RESEARCH COMMUNICATION

Fenugreek, a Naturally Occurring Edible Spice, Kills MCF-7 Human Breast Cancer Cells via an Apoptotic Pathway

Kholoud K Khoja, Gowhar Shafi, Tarique N Hasan, Naveed Ahmed Syed, Abdrohman S Al-Khalifa, Abdullah H Al-Assaf, Ali A Alshatwi*

Abstract

There is growing use of anticancer complementary and alternative medicines worldwide. *Trigonella foenum graecum* (Fenugreek) is traditionally applied to treat disorders such as diabetes, high cholesterol, wounds, inflammation, and gastrointestinal ailments. Fenugreek is also reported to have anticancer properties due to its active beneficial chemical constituents. The mechanism of action of several anticancer drugs is based on their ability to induce apoptosis. The objective of the study was to characterize the downstream apoptotic genes targeted by FCE in MCF-7 human immortalized breast cells. FCE effectively killed MCF-7 cells through induction of apoptosis,confirmed by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and RT-PCR assays. When cells were exposed to 50 µg/mL FCE for 24 hours, 23.2% apoptotic cells resulted, while a 48-hour exposure to 50 µg/mL caused 73.8% apoptosis. This was associated with increased expression of *Caspase 3, 8, 9, p53, Fas, FADD, Bax* and *Bak* in a time-and dose-dependent manner, as determined by real-time quantitative PCR. In summary, the induction of apoptosis by FCE is effected by its ability to increase the expression of pro-apoptotic genes and the spice holds promise for consideration in complementary therapy forbreast cancer patients.

Keywords: Fenugreek - apoptosis - breast cancer - gene expression

Asian Pacific J Cancer Prev, **12**, 3299-3304

Introduction

Breast cancer is the most common type of cancer found in female worldwide; approximately 10% of female are confronted with breast cancer in their life span, a disease commonly believed to be preventable (Jie et al., 2006, Lu et al., 2007, Ahmedin et al., 2011). It may be hereditary as well as sporadic. Studies on breast cancer have consistently found an increased cancer risk associated with elevated levels of endogenous and exogenous estrogens in the body (Mark et al .,2001, James et al, 2006). Differences in breast cancer mortality can be grasped among worldwide countries and is mainly attributed to the role of lifestyle, especially nutrition. Looking at the possible relation between dietary factors and breast cancer risk an essential group of molecules that deserves special attention is the phytoestrogens. Naturally occurring plant derived nonsteroidal compounds have diverse structure, which can bind to estrogen receptors and behave as weak agonist/antagonist (Francesco et al., 2005). The major classes of phytoestrogens are isoflavonoids, flavonoids, coumestans, lignans and mycoestrogens, which are known to be present in fruits, vegetables and whole grains commonly consumed by humans.

Fenugreek (*Trigonella foenum-graecum L.*) is an annual legume crop, due to its spice possessing amazing therapeutic and medical properties it is used in many parts of the world. It is one of the oldest medicinal plants known and has long been recognized as a traditional medicine in Asia, Africa and Mediterranean countries (Mebazaa et al., 2009, Naidu et al., 2011). Current research on fenugreek has shown that it contains active beneficial chemical constituents including steroidal sapogenins (Taylor et al., 1997), dietary fiber (Naidu et al., 2011), galactomannans (Wu et al., 2009), antioxidants, and amino acids such as 4-hydroxyisoleucine which possess anti-diabetic, antioxidant, hypocholesterolemic and hypoglycemic properties which have potential for use in the treatment of antipyretic (Bhatia et al., 2006), antinociceptive, antifertility activity, cure leprosy galactogogue (Bhalke et al., 2009), obesity, diabetes and cancer (Thomas et al., 2011).

