Research Journal of Biological Sciences 2 (4): 438-443, 2007

Fenugreek (Trigonella Foenum Graecum L.) Seed Extract Induces Cell Death, Growth Inhibition and Morphological Change Indicative of Apoptosis in Acute Lymphoblastic Leukemia

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Abstract: Cancer is the second leading cause of death worldwide. Conventional therapies cause serious side effects and, at best, merely extend the patient's lifespan by a few years. Cancer control may therefore benefit from the potential that resides in alternative therapies. There is thus an increasing demand to utilize alternative concepts or approaches to the prevention of cancer. The antineoplastic effect of Trigonella foenum graecum seed extract has been evaluated in human acute T Lymphoblastic leukemia cell lines (CCRF-HSB-2). After determination of toxic level of fenugreek seeds extract on each cell lines, them were treated with different doses of extract and after incubation time (24 and 48 h) results were evaluated by cell count, viability test, staining and light microscopy and finally were analyzed in term of apoptosis induction using annexin v-fitc flowcytometry kit. Then data were analyzed using SPSS 11.5 software. Results show significant effects of Fenugreek seeds extract against this cell line such as growth inhibition, cell death and morphological change. Apoptosis induction by this extract in these cell lines was little. Fenugreek seeds extract didn't change the count and morphology of normal lymphocytes. These findings suggest significant antineoplastic effects of Fenugreek seeds against CCRF-HSB-2 cell line. A strategy to selectively induce apoptosis of leukemia cells without altering healthy cells is a major goal for the development of new therapeutic techniques. To our knowledge, this is the first study that suggests significant chemo preventive effects of Fenugreek seeds against these cell lines.

Key words: Fenugreek, CCRF-HSB-2, acute T Lymphoblastic leukemia, apoptosis

INTRODUCTION

Medical herbs have been widely used in folk medicine to avoid serious side effects of available chemotherapeutic drugs (Abuharfeil *et al*., 2001). Fenugreek (Trigonella foenum graecum) is an annual herb belonging to the family Leguminosea (Alarcon-Aguilara *et al*., 1998) widely grown in India, Egypt and Middle Eastern countries. The name fenugreek comes from foenum-graecum, meaning Greek hay, as the plant was traditionally used to scent inferior hay. (Flammang *et al*., 2004). Fenugreek seeds, popularly known as 'Methi' is abundantly all over India and is used one of the common ingredients in Indian spices. Its use in the Ayurvedic system of medicine is well documented.

It has been shown to possess a hypocholesterolaemic effect in rats and dogs (Khosla *et al*., 1995a; Valette *et al*., 1984). Fenugreek seeds also are used as a traditional remedy for the treatment of diabetes and hypercholesterolemia in Ayurvedic (Indian), Unani (Arabic) and Chinese medicine (Basch *et al*., 2003; Miraldi *et al*., 2001; Southern California Evidence-Based Practice Center/RAND for Agency on Healthcare Research and Quality, 2001).

The anti-inflammatory activity of fenugreek seed extract in rats was reported. (Thakur *et al*., 1994).

The evaluation of natural killer cell activity against tumor cells by fenugreek and other wild plants from Jordan was established (Abuherfeil *et al*., 2001).

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Several compounds extracted from plants and evaluated the antitumor activity of those compounds; the efficiency of the antitumor compounds seems to be related to the propensity of tumor cells to respond to these compounds by apoptosis (Hibasami *et al*., 2003). Recently, considerable attention has been focused on the sequence of events referred to as apoptosis and the role of this process in mediating the lethal effects of antineoplastic agents in leukemia cells (Kaufman, 1989). Apoptosis is a highly regulated process that is characterized by cell shrinkage, membrane blebbing, chromatin condensationand formation of a DNA ladder with multiple fragments of 180-200 bp caused by inter-nucleosomal DNA cleavage (Steller, 1995). the identification of Protodioscin (PD) from fenugreek and showing the inhibitory effects of PD on the growth of human leukemia HL-60 cells which results from the induction of a poptosis were demonstrated (Hibasami *et al*., 2003).

