



Toxicological profile for Fenugreek extract, absolute, oleoresin, resin

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Not applicable.

1.2. Synonyms

68990-15-8: Extract of fenugreek; FEMA No. 2484; Fenugreek; Fenugreek (Trigonella foenum-graecum L.); Fenugreek absolute; Fenugreek extract; Fenugreek oil; fenugreek seed; Fenugreek seed meal; Fenugreek absolute; Foenugreek seed extract; Foenugreek tincture; Hyperabsolute funugreek; Oils, fenugreek; semen trigonellae; Trigonella; Trigonella extract; Trigonella foenum-graecium oil; Trigonella foenumgraecum extract; trigonella foenum-graecum seed oil; trigonella tibetana seed oil; trigonellae foenugraeci semen; trigonellae semen (PubChem 2023b,c, 2024)

84625-40-1: EINECS 283-415-1; FEMA No. 2485; FEMA No. 2486; Fenugreek extract; Fenugreek extract (Trigonella foenum-graecum L.); Fenugreek EXtract, 4:1, Powder; Fenugreek oleoresin (Trigonella foenum-graecum L.); Fenugreek seed; fenugreek seed extract; Fenugreek, ext.; Fenugreek, extract (Trigonella foenum-graecum L.); Trigonella; Trigonella foenum-graecum extract; Trigonella foenum-graecum seed extract (PubChem 2017, 2023a, 2021)

1.3. Molecular formula

No data available to us at this time.

1.4. Structural Formula

Not applicable

1.5. Molecular weight (g/mol)

Not applicable.

1.6. CAS registration number

68990-15-8 and 84625-40-1

1.7. Properties

1.7.1. Melting point

(°C): 42.97 (estimated) (CAS RN 68990-15-8) (EPISuite, 2017)

1.7.2. Boiling point

(°C): 288.57 (estimated) (CAS RN 68990-15-8) (EPISuite, 2017)

1.7.3. Solubility

2.228e+005 mg/L at 25°C (estimated) (CAS RN 68990-15-8) (EPISuite, 2017)

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): 74 (165°F; closed cup) (CAS RN 84625-40-1) (HSDB, 2016)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

0.000192 mm Hg at 25°C (estimated) (CAS RN 68990-15-8) (EPISuite, 2017)

1.7.11. log Kow

-0.44 (estimated) (CAS RN 68990-15-8) (EPISuite, 2017)

2. General information

2.1. Exposure

Cosmetics	Yes (Cosmetics Bench Ref. 1996)	Food	Yes (Merck 1996)
Environment	Yes (Merck 1996)	Pharmaceuticals	Yes (Martindale 1993)

Trigonella foenum symbiosome extract (CAS RN 86425-40-1) is used as an antioxidant and skin conditioning agent, Trigonella foenum-graecum fruit extract (CAS RN 84625-40-1) as a fragrance and skin conditioning agent, Trigonella foenum-graecum seed extract (CAS RN 84625-40-1) as a perfuming and skin conditioning agent, Trigonella foenum-graecum seed oil (CAS RN 84625-40-1) as a perfuming agent, Trigonella foenum-graecum seed powder (CAS RN 84625-40-1; generic) as a viscosity controlling agent and Trigonella foenum-graecum sprout juice (no CAS RN given) as a skin conditioning agent in cosmetics in the EU.

As taken from CosIng (Cosmetic substances and ingredients database).

Fenugreek absolute, oleoresin, tincture and extract (all CAS RN 68990-15-8) are listed as fragrance ingredients by IFRA.

Estimated intake from use as flavouring in the USA is 0.2203 and 0.1935 mg/kg bw/day for fenugreek extract and oleoresin, respectively (Burdock GA, 2010).

Levels used are reported as (Burdock GA, 2010):

Food category	Fenugreek extract		Fenugreek oleoresin	
	Usual (ppm)	Max (ppm)	Usual (ppm)	Max (ppm)
Alcoholic beverages	18.78	24.06	0.10	20.00
Baked goods	57.00	214.30	114.60	228.10
Chewing gum	1.15	3.96	-	-
Condiments, relishes	423.60	486.30	-	-
Frozen dairy	31.27	46.51	58.45	115.60
Gelatins, puddings	5.82	11.30	119.00	243.90
Gravies	10.00	20.00	-	-
Hard candy	85.80	85.80	154.90	154.90
Meat products	51.75	58.08	100.00	150.00
Nonalcoholic beverages	8.78	20.08	60.36	122.60
Soft candy	28.67	48.45	116.70	231.80
Sweet sauce	206.50	278.40	-	-

“Fenugreek extracts are used in certain perfume bases as well as in soaps, detergents, creams, and lotions, with maximum use level of 0.2% reported in perfumes. Used as an ingredient of curry powder and many spice blends. Its major use in the United States is in imitation maple syrups for which solid extracts are mostly employed; flavor of the extracts varies with the extent of roasting and the solvents used. Other food products in which it is used include alcoholic and nonalcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins and puddings, meat and meat products, and others. Use levels for extracts are usually below 0.05%. Has been used for millennia as a drug and a food or spice in Egypt, India, and the Middle East....Reported to be used in Java in hair tonics and to cure baldness....Extracts used in flavoring tobacco. Used extensively in foreign countries as a feed for livestock.”

As taken from Khan IA and Abourashed EA, 2010.

Fenugreek extract (no CAS RN listed) is used as a flavour enhancer and *Trigonella foenum-graecum* seed extract (no CAS RN listed) as a skin conditioning agent in non-medicinal natural health products for oral and topical use, respectively. The dried ripe seeds of *Trigonella foenum-graecum* are used as homeopathic substances (Health Canada, 2021).

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
furfural	5718729	0.92	16082032	1.37

propylene glycol	153424911	24.93	327616576	28.29
5-methylfurfural	6320088	1.03	4805047	0.42
unknown	7344548	1.19	9726579	0.84
1,4:3,6-dianhydro-alpha-D-glucopyranose	8929221	1.45	17319599	1.50
5-hydroxymethylfurfural	43380286	7.05	52476106	4.53
unknown	9785558	1.59	21694528	1.87
levoglucosan	273053344	44.37	605809156	52.31
1,6-anhydro-beta-D-glucofuranose	54636676	8.88	66671885	5.76
unknown	8598405	1.40	n.d.	n.d.
Total ion chromatogram	615490694	100	1158113470	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2005) J. Anal. Appl. Pyrolysis 74, pp. 145–170.

Ingredient Name & CAS Number	Max. cig. appln. level (ppm)	Purity of Sample (%)	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
Fenugreek Oil 84265-40-1	5	na	Diethyl tartrate (74.3) Acetic anhydride (4.7) Acetic acid (4.7) Ethyl oleate (3.9) Ethyl linoleate (2.4)	2 0.1 0.1 0.1 0.06
Fenugreek Tincture 84265-40-1	500	na	Heptanoic acid (71.4) Acetic acid (5.2) Pyridine (3.4) Vinylphenol (2.5) Phenol (2.3) Furfural (0.5)	180 13 9 6 6 1

2.3. Ingredient(s) from which it originates

No evidence of its presence in tobacco naturally (Stedman 1968; Lloyd et al 1976)

Fenugreek extract (CAS RN 84625-40-1): Reported found in Fenugreek (Burdock, 2010).

“PLANT CONCENTRATIONS:

Fenugreek extract comes from the seed of an annual herb (*Trigonella foenumgraecum* L.), unusual for a spice, because it belongs to the bean or Leguminosae family. It is native to southern Europe and now commonly cultivated in Northern Africa, India, Turkey, and Morocco. The seed is irregularly oval, up to about 0.5 cm in length and is brownish yellow; ten to twenty seeds grow in a pod much like peas(1).[(1) Grenis AT; Spices. Kirk-Othmer Encyclopedia of Chemical Technology. (1999-2016). New York, NY: John Wiley & Sons. Online Posting Date: Sep 15, 2006.] **PEER REVIEWED**”

As taken from HSDB, 2016

Trigonella foenum symbiosome extract (CAS RN 84625-40-1) is an extract of the symbiosome (root nodule) of the *Trigonella foenum*, Leguminosae.

Trigonella foenum-graecum fruit extract (CAS RN 84625-40-1) is an extract of the fruit of the fenugreek, *Trigonella foenum-graecum* L., Leguminosae.

Trigonella foenum-graecum seed extract (CAS RN 84625-40-1) is an extract of the seeds of the fenugreek, *Trigonella foenum-graecum* L., Fabaceae.

Trigonella foenum-graecum seed oil (CAS RN 84625-40-1) is an essential oil obtained from the seeds of the Fenugreek, *Trigonella foenum-graecum* L., Leguminosae.

Trigonella foenum-graecum seed powder (CAS RN 84625-4-1, generic) is the dried, ground powder obtained from the seeds of *Trigonella foenum-graecum*.

Trigonella foenum-graecum sprout juice (no CAS RN given) is the juice expressed from the sprouts of *Trigonella foenum-graecum*, Fabaceae.

As taken from CosIng (Cosmetic substances and ingredients database).

3. Status in legislation and other official guidance

There are REACH dossiers on fenugreek ext. (CAS RN 84625-40-1), concrete of *Trigonella foenum graecum* L. (Leguminosae) obtained from seeds by extraction with organic solvents (no CAS RN listed, EC RN 947-408-3) and extract from the seeds of *Trigonella foenum-graecum* (Fabaceae) obtained by extraction with polar solvents (no CAS RN listed, EC RN 950-727-0) (ECHA).

CAS RN 68990-15-8 is not registered under REACH (ECHA).

Fenugreek, ext. (CAS RN 84625-40-1), concrete of *Trigonella foenum graecum* L. (Leguminosae) obtained from seeds by extraction with organic solvents (no CAS RN listed, EC RN 947-408-3) and extract from the seeds of *Trigonella foenum-graecum* (Fabaceae) obtained by extraction with polar solvents (no CAS RN listed, EC RN 950-727-0) are not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2025).

Oils, fenugreek (CAS RN 68990-15-8) is listed in the US EPA InertFinder Database as approved for non-food use pesticide products.

Oils, fenugreek (CAS RN 68990-15-8) is listed in the US EPA Toxic Substances Control Act (TSCA) and in the US EPA 2024 CDR TSCA Inv Active inventories.

US EPA Substance Registry Services (SRS).

FDA REQUIREMENTS:

Fenugreek used as a spice, natural seasoning and flavoring in food for human consumption is generally recognized as safe when used in accordance with good manufacturing practice. [21 CFR 182.10 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 6, 2016: <http://www.ecfr.gov>] **PEER REVIEWED**

Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are generally recognized as safe for their intended use, within the meaning of section 409 of the Act.

Fenugreek is included on this list. [21 CFR 182.20 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 6, 2016: <http://www.ecfr.gov>] **PEER REVIEWED**

Fenugreek used as a spice, natural seasoning and flavoring in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding practice. [21 CFR 582.10 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 6, 2016: <http://www.ecfr.gov>] **PEER REVIEWED**

Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) in animal drugs, feeds, and related products that are generally recognized as safe for their intended use, within the meaning of section 409 of the Act. Fenugreek is included on this list. [21 CFR 582.20 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of July 6, 2016: <http://www.ecfr.gov>] **PEER REVIEWED**

As taken from HSDB, 2016

Fenugreek (*Trigonella foenum graecum* L.; FEMA no. 2484), fenugreek extract (*Trigonella foenum graecum* L.; FEMA no. 2485) and fenugreek oleoresin (*Trigonella foenum graecum* L.; FEMA no. 2486) have all been given GRAS (generally recognized as safe) status by FEMA (Hall and Oser, 1965).

Semen *Trigonellae* (no CAS RN listed) and *Trigonella foenum-graecum* (no CAS RN listed) are included on Health Canada's Natural Health Products Ingredients Database and are classified as NHPs under Schedule 1, item 1 (plant or plant material) of the Natural Health Products Regulations (Health Canada, 2021).

Fenugreek, extract (*Trigonella foenum-graecum* L.) (CAS RN 84625-40-1) is included on the FDA's inventory of "Substances Added to Food (formerly EAFUS)" as flavoring agent or adjuvant and is generally recognized as safe (GRAS) under section 182.20 (Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)) (FDA, 2024).

