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Mechanism of Action of *Trigonella* in Cancer Prevention

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Abstract

Cancer known as the most common cause of death worldwide. Synthetic medicines cause serious side effects and associated with drug resistance. Therefor utilization of natural compounds could be an alternative concept to prevent the development of tumors. Targeting the proliferation or inducing apoptosis is a major critical point in the cancer therapy. Medicinal plants have been related to fight many illnesses including cancer. They have acquired glory as inexpensive and inoffensive substitution as compared to the synthetic drugs. Consumption a diet rich in natural plant can supply an ambience of phytochemicals that possess health-prophylactic effects. *Trigonella* is one of the famous curative plants and used as hot drinks. It is used as anti-tumor agent in many researches. However, proliferation and the induction of apoptosis are regulated by several mechanisms. The current review will discuss the mechanism by which this herb gained its anti-tumor and immune-boosting activities.

Keywords

Trigonella, Mechanism of Action, Cancer Prevention

1. Introduction

Two-thirds of cancer or more may be hold back through life pattern modulation (Oliveria et al., 1997). Chemoprevention is the trial to exploit natural compounds to stop cancer early stages, and prevent invasive disease begins (Greenwald et al., 1995). Natural dietary factors such as vegetables and fruits have a great deal of regard due to their various health promoting effects including inhibition of cancers, (Aggarwal and Shishodia, 2006). Some phytochemicals derived from natural plants possess essential cancer preventive properties (Aggarwal et al., 2008). The chemoprevention factors grouped into two major classes: blocking agents and repressing agents. Blocking agents hold back carcinogenic agents from reaching or reacting with critical target locations by preventing the metabolic activation of carcinogens or tumor promoters via enhancing detoxification systems and via trapping reactive carcinogens (Wattenberg, 1996). Repressing agents prohibit the progress of the neoplastic cells that would further become malignant.

Fenugreek (*Trigonella foenum-graecum* L.; family Fabaceae), is well known as a herb and spice crop (Petropoulos, 2002). Trigonella is grown as a spice crop in parts of North Africa, Mediterranean Europe, West and South Asia and parts of Australia. In North American fenugreek is grown in Canada, Unites States and in South America it has been grown in Argentina (Acharya et al., 2008). In Egypt and India, fenugreek is consumed as a spice and used medicinally as a galactagogue by lactating mothers to increase insufficient breast milk supply (Tiran, 2003); this increase reach to 900% as reported by Fleiss (1988). A study by Muraki et al. (2011) has indicated that the feeding of up to many grams per day over several weeks of fenugreek revealed no signs of toxicity.

In addition to its traditional use, fenugreek has been reported to possess a huge number of medicinal characteristics such as anti-diabetic (Prabhakar and Doble, 2011), hypercholesterolemic, antileukemic, anti-nociceptive, antipyretic and antimicrobial (Acharya et al., 2008; Alshatwi et al., 2013), antioxidant properties (Kaviarasan et al., 2004), it proven its activity against hepatotoxicity (Ulbriht et al., 2007), against glycemia (Neelakantan et al., 2014) and cardiovascular diseases (Sirtori et al., 2009). Fenugreek has anticancer properties (Shabbeer et al., 2009) in fighting colon cancer (Raju et al., 2004) and breast cancer (Amin et al., 2005). Fenugreek also, possess stimulatory role on immune

functions of mice (Bin-Hafeez et al., 2003).

This review focuses on the mechanism of action for fenugreek, a natural herb with confirmed antitumor and immune-boosting properties.

2. Chemical Composition

The bioactive compounds isolated from fenugreek seeds are diosgenin, yamogenin, gitogenin, tigogenin, trigonelline, protodioscin, and polysaccharides (Leung and Foster, 1996). Other compounds with antitumor properties, such as eugenol, gingerol, cedrene, zingerone, and vanillin (Al-Daghri et al., 2012); where their chemical formulae are postulated in figure (1).

In a study by Chatterjee et al. (2013) who revealed positive results for carbohydrates, reducing sugars, secondary metabolites such as tannins, saponins, terpenes, flavanoids, phenols and proanthocyanidins that are found in the aqueous extract of *Trigonella foenum-graecum* L. seeds. These compounds play an important role in exhibiting the suppressing activity against the chemical mutagens and are responsible for antioxidant and anticancer properties. The medicinal properties attributed to fenugreek have been reported to be associated with its unique phytochemicals, such as complex carbohydrates (galactomannans), steroidal sapogenins (diosgenin, yamogenin, tigogenin, neotigogenin), alkaloids (trigonelline) and amino acids (4-hydroxyisoleucine) (Acharya et al., 2008). Three active compounds from fenugreek seed extracts were recognized as the steroidal saponins 26- O-β-D-glucopyranosyl-(25 R)-furost-5(6)-en-3 β,22 β,26-triol-3- O-α-L-rhamno-pyranosyl-(1"→2')-O-[β-D-glucopyranosyl-(1"'→6')-O]-β-D-glucopyranoside1, minutoside B2, and pseudoprotodioscin 3 (Kawabata et al., 2011).

