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REVIEW

A small plant with big benefits: Fenugreek (*Trigonella foenum-graecum* Linn.) for disease prevention and health promotion

Kalyan C. Nagulapalli Venkata[1], Anand Swaroop[2], Debasis Bagchi[2,3] and Anupam Bishayee[1]*

[1] Department of Pharmaceutical Sciences, College of Pharmacy, Larkin University, Miami, FL, USA
[2] Cepham Research Center, Piscataway, NJ, USA
[3] Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, Houston, TX, USA

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Plant-derived natural products have long-standing utility toward treating degenerative diseases. It is estimated that about two-thirds of world population depend on traditional medicine for primary medical needs. Fenugreek (*Trigonella foenum-graecum* Linn.), a short-living annual medicinal plant belonging to Fabaceae family, is used extensively in various parts of the world as herb, food, spice, and traditional medicine. Fenugreek is considered as one of the oldest medicinal plants and its health-promoting effects have been cited in Ayurveda and traditional Chinese medicine. The investigations into the chemical composition and pharmacological actions have seen a renaissance in recent years. Extensive preclinical and clinical research have outlined the pharmaceutical uses of fenugreek as antidiabetic, antihyperlipidemic, antiobesity, anticancer, anti-inflammatory, antioxidant, antifungal, antibacterial, galactogogue and for miscellaneous pharmacological effects, including improving women's health. The pharmacological actions of fenugreek are attributed to diverse array of phytoconstituents. The phytochemical analysis reveals the presence of steroids, alkaloids, saponins, polyphenols, flavonoids, lipids, carbohydrates, amino acids, and hydrocarbons. This review aims to summarize and critically analyze the current available literature to understand the potential of fenugreek for disease prevention and health improvement with special emphasis on cellular and molecular mechanisms. Current challenges and new directions of research on fenugreek are also discussed.

Keywords:
Cancer / Diabetes / Health benefits / Phytochemicals / *Trigonella foenum-graecum*

1 Introduction

Plants survived for millions of years on planet earth by continuously evolving and adapting. They developed defense mechanisms by biosynthesizing metabolites as a guard against external factors, such as pests and climatic conditions [1]. Medicines obtained from Mother Nature, especially derived from plants, have been well documented for several millennia [2]. Even today, according to World Health Organization, about 80% of world population from the developing and underdeveloped countries still bank on plant-derived medicines for their healthcare requirements [3].

Fenugreek (*Trigonella foenum-graecum* Linn.), is a short-living annual plant, belongs to the Fabaceae family. It is grown in many parts of Asia, Africa, and Europe as food,

Correspondence: Anupam Bishayee
E-mail: abishayee@ULarkin.org, abishayee@gmail.com

Abbreviations: Bcl-2, B-cell lymphoma-2; **CAT**, catalase; **COX**, cyclooxygenase; **DMBA**, 7,12-dimethylbenz(α)anthracene; **ERK**, extracellular signal-regulated kinase; **FSE**, fenugreek seed extract; **GLP-1**, glucagon-like peptide-1; **GPx**, glutathione peroxidase; **HCC**, hepatocellular carcinoma; **HIL**, hydroxy isoleucine; **ILs**, interleukins; **IRS-1**, insulin receptor substrate-1; **JNK**, c-jun N-terminal kinase; **LD_{50}**, lethal dose 50; **LH**, luteinizing hormone; **LPO**, lipid peroxidation; **MDA**, malonidialdehyde; **MMP**, matrix metalloproteinase; **NF-κB**, nuclear factor-κB; **NIDD**, noninsulin-dependent diabetic; **PPAR-γ**, peroxisome proliferator activated receptor-γ; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **SREBP**, sterol regulatory element binding protein; **STAT3**, signal transducer and activator of transcription 3; **STZ**, streptozotocin; **$t_{1/2}$**, half-life; **TBARS**, thiobarbituric acid reactive substances; **TGs**, triglycerides; **TIMP-3**, tissue inhibitors of metalloproteinase-3; **TNF-α**, tumor necrosis factor-α

*Additional corresponding author: Kalyan C. Nagulapalli Venkata
E-mail: KVenkata@ULarkin.org

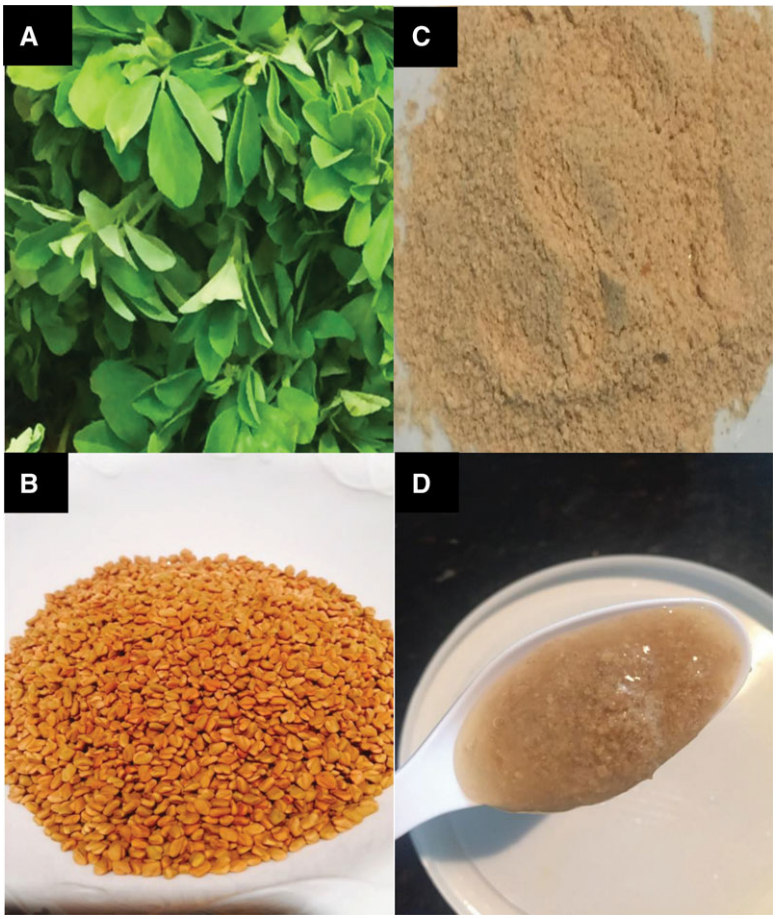

Figure 1. Several photographs of fenugreek leaves (A), seeds (B), seed powder (C), and water insoluble gum (D).

condiment, spice, and as native medicine [4, 5]. The genus *Trigonella* is named in reference to its triangular shaped flowers, and in Latin little triangle is referred to as *Trigonella* [6]. The species *foenum-graecum* gets its name from historical perspective of Romans, since referred to it as Greek-hay, symbolizing it as the common crop used as fodder for animals in Greece [7]. Fenugreek plant attains the height of 1–2 feet and bears green trifoliate leaves (Fig. 1). The flowers are white to yellow in color and the plant carries thin pods. The pods are about 15 cm in length and they contain on an average 10–20 seeds [6, 8]. Fenugreek seeds are golden yellow in color and their average height, width, and thickness are 4.01–4.19, 2.35–2.60, and 2.40–2.66 mm, respectively. Fenugreek seeds are the most important and well-studied part of fenugreek plant. The dried fenugreek seeds are grounded to obtain fenugreek seed powder which is used as condiment. Fenugreek gum is obtained from the endosperm of the seeds [9].

Fenugreek is used as a spice and herb in many culinary dishes and its green leaves and seeds are the only parts of the plant that are edible [10]. In Indian cuisine, leaves are used to flavor dishes or eaten as greens, and seeds are used for seasonings or crushed to prepare curry powders and pastes [11]. In African cuisine, fenugreek is used as supplement for making bread. The presence of galactomannan, the source of soluble dietary fiber in the endosperm of seed, improves the nutritional and physicochemical properties of the bread [12]. In addition to being used in various food preparations, fenugreek also has healing benefits. Fenugreek is one of the oldest medicinal plant and the medicinal properties are well documented in the ancient medical literature [6]. In Ayurveda, the traditional Indian medical system, fenugreek was used as a digestive aid and ancient Egyptians used it to incense and embalm mummies and also as lactation aid [13–15]. In traditional Chinese medicine, fenugreek was used to treat edema in the legs. There are number of folkloric uses of fenugreek, including the treatment of lung congestion and sinus, indigestion, baldness in men, hair tonic and conditioner and as galactogogue. Currently, a large number of studies have shed positive light on fenugreek's medicinal properties, such as antioxidant [16], anti-inflammatory [17], antidiabetic [18], antiobesity [19], anticancer [20], hepatoprotective [21], antihyperlipidemic [22], women's health- [23] and sexual health-modulating activities [24].

The health promotion and disease prevention attributes of fenugreek are due to presence of diverse array of phytochemicals and their varying pharmacological and biological activities. Although prior accounts in the literature present an overview of the ethno-medicinal aspects of fenugreek;

however, many developments occurred in fenugreek research [25, 26]. The goal of this review is to provide an update on recent findings of the nutritive value, the molecular mechanisms and pharmacological properties of fenugreek in prevention and management of acute and chronic diseases.

2 Methods for literature search

An extensive search was conducted using PubMed and Google Scholar to identify the scientific studies conducted with fenugreek and its medicinal uses related to disease preventing and health-promoting effects. No date restrictions were applied on the published literature and only English language publications or reports were considered. The criteria for the exclusion of articles were the language of reports being other than English reports with unavailable abstracts and nonpeer-reviewed articles and websites. The bibliography of the primary literature was also used as a source to find additional relevant publications.

3 Chemical constituents

The phytochemical analysis of fenugreek has revealed the presence of various categories of secondary metabolites, such as saponins, steroids, alkaloids, flavonoids, terpenes, phenolic acid derivatives, amino acids and fatty acids and their derivatives [27]. Figures 2–4 show the structural diversity of the compounds isolated from fenugreek.

3.1 Saponins and steroidal compounds

Saponins are the most abundant class of phytoconstituents present in fenugreek. Structurally, saponins contain a steroidal skeleton or a hydrophobic triterpenoid ring which is glycosylated with varying number of hydrophilic sugar units at different positions through *O*-glycosidic bond. Yoshikawa et al. [28] discovered six saponins from fenugreek. Steroidal saponins are generally divided into two classes, namely spirostanol and furostanol. The majority of saponins isolated from fenugreek belong to the furostanol class. Furostanol steroidal saponins present in fenugreek, such as protodioscin (Fig. 2), the carbon at C-26 position bears *O*-linked β-D-glucose to prevent the cyclization and formation of steroid ring F. Whereas in spirostanol saponins, such as dioscin, a spiroacetal bicyclic moiety is formed at C-22 carbon by connecting E and F steroidal rings [29]. The nonsugar aglycone portion of both the furostanol and spirostanol saponins is referred to as sapogenins.