The protective effects of T. foenum graecum against the development of breast cancer in rats using the DMBA-induced mammary tumor model was proved the main objective of cancer.

Chemoprevention research is to advance knowledge in identifying and characterizing entities that might reduce the risk of the human population developing cancer. Therefore, it is of interest to explore the possibility of using phytochemicals or other dietary chemicals as chemo preventive agents. (Amin *et al*., 2005). Further, the study of the biological effects of these phytochemicals at cellular level provides the molecular basis for their anti-disease function and helps to establish the platform for generating more potent chemo preventive and even chemotherapeutic agents (Gosslau and Yu-Chen, 2004). In theory, cancer chemoprevention can be defined as an intervention in the carcinogenic process by a chemical that either blocks neoplastic process induction or prevents transformed cells from progressing to malignant phenotype. It may also encompass a reversal of the process of progression (Chow *et al*., 2003; Kelloff *et al*., 1994). In practice, a potential intervention agent must enhance the physiological processes protecting humans against pre-neoplastic cell progression or neoplastic cell growth (Amin *et al*., 2005).

This background promoted use to look for the antitumer effect of fenugreek seeds against to leukemic cell line CCRF-HSB.

MATERIALS AND METHODS

Plant extract: Fenugreek seeds were supplied by agriculture department of Tehran University and then extracted by percolation (Lixiviation) method, then the hydroalcholic extract filtrated with 0.2µm ;7barmaxnon-pyrogenic filter(Schleicher and Schuell lot:EE1041-1) and distilled in vacuum.

The dry weight of the extract assayed 3 time that it was 90%.

Leukemic cell line and cell culture: A CCRF-HSB-2 (this cell line a T lymphoblastoid line obtained from the peripheral blood of an 11 year old Caucasian male with acute lymphoblastoid leukemia with has been passaged eight times through new born Syrian hamsters) obtained through National cell bank of Iran.

CCRF-HSB-2 cells were grown in RPMI1640 medium (sigma) with 10% fetal calf serum (Gibco) at 37°C under humidified 95% air-5% CO_2 atmosphere and passaged ever 48 h.

Chemical: Trypan blue purchased from SIGMA, USA were dissolved in normal saline.

Preparation of human lymphocyte cells: Lymphocyte separation medium (3 mL) was aseptically transferred to a centrifuge tube and the diluted blood (heparinized blood: Physiological saline = 1:1) was layered over lymphocyte separation medium in the tube. The tube was centrifuged at 400×g at room temperature for 20 min. The top layer of the clear plasma was removed and the lymphocyte layer was transferred to a new centrifuge tube. An equal volume of PBS (-) was added to the lymphocyte layer in the tube and centrifuged for 10 min at room temperature at 260×g. After the centrifugation, the precipitate lymphocyte was washed again with PBS (-) and suspended in RPMI 1640 medium containing 10% FCS.

Count and viability of the cells: The lymphocyte, control and test cells were counted using haemacytometer, then counting repeated with using trypan blue (1/2 diluted) and the percent of viability for each group were been estimated.

Staining and light microscopy: The smear from lymphocyte, control and tested cell after incubation with fenugreek seed extract prepared and stained with Wright -Giemsa and then microscopy studied for detection of destroyed cell, apoptotic cell and other morphological change.

Apoptosis detection by flowcytometery (Annexin V-FITC; IQ product): Apoptosis is characterized by a variety of morphological features such as loss of membrane

asymmetry and attachment, condensation of the cytoplasm and nucleus and internucleosomal cleavage of DNA. One of the earliest indications of apoptosis is the translocation of the membrane phospholipid Phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane. Once exposed to the extra cellular environment, binding sites on PS become available for Annexin V, a 35-36 kDa, Ca 2+-dependent, phospholipid binding protein with a high affinity for PS.

The translocation of PS precedes other apoptotic processes such as loss of plasma membrane integrity, DNA fragmentation and chromatin condensation. As such, Annexin V can be conjugated to biotin or to a fluorochrome such as FITC, PE, APC, Cy5, or Cy5.5 and used for the easy, flow cytometric identification of cells in the early stages of apoptosis.