C of E no. 460

FEMA no. 2484, 2485, 2486

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

"Fenugreek with the scientific name of *Trigonella foenum-graecum* L and with leaves consisting of 3 small obovate to oblong leaflets is an annual herbaceous plant of the Fabaceae family. It is native to the eastern Mediterranean but is cultivated worldwide. This plant has medicinal alkaloids, steroid compounds, and saponin and many uses have been mentioned for this plant in traditional medicine. This plant has been used to ease childbirth, to aid digestion, and as a general tonic to improve metabolism. Trigonelline is considered as the most important metabolite of fenugreek, which is very effective in treating diabetes and decreasing blood cholesterol. Diaszhenin is another important compound in seeds of this plant, which is used in producing medicinal steroids like contraceptive pills. Many studies have been performed on the therapeutic effects and identification of chemical compounds of this plant. In this article, the most important biological effects and reported compounds about fenugreek seed are reviewed and its therapeutic applications are investigated."

As taken from Bahmani M et al. 2016. J. Evid. Based Complementary Altern. Med. 21(1), 53-62. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25922446>

“OBJECTIVE: The seeds of *Trigonella foenum-graecum* (TFG) (family: Leguminosae) are widely consumed both as a spice in food and Traditional Medicine in India. The present study was undertaken to evaluate the inhibitory effect of standardized extract of TFG and its major constituent trigonelline (TG) on rat liver microsome (RLM) and cytochrome P450 (CYP450) drug metabolizing isozymes (CYP3A4 and CYP2D6), which may indicate the possibility of a probable unwanted interaction. MATERIALS AND METHODS: Reverse phase-high performance liquid chromatography method was developed to standardize the hydroalcoholic seed extract with standard TG. The inhibitory potential of the extract and TG was evaluated on RLM and CYP isozymes using CYP450-carbon monoxide (CYP450-CO) complex assay and fluorescence assay, respectively. RESULTS: The content of TG in TFG was found to be 3.38% (w/w). The CYP-CO complex assay showed 23.32% inhibition on RLM. Fluorescence study revealed that the extract and the biomarker had some inhibition on CYP450 isozymes e.g. CYP3A4 and CYP2D6 (IC₅₀ values of the extract: 102.65 ± 2.63-142.23 ± 2.61 µg/ml and TG: 168.73 ± 4.03-180.90 ± 2.49 µg/ml) which was very less compared to positive controls ketoconazole and quinidine. Inhibition potential of TFG was little higher than TG but very less compared to positive controls. CONCLUSIONS: From the present study, we may conclude that the TFG or TG has very less potential to inhibit the CYP isozymes (CYP3A4, CYP2D6), so administration of this plant extract or its biomarker TG may be safe.” As taken from Ahmmed SM et al. 2015. *Indian J. Pharmacol.* 47(5), 530-4. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26600643>

4.2. Absorption, distribution and excretion

“The furostanol glycoside isolated from the seed of fenugreek (SFSE-G) has an array of pharmacological activities. To date, no validated high-performance liquid chromatography (HPLC) method has been reported for quantification of SFSE-G in biological samples. Hence, the aim of the present study was to study the pharmacokinetics, tissue distribution and excretion profiles of SFSE-G after oral administration in rats. A rapid, sensitive, selective, robust and reproducible HPLC method has been developed for determination of SFSE-G in the rat biological samples. The chromatographic separation was accomplished on a reversed-phase C18 column using formic acid and acetonitrile (80:20) as mobile phase at a flow rate of 1.0 mL/min and 274 nm as a detection wavelength. The assay was linear for SFSE-G with the correlation coefficients (R(2)) >0.996. The analytes were stable during samples storage and handling, and no matrix effects were observed. After oral dosing of SFSE-G at a dose of 200 mg/kg, the elimination half-life was app. 40.10 h. It showed relatively slowly distribution and eliminated in urine and feces after 24 h, and could be detected until 108 h post-dosing. Following oral single dose (200 mg/kg), SFSE-G was detected in lung and brain which indicated that it could cross the blood-brain barrier. It is a major route of elimination is excretion through urine and feces. In conclusion, oral administration of SFSE-G showed slow distribution to tissues, such as lung and brain, but showed fast renal elimination.” As taken from Kandhare AD et al. 2015a. *Ren. Fail.* 37(7), 1208-18. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26104039>

“One nursing mother developed toxic epidermal necrolysis thought to be cause by her intake of fenugreek as a galactagogue.”

“Caution should be used in giving high dosages to women with diabetes mellitus or those taking warfarin.”

“Perhaps its most unusual side effect is the imparting an odor of maple syrup to the urine, sweat, feces, and possibly breastmilk by the sotonon in fenugreek.”

As taken from LactMed, 2021. Record for CAS RN 68990-15-8.

“Diosgenin (DI) and trigonelline (TRG) are the main bioactive compounds of fenugreek seed. A micro-extraction method using magnetic nanocomposites coupled with ion-pairing reversed phase HPLC (RP-HPLC) method was used to determine the analytes in rabbit plasma. The main PK parameters after oral administration of pure DI and the extract were: C_{max}: 0.48, 0.057 µg/ml;

t_{max} : 1, 2 h; and $t_{1/2\beta}$: 6.23, 15.04 h, respectively. Pure TRG and the extract PK parameters were: C_{max} : 0.121, 0.081 $\mu\text{g/ml}$; t_{max} : 1.30, 1.30 h; and $t_{1/2\beta}$: 20.06, 24.70 h, respectively. Generally, the PK profile of the two compounds was best fitted on the two-compartment model.” As taken from Mohamadi N et al. 2020. J. Asian Nat. Prod. Res. Epub ahead of print. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32447972/>

4.3. Interactions

“...in dosages of about 25 grams or more daily, fenugreek may cause lowering of cholesterol and blood sugar. It can also interact with warfarin to cause bleeding.”

“Cross-reaction with chickpeas, peanuts, and other legumes is possible.”

As taken from LactMed, 2021. Record for CAS RN 68990-15-8.

“CONTEXT: Herb-drug interactions are a serious problem especially for drugs with a narrow therapeutic index, taking into consideration that herbal medicines are commonly used in various parts of the world. OBJECTIVE: The present study investigates the effect of fenugreek, garden cress, and black seed on the pharmacokinetics of theophylline in beagle dogs. MATERIALS AND METHODS: Beagle dogs received theophylline (200 mg) orally and blood samples were withdrawn at different time intervals (0.33, 0.66, 1.0, 1.5, 2, 3, 4, 6, 8, 12, 24, and 30 h). After a suitable washout period, each herb was given orally at doses of 25, 7.5, and 2.5 g, twice daily for 7 d. On the eighth day, theophylline was re-administrated orally and blood samples were collected. Plasma concentrations of theophylline were determined using HPLC and pharmacokinetic parameters were calculated using a non-compartmental analysis. RESULTS: Treatment with fenugreek (25 g, orally) lead to a decrease in C_{max} and AUC_{0-t} of theophylline of about 28% ($p < 0.05$) and 22% ($p < 0.05$), respectively, with no significant changes in $T_{1/2\lambda}$ compared with the baseline values. Garden cress caused a decrease in C_{max} to a lesser extent and delayed T_{max} of theophylline (2.10 ± 0.24 h versus 3.40 ± 0.74 h), while $AUC_{0-\infty}$ increased by 37.44%. No significant effect was observed for the black seed treatment on theophylline disposition as measured by C_{max} , T_{max} , $AUC_{0-\infty}$, and CL/F . DISCUSSION AND CONCLUSION: The concurrent use of fenugreek or garden cress alters theophylline pharmacokinetic behavior in an animal model. This could represent a modulation in cytochrome P450 activity, which is responsible for theophylline metabolism in beagle dogs. Further confirmation of these results in humans will warrant changes in theophylline dosing before the co-administration of such herbs.” As taken from Al-Jenoobi FI et al. 2015. Pharm. Biol. 53(2), 296-300. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25243874>

“AIM: Combined use of herbs and drugs may result in clinically important herb-drug interactions. The majorities of these interactions are thought to be metabolism-based and involve induction or inhibition of cytochrome P450 (CYP). The current study was designed to investigate the effect of some commonly used herbs on rat CYP2C11 gene expression and metabolic activity. METHODS: Wistar rats were treated for 7 days with increasing doses of 3 herbs; Nigella sativa, Trigonella foenum-graecum, and Ferula asafoetida. Thereafter, CYP2C11 mRNA and protein levels were determined by real-time polymerase chain reaction (RT-PCR) and western blot analyses, respectively. In vitro metabolic activity of CYP2C11 was performed on rat hepatic microsomes using tolbutamide as specific substrate. RESULTS: Our results showed that all the 3 herbs significantly inhibited the mRNA and protein expression levels of CYP2C11 in a dose-dependent manner. Furthermore, the in vitro enzyme metabolic activity study showed a significant decrease in the formation of 4-hydroxy-tolbutamide, a tolbutamide metabolite, at the higher doses. The inhibitory effects of the investigated herbs on rat CYP2C11 was in the order: Nigella Sativa > Trigonella foenum-graecum > Ferula asafoetida. CONCLUSIONS: The 3 herbs are strong inhibitor of CYP2C11 expression, which can lead to an undesirable pharmacological effect of clinically used CYP2C11 substrate drugs with a low therapeutic index.” As taken from Korashy HM et al. 2015. Drug Res. (Stuttg.) 65(7), 366-72. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25099385>

"The present study was conducted to investigate the effects of some commonly used herbs namely *Nigella sativa*, *Lepidium sativum* and *Trigonella foenum-graecum* on the pharmacokinetics of sildenafil in beagle dogs. The study design involved four treatments in a non-balanced crossover design. Sildenafil was given one tablet 100 mg orally to each dog and blood samples were obtained. After a suitable washout period, animals were commenced on a specific herb treatment for 1 week. Blood samples were withdrawn at different time intervals and sildenafil was analyzed by HPLC method. Oral administration of *Nigella sativa* resulted in reduction of $AUC_{0-\infty}$, C_{max} and $t_{1/2}$ as compared to the control. Treatment of *Lepidium sativum* resulted in a significant reduction in the C_{max} and AUC. There were no significant differences between the rests of the pharmacokinetic parameters relative to those of the control. For *Trigonella foenum-graecum*, the effects were similar to those obtained in case of *Lepidium sativum*. It was concluded that concurrent use of investigated herbs alters the pharmacokinetics of sildenafil. Co-administration of investigated herbs should be cautious since their concomitant use might result in decrease in sildenafil bioavailability." As taken from Al-Mohizea AM et al. 2015. Eur. J. Drug Metab. Pharmacokinet. 40(2), 219-24. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/24719213>

"The present study investigated the effect of fenugreek seed powder on disposition of CYP3A substrates, cyclosporine and carbamazepine. Rabbits were treated with fenugreek seed powder (300 mg/kg p.o.) for 8 days and on 8th day the single dose of cyclosporine (30 mg/kg, p.o.) and carbamazepine (40 mg/kg, p.o.) were administered to the corresponding group after 1 h of fenugreek administration. Blood samples were drawn at several time points and analyzed by using UPLC-MS (cyclosporine) and HPLC (carbamazepine). Pharmacokinetic parameters were calculated by using PK Solver. The present investigation reveals that there was no statistically significant difference between pre- and post-treated pharmacokinetic parameters such as $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $C_{(max)}$, $T_{(max)}$, $T_{(1/2)}$, $K_{(el)}$, $MRT_{(0-\infty)}$, $V_{(z/F)}$, and Cl/F for cyclosporine and carbamazepine. Two tailed "P" values for all these pharmacokinetic parameters were more than 0.05, indicating insignificant impact of fenugreek treatment on the disposition of cyclosporine and carbamazepine. Further, fenugreek may also not have any significant effect on the functionality of P-glycoprotein as cyclosporine is a substrate to P-glycoprotein. The outcomes of present study suggested that fenugreek may not likely to interfere cyclosporine and carbamazepine pharmacokinetics, when co-administered with these drugs." As taken from Al-Jenoobi FI et al. 2014. Eur. J. Drug. Metab. Pharmacokinet. 39(2), 147-53. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24022709>

