank at a constant volt (100-volt) till the tracking dye reach the bottom of the gel and the current was switched off. The gel was removed by prying the glass plated apart gently, and then the separated protein gel was stained by using comassie blue stain over night.

After gel staining, the gel was transferred to destaining solution which changed several times , the destaining solution removed the background dye from the gel leaving stained proteins as visible blue bands. The protein pattern in terms of molecular weight, band matching and band density was analyzed using Gel documentation system.

2.5. Determination of DNA fragmentation according kit of promega (England).

DNA damage was analyzed by 1% agarose gel. Cancer cells were placed in a flask at a concentration of 5 × 105cell/mL. The cells were treated with IC50 concentration and incubated for 48 h. The DNA was isolated and electrophoretically analyzed on 1% agarose gel containing 10 µL ethidium bromide. **Lerman and Frisch, 1982; Lumpkin and Zimm, 1982; Stellwagen, 1983.**

2.6. RNA Extraction from HePG-2, HCT 116 and MCF7 Cell line

Cancer cells were placed in a flask at a concentration of 5 × 10^5cell/mL. The cells were treated with IC50 concentration of selected fraction with positive control and incubated for 48 h. - 1ml trizol was added to the cells pellet. The cells pellet was layside with trizol by vortex. The cells were incubated for 5min. at R.T. and 200µl of chloroform was added .Vortex was performed for 15 sec. and cells were incubated at R.T. for 3min. The cells were spin at 13000 rpm. For 15 min. at 4°C. Upper aqueous phase -colorless- containing RNA was transferred in new tube and equal volume of ice cold Isopropanol was added. The tube was vortexed briefly 3 sec. The tube was incubated at R.T. for 10min & Spin 13000 rpm at 10 min at 4oC.The pellet was washed with 1ml cold 75% Ethanol & spin7500 rpm. 10 min. The pellet was dissolved in 20µl DEPC water. Store at -80°C. The RNA was isolated and electrophoretically analyzed on 1% agarose gel containing 10 µL ethidium bromide. . **Lerman and Frisch, 1982; Lumpkin and Zimm, 1982; Stellwagen, 1983.**

2.7. RT-PCR (semiquantitative reverse transcriptase polymerase chain reaction)

RT-PCR reaction was done using two steps RT-PCR Kit RT-PCR program started while the PCR tubes still in ice bath. Annealing temperature is 55°C for all genes. The numbers of cycles were 35 cycles. After PCR amplification.10 µl of samples was loaded in the gel to detect the precise size of PCR products. The gel was running at 100 volts for 20-30 min. using submarine unit.

1. Results

The present work aimed to evaluate of anticancer effect of *Foeniculum vulgare*. The evaluation parameters include cell viability, protein analysis, DNA damage and analysis of expression of some relevant genes by RT – PCR.

1.1. Morphological changes

Morphological changes were obvious where treated cells started showing gradual cell shrinkage, cell rounding and detaching from the surface of tissue culture flasks, finally followed by cell swalling and rupture (the morphological features give an indicator of cell apoptosis).

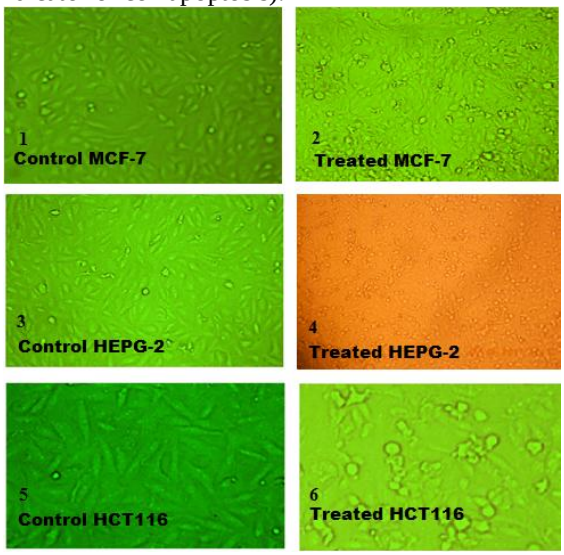

Figure (1): (1)MCF-7 cells (control) , (2) MCF-7 cells treated with *Foeniculum vulgare*, (3) HEPG-2 cells (control) , (4) HEPG-2 cells treated with *Foeniculum vulgare*, (5) HCT116 cells (control) and (6) HCT116 cells treated with *Foeniculum vulgare*

1.2. Measurement of cytotoxicity to cells by (SRB assay)

The survival fraction (% viability) was used as indicator for cell cytotoxcity HEPG-2,MCF-7,HCT116 cells were treated with different serial concentrations of *Foeniculum vulgare* extracts for 48 hrs . Surviving fraction at each concentration and The inhibition concentration (**IC50)** which is the concentration of treatment was determined for the evaluate treatment and calculated by using sigma plot program, were calculated in tables (1).