3. Mechanism of Action

The curative effects of fenugreek seeds extract (FE) on cell proliferation in animal model of hepatotoxicity were evaluated by Zargar (2014). Rats were induced liver cirrhosis by thioacetamide and FE was administered orally for 3 weeks after induction which caused reversed results for the oxidative stress and lipid peroxidation. The elevated levels of biochemical liver function enzymes as a result of liver cirrhosis including drug metabolizing enzymes were reversed. This study has entanglement in exploring a treatment for liver cirrhosis by a natural herbal drug with no side effects. Inclusion of FE in the diet of 1,2-dimethylhydrazine (DMH) feeding rats diminished the colon cancer incidence to 16.6%, inhibited the lipid peroxidation (LPO). An increased the activities of glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) in the liver; which are considered the first line of cellular defense against oxidative injury.

FE modulates DMH-stimulated hepatic oxidative stress during colon tumor (Devasena et al., 2002; 2007). The author showed that FE exerts its chemopreventive impact via reducing LPO and stimulating antioxidant levels. SOD decreases superoxide anion to form H_2O_2 and O_2. CAT removes H_2O_2 by breaking it down directly to O_2 (Silambarasan and Raja, 2012). Diosgenin (DG) holds back the inhibition in the activity of all the above enzymatic antioxidants in hepatic and renal tissues in rats with renal failure (Manivannan et al., 2013). DG revoked tumor necrosis factor-alpha (TNF-α) induced production of intracellular reactive oxygen species (ROS) and phosphorylation of mitogen activated protein kinases (MAPK) as reported by Choi et al. (2010).

Cyclophosphamide (CP) is a known antitumor drug; that causes toxicity by its reactive metabolites such as phosphoramide mustard and acrolein. The alteration of CP toxicity accompanied by the FE treatment was delineated by the measurement of antioxidants in mice urinary bladder. CP-treated mice exhibited a significant decrease in the activities of first line of cellular defense enzymes such as: CAT, GPx, GST, glutathione (GSH), and glutathione reductase (GR) as compared to controls. On the other hand, LPO level were increased. Pre-treatment of FE regained activities of all these enzymes showing a protective effect against CP (Bhatia et al., 2006). The authors reported that the increase of GSH levels by FE may play an essential role in turning over CP-induced apoptosis and free radical-mediated LPO.

Raju et al. (2004) showed that the continuous feeding of FE or DG to azoxymethane-induced rat colon cancer inhibited the number of crypt foci by comparison with the control during initiation and promotion stages. The promotion of apoptosis in HT-29 cells at least in part by decrease of Bcl-2 and stimulation of caspase-3 expression. While, Devasena et al. (2003) showed that the inhibition of beta-glucuronidase can stop the carcinogens from working on colonocytes. Animals given 1,2-dimethylhydrazine (DMH) showed an increase in the mucopolysaccharidase activity in colon and feces. This activity was inhibited as a result of FE treatment.

Protodioscin (PD) a compound extracted from fenugreek was studied by Hibasami et al. (2003), for its effects on cell viability in human leukemia HL-60 cells. PD showed strong growth repression against this cell line, by the presence of apoptotic bodies in the HL-60 cells. The treatment with 2.5, 5, and 10 microM of PD for 3 days revealed that hypodiploid nuclei were increased reaching 75.2, 96.3, and 100%, respectively. DNA was fragmented to oligonucleosomal-sized fragments; a sign of apoptosis, which was proportional with time and concentration of PD.