One such active agent is the diosgenin, which inhibits azoxymethane-induced aberrant crypt foci formation in F344 rats and induce apoptosis in HT-29 human colon cancer cells (Raju et al., 1999). Diosgenin also inhibits osteoclastogenesis, invasion and proliferation through the down-regulation of Akt, I kappa B kinase activation and NF kappa B-regulated gene expression in tumor cells (Shishodia et al., 2006). It has an antioxidant activity in

*Molecular Cancer Biology Research Lab, Dept. of Food Sciences and Nutrition, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia *For correspondence: alialshatwi@gmail.com*

HIV patients with dementia (Turchan et al., 2003). Another active agent identified in fenugreek is Protodioscin which induces cell death and morphological change indicative of apoptosis in the leukemic cell line H-60 (Hibasami et al., 2003). The chemopreventive aspects and the potential protective effect of fenugreek seeds against 7, 12-dimethylbenz[α] anthracene (DMBA) in rats has been reported (Amin et al., 2005). Some constituent of alkaloids, called 'trigonelline', has revealed potential for use in cancer therapy (Bhalke et al., 2009).

This study was aimed to evaluate the therapeutic window of chloroform extract of fenugreek plant on immortalized breast cells (MCF-7). The relative quantification of *Caspase 3, 8, 9, p53, Fas, FADD, Bax* and *Bak* gene expression on MCF-7 cell line with treatment of fenugreek extract was examined by RT-PCR.

Materials and Methods

Preparation of plant extracts

Fenugreek plant was selected on the basics of ethanopharmacology. Whole plant was shade dried ground and soaked into chloroform for extract. The quantity of solvent was taken 10 times the quantity of plant material. Extraction was performed thrice and extraction was done for 24 hours. The filtrate extract were then evaporated to dryness at 30°C under reduced pressure. Further 100 mg of each extract was dissolved in 10 mL DMEM medium (10% FCS) to obtain stock solution and was further diluted in medium to 10, 25, 50, 75 and 100 μg/mL (Haraguchi et al., 2000, Hasan et al., 2011).

Maintenance of MCF-7 cells

The MCF-7 breast cancer cell line was a kind gift by Dr. M. A. Akbarshah at the Mahatma Gandhi-Doerenkamp Center (MGDC) for alternatives to use of animals in life science education, Bharathidasan University, India. The cell line was tested and found to be free from Mycoplasma. The cell line was maintained and propagated in 90% Dulbecco's Modified Eagle's Medium (DMEM) + phenol red supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (100 Units/0.1mg, mL) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. All the studies done with the cell at ~70% to 80% confluence. Harvested cells were after being subjected to brief trypsinization. Cell viability was assayed by Trypan Blue exlusion test with slight modification (James et al., 1999). The viability of cells was greater than 95%.

Cell Titer Blue® viability assays

Cell Titer Blue® viability assay (Promega) was performed to assess the toxicity of different concentrations of chloroform fenugreek extract (FCE) on MCF-7 cells. The assay was performed according to the manufacturer's instructions. In brief, MCF-7 cells (2 x 10^4 cells/well) were plated in 96 well plates and treated with 0 - 100 μg/mL extract for 24 hours. Then 40 μL of the Cell Titer Blue solution was directly added to the wells and incubated at 37°C for 6 hours. The fluorescence was recorded with a 560/590 nm (excitation/emission) filter set using a Bio-Tek microplate fluorescence reader (FLx800™), and the IC_{50} was calculated. Quadruplet samples were run for each concentration of the FCE in three independent experiments.

FCE-treatment concentration and time-dependence

For a concentration and time dependent study, FCE of 50 μg/mL chloroform was treated with MCF-7 cells for 24 and 48 h for the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. The cells were incubated with the same FCE concentration for 24 and 48 h for real-time quantitative PCR analysis.

TUNEL assay

DeadEnd® TUNEL assay kit (Promega) was used for studying apoptosis in a time and dose dependent manner. The manufactures instructions were followed. Briefly, MCF-7 cells (1.5 x 10^6 cells/well) were cultured in 6 well plates to study apoptosis in adherent cells. Cells were treated with FCE of 50 μg/mL for 24 and 48 h. After the incubation period, the culture medium was aspirated off, and the cell layers were trypsinized. The trypsinized cells were reattached on 0.01% polylysine-coated slides, fixed with 4% methanol-free formaldehyde solution, and stained according to the DeadEnd fluorometric TUNEL system protocol. The stained cells were observed using a Carl- Zeiss (Axiovert) epifluorescence microscope using a triple band-pass filter. To determine the percentage of cells demonstrating apoptosis, 1000 cells were counted in each experiment (Shafi et al., 2009).