Table (1): The surviving fraction (% viability) of HEPG-2, MCF 7, HCT 116 cells post treatment with different concentrations of *Foeniculum vulgare*.

Concentrations µg/ml	Surviving fraction%± SE		
	HEPG-2	MCF-7	HCT116
0.00 (control)	100	100	100
0.01	97.2±0.05	88.1±0.01	93.3±0.02
0.1	92.7±0.1	87.9±0.03	91.5±0.05
1	87.5±0.05	86.5±0.09	91.3±0.04
10	85.9±0.09	82.1±0.1	87.5±0.08
100	10.8±0.2	8.31±0.06	37.0±0.03
(IC50)	28.7±.04	24.5±.08	59.8±.09

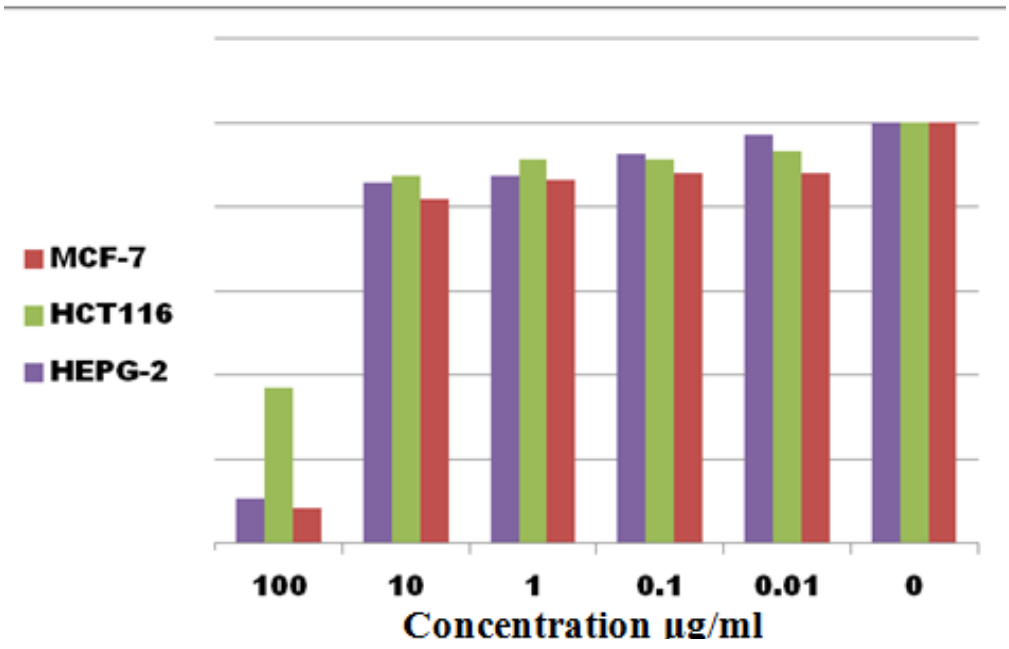

Figure (2) The surviving fraction (% viability) of HEPG-2, MCF 7, HCT116 cells post treatment with different concentrations of *Foeniculum vulgare*.

2.3.SDS polyacylamide Gel Electrophoresis.