"OBJECTIVE: The seeds of *Trigonella foenum-graecum* (TFG) (family: Leguminosae) are widely consumed both as a spice in food and Traditional Medicine in India. The present study was undertaken to evaluate the inhibitory effect of standardized extract of TFG and its major constituent trigonelline (TG) on rat liver microsome (RLM) and cytochrome P450 (CYP450) drug metabolizing isozymes (CYP3A4 and CYP2D6), which may indicate the possibility of a probable unwanted interaction. MATERIALS AND METHODS: Reverse phase-high performance liquid chromatography method was developed to standardize the hydroalcoholic seed extract with standard TG. The inhibitory potential of the extract and TG was evaluated on RLM and CYP isozymes using CYP450-carbon monoxide (CYP450-CO) complex assay and fluorescence assay, respectively. RESULTS: The content of TG in TFG was found to be 3.38% (w/w). The CYP-CO complex assay showed 23.32% inhibition on RLM. Fluorescence study revealed that the extract and the biomarker had some inhibition on CYP450 isozymes e.g. CYP3A4 and CYP2D6 (IC_{50} values of the extract: 102.65 ± 2.63 - 142.23 ± 2.61 μ g/ml and TG: 168.73 ± 4.03 - 180.90 ± 2.49 μ g/ml) which was very less compared to positive controls ketoconazole and quinidine. Inhibition potential of TFG was little higher than TG but very less compared to positive controls. CONCLUSIONS: From the present study, we may conclude that the TFG or TG has very less potential to inhibit the CYP isozymes (CYP3A4, CYP2D6), so administration of this plant extract or its biomarker TG may be safe." As taken from Ahmmed SM et al. 2015. Indian J. Pharmacol. 47(5), 530-4. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26600643>

“The combined effects of *Trigonella foenum-graecum* and *Allium sativum* extracts were evaluated for their ameliorative potential in the L-thyroxine-induced hyperthyroidic rat model to contribute to an understanding of interaction between the two extracts. The investigation was carried out using two different doses. A comparison was made with the response of individual plant extracts at the previously studied effective dose in adult Wistar rats rendered hyperthyroidic by daily injections of L-thyroxine (300 ug/kg body wt., s.c.). Propylthiouracil (PTU), an antithyroid drug, was used as a reference compound. Alterations in serum triiodothyronine (T3), thyroxine (T4), glucose, hepatic glucose-6-phosphatase (G-6-Pase) and oxygen consumption were studied as end parameters. Superoxide dismutase (SOD), catalase (CAT) activities, lipid peroxidation (LPO) and reduced glutathione (GSH) were examined to reveal any toxic effects of the drugs. The combined effects of *Trigonella* and *Allium* at 200 and 500 mg/kg body wt. respectively, were equipotent as compared to the individual extracts in lowering the serum concentrations of T3 and T4 in hyperthyroidic rats. Our findings reveal that some plant extracts in combination may not always prove to be synergistic. It is therefore suggested that *Trigonella foenum-graecum* and *Allium sativum* extracts may be used individually and not together in the regulation of hyperthyroidism. [Tahiliani P, Kar A; *Phytomedicine* 10 (8): 665-8 (2003)] **PEER REVIEWED**“

“Erythrocytes are excellent model to study the xenobiotic induced oxidative changes. Pyrethroid pesticides are increasingly being used in insecticidal preparations from the simple mosquito coils to house hold aerosols to sophisticated ultra low volume foggers and sprays. Cypermethrin a Type II pyrethroid pesticide is used widely in pest control. Fenugreek is a potent antioxidant. We have evaluated the potential of aqueous extract of germinated fenugreek seeds in counteracting cypermethrin induced oxidative changes in erythrocytes of male Wistar rats. Male Wistar rats were treated with 1/10 LD50 (25 mg/kg body weight) of cypermethrin and 10 percent aqueous extract of germinated fenugreek for 60 days. Cypermethrin treatment caused significant decrease in non enzymatic antioxidants, glutathione (GSH), vitamin E, vitamin C, increased methemoglobin formation in erythrocytes and increased their mechanical fragility. Treatment with fenugreek reversed the cypermethrin induced oxidative changes in erythrocytes and restored all the parameters to near normal levels. The overall results reveal the ameliorating effect of aqueous extract of germinated fenugreek on cypermethrin induced toxicity in erythrocytes. [Navayath S, Thiyagarajan D; *J Complement Integr Med* 8 doi:10.2202/1553-3840.1436 (2011)] **PEER REVIEWED**”

As taken from HSDB, 2016

“Twenty eight commonly used herbal supplements (HS) were evaluated for their drug interaction potential via quantification of their inhibitory effects on the activity of CYP3A4, a key intestinal drug metabolizing enzyme, in cryopreserved human enterocytes, using the novel enteric model, MetMax™ Pooled Donor Human Enterocytes. Commercially obtained formulated oral herbal nutrient products were used in the study. The HS were dissolved in protein free culture medium (HQM) followed by filtration to remove insoluble components and pH adjustment to 7.0. MetMax™ human enterocytes were thawed and added directly in a volume of 10 uL per well to 384 well plates containing 10 uL of HS and luciferin IPA (CYP3A4-specific substrate), yielding final concentrations of 1.6, 3.15, 6.25, 12.5, 25, 50 and 100% of HS, with 100% defined as the recommended oral dosage dissolved in 200 mL of HQM (based on the commonly accepted enteric fluid volume of 214 mL). After a 60 min. incubation at 37 deg. C in a cell culture incubator, CYP3A4 activity was quantified based on luciferin luminescence. Dose-dependent inhibition was observed for the following HS, in descending order of inhibition at 100% of the recommended dosage: green tea (97.6% inhibition), grapefruit juice (93.1%), St. John's Wort (91.9%), echinacea (80.1%), ginger root (74.7%), horehound (63.3%), spirulina (61.5%), milk thistle (58.7%), black elderberry (57.1%), ginkgo (50.8%), cinnamon (48.4%), ginseng (41.1%), garlic (33.4%), black cohosh (33.0%). No inhibition was observed for maca, red yeast rice, flaxseed oil, evening primrose, oregano, fenugreek, valerian root, soy isoflavone, rhodiola, tumeric, saw palmetto, wheat grass, cranberry juice, white kidney bean, and guarana. The results are consistent with clinically observed herb-drug interactions that have been reported in the literature, suggesting that MetMax™ Pooled Human

Enterocytes can be used for the evaluation of potential herb-drug and food-drug interactions." As taken from Loretz C and Li AP. 2018. *Drug Metabolism and Pharmacokinetics* 33(1Suppl.), S62. Available at <https://www.sciencedirect.com/science/article/pii/S1347436717304408>

"Dimethoate is a widely used organophosphate insecticide known to be toxic to the pancreas. The aim of this study is to detect the possible protective effects of the fenugreek seed ethanolic extract on the biochemical, histological, and ultra-structural abnormalities induced by dimethoate chronic exposure in the pancreas of adult male rats. The study was conducted on 50 adult male albino rats that were divided equally into 5 groups: (group I) negative control, (group II) vehicle control group, (group III) fenugreek-treated group that was given 400 mg/kg ethanolic fenugreek seed extract once daily, (group IV) dimethoate group received 20 mg/kg/day dimethoate, and (group V) dimethoate- + fenugreek-treated group received a combination of dimethoate and fenugreek in the same previous doses. Dimethoate treatment caused a significant increase in serum glucose, amylase, and lipase levels and a significant decrease in serum insulin. A significant increase in lipid peroxidation and pro-fibrotic cytokine (TGF- β 1) together with a significant reduction of the antioxidant {reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD)} activities and the anti-inflammatory (IL-4) in pancreatic tissues was also recorded. There was a histological and ultra-structural evidence of pancreatic acinar and islet cell injury. The recorded abnormalities were reversed in dimethoate+fenugreek treated group indicating that fenugreek ethanolic extract can serve as an antidote for dimethoate-induced pancreatic insult." As taken from Mesallam DIA et al. 2018. *Environ. Sci. Pollut. Res. Int.* 25(4), 3894-3904. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29177779>

"Liver and kidney diseases are a global concern, therefore considerable efforts to obtain fine herbs useful as drugs from medicinal plants are currently in progress. The aim of this work was to study the antioxidant effects of previous supplementation with fenugreek seeds (FS) against carbon tetrachloride (CCl₄) toxicity in the liver and kidney. CCl₄ toxicity was induced by one dose (i.g. 5ml CCl₄/kg of body weight, 50% CCl₄ in olive oil) after 7 weeks of normal diet or diet rich in 10% of grinded fenugreek seeds (20g of pellet rat food/rat/day). 24h after the treatment with CCl₄, all animals were scarified and biological analyses were performed. A phytochemical study of fenugreek seed extract (FSE) was also carried out. The phytochemical analysis of FS and FSE revealed the presence of polyphenols (5.92 \pm 0.02mg EGA/g DM), flavonoids (0.44 \pm 0.19mg ER/g DM), polysaccharides and trace elements. DPPH radical-scavenging activity of FSE showed an EC₅₀ of 285.59 \pm 2.01 μ g/ml. In vivo, CCl₄ administration significantly (p<0.05) induced an increase liver and kidney biomarkers. A significant (p<0.05) alteration of the antioxidant enzyme activities was also observed. In animals pretreated with FS, the studied parameters were much less shifted. These results indicate that the supplementation with fenugreek seeds is significantly effective in protecting the liver and kidneys from CCl₄ toxicity." As taken from Mbarki S et al. 2017. *Biomed. Pharmacother.* 88, 19-26. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28092841>

"As fenugreek is rich in fiber, it can interfere with the absorption of orally taken drugs. Prescription of medicines must be taken separately from fenugreek-containing products. Bash et al. [138] reported that the concomitant use of fenugreek with other hypoglycemic drugs may reduce serum glucose levels more than expected. It has been suggested that herbal supplements containing coumarin, e.g., fenugreek may potentially increase the risk of bleeding or potentiate the effects of warfarin therapy [139]. In fact, a potential interaction between warfarin (used for atrial fibrillation) and boldo-fenugreek [140], and between aspirin and fenugreek, resulting in bleeding [141] was also observed. In addition, it has been suggested that the consumption of fenugreek is a potential risk for patients with chronic asthma and patients known to be allergic to it, or who are allergic to chickpeas or peanuts because of possible cross-reactivity." As taken from El Bairi K et al. 2017. *Biomedicine and Pharmacotherapy.* 90: 479-491. Pubmed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/?term=28391170>

“Ethnopharmacological relevance: Diabetes mellitus is a metabolic disorder, with a large prevalence in low- and middle-income countries. Numerous studies highlighted the use of medicinal plants for diabetes in Algeria. This use is influenced by cultural considerations. The industrialization of the country leads to the transformation of the trend of the utilization of plants, and the increase of polymedication. In this context, there is no information about the profile of diabetic patients using plants and the potential of herb-drug interactions. Aim of the study: The objective was to evaluate the use of plants by diabetic patients and to search the drug interactions with the most cited species. Materials and methods: Descriptive and comparative studies were carried out among diabetic patients and traditional healers, in the west of Algeria, to inventory the plants used in diabetes. Bivariate and multivariate analysis by logistic regression were performed to evaluate the associated factors with the use, to determine the profile of users. Bibliometric research in PubMed, Google Scholar, Cochrane Library and ClinicalTrials was conducted to look for herb-drug interactions. Results: Results show a decrease in the use of medicinal plants by diabetic patients, in comparison of previous findings, some of which have not been cited by traditional healers. The patients under oral anti-diabetic agents (ODA) have twice the risk to use plants in concomitant with drugs. *Olea europea* leaves and *Trigonella foenum-graecum* seeds were the most cited herbals. Metformin was the most used drug. Several clinical trials revealed that the anti-diabetic plants had a synergistic effect with ODA, which can increase the hypoglycemia of diabetics. Non-important adverse reactions were noted. Conclusions: New trends of the use of medicinal plants by diabetic patients were noted, this is due to the diversification of information sources. Patients under ODA must be educated about the use and the risk of herb-drug interactions.” As taken from Mustapha C et al. 2021. *J. Ethnopharmacol.* Epub ahead of print. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33711438/>