Because PS translocation also occurs during necrosis, Annexin V is not an absolute marker of apoptosis. Therefore, it is often used in conjunction with vital dyes such as 7-Amino-Actinomysin (7-AAD) or Propidium Iodide (PI), which bind to nucleic acids, but can only penetrate the plasma membrane when membrane integrity is breached, as occurs in the later stages of apoptosis or in necrosis.

RESULTS

Toxicity of fenugreek extract: Normal lymphocyte treated with the hydro alcoholic extract of fenugreek (0.5μg mL^{-1} up 5μg mL^{-1}) for 48 h and didn't show a toxic effect.

Nor morphological changes in these cells could be observed neither in the count of the cells.

LD50 determination: The CCRF-HSB-2 cells treated with different doses of extract for 24 h to determine of LD50. Obtained results showed that LD50 for these cells was 7.5 μg mL^{-1}.

Assay for growth inhibition: Exponentially growing CCRF-HSB-2 were placed at 4-5×10^5cells mL^{-1} in a culture flask and cultivated in the presence of a vehicle, or fenugreek extract. After cultivating for defined time periods, the cell number was counted by a hemocytometer. These results shown in Table 1 and Fig. 1.

Assay for viability: The viability test for these cells was applied in different doses and times by trypan blue that these results shown in Table 1 and Fig. 1.

Induction of apoptosis: The significant growth inhibitory activities of fenugreek extract led us to investigate the induction of apoptosis. Morphological change showing apoptotic body and fragmentation of nucleus and vacuolization in some of cells but evaluation of apoptosis

Table 1: Cell count and viability of leukemic cell line after 12 and 24 h incubation with different doses of Fenugreek seeds extract

Extract dose(μg mL^{-1})	Control	0.5	1.0	2.0	4.0
Amount of 2000 μg mL^{-1}extract (μL)	0	0.25	0.5	1.0	2.0
Media RPMI + 10% FCS (μL)	1000	999.75	999.5	999	998
Cell count after 24 h	400	370	360	310	280
Viability after 24 h	95%	95%	90%	90%	55%
Dead cells	4.5%	4.5%	10.3%	10.7%	45%
Apoptotic cells	0.9%	0.75%	1.4%	1.4%	4%
Cell count after 24 h	510	320	300	285	240
Viability after 48 h	87%	41%	33%	28%	16%

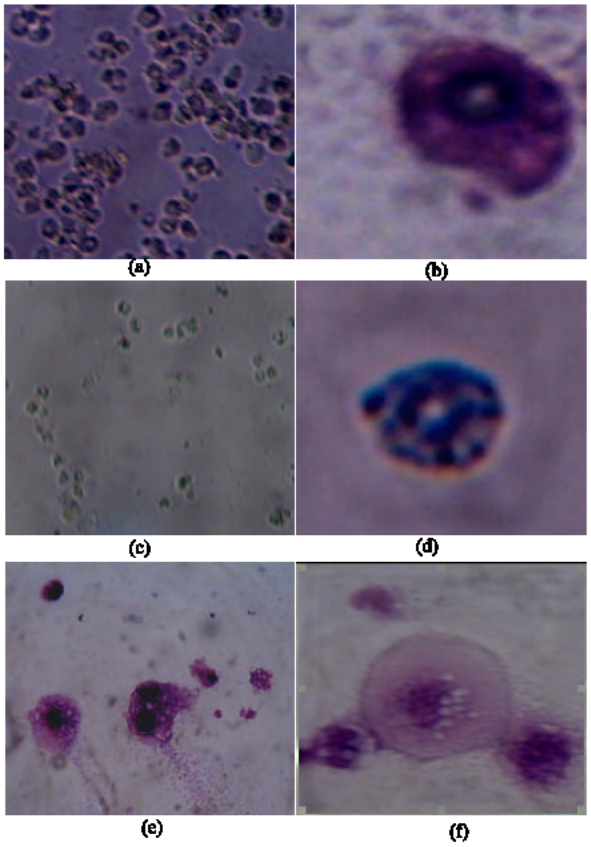

Fig. 1: a) CCRF-HSB-2 cells in culture flask before treatment with extract (40X), b) CCRF-HSB-2 cells in before treatment with extract stained with wright-Geimsa (100X), c) CCRF-HSB-2 cells in culture flask after treatment with 4.0μg mL^{-1} extract for 24 h (40X), d) CCRF-HSB-2 cells after treatment with 4.0μg mL^{-1} extract for 24 h, nucleus fragmentation is evident. (100X), e, f) CCRF-HSB-2 cells after treatment with 4.0μg mL^{-1} extract for 24 h, cell destruction and vacuolization is evident (100X)