Chatterjee et al. (2012) delineated significant reduction of papillomas due to treatment with FE (400 mg/kg). Pre-initiational, post-initiational, promotional, and throughout the experiment treatment with FE were conducted along with 7,12-dimethylbenz [a] anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA). Mice treated with DMBA+TPA+FE showed inhibition of the tumor incidence, cumulative number of papillomas, tumor yield and tumor burden when compared with control. The pre-initiation of FE were more powerful than the treatment during, and/or after

DMBA treatment. Again, FE showed a modulatory effect on hepatic antioxidant defense enzymes as GSH and LPO levels. In another study, the antimutagenic and anticarcinogenic activity of FE (2.5, 5, 10 and 20 g/100 ml) using TA98 and TA100 strains of *Salmonella* and skin papilloma model in mice, were investigated by Chatterjee et al. (2013). Inhibition in reverting colony count in FE treated mice was obtained. At 20 g/100 ml of FE, a powerful reduction rate was delineated in both strains of *Salmonella* against different mutagens. The same author tested FE with a dose level 400 mg/kg/day; this dose showed an inhibition in the total chromosomal aberrations and micronuclei score throughout all the stages of papillomagenesis. The antineoplastic effect of FE has been conducted in the Ehrlich ascites carcinoma (EAC) model. Balb-C mice were administered with FE intra-peritoneally, before and after EAC cell inoculation. FE produced more than 70% inhibition of tumor cell growth with respect to the control. FE showed anti-inflammatory and antineoplastic effects appeared by the enhancement of both the peritoneal exudate cell and macrophage cell counts (Sur et al., 2001).

Ali et al. (2013) used the murine 2-stage skin carcinogenesis model to prove the chemopreventive effect of FE for 32 weeks in mice. FE inhibited the number, incidence, and multiplicity of tumors. There was a significant reduction in the proliferating cell nuclear antigen (PCNA) positive nuclei. Tumor proliferation was decreased by the modulation of the endogenous antioxidant defense, inhibition of the signal-transducing element NF-kB, enhancement of immunosurveillance of the genetically mutated cells, induction stable p53, along with silencing of the cell cycle progression signals.

The early apoptotic and the inhibition of cell viability effect of FE appeared by its ability to decrease mitochondrial membrane potential, phosphatidylserine flipping and DNA fragmentation. Sebastian et al. (2007) showed that the presence of a subG1 apoptotic population which was prominent with cell cycle arrest at G2/M phase which induced apoptosis in MCF-7 breast cancer cell line. MCF-7 proliferation was induced by FE; showed binding to estrogen receptor (ER) that it might function as an agonist or antagonist via estrogen receptor element (ERE) as reported by Sreeja et al. (2010); Sreeja and Thampan (2004). FE induced the expression of estrogen responsive gene pS2; which is as an estrogen inducible transcript and encoded for a secretary protein from MCF-7 cells (Ahmad et al., 2001), and it is considered as an evidence for estrogenic effect of FE. The excess of estrogen can cause breast, endometrial, ovarian, and prostate cancer (Lewiecki, 2009). FE effect is similar to the effects of estradiol; inducing cell proliferation in MCF-7. This effect found to be suppressed by ER antagonist ICI 182-780. Following the same line, Sreeja and Thampan (2004) confirmed that FE bound ER and decreased the combination of labeled estradiol to ER in a concentration dependent manner.

N-Methyl-N-nitrosourea (NMU) is a mammary gland-specific carcinogen that closely mimics human breast cancer in many aspects. Jagadeesan et al. (2012) investigated the anticarcinogenic property of 20 mg/kg DG with reference to lipid peroxidation and status of antioxidants. DG treatment remarkably down regulated the peroxidation reaction and extraordinarily enhanced the indigenous antioxidant defense system. The factor for this restoration might be due to the effect of the intervention strategy on the down regulation of the peroxidation reaction through the strong antioxidant nature.

Breast cancer MCF-7 apoptosis was proved by the treatment with FE (65 μg/mL for 24 and 48 hours). The induction of apoptosis was reported by the increase of Fas receptor expression (Alshatwi et al., 2013). The mechanism by which apoptosis can be induced is the expression of anti- and pro-apoptotic proteins. There are two pathways which are responsible for apoptosis; which are the mitochondrial apoptotic pathways and death receptor pathways. The mitochondria have an essential effect in regulating the caspase cascade and apoptosis (Shafi et al., 2009). Bax and Bak are the key molecules in the mitochondrial pathways apoptosis, were interdependently stimulated by p53, causing the release of mitochondrial cytochrome-c caused an activation of procaspase-9 directly and then caspase-3 from caspase 8 & 9 indirectly (Malik et al., 2007; Shafi et al., 2009). Caspase-3 activation is key role downstream stage in the apoptotic pathway (Earnshaw et al., 1999; Alshatwi, 2010). In addition, the effector caspase-3, and the initiator caspase-8 and 9, are the main executors of apoptosis (Riedl and Shi, 2004). Caspase-8 considered as the death receptor pathway whereas caspase-9 considered as the mitochondrial pathway, and both pathways share caspase-3 (Pommier et al., 2004). Caspase-8 activates crosstalk between the death receptor pathway and the mitochondrial pathway by the cleavage of Bid to tBid, a pro-apoptotic member of the Bcl-2 family. This cleavage of Bid showed to be associated with caspase-8 activation. This activation in turn, has a central role in Fas-mediated apoptosis (Malik et al., 2007).