“Commercial formulations of 29 commonly used herbal supplements (HSs) and grapefruit juice were evaluated for drug interaction potential via quantification of their CYP3A inhibitory potential in two in vitro experimental models of human small intestine, cryopreserved human intestinal mucosa (CHIM), and cryopreserved human enterocytes (CHEs). Two CYP3A substrates were used-in the studies with CHIM, CYP3A activity was quantified via liquid chromatography tandem mass spectrometry quantification of midazolam 1'-hydroxylation, whereas in CHE, luciferin-IPA metabolism to luciferin was quantified by luminescence. Upon treatment of CHIM with the estimated lumen concentration of the HS upon each oral administration (manufacturers' recommended dosage dissolved in 200 ml of culture medium), >80% CYP3A inhibition was observed for green tea extract, St. John's wort, valerian root, horehound, and grapefruit juice. Less than 50% inhibition was observed for fenugreek, aloe vera, guarana, soy isoflavone, maca, echinacea, spirulina, evening primrose, milk thistle, cranberry, red yeast rice, rhodiola, ginkgo biloba, turmeric, curcumin, white kidney bean, garlic, cinnamon, saw palmetto berries, panax ginseng, black elderberry, wheat grass juice, flaxseed oil, black cohosh, and ginger root. The results were confirmed in a dose-response study with HSs obtained from three suppliers for the four inhibitory HSs (green tea extract, horehound, St. John's wort, valerian root) and three representative noninhibitory HSs (black cohosh, black elderberry, echinacea). Similar results were obtained with the inhibitory HSs in CHE. The results illustrate that CHIM and CHE represent physiologically relevant in vitro experimental models for the evaluation of drug interaction potential of herbal supplements. Based on the results, green tea extract, horehound, St. John's wort, and valerian root may cause drug interactions with orally administered drugs that are CYP3A substrates, as was observed for grapefruit juice. SIGNIFICANCE STATEMENT: In vitro evaluation of 29 popular herbal supplements in cryopreserved human intestinal mucosa identified green tea extract, horehound, St. John's wort, and valerian root to have CYP3A inhibitory potential similar to that for grapefruit juice, suggesting their potential to have clinically significant pharmacokinetic interaction with orally administered drugs that are CYP3A substrates. The results suggest that cryopreserved human intestinal mucosa can be used for in vitro evaluation of drug interactions involving enteric drug metabolism.” As taken from Loretz C et al. 2020. *Drug Metab. Dispos.* 48(10), 1084-1091. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32719085/>

“Treating herbal medicines, based on their long-term widespread use as anti-fertility agents, conversely it’s possible to have herbs that are effective for improving fertility. Endometrium of the uterus undergoes enormous renovation in response to systemic changes in reproductive hormones (progesterone and estradiol) during each reproductive cycle). Twenty five health mature female mice (aged 8 weeks) divided into five groups (5/each) were participants in the current to investigate the fertility effects of *Origanum vulgare* against the reproductive toxicity induced by Fenugreek. These effects examine by measuring the number of ovarian follicles, thickness of the uterus and steroid hormone levels (estrogen & progesterone) in serum. The results showed no significant differences ($p > 0.05$) in number of ovarian follicles, steroid hormones level, and lower in thickness layers of uterus tissue the experimental treated groups with Fenugreek (250 mg/kg and *O. vulgare* (90,180,270) mg/kg respectively. Depending on these outcomes, the hot aqueous of *O. vulgare* has enhancement reproductive system in female albino mice, and these results give additional scientific support to maybe use *O. vulgare* as an alternative for problems of fertility.” As taken from Ghani JM et al. 2020. *Eurasia. J. Biosci.* 14(1), 1335-1340. Available at <https://www.researchgate.net/publication/342196791>

“The objective of this investigation was to study whether *Nigella Sativa* and *Trigonella Foenum-graecum*, could modulate the losartan pharmacodynamic (PD) and pharmacokinetic (PK) in experimental L-NAME induced hypertensive rats. For in vivo study, the systolic blood pressure (SBP) of rats was measured by the "tail-cuff system" after the treatment of rats with herb alone and herb + losartan in hypertensive rats. The SBP of rats treated with L-NAME + losartan also recorded. For the PK study, blood samples were obtained for up to 12 h to determine the concentrations of the drug, and various PK parameters were calculated. The data displayed that the SBP was significantly ($p < 0.05$) decreased in the rats when administered with L-NAME + *N. Sativa* or L-NAME + *T. Foenum-graecum* in contrast to the rats administered with L-NAME alone. A more prominent decline ($p < 0.05$) in SBP was detected in rats administered with L-NAME + *N. Sativa* + losartan and L-NAME + *T. Foenum-graecum* + losartan. In a PK study, higher losartan C_{max} and AUC_{0-t} were noted in rats treated with *N. Sativa* + losartan and *T. Foenum-graecum* + losartan, although the difference was not significant in contrast to the control group. This study proposed that the interaction between *N. Sativa* & losartan and *T. Foenum-graecum* & losartan could take place on concurrent administration; consequently, the dose of losartan may need to be accustomed when they are utilized simultaneously.” As taken from Ahad A et al. 2020. *Saudi J. Biol. Sci.* 27(10), 2544-2550. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32994710/>

“Background: The present article is related to in-vitro and in-vivo herb-drug interaction studies. Objectives: This study aimed to investigate the effect of *Nigella sativa* and fenugreek on the pharmacodynamics and pharmacokinetics of amlodipine. Method: Hypertensive rats of group-I were treated with amlodipine and rats of group-II and III were treated with *N. sativa*, and *N. sativa* + amlodipine and fenugreek, and fenugreek + amlodipine, respectively. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP) of group-I, II and III rats were measured by the “tail-cuff system”. Results: *N. sativa*, as well as fenugreek, reduced the SBP, DBP and MBP. Simultaneously, administration of fenugreek + amlodipine or *N. sativa* + amlodipine showed better control of BP. Individually, fenugreek, as well as *N. sativa*, showed a surprising reduction in the heart rate. There was no remarkable effect of any of these two herbs on C_{max} , AUC_{0-t} , K_{el} , and terminal elimination half-life of amlodipine, but fenugreek altered the T_{max} of amlodipine significantly, from $2 \pm 1.2h$ in control to $7.2 \pm 1.7h$ in fenugreek treated group, probably by delaying the absorption. Conclusion: Results of pharmacodynamics and pharmacokinetics studies suggested that simultaneous administration of fenugreek or *N. sativa* with amlodipine improved the pharmacological response of amlodipine in hypertensive rats, though there was no remarkable change in pharmacokinetic parameters (C_{max} , K_{el} , elimination $t_{1/2}$, and AUC_{0-t}).” As taken from Alam MA et al. 2020. *Curr. Drug. Metab.* 21(4), 318-325. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32407268/>

5. Toxicity

5.1. Single dose toxicity

“The present study was carried out to determine the acute toxicity of the leaf glycosidic extract of *Trigonella foenum-graecum* by estimation of its medium [median] lethal dose (LD₅₀) after oral and intraperitoneal administration to mice and also to identify the target organs for its possible toxic effects. The main target organ affected among the four organs studied (liver, kidney, stomach, small and large intestine) was the liver, where early degeneration with infiltration of mononuclear and mild hepatitis was found in some animals treated with toxic doses of glycosidic extract. It is concluded that the glycosidic extract of *T. foenum-graecum* leaves is considered to be safe and have minimal adverse effect”. As taken from Abdel-Barry JA & El-Hakiem MH. 2000. *J. Ethnopharmacol.* 70, 65-68. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/10720790>.

Antihyperglycemic effect of *Trigonella foenum-graecum* (fenugreek) seed extract in alloxan-induced diabetic rats and its use in diabetes mellitus: a brief qualitative phytochemical and acute toxicity test on the extract (Abstract). The effects of ethanol extract of *Trigonella foenum-graecum* (Fenugreek) seeds on the blood glucose levels in alloxan-induced diabetic rats at different doses (2 g/kg, 1 g/kg, 0.5 g/kg and 0.1 g/kg) were studied. The hypoglycemic effect of extract was compared with that of the standard antidiabetic drug (glimepiride, 4 mg/kg) single dose. The extract showed significant activity against the diabetic state induced by alloxan but the intensity of hypoglycemic effect varied from dose to dose. The most effective dose recognized was 1 g/kg but that is still lower than the standard antidiabetic drug. No acute toxicity was observed for ethanol extract of *T. foenum-graecum* seed when it was administered orally at high dose level (3 g/kg body weight), which is higher than effective antihyperglycemic dose, and closely observed for 24 hrs for any mortality and next 10 days for any delayed toxic effects on gross behavioral activities. Phytochemical group tests were also accomplished and presence of alkaloids, steroids and carbohydrates were recognized in the extract. As taken from Mowla et al. *Afr J Tradit Complement Altern Med.* 2009; 6(3):255-61. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2816457/> (CAS 68990-15-8)

“Safety and anti-diabetic efficacy of a novel, proprietary *Trigonella foenum-graecum* seed extract [novel fenugreek extract (FE), Fenfuro™, CR0010810) enriched in furostanolic saponins (>60% w/w, HPLC) were assessed. Concerning safety, we undertook studies dealing with acute oral toxicity, 28-d sub-chronic toxicity and Ames' bacterial reverse mutation assay that revealed no toxicity. Concerning efficacy, we examined beneficial effects of the extract on rats with type 2 diabetes (T2D). Male Sprague-Dawley rats received a high-fat diet for 2 weeks followed by streptozotocin (STZ, 35 mg/kg i.p.) to produce T2D. Seven days post-STZ, rats showing ≥300 mg/dl fasting plasma glucose level (PGL) were included in the study. FE (150- or 450- mg/kg p.o.) and glipizide (5 mg/kg p.o.) were administered once daily for 20 d and then twice daily for another 10 d (total 30 d). Blood samples were collected at 0, 10, 20 and 30 d of treatment and estimated for fasting plasma triglyceride (PTG), total cholesterol and insulin levels. After 30 d, FE and glipizide-treated diabetic animals were treated in combination with or without metformin (100 mg/kg) twice daily for another 10 d. FE did not influence body weight, feed and water intake. FE (150 mg/kg p.o.) reduced PTG levels in T2D rats by 22%, 24.6% and 29% at 10, 20 and 30 d of treatment, respectively, while glipizide (5 mg/kg p.o.) reduced the PTG levels by 57.4%, 46.2% and 39.4% at these time points. FE (450 mg/kg) treatment in STZ-induced diabetic rats produced significant hypoglycemic activity (approximately 31.5%) as compared to insulin (48.2% with 1 U/kg i.p.). FE (150 mg/kg p.o.) and metformin (100 mg/kg p.o.) combined produced significant reduction (20.7%) of PGL in T2D rats. No adverse effects were observed. We conclude after extensive *in vitro* and *in vivo* safety and efficacy studies that FE is safe and effective in treating T2D.” As taken from Swaroop A et al. 2014. *Toxicol. Mech. Methods* 24(7), 495-503. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25045923>

"The objective of the present work was to study acute and subacute (28-days repeated dose) oral toxicity effect of glycosides based standardized fenugreek seed extract (SFSE-G) in vivo. SFSE-G was prepared by resin-based chromatography and standardized to glycosides namely trigoneoside Ib (76%) and vicianin 1 (15%). The acute oral toxicity (AOT) and subacute toxicity studies were performed in Swiss albino mice (5 mice/sex/group) as per OECD 425 (up-and-down procedure) and OCED 407 guidelines respectively. Acute oral administration of 5000mg/kg of SFSE-G showed 40% mortality with no mortality in lower dosages. The subacute oral administration of SFSE-G did not show observational or toxicological effects on the body or organ weights, food consumption, ophthalmic effects, locomotor activity, hematology, blood biochemistry, urinalysis, or histopathology at dose 250mg/kg. However, SFSE-G (1000mg/kg) showed mortality and minor alterations to body weight, relative liver weights, hematology and blood chemistry parameters related to treatment but it was within normal laboratory ranges. In conclusion, SFSE-G showed median lethal dose (LD50) more than 4350mg/kg and no-observed adverse effect levels (NOAEL) of 250mg/kg for both sexes during AOT and sub-acute toxicity study, respectively. " As taken from Kandhare AD et al. 2015b. Regul. Toxicol. Pharmacol. 72(2), 323-34. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25979642>

Record for *Trigonella foenum-graecum* Linn., extract (no CAS RN):

Type of Test	Route of Exposure Administration	Species/ or Test System	Dose Data	Toxic Effects	Reference
LD Lethal dose	Oral	Rodent rat	>5 gm/kg	Details of toxic effects not reported other than lethal dose value	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20-1982-37,831,1999 Volume(issue)/page/year:
LD50 Lethal dose, 50 percent kill	Intraperitoneal	Rodent rat	500 mg/kg	Vascular - BP lowering characterized in autonomic section	IJEBA6 Indian Journal of Experimental Biology. (Publications & Information Directorate, CSIR, Hillside Rd., New Delhi 110 012, India) V.1- 1963-16,228,1978 Volume(issue)/page/year:
LD50 Lethal dose, 50 percent kill	Oral	Rodent mouse	10 gm/kg	Details of toxic effects not reported other than lethal dose value	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979-58,149,1997 Volume(issue)/page/year:

As taken from RTECS, 2010a.