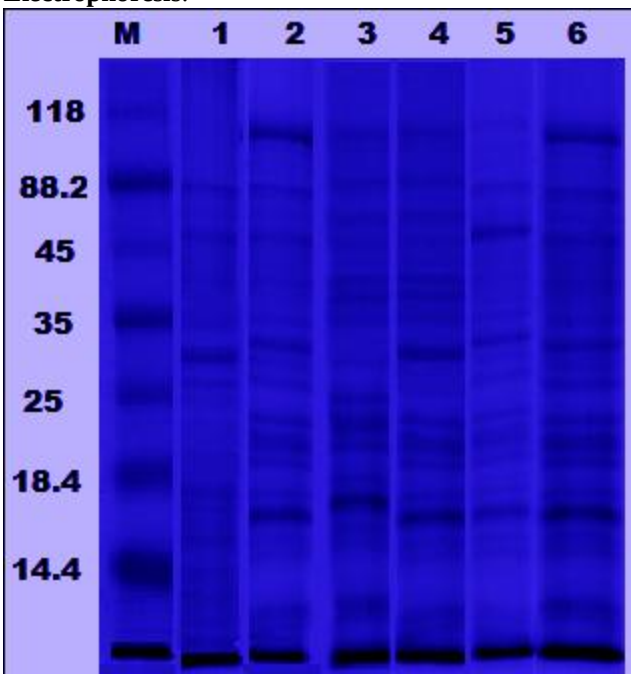

Figure (3): Effect of *Foeniculum vulgare* on protein pattern.

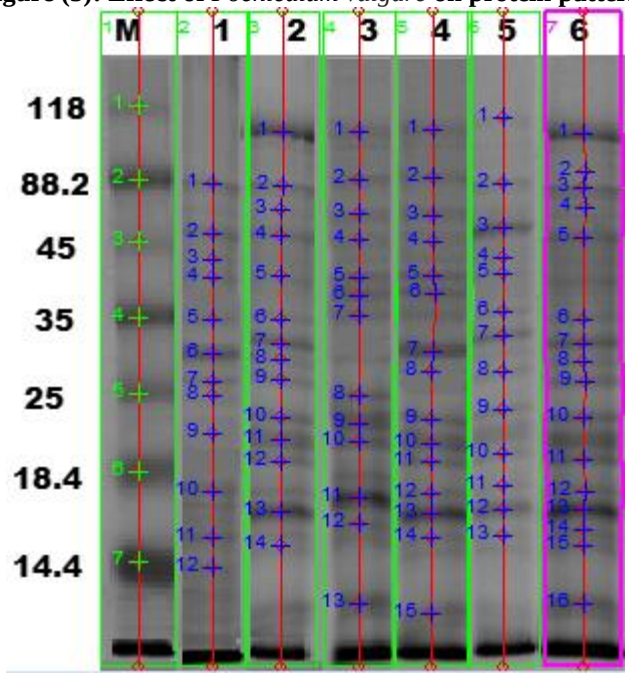

Figure (4) : Sodium Dodecyl Sulphate polyacrylamide Gel electrophoresis (SDS-PAGE) of total proteins in HEPG-2, MCF-7 and HCT116 cells line treated for 48 hrs. (M) marker , (1) non treated MCF-7 cells (control) , (2) MCF-7 cells treated with *Foeniculum vulgare*, (3) non treated HEPG-2 cells (control) , (4) HEPG-2 cells treated with *Foeniculum vulgare* , (5) non treated HCT116 cells (control) and (7) HCT116 cells treated with *Foeniculum vulgare*.

The results of scanning of the SDS-PAGE (SDS-poly acrylamide gel electrophoresis) of HEPG-2, MCF-7 and HCT116 cells in response to the treatment with *Foeniculum vulgare* were expressed as variations in molecular

weights (Mw), number of bands, bands area, bands distance , bands width and bands highest were recorded in tables (2,3 and 4) .

Table (2): Show distance, width, height, area, and molecular weight of bands of non treated MCF-7 cells (control) and treated with *Foeniculum vulgare*.