Sauvaire et al. [30] have reported that fenugreek contains no free spirostanol saponins and sapogenins, but they are formed secondarily through enzymatic reactions from furostanol saponins. Pang et al. [31] recently reported an efficient route to synthesize sapogenins from furostanol saponins through an enzymatic reaction followed by acid hydrolysis. The sapogenins mainly isolated from fenugreek seeds after acid hydrolysis are diosgenin and yamogenin. Diosgenin (25*R*-spirosten-5-en-3β-ol) is the most extensively studied steroidal sapogenin, structurally it is a C-27 steroid skeleton with a double bond at C-5 carbon, a spiroacetal moiety at C-22 position, and alcohol group at C-3 carbon. The continued biological and economic interest in diosgenin is due to its role as advanced product intermediate for the synthesis of oral contraceptives and steroid hormone drugs and also due to its wide range of pharmacological activities as anti-inflammatory and anticancer agents. Fenugreek is one of very few natural sources of diosgenin, which is currently extracted from Mexican and Asian yams along with its 25*S* epimer yamogenin [32].

3.2 Dietary fibers

Fenugreek is good source for dietary fiber. Two types of dietary fibers present in fenugreek seeds are insoluble and soluble in 32 and 13%, respectively [33]. The insoluble fibers are not digested by the enzymes present in humans and soluble fibers dissolve in aqueous medium of the gastrointestinal tract, hence the name insoluble and soluble. Insoluble fibers soften the stools and decrease the appetite; whereas soluble fibers inhibit the absorption of glucose into the blood stream and enhance its glycemic control [15]. The cell wall of fenugreek seeds contain mostly polysaccharides made of galactomannan, which provides energy for seeds during germination [34]. The fenugreek galactomannans are composed of β-(1→4) linear mannose backbone linked by α-(1→6) single galactose units (Fig. 2). The molecular weight of fenugreek galactomannan is approximately 13 000 Daltons [35]. The galactomannan in fenugreek is unique due to its 1:1 ratio of mannose to galactose monosaccharides. The presence of excess galactose minimizes the intrachain hydrogen bonding and thus increases the cold water solubility of fenugreek galactomannan (80%) when compared to other commonly used galactomannans, such as locust beans (30%) and guar (60%) [36].

3.3 Alkaloids

Trigonelline is a pyridine alkaloid and nicotinic acid betaine derivative and chemically it is a quaternary ammonium compound with zwitter ionic properties. Biologically, apart from being hypoglycemic and neuroprotective trigonelline is also classified as a phytoestrogen, due to its ability to activate estrogen receptor. Trigonelline, *N*-methylnicotinic acid, was first discovered in fenugreek seeds in 1885 [37]. Joshi and Handler [37] have elegantly demonstrated the

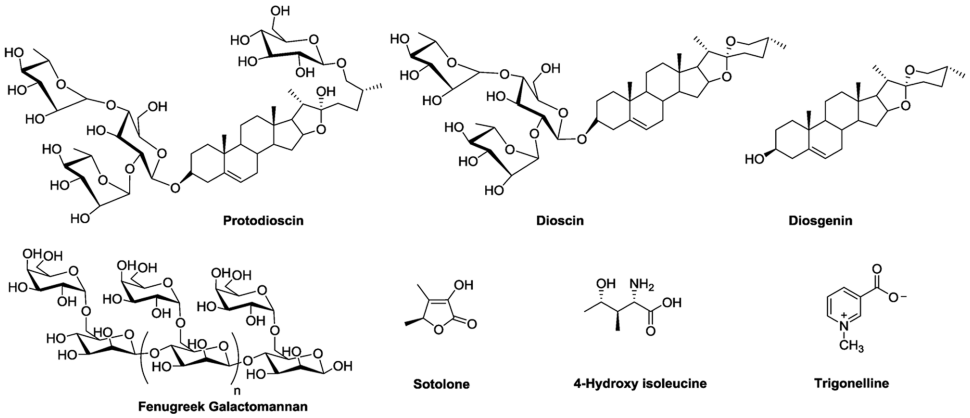

Figure 2. Structures of fenugreek phytochemicals tested for various pharmacological activities.

biosynthetic pathway for the generation of *N*-methylnicotinic acid from nicotinic acid and *S*-adenosyl methionine through methylation in green peas.

3.4 Amino acids

Fenugreek is rich source for number of nitrogen-containing amino acids, such as aspartic acid, glutamic acid, leucine, tyrosine, and phenyl alanine. However, the quantity of sulfur-containing amino acids cysteine and methionine is relatively low [38]. The most studied and biologically important free amino acid present in fenugreek is (2*S*, 3*R*, 4*S*)-4-hydroxy isoleucine (HIL). The nonproteinergic amino acid 4-HIL is about 80% of amino acid content in dry fenugreek seeds and the quantities of this amino acid increases during the seeds growth phase [39]. Sauvaire et al. [40] have reported that the quality of protein present in fenugreek is on par with soybean. The other most important nitrogen-rich nonproteinergic amino acid present in fenugreek is (*S*)-canavanine [41]. By employing GC-olfactometry, Blank et al. [42] established that the phytochemical sotolone is the principal "seasoning flavor" inducing ingredient of fenugreek. They have experimentally shown the formation of sotolone from 4-HIL through oxidative desamination reaction.

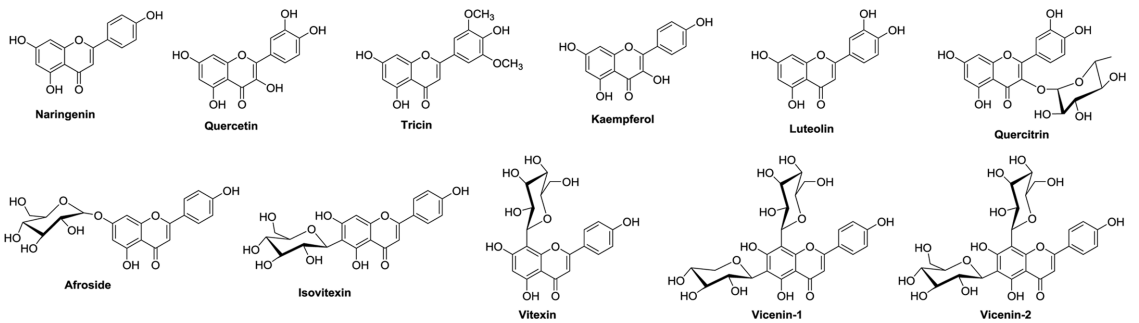

Figure 3. Flavonoids and their derivatives present in fenugreek.

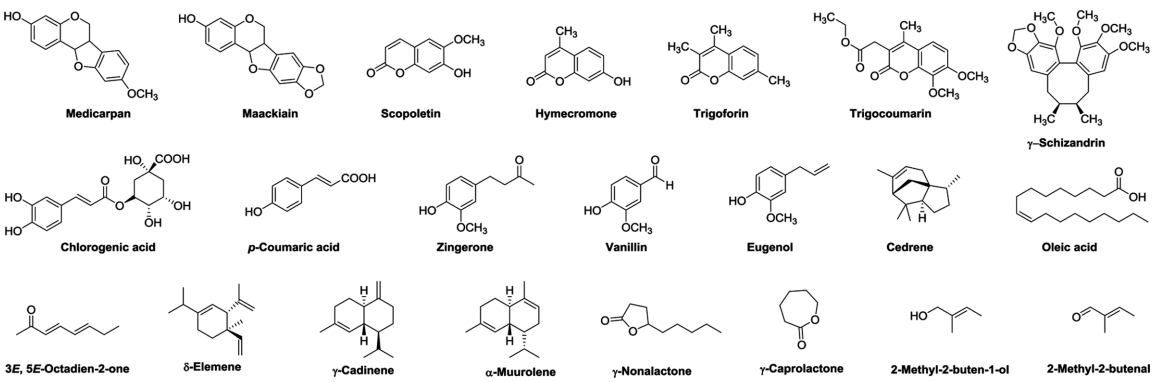

Figure 4. Structurally diverse isoflavonoids, polyphenols, and miscellaneous phytochemicals present in fenugreek.

3.5 Flavonoids

Fenugreek contains number of important, beneficial flavonoids and polyphenol compounds (Fig. 3). Parmar et al. [43] have reported the presence of wide range of flavonoids, namely quercetin, luteolin, vitexin, and 7, 4′-dimethoxy flavanones in the alcoholic extracts of the whole plant. Several other groups have reported similar findings of the presence of aglycones kaempferol, quercetin, tricin, and naringenin [27]. These compounds were isolated from the individual parts of fenugreek plant and also from the extracts and hydrolysates of leaves, flowers, and stems [44–46]. The phytochemical analysis of fenugreek revealed that the most of flavonoids are present as complex glycosides by conjugating with carbohydrates via *O*-glycosidic and *C*-glycosidic bond. Quercetin-3-*O*-rhamnoside (quercitrin), vitexin-7-*O*-glucoside (afroside), and apigenin-6-*C*-glucoside (isovitexin) are few examples of flavonol glycosides present in fenugreek [47, 48]. Seshadri et al. [49] isolated apigenin-6-*C*-glucoside (isovitexin) and apigenin-8-*C*-glucoside (vitexin) from fenugreek seeds. Similarly, Wagner et al. [50] isolated apigenin-6-*C*-xyloside-8-*C*-glucoside (vicenin-1) and apigenin-6, 8-*C*-diglucoside (vicenin-2) from the seeds of fenugreek plant. Ingham and Harborne [51] have reported the presence of isoflavonoid phytoalexins aglycones, such as medicarpan and maackiaian, in fenugreek. These are generated in response to an external insult such as microbial invasion or infection and are generally referred to as "induced isoflavonoids."

3.6 Phenolic and acid derivatives

Number of important phenolic and styrylic acid derivatives, such as *p*-coumaric acid, caffeic acid, chlorogenic acid [27], coumarin derivatives, namely hymecromone [52], trigoforin [46], trigocoumarin [53], scopoletin [27], and cyclooctane derivatives, such as γ-schizandrin [27], were isolated from different parts of fenugreek plant. Al-Daghri et al. [54] isolated polyphenol derivatives, such as zingerone, vanillin, gingerol, and eugenol, from fenugreek extract and reported anticancer activities of these compounds.

3.7 Vitamins and minerals

Fenugreek is rich in number of vitamins. Fenugreek seeds contain vitamins A, B1, B2, C, niacin, and nicotinic acid [11]. Sreeramulu et al. [55] have reported that fenugreek leaves are rich in vitamin C the ascorbic content in fresh leaves was calculated as 276 mg per 100 g, and leaves are also good source for calcium, β-carotene, and folic acid. Fenugreek plant is well stocked with essential inorganic elements and the plant is good source for major dietary elements, such as Fe, Ca, P, S, and Mg. The seeds contain adequate amounts of important trace dietary elements such as Co, Cu, Mn, Zn, and Br [27, 56, 57].

3.8 Lipids, fatty acids, and esters

Fenugreek leaves like most of other photosynthesizing plants and green microorganisms have abundant amount of mono- and di-galactodiacylglycerols and they also contain glyco and phospholipids [58]. The seeds are rich in fatty acids, namely oleic, linoleic, and linolenic acids and number of important phospholipids, such as phosphatidyl choline, phosphatidyl ethanolamine [59]. The presence of several other phospholipids in minor quantities has also been reported [58].

3.9 Miscellaneous compounds

Girardon et al. [60] have reported more than 50 volatile chemicals from fenugreek seeds and they have chemically characterized 39 of those phytochemicals. In 2002, Mazza et al. [61] employed headspace sampling coupled with GC to analyze solid phase micro extract of fenugreek seeds. Through this analysis, they identified 175 compounds in fenugreek seeds and 66 of those were identified in fenugreek for the first time. The identification analysis showed the presence of chemicals with various functional groups, such as alcohols, aldehydes, ketones, sesquiterpene hydrocarbons (cedrene), and heterocycles. Few representative compounds are shown in Fig. 4.

