REVIEW on *Trigonella foenum-graecum* Linn.

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**ABSTRACT**

The plant fenugreek (*Trigonella foenum-graecum* L.), which belongs to the Leguminosae (Fabaceae) family, is widely distributed throughout the world. The species name "foenum-graecum" means "Greek hay," stating that it has been used as a forage crop. *Trigonella foenum-graecum* was once used as a traditional medicine for a variety of ailments. In this review the pharmacognosy of the whole plant, the quantitative and qualitative phytochemistry and the pharmacological activities of the various parts of the plants were discussed. The pharmacological study includes acetylcholine esterase inhibition, analgesic, antibacterial, antifatigue, anti-inflammatory, antiulcer, antidiabetic, anti-melanogenic, antipyretic, arthritis, antianemia, anticancer, hypocholesterolemic properties, anticataract, hepatoprotective, immunomodulatory, anti-urothiasis, galactagogue, antimicrobial, neuropathy, anti-fertility, urinary anti-infectives and its toxicological study. This review article presents an overview of experimental evidence for the nutraceutical potential of fenugreek seeds.

**KEYWORDS:** Fenugreek, *Trigonella foenum-graecum* L. Antidiabetic, Hypocholesterolemic.
1. INTRODUCTION

*Trigonella foenum-graecum* Linn. is an annual plant grown mainly in India, Egypt, and Morocco, native to the countries in the Mediterranean bordering the eastern shores [1]. The name fenugreek is derived from the Greek word *foenum-graecum*, which means that the plant has historically been used to smell inferior hay. *Trigonella*, the genus name is derived from the ancient Greek name, which means 'three-angled' which refers to the triangular shape of the flowers [2]. There are two relatively different types of plants, the dwarf type, grown for culinary purposes and the tall growing type, known as Methi in Punjab, grown for fodder use [3]. Fenugreek seeds tastes bitter, and their medicinal properties have been known for a long time. For more than 2500 years, fenugreek seeds have been in use. Largest producer of fenugreek is India, and it is produced for their culinary and medicinal uses [4]. Vitamins, dietary fibres and proteins and also chemical constituents such as alkaloids, coumarins, flavonoids, saponins, and vitamins are rich in fenugreek seeds [5]. The oral use of fenugreek is for diabetes, constipation, gastroesophageal reflux disease, gastritis, dysmenorrhea, obesity, atherosclerosis, polycystic ovary syndrome (PCOS), hyperlipidaemia, and for stimulating lactation, mouth ulcers, dyspepsia, beriberi, kidney diseases, loss of appetite, fever, boils, Parkinson's disease, cellulitis, tuberculosis, chronic coughs, chapped lips, bronchitis, baldness, exercise performance, and cancer. It is topically used as a poultice for local inflammation, gout, wounds, myalgia, leg ulcers, lymphadenitis, and eczema [6-8].

2. PHARMACOGNOSTIC STUDIES

2.1 Taxonomical classification [9].

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Fabales
- Family: Fabaceae
- Genus: *Trigonella*
- Species: *Trigonella foenum-graecum*
- Botanical name: *Trigonella foenum-graecum*

2.2 Vernacular names [10-12].

- Arabic: Hhelbah, Hhelbeh, Hulba and Hulabah
Fenugreek is an annual herb [8]. The plants are spreading, moderately branched, and weak. Fenugreek is a leguminous, herbaceous and rainfed crop.

2.3 Leaf
Fenugreek leaf is pinnately trifoliate, alternate, compound, stipules triangular, petiolate, pubescent, smooth and fragrant. Leaf is dark green at adaxial surface and light green in abaxial surface. Leaflet shapes vary from ovate-orbicular to oblong-lanceolate and are toothed, with nerves extended out into the teeth. The fenugreek plant's first leaf, with its entire margin and a long petiole, is simple, sometimes weakly trifoliolate, and oval or orbicular. The stipules are big and covered with hair that is soft. The tops of the leaf petioles are thickened, and beyond the point of attachment, the lateral leaflets are attenuated. On the underside, the petioles and petioleles have simple, soft, sparse hair and are a little cartilaginous. Dentation in the upper leaves is more deeply defined than in the lower. The petioles and leaflet blades are tinged with anthocyanin to a varying degree of green [13-15].

2.4 Stem
Stem is erect, may be single or may be branched at the stem base, green, smooth, herbaceous and having strong sweet aroma. In rare cases, the stem is simple, sparsely pubescent and typically hollow. Anthocyanin is found at the base of the plant or covered by the whole plant [16].
2.5 Flower
The flowers are 1-2, axillary, sessile, racemed, triangular in shape and are whitish to lemon yellow in colour. The flowering season is June to July. Following a vegetative growth cycle, fenugreek plants enter the reproductive process. It has found two kinds of flowering shoots, indeterminate and determinate. The most popular are axillary flowers exhibiting an indeterminate growth habit. Near determinate or determinate types that is blind shoots bearing axillary and terminal flowers are not prevalent. There are two types of flowers they are cleistogamous and aneictgamous. Of these two types cleistogamous is commonly found. The flowers are bisexual, bracteates, complete, hypogynous, pedicellate and zygomorphic. The inflorescence is racemose. Sepals are five and are gamosepalous, valvate and green. The number of peats is five and they are papilionaceous polypetalous, and standard with two wings. The anterior petals, with ascending imbricate aetivation, form a boat-shaped kneel. 10 diadelphous stamens exist; around the ovary 9 stamens are united to form a tube-like structure and the remaining one diadelphous stamen is free. In the kneel, anthers are introse, basified and enclosed. The gynoecium is monocarpellary, superior ovary and unilocular. Ovules are numerous with marginal placellation. The style is long, and the stigma is terminal. Calyx is 6-8 mm and is soft, hairy with teeth. The corolla is pale yellow and is 13-19 mm long [14,16].