	Control HEPG-2					**Treated** HEPG-2				
Bands	**Dist**	**Width**	**Height**	**Area**	**Mol. weight**	**Dist**	**Width**	**Height**	**Area**	**Mol. weight**
1	**62**	**12**	**41**	**431**	**107.53**	**61**	**11**	**49**	**499**	**108.34**
2	**86**	**23**	**39**	**696**	**88.2**	**85**	**18**	**44**	**653**	**89.01**
3	**103**	**12**	**38**	**376**	**64.51**	**104**	**19**	**42**	**693**	**63.12**
4	**116**	**16**	**37**	**537**	**46.39**	**117**	**9**	**41**	**334**	**45**
5	**135**	**10**	**47**	**401**	**40.26**	**134**	**11**	**46**	**434**	**40.53**
6	**144**	**10**	**50**	**425**	**37.89**	**143**	**10**	**40**	**341**	**38.16**
7	**154**	**15**	**41**	**532**	**35.26**	**172**	**15**	**59**	**656**	**30.53**
8	**194**	**16**	**48**	**534**	**24.83**	**182**	**12**	**34**	**374**	**27.89**
9	**208**	**14**	**58**	**706**	**22.46**	**206**	**14**	**48**	**492**	**22.8**
10	**217**	**11**	**50**	**473**	**20.94**	**218**	**16**	**51**	**703**	**20.77**
11	**245**	**19**	**74**	**1033**	**17.24**	**227**	**10**	**45**	**363**	**19.25**
12	**258**	**10**	**46**	**423**	**16.09**	**243**	**11**	**44**	**416**	**17.42**
13	**298**	**19**	**54**	**822**	**11.81**	**253**	**17**	**71**	**845**	**16.53**
14	**-**					**265**	**7**	**37**	**251**	**15.47**
15	**-**					**303**	**18**	**44**	**753**	**10.5**

Table (3): Show distance, width, height, area, and molecular weight of bands of non treated HEPG-2 cells (control) and treated with *Foeniculum vulgare*.

	Control MCF-7					**Treated MCF-7**				
Bands	**Dist**	**Width**	**Height**	**Area**	**Mol. weight**	**Dist**	**Width**	**Height**	**Area**	**Mol. weight**
1	**88**	**7**	**35**	**203**	**85.41**	**62**	**18**	**69**	**833**	**107.53**
2	**113**	**15**	**36**	**456**	**50.57**	**89**	**9**	**40**	**274**	**84.02**
3	**126**	**10**	**32**	**295**	**42.63**	**101**	**13**	**29**	**346**	**67.3**
4	**135**	**11**	**34**	**337**	**40.26**	**114**	**11**	**39**	**378**	**49.18**
5	**156**	**12**	**39**	**347**	**34.74**	**134**	**27**	**34**	**846**	**40.53**
6	**173**	**18**	**54**	**633**	**30.26**	**156**	**9**	**34**	**267**	**34.74**
7	**187**	**9**	**35**	**272**	**26.58**	**168**	**14**	**48**	**497**	**31.58**
8	**194**	**12**	**29**	**302**	**24.83**	**176**	**10**	**33**	**309**	**29.47**
9	**213**	**12**	**29**	**314**	**21.62**	**186**	**13**	**35**	**341**	**26.84**
10	**242**	**7**	**42**	**270**	**17.51**	**205**	**17**	**45**	**496**	**22.97**
11	**265**	**9**	**44**	**347**	**15.47**	**216**	**15**	**49**	**633**	**21.11**
12	**280**	**8**	**41**	**294**	**13.8**	**227**	**11**	**43**	**378**	**19.25**
13	**-**					**252**	**19**	**72**	**946**	**16.62**
14	**-**					**269**	**11**	**33**	**325**	**15.11**

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Table (4): Show distance, width, height, area, and molecular weight of bands of non treated HCT116 cells (control) and treated with *Foeniculum vulgare*.

2.4. DNA Fragmentation

DNA fragmentation is a characteristic feature of apoptosis. Increased DNA fragmentation was apparent in MCF-7 , HEPG-2 and CHT 116cells after treatment with IC50values of *Foeniculum vulgare* for 48 h. (Fig. 5).

	Control HCT116					**Treated** HCT116				
Bands	**Dist**	**Width**	**Height**	**Area**	**Mol. weight**	**Dist**	**Width**	**Height**	**Area**	**Mol. weight**
1	**55**	**8**	**26**	**174**	**113.17**	**63**	**13**	**34**	**263**	**106.72**
2	**88**	**10**	**26**	**197**	**85.41**	**82**	**9**	**0**	**0**	**91.42**
3	**110**	**16**	**50**	**515**	**54.75**	**90**	**11**	**15**	**80**	**82.63**
4	**125**	**7**	**25**	**155**	**42.89**	**100**	**7**	**3**	**11**	**68.69**
5	**133**	**10**	**25**	**221**	**40.79**	**115**	**38**	**13**	**188**	**47.79**
6	**152**	**7**	**25**	**152**	**35.79**	**156**	**9**	**5**	**10**	**34.74**
7	**164**	**13**	**36**	**319**	**32.63**	**168**	**11**	**16**	**76**	**31.58**
8	**182**	**12**	**18**	**163**	**27.89**	**177**	**10**	**2**	**6**	**29.21**
9	**201**	**13**	**24**	**208**	**23.65**	**187**	**11**	**12**	**46**	**26.58**
10	**223**	**11**	**23**	**180**	**19.92**	**205**	**11**	**17**	**95**	**22.97**
11	**239**	**8**	**18**	**114**	**17.78**	**226**	**23**	**7**	**160**	**19.42**
12	**251**	**18**	**44**	**467**	**16.71**	**242**	**8**	**6**	**28**	**17.51**
13	**264**	**16**	**20**	**286**	**15.56**	**251**	**15**	**33**	**245**	**16.71**
14	**-**					**261**	**7**	**4**	**18**	**15.82**
15	**-**					**269**	**12**	**4**	**19**	**15.11**
16	**-**					**298**	**12**	**25**	**199**	**11.21**