4 Bioavailability of active compounds

Kandhare et al. [62] studied the bioavailability of furastanol glycoside isolated from fenugreek seed extract (FSE) in rats. They studied the pharmacokinetics, tissue distribution, and excretion following oral administration of 200 mg/kg of furastanol glycoside extract. They noted that after single oral administration, the area under the curve was 0.177 μg/mL h; maximum concentration time period (T_{max}) was 72 h and half-life ($t_{1/2}$) was 40.10 h. The extract was absorbed slowly through the intestine and had comparatively slow distribution. The extract was also detected in brain and lung tissues, indicating its passage through blood–brain barrier. Elimination through urine and feces was observed after 24 h and could be noticed in urine and feces even after 108 h post oral administration. In another study, Kandhare et al. [63] reported the pharmacokinetics and tissue distribution of flavonol glycoside, vicenin-1. They reported after single oral administration of 60 mg/kg of vicenin-1, the C_{max} was 7.039 μg/mL, area under the curve was 0.044 μg/mL h, and $t_{1/2}$ was 11.60 h. They also observed the distribution of vicenin-1 in various rat tissues, the flavonol glycoside was detected in higher concentrations in the liver and lungs and very low concentrations in the brain, kidneys, and adrenal glands. Buqui et al. [64] utilized nonlinear mixed effects model to study the absorption pharmacokinetic parameters of vicenin-2, with the assumption of first-order absorption and zero-order secretion. They reported the first-order absorption rate constant as 0.274 min^{-1} and zero-order rate constant as 16.3 min^{-1}.

The investigators also noted that approximately 40% of the original dose of vicenin-2 was rapidly absorbed in the small intestine [64]. The pharmacokinetic studies of fenugreek and its safety profile, either used alone or in combination with other natural products, have been reported in literature [65]. Although no bioavailability studies are available, fenugreek is widely used for centuries around the world, especially in the Asian and African subcontinents, several clinical studies have been conducted in the recent past and the fenugreek-based nutraceuticals are becoming increasingly popular worldwide. These advances confirm the benefits and usefulness of fenugreek in diverse disease conditions.

5 Biochemical, biological, and pharmacological activities

5.1 Antioxidative effect

Oxygen is an essential and integral component of life for all aerobic living organisms. However, oxygen is also a highly reactive atom that undergoes metabolism to generate the reactive oxygen species (ROS), which are free radicals containing oxygen atom [66]. ROS include the hydroxyl radicals ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), and superoxide anions (O_2^{-}), all of which are chemically capable of reacting with various macromolecules, such as lipids, proteins, and nucleic acids. ROS induce membrane lipid peroxidation (LPO) and trigger the loss of membrane fluidity, thus impairing cellular functions. Protein oxidation exerted by ROS leads to fragmentation of amino acids residues, oxidation of protein backbone, and loss of protein function. Additionally, ROS-evoked oxidation leads to DNA damage which, in turn, alters replication and transcription and results in mutations and genetic defects. ROS play an important beneficial role in homeostasis and cell signaling [67]. Normally all living beings are equipped with endogenous defense mechanisms, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) to metabolize the toxic intermediates and protect against ROS-induced damage [68]. The pro-oxidation and antioxidation are highly regulated in normal cells, but when there is imbalance it leads to oxidative stress [69]. The oxidative stress is implicated in more than 50 diseases, including inflammation, cancer, and diabetes [70]. Whenever there is oxidative stress, there is a critical need for antioxidants. The best source for antioxidants is foods rich in polyphenols, such as fruits and vegetables [71]. The phytochemical analysis of fenugreek revealed the presence of number of polyphenols which were investigated by several groups worldwide to study the antioxidant properties of fenugreek [72].

Sharma et al. [73] reported that administration of FSE reduced the in vitro LPO induced by ferrous sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), and carbon tetrachloride in a concentration-dependent manner. The LPO was further reduced when FSE and glibenclamide were used in combination. Dixit et al. [16] observed that due to the presence of polyphenols, flavonoids, and other water-soluble components in aqueous extracts showed elevated antioxidant activity when compared to ethanol extracts. The aqueous extracts exhibited enhanced iron-reducing capacity. The extracts displayed protective activity against Fe^{2+}-ascorbate-induced LPO. The authors hypothesized the possible mechanism of action might be due to the fenugreek's ability to scavenge free radicals and form iron complexes as evidenced by ferric-reducing ability of plasma assay. In another study, Naidu et al. [74] have experimentally shown the direct correlation between the presence of polyphenols in fenugreek seeds and their concentration on antioxidant activity. The concentration of polyphenols in husk is greater than seed which is greater than endosperm and their IC_{50} values for scavenging 1,1-diphenyl-2-picrylhydrazyl radical were 138, 158, and 176 μg, respectively. Additionally, polyphenols in fenugreek also help to protect the RBC from oxidative change inflicted by the peroxide treatment [75]. Thirunavukkarasu et al. [76] reported the protective activity of fenugreek aqueous extract against ethanol toxicity. They observed that aqueous extract inhibited the promoter of LPO by blocking the production of thiobarbituric acid reactive substances (TBARS). The antioxidative potential of fenugreek is comparable to other well-known antioxidants, such as glutathione and α-tocopherol.

Genet et al. [77] reported similar findings regarding the combination therapy. When fenugreek and vanadate were administered to alloxan-induced diabetic rats, the antioxidant enzymes, such as SOD, GPx, and CAT returned to the normal levels from the lower levels inflicted by diabetes. Tripathi and Chandra [78] reported similar results based on the observation that fenugreek seeds imparted antioxidation activity even when they were used alone without any co-oxidant present in alloxan-induced diabetic rats. Fenugreek displayed protective effects when it was administered in combination with insulin. The combination normalized the mono amino oxidase enzyme levels in alloxan-induced diabetic rat brain and also prevented the genomic DNA fragmentation [79].

The role of fenugreek in ameliorating the hepato- and nephro-toxicity induced by cypermethrin in rats was reported by Sushma and Devasena [80]. They showed that fenugreek reduced the levels of LPO by-product, TBARS, and the levels of antioxidant enzymes, such as SOD, GPx, and GST, were found to be increased in the liver and kidneys. Authors postulate mechanism of action might be due to the flavonoids present in fenugreek and their ability to scavenge the free radicals induced by cypermethrin metabolism. Fenugreek ethanol extract significantly reduced high LPO levels in Freund's complete adjuvant-induced arthritic rats [81]. According to the investigators, the antiarthritic effect of fenugreek extract was due to its ability to decrease the levels of interleukins (ILs) and tumor necrosis factor-α (TNF-α).

Fenugreek imparted its protective effect in rats against the changes induced by fungicide carbendazim, such as testicular toxicity and increase in oxidative stress. Fenugreek ameliorated these changes and improved the function of SOD and CAT and decreased the levels of biomarker for oxidative

stress, namely malonidialdehyde (MDA) [82]. When fenugreek extract was used in tandem with swimming exercise there was a significant increase in SOD, CAT, and GPx activities in the heart tissue of diabetic rats [83]. Also, fenugreek extract caused significant reduction in LPO and increase in SOD, CAT and GST when used in combination with *Momordica charantia* (MC) in alloxan-induced diabetic rats. [84]. Fenugreek significantly reduced the LPO of white blood cells in the livers of alloxan-induced diabetic rats. The antioxidant activity is concentration dependent and its activity was comparable to known antidiabetic drug, glibenclamide [85].

Anuradha and Ravikumar [86] reported the ability of fenugreek extract to reduce the LPO and oxidative stress in alloxan-induced diabetic rats. They postulated the pharmacological effects may be due to fenugreek's ability to protect the Ca^{2+}-ATPase activity in the liver. Fenugreek presented its role as a diabetic neuropathic agent [87]. Fenugreek aqueous extract helped to repair the morphological changes caused by diabetes. It restored the uneven thickening of glomerulus and decreased the levels of MDA in the serum and kidneys and 8-hydroxy-2′-deoxyguanosine levels in renal cortex DNA and urine. Additionally, fenugreek extract upregulated the key enzymes required for antioxidation, SOD and CAT, in the serum and kidneys. The authors postulated the beneficial effects may be due to fenugreek's ability to increase the activities of antioxidants and decrease the accumulation of oxidized DNA in the kidneys. In another study, Abdel-Daim et al. [88] evaluated the role of fenugreek oil as a protective agent against deltamethrin-induced toxicity in rats. They observed that fenugreek oil maintained the hematological parameters, namely red blood cells ranges, platelet counts, hemoglobin, and hematocrit values. Fenugreek oil restored the biochemical changes such as cholesterol, triglycerides (TGs), urea, uric acid, creatinine, and enzyme alanine amino transferase to normal levels. Additionally, fenugreek oil also prevented the LPO and oxidative stress in a dose-dependent manner [88].

5.2 Anti-inflammatory effect

Maurya et al. [89] reported the anti-inflammatory activity of HIL present in fenugreek. They noted that HIL ameliorated the ROS-induced inflammation by reducing the activation of nuclear factor-κB (NF-κB), c-jun N-terminal kinase (JNK 1/2), extracellular signal-regulated kinases (ERK 1/2), mitogen-activated protein kinase (P38MAPK), and palmitate-induced generation of ROS. Additionally, HIL significantly inhibited inflammation-stimulated insulin receptor (insulin receptor substrate-1 (IRS-1) serine phosphorylation and restored palmitate-effected IRS-1 tyrosine phosphorylation, which in turn, increased insulin sensitivity. In another study, anti-inflammatory activity of HIL on TNF-α-mediated insulin resistance was analyzed [17]. HIL activated adenosine monophosphate-activated protein kinase in a dose-dependent manner and increased the insulin-stimulated glucose transport. Also, HIL enhanced the phosphorylation of insulin receptor IR-β and IRS-1, decreased the suppressor of cytokine signaling-3 co-immunoprecipitation with IR-β and IRS-1. HIL reversed the effects of TNF-α and normalized the glucose uptake that was lowered by insulin resistance induced by TNF-α activity. The anti-inflammatory and antinociceptive activity of alkaloid and flavonoid fractions of fenugreek seeds is probably due to their ability to inhibit proinflammatory enzymes, namely cyclooxygenases (COXs) and lipooxygenases [90]. The furastanol and spirostanol saponins are shown to induce anti-inflammatory activity by inhibiting the production of inflammatory cytokines, such as IL-1β and TNF-α [91]. Fenugreek mucilage significantly reduced the activities of inflammatory enzymes COX, lipoxygenase, and myeloperoxidase in arthritic rats [92]. Liu et al. [93] conducted bio-assay-guided purification of various extracts of fenugreek seeds utilizing hexanes, ethyl acetate, methanol, and water and isolated several important classes of phytochemicals present in fenugreek, such as TGs, fatty acids, polysaccharides, and flavonoid *C*-glycosides. They reported that hexane and aqueous extracts inhibited COX-2 enzyme better than other extracts. The ethyl acetate fraction exhibited significant inhibition of proinflammatory enzyme COX-1. The investigators also isolated three key flavonoid-*C*-glycosides, namely apigenin 8-*C*-β-glucopyranoside, apigenin 6-*C*-β-glucopyranoside, and apigenin 6, 8-*C*-di-β-glucopyranoside, which inhibited COX-1 and COX-2. Among the three aforementioned apigenins, apigenin 8-*C*-β-glucopyranoside showed superior COX-2-inhibitory activity compared to two other apigenins. HIL, alkaloids, apigenins, and saponins isolated from fenugreek have displayed remarkable anti-inflammatory activity. However, further research is needed to determine the underlying mechanisms by which fenugreek products inhibit inflammatory signals.

