Available online on www.ijcpr.com

International Journal of Current Pharmaceutical Review and Research, 2(4), ISSN: 0976-822X

Fenugreek (*Trigonella foenum-graecum* L.): An Overview

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ABSTRACT:

Fenugreek (*Trigonella foenum-graecum* L.), plant is widely distributed throughout the world and which belongs to the family Fabacecae. The yields can be significant increase in quantity and quality through the suitable management of cultivation, irrigation and harvesting. The plant contains active constituents such as alkaloids, flavonoids, steroids, Saponins etc. It is an old medicinal plant. It has been commonly used as a traditional food and medicine. Fenugreek is known to have hypoglycemic, and hypocholesterolaemic, effects, Anti-inflammatory effects. Recent research has identified fenugreek as a valuable medicinal plant with potential for curing diseases and also as a source for preparing raw materials of pharmaceutical industry, like in steroidal hormones. Since fenugreek is a self-pollinated crop, a mutation breeding method can be used to generate mutants with a determinate growth habit. Irradiation and chemical mutagens can be used to produce point mutations in fenugreek. This review gives view mainly on the biological activities of some of the fenugreek compounds isolated, pharmacological actions of the fenugreek extracts, clinical studies and genetic, bredding and biotechnological studies.

KEYWORDS: *Trigonella Foenum-groecum* L., Seeds, Cultivation, saponin.

INTRODUCTION:

Fenugreek, *Trigonella Foenum-groecum* Linne , is an annual herb indigenous to the countries bordering on the eastern shores of the Mediterranean and largely cultivated in India, Egypt, and Morocco [1]. The name fenugreek comes from foenum-graecum, meaning Greek hay, as the plant was traditionally used to scent inferior hay [2]. The name of the genus, Trigonella, is

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derived from the old Greek name, denoting 'three-angled', [3, 4] probably refering to the triangular shape of the flowers. The first recorded use of fenugreek is described on an ancient Egyptian papyrus dated to 1500 B.C. Fenugreek seed is commonly used in cooking [5].

Fenugreek has strong flavor and aroma. The plants leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriental countries as a spice in food preparations, and as an ingredient in traditional medicine [6]. A wide range of uses were found for fenugreek in ancient times. Medicinally it was used for the treatment of wounds, abscesses, arthritis, bronchitis, ulcer and digestive problems. Traditional Chinese herbalists used it for kidney problems and conditions affecting the male reproductive tract. Fenugreek was, and remains, a food and a spice commonly eaten in many parts of the world [3].

Botany:

The taxonomical position is as follows:

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Fabales

Family : Fabaceae

Genus : *Trigonella*

Species : *foenum-graecum* Linn. [2, 7, 8]

The exact number of species of Fenugreek has been debated. Taxonomists such as Linnaeus suggested that as many as 260 species of Fenugreek may exist, of which a total of only 18 species of *Trigonella* are currently recognized. Most species, including *Trigonella joenum-graecum* L., are diploids with *2n* = 16 chromosomes. However, some species of *Trigonella* may contain 18, 28, 30, 32 or 44 chromosomes [9].

Common Names:

Latin: Trigonella foenum-graecum L.; Foenugraeci semen (for the seed)

Italian: Fieno Greco

Arabic: Hulba

Chinese: Hu-lu-ba,Hu-lu-pa, K'u-Tou

Norwegian: Bukkehonrkler

Dutch: Fenegriek

Portuguese: Alforva,Feno-grego

Farsi: Sambelil

Russian: Pazhitnik,Pazhitnik grecheskiy, Sambala

Finnish: Sarviapila

Sanskrit: Methi,Methika, Peetbeeja

French: Fenugrec,Trigonelle

Spanish: Alholva,Fenogreco

German: Bockshornsamen (seed),Bockshorklee.

