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Anticancer potential of Trigonella foenum graecum: Cellular and molecular targets

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ABSTRACT

A growing body of evidence supported by numerous studies on tumorigenesis confirms that it is possible to target various hallmarks of cancer. Recent studies have shown that plant-derived molecules may be used in targeting different signaling pathways for cancer drug discovery. The present paper gives an insight into the anticancer potential of fenugreek and lists the existing studies that have been carried out to demonstrate the advantages of the use of fenugreek in cancer treatment and prevention. It also aims at opening up new perspectives in the development of new drugs of natural origins in the future clinical trials. This review article will discuss; (1) the chemical constituents and bioactive compounds of fenugreek; (2) effects on oxidative stress and inflammation; (3) effects on proliferation, apoptosis, and invasion; (4) toxicity of fenugreek; and 5) future directions in cancer drug development. All of the experimental studies discussed in this paper suggest that multiple signaling pathways (hallmarks) are involved in the anticancer activities of fenugreek, but their efficacy is still unclear, which requires further investigation.

Contents

Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; Akt, Ak strain thymoma (serine/threonine-specific protein kinase); ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; Bak, Bcl-2 homologous antagonist/killer; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; Cdc42, cell division cycle 42; C-myc, avian myelocytomatosis virus oncogene cellular; CNS, central nervous system; COX-2, cyclooxygenase-2; C-Src, rous sarcoma oncogene cellular homolog; DNA, deoxyribonucleic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ED_{50}, median effective dose; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ERK, extracellular signal-regulated kinase; FADD, fas-associated protein with death domain; Fas, factor ligand superfamily (tumor necrosis factor ligand super-family); γ-GT, gamma-glutamyltransferas; FRAP, ferric reducing antioxidant power; HER2, human epidermal receptor 2; IKK-β, inhibitor of nuclear factor kappa-B kinase subunit beta; IL-6, interleukin-6; IL-1β, interleukin-1beta; iNOS, inducible nitric oxide synthase; JAK, janus kinase; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; microRNA, microRibonucleic acid; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; MTT, 4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf-2, nuclear factor (erythroid-derived 2)-like 2; ORAC, oxygen radical absorbance capacity; p21, cyclin-dependent kinase inhibitor 1; p53, phosphoprotein 53; PARP, poly (ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; PKC, protein kinase C; Raf, v-Raf murine sarcoma viral oncogene homolog B; Ras, Kirsten rat sarcoma; RT-PCR, reverse transcription polymerase chain reaction; STAT-3, signal transducer and activator of transcription; TGF-β, transforming growth factor-β; TIMP-2, tissue inhibitor of metalloproteinases 2; TNF-α, tumor necrosis factor-α; TRAIL, TNF-related apoptosis-inducing ligand; Vav2, guanine nucleotide exchange factor VAV2; VEGF, vascular endothelial growth factor.

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1. Introduction

Cancer is a complex and heterogeneous disease developing through a multi-stage and progressive process [1–4]. The model proposed by Hanahan and Weinberg is widely accepted as a major advance in the understanding of the biology of cancer cells [5–8]. The proposed hallmarks include (1) resisting cell death, (2) deregulating cellular energetics, (3) sustaining proliferative signaling, (4) evading growth suppressors, (5) avoiding immune destruction, (6) inducing angiogenesis, (7) enabling replicative immortality, (8) activating invasion and metastasis, (9) genome instability, and (10) tumor promoting inflammation. Therefore, signaling pathways underlying these organizing principles were extensively investigated in preclinical and clinical drug development [6,9–11]. In spite of the fact that there is an enormous arsenal of synthetic, semi-synthetic, and naturally-occurring anticancer agents, high toxic events and chemoresistance are a major problem in oncology practice. Emerging anticancer approaches should involve the determination of novel drug targets that must be highly effective and specific against cancer development and growth with limited toxic side-effects. In recent decades, the discovery of cancer drugs has grown rapidly and hundreds of new active compounds of natural origin were examined to be used in clinical trials, especially with the emergence of high throughput technologies for the screening of natural compounds [12–14]. Those natural anticancer products are not only regarded as necessarily cytotoxic but also as cytostatic [14]. Moreover, many potential chemopreventive secondary metabolites in plant extracts, as well as in purified molecules isolated from teas, herbs, spices, fruits, vegetables, and marine sources have been explored [15–19].

Fenugreek (*Trigonella foenum graecum*) is one of these medicinal plants used by cancer patients and the general population and is considered one of the oldest traditional medicinal herbs, cultivated in India, the Mediterranean region, and North Africa [20–22]. Bibliometric data indicates that the number of publications and clinical trials about fenugreek have steadily increased in the era of re-emergence of natural products in drug development (Supplementary Table S1, Fig. 1). Fenugreek seeds contain more biologically active substances which have widely described for their pharmacological activities (Supplementary Table S2) [23,24]. Fenugreek extracts and compounds were found to target at least five hallmarks of cancer including, proliferation, inflammation, angiogenesis, invasion, and metastasis. This review aims to discuss the anticancer potential of fenugreek and lists the existing studies that have been carried out to demonstrate the advantages of the use of fenugreek in targeting hallmarks of cancer.

2. Chemical constituents and bioactive compounds

Table 1 summarizes the chemical composition of fenugreek. Fenugreek seeds contain simple alkaloids consisting mainly of trigonelline, choline, gentianine, and carpaine. Trigonelline (an important marker with estrogenic, anti-diabetic, and anti-invasive properties) is degraded during roasting to nicotinic acid and other pyridines and pyrroles [25,26]. Other constituents include saponins that yield on hydrolysis, steroid sapogenins; yamogenin tetrosides B and C; flavonoids such as quercetin, luteolin, vitexin cinnamate, vicenin and isovitexin suggested to support the anticancer activity of fenugreek (for chemical structures see Fig. 2) [27,28].

Fenugreek contains also a considerable amount of mucilage rich in galactomannan and proteins [29,30]. Diosgenin is a major bioactive steroidal saponin of various edible pulses and roots, well characterized in the seeds of fenugreek known as a chemopreventive/therapeutic agent against cancers of several organ sites [31]. Many components of fenugreek possess also significant anticancer activities. These include gingerol, cedrene, zingerone, vanillin, and eugenol [32]. A recent Chromatography-Mass Spectrometry (GC–MS) analysis of fenugreek seeds showed the presence of fourteen bioactive compounds such as terpenoids and flavonoids, including two main constituents with anticancer activity, squalene, and naringenin (27.71% and 24.05%, respectively) [33]. Importantly, fenugreek seeds are also rich sources of vitamins, minerals, and antioxidants, which help to protect the cells from free radical-induced oxidative injury [34].

3. Effects on oxidative stress and inflammation

Oxidative stress is a biochemical disturbance between the production of free radicals and the highly reactive metabolites, known as reactive oxygen species (ROS), and their turnover by protective antioxidants [35,36]. Oxidative stress is linked to a wide variety of human diseases including cancer [37–39]. Chronic inflammation causes release of chemical mediators by immune cells, in particular, ROS, which can damage intracellular molecules and promote cancer promotion and progression by altering many cell signaling pathways [36,40].