2.6 Pod
Pods are thin, sword-shaped and at the time maturity the pod changes from green colour or light purple colour to brown colour or yellow brown colour. Pods are 9.5-18.6 cm long, 0.2-10.4 cm broad, curved, and sometimes straight with transient hairs with a beak. There are about 10–20 seeds in each pod [14].

2.7 Seed
Seeds are small, hard, oblong, rhomboidal with a deep furrow extending from one side obliquely. And they are in brownish yellow colour with bitter taste and pleasant odour. Seeds are about 0.2-0.5 cm long and 0.15-0.35 cm broad. Hilum and micropyle are nearly in the middle of one of the long, narrow sides of a small depression, the former being clearly visible as a whitish point. The seed is then divided into two unequal lobes by a furrow that runs diagonally along part of each of the adjoining sides. Two accumbent cotyledons—the smaller, the radical—are found in the larger lobe if the seed is split transversely to the side of the hilum in order to pass across both seed lobes. Both are yellowish in colour and the radicle is separated
from the cotyledons by a darker, hornier, translucent endosperm. The endosperm swells, producing mucilage when immersed in water [9,16].

2.8 Root
A mass of fingery structures makes up the root. The plant's taproot grows at first, followed by a great number of secondary roots. Root nodules are produced by the roots and create a symbiotic relationship with the bacterium *Rhizobium* [14].

3. ISOLATION AND CHARACTERISATION
The presence of an artefact, 25α-spirosta-3,5-diene, was reported, together presence of gitogenin, and evidence for the isolation of their 25β-epimers was obtained. The powdered seeds yielded fixed oil (7%) after extraction with petroleum, which contained squalane-like hydrocarbons. β-sitosterol and cholesterol were among the sterols identified. Gitogenin was not present in the leaf, stem, or root, which would otherwise provided the seed's compounds. Soliman and Mustafas isolated and named trigonellagenin, a compound with m.p. 189-190" and acetate m.p. 1% 158". Trigonellagenin, is a combination of diosgenin and yamogenin, rather than a new compound [17]. The highest amount of free amino acid found in *Trigonella foenum-gracecum* seed has been reported as (2S, 3R, 4R)-4-hydroxyisoleucine. The (2R, 3R, 4R)-isomer forms a minor component of *Trigonella* seed. The content of 4-hydroxyisoleucine in fenugreek increases as seedlings and plants grow, and 14C-isoleucine was found to be an effective biosynthetic precursor [18].

Fenugreekine, a new C₃-steroidal sapogenin-peptide ester, has been isolated from *Trigonella foenum-gracecum* seeds. It yielded diosgenin, yamogenin, (25R)-spirola-3, 5-diene, a mixture of three isomeric (2S,3R,4R-, 2S,3R,4S-, 2S,3S, 4R-) -4-hydroxyisoleucine lactones, 4'-hydroxyisoleucyl-4-hydroxyisoleucine lactone, and a partly characterised C₁₄-dipeptide [19]. A new glycoside has been isolated and shown to have the structure from fenugreek seed (25S)-22-O-methyl-5α-furostan-3β,22,26-triol 3-O-a-rhamnopyranosyl(1→2) [-β-D-glucopyranosyl (1→31)] - β-D-glucopyranoside-26-O-β-D-glucopyranoside [20]. Trigocoumarin (C₁₆H₁₈O₆ from analytical data and M⁺ peak at m/e 306 in its MS) was obtained as colourless needles, mp 87-88 °C. Under UV light it gave a blue fluorescence and exposed yellowish orange color with NaOH. And it did not react to any metal/acid reduction test. Significant absorptions were observed in the IR range at 1605, 1700,
and 1730 cm⁻¹; these colour reactions and spectral results are attributed to a coumarin system [21].

The methyl ethers of two new furostanol glycosides, trigofoenosides F and G, were isolated from the methanolic extract of *Trigonella foenum-graecum* seeds [22]. *Trigonella foenum-graecum* seeds yielded two new furostanol glycosides, trigofoenosides A and D, as well as their methyl ethers, A-I and D-I [23]. *Trigonella foenum-graecum* seeds yielded two new furostanol glycosides, trigofoenosides B and C, as well as their methyl ethers, B-I and C-I, respectively [24]. Preparative Thin Layer Chromatography (TLC) on an argentized Silica gel divided the three fractions (A-C) into their individual components. Smilagenin (20 mg) and sarsasapogenin (28 mg) were classified as two crystalline compounds obtained from fraction A; fraction B only contained diosgenin, yamogenin, tigogenin, and neotigogenin. Fraction C yielded three pure compounds: yuccagenin (9 mg), gitogenin (65 mg), and neogitogenin (13 mg) [25].

Quercetin glycoside [quercetin 3-O-β-d-glucosyl (1→2)-β-D-galactoside 7-O-β-D-glucoside] as well as the [kaempferol 3-O-β-d-glucosyl(1→2)-β-d-galactoside 7-O-β-d-glucoside and kaempferol 3-O-β-D-glucosyl(1→2)-(6″-O-acetyl)-β-d-galactoside 7-O-β-d-glucoside] are the two two kaempferol glycosides which were isolated from the stems of *Trigonella foenum-graecum* L. along with a known kaempferol glycoside, lilyn [kaempferol 3-O-β-D-glucosyl(1→2)-β-D-galactoside]. Their structures were established by analysis of chemical and spectral evidence. By extracting the concentrated methanolic extract from the stems of *Trigonella foenum-graecum* with petroleum ether, ethyl acetate, and n-butanol in order, the concentrated methanolic extract from the stems of *Trigonella foenum-graecum* was fractionated. To obtain compound 1, the n-butanol extract was subjected to column chromatography (CC) on silica gel followed by CC on Sephadex LH-20. Compounds 2, 3 and 4 were obtained from the aqueous fraction using a mixture of CC on silica gel and Diaion HP-20, followed by preparative High Performance Liquid Chromatography (HPLC) [26].