Swedish: Bockshornklee [9, 10, 11]

GENERAL DESCRIPTION:

It is an erect hairy annual of the bean family, reaching 30-60 cm (1-2 ft.). The plant grows to a height of about three feet, has three part leaves, the long slender stems bear tripartite, toothed, grey-green obovate leaves, 20-25 mm (3/4-1 in) long. *Trigonella foenum-graecum* has long stalked leaves up to 5 cm long stipules triangular, lanceolate, leaflets about 2.5 cms long, obovate to obanceolate. The root is a mass of fingery structures. The sissile axillary flowers are white or pale yellow. The thin, sword-shaped pods are 10-15 cm (4-6 in), with a curved beak-like tip, each carrying 10-20 seeds. The plant radiates a spicy odour which persists on the hands after touching. Wild and cultivated varieties exist. . Flowers are 1-2, axillary, sessile, racemed, whitish or lemon yellow that bloom from June to July. Pod 5.7 cm long with a persistent beak, hairy with 10-20 seeds. Mild Mediterranean climates are most suitable. Plants mature in about four months. [12, 13, 14, 15, 16]. The flowering season for the herb fenugreek is generally midsummer [14].

Fenugreek seeds are small (5 mm. long), hard, and brownish yellow the colour may varies. They are flattened and have a very characteristic rhomboidal outline. Nearly in the centre of one of the long, narrow sides is a small depression in which hilum and micropyle are situated, the former being distinctly visible as a whitish point; this depression is continued in the form of a furrow running diagonally across part of each of the adjoining sides, thus dividing the seed into two unequal lobes. If the seed is cut in a direction transverse to the side in which the hilum lies, so as to pass through both lobes of the seed, it will be found that the larger lobe contains two accumbent cotyledons - the smaller, the radical. Both are yellowish in colour, and surrounded by a darker, horny, translucent endosperm, which separates the radicle from the cotyledons. When it is soaked in water the endosperm swells and yields mucilage to the surrounding liquid. Entire seeds macerated in warm water burst their seed-coats by the swelling of the mucilage, and disclose the structure of the seed [1, 15].

Cultivation:

Fenugreek is best grown as an annual crop from seeds, by the line sowing method. The land should be prepared but related ploughiong and harrowing [3]. In India, it is used as green leafy vegetable as well as spice. The plant is cultivated as a semi-arid crop. It is cold season crop and is fairly tolerant to frost and very low temperature. It can also grow on black cotton soils [17, 18]. Fenugreek requires well-drained, good soil of medium texture. Tolerated pH range is 5.3 to 8.2. Needs full sunlight, and requires watering during dry periods [19, 20].

Interculture:

Fenugreek is a broadcasted crop. The plant distance should be maintain about 8-10 cm. 2 to 3 weeding are required to fenugreek crop. One weeding and hoeing should be done about 20-25 days after sowing. Second weeding should be done 45-50 days after sowing [21, 22].

Irrigation:

To the fenugreek crop 4-6 irrigations should be given depending on soil type and climate. if moisture level of the soil is not optimum for seed germination, pre-sowing irrigation also be given. Seeds should be soaked in water for 6 to 8 hrs and dried in shade before sowing to hasten germination [23]. First irrigation should be given at the time of thinning and subsequent irrigation is given at an interval of 20-25 days. For an irrigated crop irrigation channels are made along the alternate rows of bed. The seeds should be treated with rhizobium culture before sowing. The number of irrigation required depends upon the type of the soil and evaporate transpiration potential prevailing during the season. Two hoeings and weedings are enough to keep the crop well aerated and weed free [3].

Manures & Fertilizers:

F.Y.M. (10-15 MT), Nitrogen (40 kg) and Phosphorous (20 kg) per hector is required for fenugreek. FYM should be mixed in soil at the time of land preparation. Half dose of Nitrogen and full dose of Phosphorous should be applied as basal dose and remaining 20 kg Nitrogen applied at an interval of 30 days after sowing.

Weed control:

First hoeing and weeding is recommended at the time of thinning i.e. 25–30 days after sowing and second weeding is recommended at 50–60 days after sowing. Herbicides may also be used for weed control. Pre-plant application of Fluchloralin at 0.75 kg/ha supplemented with hand-weeding 50 days after sowing is proved to be highly effective for complete weed control in the field [23].

Harvesting:

Crop becomes ready for harvest in about 120-150 days. At the time of ripening or maturity, leaves and pods become yellowish and leaves start falling. Timely harvesting is very important for this crop as late harvest leads to seed losses due to pod bursting, while in early harvest, the grains remain immature and small. Harvesting should be done early in the morning. After harvest, plants should be dried in threshing yard and threshed by trampling under the feet of bullocks. Seeds should be separated and cleaned by winnowing [21, 22].