During ROS-induced carcinogenesis; an increase in DNA mutations, genome instability, and cell proliferation is observed [41–43]. Oxidative stress affects various biochemical pathways related to cancer initiation and progression such as TNF-α, EGFR, VEGF, Nrf2, Ras, Raf, MAPK, MEK, MMP, p53, PKC, and mTOR; all known as key regulators in cancer cell proliferation, metabolism, survival, motility, and invasion [37,42]. In turn, tumors induce inflammation and contribute to multiple capabilities acting on the tumor stroma by release of growth factors, enzymes, pro-angiogenic and inductive signals to promote invasion, epithelial mesenchymal transition and metastasis. This is a major hallmark of cancer considered to be a promising druggable target for future cancer therapy development [6,44,45]. Interestingly, supplemental antioxidants have become an important strategy in ROS-scavenging, and therefore cancer chemoprevention (or pro-oxidants sensitizing cancer cells to chemotherapy) [46–50]. In this direction, plant compounds such as polyphenols have an important place as

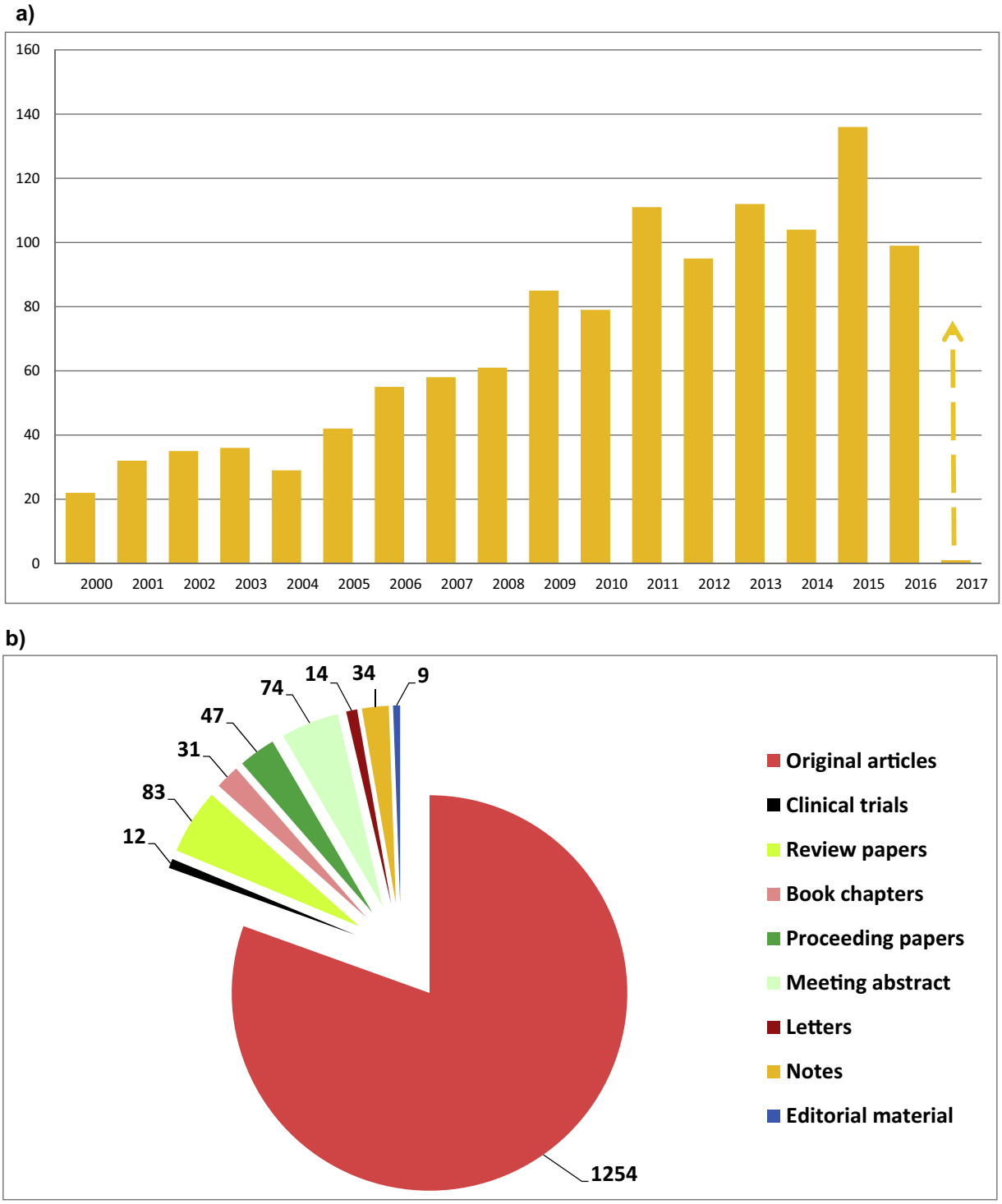

Fig. 1. Evolution of research on Fenugreek. (a) Evolution of published Web of Science (WOS-Thomson Reuters™)-indexed biomedical articles from 2000 to 2017, related to several areas of fenugreek's research; data for this figure were extracted from WOS by searching the term "fenugreek". (b) Type of publications indexed in Web of Science (WOS-Thomson Reuters™) and ClinicalTrials, related to several areas of fenugreek's research; data for this figure were extracted from WOS and *ClinicalTrials.gov* by searching the term "fenugreek".

preventive agents. Moreover, phytocompounds can decrease adverse effects caused by oxidative stress related to current anticancer regimens; and their combination with chemotherapeutic agents is crucial [51–54].

Among plants with antioxidant activity, fenugreek has been extensively studied in several *in vivo* and *in vitro* studies. Dixit et al. found a significant antioxidant potential of germinated fenugreek seeds using various *in vitro* assays, including inhibition of lipid peroxidation, FRAP, DPPH, ABTS, ORAC, and pulse radiolysis [55]. Also, fenugreek ethyl acetate crude extract demonstrated high antioxidant activities *in vitro*, using FRAP and DPPH assays [22]. In a cataract model induced by selenite (selenite-induced oxidative stress transparent cultured lenses), fenugreek significantly restored the activities of antioxidative enzymes such as superoxide dismutase (SOD), glutathione-S-transferase (GST), catalase (CAT), and glutathione peroxidase [56]. Several recent *in vivo* studies have

Table 1
Main chemical components of Fenugreek.

Class of chemical constituents	Chemical components
Saponins and saponisides	Foenugraecine, trigofoenoside A, other glycosides of diosgenin, trigogenin and yangogenin, many steroidal sapogenins
Carbohydrates	Cellulose, hemicellulose; mucilage: galactomannan; phytin
Flavonoids	Quercetin, rutin, vitexin, isovitexin, vincennes, derivatives of orientin
Amino acids	Isoleucine, 4-hydroxyisoleucine, histidine, leucine, lysine, L-tryptophan, arginine
Coumarins	Scopoletin, lactone orthodihydroxycinnamic acid, methyl coumarin, trigocoumarin, trimethylcoumarin
Alkaloids	Trigonelline, choline, gentianine, carpaine, betain, neurin
Fibers (50%)	Gum, neutral detergent fiber

demonstrated the hepatoprotection of the phytoconstituents of fenugreek extracts against oxidative stress, lipid peroxidation and morphological changes in the diabetic rats, suggesting its preventive and antioxidant effect against liver damages [57–59]. Sakr and Abo-El-Yazid evaluated the effect of the aqueous extract of fenugreek seeds (0.4 g/kg body weight (bw)) against hepatotoxicity induced by adriamycin in albino rats. An increase in SOD and CAT was observed, very important enzymes in protecting tissues from oxidative damage by ROS [60].