Real-time quantitative PCR analysis

The expression of apoptotic genes was analyzed by the reverse transcription-PCR (RT-PCR; Applied Biosystems 7500 Fast) using a real-time SYBR Green/ROX gene expression assay kit (QIAGEN). The cDNA was directly prepared from cultured cells using a Fastlane® Cell cDNA kit (QIAGEN), and the mRNA levels of *Caspases 3, 8, 9, p53, Fas, FADD, Bax* and *Bak* as well as the reference gene *GAPDH*, were assayed using gene-specific SYBR Green-based QuantiTect® Primer assays (QIAGEN). Quantitative real-time RT-PCR was performed in a reaction volume of 25 μL according to the manufactures instruction. In brief, 12.5 μL of master mix, 2.5 μL of primer assay (10x) and 10 μL of template cDNA (100 μg) were added to each well. After a short centrifugation, the PCR plate was subjected to 35 cycles of the following conditions: PCR activation at 95°C for 5', denaturation at 95°C for 5" and annealing/extension at 60°C for 10". All samples and controls were run in triplicates on an ABI 7500 Fast Real-time PCR system. The quantitative RT-PCR data was analyzed by the comparative threshold (Ct) method, and the fold inductions of samples were compared with the untreated samples. *GAPDH* was used as an internal reference gene to normalize the expression of the apoptotic genes. The Ct cycle was used to determine the expression level in the control cells and MCF-7 cells treated with FCE for 24 and 48 h. The gene expression level was then calculated as described earlier (Yuan et al). The results were expressed as the ratio of reference gene to target gene by using the following formula: $^{\Delta}Ct$ = Ct (apoptotic genes) - Ct (*GAPDH*). To determine the relative expression levels,

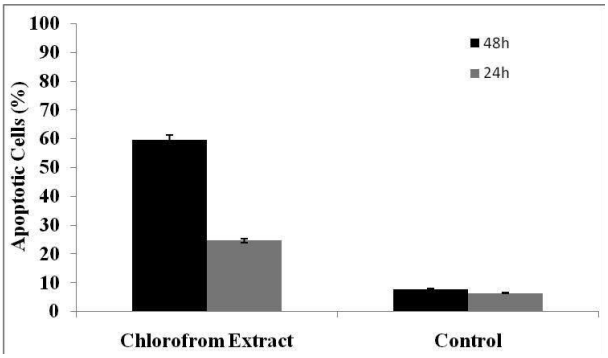

Figure 1. Percentage of TUNEL Positive Cells with or without FCE Extract (50 µg/mL)