5.3 Antimicrobial activity

Haouala et al. [94] studied the antifungal activity of the aqueous and organic extracts derived from different aerial parts of the fenugreek plant. They studied the antimycotic activity against *Botrytis cinerea*, *Fusarium graminearum*, *Pythium aphanidermatum*, *Alternaria* sp., and *Rhizoctinia solani* pathogenic fungi. They observed that aqueous extract and organic extracts derived from different solvents displayed antifungal activity. However, the range of inhibition depended upon the species and the part of the plant used. The methanolic fraction of the nonground seeds completely inhibited the growth of *Alternaria* sp., and *R. solani* and the minimum inhibitory concentration was 60 μg/mL. Randhir et al. [95] reported the antimicrobial activity of fenugreek sprout extract against the peptic ulcer causing bacteria, *Heliobacter pylori*. They suggested that less-polymerized free phenols act as the main active constituents for antimicrobial activity. Apart from free phenols, the presence of phytochemical scopoletin (coumarin derivative) that interferes with the

prokaryotes electron chain transport mechanism is the other main ingredient for the antimicrobial action.

The hydroalcoholic extracts of fenugreek fraction rich in polyphenols and flavonoids displayed dose-dependent antifungal activity against fluconazole resistant *Candida albicans* [96]. The ethanolic extract of fenugreek seeds inhibited both the positive and negative strains of bacteria with minimum inhibitory concentration against test bacteria, such as *Escherichia coli, Salmonella typhi, Vibrio cholerae, Staphylococcus aureus, Micrococcus lutea, and Bacillus subtilis*, was 400 mg/mL [97]. Additionally, the aqueous extract of germinated fenugreek seeds inhibited *E. coli, S. aureus,* and *B. subtilis* [98]. Fenugreek leaf extracts displayed larvicidal activity against *anopheles, culex,* and *aedes* genera of mosquitoes [99].

5.4 Antidiabetic effect

Naicker et al. [100] recently demonstrated that FSE increased the glucose uptake through upregulation of mRNA expression levels of glucose transporter (GLUT-2) and sterol regulatory element binding protein (SREBP1C; Table 1). Also, FSE increased the activities of glycogen kinase and glycogen synthase enzymes by imparting modifications to downstream insulin signaling pathways. FSE imparted insulin mimicking properties by increasing the intracellular creatinine levels in L6C11 muscle cells [101]. Uemura et al. [102] reported that major saponin, diosgenin, present in fenugreek seeds ameliorated the hyperglycemia and diabetic condition by promoting the adipocyte differentiation of 3T3-L1 cells isolated from kk-Ay mice. Diosgenin increased the mRNA expression levels of CCAAT/enhancer-binding protein (C/EBPδ), peroxisome proliferator activated receptor-γ (PPAR-γ), and its target genes adipocyte protein 2, lipoprotein lipase, and glucose transporter-4.

Sauvaire et al. [103] reported the in vitro insulinotropic activity of HIL from fenugreek seeds. They observed that HIL increased glucose-induced insulin release by directly acting on islets of Langerhans isolated from rats and humans. However, HIL showed no effect on other insulin secretion agonists, such as leucine, arginine, and glyceraldehyde. HIL has significant impact on insulin signaling pathway. Gao et al. [104] observed that HIL downregulated the TNF-α and TNF-α converting enzyme expression and upregulated the expression of tissue inhibitors of metalloproteinase-3 (TIMP3) in hepatic cells in a dose-dependent manner. Additionally, HIL downregulated pIRS-1, upregulated the expression levels of IRS-1 and glucose transporter-4, and also increased the uptake of glucose by insulin-resistant hepatic cells. The administration of HIL to rat islets isolated from noninsulin-dependent diabetic rats triggered the insulin response and displayed antihyperglycemic activity by improving glucose tolerance [105]. Additionally, HIL ameliorated the TNF-α-induced insulin resistance conditions in hepatic cells.

Lu et al. [106] observed that HIL increased the phosphorylation of protein kinase B and glycogen synthase kinase and decreased the phosphorylation of JNK and IRS-1. In a study, Jaiswal et al. [107] have shown that HIL can significantly increase the uptake of glucose, they postulated that HIL imparts its activity through PI3 kinase/Akt-dependent mechanism. HIL ameliorates the insulin resistance condition by promoting the insulin-sensitizing effects. It does by increasing glucose utilization and decreasing hepatic glucose synthesis in obese rats [108].

Sotolone, the volatile lactone ameliorated the dysfunction caused by palmitate-induced apoptosis in pancreatic NIT β-cells. Sotolone reversed the oxidative stress in NIT cells and decreased the expression and phosphorylation of protein kinase C-α and p47PHOX gene expression. Lactone also inhibited the protein kinase C-α/NADPH oxidase pathway, induced insulin secretion and prevented the apoptosis of NIT β-cells [109]. King et al. [110] have recently identified a new compound, *N*-linoleoyl-2-amino-γ-butyro lactone (Fig. 2), from the ethanolic extract of fenugreek seeds. The novel compound is shown to induce glucagon-like peptide-1 (GLP-1)-dependent cAMP production and GLP-1R endocytosis. The authors postulate that novel lactone binds to GLP-1 peptide and catalyzes the trypsin-mediated GLP-1 inactivation. Broca et al. [111] studied the structure activity relationship and insulinotropic activities of HIL, its isomers, and synthetic analogs. They noted that only the major isomer (2*S*, 3*R*, 4*S*)-4-hydroxyisoleucine imparted the desired insulin-releasing activity. The authors also observed that the structural features required for activity were (i) *S*-configurations at C-2 carbon, (ii) methylated at C-3 and C-4 carbons, and (iii) hydroxyl group in *S*-configuration at C-4 carbon. In a recent study, Korthikunta et al. [112] reported the design and synthesis of novel HIL analogs. They demonstrated that synthetic analogs exhibited superior glucose uptake activity compared to HIL in an in vitro study.

HIL reversed the inhibition of glycogen levels affected by TNF-α activity. In addition to HIL, the fiber-rich fraction of fenugreek seeds demonstrated antidiabetic effect by decreasing hyperglycemia, glycosuria, high plasma glucagon levels, and somastatin levels in alloxan-induced diabetic dogs (Table 2) [113]. An in vivo study in rats demonstrated the antidiabetic potential FSE. The extract selectively decreased serum glucose and increased serum insulin only in STZ-induced diabetic rats, but did not impart any changes in normal rats. The antidiabetic potential was equivalent to the known antidiabetic drug, glibenclamide [114]. Oral administration of fenugreek soluble dietary fiber helped lower the serum fructosamine and inhibited the platelet aggregation without effecting insulin levels in diabetic rats [115]. In a different study, Hannan et al. [116] observed that administration of fenugreek soluble dietary fiber lowered the serum glucose levels and increased liver glycogen levels and antioxidant capability. Also, increased the glucose uptake by 3T3-L1 adipocytes.

Jin et al. [117] demonstrated the nephroprotective and antidiabetic potential of fenugreek. They noted that fenugreek ameliorated the diabetic neuropathic conditions induced by STZ. Fenugreek decreased the blood glucose levels and

Table 1. In vitro antidiabetic effects of fenugreek products and pure compounds

Materials tested	Cell type	Concentration	Mechanisms	References
Fenugreek seed extract	HepG2 Cells	100 ng/mL	↑GLUT-2; ↑SREBP1C; ↑GK; ↑GS; ↑glucose uptake	Naicker et al. [100]
Fenugreek seed extract	L6C11 myotubes	5–20 μg/mL	↑Intracellular creatinine	Tomcik et al. [101]
Diosgenin	3T3-L1 cells	1–10 μM	↑PPAR-γ; ↑ aP2; ↑ LPL; ↑ C/EBPδ; ↑ GLUT-4	Uemura et al. [102]
4-Hydroxyisoleucine	Male Wistar rat islets	200 μM/L	↑Insulin	Sauvaire et al. [103]
4-Hydroxyisoleucine	HepG2 cells	5–40 μmol/L	↓TNF-α; ↓TECE; ↑TIMP3; ↓phosphorylation IRS-1; ↑IRS-1; ↑GLUT-4; ↑glucose uptake	Gao et al. [104]
4-Hydroxyisoleucine	NIDD rat islets	200 μM	↑Insulin secretion	Broca et al. [105]
4-Hydroxyisoleucine	HepG2 cells treated with TNF-α	20 μM	↑Glycogen; ↑phosphorylation Akt; ↑phosphorylation GSK; ↑phosphorylation JNK; ↓phosphorylation IRS-1	Lu et al. [106]
4-Hydroxyisoleucine	L6-GLUT4 myc myotubes	5–25 μM	↑Glucose uptake; ↑GLUT-4 translocation; ↑phosphorylation Akt	Jaiswal et al. [107]
4-Hydroxyisoleucine	Male Wistar rat islets	10 μM to 1 mM	↑Insulin release	Broca et al. [108]
Fenugreek lactone (sotolone)	Pancreatic NIT-1 β-cells	1 μmol/L	↓PKC-α; ↓phosphorylation PKC-α; ↓apoptosis; ↑insulin secretion; ↓PKC-α/NADPH	Gong et al. [109]

aP2, adipocyte protein 2; GLUT-4, glucose transporter-4; GSK, glycogen synthase kinase; LPL, lipoprotein lipase; PKC, protein kinase C; C/EBP, CCAAT/enhancer-binding protein.

improved the renal functions, such as, albuminuria, glycated hemoglobin (HbA1c), blood urea nitrogen, and kidney index in neuropathic rats. Raju and his group [18] have made similar observations that fenugreek seed powder normalized the aberrations caused by diabetes, such as increase in lipogenic enzymes and gluconeogenic enzymes, in the liver and kidneys and returned them to normal levels.

Vijayakumar and Bhat [118] reported that FSE corrected the metabolic deviations caused by diabetes. They demonstrated that FSE imparted extra pancreatic activity and exhibited insulin like properties by increasing glucose tolerance levels and decreasing serum insulin levels. Also, FSE upregulated the glucokinase and hexokinase enzymes activities in diabetic mice. In a different study, Vijayakumar et al. [119] reported the hypoglycemic effect of FSE in vivo. They noted that the beneficial effect was due to the activation of insulin signaling pathway in hepatic cells and adipocytes.

Significant reduction in glycemic response following oral glucose tolerance test was observed in rats that were administered soluble dietary fiber (galactomannan). Additionally, the plasma insulin, TGs, and total cholesterol were also noticeably lowered following the diet rich in soluble dietary fiber [120]. The nonlipid portion of the fenugreek extract inhibited the glucose absorption across brush border membrane of intestinal vesicles of high fat fed obese mice. When mice fed with high-fat diet were given fenugreek extract, there were significant changes in the levels of plasma glucose, plasma insulin and insulin resistance conditions when compared to control group mice that were fed high-fat food alone [121]. Beneficial effects, such as decrease in fasting glucose and increase in insulin, were observed in STZ-induced diabetic rats on diet supplemented with fenugreek leaves [122].