The known flavone C-glycosides, apigenin 6,8-C-di-β-galactopyranoside (1), apigenin 6-C-β-arabinopyranosyl-8-C-β-galactopyranoside (3), apigenin 6-C-β-glucopyranoside (9), apigenin 6-C-β-xylopyranosyl-8-C-β-galactopyranoside (2), luteolin 8-C-β-glucopyranoside (4), luteolin 6-C-β-glucopyranoside (5), apigenin 8-C-β-glucopyranoside (8), luteolin 8-C-(200-O-(E)-p-coumaroyl-β-glucopyranoside) (10), and apigenin 8-C-(200-O-(E)-p-coumaroylβ-glucopyranoside) (11) in addition to the flavone C-glycosides, apigenin 6-C-β-xylopyranosyl-8-C-(6000-O-(3-hydroxy-3-methylglutaroyl)-β-glucopyranoside)
and apigenin 6-C-β-chinovopyranosyl-8-C-β-galactopyranoside (6) were isolated from fenugreek seeds. For the first time in this species, compounds 1, 5, and 10 were identified [27]. The ethyl acetate-soluble fraction of the ethanol extract of the *Trigonella foenum-gracecum* seeds yielded ten flavonoids and their structures have been elucidated by spectroscopic methods 5,7,30-trihydroxy-50-methoxylisoflavone (1), formononetin (3), biochanin A (2), tricin (5), daidzein (6), irilone (4), calycosin (7), vitexin-200-O-p-trans-coumarate (9), orientin-200-O-p-trans-coumarate (8), and tricin-7-O-b-D-glucopyranoside (10). Compounds 1 and 8 are new flavonoids, and they strongly promoted H2O2 induced 2BS cell proliferation [28].

4. PHYTOCHEMISTRY STUDIES

4.1 QUALITATIVE ANALYSIS OF FENUGREEK [29,30]

The test for carbohydrates and proteins are done and the result revealed the presence of carbohydrates and proteins in leaf stem and seed. The presence of carbohydrate and protein is moderate in leaf and strongly present in stem and seed. The test for phenol, catechol and sterol is performed. And it was showed that phenol was slightly present in leaf, absent in stem and abundantly present in seed. Catechol was absent in leaf, stem, and seed. Sterol is strongly present in leaf, stem, and seed. The test for flavonoid an alkaloid reported the presence of flavonoid and alkaloid in *Trigonella foenum-gracecum*. Flavanoids was absent in stem but moderately found in leaf and seed. The strong presence of alkaloid was found in leaf, stem, and seed. Glycoside was present in stem and seed and it was absent in leaf. Saponin was mildly present in all the parts. Strong presence of quinones was found in stem, leaf, and seed. The terpenoid is slightly present in leaf but strongly present in stem and seed. Tannin is slightly present in leaf, stem, and seed.

4.2 QUANTITATIVE PHYTOCHEMISTRY

4.2.1 Total phenolic content

With gallic acid as standard by Folin-Ciocalteu method the total phenolic content is estimated. Total phenol content increased in the extract with rising solvent polarity. A high level of total phenol content from acetone extract was obtained. Methanol 50% had a high phenolic content in the extract after acetone 50%. It is evident that the addition of a certain amount of water increases the efficiency of extraction [31]. Compared to petroleum ether methanolic extract has the highest phenolic content [32].
4.2.2 Total flavonoid content
By aluminium chloride colorimetric assay, the flavonoid content of methanolic extract of seed was estimated and it was found to have 424.951 mg/l. The hydroalcoholic extract was found to have 0.489 mg/g of flavonoids by the same method [32,33].

4.2.3 Total tannin content
Using FeCl₃ and the gelatin test, the tannin content was determined. Fenugreek methanolic extract with a tannin content of 116.259 mg/l measured as tannic acid equivalent of tannins. In comparison with maceration, the tannin content of the decoction was significantly elevated [32,34].

4.2.4 Total alkaloid content
The total alkaloid content was estimated by Harborne method. The content of alkaloids in aqueous maceration extract was 1.71 ± 0.02% and for decoction was 2.12 ± 0.015% [34].

4.2.5 Total saponin content
Using diosgenin as standard the total saponin content is determined using UV-Vis spectrophotometric device. The total saponin content was 195.89 ± 1.07 (mg DE/g d.w) at optimum condition [35].

4.2.6 Determination of mineral elements
As a result of the mineral analysis, fenugreek seeds contain various concentrations of copper, zinc, manganese, and calcium. And the concentration of copper, zinc, manganese and calcium was found to be 0.734 mg/100 ml, 0.961 mg/100 ml, 0.503 mg/100 ml and 0.403 mg/100 ml respectively [36].

5. PHARMACOLOGY PART OF FENUGREEK
5.1 Acetylcholine esterase inhibition
TLC profile and High Performance Thin Layer Chromatography (HPTLC) fingerprints were developed for the hydro alcoholic extract and the percentage of the active marker was determined using HPTLC peak area and the trigonellin content in Trigonella foenum-gracecum hydroalcoholic extract was found to be no less than 13 mg g⁻¹ w/w. The AChE inhibitory activity of crude fenugreek seed extracts, fractions, and trigonelline was determined using the Ellman's method in a 96-well micro plate assay with TLC bioassay detection. AChE inhibition was
observed in both the alcohol extract fraction of ethyl acetate and the complete alkaloids [37].

5.2 Analgesic

From the partially purifies extract of *Trigonella foenum-gracecum* analgesic and anti-inflammatory effect were performed. Analgesic effect was performed by two methods thermally induced pain-hot-plate method using pentazocin as standard and chemically induced pain-acetic acid induced writhing using diclofenac sodium as standard. In the acetic acid induced writhing method the methanolic extract at 40 mg/kg showed higher analgesic effect when compared to the standard diclofenac sodium and in hot plate method fenugreek showed significant effect when compared to control but showed lower effect when compared with the standard pentazocine [38]. Aqueous extract of *Trigonella foenum-gracecum* exhibited analgesic effects in intraperitoneal at dose (1 g/kg) as well as intrathecal at dose (0.5, 1 and 2 mg/rat) but not in intracerebroventricular administrations. (1 and 3 mg/rat). It was identified that the central action of the extract of *Trigonella foenum-gracecum* and the partial involvement of the spinal 5-HT system in the analgesia caused by it during the second stage of the formalin test and suggests that other analgesic mechanisms coexist [39].