Promisingly, the effects of fenugreek seed extract, trigonelline, and diosgenin against hepatic induced-oxidative stress in rats were found to exhibit protective effects individually. They also reduced significantly the expression of liver endoplasmic reticulum stress biomarkers, and increased hepatic antioxidants [58]. In another study, supplementation by seed essential oil of fenugreek was tested against acrylamide-induced hepatotoxicity in male Wistar rats. It was found to normalize altered status of many biomarkers related to oxidative stress, including TNF-α, IL-1β, IL-6 LDH, AST, ALT, APL, and γ-GT by its potent scavenging properties [61]. Xue et al. showed that the seed aqueous extract of fenugreek re-established the kidney function of diabetic rats via its antioxidant property; the ultra-morphologic alterations in the kidney of diabetic rats, including the unequal thickening of the glomerular basement membrane, were improved by fenugreek extract treatment. This nephroprotection was correlated with the richness of fenugreek in antioxidants [62]. *In vivo* studies of the ethanolic and the methanolic seed extracts revealed their anti-inflammatory effect on a number of animal models [63–65]. Yet, another study by Mandegary et al. done on flavonoid rich fractions of fenugreek seeds showed its inhibitory effect on the carrageenan-induced paw edema test in male rats [66]. Kawabata et al. conducted an *in vitro* study to assess the anti-inflammatory effect of the methanol extract of the fenugreek seeds using human monocytic cell line (THP-1); a suppression of TNF-α production was observed [67]. Another study conducted by Liu et al. found an inhibitory effect of the methanol extract of fenugreek seeds against lipid peroxidation and cyclooxyganase enzyme [68]. Additionally, diosgenin, a major component of fenugreek was found to significantly inhibit TNF-α-induced tissue factor and expression in monocytes by down-regulation of the phosphorylation of ERK, Akt, NF-κB/p65, IKK-β, and JNK [69]. Therefore, fenugreek can be considered to be a preventive agent against inflammation and ROS-induced cancer in which free radicals have been implicated.

4. Effects on proliferation, apoptosis, and invasion

4.1. Fenugreek extracts

4.1.1. In vitro studies

Many studies have demonstrated the anticancer effect of fenugreek extracts in experimental models of cancer using cell lines (Table 2). An early study in cell lines has shown that the ethanolic extract of fenugreek, with an ED_{50} less than 10 μg/mL, possesses antineoplastic effects in A-549 male lung carcinoma, MCF-7 female breast cancer, and HT-29 colon adenocarcinoma cell lines [81]. Later, Sebastian and Thampan [78] examined the effect

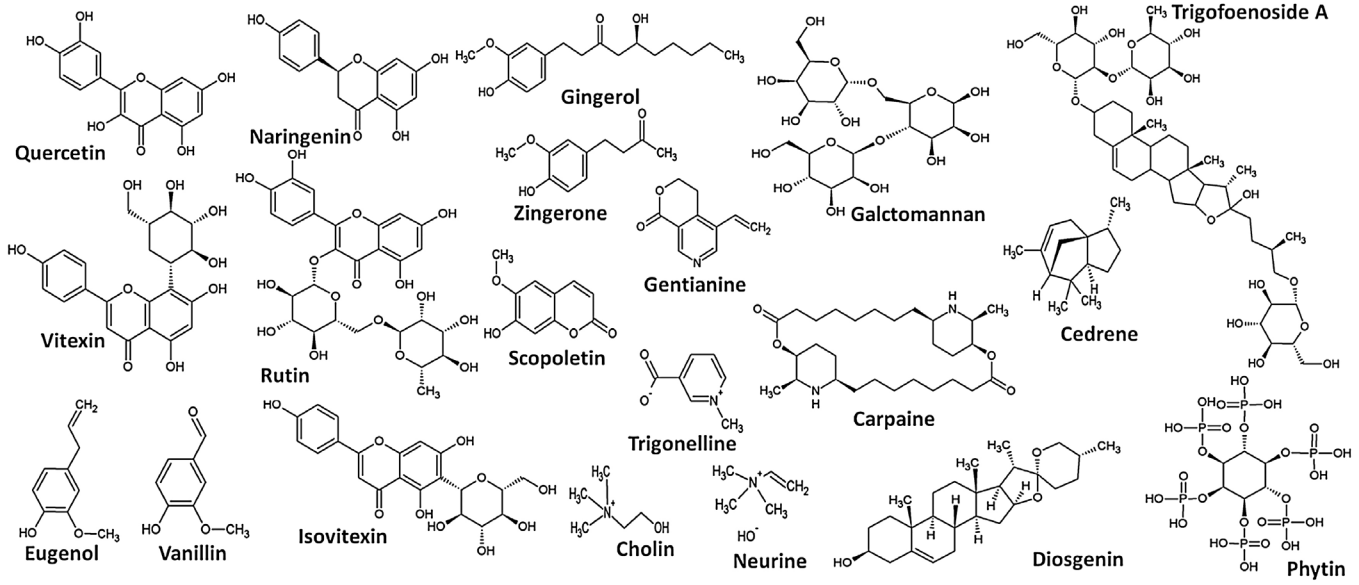

Fig. 2. Chemical structures of fenugreek bioactive compounds.

of extracts of fenugreek (aqueous and ethanol) on the growth of MCF-7 cancer cells, an estrogen receptor positive breast cancer cell line, and reported that the ethanol extract of fenugreek decreased the viability and induced apoptotic modifications such as inversion of phosphatidylserine and decreased mitochondrial membrane potential. Moreover, degradation of DNA into multiple fragments (approximately 180–200 base pair) has also been observed. Findings of this study revealed a cell cycle arrest at G2/M phase in apoptotic populations after analysis of the cell cycle of fenugreek extract treated cells implicating its role in inducing apoptosis [78].

Moreover, Shabbeer et al. [20] showed that treatment with fenugreek seed extract (10–15 ug/mL for 72 h) inhibited the growth of prostate, pancreatic and mammary breast cancer cell lines. Yet, primary prostate or immortalized prostate cells remained unaffected. Notably, this study suggests that this anticancer activity is due to some molecular changes induced in cancer cells lines: down-regulation of mutant p53 in DU-145 cells, and up-regulation of p21 and inhibition of TGF-β induced phosphorylation of Akt in PC-3 cells.