Plant Protection:

It is pest-free, but susceptible to Cercospora leaf spot, a fungus disease [19, 20]. Fenugreek appears very resistant to attacks by insects and animal enemies. The seeds of fenugreek can be store for more than 10 years without any treatment. The peculiar smell of the fenugreek plants and seeds may be a possible factor for their resistance to the attack of insects [3].

a) Pest:

Aphid: Aphid sucks the sap of tender parts of plants and affects the growth, adversely. Spraying of 0.03% solution of Dimethoate or 0.025% solution of Methyl demetone or 0.04% solution of Monocrotophos is recommended to control the aphid. If the crop is grown for green (vegetable) purpose then spray Malathion.

b) Diseases:

i) Powdery mildew: This disease appears in later stage of crop and becomes serious, when pod formation takes place. In this disease, white powdery patches appear on the lower and upper surface of leaves and other parts of plant. To control this disease Crop should be dusted with 300 mesh Sulphur dust (25 kg/hector) as soon as the symptoms are noticed. Spraying of wet table Sulphur or Dinocap (Kerathan or Thiowet) can also be used to control the disease at 20-25 g per 10 liter of water at the initial stage of this disease. If needed two more sprays should be given at an interval of 15 days after first spray.

ii) Downy Mildew: This disease occurs in February and March month. In this disease yellow patch on the upper surface of leaves appear in the infected plants and white cottony myceliums appear on the lower surface of leaves. This disease can be controlled by spraying of 0.2% solution of Difoltan or any other copper fungicide [21].

A list of the medicinal species of the genus *Trigonella* [24]

Genus	Species
Trigonella	*T. foenum-graecum, T. balansae, T. corniculata, T. maritima, T. spicata, T. occulta, T. polycerata, T. calliceras, T. cretica, T. caerulea, T. lilacina, T. radiata, T. spinos*

PHYTOCHEMISTRY

Stem:

Fenugreek contains a number of steroidal sapogenins. The diosgenin were found in the oily embryo. Two furastanol glycosides, F-ring opened precursors of diosgenin have been reported, as also hederagin glycosides. The alkaloid trigonelline, trigocoumarin, trimethyl coumarin and nicotinic acid are present in stem. Mucilage is a prominent constituent of the seeds [25]. About 28 % mucilage; 5 % of a stronger-smelling, bitter fixed oil, 22 % proteins; a volatile oil; two alkaloids, Trigonelline and Choline, and a yellow colouring substanceare present in stem[26].

Leaves:

The leaves contain 7 saponins, known as graecunins. These compounds are glycosides of diosgenin. Leaves contain moisture 86.1%, protein 4.4%, fat 0.9%, minerals 1.5%, fiber 1.1%, and carbohydrates 6%. The mineral and vitamins contents are calcium, iron, phosphorous, carotene, thiamine, riboflavin, niacin and vitamine C [27].

Seed:

Fenugreek Seeds are aromatic, bitter, carminative, galactogouge, antibacterial and may be eaten raw or cooked. Bulk of the seed is dietary fiber (50%) and protein (30%) both of which have no taste or flavor. The chemical components of fenugreek seeds include a large carbohydrate fraction (mucilaginous fiber, galactomannan); 20-30% proteins high in tryptophan and lysine; pyridine-type alkaloids; flavonoids; free amino acids (4-hydroxyisoleucine, arginine, lysine, histidine); saponins; glycosides; vitamins, minerals, (28%) mucilage, (22 %) proteids, 5 % of a stronger-smelling, bitter fixed oil. volatile oils. Bitterness is mainly due to the oil, steroidal saponins and alkaloids. Historically used as a culinary and medicinal herb, recent research studies have shown its effectiveness in reducing blood glucose levels, promoting lean body mass, lowering cholesterol, and treating gastrointestinal disorders. Studies with type 2 diabetics have shown a blood glucose normalizing effect and decreased insulin resistance. Preliminary research with type-1 diabetics suggest that fenugreek may aid insulin secretion and may reduce total cholesterol and LDL cholesterol levels [26, 27, 28, 29].