Das et al. (2012) investigated the antineoplastic activity of DG against A431 and Hep2 cell carcinoma in vitro and sarcoma 180-induced tumors. DG induced cytotoxicity by inhibited cell proliferation in A431 and Hep2 cells. Many factors proved the ability of DG induced apoptosis and suppress cell proliferation. These factors are the 1) increasing the subG1 population, Live/Dead cytotoxicity, chromatin condensation, and finally DNA laddering. 2) increasing of Bax/Bcl-2 ratio which means the inhibition of Bcl-2 and the increase of Bax, activation of caspases and cleavage of poly ADP ribose polymerase were observed in treated cells. 3) decreasing of Akt and c-Jun N-terminal kinase (JNK) phosphorylations. 4) activation of p53, stop the cell cycle, and releasing caspase-3 (Corbiere et al., 2004). Another report suggested that DG apoptotic activity is due to the suppression of the extracellular signal regulating kinase (ERK), JNK and phosphoinositide 3-kinase (PI3K)/Akt signaling pathways, nuclear factor kappa B (NF-κB) activity and NF-κB–regulated gene expression, inhibiting matrix metalloproteinases and osteoclastogenesis (Chen et al., 2011).

Bcl-2 family plays an essential role in controlling

activation of caspases. Data reported by Das et al. (2012) showed that Bcl-2 protein inhibition and Bax stimulation, a mechanism by which DG-induced apoptosis in A431 and Hep2 cells. The elevated Bax/Bcl-2 ratio provides an apoptotic stimulus via mitochondrial cytochrome c releasing, that activate caspase-3 and cleavage of PARP cleavage. Caspase-3 is partially or totally responsible for cleavage of many key proteins, including the nuclear enzyme PARP. Cleavage of PARP correlated well with chromatin condensation in apoptotic cells leading to DNA fragmentation; the hallmarks of apoptosis. DG inhibited pAkt expression; a survival signaling in these cells in a time-dependent manner. Following the same line, Chiang et al. (2007), indicated that DG inhibited Akt and JNK phosphorylation in cases of HER2 breast cancer; which involved in cell cycle control and leading to apoptosis. Suppression of the MAPK and PI3K/Akt pathway (Chien et al., 2010) and inhibition of Ki-67 and CD31 positive cells (Yang et al., 2008) due to the treatment of DG; may have the potential to prevent angiogenesis, proliferation, invasion and metastasis of tumors.

DG suppresses proliferation, osteoclastogenesis and inhibits via the suppression of NF-κB gene and TNF-induced activation of Akt (Shishodia and Aggarwal, 2006). Cellular chemoprotectors worked against cancer evoke large inductions of phase II enzymes of carcinogens metabolism and increase GSH levels in tissues. Significant increase in GSH and decrease in LPO in the group with FE extract treatment before DMBA and TPA application were also reported. GSH provide the protection to the intracellular proteins against oxidation by glutathione redox cycle and detoxifying ROS and/or neutralizing reactive intermediate species generated from exposure to carcinogens (Bhatia et al., 2006). GSH has a key role in preserving the suppressed state of cellular environment, in addition to its binding ability owing to nucleophilic center and its involvement in detoxification of carcinogens. This mechanism would inhibit the reactive electrophiles available to combine with DNA, causing the reducing of DNA damage and possible induction of tumor incidence (Seo et al., 2000).

Incubation of Jurkat cells (leukaemic cell lines) with FE ranging from 30 μg/mL to 1500 μg/mL for 3 days caused death of cancerous cells. This effect was proportional to the time and the doses used of FE. This effect was proved by the appearance of Jurkat cells with cytoplasmic vacuoles, membrane degeneration, and increased LC3 expression transcripts; (a marker of autophagy process). Therefore, the stimulation of autophagy considered as another mechanism by which FE could fighting cancer, beside its apoptotic properties (Al-Daghri et al., 2012). Apoptosis and autophagy are two pathways responsible for programmed death of cancerous and stressed cells. Autophagy is the process of transport of cytoplasmic contents to the lysozomes (Klionsky and Emr, 2000); a physiological response to stress and has been suggested to enable cells to adapt and survive. Therefore, this process is considered to be as a pro-survival mechanism (Chen and Debnath, 2010). Autophagy disturbances in cancers revealed that it will be a true tumor-suppressor pathway (Levine and Kroemer, 2008).