In another study, fenugreek extract was found to reduce cancer stem cell properties and to increase sensitivity to doxorubicin treatment (one of the effective agent for breast cancer treatment), by directly targeting mutant p53, Notch4 and microRNAs [82]. In addition, fenugreek (50 μg/mL for 24 h) exhibits anticancer effects by blocking the proliferation of MCF-7 cells and inducing apoptosis by modulating expression levels of caspase-3, 8, 9, p53, Fas, FADD, Bax and Bak using RT-PCR [75]. Later, this team demonstrated that the methanol fenugreek extract at 65 μg/mL for 24 and 48 h induced breast cell apoptosis (MCF-7 cells). This team further demonstrated that the methanol fenugreek extract at 65 μg/mL for 24 and 48 h induced apoptosis by activation of the extrinsic death pathway (Fas and FADD), caspase 8 or 3 and as well as p53 in a dose and time-dependent manner [73].

Recently, Alsemari et al. [72] have observed selective cytotoxic effects of fenugreek extract *in vitro* to a panel of cancer cell lines (T-cell lymphoma-TCP, and human Thyroid papillary carcinoma-FRO). They measured the apoptosis and necrosis of normal and cancer cells using Annexin V apoptosis assay kit, viable cells, necrotic cells,

Table 2
Anticancer activities of fenugreek extract *in vivo* and *in vitro*.

Cellular and molecular targets	Extract	Cancer cell type	Author/year
Anti −proliferative effect reducing ascites development and cancer transformation to solid mass	Boiled water extract of fenugreek seeds 20% (w/v)	Mouse Lymphocytic Leukemia (L1210)	Zailei et al. [70]
Apoptosis by up-regulation of p53, Bax and PCNA-dependent pathway, and G1 phase arrest	Crude methanol extract of fenugreek	Hepatocellular Carcinoma Cell Line, HepG2	Khalil et al. [33]
Decrease in NF-κB expression in nuclei of cells, and NF-κB-dependent genes expression (C-myc, Bcl-xL and Cox-2)	Fenugreek powder (250 mg/kg of bw)	Ca755 mammary carcinoma and Lewis lung carcinoma in intracranial and subcutaneously grafeted rats	Bentrad et al. [71]
Selective cytotoxic effects	100 ug/ml, 200 ug/ml and 300 ug/ml of aqueous fenugreek extract for 0, 24, 48, 72 and 96 h	T-cell lymphoma (TCP), B-cell lymphomas, Thyroid Papillary carcinoma (FRO) and human breast cancer (MCF7)	Alsemari et al. [72]
Apoptosis by Fas receptor activation	Methanol fenugreek extract at 65 μg/mL for 24 and 48 h	Breast Cancer MCF-7Cells	Alshatwi et al. [73]
Membrane disintegration, appearance of large vacuoles, and increase in the expression of autophagic marker LC3 transcripts	Ethanolic extract of dry fenugreek seeds at concentrations ranging from 30 to 1500 μg/mL for up to 3 days	Jurkat cell line	Al-Daghri et al. [32]
Reduction in tumor rate/tumor incidence, tumor yield, cumulative number of papillomas, average weight of tumors	Methanolic extract of fenugreeek 400 mg/kg b.wt orally	Skin papillomas in Swiss albino mice	Chatterjee et al. [74]
Inhibition of proliferation and inducing apoptosis by modulating expression levels of caspase-3, 8, 9, p53, Fas, FADD, Bax and Bak	Fenugreek (50 μg/mL for 24 h)	Breast Cancer MCF-7Cells	Khoja et al. [75]
Increase in lifespan	Ethanol leaf extract of fenugreek	Ehrlich ascites carcinoma cells in Swiss albino mice	Prabhu and Krishnamoorthy [76]
Alterations of Ehrlich ascites cells and inhibition of tumor growth mediated through enhancement and activation of macrophages	Alcohol extract of fenugreek seeds (100 mg/kg and 200 mg/kg daily for 5 days)	Ehrlich ascites carcinoma model on mice	Ardelean et al. [77]
Inhibition of growth, down-regulation of mutant p53 in DU-145 cells, and up-regulation of p21 and inhibition of TGF-β induced phosphorylation of Akt in PC-3 cells	Fenugreek seed extract (10–15 ug/mL for 72 h)	Prostate (DU-145, LNCaP and PC-3), pancreatic (MiaPaCa, HS766T, Panc1, L3.6PL and BXPC3) and breast cancer (MDA-MB-231, MCF-7, T47D and SKBR3) cell lines	Shabbeer et al. [20]
Ethanol extract decreased the viability and induced apoptotic modifications. Degradation of DNA, cycle arrest at G2/M phase in apoptotic populations.	Extracts of fenugreek (aqueous and ethanol)	MCF-7 cancer cells	Sebastian and Thampan [78]
Decrease in colon tumor incidence and increase in activities of antioxidant hepatic enzymes such as GST, SOD and catalase	Diet containing fenugreek seed powder	DMH-treated rats	Devasena and Menon [79]
Inhibition of DMBA and decrease the incidence of mammary hyperplasia.	Fenugreek seed extract	Mammary hyperplasia in female Wistar rats	Amin et al. [80]
Anti-tumor activity	Ethanolic extract of Trigonella foenum-graecum, with an ED_{50} less than 10 g/mL	A-549 male lung carcinoma, MCF-7 female breast cancer and HT-29 colon adenocarcinoma cell lines	Alkofahi et al. [81]

Abbreviations: Akt: Ak strain thymoma (serine/threonine-specific protein kinase); Bak: Bcl-2 homologous antagonist/killer; Bax: Bcl-2-associated X protein; Bcl-xL: B-cell lymphoma-extra large; C-myc: Avian myelocytomatosis virus oncogene cellular; COX-2: Cyclooxygenase-2; DMBA: 7,12-dimethylbenz(a)anthracene; DNA: Deoxyribonucleic acid; ED_{50}: median effective dose; FADD: Fas-associated protein with death domain; GST: Glutathione-S-transferase; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; p21: cyclin-dependent kinase inhibitor 1; p53: Phosphoprotein 53; PCNA: Proliferating cell nuclear antigen; SOD: Superoxide dismutase; TGF-β: Transforming growth factor-β.

and apoptotic cells by using Annexin V immune-staining. They reported significant fractions of apoptotic cells when normal T-cell lymphocytes and cancer cells (T-cell lymphoma-TCP, and human Thyroid papillary carcinoma-FRO) were treated with 300 μg/mL of fenugreek seed extracts for 24 and 72 h respectively. Using a Jurkat cell line, Al-Daghri et al. [32] demonstrated that incubation with an ethanolic extract of dry fenugreek seeds at concentrations ranging from 30 to 1500 μg/mL for up to 3 days, caused distinct histological changes involving membrane disintegration, appearance of large vacuoles, and increased expression of LC3 transcripts indicates that fenugreek extract induced autophagy and autophagy-associated death of Jurkat cells as new antineoplastic activity of fenugreek, in addition to already known apoptosis activation. Therefore, autophagy may be an additional mechanism underlying the

Table 3
Anticancer activities of active compounds *in vivo* and *in vitro*.