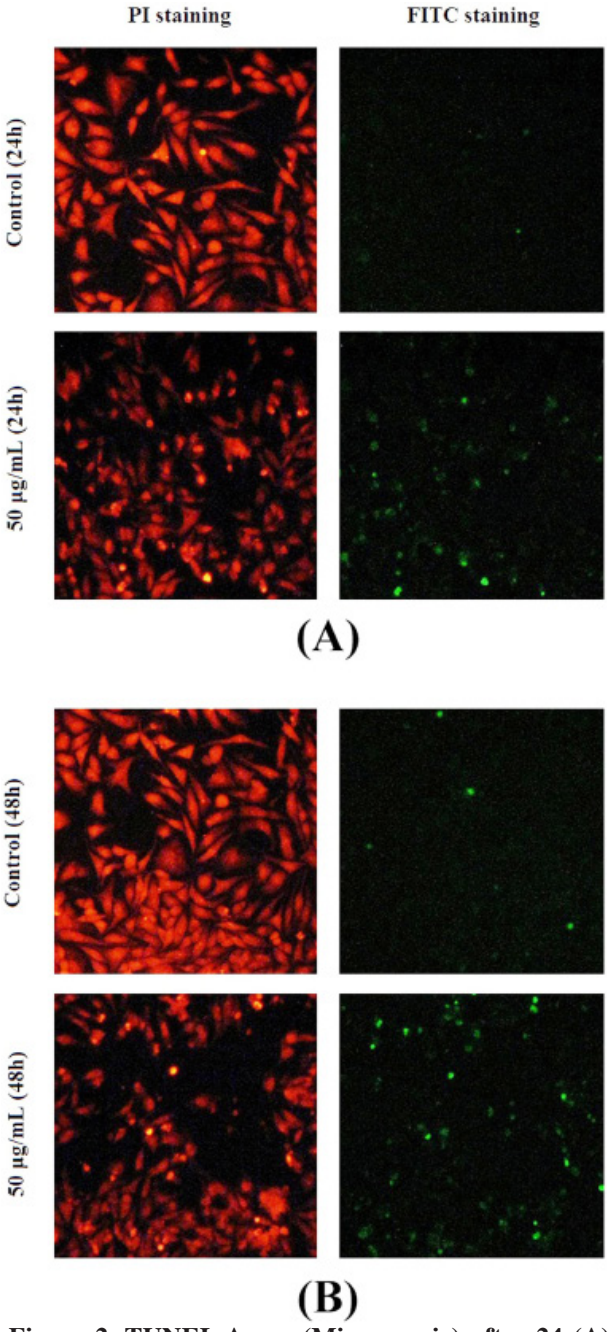

Figure 2. TUNEL Assay (Microscopic) after 24 (A) and 48 (B) Hours Incubation of MCF-7 Treated with or without 50 µg/mL FCE. Red fluorescence is due to propidium iodide staining and observed under green filter while green fluorescence is due to FITC staining and observed under blue filter. Observations were made at 200× magnification

the following formula was used: $^{\Delta\Delta}Ct = {}^{\Delta}Ct$ (treated) - $^{\Delta}Ct$ (control). In short, the expression levels were expressed as n-fold differences relative to the calibrator. The value was used to plot the expression of apoptotic genes using the expression of $2^{-\Delta\Delta Ct}$.

Results

Determination of FCE toxicity on MCF-7 cells

The cytotoxic effect of 0 to 100 µg/mL concentration of different FCE on MCF-7 cells was examined using the Cell Titer Blue® viability assay (Promega). A dose-dependent reduction in color was observed after 24 h of treatment with different FCE. In brief, 64.5% of the cells were found dead at the highest concentration of FCE tested (100 µg/mL), whereas the IC50 of FCE was achieved at 41.6 µg/mL.

Quantification of apoptosis by a TUNEL assay

To determine whether the inhibition of cell proliferation by FCE was due to the induction of apoptosis, a TUNEL assay was used. Figure 1 and 2 summarize the effect of FCE on MCF-7 cells. A dose and time dependent increase in the induction of apoptosis was observed when MCF-7 cells were treated with FCE. When compared to the control cells at 24 h, 23.2% of cells treated with 50 µg/mL of FCE, respectively underwent apoptosis. Similarly, 73.8% of cells treated with 50 µg/mL of FCE, respectively, for 48 h underwent apoptosis.

Quantification of mRNA levels of apoptotic related genes

To investigate the molecular mechanism of FCE induced apoptosis in MCF-7 cells, the expression levels of several apoptosis related genes were examined. The relative quantification of *Caspase 3, 8, 9, p53, Fas, FADD, Bax* and *Bak* mRNA expression levels was performed by SYBR Green based quantitative real-time PCR (RT-PCR) using a 7500 Fast Real Time System (Applied Biosytem).

In most of the expression FCE increased the transcripts of Caspase 3, 8, 9, p53, Fas, FADD, Bax and Bak by several fold. The expression levels of these genes in MCF-7 cells treated with 50 µg/mL of FCE for 24 and 48 h increased by as follows: 3.5 fold in *Caspase-3,* 5.5 fold in *Caspase-8*, 3.7 fold in *Caspase-9*, 1.4 fold in *p53*, 1.7 fold in *Fas*, 2.6 fold in *FADD*, 3.2 fold in *Bax* and 4.4 fold in *Bak* respectively as compared to the levels in untreated control cells. All together these data advocates that these *caspases, p53, Fas, FADD, Bax* and *Bak* were induced by FCE in a dose and time dependent manner.