The aqueous extract of fenugreek leaf was checked for antinociceptive activity by tail flick test. In this experiment sodium salicylate (100 mg/kg and 300 mg/kg) is used as a positive control and the extract was given at the concentration of 500 mg/kg, 1000 mg/kg and 2000 mg/kg. The antinociceptive effects of the 300 mg/kg sodium salicylate is less effective than the extract 2000 mg/kg [40]. Different extracts of fenugreek were used in the evaluation of peripheral and central analgesic activity by acetic acid induced writhing test and hot plate method respectively. For hot plate and writhing model, pentazocin and paracetamol were taken as standard drug respectively. The methanol extract was more significant compared to the various extracts, such as petroleum ether, chloroform, ethyl acetate and methanol extracts from leaves and seeds of *Trigonella foenum-gracecum*. All the *Trigonella foenum-gracecum* extracts showed greater peripheral and central analgesic activity intraperitoneally at dose 50 mg/kg [41]. The antinociceptive and anti-inflammatory effects were evaluated in the six major fractionation of fenugreek seed methanolic extracts. Formalin test was done to evaluate antinociceptive effect in which ibuprofen and morphine were used as standard. Both antinociceptive and anti-inflammatory effects were found in methanolic extract at a dosage of 100 mg/kg, and the alkaloid positive alkaline chloroform fraction, had the most analaciceptive effect [42].
5.3 Antibacterial
Fenugreek was well known for its many pharmacological activities, here the fenugreek extract was tested for antibacterial activity. In this study the crude methanol extract is further fractioned using petroleum ether, dichloromethane and ethyl acetate. In the extended spectrum beta lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* the extracts were checked for antibacterial activity. But only the aqueous extract showed inhibition and the lowest minimum inhibitory concentration was reported at 10 μg/μl with Ec021SGH [43]. Fenugreek seed was reported for many antimicrobial property, here the fenugreek leaf was checked for antibacterial activity against the UTI causing bacteria such as *Klebsiella* spp., *Pseudomonas aeruginosa*, *Enterobacter aerogens*, *Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis*. Among ethanol, methanol, hexane, chloroform and aqueous extracts the methanol extract has been reported to inhibit all the strains of bacteria mentioned above. The aqueous extract of fenugreek leaf showed the minimum zone of inhibition [44].

5.4 Anti-fatigue
Anti-fatigue potential of *Trigonella foenum-graecum* was studies by weight loaded forced swim test. The dosage given was 10 mg/kg. The fenugreek treated rats and control rats were allowed to swim with the constant weight of 5% of its body weight for alternative days for three weeks. For the analysis blood was taken from the heart immediately after the last exercise into a heparinised syringe into centrifuge tubes. The experiment showed significant results in fenugreek extract treated groups when compared with the control group [45].

5.5 Anti-inflammatory
By using carrageenan-induced oedema methodin fenugreek the anti-inflammatory study was done. The methanolic extract was given through intraperitoneal route using dexamethasone and ibuprofen as standard. Fenugreek cream was formulated at various concentration and its anti-inflammatory effect was compared with 1% hydrocortisone ointment. When compared to control both the methods gave significant results [46]. In another study using the fenugreek mucilage the anti-inflammatory study was performed by inducing arthritis by Freund's adjuvant. On 21st day of adjuvant arthritis the group treated with fenugreek mucilage showed maximum percentage of oedema inhibition. And the effects of fenugreek mucilage were higher than the standard drug indomethacin. In paw tissue histopathology,
supplementation with fenugreek mucilage showed reduced oedema formation and cellular infiltration [47].

5.6 Antiulcer
The gastroprotective effect of fenugreek was studied using aqueous extract of fenugreek and a gel extracted from fenugreek in ethanol induced ulcer rats. The anti-secretory action rises in lipid peroxidation and enhancement in oxidation potential of the gastric mucosa by fenugreek showed it has a considerable gastroprotective effect. Histological tests showed that the seed-derived soluble gel fraction was more effective in preventing lesion formation than omeprazole which is taken as a standard drug [48]. In this study honey with fenugreek extract in combination is given to rats against ethanol induced gastric damage. Honey in combination with cimetidine, aqueous and alcoholic extract of fenugreek reduced gastric lesion macroscopically. Honey with aqueous extract of fenugreek was more effective than all combinations and is it is confirmed that the aqueous extract has the maximum gastroprotective effect [49].

5.7 Antidiabetic
Both *Trigonella foenum-gracecum* seed powder and their extracts when fed together with glucose, the soluble dietary fibre fraction had a significant hypoglycemic effect in NIDDM model rats [50]. Control and alloxan-induced diabetic rats were given oral doses of 2 and 8 g/kg of *Trigonella foenum-gracecum*. There was a dose-related hypoglycemic impact in control and diabetic rats [51]. Aqueous and ethanolic extract of fenugreek leaf was administered to control and alloxan induced diabetic rat it showed significant reduction in glucose level. The study reveals that in normoglycemic and alloxan-induced hyperglycaemic rats, the aqueous extract of *Trigonella foenum-gracecum* leaves administered both orally and intraperitoneally has a hypoglycaemic impact [52]. Creatine kinase activities were found to decrease significantly in the tissues during experimental diabetes compared to control rats. The decreased activity was normalised to almost control values by all the antidiabetic compounds used, namely insulin, vanadate, and Fenugreek seed powder [53].

By HOMA model glycaemic control and insulin resistance of fenugreek was studied. Insulin resistance derived from the HOMA model showed a decrease in beta-cell secretion percentage and improvement in insulin sensitivity in fenugreek treated Group [54]. Oral administration of methanolic and aqueous extract of fenugreek to normoglycemic rats had concluded the dose of 1 g/kg body weight only has a
significant result [55]. The blood glucose level was brought under control by 21 days treatment of fenugreek powder in type 1 diabetes induced rats by alloxan [56].