Cellular and molecular targets	Active compound and dose	Experimental model	Author/year
Inhibition of proliferation	Diosgenin 40, 80 and 100 μM	1547 Osteosarcoma cells	Moalic et al. [85]
	Diosgenin 40 μM		Corbiere et al. [86]
	Diosgenin 40 μM	HEL Erythroleukemia cells	Leger et al. [87]
	Diosgenin 15 mg/kg	Adenocarcinomas in rat	Malisetty et al. [88]
	Diosgenin 10 mmol/L	Human cancer cells, A431, A2780, A549, K562, and HCT-15	Wang et al. [89]
	Diosgenin 20 and 30 μM	Human breast cancer MCF-7 and MDA 231 tumor xenografts in mice	Srinivasan et al. [90]
	Diosgenin 200 mg/kg	Mouse LA795 lung adenocarcinoma tumors	Yan et al. [91]
	Diosgenin 5,10 and 20 μM	PC-3 human prostate cancer cells	Chen et al. [92]
	Diosgenin 20 mg/kg	A431 and Hep2 cells	Das et al. [93]
	Diosgenin 20, 100 and 500 mg/kg in the diet	Colon adenoma + adenocarcinoma in mice	Miyoshi et al. [94]
p53 activation	Diosgenin 40 μM	M4Beu Melanoma cells	Corbiere et al. [95]
	Diosgenin 40 μM	HEp-2 Laryngocarcinoma	Corbiere et al. [95]
	Diosgenin 40 μM	1547 Osteosarcoma cells	Corbiere et al. [86]
	Diosgenin 20, and 40 μM	MCF-7 Breast carcinoma cells	Sowmyalakshmi et al. [96]
PARP cleavage	Diosgenin 50 μM	Human hepatocellular carcinoma cells HCC cells	Li et al. [97]
Inhibition of STAT-3 signaling pathway, suppression of the activation of c-Src, JAK1 and JAK2	Diosgenin 100 μM	Human hepatocellular carcinoma cells HCC cells	Li et al. [97]
Caspase-3 activation	Diosgenin 20 μM	K562 Leukemia cells	Liu et al. [98]
	Diosgenin 50 μM	Human hepatocellular carcinoma cells HCC cells	Li et al. [97]
NF-κB activation	Diosgenin 40 μM	1547 Osteosarcoma cells	Corbiere et al. [86]
	Diosgenin 50 and 100 μM	Multiple myeloma (U266), leukemia (U937), and breast cancer (MCF-7)	Shishodia and Aggarwal [78]
	Diosgenin 5, 10 and 20 μM	PC-3 human prostate cancer cells	Chen et al. [92]
Down-regulation of Bcl-2	Diosgenin 20, 40, and 60 μM	MDA breast carcinoma cells	Raju et al. [99]
Inhibition of Akt and JNK phosphorylations	Diosgenin 20 mg/kg	A431 and Hep2 cells	Das et al. [93]
Modulation of Akt, mTOR, and JNK phosphorylation	Diosgenin 5–20 μM	HER2-overexpressing cancer cells	Chiang et al. [100]
protein kinase Cα (PKCα) and Raf/ERK/Nrf2 signaling pathway and MMP-7 gene expression were involved in the trigonelline-mediated migration inhibition of Hep3 B cells	Trigonelline 75–100 μM	Hepatocarcinoma Hep3 B cells	Liao et al. [101]
Suppress the ROS-induced increase in invasive capacity.	Trigonelline 2.5–40 μM	Hepatoma cell line of AH109A	Hirakawa et al. [25]
Cytotoxic effects	4-hydroxyisoleucine 0.5–64 mM	HepG2 and Huh-7 hepatoma cell lines	Babaei et al. [102]

Abbreviations: Akt: Ak strain thymoma (serine/threonine-specific protein kinase); Bcl-2: B-cell lymphoma 2; C-Src: Rous sarcoma oncogene cellular homolog; ERK: Extracellular signal-regulated kinase;JAK: Janus kinase; JNK: c-Jun N-terminal kinase; MMP: Matrix metalloproteinase; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf-2:Nuclear factor (erythroid-derived 2)-like 2; p53: Phosphoprotein 53; PARP: Poly (ADP-ribose) polymerase; Raf: v-Raf murine sarcoma viral oncogene homolog B; STAT-3: Signal transducer and activator of transcription.

anticancer properties of fenugreek [32]. According to Khalil et al. [33], GC–MS analysis of the methanol extract of fenugreek contains many important phytochemicals known for their anticancer potential, such as flavonoids, tricin, naringenin, quercetin and squalene. In their study, the crude methanol extract of fenugreek induced cell death in hepatocarcinoma cell line (HepG2) via inducing apoptosis by up-regulation of p53, Bax and PCNA-dependent pathway, and G1 phase arrest that was confirmed by cell cycle analysis.

4.1.2. In vivo studies

In animal models (Table 2), Sur et al. [83] showed that intraperitoneal administration of the alcohol extract of fenugreek seeds before as well as after inoculation of Ehrlich ascites carcinoma (EAC) cells in Balb-C mice decreased tumor cell growth by more than 70%, compared to the control. This result was supported by two recent studies; the first one, conducted by Prabhu and Krishnamoorthy [76] reported the anticancer activity in EAC cells in Swiss albino mice orally treated by ethanol extract of fenugreek leaf at 100, 200 and 400 mg/kg (bw) for ten consecutive days. The other study, conducted by Ardelean et al. [77], investigated the intraperitoneal treatment of mice with an alcohol extract of fenugreek seeds (100 mg/kg and 200 mg/kg daily for 5 days).They found that fenugreek produced alterations of Ehrlich ascites cells and inhibition of tumor growth. Furthermore, Amin et al. [80] also demonstrated that fenugreek seed extract inhibited 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary hyperplasia in female Wistar rats, decreased its incidence. They suggested that fenugreek's anti-breast cancer protective effects could caused by its richness in flavonoids and may cause increased cell death. A diet containing fenugreek seed powder reduced colon tumor incidence in 1,2-Dimethylhydrazine (DMH)-treated rats and increased activities of antioxidant hepatic enzymes such as GST, SOD, and CAT [79]. In addition, the effect of oral administration of methanolic extract of fenugreek seeds (400 mg/kg bw) on two-stage mouse skin carcinogenesis, shows a reduction in tumor rate, tumor incidence, tumor yield, cumulative number of papillomas, average weight of tumors as well as a modulatory effect on mouse hepatic antioxidant status [74].