Discussion

Currently there is a growing interest in the use of phytocompounds to develop safe and more effective therapeutic agents for cancer treatment throughout the world (Panchal et al., 1998). Which is due to the unfavourable side effects and resistance of many anticancer agents that have been developed are severe problems (Khan et al., 1999). The mechanism of action of many anticancer drugs is based on their ability to induce

apoptosis (Sen et al., 1992, Motomura et al., 2008). It is a vital process in human development, immunity, and tissue homeostasis (Travis et al, 2008). There are many mechanisms through which apoptosis can be enhanced in cells. This may be due to sequence communication between the caspases, genes and death receptors of intrinsic and extrinsic pathways (Hengartner, 2002). Further, *Bax* and *Bak* are the two key molecules in the mitochondrial pathway of apoptosis, were interdependently activated by *p53*, leading to cytochrome *c* release and followed by apoptosis, which may be indirect activation of *caspase 3* from *caspase 8, 9* or direct by *caspase 9*. Concerning this, the side effects of fenugreek plant are modest and well tolerated, draws attention in the application for cancer prevention and treatment.

Fenugreek seed protect against experimental cataract by virtue of antioxidant properties. It facilitates slow absorption of carbohydrates, thus resulting in feeling of fullness and aid in relieving stomach disorders. These natural antioxidants help to strengthen the immune system, improve cellular health and reduce signs of ageing (Kaviarasan et al., 2004). Its seeds are used for medicinal purposes; restrain a large amount of saponins (4.8%) and the alkaloid trigonelline (0.37%), which may have a role, in hypocholesterolemic activity (Srinivasan, 2006). An unusual amino acid isolated from fenugreek, 4-hydroxyisoleucine, was shown to exert significant insulin secretagogue property (Narender et al., 2006). In traditional Chinese and Indian medicine fenugreek seeds were used as a tonic as well as a cure for weakness and edema (Yoshikawa et al., 1997).

According to the GC/MS study carried out by Wesley et al (1997) on the chloroform/petroleum ether extract from amber fenugreek for the analysis of steroidal sapogenins. Diosgenin was the major component in seed, Yamogenin, Tigogenin, Neotigogenin, Smilagenin, and Sarsasapogenin were identified in the extracts. Dihydroxy steroidal sapogenins, tentatively identified as Yuccagenin, Gitogenin, and Neogitogenin, were detected as minor components. The spice seeds contain 0.1–0.9% diosgenin and are extracted on a commercial basis (Baccou et al., 1978; Sulieman et al., 2000). Two minor membrane lipid components implicated in lipid signalling pathway are the N-acylethanolamines that have been acknowledged from the chloroform extract of fenugreek as phospholipid constituents in desiccated seeds as well as fatty acid amides with a physiological role in mammalian nervous system. Especially in the perception of pain and in control of appetite (Chapman, 2004). Some of them have anti-inflammatory and anti-cancer properties and help regulate many physiological and pathological processes in the reproductive system. Oleoylethanolamine is an endogenous regulator of food intake and is suggested as a potential anti-obesity drug (Suchandra et al., 2010). Steroidal sapogenin is considered as a basic compound in the hemisyntheis of steroid drugs such as cortisone and sexual hormones (Brenac et al., 1996).

Diosgenin used for medicinal steroids synthesis, steroidal saponins which are for the hypochlolesterolemic activity of fenugreek, as well as the free 80% of aminoacids present in it. Diosgenin, furanones, dioscin, protodioscin and trigonelline have been shown to have anticancer activity in mice, breast cancer, and colon cancer (Amin et al., 2005, Raju et al., 2006). Diosgenin has induce apoptosis in human rheumatoid arthritis, human osteosarcoma 1547 cell line, HT-29 human colon cancer cells (Raju et al, 2004) and leukemic cell line H-60 by various carcinogens in the form of cancer therapy (Hibasami et al, 2003). Dioscin were also shown to include antifungal, antivirus and antitumor activities. In cell culture studies dioscin exerted apoptosis-inducing effects against human myeloblast leukemia HL-60 cells, human cervical cancer HeLa cells, Caco-2, HCT-116, HepG2, K562 and A-549 (Yum et al., 2010).