Record for *Trigonella foenum-graecum* L. (Papilionaceae), seed, water extract (no CAS RN):

Type of Test	Route of Exposure Administration	Species/ or Test System	Dose Data	Toxic Effects	Reference
TDLo Lowest published toxic dose	Oral	Rodent rat	15 mL/kg	Gastrointestinal - alteration in gastric secretion Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - catalases Biochemical - Enzyme inhibition, induction, or change in blood or tissue	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979-81,393,2002 Volume(issue)/page/year:

				levels - other oxidoreductases	
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As taken from RTECS, 2014.

Record for *Trigonella foenum-graecum* L., seed, methanol extract (no CAS RN):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent mouse	- 100 mg/kg	Behavioral analgesia - Biochemical Metabolism (Intermediary) - effect on or of inflammation mediation inflammation	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20-1982- Volume(issue)/page/year: 50,2503,2012

As taken from RTECS, 2017a

“LABORATORY ANIMALS: Acute Exposure/ There are some reports concerning the antinociceptive effects of the plant *Trigonella foenum-graecum* (TFG) in Iranian traditional medicine. Because of the side effects of nonsteroidal anti-inflammatory and antinociceptive drugs, and in search for more potent and less harmful compounds, we tried to study the antinociceptive effects of TFG leaves by using tail-flick and formalin tests. Intraperitoneal (i.p.) administration of 500 mg/kg of TFG extract and 100 and 300 mg/kg of sodium salicylate (SS), as a positive control, did not show any effect in the tail-flick test, but the 1000 and 2000 mg/kg of the extract produced significant increase in the tail-flick latency. SS (300 mg/kg, i.p.) induced antinociception in the second phase of the formalin test. TFG (500 mg/kg, i.p.) demonstrated antinociception only in the first phase, but 1000 and 2000 mg/kg, i.p. doses alleviated the pain in both phases. Preliminary LD50 of the extract was very close to 4000 mg/kg, i.p. We conclude that: (1) the extract of TFG leaves produces antinociceptive effects through central and peripheral mechanisms; (2) the antinociceptive effects of 2000 mg/kg of the extract was more potent than 300 mg/kg of SS.[Javan M et al; J Ethnopharmacol 58 (2): 125-9 (1997)] **PEER REVIEWED**”

As taken from HSDB, 2016

“OBJECTIVE: To evaluate acute oral toxicity (AOT), subchronic (90-day repeated dose) toxicity, mutagenicity, and genotoxicity potential of IDM01, the botanical composition of 4-hydroxyisoleucine- and trigonelline-based standardized fenugreek (*Trigonella foenum-graecum* L) seed extract in laboratory rats. MATERIALS AND METHODS: The AOT and subchronic (90-day repeated dose) toxicity were evaluated using Sprague-Dawley rats as per the Organisation for Economic Co-operation and Development (OECD) guidelines No. 423 and No. 408, respectively. During the subchronic study, the effects on body weight, food and water consumption, organ weights with hematology, clinical biochemistry, and histology were studied. The mutagenicity and genotoxicity of IDM01 were evaluated by reverse mutation assay (Ames test, OECD guideline No. 471) and chromosome aberration test (OECD guideline No. 473), respectively. RESULTS: The IDM01 did not show mortality or treatment-related adverse signs during acute (limit dose of 2000 mg/kg) and subchronic (90-day repeated dose of 250, 500, and 1000 mg/kg with 28 days of recovery period) administration. The IDM01 showed oral median lethal dose (LD50) >2000 mg/kg during AOT study. The no-observed adverse effect level (NOAEL) of IDM01 was 500 mg/kg. IDM01 did not show mutagenicity up to a concentration of 5000 µg/plate during Ames test and did not induce structural chromosomal aberrations up to 50 mg/culture. CONCLUSIONS: IDM01 was found

safe during preclinical acute and subchronic (90-day repeated dose) toxicity in rats without mutagenicity or genotoxicity. SUMMARY: Acute oral toxicity, subchronic (90-day) oral toxicity, mutagenicity and genotoxicity of IDM01 (4-hydroxyisoleucine- and trigonelline-based standardized fenugreek seed extract) was evaluated. The median lethal dose, LD50, of IDM01 was more than 2000 mg/kg of body weight in rats.No observed adverse effect level (NOAEL) of IDM01 was 500 mg/kg of body weight in rats.IDM01 was found safe during acute and subchronic oral toxicity studies in rats without mutagenicity or genotoxicity potential.” As taken from Deshpande PO et al. 2017a. Pharmacognosy Res. 9(2), 138-150. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28539737>

“The present work is aimed at studying acute oral toxicity (AOT), subchronic oral toxicity, mutagenicity, and genotoxicity of furostanol glycosides-based standardized fenugreek seed extract (Fenu-FG) using the Organization for Economic Co-operation and Development (OECD) guidelines. The AOT and subchronic (90-day repeated dose) toxicity studies were performed on Wistar rats as per OECD 423 and OECD 408 guidelines, respectively. The mutagenicity (reverse mutation assay, Ames test) and genotoxicity (mammalian chromosome aberration test) were assessed in vitro using OECD 471 and OECD 473 guidelines, respectively. At an acute oral limit dose of 2,000 mg/kg, Fenu-FG did not show any mortality or treatment-related adverse signs. Ninety days of subchronic oral administration of Fenu-FG (250, 500, or 1,000 mg/kg) in rats did not induce any treatment-related significant changes with respect to body weight, hematology, blood biochemistry, urinalysis, gross pathology, or histopathology. The no-observed-adverse-effect-level of Fenu-FG was 1,000 mg/kg/day. Furthermore, Fenu-FG did not demonstrate mutagenic potential up to a concentration of 5,000 µg/plate (Ames test) and did not induce structural chromosome aberrations up to 2,000 µg/ml (in human lymphocyte cells in vitro). In conclusion, Fenu-FG was found safe during preclinical safety assessments.” As taken from Deshpande P et al. 2017b. J. Diet. Suppl. 14(5), 521-541. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28156165>

Record for *Trigonella foenum-graecum* Linn., leaf, water extract (no CAS RN given):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent mouse	1.9 gm/kg	Details of toxic effects not reported other than lethal dose value	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 58,149,1997
LD50 - Lethal dose, 50 percent kill	Oral	Rodent mouse	10 gm/kg	Details of toxic effects not reported other than lethal dose value	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 58,149,1997
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - rat	0.5 gm/kg	Endocrine - hypoglycemia	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 58,149,1997
TDLo -	Oral	Rodent - rat	1	Endocrine -	JOETD7 Journal of

Lowest published toxic dose			gm/kg	hypoglycemia	Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979-Volume(issue)/page/year: 58,149,1997
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - rat	1 gm/kg	Behavioral analgesia -	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979-Volume(issue)/page/year: 95,13,2004
TDLo - Lowest published toxic dose	Intraspinal	Rodent - rat	2.2 mg/kg	Behavioral analgesia -	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979-Volume(issue)/page/year: 95,13,2004

As taken from RTECS, 2018a

“The present study investigated the safety of a saponin-rich standardized extract of fenugreek seeds (FenuSMART®; FHE), that has been clinically shown to be effective in ameliorating the postmenopausal discomforts and establishing hormonal balance. The safety was assessed by oral acute (2500 mg/kg b. wt. for 14 days) and subchronic (250, 500 and 1000 mg/kg b. wt. for 90 days) toxicity studies on Wistar rats and mutagenicity studies employing Salmonella typhimurium strains. Administration of FHE did not produce any toxicologically significant changes in clinical/behavioral observations, ophthalmic examinations, body weight, organ weight, feed consumption, urinalysis, hematology and clinical biochemistry parameters when compared to the untreated control group of animals. Highest dose recovery group (1000 mg/kg b. wt.) of animals also showed no mortality or adverse events; with hematological and biochemical parameters at par with those of controls. Terminal autopsy revealed no alterations in relative organ weight or any treatment-related histopathology changes. FHE also showed no mutagenicity upon Ames test employing TA-98, TA-100 and TA-102 Salmonella typhimurium strains with or without metabolic activation. Based on the results of the study, the no observed-adverse-effect level (NOAEL) of FHE was determined as 1000 mg/kg b. wt./day, the highest dose tested.” As taken from Sureshkumar D et al. 2018. Toxicol. Rep. 5, 1060-1068. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30416976>

5.2. Repeated dose toxicity

“We previously reported that fenugreek (*Trigonella foenum-graecum* L.) improved diet-induced metabolic disorders in rats. The purpose of the present study was to examine the dose-dependent effects, safety and tolerability of fenugreek. METHODS: The diets used in this study were the high-fat high-sucrose diet (HFS; lard 50%kcal, sucrose 25%kcal) as a control (Ctrl group) or the HFS containing 0.25% (VL group), 1.25% (L group), 2.50% (M group), 5.00% (H group) or 12.30% (VH group) fenugreek based on the modified version of the AIN-93G purified diet. RESULTS: Fenugreek dose-dependently reduced the hepatic triglyceride and total cholesterol levels. Fenugreek also dose-dependently increased the excretion of cholesterol and total bile acids into the feces. However, the glucose tolerance showed no significant change by fenugreek administration. The VL and L groups did not significantly change triglyceride or total cholesterol levels in the liver. The VL group showed no increase in excretion of triglyceride, total cholesterol or bile acids in the feces. The VH group showed appetite reduction and diarrhea, while no adverse effect or symptoms were observed in the M group. CONCLUSION: These results suggest that fenugreek inhibited lipid accumulation in the liver by increasing the lipid excretion in the feces. The

effective, safe and tolerable dose of fenugreek was found to be around 2.50% (w/w)". As taken from Muraki E et al. 2011. *Lipids Health Dis.* 10, 240. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3292492/>.