Recently, Bentrad et al. [71] have conducted an *in vivo* study to evaluate the molecular mechanisms of anticancer activity of fenugreek using Wistar rats with intracranial grafted C6 glioma, rats with subcutaneously grafted Guerin carcinoma and Guerin carcinoma substrains resistant to doxorubicin and cisplatin, as well as C57Bl/6 mice with grafted Lewis lung carcinoma and grafted Ca755 mammary carcinoma. Administration of the fenugreek powder (250 mg/kg of bw) increased the lifetime of animals by 15–50%. Also, a decrease of the average volume of metastases by 18–86% was observed. A mechanistic analysis of these results found that fenugreek increased the level of global DNA methylation, reduced the NF-κB expression in cells nuclei and decreased the NF-κB-dependent genes expression (C-myc, Bcl-xl and Cox-2) [71]. This study suggests that the mechanisms of the antitumor action of fenugreek maybe mediated by NF-κB-dependent signaling pathways and their influence on DNA methylation. More recently, an anti-proliferative effect of water extract of fenugreek seeds on the growth of Leukemia L1210 cells induced ascities in experimental mice has been shown to reduce ascites development and cancer transformation to a solid mass [70]. Interestingly, the anticancer activities of fenugreek seed oil (10–1000 μg/mL for 24 h) against cancer cell lines, including human epidermoid cancer cells (HEp2), human breast adenocarcinoma cells (MCF-7), human amniotic epithelial cells (WISH), and a normal cell line African green monkey kidney cells (Vero) have shown that fenugreek seeds oil significantly reduced the cell viability, and altered the cellular morphology in a dose-dependent manner [84].

4.2. Fenugreek bioactive compounds

Several studies have investigated the potential anticancer effects of fenugreek bioactive compounds such as trigonelline and diosgenin on various *in vitro* and *in vivo* models (Table 3). These compounds were found to be effective against a large variety of cancer lines and animal models as well as to target many signaling pathways involved in cancer hallmarks.

4.2.1. In vitro studies

Trigonelline, the major component of alkaloids in fenugreek, is also considered an anticarcinogenic agent [103]. Hirakawa et al. suggested that trigonelline suppresses the ROS-induced increase in the invasive capacity of hepatoma cells AH109A without affecting the proliferation of the cells [25]. In a recent study, Liao et al. [101] have shown that the trigonelline-mediated migration inhibition of Hep3 B cells through PKCα, the Raf/ERK/Nrf2 signaling pathway as well as MMP-7 gene expression. They have also demonstrated that trigonelline inhibits Hep3 B cell migration through down-regulation of nuclear factor E2-related factor 2–dependent antioxidant enzymes activity. In addition, 4-hydroxyisoleucine at different concentrations (0.5–64 mM) has been reported to have cytotoxic effects on HepG2 and Huh-7 hepatoma cell lines [102].

Diosgenin, a major sapogenin found in fenugreek seed, has been shown to suppress inflammation, inhibit proliferation, and induce apoptosis in a variety of tumor cells [78]. Several preclinical studies have demonstrated its anticancer properties. In a study by Shishodia and Aggarwal [78], it has been reported that diosgenin effects causes the suppression of osteoclastogenesis in RAW-264.7 cells following a pro-apoptotic mechanism through interruption of NF-κB pathway, and suppresses TNF-induced invasion. In HER2-overexpressing breast cancer cells, diosgenin was reported to suppress fatty acid synthase expression through modulating Akt, mTOR, and JNK phosphorylation [100], suggesting a new role of diosgenin as chemopreventive or chemotherapeutic agent for cancers that overexpress HER2.

Interestingly, Li et al. [97] showed that diosgenin could modulate the signaling pathway of STAT3 in hepatocellular carcinoma by suppressing the activation of c-Src, JAK1, and JAK2. Also, diosgenin down-regulated the expression of STAT3-regulated genes, inhibited proliferation and potentiated the apoptotic effects of induced apoptosis by doxorubicin and paclitaxel, suggesting that diosgenin could be a novel and potential bioactive compound for the treatment option in hepatocellular carcinoma and other cancers [97]. More recent evidence shows that HT-29 colon cancer cells died immediately after treatment with dioscin (a bioactive compound from fenugreek analog to diosgenin) above 7.5 μM [104]. Dioscin at 5 μM increased sub-G1 phase cells when assessed by flow cytometric analysis. Since caspase-3 activity was increased by dioscin, cytotoxicity of dioscin might be related to apoptosis [104]. Hibasami et al. [105] revealed that protodioscin, a steroidal saponin bioactive compound of fenugreek, exhibited a strong inhibitory activity against the leukemic cell line HL-60 by activating apoptosis as well as a weak proliferative inhibitory effect on the gastric cancer cell line KATO-III. In skin cancer, thymoquinone and diosgenin have antiproliferative and apoptotic properties mediated through the caspase, JNK and Akt pathway in A431 and Hep2 squamous cell carcinoma cell lines [93]. The synergistic effects of this combination show potential in suppressing sarcoma 180–induced solid tumors in mice [93].

Very recently, Mohammad et al. have evaluated the antiproliferative effects of diosgenin and its synthesized triazole derivatives against several human cancer cells lines including A549 (lung), viz. HBL-100 (breast), HT-29 (colon) and HCT-116 (colon) using MTT assay [106]. In this structure-activity relationship study, both diosgenin and synthetic analogues with a simple phenyl R moiety were found to exhibit potent antiproliferative properties against all these cancer cells lines. Furthermore, diosgenin has been reported to induce cell death in the HCT-116 resistant colorectal cancer cell line via TRAIL-induced apoptosis. This pro-apoptotic action was associated with the involvement of p38/MAPK signaling pathway, which explains the induced-cell death [107]. Furthermore, Romero-Hernández et al. developed diosgenin-based glycoconjugates as potent antiproliferative agents against HeLa cells, MDA-MB-231, MCF-7 and HepG2 cancer cell lines; apoptosis was found to be involved in this anticancer effect [108]. Importantly; a conjugation of diosgenin to methotrexate (MTX) was investigated in the MDA-MB-231 cell line (transport-resistant breast cancer cell line) [109]. All of the substituted MTX exhibited an inhibition of dihydrofolate reductase (a key enzyme in DNA synthesis), and have antiproliferative effect. This strategy provided a promising approach to overcome resistance in many cancers treated by MTX. Diosgenin has also been shown to induce apoptosis and autophagy by examining autophagic flux, including autophagosomes accumulation, autophagosome-lysosome fusion, and degradation of autophagosomes [110]. This diosgenin-induced anticancer activity was accompanied by an inhibition of mTOR pathway and ROS release, which explains the cytotoxicity against chronic myeloid leukemia cells. Another study on a human mammary carcinoma cell line MCF-7 also advocates the suppression of proliferation in a dose-dependent manner [111].

Another hallmark involved in invasion and metastasis is angiogenesis that occurs excessively in some cancers, such as ovarian and colorectal cancers [112–116]. Thus, the inhibition of angiogenesis is an important target for natural compounds in cancer drug discovery [117]. Several recent studies have demonstrated the effects of diosgenin against cancer cell motility, invasion and angiogenesis (major cancer hallmar) [118]. He et al. performed *in vitro* experiments to investigate the metastatic effects of diosgenin on human breast cancer MDA-MB-231 cell line. In this study, diosgenin caused a significant inhibition of cell migration under real-time observation. In addition, the authors found that diosgenin significantly inhibited polymerization of actin and phosphorylation of Vav2 and activation of Cdc42 oncoproteins known for their action during invasion and metastasis [119].