Zhou and Zhou [123] made similar observations when STZ-induced diabetic neuropathic and high fat fed diabetic rats were treated with trigonelline. They observed that alkaloid ameliorated the changes imparted by diabetes. Trigonelline improved the serum glucose, serum insulin, body weight, and sciatic nerve conduction velocity. They hypothesized that the beneficial effects of trigonelline might be through its action on GLP-1/p38 MAP kinase signaling pathway. In a study on the furanostolic saponin-rich fenugreek extract (Fenfuro™), Swaroop et al. [124] demonstrated the antidiabetic potential. They demonstrated Fenfuro™ reduced the plasma glucose and decreased the plasma TG levels in STZ-induced diabetic rats. Fenugreek seed powder restored the altered levels of neurolipofuscin accumulation and membrane bound enzymes as well as revived the glycemic conditions to normalcy in diabetic rat brain. Fenugreek powder significantly decreased the blood glucose levels and increased serum insulin levels. The beneficial effects were more significant when fenugreek seed powder was used in combination with sodium orthovanadate [125].

Table 2. In vivo antidiabetic effects of fenugreek extracts and pure compounds

Materials tested	Animal models	Dose	Duration	Mechanisms	References
Fenugreek seed extract	STZ-induced diabetic male Wistar rats	0.1–0.5 g/kg (p.o.)	14 days	↓Glucose; ↓urea; ↓uric acid; ↓AST; ↓ALT; ↓creatinine; ↑insulin	Eidi et al. [114]
Fenugreek seed extract	Alloxan-induced diabetic dogs	1.126–1.145 g/kg (diet) twice daily	21 days	↓Blood glucose; ↓plasma glucagon; ↓plasma somatostatin	Ribes et al. [113]
Fenugreek seed extract	Alloxan-induced diabetic BALB/CJ mice	15 mg/kg (i.p.)	5 days	↓Hyperglycemia; ↓serum insulin; ↑glucose tolerance; ↑glucokinase; ↑hexokinase	Vijaykumar et. al. [118]
Fenugreek seed extract	Alloxan-induced diabetic Swiss albino mice	1–15 mg/kg (i.p.)	Single dose	↑IR-β; ↑IRS-1; ↑PI3K; ↑glucose uptake	Vijaykumar et al. [119]
Fenugreek seed extract aqueous solution	High fat diet fed obese C57BL/6J mice	2 g/kg/day (gastric tube)	20 weeks	↓Plasma glucose; ↓plasma insulin; ↓insulin resistance	Hamza et al. [121]
Fenugreek seed powder	STZ-induced diabetic Sprague–Dawley rats	9 g/kg (diet)	12 weeks	↓Glucose; ↓albuminuria; ↓HbA1C; ↓BUN; ↓serum creatinine; ↓TGF-β1; ↓CTGF	Jin et al. [117]
Fenugreek seed powder	Alloxan-induced diabetic female albino Wistar rats	5% diet	21 days	↓Glucose-6-phosphate; ↓fructose-1,6-biphosphate; ↑pyruvate kinase; ↑phosphofructokinase; ↑lactate dehydrogenase	Raju et al. [18]
Fenugreek seed powder	Alloxan-induced female albino Wistar rats	5% (diet)	21 days	↓Blood glucose; ↑serum insulin	Kumar et al. [125]
Fenugreek leaf powder	STZ-induced diabetic female albino Wistar rats	0.5–1.0 g/kg (diet)	45 days	↓Fasting glucose; ↑insulin; ↑body weight; ↑pancreas weight	Balakrishnan et al. [122]
Fenugreek soluble dietary fiber	STZ-induced diabetic long Evans male rats	0.5 g/kg (diet)	28 days	↓Serum fructosamine; ↓platelet aggregation; ⊥insulin	Hannan et al. [115]
Galactomannan	High sucrose fed male Sprague–Dawley rats	5% (diet)	28 days	↓↓Body weight gain; ↓glycemic response; ↓plasma insulin; ↓↓epididymal adipose weight	Srichamreaon et al. [120]
4-Hydroxyisoleucine (HIL)	Noninsulin-dependent diabetic rats	50 mg/kg (i.v.) twice daily	6 days	↓Hyperglycemia; ↑insulin; ↑glucose tolerance	Broca et al. [105]
4-Hydroxyisoleucine (HIL)	Obese Zucker fa/fa rats	100 mg/kg (diet)	21 days	↑Glucose tolerance; ↓insulinemia; ↑peripheral glucose uptake	Broca et al. [108]
4-Hydroxyisoleucine (HIL)	STZ-induced diabetic male Wistar rats	50 mg/kg (i.p.)	28 days	↑PI3K; ↑↑insulin sensitivity	Broca et al. [108]
Trigonelline	STZ-induced diabetic male Wistar rats	40 mg/kg (diet)	48 weeks	↑GLP-1; ↓GLP-1 mRNA; ↓p38 MAPK; ↓HbA1C	Zhou et al. [123]
Fenfuro™	STZ-induced diabetic male Sprague–Dawley rats	450 mg/kg (p.o.)	30 days	↓Plasma glucose; ↓plasma triglycerides	Swaroop et al. [124]

BUN, blood urea nitrogen; CTGF, connective tissue growth factor; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide-3-kinase.

Raghuram et al. [126] carried out a clinical metabolic study in humans. Ten noninsulin-dependent diabetic (NIDD) subjects were randomly administered diets with and without 25 g of fenugreek seeds for 15 days in a crossover design. They reported their results from the intravenous glucose tolerance test and they noted there was significant reduction in plasma glucose and increase in erythrocyte insulin receptors. Also, reduction in plasma glucose curve, $t_{1/2}$, and increased metabolic clearance were observed. In another clinical study, diets with and without 100 g of defatted fenugreek seed powder were given randomly to 15 NIDD patients for 10 days in a crossover design to evaluate their hypoglycemic activity. Fenugreek powder caused significant reduction in fasting blood glucose, insulin response, and decreased glucose excretion through urine by 64% [127]. Gaddam et al. [128] recently conducted a 3-year randomized controlled clinical study in nondiabetic subjects with prediabetic conditions to evaluate the potency of fenugreek to prevent type 2 diabetes mellitus. They reported that fenugreek decreased the cumulative incidence of diabetes, fasting plasma glucose, postprandial plasma glucose and increased the serum insulin levels. Verma et al. [129] carried out a multicenter, randomized, placebo-controlled, double-blind clinical study of Fenfuro™ in 154 patient with type 2 diabetes mellitus for 90 days. The researchers noted that Fenfuro™ at doses 500 mg twice a day significantly reduced fasting plasma sugar and postprandial sugar levels without causing any serious adverse effects. Results clearly indicate that fenugreek is an inexpensive, low-risk dietary supplement that has potential to improve glycemic control in diabetes.

5.5 Antihyperlipidemic effect

Fenugreek inhibited the fat accumulation in 3T3-L1 cells by decreasing the expression levels of adipogenetic factors, namely PPAR-γ, SREBP1C, and CAAT element binding protein [130]. Additionally, fenugreek extract decreased the cellular TGs and cholesterol concentration in HepG2 cells and increased the uptake of LDL by upregulating LDL receptor. Diosgenin extracted from fenugreek was shown to reduce the hepatic and plasma TG and mRNA expression levels [90]. Trigonelline, an alkaloid isolated from fenugreek insuch as, leptin, resistin, and adiponectin [131].

Chaturvedi et al. [22] recently reported the antidyslipidemic effect of alcoholic FSE on the high fat fed diet- and triton-induced hyperlipidemic rats. They noted that at doses 200 mg/kg body weight, plasma cholesterol and TGs were lowered by 26.2 and 36.6%, respectively. Also, the chronic feeding of the extract reduced the hepatic lipid levels. The authors hypothesized that the mechanism of action might be due to the activation of several enzymes, such as lecithin-cholesterol acyltransferase, TG lipase, and lipoprotein lipase. Additionally, diosgenin inhibited the accumulation of TG and decreased the lipogenetic gene expression in HepG2 cells and also suppressed the liver X receptor-α transactivation in diabetic obese mice [132]. Fenugreek lowered the hepatic TGs and cholesterol levels by increasing the excretion of bile acids and cholesterol from the feces of rats in a dose-dependent manner [133].

Reddy and Srinivasan [134] reported antilithogenic effect when fenugreek was incorporated in diet. They noted that the rats on fenugreek diet had decreased cholesterol saturation, cholesterol content, cholesterol crystallization, and total protein in bile and increased cholesterol nucleation time. Fenugreek induced hypolipidemic effect in hyperlipidemic and diabetic rats by significantly reducing serum total cholesterol, LDL, lowered blood platelet aggregation, and by increasing HDL [115, 135]. Petit et al. [136] studied the feeding behavior in rats and reported that chronic administration of an FSE increased the food consumption and motivation to eat in rats and also induced hypocholesterolemia and hyperinsulinemia. Also, the increase in plasma insulin levels and decrease in LDL and VLDL levels were observed.

Fenugreek fiber lowered the TGs and total cholesterol by reducing the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme and increasing the excretion of neutral sterols in obese rats [137]. Fenugreek fiber both alone or in combination with garlic alleviated the lipid abnormalities and heart tissue changes. It also exhibited cardioprotective effect in myocardial infarction [138]. Similarly, steroidal saponins isolated from fenugreek selectively decreased the total plasma cholesterol without altering the TG levels in diabetic rats. Also, no change was observed in plasma insulin and blood glucose levels in normal rats [139]. Fenugreek seeds supplementation induced lipid modulatory effects by reducing the abnormal levels of total cholesterol, TG, and LDL [140]. Similarly, fenugreek leaves [141] and fenugreek ethanolic extract [102] decreased the levels of total cholesterol, TG, and free fatty acids in STZ-induced diabetic rats. Raju et al. [18] observed that fenugreek seed powder restored the altered gluconeogenic and lipogenic enzymes to normal levels and also stabilized glucose homeostasis in the liver and kidneys of rats.

Sowmya and Rajyalakshmi [142] reported that consumption of germinated seeds by human volunteers lowered the total cholesterol and LDL. However, no change was observed in HDL and TG levels. In another study, Sharma et al. [143] reported that total cholesterol decreased by 14% in noninsulin-dependent diabetic patients. Also, the reduction in LDL, VLDL, and TG levels was reported.

5.6 Antiobesity effect

Gao et al. [19] elucidated the mechanism by which HIL ameliorated obesity-induced insulin resistance. They postulated that HIL downregulated the TNF-α converting enzyme which blocks the conversion of mTNF-α to sTNF-α. These events blocked the signal transduction pathway and improved the obesity-induced insulin resistance in 3T3-L1 adipocytes. Raju and Bird [144] have reported similar findings in Zucker obese

rats fed with fenugreek seeds. They observed the drop in TNF-α levels and significant increase in membrane receptors and tumor necrosis factor receptor 2 in the livers of obese rats.