"This study investigated the inhibitory effect of aqueous extract of *Trigonella foenum-graecum* seeds (AqE-TFG) on fat accumulation and dyslipidemia in high fat diet- (HFD-) induced obese rats. Female Wistar rats were fed with HFD ad libitum, and the rats on HFD were treated orally with AqE-TFG or orlistat ((HFD for 28 days+AqE-TFG (0.5 and 1.0 g/kg) or orlistat (10 mg/kg) from day 8 to 28), respectively. Treatment with AqE-TFG produced significant reduction in body weight gain, body mass index (BMI), white adipose tissue (WAT) weights, blood glucose, serum insulin, lipids, leptin, lipase, and apolipoprotein-B levels and elevation in adiponectin levels. AqE-TFG improved serum aspartate amino transferase (AST), alanine amino transferase (ALT), and lactate dehydrogenase (LDH) levels. AqE-TFG treatment reduced the hepatic and cardiac thiobarbituric acid reactive substances (TBARS) and elevated the antioxidant enzyme (glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT)) levels. In addition, liver and uterine WAT lipogenic enzyme (fatty acid synthetase (FAS) and glucose-6-phosphate dehydrogenase (G6PD)) activities were restored towards normal levels. These findings demonstrated the preventive effect of AqE-TFG on fat accumulation and dyslipidemia, due to inhibition of impaired lipid digestion and absorption, in addition to improvement in glucose and lipid metabolism, enhancement of insulin sensitivity, increased antioxidant defense, and downregulation of lipogenic enzymes." As taken from Kumar P et al. 2014. *Biomed. Res. Int.* 2014, 606021. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24868532>

"Safety and anti-diabetic efficacy of a novel, proprietary *Trigonella foenum-graecum* seed extract [novel fenugreek extract (FE), Fenfuro™, CR0010810) enriched in furostanolic saponins (>60% w/w, HPLC) were assessed. Concerning safety, we undertook studies dealing with acute oral toxicity, 28-d sub-chronic toxicity and Ames' bacterial reverse mutation assay that revealed no toxicity. Concerning efficacy, we examined beneficial effects of the extract on rats with type 2 diabetes (T2D). Male Sprague-Dawley rats received a high-fat diet for 2 weeks followed by streptozotocin (STZ, 35 mg/kg i.p.) to produce T2D. Seven days post-STZ, rats showing ≥ 300 mg/dl fasting plasma glucose level (PGL) were included in the study. FE (150- or 450- mg/kg p.o.) and glipizide (5 mg/kg p.o.) were administered once daily for 20 d and then twice daily for another 10 d (total 30 d). Blood samples were collected at 0, 10, 20 and 30 d of treatment and estimated for fasting plasma triglyceride (PTG), total cholesterol and insulin levels. After 30 d, FE and glipizide-treated diabetic animals were treated in combination with or without metformin (100 mg/kg) twice daily for another 10 d. FE did not influence body weight, feed and water intake. FE (150 mg/kg p.o.) reduced PTG levels in T2D rats by 22%, 24.6% and 29% at 10, 20 and 30 d of treatment, respectively, while glipizide (5 mg/kg p.o.) reduced the PTG levels by 57.4%, 46.2% and 39.4% at these time points. FE (450 mg/kg) treatment in STZ-induced diabetic rats produced significant hypoglycemic activity (approximately 31.5%) as compared to insulin (48.2% with 1 U/kg i.p.). FE (150 mg/kg p.o.) and metformin (100 mg/kg p.o.) combined produced significant reduction (20.7%) of PGL in T2D rats. No adverse effects were observed. We conclude after extensive in vitro and in vivo safety and efficacy studies that FE is safe and effective in treating T2D." As taken from Swaroop A et al. 2014. *Toxicol. Mech. Methods* 24(7), 495-503. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25045923>

"The objective of the present work was to study acute and subacute (28-days repeated dose) oral toxicity effect of glycosides based standardized fenugreek seed extract (SFSE-G) in vivo. SFSE-G was prepared by resin-based chromatography and standardized to glycosides namely trigoneoside Ib (76%) and vicenin 1 (15%). The acute oral toxicity (AOT) and subacute toxicity studies were performed in Swiss albino mice (5 mice/sex/group) as per OECD 425 (up-and-down procedure) and OCED 407 guidelines respectively. Acute oral administration of 5000mg/kg of SFSE-G showed 40% mortality with no mortality in lower dosages. The subacute oral administration of SFSE-G did not show observational or toxicological effects on the body or organ weights, food consumption, ophthalmic effects, locomotor activity, hematology, blood biochemistry, urinalysis, or histopathology

at dose 250mg/kg. However, SFSE-G (1000mg/kg) showed mortality and minor alterations to body weight, relative liver weights, hematology and blood chemistry parameters related to treatment but it was within normal laboratory ranges. In conclusion, SFSE-G showed median lethal dose (LD50) more than 4350mg/kg and no-observed adverse effect levels (NOAEL) of 250mg/kg for both sexes during AOT and sub-acute toxicity study, respectively” As taken from Kandhare AD et al. 2015b. Regul. Toxicol. Pharmacol. 72(2), 323-34. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25979642>.

“BACKGROUND: Several experimental and clinical studies support beneficial effects of *Trigonella foenum-graecum* (fenugreek) in the management of metabolic diseases and inflammatory disorders. OBJECTIVES: The purpose of this study was to examine the effect of *T. foenum-graecum* seed extract in reducing the metabolic and inflammatory alternations associated with menopause. MATERIALS AND METHODS: In this experimental study, 49 rats were divided into seven groups: (I) sham-control, (II) ovariectomized-control, (III and IV) ovariectomized treated with 50 and 150 mg/kg of *T. foenum-graecum* seed ethanolic extract, (V and VI) ovariectomized treated with 50 and 150 mg/kg of *T. foenum-graecum* hexanic extract, (VII) ovariectomized-positive control treated with 10 µg/kg of estradiol. The extracts were injected intraperitoneally one day after ovariectomy and the treatments were lasted for 42 days. RESULTS: Fasting blood glucose and body weight gain increased significantly in the ovariectomized-control group compared with that in the sham animals ($P < 0.05$). Administration of estradiol and *T. foenum-graecum* (50 and 150 mg/dL of hexanic extract and 150 mg/kg of ethanolic extract) significantly diminished the increase in glucose and body weight ($P < 0.05$). The serum level of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) in the ovariectomized control group was significantly higher than those in the sham animals ($P < 0.05$). Both hexanic and ethanolic extracts as well as estradiol were able to decrease level of these cytokines in the serum of ovariectomized rats ($P < 0.05$). CONCLUSIONS: The results of the present study show that administration of *T. foenum-graecum* corrects metabolic and inflammatory alterations associated with ovariectomy and has a potential for the management of menopause.” As taken from Abedinzade M et al. 2015. Iran. Red Crescent Med. J. 17(11), e26685. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26732240>

“A study in mothers of preterm infants less than 31 weeks gestation compared the use of fenugreek 1725 mg 3 times daily for 21 days to placebo. No adverse effects were noted in the infants given the breastmilk.”

As taken from LactMed, 2021. Record for CAS RN 68990-15-8.

“BACKGROUND/OBJECTIVE: The purpose of the present study was to evaluate the effect of fenugreek seed extract in combination with swimming exercise compared to glibenclamide consumption on type 2 diabetic rats. DESIGN: The acute toxicity test was carried out to choose the safe doses and identify the toxicity effects of the fenugreek seed extract. To investigate the hypoglycemic effect of the extract and its effect in combination with swimming training, 80 Wistar Kyoto male streptozotocin-induced diabetic rats were divided randomly into eight groups: diabetic control (C); fenugreek seed extract 0.8 g/kg (F1); fenugreek extract 1.6 g/kg (F2); swimming training (S); swimming training plus fenugreek extract 0.8 g/kg (SF1); swimming training plus fenugreek extract 1.6 g/kg (SF2); glibenclamide (G) and swimming training plus glibenclamide (SG). The rats were orally administrated with the treatments once a day with the respective treatment, and the training groups were subjected to swimming training every day for 60 min. Fasting blood samples were collected to measure fasting blood glucose, lipid profile, adiponectin, leptin, and insulin concentrations. RESULTS: The results obtained from acute toxicity study showed no toxicity effect of fenugreek seed extract on the tested dose. Biochemical analysis showed significant improvements in all of the groups compared to the control group ($p < 0.05$). Plasma insulin concentration and insulin resistance (HOMA-IR) was significantly reduced in treated groups compared with the diabetic control group. Plasma leptin were significantly decreased in treated groups compared with the control group; while adiponectin had markedly increased ($p < 0.05$). CONCLUSION: The findings suggest that fenugreek seed consuming, alongside swimming

exercise, has a strong therapeutic effect on the improvement of diabetic parameters.” As taken from Arshadi S et al. 2015a. Food Nutr. Res. 59, 29717. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26699937>

“/EPIDEMIOLOGY STUDIES/ The safety and efficacy of Trigonella foenum-graecum extract was investigated using 20 male volunteers aged 20-30 years. They were randomly treated with either 40 mg/kg aqueous extract powder in 10 mL distilled water or 10 mL distilled water in which coffee simulated the extract. The extract significantly lowered blood glucose level by 13.4% 4 hours after ingestion. A significant change of 14.1% was observed in potassium levels. No significant alteration in serum cholesterol, total serum protein and blood urea occurred. Approximately one-third experienced feelings of hunger, frequency of micturition or dizziness during the 24 hours after ingestion. The aqueous extract effectively reduced blood glucose in normal subjects safely. Its hypokalemic effect merits further investigation. [Abdel-Barry JA et al; East Mediterr Health J 6 (1): 83-8 (2000)] **PEER REVIEWED**”

“/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ Fenugreek seeds (Trigonella foenum graecum L.) are assumed to have restorative and nutritive properties. The present work was designed to investigate the effects of a fenugreek seed extract on feeding behavior. Experiments were performed to determine food consumption and motivation to eat as well as metabolic-endocrine changes in chronically treated animals. Male Wistar rats were given the seed extract orally (10 and 100 mg/day per 300 g body weight), mixed together with food, and control animals were monitored in parallel. The results show that chronic oral administration of the fenugreek extract significantly increases food intake and the motivation to eat. The treatment, however, does not prevent the anorexia nor the decreased motivation to eat induced by d-fenfluramine (2 mg/kg, IP). An increase in plasma insulin and a decrease in total cholesterol and very low-density lipoprotein (VLDL)-low-density lipoprotein (LDL) total cholesterol were also observed. In conclusion, chronic administration of a fenugreek seed extract enhances food consumption and motivation to eat in rats and also induces hyperinsulinemia as well as hypocholesterolemia.[Petit P et al; Pharmacol Biochem Behav 45 (2): 369-74 (1993)] **PEER REVIEWED**”

As taken from HSDB, 2016

Record for Trigonella foenum-graecum Linn., seed, alcohol extract (no CAS RN given):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	230 gm/kg/115D (intermittent)	Endocrine - other changes Sense Organs and Special Senses (Eye) - effect, not otherwise specified	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 93,289,2004
TDLo - Lowest published toxic dose	Oral	Rodent - rat	3300 mg/kg/15D (intermittent)	Endocrine - hypoglycemia Endocrine - other changes	PYTOEY Phytomedicine. (Gustav Fischer Verlag, Postfach 720143, D-70577 Stuttgart, Germany) V.1- 1994- Volume(issue)/page/year: 10,665,2003
TDLo - Lowest published	Oral	Rodent mouse	252 gm/kg/18W	Endocrine - hypoglycemia Endocrine - other changes Biochemical	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub.

toxic dose			(intermittent)	Metabolism (Intermediary) - lipids including transport	Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 142,516,2012
TDLo - Lowest published toxic dose	Oral	Rodent mouse	14000 mg/kg/28D (intermittent)	Blood - changes in erythrocyte (RBC) count	RTOPDW Regulatory Toxicology and Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1-1981- Volume(issue)/page/year: 72,323,2015
TDLo - Lowest published toxic dose	Oral	Rodent mouse	28000 mg/kg/28D (intermittent)	Liver - hepatitis (hepatocellular necrosis), zonal Liver - changes in liver weight Kidney/Ureter/Bladder changes primarily in glomeruli	RTOPDW Regulatory Toxicology and Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1-1981- Volume(issue)/page/year: 72,323,2015
TDLo - Lowest published toxic dose	Oral	Rodent mouse	28000 mg/kg/28D (intermittent)	Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol) Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - transaminases Related to Chronic Data - death	RTOPDW Regulatory Toxicology and Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1-1981- Volume(issue)/page/year: 72,323,2015