The oxidative enzyme damage in alloxan induced diabetes rats were reversed on administration of Vanadate and fenugreek exhibiting its antioxidant property [57]. Soluble dietary fibre of fenugreek was found to reduce the serum fructosamine level without affecting the insulin level and atherogenic lipids level were also reduced [58]. Sodium-orthovanadate and *Trigonella foenum-gracecum* were given to alloxan induced diabetes rats to investigate their effect in ameliorate altered lipid metabolism. The short-term effect of oral administration of fenugreek maintained the lipid profile at near normal [59].

In addition to metabolic normalisation, the role of *Trigonella* seed powder in reversing the diabetic state at the cellular level further proves its ability as an antidiabetic agent [60]. On administration of fenugreek powder the imbalance of liver pyruvate kinase, phosphoenolpyruvate carboxykinase and skeletal muscle glucose transporter enzymes was restored and there was significantly reduction in blood glucose level [61]. Administrations of the extract significantly decreased serum glucose, total cholesterol, triacylglycerol, urea and many parameters which is similar to the drug glibenclamide [62]. The hemorheological parameters like glycated haemoglobin, plasma viscosity, whole blood viscosity of high shear rate and low shear rate were reduced on oral intragastric intubation of fenugreek in streptozotocin induced diabetic rats [63].

In alloxan induced diabetics rats when fenugreek ethanol extract was administered in different doses 2g/kg showed the maximum effect [64]. In vitro intestinal glucose uptake in jejunum and ileum of lean and obese rats on administration of galactomannan from Canadian fenugreek revealed that increasing in the concentration of galactomannan the uptake of low or high concentration of glucose was reduced progressively [65]. There was significant decrease in lipid peroxidation and increase in antioxidant enzymes in the heart tissue of alloxan induced diabetic rats on administration of fenugreek extract [66]. GII an antihyperglycemic compound isolated from aqueous extract of fenugreek has the potential to reduce Glycosylated haemoglobin and increase the insulin in rabbits on intermittent therapy of GII and it was more potent in reducing the fasting blood glucose level [67].

When used to lower blood glucose levels, the addition of *Trigonella* to vanadium greatly eliminated vanadium toxicity [68]. In alloxan induced diabetes in female wistar rats there was a reverse in hyperglycaemia in rat brain and also improving the diabetic complications on fenugreek administration [69]. Administration of fenugreek to alloxan induced diabetic adult male rabbits showed significant decrease
in blood glucose level and serum cholesterol level and increase in insulin level [70]. The hydro alcoholic extract of fenugreek has been found to lower the fasting plasma glucose, plasma insulin, insulin resistance, triglycerides and total cholesterol in high fat diet C57/BL6J mouse model [71]. The total saponin of *Trigonella foenum-gracecum* in combination with sulfonylureas when given to type 2 diabetes mellitus patients were found to be effectively reduce the fast blood glucose, 2-h post-parandial blood glucose, Glycosylated haemoglobin, clinical symptomatic quantitative scores [72].

5.9 Anti-melanogenic
The steroidal saponin glycosides were isolated from fenugreek and they are tested for various pharmacological activities. In this article the 3 steroidal saponin glycosides pseudoprotodioscin (3), 26-O-β-D-glucopyranosyl-(25R)-furost-5(6)-en-3β,22β,26-triol-3-O-α-L-rhamnopyranosyl-(1”′→2′)-O-[β-D-glucopyranosyl-(1’’” →6′)-O]-β-D-glucopyranoside (1) , and minutoside B (2) were isolated from fenugreek were selected and studied for anti-melanogenic activity in B16F1 cells. Melanogenesis was was weakly suppressed by 2 and strongly suppressed by 1 and 3 in B16F1 cells [73].

5.10 Antipyretic
To examine the anti-pyretic activity of *Trigonella foenum-gracecum* the brewer’s yeast 20% (w/v) was administered to induce hyperthermia and sodium salicylate was used as standard drug. The aqueous extract showed significant result after 1 and two hours of administration [74].

5.11 Arthritis
Freund's adjuvant-induced arthritis in albino rats were subjected to the study of effect of ethanolic extract of fenugreek on arthritis rats. The paw volume and body weight of the animals were checked on 4th, 8th, 14th and 21st days. On 22nd day the animals were sacrificed and checked for biochemical parameters and inflammatory mediators. Cartilage tissue was isolated for the estimation of lipid peroxidation, superoxide dismutase and glutathione. The IL-1α, IL-1β, IL-2, IL-6 and TNF-α levels and lipid peroxidation levels decreased significantly in *Trigonella foenum-gracecum*. It increased the levels of glutathione and superoxide dismutase in cartilage tissue and it has been found that 400 mg/kg dose of foenum graecum showed more prominent results than a 200-mg / kg dose of *T.Graecum foenum* [75].
5.12 Antianemia
The effect of ginger and fenugreek on blood lipids, blood sugar, platelet aggregation, fibrinogen and fibrinolytic activity was studied in a placebo-controlled study. Blood lipids and blood sugar level were not affected when fenugreek is given at a dose of 2.5 g twice a day but there was a slight decrease in blood lipids when it was administered to coronary artery disease patients with NIDDM. When administered to mild NIDDM patients’ blood sugar level was reduced significantly and there was mild reduction in blood sugar level when administered to severe NIDDM patients. Fenugreek administration had no effect on the aggregation of platelets, fibrinolytic function, and fibrinogen [76]. Fenugreek galactomannan fractions that is polysaccharide from cold water extraction and polysaccharide B from alkali extraction and its fractions fraction 1 and fraction 2 were used to estimate the activation of phagocytosis and proliferation, secretion of IgM in human hybridoma HB4C5 cells. The alkali extracted polysaccharide B showed 40% of activation of phagocytosis at 10µg/ml. The alkali extracted polysaccharide showed inhibited IgM secretion effect but fraction 1 showed 9.8% activation at the concentration 5 µg/ml. And also, at the concentration 2.5 µg/ml of fraction 1 and fraction 2 it expressed an inhibition of 2.5 % and 24.7% respectively [77]. Anaemia may be identified by calculating the amount of haemoglobin that determines the capacity of oxygen to carry the tissues of the body. A phase I randomised clinical trial was conducted to observe its effect on haemoglobin levels by giving women of childbearing age a daily oral medicinal dose of powdered fenugreek seeds for a given period. A substantial increase of 2.24 gm percent haemoglobin was observed at the end of the third month, in subjects supplemented with the medicinal dosage of powdered fenugreek seeds [78].