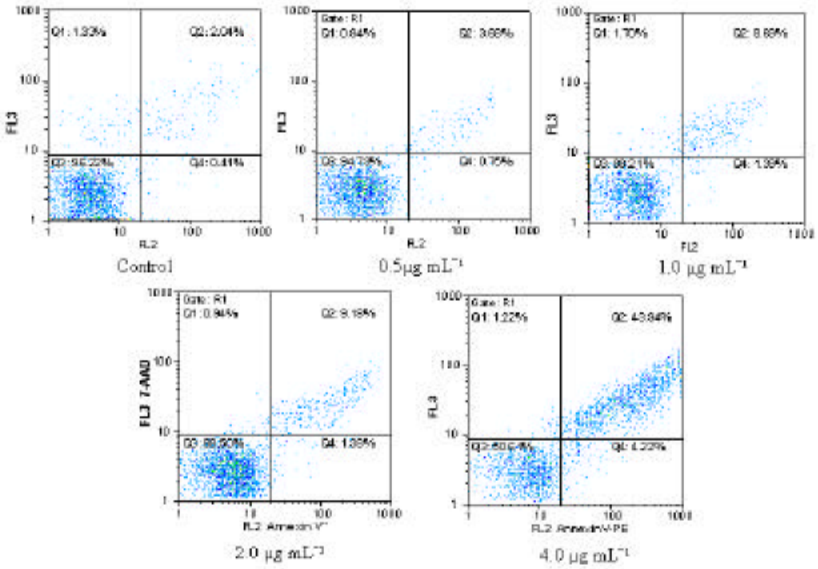

Fig. 2: Evaluation of apoptosis induction by ANNEXIN V-FITC (flowcytometery) 4.0µg mL^{-1}

induction by ANNEXIN V-FITC kit showed that apoptosis occur only in few cells (Fig. 2).

Analysis of data: the data analyzed with SPSS 11.5 software. The KS test was 0.954 (significant), correlation coefficient (R=0.966 high relation between count and dose), determination coefficient (R^2 =0.934), ANOVA = 0.007(p-value<0.05 so significant) and count equation is: Count = -29.5(dose) +388.25.

DISCUSSION

Fenugreek has primarily been described as an anti-hyperglycemic herb in humans and in laboratory animals (Bordia *et al*., 1997; Sharma *et al*., 1990). Its cholesterol-reducing effect is also well established (Sharma, 1984). Fenugreek has also shown an overall stimulatory effect on the pecific as well as non-specific immune functions in mice (Bin-Hafeez *et al*., 2003). The main chemical constituents of T. foenum graecum are fibers, flavonoids, polysaccharides and saponins (Jayaweera, 1981; Yoshikawa *et al*., 1997). Some of the constituents might be having mitogenic effects, which in turn lead to stimulatory effects on the immunocompetent cells. Some of these constituents also possess antioxidant properties and they may induce the immunostimulant effects (Fuente and Victor, 2000; Ruby *et al*., 1995; Devasagayam and Sainis, 2002). Both pro-oxidant and antioxidant effects of flavonoids have previously been identified (Shen *et al*., 2004; Rice-Evans, 2001; Ross and Kasum, 2002; Shen *et al*., 2002).

Apoptosis is a type of cell death and agents with the ability to induce apoptosis in tumors have the potential to be used for anti-tumor therapy. Flavonoids produce several biological effects and the apoptosis-inducing activities of flavonoids have been identified in several previous studies (Chen *et al*., 2003; Shen *et al*., 2003).