As taken from RTECS, 2018b

“OBJECTIVE: To evaluate acute oral toxicity (AOT), subchronic (90-day repeated dose) toxicity, mutagenicity, and genotoxicity potential of IDM01, the botanical composition of 4-hydroxyisoleucine- and trigonelline-based standardized fenugreek (*Trigonella foenum-graecum* L) seed extract in laboratory rats. MATERIALS AND METHODS: The AOT and subchronic (90-day repeated dose) toxicity were evaluated using Sprague-Dawley rats as per the Organisation for Economic Co-operation and Development (OECD) guidelines No. 423 and No. 408, respectively. During the subchronic study, the effects on body weight, food and water consumption, organ weights with hematology, clinical biochemistry, and histology were studied. The mutagenicity and genotoxicity of IDM01 were evaluated by reverse mutation assay (Ames test, OECD guideline No. 471) and chromosome aberration test (OECD guideline No. 473), respectively. RESULTS: The IDM01 did not show mortality or treatment-related adverse signs during acute (limit dose of 2000 mg/kg) and subchronic (90-day repeated dose of 250, 500, and 1000 mg/kg with 28 days of recovery period) administration. The IDM01 showed oral median lethal dose (LD50) >2000 mg/kg during AOT study. The no-observed adverse effect level (NOAEL) of IDM01 was 500 mg/kg. IDM01 did not show mutagenicity up to a concentration of 5000 µg/plate during Ames test and did not induce structural chromosomal aberrations up to 50 mg/culture. CONCLUSIONS: IDM01 was found safe during preclinical acute and subchronic (90-day repeated dose) toxicity in rats without mutagenicity or genotoxicity. SUMMARY: Acute oral toxicity, subchronic (90-day) oral toxicity, mutagenicity and genotoxicity of IDM01 (4-hydroxyisoleucine- and trigonelline-based standardized fenugreek seed extract) was evaluated. The median lethal dose, LD50, of IDM01 was more than 2000 mg/kg of body weight in rats. No observed adverse effect level (NOAEL) of IDM01 was 500 mg/kg of body weight in rats. IDM01 was found safe during acute and subchronic oral toxicity

studies in rats without mutagenicity or genotoxicity potential.” As taken from Deshpande PO et al. 2017a. Pharmacognosy Res. 9(2), 138-150. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28539737>

“The present work is aimed at studying acute oral toxicity (AOT), subchronic oral toxicity, mutagenicity, and genotoxicity of furostanol glycosides-based standardized fenugreek seed extract (Fenu-FG) using the Organization for Economic Co-operation and Development (OECD) guidelines. The AOT and subchronic (90-day repeated dose) toxicity studies were performed on Wistar rats as per OECD 423 and OECD 408 guidelines, respectively. The mutagenicity (reverse mutation assay, Ames test) and genotoxicity (mammalian chromosome aberration test) were assessed in vitro using OECD 471 and OECD 473 guidelines, respectively. At an acute oral limit dose of 2,000 mg/kg, Fenu-FG did not show any mortality or treatment-related adverse signs. Ninety days of subchronic oral administration of Fenu-FG (250, 500, or 1,000 mg/kg) in rats did not induce any treatment-related significant changes with respect to body weight, hematology, blood biochemistry, urinalysis, gross pathology, or histopathology. The no-observed-adverse-effect-level of Fenu-FG was 1,000 mg/kg/day. Furthermore, Fenu-FG did not demonstrate mutagenic potential up to a concentration of 5,000 µg/plate (Ames test) and did not induce structural chromosome aberrations up to 2,000 µg/ml (in human lymphocyte cells in vitro). In conclusion, Fenu-FG was found safe during preclinical safety assessments.” As taken from Deshpande P et al. 2017b. J. Diet. Suppl. 14(5), 521-541. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28156165>

Record for *Trigonella foenum-graecum* Linn., leaf, water extract (no CAS RN given):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - rat	3200 mg/kg/30D (intermittent)	Vascular contraction (isolated tissues) - Vascular relaxation (isolated tissues) - Endocrine hypoglycemia -	INJPD2 Indian Journal of Pharmacology. (Dept. of Pharmacology, Baranas Hindu Univ., Varanasi 221 005, India) V.1- 1968(?) - Volume(issue)/page/year: 40,59,2008

As taken from RTECS, 2018a

“The present study investigated the safety of a saponin-rich standardized extract of fenugreek seeds (FenuSMART®; FHE), that has been clinically shown to be effective in ameliorating the postmenopausal discomforts and establishing hormonal balance. The safety was assessed by oral acute (2500 mg/kg b. wt. for 14 days) and subchronic (250, 500 and 1000 mg/kg b. wt. for 90 days) toxicity studies on Wistar rats and mutagenicity studies employing *Salmonella typhimurium* strains. Administration of FHE did not produce any toxicologically significant changes in clinical/behavioral observations, ophthalmic examinations, body weight, organ weight, feed consumption, urinalysis, hematology and clinical biochemistry parameters when compared to the untreated control group of animals. Highest dose recovery group (1000 mg/kg b. wt.) of animals also showed no mortality or adverse events; with hematological and biochemical parameters at par with those of controls. Terminal autopsy revealed no alterations in relative organ weight or any treatment-related histopathology changes. FHE also showed no mutagenicity upon Ames test employing TA-98, TA-100 and TA-102 *Salmonella typhimurium* strains with or without metabolic activation. Based on the results of the study, the no observed-adverse-effect level (NOAEL) of FHE was determined as

1000 mg/kg b. wt./day, the highest dose tested.” As taken from Sureshkumar D et al. 2018. Toxicol. Rep. 5, 1060-1068. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30416976>

5.3. *Reproduction toxicity*

“The research for an antifertility drug is not only a matter of great interest but of great need with uncontrolled population explosion. Many synthetic as well as indigenous drugs have been introduced but none of them is a completely satisfactory drug at present. Keeping this in view, 8 indigenous medicinal plants, *Trigonella foenum graecum*, -- were tested for antifertility activity in Charles Foster rats. Antifertility activity between 14% to 65.78% was observed. It was obvious that the plants did not show much high activity. Interestingly, in failure cases where pregnancy was not terminated inspite of feeding of plant extracts, several congenital anomalies were recorded in neonates. These were gross, visceral and skeletal anomalies including mouth flattened, inverted claw, everted claw, kinking of tail, shoulder joint defect, syndactyly, clubbing of limbs, cleft palate, liver and neural canal enlarged, nonossified skull bones, etc. In India, a large number of people are exposed to above mentioned plant extracts unknowingly. So it is very important that every practitioner should keep in mind the side effects of above plants, while prescribing them in one form or the other ...”. As taken from Sethi N. 1991. *Int. J. Toxic. Occup. Environ. Hlth* 1(1), 131.

“Fenugreek, maple syrup and the urine of maple syrup urine disease (MSUD) patients all share a characteristic odour originating from a common component, sotolone. Ingestion of fenugreek by mothers during labour resulted in a maple syrup-like odour in their newborn infants, leading to a false suspicion of MSUD.” As taken from Korman SH et al. *J Paediatr Child Health*. 2001 Aug; 37(4):403-4. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11532065&query_hl=31&itool=pubmed_docsum

“The objective of this study was to evaluate the potential antifertility activity of feeding diets containing 30% fenugreek seeds to male and female white New Zealand rabbits. RESULTS: The data presented in this study clearly demonstrate an antifertility effect of fenugreek seeds in the female rabbits and more of a toxicity effect in the male rabbits. In males, testis weight was reduced, with evident damage to the seminiferous tubules and interstitial tissues as shown by the histopathology of testis tissue sections. In addition, the plasma concentration of the androgen hormone and sperm concentrations were halved in the treated animals. In the case of the females, there was evidence of a significant reduction of developing fetuses as observed by reductions of both fetal and placental weights at 20 days of gestation and of the litter size. This was further supported histopathologically by the observed proliferative changes of the endometrial glands. The circulating plasma progesterone concentrations at 10 and 20 days of gestation significantly increased with no significant effect on the prebreeding estrogen concentrations in the treated animals.” As taken from Kassem A et al. *Contraception*. 2006 Mar; 73(3):301-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16472574&query_hl=34&itool=pubmed_DocSum

“Kamal et al, (1993) treated male rats with the steroidal fraction of fenugreek seed extract for 2 months. The sperm count and motility of treated animals were decreased. In addition, the weight of reproductive tissues and androgen-dependent parameters (protein, sialic acid and fructose) were lower, thus indicating reduced levels of circulating androgens. These findings were shown to have histological correlates (arrest of spermatogenesis, degeneration of seminiferous tubules and epididymis). Cholesterol levels were higher in treated vs. control animals in serum and testis so that the authors concluded that it may be co-related with its non-utilisation thus leading to decreased circulating androgen and altered testicular histoarchitecture. The functional consequence was a loss of fertility for 20/20 treated males. They conclude that the test-article exerts both anti-fertility and antiandrogenic activities” (EMA, 2011).

“Test system: Isolated uterus pieces (4 cm) from pregnant and non-pregnant guinea pig Test solution (2 ml from water or ethanol extract) or control (either water or ethanol) added to a bath containing duodenum pieces in oxygenated Dale’s solution. Uterine motility was recorded by means of a light lever on a smoked drum paper moving at slow speed. Water extract: stimulating effect on uterine contractility; the effect is markedly increased on tissues obtained from pregnant animals. Ethanol extract: same results as those obtained with water extract” (EMA, 2011).

“Sethi et al, (1990) administered fenugreek seed powder to rats during the first ten days of gestation at 175 mg/kg a day. The number of resorptions was increased. This is coherent with the results published by Elbetieha et al, (1996) and Adhikary (1990) with fenugreek seed extracts administered from the beginning up to the 6th or 10th day of pregnancy, respectively. In addition, some gross and visceral anomalies were reported in the study published by Sethi et al, (1990). The only negative study was conducted by Mital and Gopaldas (1986) by administration of up to 20% fenugreek seed powder in the diet of rats for the whole gestation period” (EMA, 2011).

“The use of medicinal plant products to treat various ailments is a common practice in many developing countries. However, a lack of information on the adverse effects of these plants raises questions on their safety and possible adverse side effects. This study was undertaken to evaluate the potential toxic effects of fenugreek seeds on pregnant mice and foetal development. MATERIALS AND METHODS: Lyophilized aqueous extract from fenugreek seeds (LAE-FS) was administered to mated female mice during the entire period of pregnancy, at doses of 500 and 1000 mg/kg/day. Females were examined for standard parameters of reproductive performance. Foetuses were weighed and examined for externally visible malformations. RESULTS: In pregnant females, there were no obvious symptoms of toxicity, LAE-FS-related deaths or macroscopic abnormalities. Developmental toxicity in offspring included an increase in the foetal death rate, a decrease in the litter size, and a reduction in the foetal body weight. In addition there was an increase in the incidence of morphological abnormalities. CONCLUSIONS: Based on these results, it was concluded that fenugreek seeds extract may have deleterious toxic effects on reproductive performance and potential teratogenic effects in foetuses”. As taken from Khalki L et al. 2010. J. Ethnopharmacol. 131, 321-325 PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/20600755?dopt=AbstractPlus>.

“Fenugreek (*Trigonella foenum graecum* (L.)), is a medicinal plant whose seeds and leaves are widely used in Moroccan traditional medicine. Consumption of fenugreek seeds during pregnancy has been associated with a range of congenital malformations, including hydrocephalus, anencephaly and spina bifida. In previous work we have shown that exposure of pregnant mice to aqueous extract of fenugreek seeds (AEFS) leads to reduced litter size, intrauterine growth retardation, and malformations. However, there have been no studies to date of its longer-term neurobehavioral effects. We investigated these effects in prenatally exposed mice. MATERIALS AND METHODS: Pregnant females were exposed to 0, 500 or 1000 mg/kg/day AEFS, by gavage, for the whole period of gestation. Pups body weight was measured at 1, 7, 14, 21 and 28 day of age. Behavior of progeny was evaluated three weeks after birth using the open field, the rotarod test and the continuous alternation task by the T-maze. At 28 postnatal day age, brain of progeny was removed and cut for histological evaluation. RESULTS: The progeny of exposed mice displayed reduced body weight at birth (1000 mg/kg group: 27%; 500 mg/kg group: 32%) and reduced brain weight (10% in both treated groups). Both males and females mice prenatally exposed to AEFS displayed a significant decrease in the locomotor activity, in the boli deposits during the open field test and in motor coordination. These results seem to show that exposure to AEFS induces a depressive effect in the offspring. Assessment on a continuous alternation T-maze test showed a significant reduction in successful spontaneous alternations in males and females but only in the 1000 mg/kg group. CONCLUSION: These results suggest that prenatal exposure of mice to high dose of fenugreek seeds causes growth retardation and altered neurobehavioral performance in the post-weaning period in both male and female”. As taken from Khalki L et al 2012. J. Ethnopharmacol. 139, 672-677 PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22178172?dopt=AbstractPlus>.