DG stopped proliferation in different human prostate cancer PC-3 cells lines; in a dose-dependent manner. Tumor invasion was markedly suppressed by the reduction of of matrix metalloproteinase-2 (MMP-2), MMP-9, mRNA level of MMP-2, -9, -7 and extracellular matrix metalloproteinase (EMMPRIN), while tissue inhibitor of metalloproteinase-2 (TIMP-2) was increased. DG cancelled the increase of vascular endothelial growth factor (VEGF) in PC-3 cells and tube formation of endothelial cells. DG potently suppressed the phosphorylation of phosphatidylinositide-3 kinase (PI3K), Akt, ERK and JNK and nuclear NF-κB (Samuels and Ericson, 2006; Chen et al., 2011). The suppression role of DG on MMPs, nuclear NF-κB, and the induction role of IκBα responsible for its anti-metastatic potential.

A key step in cancer growth and its metastasis, is the angiogenesis which is regulated via VEGF (Carmeliet and Jain, 2000). DG suppressed endothelial cell tube formation, which is the first step in angiogenesis. This effect could be mediated by the inhibition of VEGF expression in different prostate PC-3 cell lines. Therefore, leading to the suppression of tumor growth. Prostate cancer cell lines (PCa) treated with 10-15 ug/mL of FE, the experiment lased for 72 h revealed an inhibitory effect on tumor growth. This effect represented by the appearance of molecular changes in DU-145 cell lines by the inhibition of mutant p53, and by the stimulation of p21 and the suppression of transforming growth factor-beta (TGF-β) induced phosphorylation of Akt in PC-3 cells. Shabbeer et al. (2009) reported that cancer cell death occurred although the stimulation of tumor growth pathways which being simultaneously up regulated by FE. Again, the anticancer role of FE and/or its constituents may be involved by decreased nitric oxide (NO) and prostaglandin production via inhibiting COX-2 and iNOS, in osteosarcoma cell line (Varjas et al., 2011). FE and DG blocked activation of NF-KB, I kappa B kinase, Akt, suppressed the pro-inflammatory cytokines like IL-1, IL-6 and TNF-α production and blocked tumor cells proliferation (Srinivasan et al., 2009). Feeding on FE to DBMA treated rats caused the formation of cytoplasmic vacuoles in breast tumor tissue (Amin et al., 2005).

4. Conclusion

Fenugreek extract could control many types of cancers via different mechanisms. The following scheme summarized the mechanism of action pathway by which fenugreek and their constituents control cancer. The fighting mechanisms can be concluded in the following steps:

1. The modulation of the antioxidant enzymes.
2. Stimulation Bax and decreasing Bcl-2 expression, which increased Bax/Bcl-2 ratio.
3. Suppress TNF-α, JNk phosphorylation, ERK, Akt, Ki-67, CD31, IL-6, IL-1, TGF-β, iNOS, COX-2, VEGF and NF-kB.
4. Stimulation of caspase-3 & 9, cleavage of PARP, p21, p53, cytochrome c,

5. Increase chromatin condensation and DNA fragmentation leading to apoptosis in the tumor cells, by increasing the subG1 population with cell cycle arrest at G2/M phase.

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References

[1] Acharya SN, Thomas JE, Basu SK. (2008). Fenugreek (*Trigonella foenum-graecum* L.) an alternative crop for semiarid regions of North America. Crop Sci; 48:841-853.

[2] Aggarwal BB, Kunnumakkara AB, Harikumar KB, Tharakan, ST, Sung B. (2008). Potential of spice derived phytochemicals for cancer prevention. Planta Med; 74:1560-1569.

[3] Aggarwal BB, Shishodia S. (2006). Molecular target of dietary agents for prevention and therapy of cancer. Biochem Pharmacol; 71:1397-1421.

[4] Ahmad N, Adhami VM, Afaq F, Feyes DK, Mukhtar H. (2001). Resveratrol causes waf-1/p21-mediated phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. Clin Cancer Res; 7: 1466 -1473.

[5] Al-Daghri NM, Alokail MS, Alkharfy KM, Mohammed AK, Abd-Alrahman SH, Yakout SM, Amer OE, Krishnaswamy S. (2012). Fenugreek extract as an inducer of cellular death via autophagy in human T lymphoma Jurkat cells. BMC Complement Altern Med; 12:202.