Flavonoids and catechins were first shown to be apoptotic in human carcinoma cells (Ahmad *et al*., 2000). Similar observation has since been extended to lung tumor cell lines (Yang *et al*., 1998), colon cancer cells, breast cancer cells, prostate cancer cells (Paschka *et al*., 1998) stomach cancer cells (Okabe *et al*., 1999) brain tumor cells (Yokoyama *et al*., 2001), head and neck squamous carcinoma (Masuda *et al*., 2001) leukemia (Hibasami *et al*., 2003) and cervical cancer cells (Ahn *et al*., 2003).

Genistein, quercetin, rutin and other food flavonoids have been shown to inhibit carcinogenesis in animal models (Gee *et al*., 2002). They all induce apoptosis in tumor cells (Katdare *et al*., 2002; Upadhyay *et al*., 2001; Choi *et al*., 2001; Iwashita *et al*., 2000). It appears that these flavonoids can also differentially induce apoptosis in cancer cells, but not in their normal counterparts.

Recently, alternative cell death processes have been recognized in epithelial cells, including autophagy and para-apoptosis (Bursch *et al*., 2000; Leist and Jaattela, 2001; Sperandio *et al*., 2000). These pathways can be activated in parallel with apoptosis and significant crosstalk between apoptotic and alternative death pathways may exist (Lee and Baehrecke, 2001). Thus, herbal-induced autophagic or ‘‘type II’’ cell death may also contribute to the cell death. The present study establishes that T. foenum graecum has appreciable anti-cancer activity. It is not possible to identify the most effective anti-cancer constituent of T. foenum graecum at this point. However, based on the published studies,

flavonoids seem to be most likely candidates eliciting anti-tumorigenic effect. Administration of Fenugreek to man is simple, since its seeds and leaves are used as common dietary constituents in many parts of the world. Further investigations are underway to unravel the molecular mechanism that mediates the legume's anti-cancer protective effects. In addition, further studies are underway to isolate and characterize the Fenugreek's active ingredients that contribute to its preventive effects.

A strategy to selectively induce apoptosis of leukemia cells without altering healthy cells is a major goal for the development of new therapeutic techniques.

REFERENCES

Abuharfeil, N.M., Maher Salim and S. Von Kleist, 2001. Augmentation of Natural Killer cell activity *in vivo* against tumor cells by some wild plants from Jordan. Phytother. Res., 15: 109-113.

Ahmad, N., S. Gupta and H. Mukhtar, 2000. Green tea polyphenol epigallocatechin 3-gallate differentially modulates nuclear factor kappa B in cancer cells versus normal cells. Arch. Biochem. Biophys., pp: 376-338.

Ahn, W., S. Huh and S. Bae, 2003. A major constituent of green tea, EGCG, inhibits the growth of a human cervical cancer cell line, CaSki cells, through apoptosis, G (1) arrest and regulation of gene expression. DNA Cell. Biol., pp: 22-217.

Alarcon-Aguilara, F.J., R. Roman-Ramos, S. Perez-Gutierrez, A. Aguilar-Contreras, C.C. Contreras-Weber and J.L. Flores-Saenz, 1998. Study of the anti-hyperglycemic effects of plants used as antidiabetics. J. Ethnopharmacol., 61: 101-110.

Amr Amin, Aysha Alkaabi, Shamaa Al-Falasi, Sayel A. Daoud, 2005. Chemopreventive activities of Trigonella foenum graecum (Fenugreek) against breast cancer. Cell. Biol. Int., 29: 687-694.

Basch, E., C. Ulbricht, G. Kuo, P. Szapary and M. Smith, 2003. Therapeutic applications of fenugreek. Alternative Med. Rev., 8: 20-27.

Bin-Hafeez, B., R. Haque, S. Parvez, S. Pandey and I. Sayeed, 2003. Raisuddin S. immunomodulatory effects of fenugreek (Trigonella foenum graecum L.) extract in mice. Int. Immunopharmacol., 3: 257-65.

Bordia, A., S.K. Verma and K.C. Srivastava, 1997. Effect of ginger (Zinger officinale Rosc) and fenugreek (Trigonella foenum graecum L.) on blood lipids, blood sugar and platelet aggregation in patients with coronary heart disease. Prostaglandins Leukot Essent Fatty Acids, 56: 379-84.