Seeds contain 0.1% to 0.9% diosgenin and are extracted on a commercial basis. Plant tissue cultures from seeds grown under optimal conditions have been found to produce as much as 2% diosgenin with smaller amounts of gitongenin and trigogenin. The seeds also contain the saponin fenugrin B. Several coumarin compounds have been identified in fenugreek seeds as well as a number of alkaloids (eg, trigonelline, gentianine, carpaine). A large proportion of

the trigonelline is degraded to nicotinic acid and related pyridines during roasting. These degradation products are, in part, responsible for the flavor of the seed. The seeds also yield as much as 8% of a fixed, foul-smelling oil. Three minor steroidal sapogenins also have been found in the seeds: smilagenin, sarsapogenin, and yuccagenin [26, 30, 31].

TRADITIONAL USES:

The nourishing seeds are given during convalescence and to encourage weight gain, especially in anorexia. Helpful in lowering fever, it is compared to quinine by some authorities. The seeds' soothing effect makes them of value in treating gastritis and gastric ulcers. The seeds freshen bad breath and help restore a dulled sense of taste. The oil in the seeds is used as a skin softener and emollient.

In China, the fenugreek seeds are used as a pessary to treat cervical cancer. In the Middle East and the Balkans, the aerial parts of plant are a folk remedy for abdominal cramps associated with both menstrual pain and diarrhea or gastroenteritis. They are also used to ease labour pains [17]. Traditional Chinese herbalists used plant for kidney problems and conditions affecting the male reproductive tract. The seeds also function as a preservative and are added to pickles, chutneys and other similar products [32]. In modern food practice, the seeds or the extract are used in bakery products, frozen dairy products, meat products, relish, condiments, candy, gravy sauces, gelatin puddings and in alcoholic and non-alcoholic beverages [33].

Fenugreek has a beneficial action on cleansing the blood. As a diaphoretic it is able to bring on a sweat and to help detox the body. This takes place through the pores of the skin. The pungent aroma of fenugreek may be smelt on the skin and in under-arm perspiration. After using the sprouts for a while, this fenugreek body aroma, does not seem to be so apparent, maybe, the sprouts have done a pretty good cleanse. Fenugreek also has the reputation as a lymphatic cleansing herb. The lymphatic system is the vacuum cleaner of the body. It has the vital role to irrigate the cells with nutrients and to remove toxic wastes, dead cells and trapped proteins. The fluid is cleaned through the lymph nodes, before the body's 13 litres of filtered lymph fluid recycles again, via the subclavian vein near the heart. A blocked lymphatic system can mean poor circulation, fluid retention, pain, loss of energy and disease, anywhere in the body. Fenugreek is a practical herb for all mucus conditions of the body, particularly the lungs, by helping to clear congestion. It is a powerful antioxidant and it acts as a mucus solvent and throat cleanser, which also eases the urge to cough. Even drinking the water that seeds have soaked in and been rinsed with, helps to soften and dissolve, accumulated and hardened masses of cellular debris. Use fenugreek for head colds, influenza,

catarrh, constipation, bronchial complaints, asthma, emphysema, pneumonia, pleurisy, tuberculosis, sore throat, laryngitis, hay fever and sinusitis [34, 35].

Fenugreek has been used to treat peptic ulcers and inflamed conditions of the stomach and bowel, it absorb toxic material and eliminate it. The healing and soothing action creates a protective coating, like a lubricant, over inflamed areas. The slightly bitter properties of the seed are beneficial for digestion. Fenugreek has a powerful demulcent action, as it is rich in mucilage and it can soothe irritated or inflamed tissue. For relief from the agonizing symptoms of irritable bowel syndrome, colitis and diverticulitis, the 'soak-and-rinse water' is drunk and the sprouts blended to a liquid. It has been called the herb for 'every ailment under the sun'! [34, 36, 37] . The Fenugreek herb has been known to help reduce fever when taken with lemon and honey, since it nourishes the body during an illness. Some health food stores also sell herbal Fenugreek teas, which can be used instead of the green tea. Fenugreek is often used in many teas and other products that help balance women's hormones and/or enlarge the breasts. Remedy to Ease Child Birth for Pregnant Women: Fenugreek stimulates uterine contractions and can be helpful to induce childbirth. However, pregnant women should only use Fenugreek for inducing labor after consulting with their doctor [36, 37, 38].

Lactation Aid: Fenugreek seeds contain hormone precursors that increase milk supply. Some scientists believe it is possible because breasts are modified sweat glands, and fenugreek stimulates sweat production. It has been found that fenugreek can increase a nursing mother's milk supply within 24 to 72 hours after first taking the herb.