[6] Ali F, Khan R, Khan AQ, Lateef MA, Maqbool T, Sultana S. (2013). Assessment of Augmented Immune Surveillance and Tumor Cell Death by Cytoplasmic Stabilization of p53 as a Chemopreventive Strategy of 3 Promising Medicinal Herbs in Murine 2-Stage Skin Carcinogenesis. Integr Cancer Ther; 13(4):351-367

[7] Alshatwi AA. (2010). Catechin hydrate suppresses MCF-7 proliferation through TP53/Caspase-mediated apoptosis. J Exp Clin Cancer Res; 17: 167.

[8] Alshatwi AA, Shafi G, Hasan TN, Syed NA, Khoja KK. (2013). Fenugreek induced apoptosis in breast cancer MCF-7 cells mediated independently by fas receptor change. Asian Pac J Cancer Prev; 14(10):5783-5788.

[9] Amin A, Alkaabi A, Al-Falasi S, Daoud SA. (2005). Chemopreventive activities of *Trigonella foenum graecum* (Fenugreek) against breast cancer. Cell Biol Int; 29(8):687-694.

[10] Bhatia K, Kaur M, Atif F, Ali M, Rehman H, Rahman S, Raisuddin S. (2006). Aqueous extract of *Trigonella foenum-graecum* L. ameliorates additive urotoxicity of buthionine sulfoximine and cyclophosphamide in mice. Food Chem Toxicol.; 44(10):1744-1750.

[11] Bin-Hafeez B, Haque R, Parvez S, Pandey S, Sayeed I, Raisuddin S. (2003). Immunomodulatory effects of fenugreek (*Trigonella foenum graecum* L.) extract in mice. Int Immunopharmacol; 3(2):257-265.

[12] Carmeliet P, Jain RK. (2000). Angiogenesis in cancer and other diseases. Nature; 407:249–257.

[13] Chatterjee S, Goswami N, Bhatnagar P, Kumar M, Kumar A. (2013). Antimutagenic and chemopreventive potentialities of fenugreek (*Trigonella foenum-graecum*) seed extract. Oxid Antioxid Med Sci; 2(1):45-53

[14] Chatterjee S, Kumar M, Kumar A. (2012). Chemomodulatory Effect of *Trigonella foenum graecum* (L.) Seed Extract on Two Stage Mouse Skin Carcinogenesis. Toxicol Int; 19(3):287-294.

[15] Chen N, Debnath J. (2010). Autophagy and tumorigenesis. FEBS Lett; 584(7):1427-1435.

[16] Chen PS, Shih YW, Huang HC, Cheng HW. (2011). Diosgenin, a steroidal saponin, inhibits migration and invasion of human prostate cancer PC-3 cells by reducing matrix metalloproteinases expression. PLoS One; 6(5):e20164.

[17] Chiang C-T, Way T-D, Tsai S-J, Lin J-K. (2007). Diosgenin, a naturally occurring steroid, suppresses fatty acid synthase expression in HER2-overexpressing breast cancer cells through modulating Akt, mTOR and JNK phosphorylation. FEBS Lett; 581: 5735–5742.

[18] Chien CS, Shen KH, Huang JS, Ko SC, Shih YW. (2010). Antimetastatic potential of fisetin involves inactivation of the PI3K/Akt and JNK signaling pathways with downregulation of MMP-2/9 expressions in prostate cancer PC-3 cells. Mol Cell Biochem; 333: 169–180.

[19] Choi KW, Park HJ, Jung DH, Kim TW, Park YM, Kim BO, Sohn EH, Moon EY, Um SH, Rhee DK, Pyo S. (2010). Inhibition of TNF-α-induced adhesion molecule expression by diosgenin in mouse vascular smooth muscle cells via downregulation of the MAPK, Akt and NF-κB signaling pathways. Vascul Pharmacol; 53:273-280

[20] Corbiere C, Liagre B, Terro F, Beneytout JL. (2004). Induction of antiproliferative effect by diosgenin through activation of p53, release of apoptosis-inducing factor (AIF) and modulation of caspase-3 activity in different human cancer cells. Cell Res; 14: 188–196.

[21] Das S, Dey KK, Dey G, Pal I, Majumder A, MaitiChoudhury S, kundu SC, Mandal M. (2012). Antineoplastic and apoptotic potential of traditional medicines thymoquinone and diosgenin in squamous cell carcinoma. PLoS One; 7(10):e46641.

[22] Devasena T, Menon VP. (2002). Enhancement of circulatory antioxidants by fenugreek during 1,2-dimethylhydrazine-induced rat colon carcinogenesis. J Biochem Mol Biol Biophys; 6(4):289-292.