Bursch, W., A. Ellinger, C. Gerner, U. Frohwein, R. Schulte-Hermann, 2000. Programmed cell death. Apoptosis, autophagic PCD, or others, Ann. N.Y. Acad. Sci., 926: 1-12.

Chen, Y.C., S.C. Shen and H.Y. Lin, 2003. Rutinoside at C7 attenuates the apoptosis-inducing activity of flavonoids. Biochem. Pharmacol., 66: 1139-1150.

Choi, J., J. Kim and J. Lee, 2001. Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. Int. J. Oncol., 19: 837.

Chow, L.W.C., M.N.B. Cheung, W.T.Y. Loo and X.Y. Guan, 2003. A rat cell line derived from DMBA-induced mammary carcinoma. Life Sci., 73: 27-40.

De La Fuente, M. and V.M. Victor, 2000. Anti-oxidants as modulators of immune function. Immunol. Cell. Biol., 78: 49-54.

Devasagayam, T.P.A. and K.B. Sainis, 2002. Immune system and antioxidants, especially those derived from Indian medicinal plants. Indian J. Exp. Biol., 40: 639-55.

Flammang, A.M., M.A. Cifone, G.L. Erexson and L.F. Stankowski, 2004. Genotoxicity testing of a fenugreek extract. Food and Chem. Toxicol., 42: 1769-1775.

Gee, J., H. Hara and I. Johnson, 2002. Suppression of intestinal crypt cell proliferation and aberrant crypt foci by dietary quercetin in rats. Nutr. Cancer, 43: 193.

Gosslau, A. and K. Yu-Chen, 2004. Nutraceuticals, apoptosis and disease prevention. Nutrition, 20: 95-102.

Hiroshige Hibasami, Hiroyuki Moteki, Kengo Ishikawa, Hirotaka Katsuzaki,kunio Imai, Kazumi Yoshioka1 and Yaeko Ishii, 2003. Takashi Komiya. Protodioscin Isolated from Fenugreek (*Trigonella foenumgraecum* 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. Molecular Med., 11: 23-26.

Iwashita, K., M. Kobori, K. Yamaki and T. Tsushida, 2000. Flavonoids inhibit cell growth and induce apoptosis in B16 melanoma 4A5 cells. Biosci. Biotech. Biochem., 64: 1813-20.

Jayaweera, D.M.A., 1981. Medicinal plant: Part III. Peradeniya, Sri Lanka: Royal Botanic Garden, pp: 255.

Katdare, M., M. Osborne and N. Telang, 2002. Soy isoflavone genistein modulates cell cycle progression and induces apoptosis in HER-2/neu oncogene expressing human breast epithelial cells. Int. J. Oncol., 21: 809.

Kaufman, S.N., 1989. Induction by endonucleolytic DNA cleavage in human acute myelogenous leukemia by etoposide, camptothecin and other cytotoxic anticancer drugs: A cautionary note. Cancer Res., 49: 5870-5878.

Kelloff, G.J., C.W. Crowell, J.A. Stele, V.E. Lubet and C.C. Sigman, 1994. Cancer Epidemiol. Biomarkers, 3: 85.

Khosla, P., D.D. Gupta and R.K. Nagpal, 1995. Effect of Trigonella foenumgraecum L. (Fenugreek) on serum lipids in normal and diabetic rates. Indian J. Pharmacol., 27: 89-93.

Lee, C.Y. and E.H. Baehrecke, 2001. Steroid regulation of autophagic programmed cell death during development. Development, 128: 1443-55.

Leist, M. and M. Jaattela, 2001. Four deaths and a funeral: from caspases to alternative mechanisms. Natl. Rev. Mol. Cell. Biol., 2: 589-98.

Masuda, M., M. Suzui and I. Weinstein, 2001. Effects of epigallocatechin-3-gallate on growth, epidermal growth factor receptor signaling pathways, gene expression and chemosensitivity in human head and neck squamous cell carcinoma cell lines. Clin. Cancer Res., 7: 4220.