Immunological Activity: An Immunomodulatory effect of fenugreek extract in mice has been investigated. Overall, Fenugreek showed a stimulatory effect on immune functions in mice. As it is used for a variety of medicinal purposes, its immunostimulatory effect, as reported in this study, strengthens the rationale of its use in several Unani and Ayurvedic drugs. For the removal of Kidney Stones, a study was undertaken to investigate the effect of Fenugreek (*Trigonella foenum-graecum*) seed on experimentally-induced kidney stones in rats. Oxalate urolithiasis in male rats was produced by the addition of 3% glycolic acid to their diet. After 4 weeks, highly significant deposition in the kidneys was noticed and changes in water intake and body weight recorded. Daily oral treatment with *T. foenum-graecum* significantly decreased the quantity of calcium oxalate deposited in the kidneys thus supporting its use in Saudi folk medicine [33].

CLINICAL STUDIES:

Antimicrobial Effects: The seeds of the fenugreek herb possess toxic oils, and other constituents of the fenugreek leaf have been shown to be toxic to bacteria, parasites and fungi. A 2007 issue of Current Science journal noted the antifungal properties of fenugreek. The research attempted to clone the substance defensins which are native to plants such as fenugreek to test their effects in the petri dish. The defensins protect the plant from fungi which was extracted from leaf tissue. As an antiparasitic agent, fenugreek was pitted in a 2008 Oxford Journals article against the malaria-causing organism Plasmodium. In vitro studies found that fenugreek extracts were effective against resistant species of Plasmodium. The 2004 Asia Pacific Journal of Clinical Nutrition article also noted that germination or sprouting of fenugreek seeds increased their antioxidant profile and antimicrobial activity against *H-pylori*. Finally, a 2006 African Journal of Biotechnology article compared the effectiveness of fenugreek against two common pathogenic bacteria. Fenugreek was found to strongly inhibit the growth of *Staphylococcus aureaus* and *Pseudomonus aeruginosa* in a petri dish [39].

Animal Studies have clearly demonstrated the cholesterol-lowering activity of fenugreek in animals. In a typical study, fractions of fenugreek seeds were added to the diets of diabetic hypercholesterolemic and normal dogs. The defatted fraction, which contains about 54% fiber and about 5% steroidal saponins, lowered plasma cholesterol, blood glucose, and plasma glucagon levels from pretreatment values in both groups of dogs. The hypocholesterolemic effect has been reproduced in rats. Administration of the fiber-rich fraction of fenugreek to diabetic rats lowered total cholesterol, triglycerides, and low density lipoprotein (LDL). The level of high density lipoprotein (HDL) was increased. In the Clinical study Serum triglycerides were reduced from baseline in patients with newly-diagnosed, mild, type-2 diabetes mellitus who received a hydroalcoholic extract of fenugreek seeds 1 g/day. Total cholesterol and proportions of LDL and HDL fractions were not altered by treatment. A systematic review identified 5 other randomized clinical trials (N = 140) investigating the cholesterol-lowering effects of fenugreek seeds. Reductions (15% to 33%) of serum cholesterol from baseline were reported in all the trials identified. Total serum cholesterol and LDL cholesterol were reduced, while HDL cholesterol remained unchanged.

The galactomannan-rich soluble fiber fraction of fenugreek may be responsible for the antidiabetic activity of the seeds. An Animal study evaluated the hypoglycemic effects of the seeds in dogs. The defatted fraction of the seeds lowered blood glucose levels, plasma glucagons and somatostatin levels; carbohydrate-induced hyperglycemia also was reduced. Clinical data shows that Glycemic control was improved in a small study of patients with

mild type-2 diabetes mellitus. A reduction in glycosylated hemoglobin (HbA_{1c}) levels and increased insulin sensitivity were observed in fenugreek recipients. An anti-inflammatory effect was observed in an Animal study which shows that when rats treated with a single dose of fenugreek extract (100 or 200 mg/kg). Inhibition of inflammatory swelling was 45% and 62% in the lower and higher dose groups, respectively, compared with 100% in untreated animals.

Antitumor activity: The Pretreatment with a fenugreek extract was found to enhance macrophage cell counts in rats. When these rats were subsequently inoculated with tumor cells, tumor cell growth was inhibited [40].