5.13 Cancer
The alcohol extract of fenugreek when administered by intraperitoneal route to ehrlichascites carcinoma model in Balb-C mice it showed 70% inhibition of tumour cell growth. And boosted the peritoneal exudate cell and macrophages cell counts [79]. In 7,12-dimethylbenz(a)anthracene induced breast cancer in rats, fenugreek inhibited the mammary hyperplasia and reduced its occurrence. Epidemiological studies also include apoptosis as a mechanism that can mediate the protective effect of the anti-breast cancer of Fenugreek [80]. Protodioscin purified from fenugreek showed strong inhibition against HL-60 cells, the human leukaemia cells. And morphological modification showing apoptotic bodies was observed in these cells. But protodioscin did not show significant effect in KATO III the human stomach
cancer cells [81]. The growth inhibitor of breast, pancreatic and prostate cancer cell lines was 10-15 µg/ml of fenugreek treatment for 72 hours. FE tended to be growth inhibitory for PCa cell lines when tested at higher doses (15–20 µg/ml), but not for either primary prostate or hTert-immortalized prostate cells. The surprising finding was that cancer cell death occurs despite growth stimulatory pathways being upregulated (phosphorylated) by fenugreek at the same time [82]. The selective cytotoxic effects of fenugreek extract in vitro to a panel of cancer cell lines, including T-cell lymphoma was observed. Furthermore, the proteomics data cluster analysis showed that the protein profile of the specific fenugreek used by the patient differs significantly from three other regional fenugreek extract subtypes [83]. The in vitro experiments indicated that diosgenin inhibits cell growth and induces dose-dependent apoptosis in the human colon cancer cell line of HT-29. In addition, diosgenin induced apoptosis in HT-29 cells at least partially by bcl-2 inhibition and caspase-3 protein expression induction [84].

5.14 Hypercholesterolaemic
A haemolytic isolate from the deffated fenugreek ethanol extract was studied for hypocholesterolaemic property. The isolate was purified from the extract by dialysis and the dialysate found to contain saponins by tlc. After 4 weeks administration of fenugreek extract of dose 30-50 g/kg for hypercholesterolaemic rats in 2 separate feeding experiments, it was concluded that there was reduction in plasma cholesterol level at the range of 18% to 26%. And there was a tendency to lower liver cholesterol concentrations. These studies indicate that there were hypocholesterolaemic components in the ethanol extract from fenugreek seeds, which tend to be saponins that bind with bile salts in the digestive tract [85]. In two groups of rabbit hypercholesterolemia was induced by feeding cholesterol 100 mg/kg/day for one week. Then they were divided into 2 groups, group 1 and group 2. Group 1 animals were treated with same amount of cholesterol diet while the group 2 animals were treated with fenugreek principle along with the cholesterol diet. In addition to preventing the elevation of serum cholesterol, (LDL+VLDL) c, triacylglycerols, and total cholesterol / HDLc and (LDL+VLDL) c / HDLc ratios, the Fenugreek principle has decreased almost all of these values [86]. *Trigonella foenum-gracecum* was administered to normal and alloxan-induced diabetic rats in low (2 g/kg) and high (6 g/kg) doses in the form of unroasted and roasted powdered seeds. In various serum lipids such as total cholesterol, triglycerides, LDL and VLDL cholesterol in ordinary rats, both the unroasted and roasted forms produced a significant decrease in their elevated levels and increased HDL cholesterol in diabetic rats. Its hypolipidemic
effect may constitute a protective mechanism against atherosclerosis growth as it associated with hyperlipidemic and the incidence of atherosclerosis is greatly increased in diabetics [87]. A soluble dietary fibre extracted from Canadian grown fenugreek Galactomannan (GAL) was administered to three high sucrose diet male Sprague-Dawley rats. The 3 groups are 10% cellulose, 7.5% cellulose + 2.5% GAL and 5% cellulose and 5% GAL. Oral glucose tolerance test was performed after 3 weeks and blood samples were collected one week later. There was significant fall in the level of triglycerides and total cholesterol in all GAL fed rats with reduction in epididymal adipose weight. This shows the GAL has the potential of decreasing the lipidemic status along with the reduction of abdominal fat [88]. The monosodium induced dyslipidaemia and oxidative rats when treated with fenugreek showed reduction in serum total cholesterol, triglycerides, lactate dehydrogenase, aspartate amino transferase, alanine amino transferase, hepatic and cardiac lipid peroxides levels. And there was increase in serum high density lipoprotein, hepatic and antioxidant enzymes and superoxide dismutase and catalase levels which is analogous to the standard obesity drug orlistat [89].

5.15 Anticataract
There was decrease in opacity index which is an anti-cataract effect was exhibited by the alcoholic extract of fenugreek in alloxan induced diabetic rats with the development of cataract [90]. The blood glucose, glycosylated haemoglobin level, poly pathways enzymes like aldose reductase and sorbitol dehydrogenase and fructose in diabetic lens was reduced when treated with fenugreek. And modified the rat lens activity of hexokinase, aldose reductase, sorbitol dehydrogenase, glucose-6-phosphate dehydrogenase, glutathione peroxidase, glutathione reductase to control values. The histopathological changes like disintegration of the inner nuclear layer cells with reduction in rough endoplasmic reticulum and swelling of mitochondria in bipolar cells were restored by the treatment of fenugreek [91]. Fenugreek has greatly restored glutathione and decreased the level of malondialdehyde in selenite induced cataract in rat eye. Compared to control, a substantial restoration in the activity of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase was observed in the supplemented fenugreek group [92].