Together, the data presented in this study suggest that FCE induced apoptosis is mediated by the death receptor and mitochondrial apoptotic pathways as demonstrated by the increased levels of *caspases -3, 8, 9* expression after FCE treatment which is due to the wide distribution of sterols and steroidal sapogenins of fenugreek. In addition, this study advocates that FCE activate the extrinsic death pathway (*Fas* and *FADD*-mediated apoptosis) as demonstrated by increased expression levels of *caspase-8*. *p53* is the most commonly mutated gene associated with cancer, helps to regulate the cell cycle and has a key role in ensuring that damaged cells are destroyed by apoptosis. The data obtained in this study indicates that the expression levels of *caspases-3, 8, 9, p53, Fas, FADD, Bax* and *Bak* was found to be very high in FCE.

The possibility that *p53*-mediated apoptosis may be associated with the activation of *caspase-3, 8, 9* is suggested by the ability of *p53* to activate both the extrinsic and intrinsic apoptotic pathways. *p53* enhances cancer cell apoptosis, and it prevents cell replication by stopping the cell cycle at G1 or interphase. By inducing the release of mitochondrial cytochrome c through Bax/Bak activation, *p53* might be able to activate effector caspases including *caspase-3*. *Caspase-3, 8, 9* may be the apoptotic effector machinery engaged by *p53* to mediate teratogen-induced apoptotic pathways.

In conclusion, to our knowledge, the results presented in this study show that FCE exhibits anticancer effects by blocking the proliferation of MCF-7 cells and inducing apoptosis in part by modulating expression levels of *caspase-3, 8, 9, p53, Fas, FADD, Bax* and *Bak*. The induction of apoptosis by FCE is affected by its ability to regulate the expression of pro-apoptotic genes such as *caspase-3, 8, 9, p53, Fas, FADD, Bax* and *Bak*. Considering this, it is most likely that FCE induced, at least in part, *p53, Fas, FADD, Bax, Bak* and *caspases* mediated apoptosis in MCF-7 cells. Therefore, the present study demonstrates that FCE significantly inhibits the growth of MCF-7 human breast cancer cells *in vitro*, and it provides the underlying mechanism for the anticancer activity.

These studies support the use of FCE for breast cancer chemoprevention due to its its influence in suppressing growth of immortalized breast cells without significant toxicity. These finding provide a strong rationale for preclinical and clinical evaluation of FCE for breast cancer therapies.

Acknowledgements

THis research project was supported by a grant from the research centre of the centre forfemale scientific and medical college, King Saud University, Riyadh, Saudi Arabia for their financial support. The authors declare that there are no conflicts of interest.

References

Ahmedin J, Freddie B, Melissa MC, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Amin A, Alkaabi A, Al-Falasi S, Daoud SA (2005). Chemopreventive activities of Trigonella foenum graecum (Fenugreek) against breast cancer. *Cell Biol Int*, **29**, 687-94.

Baccou JC, Sauvaire Y, Olle M, Petit J (1978). L'huile de Fenugreek: Composition, properties, possibilities d'utilisationdsans I'indust rie des peintures et vernis. *Revue Francaise des Corps Gras*, **25**, 353-9.

Bertrand L, Pascale V, Cecile C, Jean LC, Jean B (2004). Diosgenin, a plant steroid, induces apoptosis in human rheumatoid arthritis synoviocytes with cyclooxygenase-2 overexpression. *Arthritis Res Ther*, **6**, 373-83.

Bhalke RD, Anarthe SJ, Sasane KD, et al (2009). Antinociceptive Activity of Trigonella foenum-graecum Leaves and Seeds (Fabaceae). *IJPT*, **8**, 57-9.

Bhatia K, Kaur M, Atif F, et al (2006). Aqueous extract of Trigonella foenum-graecum L. ameliorates additive urotoxicity of buthionine sulfoximine and cyclophosphamide in mice. *Food and Chem Toxicol*, **44**, 1744-50.

Brenac P, Sauvair Y (1996). Accumulation of Sterols and Steroidal Sapogenins In Developing Fenugreek Pods: Possible Biosynthesis In Situ. *Phytochemistry*, **41**, 415-22.

Chapman KD (2004). Occurrence, metabolism, and prospective functions of Nacylethanolamines in plants. *Prog Lipid Res*, **43**, 302-27.