In 2008 Bukhari *et* al reported that Fenugreek seeds extract such as methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate have a chelating activity, reducing power and antioxidant/radical scavenging activity (1,1-diphenyl-2-picryl-hydrazyl (DPPH°) free radical scavenging activity) [6]. In 2004 Flammang *et al* studied the potential genotoxicity of a fenugreek seed extract (THL), containing a minimum of 40% 4-OH-ILE, using the standard battery of tests (reverse mutation assay; mouse lymphoma forward mutation assay; mouse micronucleus assay). THL was determined not to be genotoxic under the conditions of the tested genetic toxicity battery. The negative assay results provide support that addition of THL to foodstuffs formulated for people with diabetes is expected to be safe [2].

In 2006 Bhatia *et al* reported protective effect of fenugreek, on lipid peroxidation (LPO) and on enzymatic anti-oxidants. Cyclophosphamide treated animals exhibited a significant decrease in the activities of glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GP) and catalase (CAT) when compared to the controls. Level of reduced glutathione (GSH) was also reduced with an increase in LPO in CP-treated animals. L-buthionine-SR-sulfoximine (BSO) treatment depicted an additive toxic effect in CP-treated animals. Pre-treatment of herbal extract restored activities of all the enzymes and thus showed an overall protective effect on additive effect of CP and BSO. Restoration of GSH by extract treatment may play an important role in reversing CP-induced apoptosis and free radical mediated LPO in urinary bladder [41].

In 2010 Naidu reported that **t**he proximate composition of fenugreek seeds, husk and cotyledons had the highest saponin (4.63 g/100 g) and protein (43.8 g/100 g) content. In contrast, husk had higher total polyphenols (103.8 mg of gallic acid equivalent/g, and TDF (77.1 g/100 g), comprising IDF (31.9 g/100 g) and SDF (45.2 g/100 g). At 200 µg concentration, extracts of husk, fenugreek seed, and endosperm exhibited 72%, 64%, and 56% antioxidant activity respectively by free-radical scavenging method. The study indicated

that separation of fenugreek seeds into husk and endosperm could have advantage of process viability with respect to prior selective fractionation of bioactive components for their effective isolation [42].

For the determination of dry matter, protein and free amino acid content, samples were harvested at different stages of ripeness. During maturation, reserves of solutes are established in the pod wall before the seeds begin their exponential phase of growth. Later, these reserves disappear, providing about 20% of the seeds requirements for nitrogen. SDS-electrophoresis was used to follow the formation of proteins and it was shown that the synthesis of storage proteins takes place prior to dehydration of the seed. Product soluble nitrogenous compounds precede protein accumulation [43].

In 2010 Babu *et al* studied antidiabetic and histopathological analysis of fenugreek extract on alloxan induced diabetic rats**.** A comparable hypoglycemic effect was evidenced from the data obtained after 7 and 21 days of oral administration of the extract. The extract lowered the total cholesterol and serum triglycerides. Histopathological analysis of pancreas showed normal acini, and normal cellular in the islets of langerhans in the pancreas of normal control and Extensive damage to islets of langerhans and reduced dimensions of islets in alloxan induced diabetes. Restoration of islets of langerhans seen in diabetic rats treated with fenugreek extract. The results of this study clearly show the hypoglycaemic activity of the extract [44]. In 2007 Thaakur *et al* studied the inhibition of carbon tetra chloride (CCl_4)-induced liver fibrosis by Trigonella foenum-graecum Linn. Liver fibrosis was induced in rats by CCl_4 orally 1mL/kg for 28 days. The extent of liver fibrosis assessed by measuring the level of liver hypdroxyproline (HP), serum enzyme and total bilirubin (TBL) levels which is due to deposition of collagen. The weight of liver was increased by administrating CCl4. The administration of ethanolic extract of plant reduced HP, serum enzyme and inhibits the liver fibrosis [45].