[23] Devasena T, Menon VP. (2003). Fenugreek affects the activity of beta-glucuronidase and mucinase in the colon. Phytother Res; 17(9):1088-1091.

[24] Devasena T, Menon VP. (2007). Fenugreek seeds modulate 1,2-dimethylhydrazine-induced hepatic oxidative stress during colon carcinogenesis. Ital J Biochem; 56(1):28-34.

[25] Earnshaw WC, Martins LM, Kaufmann SH. (1999). Mammalian caspases Structure, activation, substrates, and functions during apoptosis. Ann Rev Biochem; 68: 383-424.

[26] Fleiss P. (1988). Herbal remedies for the breastfeeding mother. Mothering Summer: 68-71.

[27] Greenwald P, Kelloff G, Burch-Whitman C. (1995). Chemoprevention. CA Cancer J. Clin. 45:31-49.

[28] Hibasami H, Moteki H, Ishikawa K, Katsuzaki H, Imai K, Yoshioka K, Ishii Y, Komiya T. (2003). 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 Mol Med; 11(1):23-36.

[29] Jagadeesan J, Nandakumar N, Rengarajan T, Balasubramanian MP. (2012). Diosgenin, a steroidal saponin, exhibits anticancer activity by attenuating lipid peroxidation via enhancing antioxidant defense system during NMU-induced breast carcinoma. J Environ Pathol Toxicol Oncol; 31(2):121-129.

[30] Kaviarasan S, Vijayalakshmi K, Anuradha CV. (2004). Polyphenol-rich extract of fenugreek seeds protect erythrocytes from oxidative damage. Plant Food Hum Nutr; 59(4): 143-147.

[31] Kawabata T, Cui MY, Hasegawa T, Takano F, Ohta T. (2011). Anti-inflammatory and anti-melanogenic steroidal saponin glycosides from Fenugreek (*Trigonella foenum-graecum* L.) seeds. Planta Med; 77(7):705-710.

[32] Klionsky DJ, Emr SD. (2000). Autophagy as a regulated pathway of cellular degradation. Science; 290(5497):1717-1721.

[33] Leung AY, Foster S. (1996). Encyclopaedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics. 2nd ed, John Wiley & Sons, New York, NY, USA.

[34] Levine B, Kroemer G. (2008). Autophagy in the pathogenesis of disease. Cell; 132(1):27-42.

[35] Lewiecki EM. (2009). Phytoestrogens and their role in the management of postmenopausal osteoporosis. South Med J; 102: 111-122.

[36] Malik F, Kumar A, Bhushan S, Khan S, Bhatia A, Suri KA, Qazi GN, Singh J. (2007). Reactive oxygen species generation and mitochondrial dysfunction in the apoptotic cell death of human myeloid leukemia HL-60 cells by a dietary compound with aferin A with concomitant protection by N-acetylcysteine. Apoptosis; 12(11): 2115-2133.

[37] Manivannan J, Arunagiri P, Sivasubramanian J, Balamurugan E. (2013). Diosgenin prevents hepatic oxidative stress, lipid peroxidation and molecular alterations in chronic renal failure rats. Int J Nutr Pharmacol Neurol Dis; 3(3):289-293

[38] Muraki E, Hayashi Y, Chiba H, Tsunoda N, Kasono K. (2011). Dose-dependent effects, safety and tolerability of fenugreek in diet-induced metabolic disorders in rats. Lipids Health Dis; 10:240.

[39] Neelakantan N, Narayanan M, de Souza RJ, van Dam RM. (2014). Effect of fenugreek (Trigonella foenum-graecum L.) intake on glycemia: a meta-analysis of clinical trials. Nutr J; 13:7

[40] Oliveria SA, Christos PJ, Berwick M. (1997). The role of epidemiology in cancer prevention. Proc Soc Exp Biol Med; 216:142-150.

[41] Petropoulos GA. (2002). Fenugreek: The genus *Trigonella*. Taylor and Francis, London, UK.

[42] Pommier Y, Sordet O, Antony S, Haywrd RL, Kohn KW. (2004). Apoptosis defects and chemotherapy resistance: molecular interaction maps and networks. Oncogene; 23: 2934-2949.

[43] Prabhakar PK, Doble M. (2011). Mechanism of action of natural products used in the treatment of diabetes mellitus. Chin J Integr Med; 17(8):563-574.

[44] Raju J, Patlolla JM, Swamy MV, Rao CV. (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(8):1392-1398.