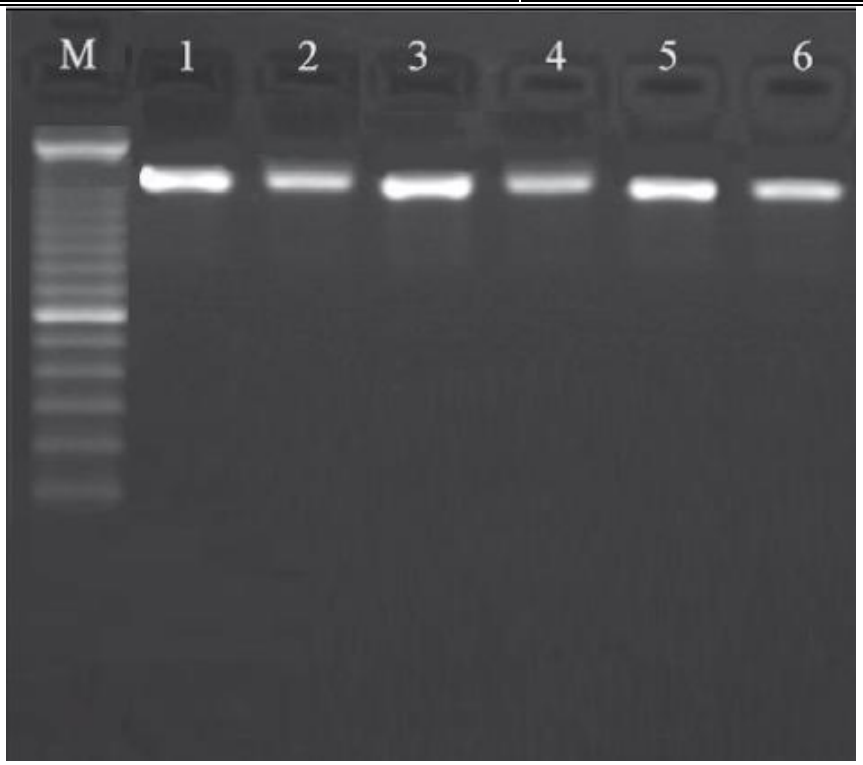

Figure (5) DNA fragmentation (M) Marker (1) non treated MCF-7 cells (control) , (2) MCF-7 cells treated with *Foeniculum vulgare*, (3) non treated HEPG-2 cells (control) , (4) HEPG-2 cells treated with *Foeniculum vulgare* , (5) non treated HCT116 cells (control) and (7) HCT116 cells treated with *Foeniculum vulgare*.

2.5. **Semi-quantitave RT-PCR analysis**

The expression profile of MCF-7, HEP-2 and HCT 116 cells treated with IC50 values of *Foeniculum vulgare* for 48 h. was examined by RT-PCR. RNA was extracted from the cells and the expression of genes; (P53 and Bax genes) were detected using semiquantitive analysis RT- PCR (Figure 6, 7).