Importantly, in another study investigating the antimetastatic properties of non toxic doses of diosgenin against human prostate cancer PC-3; cell migration and invasion were found to be significantly suppressed using *in vitro* Boyden chamber invasion and wound healing assays [92]. Moreover, diosgenin reduced the activities of matrix metalloproteinase-2 (MMP-2) and MMP-9, key enzymes in matrix degradation and stroma invasion. Not only this, but the authors also demonstrated that diosgenin suppressed the mRNA levels of these enzymes and increased the levels of tissue inhibitor of metalloproteinase-2 (TIMP-2). Expression of VEGF was found abolished in tube formation of endothelial cells and PC-3 prostate cancer cells. Furthermore, an inhibition of ERK, JNK, PI3 K/Akt, NF-κB activity was observed [92]. Mao et al. demonstrated in a hypoxia-sensitive gastric cell line BGC-823 using invasion cell assay (Chemicon QCM™ 24-well Invasion Assay Kit) that diosgenin inhibited invasion, suggesting that this effect may be related to the expression of E-cadherin, integrinα5 and integrin β6, incriminated in cell adhesion and invasion of the tumor microenvironment [120].

4.2.2. In vivo studies

Using the azoxymethane-induced rat colon carcinogenesis model (HT-29 human colon cancer model), Raju et al. [99] showed that dietary fenugreek seed and diosgenin reduced and retarded the appearance of colonic aberrant crypt foci (ACF) during the initiation/progression stages of colon carcinogenesis and even when given only during the promotional stage. In addition, diosgenin inhibits cancer cell proliferation and induces apoptosis by suppressing the expression of the antiapoptotic Bcl-2 while increasing the expression of the proapoptotic enzymes such as caspase-3 [99].

In a double-blind study designed to assess the potential chemopreventive properties of diosgenin on azoxymethane (AOM)-induced rat colon carcinogenesis, Malisetty et al. [88] showed that 0.1% of diosgenin suppressed significantly the incidence of both invasive and non-invasive colon adenocarcinomas by up to 60% and suppressed colon tumor multiplicity (adenocarcinomas/rat) by up to 68%. By contrast in mice, diosgenin at doses of 20, 100 and 500 mg/kg (bw) in the diet did not alter the incidence of colon tumors (adenoma + adenocarcinoma) induced by AOM/dextran sodium sulfate, but reduced the tumor multiplicity significantly at all the three tested doses [94]. It has been reported that 10 mg/kg (bw) of diosgenin, administered intratumorally, significantly inhibited the growth of human breast cancer MCF-7 and MDA 231 tumor xenografts in mice [90]. Diosgenin induced p53 tumor suppressor protein in ER positive MCF-7 human breast cancer cells, and the activation of caspase-3 and down-regulation of Bcl-2 in ER-negative MDA human breast carcinoma cells [90]. In addition, oral administration of diosgenin significantly inhibited the growth of mouse LA795 lung adenocarcinoma tumors by 33.94% in T739 inbred mice [91]. Similarly, Jagadeesan et al. investigated the therapeutic potential of diosgenin against DMBA-induced hamster buccal pouch carcinogenesis and found that diosgenin administered orally (80 mg/kg bw) significantly reduced the formation of oral tumors [121].

This suggests that fenugreek compounds affect multiple targets, acting on cell cycle activation, apoptosis, autophagy, angiogenesis, invasion and metastasis. So, it is undoubtedly clear from these various studies that fenugreek compounds appear to be the most promising candidates for developing anticancer agents in future clinical trials.

5. Effects on anticancer drug–induced toxicities

Every cancer therapy is associated with some adverse events. The list of possible toxicities is extensive; some are tissue-specific such as kidney and pulmonary fibrosis, hepatic cytolysis, mucositis, azoospermia, and myelosuppression, while others are more systemic like behavioral dysfunction, cachexia, malaise, anorexia, and fatigue [122] (Further information can be found in [123]). For example, the clinical application of cisplatinum (a highly effective chemotherapeutic agent used to treat many types of solid tumors) is limited by its deleterious side effects, in the male reproductive system [124,125]. In order to best use anticancer drugs, an addition of pharmacological management such as corticosteroids, analgesics, and hystamine-2 antagonists can attenuate chemotherapy-induced toxicities [122]. In view of these considerations, a recent study provides evidence that treatment with fenugreek could effectively alleviate cisplatin-induced testicular toxicity through both the inhibition of apoptosis and the abrogation of oxidative stress and iNOS and NF-κB inflammatory response associated with cisplatin treatment [126]. The use of adriamycin (another potent and effective chemotherapeutic agent in the treatment of several forms of cancer) is also limited by the development of cardiotoxicity, nephrotoxicity, and hepatotoxicity. Administration of fenugreek seeds extract has been shown to have

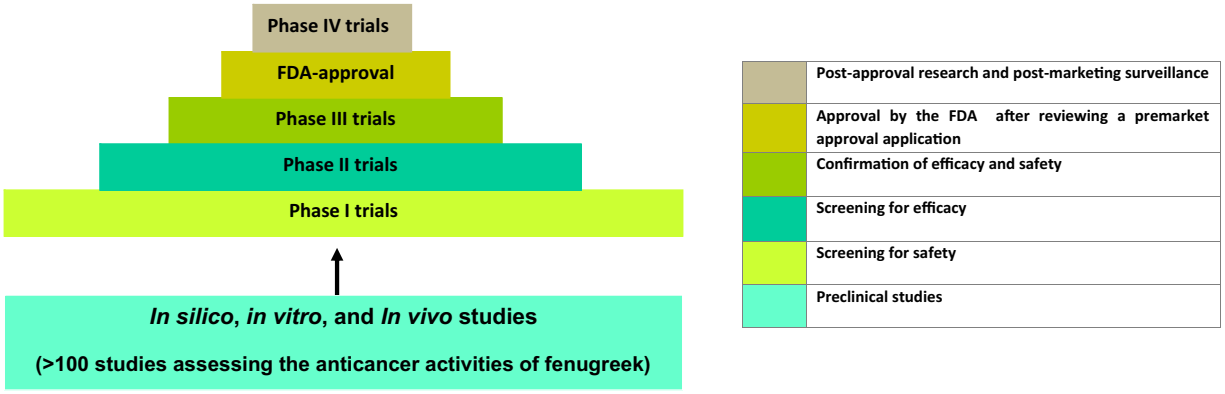

Fig. 3. The drug development process.

ameliorative effects against adriamycin-induced cytogenetic toxicity and testicular damage in albino rats by the enhancement of the activities of the antioxidant enzymes (CAT and SOD) and the reduction of lipid peroxidation [127]. However, these studies are still unclear and remain to be fully elucidated.

6. Toxicity of fenugreek

In our extensive review of the toxicological properties of fenugreek, we provided a lot of information about teratogenic, reproductive, neurodevelopmental, neurobehavioral and neuropathological abnormalities associated with fenugreek use in prospective and animal model studies [128]. In fact, many teratogenic effects of fenugreek, such as major malformations [129], growth alterations, functional developmental deficits [130], and significant reduction of total and live implants per pregnant female animal models [131], were largely reported. In addition, the developing nervous system appears to be particularly susceptible to fenugreek toxicity as reported by retrospective and animal model studies [132–134]. Otherwise, the anti-fertility effects of fenugreek in rats, mice, and rabbits, as well as an anti-implantation and abortifacient activity related to saponin compound of fenugreek, have been demonstrated in long-term daily use [128,135]. However, low to moderate doses of fenugreek have been reported to be safe for the nervous system [136] without any sign of toxicity over several weeks use in feeding studies [137]. Moreover, no evidence of mutagenicity or genotoxic activity of fenugreek was reported.