In another study, Mathern et al. [145] reported that fenugreek fiber significantly increased satiety and decreased hunger in obese subjects. They noted, the supplementation may be good short-term solution for weight loss. When obese rats were fed with fenugreek powder for 14 weeks, they observed a loss in body weight, changes in body measurements and nutritional values. The authors postulated that galactomannan present in seeds flushes out the sugars from the body before it enters the blood stream, thus resulting in weight loss. Kumar et al. [146] reported significant loss in body weight, BMI, and reduction in serum lipids and cardiac risk factors when high-fat fed rats where administered aqueous fenugreek extract. The fenugreek regulated the appetite by decreasing the levels of leptin in the adipose tissue. Similar observations were made in monosodium glutamate-induced obese rats [147].

Hua et al. [148] evaluated the role of furanostolic saponins (Fenfuro™) from fenugreek plant on insulin resistance in mice. They observed that Fenfuro™ reduced the elevated serum TG levels induced by insulin resistance and decreased the insulin-stimulated phosphorylation of protein kinase B in mice fed with high-fat diet. Fenfuro™ lowered the fat accumulation and improved glucose tolerance and insulin sensitivity.

Chevassus et al. [149] carried out a 6-week double-blind randomized placebo-controlled clinical trial with a fixed dose of FSE. They reported a significant drop in insulin to glucose ratio in overweight subjects, who were on diet administered with FSE. Robert et al. [150] reported that mixing untreated fenugreek seeds with rice or bread had positive impact in overweight and obese subjects. Fenugreek reduced the postprandial glucose and increased satiety. They speculate that HIL and galactomannan might be responsible for the beneficial effects. Number of studies reported similar findings on the role of fenugreek in reducing body weight and anthropometrical parameters [151, 152].

5.7 Sexual health-promoting effect

Aswar et al. [24] reported that furastanol glycoside fraction of fenugreek increased the muscle mass by increasing anabolic activity and with no change in the serum testosterone levels in immature castrated and immature noncastrated male rats. They postulated the beneficial effects may be due to the increased availability of free testosterone. A randomized double-blind and placebo-controlled clinical study on a proprietary generally recognized as safe affirmed FSE standardized to include 50% furostanol and steroidal saponins, Testofen™ involving 120 healthy aging males was carried out to study the androgen deficiency, sexual function, and androgen concentration [153]. The results suggest that Testofen™ significantly increased serum and free testosterone levels and improved sexual function. Additionally, subjects recorded lower aging male symptoms questionnaire score, implying decrease in symptoms of androgen deficiency. The beneficial effects are postulated as fenugreek's ability to stimulate GnRH/luteinizing hormone (LH), enhance the testicular sensitivity to LH, increase the synthesis of testosterone and decrease testosterone catabolism. Investigators have also noted that Testofen™ may incompletely inhibit 5-alpha reductase and aromatase enzymes. Testofen™ promoted positive effects on male libido and maintained normal testosterone levels. Also, fenugreek extract enhanced the physiological aspects of libido and improved the overall quality of life [154].

In a different study, Rao et al. [155] reported the positive effects of standardized proprietary FSE Libifem™ in enhancing the sexual function in healthy menstruating women with self-reported decreased sexual function. Authors noted that Libifem™ increased the levels of estradiol E2. The postulated mechanism of action that the authors have suggested is increased activity of aromatase enzyme, which catalyzes the conversion of testosterone to estradiol. Fenugreek extract was shown to have estrogenic activity through its ability to bind estrogen receptor and act as agonist for estrogen receptor transcription [156]. In another trial, glycoside fraction of fenugreek seeds (Fenu-FG) was given as supplementation to healthy male subjects enduring resistance training and there was significant increase in anabolic and androgenic activity and reduction in body fat around thighs, tricep, and chest regions without any changes to baseline biochemical and hematological parameters [157]. Maheshwari et al. [158] recently conducted an open-arm, open-label, multicenter clinical study on Furosap™ in 50 male volunteers for 12 weeks. The study results suggest that Furosap™ had several benefits, namely improved testosterone levels, increased levels of sperm counts, and also subjects demonstrated significant improvements in mental alertness and mood behavior. Additionally, Furosap™ significantly improved cardiovascular health and libido without causing any changes to blood chemistry.

5.8 Women's health-promoting effect

Swaroop et al. [159] conducted an open-label, one-arm, nonrandomized, postmarket surveillance study with fenugreek extract, Furocyst™ on 50 women diagnosed with polycystic ovary syndrome. The authors observed that Furocyst™ alleviated the symptoms associated with polycystic ovary syndrome by significantly increasing LH and follicle-stimulating hormone levels. Additionally, the extract reduced the cyst size and ovarian volume also normalized the menstrual cycle and ameliorated infertility in 12% of subjects. In another clinical study, Bashtian et al. [160] reported similar findings. They noted that fenugreek restored eumenorrhea in 55% of subjects and significantly decreased the polycystic ovaries following 2-month treatment. Fenugreek powder administered during menstruation ameliorated the symptoms associated with

dysmenorrhea, such as fatigue, headache, lack of energy, and syncope [161]. Fenugreek powder, rich in proteins, minerals, and nutrients, given to women of child bearing age fostered significant rise in hemoglobin and decreased the probability of subjects becoming anemic [23]. Overall, the human studies demonstrate the efficacy and safety of fenugreek products in the improvement of women's health.

5.9 Antitumor effects

5.9.1 Breast cancer

Sebastian and Thampan [162] investigated anticancer potential of aqueous and ethanolic extracts of fenugreek seeds using estrogen receptor-positive MCF-7 breast cancer cells (Table 3). The ethanolic extract showed superior antiproliferative effect to that of the aqueous extract. The ethanolic extract also induced early apoptotic changes, including flipping of phosphatidylserine and decrease of mitochondrial membrane potential ($\Delta\Psi_m$). Ancillary studies revealed the presence of a subG1 apoptotic cell population and cell cycle arrest at G2/M phase. An ethanolic extract of fenugreek seeds displayed antiproliferative effects against a panel of breast cancer cells, namely MCF-7, MDA-MB-231, T47D, and SKBR3 [20]. Alsemari et al. [163] observed similar cytotoxic effect of fenugreek seed aqueous extract against MCF-7 cells. A methanolic extract of fenugreek whole plant was found to exert growth-inhibitory effect against MCF-7 cells, possibly via apoptosis mediated by the death receptor pathway [164]. Fenugreek seed oil significantly diminished the viability of MCF-7 in a concentration-dependent manner [165]. However, the mechanism of such effect was not studied. Diosgenin, a steroidal saponin present in fenugreek, caused G1 cell cycle arrest by downregulating the expression of cyclin D1, cyclin-dependent kinase-2, and cyclin-dependent kinase-4, resulting in the inhibition of proliferation and induction of apoptosis in MCF-7 and MDA-MB-231 cells. Mechanistically, diosgenin inhibited pAkt expression and Akt kinase activity without affecting PI3 kinase level, resulting in the inhibition of its downstream targets, such as NF-κB, B-cell lymphoma-2 (Bcl-2), survivin, and X-linked inhibitor of apoptosis protein. The Raf/MEK/ERK pathway, another downstream target of Akt, was found to be inhibited by diosgenin in MCF-7, but not in MDA-MB-231cells [166].

The antineoplastic potential of fenugreek was evaluated in Ehrlich ascites carcinoma model in mice. Intraperitoneal administration of ethanolic extract of fenugreek seeds before or after inoculation of tumor cells produced more than 70% inhibition of tumor cell growth which could be due to anti-inflammatory mechanisms (Table 4) [167]. In another in vivo study, MCF-7 and MDA-MB-231 cells were implanted subcutaneously in female nude mice and upon reaching a volume of 50 mm^3, the tumors were injected with diosgenin (10 mg/kg) and tumor growth was monitored for 4 weeks. Diosgenin-treated tumors exhibited significant regression in both xenograft tumor models [166]. Amin et al. [168] reported for the first time the chemopreventive effects of an aqueous extract of fenugreek seeds against 7,12-dimethylbenz(α)anthracene (DMBA)-initiated breast cancer in rats. The fenugreek extract (200 mg/kg) significantly inhibited DMBA-induced mammary hyperplasia as evidenced by tumor incidence, tumor multiplicity (number of tumors/tumor-bearing animal) as well as cellular and ultrastructural analyses.

5.9.2 Connective tissue cancers

In a xenograft model, mice bearing sarcoma 180 tumors were given tail vein injections of diosgenin (20 mg/kg/day) for 27 days. Treatment with diosgenin significantly reduced tumor size and tumor mass compared to control animals. Immunohistochemical analysis of tumor sections showed antiproliferative, proapoptotic, and antiangiogenic mechanisms of action of diosgenin. Interestingly, thymoquinone, an active constituent of black cumin, potentiated the effects of diosgenin [169].

5.9.3 Gastrointestinal tract and associated cancers

A dose-dependent cytotoxic effect of an ethanolic FSE was observed in a battery of pancreatic cancer cell lines [20]. However, a fenugreek whole plant methanolic extract did not exhibit any cytotoxic activity against human epithelial type 2 liver cancer cells [170]. Khalil et al. [171] prepared a crude methanolic extract of fenugreek seeds and investigated its anticancer effect and molecular mechanisms using HepG2 human hepatocellular carcinoma (HCC) cells. The results indicated that the extract treatment for 48 h showed a cytotoxic effect and apoptosis induction which was mediated by upregulation of p53, Bcl-2-associated X-protein, and proliferating cell nuclear antigen as well as caspase-3 activation. The GC-MS analysis of the extract revealed the presence of 14 bioactive phytochemicals, including squalene and naringenin. Fenugreek plant extract was found to be cytotoxic against colon cancer cell lines, such as 502713 and HT-29 without any mechanistic insight [170].

Diosgenin exhibited anticancer activity by blocking the proliferation of HT-29 human colon cancer cells and induced apoptosis, at least in part, by inhibition of Bcl-2 and activation of caspase-3 [172]. Diosgenin suppressed constitutive and inducible activation of signal transducer and activator of transcription 3 (STAT3) in human HCC cells with concomitant inhibition of c-Src, Janus kinase 1, and Janus kinase 2 activation. Diosgenin also downregulated the expression of STAT3-regulated genes, such as cyclin D1, Bcl-2, Bcl-xL, survivin, Mcl-1, and vascular endothelial growth factor. All the molecular events may be responsible for the inhibition of proliferation, increased accumulation of cells in G1/G0 phase, and potentiation of the apoptotic effects of doxorubicin and

Table 3. Antitumor effects of fenugreek fractions and phytoconstituents based on in vitro studies