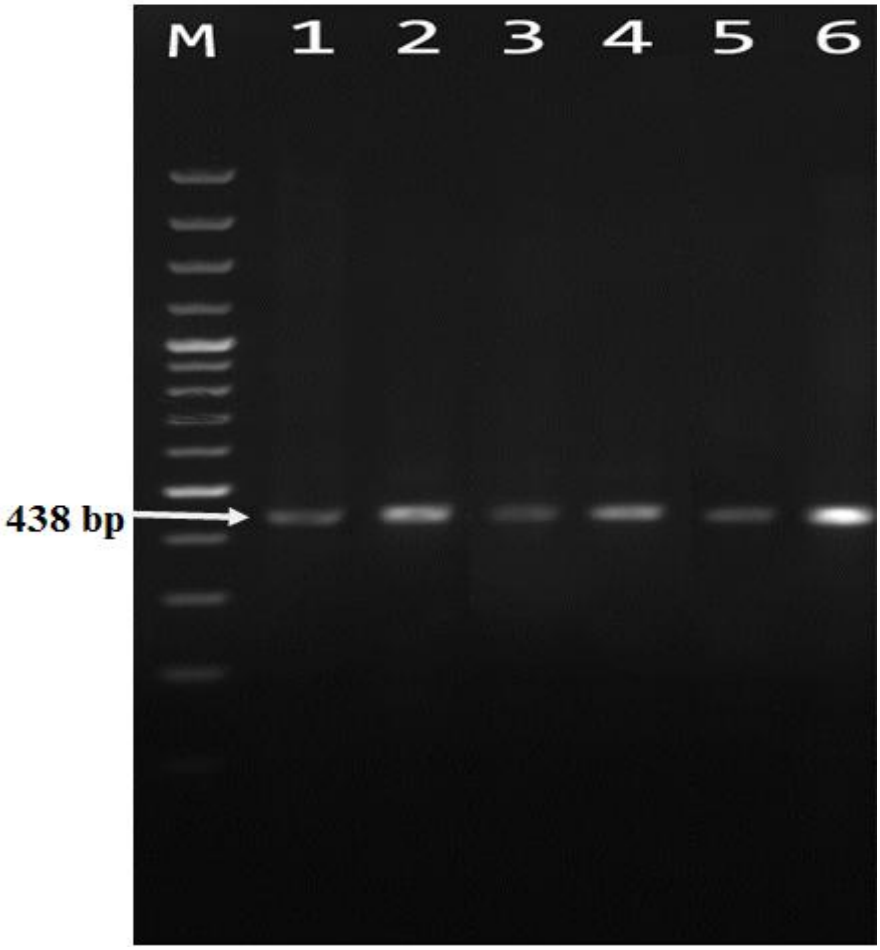

Figure (6) Expression level of P53 gene (M) Marker (1) non treated MCF-7 cells (control) , (2) MCF-7 cells treated with *Foeniculum vulgare*, (3) non treated HEPG-2 cells (control) , (4) HEPG-2 cells treated with *Foeniculum vulgare* , (5) non treated HCT116 cells (control) and (7) HCT116 cells treated with *Foeniculum vulgare*.