In 2008 Palaniswam *et al* studied the In vitro anti-plasmodial activity of *Trigonella foenum–graecum L.* In vitro anti-plasmodial assay of the extracted fractions of fenugreek leaves was carried out using chloroquine sensitive and resistant *Plasmodium falciparum* isolates. Schizont maturation inhibition assay was used to analyze the potential of the extracts. Ethanol extract (50%) possess profound anti-plasmodial activity with IC50 value of 8.75-0.35 $mgml^{-1}$ and 10.25-0.35 $mgml^{-1}$ against *P. falciparum* isolates, respectively. Among the investigated six fractions of the plant extracts, two were found to have significant anti-plasmodial activity with IC50 values510 $mgml^{-1}$, namely ethanol and butanol extracts. Two extracts chloroform and ethyl acetate showed moderate activity with IC50 values ranging from 10 to 20 $mgml^{-1}$,

and the other two extracts, hexane and water appeared to be inactive with IC50 values 485 $mgml^{-1}$[46].

In 2010 **Navayath *et al* reported that the a**queous extract of *Trigonella foenum graecum* (fenugreek) prevents cypermethrin-induced hepatotoxicity and nephrotoxicity. Male Wistar rats were treated with 1/10 LD_{50} (25 mg/kg body weight) of Cypermethrin (CM) and 10% aqueous extract of fenugreek (GFaq) for 60 days. CM treatment caused increased thiobarbituric acid reactive substances (TBARS), depletion in glutathione (GSH) and reduction in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in liver and kidneys. They observed that a significant reduction in total phospholipids and increased activities of phospholipases A (PLA) and C (PLC) in liver and kidneys and increased activities of serum marker enzymes, aspartate transaminase (AST), alanine tansaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT). Treatment with 10% GFaq showed replenishment of antioxidant status and brought all the values to near normal, indicating the protective effect of fenugreek [47].

In 2007 Laroubi *et al* studied the Prophylaxis effect of *Trigonella foenum graecum* L. seeds on renal stone formation in rats. The inhibitory effect of the aqueous extract of fenugreek seeds was examined on the formation of calcium oxalate renal stones induced by ethylene glycol (EG) with ammonium chloride. At the end of the experiment all kidneys were removed and examined microscopically for possible crystal/stone locations and the total calcium amount in the renal tissue was evaluated. The blood was recovered to determine the levels of calcium, phosphorus, creatinine and urea. The results showed that the amount of calcification in the kidneys and the total calcium amount of the renal tissue in rats treated with fenugreek was significantly reduced compared with the untreated group. The fenugreek can be used in the treatment of patients with calcic urolithiasis [48]. In 2010 Chauhan *et al* reported an anti-inflammatory potential of fenugreek [49].

GENETIC AND BREEDING:

Fenugreek according to Darlington and Wylie has 2n=16 chromosomes, while Joshi and Raghuvanshi have investigated the presence of B-chromosomes [50]. Singh and Singh [50] isolated five double trisomics along with primary trisomics from the progenies of autotriploids, which had 2n+1+1=18 chromosomes. There are three methods namely selection, hybridisation and mutation used separately or in combination, which may be involved in the development of an improved variety of fenugreek. Fenugreek is self-

pollinated, but there are opportunities for natural out crossing. The inherent variation in fenugreek is quite immense and so it is grown today in the wide range of climatic conditions of all continents. Allard has suggested that legumes are considered cross-pollinated when more than 10 % of them are "outcrossed". On this basis Petropoulos described fenugreek as a rarely cross pollinated plant as its stigma becomes receptive before the anthers mature. Because of this Petropoulos has suggested that cross 1 Mannose/Galactose; the ratio of mannose to galactose varies in the different plant genus. The selections among world accessions and mutation breeding have been advocated as the best ways to improve the crop, and much of the breeding with fenugreek has utilized these two approaches [44, 51].

On the basis it, the first North American fenugreek forage cultivar "Tristar" was released for use in western Canada in 2004 by Agriculture and Agri-Food Canada (AAFC). Tristar fenugreek was developed from a line L3314 (formerly PI-138687), originally collected in 1940 from Iran. A lot of fenugreek mutants have been isolated by the treatment of dry seeds with different chemical mutagens, while shoot apexes of fenugreek treated by colchicine produced tetraploid plants with promising economic characteristics [44]. The effect of mutagens on tissue cultures of fenugreek with UV-irradiation, ethyl-methanesulphonate (EMS), methyl-methanesulphonate (MMS), and sodium azide (NaN3) increased steroidal sapogenin about two- to three-fold [44, 52].