Earnshaw WC, Martins LM, Kaufmann SH (1999) Mammalian caspases Structure, activation, substrates, and functions during apoptosis. *Ann Rev Bioche*, **68**, 383-424.

Franc¸ois R, Tzong L, Rui-Hai Z, et al (2004). The role of the mitochondrial pathway in *Annulus fibrosus* cell apoptosis induced by overload. *Am J Pathol*, **164**, 915-24.

Francesco B, Stefano L (2005). Health effects of phytoestrogens. *Forum Nutr*, **57**, 100–111.

Haraguchi M, Mimaki Y, Motidome M, et al (2000). Steroidal saponins from the leaves of cestrum sendtenerianum. *Phytochemistry*, **55**, 715-20.

Hasan TN, Leena GB, Gowhar S, Amal AA, Ali AA (2011). Anti-proliferative Effects of Organic Extracts from Root Bark of Juglans Regia L. (RBJR) on MDA-MB-231 Human Breast Cancer Cells: Role of Bcl-2/Bax, Caspases and Tp53. *APJCP*, **12**, 525-30.

Hengartner MO (2002). The biochemistry of apoptosis. *Nature*, **407**, 770-6.

Hibasami H, Moteki H, Ishikawa K (2003). Protodioscin isolated from fenugreek (Trigonella foenum graecum L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. *Int J Mol Med*, **11**, 23-6.

James DY, Nancy ED (2006). Estrogen carcinogenesis in breast cancer. *N Engl J Med*, **354**, 270–82.

James R, Warburton S (1999). Hemocytometer Cell Counts and Viability Studies: Cell Quantification. In Cell and Tissue Culture: Laboratory Procedures in Biotechnology. 1 edition. Edited by: Doyle A, Grifith JB. England: John Wiley, 57-61.

Jie S, Rui HL (2006). Cranberry phytochemical extracts induce cell cycle arrest and apoptosis in human MCF-7 breast cancer cells. *Cancer Lett*, **241**, 124-34.

Khan MR, Mlungwana SM (1999). c-Sitosterol, a cytotoxic sterol from *Markhamia zanzibarica* and *Kigelia africana*. *Fitoterapia*, **70**, 96-7.

Lu X, Kang Y (2007). Organotropism of breast cancer metastasis. *J Mammary Gland Bio Neoplasia*, **12**, 153-62.

Malik F, Kumar A, Bhushan S, et al (2008). Reactive oxygen species generation and mitochondrial dysfunction in the apoptotic cell death of human myeloid leukemia HL-60 cells by a dietary compound withaferin A with concomitant protection by N-acetylcysteine. *Apoptosis*, **12**, 2115-33.

Mark C, Paul G (2001). Estrogen and the Risk of Breast Cancer. *N Engl J Med*, **344**, 276-85.

Mebazaa R, Amine M, Marine F, et al (2009). Characterisation of volatile compounds in Tunisian fenugreek seeds. *Food Chem*, **115**, 1326-36.

Motomura M, Kwon KM, Suh SJ, et al (2008). Propolis induces cell cycle arrest and apoptosis in human leukemic U937 cells through Bcl-2/Bax regulation. *Environ Toxicol Pharmacol*, **26**, 61-7.

Naidu MM, Shyamala BN, Naik JP, Sulochanamma G, Srinivas P (2011). Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. *J Food Sci Technol*, **44**, 451-6.

Narender T, Puri A, Shweta S, et al (2006). 4-hydroxyisoleucine an unusual amino acid as antidyslipidemic and antihyperglycemic agent. *Bioorg Med Chem Lett*, **16**, 293-6.

Panchal RG (1998). Novel therapeutic strategies to selectively kill cancer cells. *Biochem Pharmacol*, **55**, 247-52.

Pommier Y, Sordet O, Antony S, Haywrd RL, Kohn KW (2004). Apoptosis defects and chemotherapy resistance: molecular interaction maps and networks. *Oncogene*, **23**, 2934-49.

Raju J, Bird RP (2006). Alleviation of hepatic steatosis accompanied by modulation of plasma and liver TNF-alpha levels by *Trigonella foenum graecum* (fenugreek) seeds in Zucker obese (fa/fa) rats. *Int J Obes*, **30**, 1298-307.