5.16 Hepatoprotective
The prevention of enzymatic leakage rise in lipid peroxidation and antioxidant property were seen in ethanol induced toxic rats when treated with aqueous extract
of fenugreek [93]. To study the hepatoprotective effect of polyphenolic extract of fenugreek, the Chang liver cells were incubated with 30 mM ethanol to induce toxicity. There was significant increase in cell viability, reduction in lactate dehydrogenase leakage and normalised glutathione ratio both reduced and oxidised, when treated and incubated with fenugreek extract. Formation of thiobarbituric acid was reduced by the extract and reduced apoptosis was noticed in fenugreek treated cells [94]. Zucker obese rats with hepatic steatosis when treated with fenugreek showed reduction in liver weight. And there was reduction in both bound and soluble forms of TNF-α protein and an elevation in TNFR-II in liver of obese rats [95]. Increase in markers of liver dysfunction like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin and g-glutamyl transferase (GGT) in plasma and reduction in liver glycogen was noticed when rats were continuously administered with ethanol. When this ethanol treated animals were treated with polyphenolic extract of fenugreek it restored levels of markers of liver injury and mitigated alterations in the metabolising and detoxifying enzymes of alcohol and the cytochrome-c reductase electron transport portion [96].

The research aims to examine the impact of fenugreek polyphenol seed extract on liver lipids and collagen in ethanol induced hepatotoxic experimental rats. On Fenugreek administration to alcohol-fed rats substantially increased the lipid profile and decreased the content of collagen, crosslinking, aldehyde and peroxidation [97]. Fenugreek polyphenol extract reduced the lipid peroxidation products and protein carbonyl content in ethanol induced liver damaged rats. And increased the activities of antioxidant enzymes and restored the level of thiol groups when compared to the positive control silymarin [98].

The ethanolic extract of leaf when examined for H₂O₂ and CCL₄ induced hepatotoxic rats it showed significant activity in superoxide radical and nitric oxide radical scavenging. Also showed significant anti lipid peroxidation effect in invitro goat liver model [99]. Cypermethrin induced hepatic toxicity cause increased thiobarbituric acid reactive substances, glutathione depletion and reduction of superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase activities in the liver and kidneys. Total phospholipids and increased phospholipase A and C activity in the liver and kidneys and increased serum marker enzyme activity, aspartate transaminase, alanine tansaminase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase were significantly decreased. Treatment with 10% fenugreek extract exhibited antioxidant status replenishment and nearly normalised all values, suggesting the protective effect of fenugreek [100].
5.17 Immunomodulatory

Body weight, relative organ weight, lymphoid organ cellularity, delayed type of response to hypersensitivity (DTH), plaque-forming cell (PFC) assay, haemagglutination titre (HT), SRBC (QHS) quantitative haemolysis assay, phagocytosis and lymphoproliferation have been evaluated in various group of animal classes. After the administration of fenugreek extract for 10 days at 50, 100 and 250 mg/kg there was significant increase in organ weight of thymus and liver but not in spleen and kidney. In the lymphoid organ cellularity study, there was a significant increase in thymus and bone marrow but not in spleen. Increased DTH response at doses of 50 and 100 mg/kg but not statistically significant changes at higher doses of 250 mg/kg were observed. As evaluated by PFC, humoral immunity showed an elevated response at a dosage of 100 mg/kg, but no significant effect was observed at 50 and 250 mg/kg. The plant extract also demonstrated a modulatory effect at all doses in the HT test. A significant increase in the phagocytic index and phagocytic ability of macrophages was produced by plant extract. Is has been concluded that immunostimulatory effect was showed by the *Trigonella foenum-gracecum* [101].

Phagocytic activity, cell mediated and humoral immune system on mice was studied for the ethanolic extract of Fenugreek. In animals treated with methi at doses of 200 and 400 mg/kg body weight, immunomodulatory effects were assessed in the carbon clearance test, delayed form of hypersensitivity (DTH), T cell population test, and sheep erythrocyte agglutination test (SEAT). High phagocytic index was showed by fenugreek in carbon clearance test and there was decrease in foot paw thickness in delayed type of hypersensitivity. And there was significant increase in both antibody titer and t cell population test when compared to control [102]. To study the immunomodulatory effect of *Trigonella foenum-gracecum* the sharptooth catfish were selected and are divided into control, 1% thyme and 1% fenugreek. After 30 days of administration the fishes were divided into 2 half, one half was subjected to study the immune parameters as differential leucocytic counts, serum globulins, phagocytic activities, phagocytic index, catalase and glutathione peroxidase and the other half of fish was subjected to challenge infection with *Aeromonas hydrophila* to investigate the disease resistance ability. The serum protein, glutathione peroxidase and catalase increased significantly in groups administered with thyme and fenugreek when compared to control group. Serum globulin, phagocytic index and percent were higher in fenugreek treated group compared to thyme and control group. Monocytes and lymphocytes were significantly high in all treated groups. In fishes
challenged with *A. hydrophila*, the total mortalities in diet-fed fish supplemented with 1% thyme and fenugreek were 40% and 26.67%, respectively, compared with 66.7% in the control group. It has been concluded that thyme and fenugreek will positively stimulate the immune system of *C. gariepinus* and reduce the mortality rate of *A. hydrophila* challenged fish [103].

### 5.18 Anti-urolithiasis

In 3% glycolic acid induced oxalate urolithiasis Sprague-Dawly rats the oral treatment of fenugreek on a daily basis reduced the calcium oxalate deposition in kidney. This may be due to the mild diuretic activity possessed by fenugreek [104]. In ethylene glycol with ammonium chloride induced calcium oxalate renal crystals rat the administration of fenugreek showed reduction in the amount of calcification and total calcium amount in renal tissue [105].