As fenugreek is rich in fiber, it can interfere with the absorption of orally taken drugs. Prescription of medicines must be taken separately from fenugreek-containing products. Bash et al. [138] reported that the concomitant use of fenugreek with other hypoglycemic drugs may reduce serum glucose levels more than expected. It has been suggested that herbal supplements containing coumarin, e.g., fenugreek may potentially increase the risk of bleeding or potentiate the effects of warfarin therapy [139]. In fact,

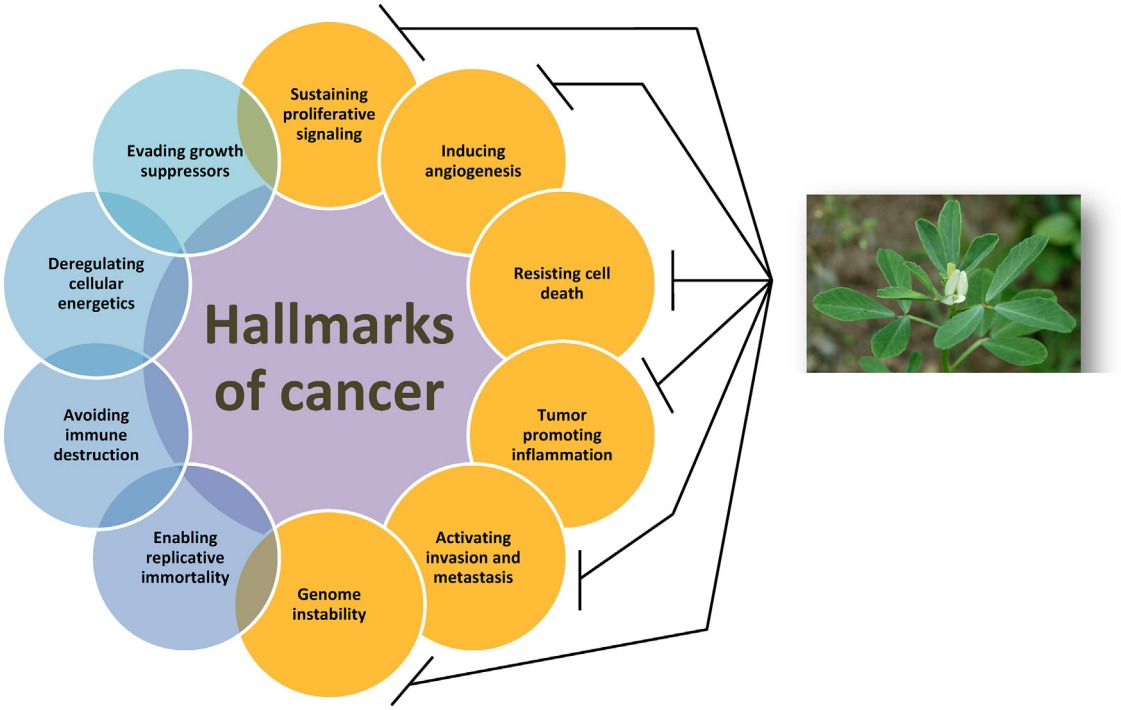

Fig. 4. Major hallmarks of cancer targeted by Fenugreek extracts and bioactive compounds.

a potential interaction between warfarin (used for atrial fibrillation) and boldo-fenugreek [140], and between aspirin and fenugreek, resulting in bleeding [141] was also observed. In addition, it has been suggested that the consumption of fenugreek is a potential risk for patients with chronic asthma and patients known to be allergic to it, or who are allergic to chickpeas or peanuts because of possible cross-reactivity (More details on this topic can be found in [128]).

7. Future directions

In the era of evidence-based medicine, anticancer drug development is a long process that involves several and obligatory steps (Fig. 3). Plant-derived molecules constitute an important source of successful anticancer drugs in various clinical trials (i.e, taxanes, vinca-alkaloids, combretastatin, trabectedin, etc.). According to this review, fenugreek presents important pharmacological properties to provide new opportunities for cancer drug discovery. A large number of preclinical data showed antitumor effects of fenugreek extracts and compounds, targeting various hallmarks of cancer (Fig. 4). Only one clinical report of fenugreek use in a human case (a 10-year-old Saudi Arabian girl) in which established malignant CNS cancer (Primary CNS T cell lymphoma) was published. The experiment consisted in a daily administration of fenugreek seeds at 8 g, over the course of 6 months. The results reported showed regression, then disappearance of the cancer lesion [72,142]. But until now, there have been no clinical trials for cancer conditions. Some clinical studies investigating different pharmacological benefits of fenugreek have been of limited quality (Supplementary Table S1). However, they presented important results with regard to its safety use. In this direction, Nathan et al. conducted a double-blind placebo-controlled clinical trial using capsules of 300 mg of standardized extract of fenugreek (twice daily) in 50 patients with Parkinson's disease treated by L-Dopa therapy and found it safe and well tolerated [143]. Later, in a randomized, double-blind, and placebo-controlled study, fenugreek dietary fiber (associated to curcumin) was found safe in 60 subjects experiencing occupational stress-related anxiety and fatigue [144].

The following points may be considered for future trials: (i) bioactive fenugreek compounds show great potential for modulating cancer cell signaling pathways associated with the proposed hallmarks of cancer such as inflammation, apoptosis, invasion, angiogenesis, cancer cell motility, and metastasis, (ii) more mechanistic studies are awaited to clarify the structure-activity relationship of anticancer properties of fenugreek compounds (example of diosgenin), (iii) cautions need to be recommended to potential risks related to allergic potential, pregnancy, and to fetal development (teratogenic effects), (iv) well-designed, controlled and randomized safety-efficacy studies are needed before recommending the use of fenugreek in modern oncology, (v) clinical use of fenugreek is not suitable in cancer patients without clinical evidence from phase III trials. Indeed, advanced mechanistic *in vivo* studies, accompanied by a meticulous determination of their mechanism of action, safety, and pharmacokinetic evaluation will be critical for translational research and precision oncology, which are urgently needed.

8. Conclusions

During the last decade, considerable data regarding the beneficial effect of fenugreek on prevention and treatment of cancer in animal models and *in vitro* have been made available. In fact, a substantial number of studies have shown that fenugreek extracts and their isolated compounds possess significant anticancer effects. To date, the most powerful anticancer components of fenugreek identified are trigonelline, diosgenin, protodioscin and dioscin. However, signaling molecules targeted by fenugreek compounds and extracts need further clarification, which requires further investigation. The efficacy of fenugreek in the prevention of cancer in clinical use requires dedicated attention to establish physiologically relevant concentrations and chronic exposures, and should be confirmed by future clinical trials.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biopha.2017.03.071.

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