Raju J, Jagan MR, Patlolla, Malisetty VS (2004). Diosgenin, a steroid saponin of *Trigonella foenum graecum* (Fenugreek), inhibits azoxymethane-induced aberrant crypt foci formation in F344 rats and induces apoptosis in HT-29 human colon cancer cells. *Cancer Epidemiol Biomarkers Prev*, **13**, 1392-8.

Riedl SJ, Shi Y (2004). Molecular mechanisms of caspase regulation during apoptosis. Nature Review: *Mol Cell Bio*, **5**, 897-907.

Sen S, D'Incalci M (1992). Biochemical events and relevance to cancer chemotherapy. *FEBS Letters*, **307**, 122-7.

Shafi G, Munshi A, Hasan TN, et al (2009). Induction of apoptosis in HeLa cells by chloroform fraction of seed extracts of Nigella sativa. *Cancer Cell Int*, **9**, ?-?.

Shah S, Gapor A, Sylvester PW (2003). Role of caspase-8 activation in mediating vitamin E-induced apoptosis in murine mammary cancer cells. *Nutr Cancer*, **45**, 236-46.

Shishodia S, Aggarwal BB (2006). Diosgenin inhibits osteoclastogenesis, invasion, and proliferation through the down regulation of Akt, IKB kinase activation and NF-KB-regulated gene expression. *Oncogene*, **25**,1463-73.

Srinivasan K (2006). Fenugreek (*Trigonella foenum-graecum*): a review of health beneficial physiological effects. *Food Rev Int*, **22**, 203-24.

Suchandra C, Prasad SV, Arun S (2010). Bioactive lipid constituents of fenugreek. *Food Chem*, **119**, 349-53.

Sulieman AME, Ali AO, Hemavathy J (2000). Lipid content and fatty acid composition of fenugreek (*Trogonella foenum-graceum* L.) seeds grown in Sudan. *Food Sci Technol Int*, **43**, 380-2.

Taylor WG, Zaman, MS, et al (1997). Analysis of steroidal

sapogenins from amber fenugreek (*Trigonella foenum-graceum*) by capillary gas chromatography and combined gas chromatography/mass spectrometry. *J Agric Food Chem*, **45**, 753-9.

Thomas JE, Manjula B, Lee EL, Darcy D, Surya A (2011). Biochemical monitoring in fenugreek to develop functional food and medicinal plant variants. *New Biotechnology*, **28**, 110-7.

Travis WD, Su H, Ahmad RS (2008). c-FLIP knockdown induces ligand-independent DR5-, FADD, caspase-8-, and caspase-9-dependent apoptosis in breast cancer cells. *Biochem Pharmacol*, **76**, 1694-704.

Turchan J, Pocernich CB, Gairola C, et al (2003). Oxidative stress in HIV demented patients and protection ex vivo with novel antioxidants. *Neurology*, **60**, 307-14.

Wesley GT, Zaman MS, Mir Z, et al (1997). Analysis of Steroidal Sapogenins from Amber Fenugreek (Trigonella foenum-graecum) by Capillary Gas Chromatography and Combined Gas Chromatography/Mass Spectrometry. *J Agric Food Chem*, **45**, 753-9.

Wu Y, Cui W, Eskin NAM, Goff HD (2009). An investigation of four commercial galactomannans on their emulsion and rheological properties. *Food Res Int*, **42**, 1141-6.

Yoshikawa M, Murakami T, Komatsu H (1997). Medicinal foodstuffs. IV. Fenugreek seed. (1): structures of trigoneosides Ia, Ib, IIa, IIb, IIIa, and IIIb, new furostanol saponins from the seeds of Indian *Trigonella foenumgraecum* L. *Chem Pharm Bull*, **45**, 81-7.

Yuan JS, Reed A, Chen F, Stewart CN (2006): Statistical analysis of real-time PCR data. *BMC Bioinformatics*, **7**, 85.

Yum CH, You HJ, Ji GE (2010). Cytotoxicity of dioscin and biotransformed Fenugreek. *J Korean Soc Appl Biol Chem*, **53**, 470-7.