Materials tested	Cell lines	Effects	Mechanisms	Concentration	References
Breast cancer					
Fenugreek seed aqueous and ethanolic extracts	MCF-7	Showed antiproliferative effects	↑Apoptosis; ↓$\Delta\Psi_m$; ⊥G2/M	25–100 μg/mL	Sebastian and Thampan [162]
Fenugreek seed ethanolic extract	MCF-7, MDA-MB-231, T47D, and SKBR3	Exhibited growth inhibition		0.1–100 μg/mL	Shabbeer et al. [20]
Fenugreek seed aqueous extract	MCF-7	Inhibited proliferation	↑Apoptosis	100–300 μg/mL	Alsemari et al. [163]
Fenugreek whole plant methanolic extract	MCF-7	Displayed reduced cell viability	↑Apoptosis; ↑Fas	10–100 μg/mL	Alshatwi et al. [164]
Fenugreek seed oil	MCF-7	Decreased cell viability		10–1000 μg/mL	Al-Oqail et al. [165]
Diosgenin	MCF-7 and MDA-MB-231	Inhibited cell viability	↑Apoptosis; ⊥G1; ↓cyclin D1; ↓cdk-2; ↓cdk-4; ↓pAkt ↓Akt kinase; ↓NF-κB, ↓Bcl-2; ↓survivin, ↓XIAP	5–30 μM	Srinivasan et al. [166]
Gastrointestinal tract and associated cancers					
Fenugreek seed ethanolic extract	MiaPaca, HS766T, Panc 1, L3.6PL, and BXPC3	Inhibited cellular growth		1–100 μg/mL	Shabbeer et al. [20]
Fenugreek seed methanolic extract	HepG2	Retarded cell growth	↑Apoptosis; ↑p53; ↑Bax; ↑PCNA; ↑caspase-3	50–2000 μg/mL	Khalil et al. [171]
Fenugreek whole plant methanolic extract	502713 and HT-29	Inhibited proliferation		100 μg/mL	Verma et al. [170]
Diosgenin	HT-29	Inhibited cell growth	↓Bcl-2; ↑caspase-3	20–100 μM	Raju et al. [172]
Diosgenin	HepG2, HUH-7, and C3A	Suppressed proliferation	↑Apoptosis; ⊥G1/G0; ↓STAT3; ↓c-Src; ↓JAK1; ↓JAK2; ↓cyclin D1; ↓Bcl-2; ↓Bcl-Xl; ↓survivin; ↓ Mcl-1; ↓VEGF	10, 50 μM	Li et al. [173]
Hematological cancer					
Fenugreek seed ethanolic extract	Jurkat cells	Exhibited cytotoxicity	↑Autophagy; ↑LC3	30–1500 μg/mL	Al-Daghri et al. [54]
Fenugreek seed aqueous extract	T-cell and B-cell lymphoma	Displayed cytotoxic effects	↑Apoptosis	100–300 μg/mL	Alsemari et al. [163]
Protodioscin	HL-60	Induced reduction of cell growth	↑Apoptosis	2.5–10 μM	Hibasami et al. [175]
Diosgenin	K562	Reduced cell viability	↑Apoptosis; ⊥G2/M; ↓cyclin D1; ↓$p21^{Cip1/Waf1}$; ↑cdc2; ↑caspase-3; ↓Ca^{2+}; ↑ROS; ↓Bcl-2; ↓Bcl-xL; ↑Bax	6.25–66.7 μM	Liu et al. [176]

Table 3. Continued

Materials tested	Cell lines	Effects	Mechanisms	Concentration	References
Diosgenin	KBM-5	Suppressed proliferation	↓NF-κB; ↓IκBα kinase; ↓pIκBα; ↓IκBα; ↓Akt; ↓cyclin D1; ↓COX-2; ↓c-myc; ↓IAP1; ↓Bcl-2; ↓Bcl-XL; ↓Bfl-1/A1; ↓TRAF1; ↓cFLIP; ↓MMP-9	1–50 μM	Shishodia and Aggarwal [177]
Lung cancer					
Fenugreek whole plant methanolic extract	A-549	Inhibited proliferation		100 μg/mL	Verma et al. [170]
Fenugreek seed ethanolic extract and diosgenin	A-549	Retarded tumor cell growth	↓hTERT	1–60 μM	Rahmati-Yamchi et al. [178, 179]
Prostate cancer					
Fenugreek seed ethanolic extract	DU-145, PC-3, and LNCaP	Inhibited cellular growth	↑Apoptosis; ⊥G2/M; ↓p53; ↑p21; ↓pEGFR ↓pAkt	5–35 μg/mL	Shabbeer et al. [20]
Diosgenin	PC-3	Inhibited proliferation, migration, and invasion	↓MMP-2; ↓MMP-9; ↓MMP-7; ↓EMMPRIN; ↑TIMP-2; ↓VEGF; ↓pERK; ↓pJNK 1&2; ↓pPI3K; ↓pAkt; ↓NF-κB	5–30 μM	Chen et al. [180]
Skin cancer					
Fenugreek seed oil	HEp-2	Showed cytotoxicity		10–1000 μg/mL	Al-Oqail et al. [165]
Diosgenin	A431 and Hep2	Induced cytotoxicity	↑Apoptosis; ↑sub-G1; ↑Bax-Bcl-2; ↑Cyt. c; ↓pJNK; ↓pAkt	10–100 μM	Das et al. [169]

Bax, Bcl-2-associated X-protein; cdk, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; HEp-2, human epithelial type 2; JAK1, hTERT, human telomerase reverse transcriptase; Janus kinase 1; JAK2, Janus kinase 2; PCNA, proliferating cell nuclear antigen; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; XIAP, X-linked inhibitor of apoptosis protein.

Table 4. In vivo anticancer and chemopreventive effects of fenugreek fractions and pure compounds

Materials tested	Animal models	Effects	Mechanisms	Dose (route)	Duration	References
Breast cancer						
Fenugreek seed ethanolic extract	Balb C mice inoculated with Ehrlich ascites cells	Inhibited tumor cell count	Activation of macrophages	100, 200 mg/kg (i.p.)	3–7 days	Sur et al. [167]
Fenugreek seed aqueous extract	DMBA-induced mammary carcinogenesis in female Wistar rats	Reduced tumor incidence, multiplicity and weight		200 mg/kg (p.o.)	Once daily for 2 weeks	Amin et al. [168]
Diosgenin	MCF-7 and MDA-MB-231 xenografts in female nude mice	Diminished tumor growth		10 mg/kg (intratumor)	Once	Srinivasan et al. [166]
Colon cancer						
Fenugreek seed powder	DMH-induced colon carcinogenesis in male Wistar rats	Reduced tumor incidence and frequency	↓β-Glucuronidase; ↓mucinase	2 g/kg (diet)	30 weeks	Devasena and Menon [174]
Fenugreek seed powder or Diosgenin	AOM-induced colon carcinogenesis in male F344 rats	Suppressed total colonic ACF and reduced the number of multicrypt foci		Powder: 1% (diet) Diosgenin: 0.05%, 0.1% (diet)	4–8 weeks	Raju et al. [172]
Connective tissue cancers						
Diosgenin	Sarcoma 180 xenografts in Swiss albino mice	Suppressed tumor mass and volume	↑Apoptosis; ↓Ki-67; ↓CD31	20 mg/kg/day (i.v.)	27 days	Das et al. [169]
Skin cancer						
Fenugreek seed ethanolic extract	DMAB-initiated and TPA-promoted skin tumorigenesis in male Swiss albino mice	Reduced tumor incidence, tumor yield and tumor burden	↑GSH; ↓LPO	400 mg/kg/day (p.o.)	1–17 weeks	Chatterjee et al. [181]
Fenugreek seed methanolic extract	DMAB-initiated and croton oil-promoted skin tumorigenesis in female Swiss albino mice	Reduced the number, incidence, and multiplicity of tumors	↓LPO; ↑GSH; ↑GPx; ↑GR; ↓COX-2; ↓MPO; ↓NF-κB; ↓p38 MAPK; ↑p53	100 μL (topical)	Twice weekly for 32 weeks	Ali et al. [182]

ACF, aberrant crypt foci; DMH, 1,2-dimethylhydrazine; GSH, reduced glutathione; MAPK, mitogen-activated protein kinase; LPO, lipid peroxidation; TPA, 12-*O*-decanoylphorbol-13-acetate.

paclitaxel in HCC cells [173]. All these results suggest that diosgenin is a novel inhibitor of STAT3 activation with potential in the treatment of HCC.

Supplementation of fenugreek seed in the diet inhibited tumor incidence in colon and reduced tumor frequency in colon and intestine of rats subjected to 1,2-dimethylhydrazine-induced colon carcinogenesis. This beneficial effect of fenugreek seed may be attributed to decrease in the activity of β-glucuronidase and mucinase in various tissues studied [174]. Raju et al. [172] evaluated the preventive efficacy of dietary fenugreek seed and its major bioactive constituent diosgenin against azoxymethane-induced rat colon carcinogenesis during initiation and promotion phases. Continuous feeding of fenugreek seed powder or diosgenin suppressed preneoplastic colonic lesions, known as aberrant crypt foci. Dietary fenugreek powder or diosgenin during the promotional stage also inhibited the formation of aberrant crypt foci.

5.9.4 Hematological cancers

Incubation of human T-cell lymphoma Jurkat cells with 50% ethanolic extract of dry fenugreek seeds resulted in cell death in a concentration- and time-dependent fashion. Distinct morphological alterations involving appearance of large vacuoles, membrane disintegration, and elevated transcriptional expression of LC3 indicated that fenugreek extract induced autophagy in Jurkat cells. Interestingly, GC-MS analysis of fenugreek extract revealed the presence of cedrene, eugenol, gingerol, vanillin, and zingerone [54]. In another study, an aqueous extract of fenugreek seeds displayed selective cytotoxic effects against T-cell and B-cell lymphoma in vitro. In T-cell lymphoma, the anticancer effect was mediated through induction of apoptosis [163].

Protodioscin, a phytochemical isolated from fenugreek, displayed strong growth-inhibitory activity against HL-60 human leukemia cells. Morphological, flow cytometric, and molecular analyses revealed induction of apoptosis of tumor cells [175]. Liu et al. [176] investigated potential antiproliferative activity and underlying molecular mechanisms of diosgenin using K562 human chronic myelogenous leukemia cells. Diosgenin inhibited the proliferation of K5U62 cells via cell cycle arrest at G2/M phase and apoptosis with disruption of Ca^{2+} homeostatsis and mitochondrial dysfunction playing important roles. In an elegant study, diosgenin induced cytotoxicity and inhibited proliferation of KBM-5 human chronic myelogenous leukemia cells. Diosgenin suppressed TNF-induced NF-κB activation, activation of IκBα kinase, IκBα phosphorylation, IκBα degradation, p65 phosphorylation, and p65 nuclear translocation through inhibition of Akt activation. TNF-induced expression of NF-κB-regulated gene products involved in cell proliferation (cyclin D1, COX-2, and c-myc), antiapoptosis (IAP1, Bcl-2, Bcl-XL, Bfl-1/A1, TRAF1, and cFLIP), and invasion (matrix metalloproteinase (MMP)-9) were also downregulated by diosgenin. Diosgenin also potentiated the apoptosis induced by TNF and chemotherapeutic drugs, such as paclitaxel and doxorubicin [177].

5.9.5 Lung cancer

Verma et al. [170] reported in vitro anticancer effect of fenugreek whole plant extract against A-549 human lung cancer cells. Treatment of A-549 cells with fenugreek extract diosgenin and pure diosgenin inhibited the growth of cancer cells and caused downregulation of human telomerase reverse transcriptase expression, indicating abrogation of telomerase activity [178, 179].

5.9.6 Prostate cancer

Fenugreek seed ethanolic extract registered cytotoxic effects against various prostate cancer cell lines, namely LNCaP, DU-145, and PC-3. The extract increased sub-G1 cell population in LNCaP and PC-3 cells and imparted G2/M cell cycle arrest in PC-3 cells. The extract treatment caused a downregulation of mutant p53 in DU-145 cells and upregulation of p21, inhibition of phosphorylation of epidermal growth factor receptor as well as transforming growth factor-β-induced phosphorylation of Akt in PC-3 cells [20].