### 5.19 Galactagogue

To study the effect of fenugreek on lactation 60g of fenugreek seed powder was administered to lactating zumeri breed goats at early lactation stage. On the daily basis the milk yield was recorded and twice a week the blood samples were collected. There was significant decrease in the plasma glucose level and urea in fenugreek treated goats. The growth hormone in plasma was increased during six hours of bleeding in fenugreek treated goats and the milk production was also increased by 13%. The increased milk production may be due to the growth hormone stimulation [106].

### 5.20 Antimicrobial

Fenugreek seed oil showed strong inhibition against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Aspergillus fumigatus*. Hence this oil can be used as antibiotic and as antimycotics at lower concentration [107]. When investigated for antifungal property against *Pythium aphanidermatum*, *Botrytis cinerea*, *Alternaria sp.*, *Fusarium graminearum* and *Rhizoctinia solani* not grounded seed extraction showed the strongest inhibition followed by ground seed extraction and leaf and stem extraction [108]. The antimicrobial activity when studied by diffusion method with mature leaf methanol extract, early leaf methanol extract and ethanolic seed extract, the mature leaf methanol extract showed the maximum effect [109]. The titanium dioxide fenugreek nanoparticles were investigated by Kirby-Bauer method and it showed an excellent antimicrobial activity. Owing to the presence of hydroxyl groups leading to the death of cells, titanium nanoparticles can
dissolve the outer membranes of bacteria. The antibacterial activity against Gram-negative bacteria was lower than that of Gram-positive bacteria [110].

5.21 Neuropathy
IND01 prepared from fenugreek seeds showed sustain protection against thermal hyperalgesia and deranged motor function test. Motor nerve conduction velocity reduction in rats with sciatic nerve crush injury but not with partial sciatic nerve ligation was restored fifteen days after daily oral administration of IND01. And in rat models of painful peripheral neuropathy, IND01 was found to be efficient [111].

5.22 Anti-fertility
In immature castrated male wistar rats the anabolic and androgenic activity was studied on administration of furostanol glycosides of fenugreek. The glycosides significantly increased the weight of the levator muscle but did not change the testosterone level. Anabolic activity without androgenic activity was reported by fenugreek [112]. The chloroform extract showed proliferation of positive breast cancer cells, MCF-7 cells. And it mimicked the estrogen and pS2 gene was induced in MCF-7 cells [113]. A double blind randomized placebo-controlled study was made to evaluate standardised fenugreek extract called testofen on male libido. It resulted in overall positive effect and there was raise in subdomains of sexual arousal and orgasm. And there was positive effect in QOL and physiological aspects of libido [114]. When saponin of fenugreek was studied for anti-fertility activity it showed effective anti-implantation and abortifacient activity but weak estrogenic activity in immature ovariectomized rats [115]. A novel fenugreek seed extract Furocyst, on PCOS women showed significant reduction in cyst size, dissolution of cyst, regular menstrual cycle and increase in chance of pregnancy. And there was also increase in the secretion of luteinizing hormone and follicular stimulating hormone [116].

5.23 Urinary anti-infectives
The glutathione glutathione reductase, S-transferase, catalase level and glutathione peroxidase were restored in cyclophosphamide and d L-buthionine-SR-sulfoximine induced toxicity rats by pre-treating them with fenugreek seed aqueous extract. Extract treatment to restore GSH can play an important role in the reversal of cyclophosphamide -induced apoptosis and free radical lipid peroxidation in the urinary bladder. So, in cancer patients under chemotherapy fenugreek can be given as a complementary treatment [117].
5.24 Toxicology
The leaf glycosidic extract when studied for acute toxicity by estimating its medium lethal dose after oral and intraperitoneal administration, liver was the most affected organ. Early degeneration with mononuclear and mild hepatitis infiltration has been observed in some animals treated with toxic glycoside extract doses [118]. The mated female mice were treated with lyophilized aqueous extract of fenugreek for the study of toxicity but there was no evident sign of toxicity. But there was a developmental toxicity in the offspring caused a rise in the rate of foetal mortality, reduction of size of the litter and a decrease in the weight of the foetal body. Furthermore, the occurrence of morphological anomalies has increased [119]. Deltamethrin induced toxicity caused an imbalance in haematological and biochemical parameters. And resulted in lipid peroxidation, oxidative stress and increase in serum lactate dehydrogenase, aspartate amino transferase, alanine aminotransferase, gamma glutamyl transferase, triglycerides, cholesterol, uric acid, urea and creatinine. All these deltamethrin induced imbalance was restored on fenugreek oil administration [120]. Acrylamide induced toxicity resulted in increase in lactate dehydrogenase alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, urea, uric acid, cholesterol, creatinine, interleukin 1 beta, 8-Oxo-2'-deoxyguanosine, interleukin 6 and tumour necrosis factor alpha. It increased the peroxidation of brain, hepatic and renal lipids while hindering the antioxidant biomarkers' activities and concentrations. Fenugreek oil supplementation stabilised altered serum parameters, prevented lipid peroxidation, and increased antioxidant biomarker concentrations and behaviours in the hepatic, renal, and brain tissues of dose-dependently intoxicated acrylamide rats [121].

6. CONCLUSION
In this review, attempts were made to explain the reported pharmacognosy, phytochemistry and its pharmacological uses. Traditional breeding, combined with modern biotechnological methods such as molecular characterization, plant tissue culture protocols, and genetic transformation, has recently made significant progress in improving the genetics of fenugreek. The key focus, however, remains on improving the secondary metabolite content rather than on improving it as an edible plant or as an agronomic practise or yield. It is concluded that fenugreek in the modern world has tremendous significance for humans, but further attempts are needed to cultivate it as a large edible plant rather than a substitute for cereals or by-products.
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CONFLICT OF INTEREST
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