[45] Riedl SJ, Shi Y. (2004). Molecular mechanisms of caspase regulation during apoptosis. Nat Rev Mol Cell Biol; 5: 897-907.

[46] Samuels Y, Ericson K. (2006). Oncogenic PI3K and its role in cancer. Curr Opin Oncol;18:77–82.

[47] Sebastian KS, Thampan RV. (2007). Differential effects of soybean and fenugreek extracts on the growth of MCF-7 cells. Chem Biol Interact; 170(2):135-143.

[48] Seo KW, Kim GJ, Park M, Kim TW, Kim HJ. (2000). Effects of phenethylisothiocyanate on the expression of glutathione S transferase and hepatotoxicity induced by acetaminophen. Xenobiotica; 30:535-543.

[49] Shafi G, Munshi A, Hasan TN, Alshatwi AA, Jyothy A, Lei DK. (2009). Induction of apoptosis in HeLa cells by chloroform fraction of seed extracts of *Nigella sativa*. Cancer Cell Int; 27: 9-29.

[50] Shabbeer S, Sobolewski M, Anchoori RK, Kachhap S, Hidalgo M, Jimeno A, Davidson N, Carducci MA, Khan SR. (2009). Fenugreek: a naturally occurring edible spice as an anticancer agent. Cancer Biol Ther; 8(3):272-278.

[51] Shishodia S, Aggarwal BB. (2006). Diosgenin inhibits osteoclastogenesis, invasion, and proliferation through the downregulation of Akt, I kappa B kinase activation and NF-kappa B-regulated gene expression. Oncogene; 25(10):1463-1473.

[52] Silambarasan T, Raja B. (2012). Diosmin, a bioflavonoid reverses alterations in blood pressure, nitric oxide, lipid peroxides and antioxidant status in DOCA-salt induced hypertensive rats. Eur J Pharmacol; 679:81-89

[53] Sirtori CR, Galli C, Anderson JW, Sirtori E, Arnoldi A. (2009). Functional foods for dyslipidaemia and cardiovascular risk prevention. Nutr Res Rev; 22(2):244-261.

[54] Sreeja S, Thampan RV. (2004). Proteins which mediate the nuclear entry of goat uterine non activated estrogen receptor (naER) following naER internalization from the plasma membrane. Mol Cell Biochem; 259: 141-148.

[55] Sreeja S, Anju VS, Sreeja S. (2010). In vitro estrogenic activities of fenugreek *Trigonella foenum graecum* seeds. Ind J Med Res;131:814-819.

[56] Srinivasan S, Koduru S, Kumar R, Venguswamy G, Kyprianou N, Damodaran C. (2009). Diosgenin targets Akt-mediated prosurvival signaling in human breast cancer cells. Int J Cancer; 125(4):961-967.

[57] Sur P, Das M, Gomes A, Vedasiromoni JR, Sahu NP, Banerjee S, Sharma RM, Ganguly DK. (2001). *Trigonella foenum graecum* (fenugreek) seed extract as an antineoplastic agent. Phytother Res; 15(3):257-259.

[58] Tiran D. (2003). The use of fenugreek for breast feeding women. Complem Therap Nursing Midwifery; 9: 155-156.

[59] Ulbricht C, Basch E, Burke D, Cheung L, Ernst E, Giese N, Foppa I, Hammerness P, Hashmi S, Kuo G, Miranda M, Mukherjee S, Smith M, Sollars D, Tanguay-Colucci S, Vijayan N, Weissner W. (2007). Fenugreek (*Trigonella foenum-graecum* L. Leguminosae): an evidence-based systematic review by the natural standard research collaboration. J Herb Pharmacother; 7(3-4):143-177.

[60] Varjas T, Nowrasteh G, Budan F, Horvath G, Cseh J, Gyongyi Z, Makai S, Ember I. (2011). The effect of fenugreek on the gene expression of arachidonic acid metabolizing enzymes. Phytother Res; 25(2):221-227.

[61] Wattenberg LW. (1996). Chemoprevention of cancer. Prev Med; 25:44-55.

[62] Yang SX, Steinberg SM, Nguyen D, Wu TD, Modrusan Z, Swain SM. (2008). Gene expression profile and angiogenic marker correlates with response to neoadjuvant bevacizumab followed by bevacizumab plus chemotherapy in breast cancer. Clin Cancer Res; 14: 5893–5899.

[63] Zargar S. (2014). Protective effect of *Trigonella foenum-graecum* on thioacetamide induced hepatotoxicity in rats. Saudi J Biol Sci; 21 (2):139–145.