Miraldi, E., S. Ferri and V. Mostaghimi, 2001. Botanical drugs and reparations in the traditional medicine of West Azerbaijan (Iran). J. Ethnopharmacol., 75: 77-87.

Okabe, S., Y. Ochiai and M. Aida, 1999. Mechanistic aspects of green tea as a cancer preventive: Effect of components on human stomach cancer cell lines. Jpn. J. Cancer Res., 90: 733.

Paschka, A., R. Butler and C. Young, 1998. Induction of apoptosis in prostate cancer cell lines by the green tea component, (_)-epigallocatechin3-gallate. Cancer Lett., 130: 1.

Rice-Evans, C., 2001. Flavonoid antioxidants. Curr. Med. Chem., 8: 797-807.

Ross, J.A. and C.M. Kasum, 2002. Dietary flavonoids: Bioavailability, metabolic effects and safety. Ann. Rev. Nutr., 22: 19-34.

Ruby, A.J., G. Kuttan, K.D. Babu, K.N. Rajasekharan and R. Kuttan, 1995. Antitumor and anti-oxidant activity of natural curcuminoids. Cancer Lett., 94: 79-85.

Shen, S.C., Y.C. Chen, F.L. Hsu and W.R. Lee, 2003. Differential apoptosis-inducing effect of quercetin and its glycosides in human promyeloleukemic HL-60 cells by alternative activation of the caspase 3 cascade. J. Cell. Biochem., 89: 1044-55.

Shen, S.C., C.H. Ko, S.W. Tseng, S.H. Tsai and Y.C. Chen, 2004. Structurally related antitumor effects of flavanones *in vitro* and *in vivo*: Involvement of caspase 3 activation, p21 gene expression and reactive oxygen species production. Toxicol. Applied Pharmacol., 197: 84-95.

Shen, S.C., W.R. Lee, H.Y. Lin, H.C. Huang, C.H. Ko and L.L. Yang, *et al*. 2002. *In vitro* and *in vivo* inhibitory activities of rutin, wogonin and quercetin on lipopolysaccharide-induced nitric oxide and prostaglandin E2 productions. Eur. J. Pharmacol., 446: 187-94.

Southern California Evidence-Based Practice Center/RAND for Agency on Healthcare Research and Quality, 2001. D.o.H.a.H.SAyurvedic interventions for diabetes mellitus: A systematic review. AHRQ Publication. No. 01-E040.

Sperandio, S., I. de Belle and D.E. Bredesen, 2000. An alternative, nonapoptotic form of programmed cell death. Proc. Natl. Acad. Sci. USA., 97: 14376-81.

Steller, H., 1995. Mechanisms and genes of cellular suicide. Science, 267: 1445-1449.

Thakur, A.M., J.G. Sarvaiya, S.K. Bhavsar and J.K. Malik, 1994. Studies on anti-inflammatory activities of Trigonella in rates. In update Ayureveda, Bombay, India, pp: 75.

Upadhyay, S., M. Neburi and 2001. S. Chinni, Differential sensitivity of normal and malignant breast epithelial cells to genistein is partly mediated by p21 (WAF1). Clin. Cancer Res., 7: 1782.

Valette, G., Y. Sauvaire, J.C. Beccou and G. Ribes, 1984. Hypocholesterolaemic effect of fenugreek seeds in dogs. Atherosclerosis, 50: 105-111.

Yang, G., J. Liao, K. Kim, E. Yurkow and C. Yang, 1998. Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. Carcinogenesis, 19: 611.

Yokoyama, S., H. Hirano, N. Wakimaru, K. Sarker and J. Kuratsu, 2001. Inhibitory effect of epigallocatechin-gallate on brain tumor cell lines *in vitro*. Neuro-oncology, 3: 22.

Yoshikawa, M., T. Murakami, H. Komatsu, N. Murakami, J. Yamahara and H. Matsuda, 1997. Medicinal foodstuffs: IV. Fenugreek seeds. (1): Structures of trigoneosides Ia, Ib, IIb, IIIa and IIIb, new furostanol saponins from the seeds of Indian Trigonella foenum graecum L. Chem. Pharmacol. Bull., 45: 81-87.