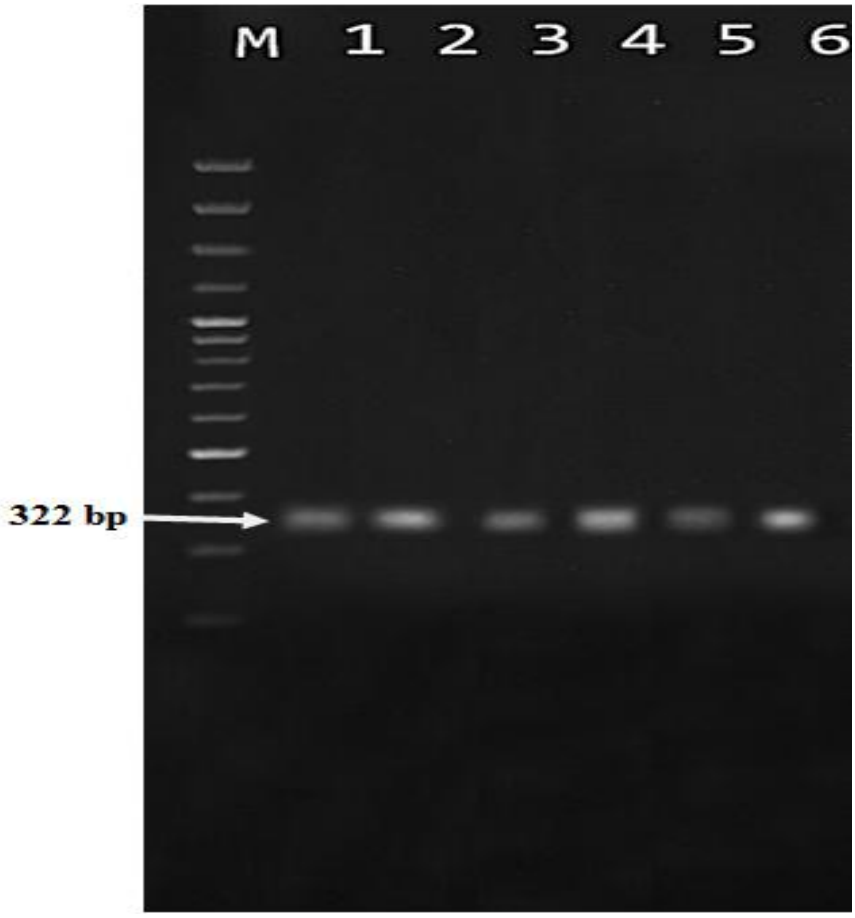

Figure (7) Expression level of bax gene (M) Marker (1) non treated MCF-7 cells (control) , (2) MCF-7 cells treated with *Foeniculum vulgare*, (3) non treated HEPG-2 cells (control) , (4) HEPG-2 cells treated with *Foeniculum vulgare* , *(*5) non treated HCT116 cells (control) and (7) HCT116 cells treated with *Foeniculum vulgare*.

3-**Discussion**

In this study Fennel extracts was chosen to determine their anticancer activity due to their bioactive components which have the ability to reduce the risk of cancer through their antimicrobial, antioxidant, antiinflammatory activity and scavenge free radical. Aqueous and organic extracts of F. vulgare have been reported to show antibacterial activity against some bacterial strains **Kaur and Arora, 2008**. The fennel essential oil has been reported to exhibit antifungal effect. The fennel essential oil and its seed extracts have been reported to exhibit antimycobacterial and anticandidal activity **Abed, 2007**. Fennel extracts were reported to have effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging activities. **Shahat et al., 2011** The essential oil of F. vulgare and its main component, anethole has been shown to have a safe antithrombotic activity that originates due to their broad-spectrum antiplatelet activity, and vasorelaxant action. **Tognolini et al., 2007**.

Therefore the fennel possess potential anticarcinogenic activity. After performing SRB cytotoxicity assay it investigated to determine their ability to cause cell death and it was observed that in all cell lines morphological changes after exposure to various concentrations of the fennel extracts.

P53 tumor-suppressor gene is involved in cell growth control, arrest and apoptosis. Nevertheless cell cycle arrest and apoptosis induction can be observed in p53-defective cells after exposure to DNA-damaging agents suggesting the importance of alternative pathways via p53-independent mechanisms. In order to establish relationship between p53 status, cell cycle arrest, Bcl-2/Bax regulation and DNA damage agents Sensitivity. **Mirjolet et al.,2000; Hwang et al., 2001**. In our results we observed that methanolic extract of *Foeniculum vulgare* effects on cancer cell lines by inducing DNA damage and this appears in MCF-7 cell line highly than HEPG-2 & HCT 116 cell lines. Also our result indicated the effect of *Foeniculum vulgare* to induce p53 gene expression in cancer cell lines which treated with IC50 of *Foeniculum vulgare* more than untreated cancer cell lines (control).

Bax protein has been established as a tumor suppressor, because Bax inactivation leads to rapid tumor growth and to a decrease in the extent of spontaneous apoptosis of tumor cells **Atsushi et al., 2001**. The Bax gene is localized in the cytoplasm of living cells after apoptotic stimulation, Bax translocates to the outer mitochondrial membrane where it oligomerizes and induces the formation of protein-conducting pores that release apoptogenic proteins. **Tsujimoto, 2003**. Bax gene aproapoptic member of the Bcl-2 family is the best characterized mediator of p53 dependent apoptosis. The Bax gene plays a direct role in increasing mitochondrial membrane permeability, leading to release of apoptogenic protein. Also Bax gene also my play important role in apoptosis. **Theodorakis et al., 2002**. The present study showed the expression of apoptotic gene Bax was investigated and the results demonstrated that, there is difference in the expression of apoptotic gene Bax in cancer cells after treatment with *Foeniculum vulgare*. There are relationship between gene expression and number of proteins so in our results we observed in SDS polyacrylamide gel an increasing in number of proteins in treated cancer cells with *Foeniculum vulgare* more than untreated cancer cells (control).

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