Diosgenin inhibited the proliferation of PC-3 cells in a concentration-dependent manner. When exposed to nontoxic concentrations of diosgenin, PC-3 cell migration and invasion were suppressed considerably. Diosgenin inhibited the activities of MMP-2 and MMP-9. The mRNA level of MMP-2, MMP-9, and MMP-7 as well as extracellular inducer of MMP (EMMPRIN) were also suppressed, whereas tissue inhibitor of metalloproteinase-2 (TIMP-2) was increased by diosgenin. Additionally, diosgenin abolished the expression of vascular endothelial growth factor in PC-3 cells and tube formation of endothelial cells. Finally, diosgenin inhibited ERK, JNK and phosphoinositide-3-kinase/Akt signaling pathways and NF-κB activity [180]. These encouraging results underscore a new therapeutic potential of diosgenin for antimetastatic therapy.

5.9.7 Skin cancer

Exposure of human epithelial type 2 cells to fenugreek seed oil decreased cell viability and altered the cellular morphology [165]. Das et al. [169] investigated the antineoplastic activity of diosgenin against squamous cell carcinoma in vitro. Diosgenin inhibited the proliferation and induced apoptosis by increasing the sub-G1 cell population, chromatin condensation, and DNA laddering in A431 and Hep2 cells. Additional studies showed increased Bcl-2-associated X-protein/Bcl-2 ratio, activation of caspases, and cleavage of poly ADP ribose

polymerase as well as inhibition of phosphorylation of Akt and JNK.

Chatterjee et al. [181] investigated chemopreventive potential of a methanolic extract of fenugreek seed against two-stage mouse skin carcinogenesis initiated by DMBA and promoted with 12-*O*-decanoylphorbol-13-acetate. Treatment of animals with the extract during various stages of the carcinogenic process (preinitiation, postinitiation, promotion, and throughout the study) reduced cumulative number of skin papillomas, tumor yield, and tumor burden. Nevertheless, the extract treatment was mostly effective when given during all stages of skin tumorigenesis. Ancillary studies showed modulatory effects of the extract on hepatic antioxidant defense system of experimental animals. Ali et al. [182] conducted an in vivo study in which topical application of fenugreek seed methanolic extract suppressed the number, incidence, and multiplicity of DMBA-initiated and croton oil-promoted skin tumors in mice. Mechanistically, the extract diminished cell proliferation (indicated by proliferating cell nuclear antigen expression), induced stable expression of p53 (apoptosis), restored endogenous antioxidant defense, enhanced immunosuvelliance, and inhibited inflammatory response possibly by suppressing NF-κB.

6 Toxicology and safety profile

Fenugreek preparations have been used since the ancient times and the normal usage of products are regarded as absolutely safe [6]. As per code of federal regulations, essential oils, oleoresins, and natural extractives obtained from fenugreek are generally recognized as safe [183]. Flammang et al. [184] subjected FSE containing 40% 4-hydroxyisoleucine to standard battery of tests advocated by FDA. The tests such as reverse mutation assay, mouse lymphoma forward mutation assay, mouse micronucleus assay gave negative results indicating that fenugreek extract is not genotoxic and is safe to use in patients with diabetes. Swaroop et al. [124] reported FenFuro™ the furanostolic saponins enriched FSE did not induce toxicity at dose of 5 g/kg body weight in female rats. In mice, lethal dose 50 (LD_{50}) of fenugreek aqueous extract was 10 g/kg body weight [185]. The oral administration of glycosidic fenugreek extract to mice for 28 days showed the median LD_{50} as 4.25 g/kg body weight and no adverse effects were observed in both sexes [186]. When administered intraperitoneally, the fenugreek ethanol extract, the LD_{50} was 5 g/kg body weight [187]. Recently, Ouzir et al. [188] reported the human equivalent dose for oral administration for adult weighing 60 kg ranged from 21.16 to 48.64 g. In diabetic patients, 25 g/day of fenugreek seed powder for 24 weeks did not elicit any hepatic or renal toxicity and no hematological aberrations were reported. Debitterized fenugreek powder did not induce toxicity or mortality in mice at 2 g/kg body weight and in rats at 5 g/kg body weight [189]. An extreme caution is advised for individuals who are allergic to chickpeas in using fenugreek as their might be possibility of cross-reactivity [190, 191]. Ulbricht et al. [21] presented an evidenced based review on fenugreek dosage, interactions, and adverse reactions. They summarized that patients who are on hypoglycemic agents should monitor their blood sugar levels very closely when using fenugreek products. Also, pregnant women should exercise caution and not use doses higher than found in foods as fenugreek might exhibit abortifacient effects. However, no human clinical studies or data exist in this regard. Testofen™ when tested in a clinical study on 60 volunteers was well tolerated without any changes to baseline hematological and biochemical parameters [153]. In another clinical study involving Libifem™ no major adverse events were observed. However, minor events such as increased incidences of migraines and indigestion were reported in four out of 80 women participated in the trial [155].

7 Conclusion and future perspectives

From the studies outlined in this review, it is clear that the ethnomedicinal plant, fenugreek (*T. foenum-graecum*), has the potential for not only serving as herb, spice, and food additive, but also has the ability to prevent and treat wide array of human diseases. In terms of structural diversity, the secondary metabolites of fenugreek are enriched with some unprecedented phytochemicals, namely 4-hydroxyisoleucine and trigonelline. The health-promoting and disease-preventive therapeutic effects of fenugreek extracts and its compounds are explained by the underlying cellular and molecular mechanisms, namely free radical scavenging, hypocholesterolemia, hyperinsulinemia, insulinotropic, enhanced anabolic activity, anti-inflammatory, cell cycle alteration, apoptosis and autophagy, antimetastatic activities, modulate dysregulated cellular signaling pathways, and antimicrobial activity.

The in vitro, in vivo, and clinical studies presented in this review show that fenugreek extracts and phytoconstituents are effective in preventing and treating many health conditions including diabetes, inflammation, cancer, obesity, hyperlipidemia, and microbial infections (Fig. 5). Also, extensive blood chemistry analysis of all the clinical studies did not demonstrate any signs of hepatotoxicity, cardiotoxicity, and nephrotoxicity, which strengthen the evidence of safety of fenugreek seeds. Despite some progress, further studies are needed to specify the mechanism of action of fenugreek products and isolated pure compounds at molecular level. By applying advanced analytical techniques, number of novel phytochemicals from fenugreek plant have been isolated and characterized. However, since these compounds could be obtained in small amounts, one of the challenges is the synthesis of compounds for further biological studies. Additionally, effective quality control measures and improved standards for production of fenugreek-derived phytochemicals are paramount, as these components could vary greatly due to variations in cultivation and extraction processes as well

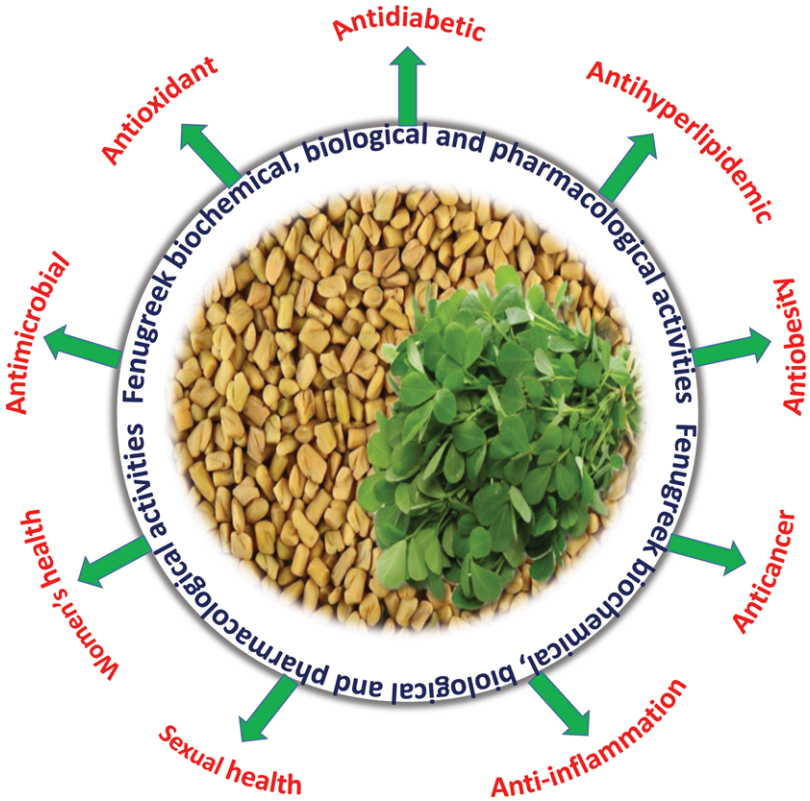

Figure 5. Summary of pharmacological activities of fenugreek plant.

as raw materials used for isolation of pure chemicals and extracts.

Fenugreek has plethora of phytoconstituents which include alkaloids, flavonoids, glycosides, phenolic compounds, saponins, and dietary fibers along with proteinergic and nonproteinergic amino acids. Even though many chemical classes of fenugreek plant have been acknowledged for their beneficial effects, the activity of only few compounds, namely 4-hydroxyisoleucine and diosgenin, has been studied and there is an urgent need to study other phytochemicals. It is very likely that fenugreek has phytochemical synergy between various molecules to exert broad pharmacological activities. The knowledge of synergistic effect and the underlying mechanisms will enable us to choose the best phytochemical constituent(s) required to prevent or treat disease.

Another significant area for development for fenugreek products is to improve their therapeutic efficacy by applying novel methods such as nano-formulation and liposomal drug delivery. At present, limited information is available on pharmacokinetic properties of fenugreek products. These studies are very critical toward the advancement of fenugreek-based drug development and extensive research is required in this area. The studies reported in the review highlight a feasible approach toward screening fenugreek products as modulators for various diseases and suggest that underlying mechanisms might provide viable targets for drug discovery. Fenugreek phytochemicals may serve as lead molecules toward the development of chemically and metabolically stable molecules with superior pharmacokinetic properties.

Regarding pharmaceutical information and commercial viability, standardized fenugreek extract is becoming popular as a botanical drug, medical food, and nutraceutical in the United States and around the world. It is very popular as nutraceutical supplement in the United States marketplace and around the world since late 1980s (http://www.usanews-today.com/health/2016/09/19/whats-fenugreek-good-for/). A meta-analysis of ten clinical trials has demonstrated that fenugreek significantly ameliorates fasting blood glucose [192]. An earlier meta-analysis demonstrated that high doses (at least 5 g of fenugreek seed powder) were associated with significant reductions in fasting blood glucose levels in diabetics [193]. Incidentally, both fenugreek seed and standardized FSE are now considered as promising and cost-effective complimentary options for clinical management of diabetes (http://www.nutraingredients-usa.com/Markets/The-key-ingredients-for-blood-sugar-management/(page)/7). Several popular and patented brands, including Testofen®, Libifem®, Fenfuro®, Furocyst®, Furasap® and other generic products, are available in the marketplace.

In conclusion, while fenugreek shows significant promise for preventing and treating numerous diseases, additional studies are needed to ascertain the real potential of fenugreek products as efficacious nutraceutical supplements, medical foods, botanical drugs, or over the counter drugs. The studies

presented and analyzed in this review highly suggest that fenugreek is a unique medicinal plant with versatile health benefits.

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