BIOTECHNOLOGY:

Fenugreek tissue and cell cultures have been used for either plant regeneration or for the production of secondary products such as diosgenin and trigonelline: a saponin and an alkaloid [44, 53]. The first report on the production of spirostane derivatives by *Trigonella* tissue cultures was published by Khanna and Jain and concerned the establishment of static cultures grown on solid Murashige and Skoog (MS) medium supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D). They reported the production of diosgenin, gitogenin and tigogenin along with other sterols. Six-week-old cultures showed a high growth index (GI= final wet weight – initial wet weight / initial wet weight) and the total steroidal content was higher than in the seeds. In other study, the highest diosgenin and tigogenin content was found in 8-week-old calli (0.40 and 0.15%, respectively).

Khanna *et al* demonstrated that suspension cultures of *Trigonella foenumgraecum* L. grown on media supplemented with various concentrations of cholesterol produced higher sapogenin contents than those grown on medium without cholesterol. There are alternative pathways from sterol to diosgenin [44, 54]. A first pathway is the incorporation of cholesterol and this

pathway is predominant when the precursor is added at subculture. The second pathway involves sidechain cleavage before incorporation and takes place when the sterol is added 10 days after subculture. In a similar way, Trisonthi *et al.* demonstrated that mevalonic acid promotes the synthesis of steroidal sapogenins in fenugreek tissue, particularly in suspension cultures grown on Miller medium (MS). Oncina et al. reported on the production of diosgenin by callus cultures of *Trigonella foenum-graecum* L. Leaf, stem and root calli were established and cultured on different soild growth media (MS, White's basal medium, Gamborg's B5) [45, 55] supplemented with coconut milk, malt extract and NAA. In all cases, MS medium supplemented with 15% (v/v) coconut milk and 3×10-6M NAA was the most suitable medium for callus growth. Diosgenin levels were higher in leaf calli than in stem and root calli and corresponded to about three to five times the levels observed in the calli from other plant organs. Maximum diosgenin levels were attained after 45 days and reached 2.2 mg/g dry weight in leaf calli, 0.74 mg/g in stem calli and 0.60 mg/g in root calli, which represents 22, 10 and 27% of the levels detected in the corresponding organs of the mother plant at 45 days.

The pyridine alkaloid, trigonelline, is widely distributed in the plant kingdom and is known for its hypoglycaemic and hypocholesterolaemic activity. Joshi and Handler demonstrated the biosynthesis of trigonelline from nicotinic acid and Sadenosylmethionine. Cell-free extracts of root callus cultures have been reported to catalyze the conversion of nicotinic acid and Sadenosylmethionine to trigonelline in the presence of ATP and MgCl2. Callus cultures of *Trigonella foenumgraecum* contained 3 to 4 times more trigonelline than the seeds of this plant and 12 to 13 times more than the roots and shoots. Even higher levels of this alkaloid were produced by suspension cultures. This high productivity was maintained during successive subculturing of calli and cell suspensions for eight months. Trigonelline accumulated in callus and suspension cultures with aging [44, 52]. Eight-week-old callus tissue cultures of fenugreek, established from seeds on solid Revised Tobacco (RT) medium and supplemented with 1 mg/l 2,4-D, produced 4.5% trigonelline. In the presence of 0.5 and 1.0 mg/l nicotinic acid, trigonelline increased to 5.25 and 5.01% respectively.

The comparison of trigonelline contents of seeds, roots, shoots and in vitro cultures was carried out by Radwan and Kokate[56]. Four-week-old callus cultures of *Trigonella foenum-graecum* L. produced 15.6 mg/g dry wt. of trigonelline, which represents 3 to 4 times more trigonelline than the seeds and 12 to 18 times more than the roots and shoots of the parent plants. Four- and 6-week-old suspension cultures produced 38.2 and 44.2 mg/g dry wt., respectively, of trigonelline which is more than twice the amount found in the calli.

Proportions of 9- 12% of the trigonelline were released into the solid medium. In suspension cultures, one third or more of the trigonelline was dissolved in the liquid medium. Therefore, it appears that the biosynthesis of this compound is favored by its enhanced removal from the site of its biosynthesis in the cells, into the medium. Cultures grown in the presence of 50 mg/l nicotinic acid contained more trigonelline than those grown in the presence of 1 mg/l only of this substrate. At the same time, the proportion of the alkaloid released into the liquid medium increased from 31%, with a low level of nicotinic acid, to 37%, with a high level of the same compound [44].

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