“Cyclophosphamide (CPA) is an anticancer drug used in the treatment of a variety of neoplastic lesions. On the other hand, treatment with CPA was accompanied by different toxic effects on different body organs. The present work was conducted to study the effect of fenugreek seed extract on histomorphometrical and ultrastructural changes induced by CPA in testes of albino mice. Twenty animals were given CPA (7.0 mg/kg body weight) three times/week orally for 8 weeks and were killed after 4 and 8 weeks. Testis of CPA-treated mice showed many histological alterations including appearance of irregular seminiferous tubules, reduction in the number of all spermatogenic cells, degeneration of Leydig cells and appearance of intertubular hemorrhage. Concerning the ultrastructural changes, abnormalities in spermatogonia (A and B), spermatocytes, round and elongated spermatids were observed. Degenerated Sertoli cells and degenerated interstitial tissue with abnormal Leydig cells were also seen. Moreover, administration of CPA to animals significantly increased malondialdehyde (MDA, lipid peroxidation marker) and decreased superoxide dismutase (SOD) and catalase (CAT). These changes were time-dependent. Treating animals with CPA and fenugreek seed extract (0.4 g/kg body weight) led to an improvement in the histological and ultrastructural pictures of the testis together with reduction in the level of serum MDA and increase in the activities of serum SOD and CAT. In conclusion, the results of the present work indicated that fenugreek had ameliorative effect against testis damage induced by CPA and this may be mediated by its potent antioxidant activities”. As taken from Sakr SA et al 2012a. *Reprod. Sci.* 19, 70-80. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22051850?dopt=AbstractPlus>.

“The present work studied the effect of fenugreek seed extracts on cytotoxicity and testicular damage induced by adriamycin (ADR) in albino rats. Administering animals with ADR caused significant increase in the percentage of chromosomal aberrations, decreased the mitotic index, and induced DNA damage in bone marrow. Testes of ADR-treated rats showed many histopathological alterations and the number of sperm head abnormalities increased. Moreover, the concentration of malondialdehyde (MDA) increased and the activity of catalase (CAT) and superoxide dismutase (SOD) decreased in the testis. Treating animals with ADR and aqueous seed extracts of fenugreek led to an improvement in the cytogenetic effect and testicular alterations induced by ADR. Lipid peroxidation was reduced and the activities of CAT and SOD were increased. In conclusion, the results indicated that fenugreek seeds ameliorated the cytotoxicity and testicular alterations induced by ADR in albino rats and this may be mediated by its potent antioxidant effects”. As taken from Sakr SA et al. 2012b. *Toxicol. Ind. Health.* 28(3), 276-88. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/21949087?dopt=AbstractPlus>.

“Polycystic ovary syndrome (PCOS) is one of the most prevalent hormonal disorders among women of reproductive age causing irregular menstrual cycles, excessive body or facial hair, miscarriage and infertility. The latter being a most common PCOS symptoms. Because the symptoms are seemingly unrelated to one another, PCOS is often overlooked and undiagnosed. The present study is an open label, one-arm, non-randomized, post-marketing surveillance study in 50 premenopausal women (18-45 years, BMI<42) diagnosed with PCOS using a novel *Trigonella foenum-graecum* seed extract (fenugreek seed extract, Furocyst, 2 capsules of 500 mg each/day) extract, enriched in approximately 40% furostanolic saponins, over a period of 90 consecutive days. The study was conducted to determine its efficacy on the reduction of ovarian volume and the number of ovarian cysts. Ethical committee approval was obtained for this study. Furocyst treatment caused significant reduction in ovary volume. Approximately 46% of study population showed reduction in cyst size, while 36% of subjects showed complete dissolution of cyst. It is important to mention that 71% of subjects reported the return of regular menstrual cycle on completion of the treatment and 12% of subjects subsequently became pregnant. Overall, 94% of patients benefitted from the regimen. Significant increases in luteinizing hormone (LH) and follicular stimulating hormone (FSH) levels were observed compared to the baseline values. Extensive blood chemistry, hematological and biochemical assays demonstrated the broad-spectrum safety. Furocyst caused significant decrease in both ovarian volume and the number of ovarian cysts. Serum ALT, BUN and CK were assessed to demonstrate the broad-spectrum safety of Furocyst. No

significant adverse effects were observed. In summary, Furocyst was efficacious in ameliorating the symptoms of PCOS.” As taken from Swaroop A et al. 2015. Int. J. Med. Sci. 12(10), 825-31. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26516311>

Record for *Trigonella foenum-graecum* L. (Papilionaceae), seed, water extract (no CAS RN):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Sex/Duration	Toxic Effects
TDLo Lowest published toxic dose	Oral	Rodent mouse	10500 mg/kg	female 1-21 day(s) after conception	Reproductive - Fertility - litter size (e.g. # fetuses per litter; measured before birth) Reproductive - Specific Developmental Abnormalities - Central Nervous System Reproductive - Effects on Newborn - other neonatal measures or effects
TDLo Lowest published toxic dose	Oral	Rodent mouse	21000 mg/kg	female 1-21 day(s) after conception	Reproductive - Fertility - female fertility index (e.g. # females pregnant per # sperm positive females; # females pregnant per # females mated) Reproductive - Specific Developmental Abnormalities - other developmental abnormalities Reproductive - Effects on Newborn - live birth index (measured after birth)
TDLo Lowest published toxic dose	Oral	Rodent mouse	10500 mg/kg	female 1-21 day(s) after conception	Reproductive - Effects on Newborn - growth statistics (e.g.%, reduced weight gain) Reproductive - Effects on Newborn - behavioral Reproductive - Effects on Newborn - other postnatal measures or effects

As taken from RTECS, 2014.

“Constipation appears in the 2nd and 3rd trimester of pregnancy, while nausea is the strongest in the 1st trimester. This review summarizes the applicability of herbal laxatives and antiemetics in pregnancy. A human study has shown that flax oil as laxative is safe from 2nd trimester. Human data is not available about the rhubarb, but animal studies reveal that its emodin content induces fetal abnormalities. Fenugreek induces teratogenic malformation both in human and animals. Senna seed is proved as a safe laxative in pregnancy. The antiemetic ginger is safe during 1st trimester, but it reduces the gestational period when applied from the 2nd trimester. Cannabis induces fetal neurological disorders while fennel can shorten the gestational age. There is herbal alternative for laxative therapy (senna) for the whole length of pregnancy, but nausea and vomiting might be reduced by herbal medicine (ginger) safely in the 1st trimester, only.” As taken from Samavati R et al. 2017. *Reprod. Toxicol.* 72, 153-158. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28610933>

“CONTEXT: Glycoside-based standardized fenugreek seed extract (SFSE-G) demonstrated promising efficacy in animal models of immune-inflammatory conditions. AIM: The present study was aimed at embryo-fetal development toxicity evaluation of SFSE-G in Wistar rats as per guideline No. 414 of the Organization for Economic Co-operation and Development (OECD). MATERIAL AND METHODS: Mated female rats were randomized into four groups of 30 each and received oral doses of either SFSE-G at 250, 500, and 1000 mg/kg or vehicle (water) during the period of gestation (postconception) from gestational day 5 (GD5, an implantation day) until 1 day before cesarean sections (GD19). Maternal food consumption, body weights, and clinical signs were monitored throughout gestation. Cesarean sections were performed on GD20 and fetal

observations (gravid uterine weight, implantation sites, early and late resorptions, live and dead fetuses) were recorded. Live fetuses were weighed and examined for external, visceral, and skeletal variations and malformations. RESULTS: None of the SFSE-G-treated groups showed maternal and embryo-fetal toxicity. Occasional and incidental skeletal and visceral malformations were observed and found to be spontaneous and unrelated to the treatment. CONCLUSION: Oral exposure of SFSE-G during the prenatal period did not show significant maternal and embryo-fetal toxicity up to a dose of 1000 mg/kg in rats. Therefore, the no-observed-adverse-effect level for SFSE-G for prenatal oral exposure was considered to be 1000 mg/kg. SUMMARY: Prenatal toxicity of glycoside-based standardized fenugreek seed extract (SFSE-G) was evaluated. SFSE-G was orally gavaged to rats on gestational days 5-19 with a limit dose of 1000 mg/kg. SFSE-G did not show maternal or developmental toxicity. SFSE-G showed NOAEL of 1000 mg/kg for prenatal exposure in female rats." As taken from Deshpande PO et al. 2017c. Pharmacogn. Mag. 13(Suppl. 1), S135-S141. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28479738>

"The seeds of fenugreek (*Trigonella foenum — graecum* L) are rich source of steroidal saponin. The steroidal fraction was extracted from fenugreek seeds by the modified method of Sarin et al. (1974) and was used for determination of its contraceptive efficacy in female rats. Normal cyclic female rats (*Rattus norvegicus*) were kept for mating with proven fertile male in the ratio of 2:1, next day in the morning the vaginal smear was checked for the presence of spermatozoa. The female rats with positive mating were divided into four groups. Group I-control received only vehicle and Group II and III treated with plant extract (50mg and 100mg) for 12 days respectively and Group IV, treated with phytodrug (100mg) and ascorbic acid (100 mg) for 12 days. The data revealed that lower dose (50 mg) of phytodrug resulted in reduction of total embryo implantation and increased in number of reabsorbed embryo as compared to control; thereby inhibit fertility upto 50%. However, higher dose (100 mg) of seed extract showed no implantation site and reabsorbed embryo in all the rats studied, revealing 100% negative fertility in female rats studied. Ascorbic acid is known for its antioxidant properties and it was given orally to female rats along with phytodrug. The observations revealed a negative impact of it i.e. the fertility rate remained 100% negative. The data suggests that steroidal fraction of fenugreek exhibited remarkable anti-implantation and early abortifacient activities in female rats leading to negative fertility. So the phytodrug can act as a potent post-coital emergency contraceptive agent in females." As taken from Anjula B and Sharma JD. 2018. Global Journal of Advanced Research 5(3), 70-75. Available at <http://gjar.org/publishpaper/vol5issue3/d803r74.pdf>

"In our extensive review of the toxicological properties of fenugreek, we provided a lot of information about teratogenic, reproductive, neurodevelopmental, neurobehavioral and neuropathological abnormalities associated with fenugreek use in prospective and animal model studies [128]. In fact, many teratogenic effects of fenugreek, such as major malformations [129], growth alterations, functional developmental deficits [130], and significant reduction of total and live implants per pregnant female animal models [131], were largely reported. In addition, the developing nervous system appears to be particularly susceptible to fenugreek toxicity as reported by retrospective and animal model studies [132–134]. Otherwise, the anti-fertility effects of fenugreek in rats, mice, and rabbits, as well as an anti-implantation and abortifacient activity related to saponin compound of fenugreek, have been demonstrated in long-term daily use [128,135]. However, low to moderate doses of fenugreek have been reported to be safe for the nervous system [136] without any sign of toxicity over several weeks use in feeding studies [137]. Moreover, no evidence of mutagenicity or genotoxic activity of fenugreek was reported." As taken from El Bairy K et al. 2017. Biomedicine and Pharmacotherapy. 90: 479-491. Pubmed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/?term=28391170>

"Different types of glycosides extract of fenugreek have shown androgenic and anabolic effect in male. The aim of the study was to evaluate the effect of fenugreek extract on total testosterone levels in male. Medline via PubMed, Scopus databases, Cochrane Library, Web of Science, and Google Scholar were searched up to November 2018 for randomized clinical trials comparing intake of fenugreek extract with control group. Data on change in serum total testosterone were

pooled using random-effects models. A total of four trials were included. Fenugreek extract has a significant effect on total serum testosterone. Results from clinical trials suggest that fenugreek extract supplement has an effect on serum total testosterone levels in male.” As taken from Mansoori A et al. 2020. *Phytother. Res.* 34(7), 1550-1555. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32048383/>

“Objectives: Estradiol (E2) plays an important role in the pathophysiology of ovarian hyperstimulation syndrome (OHSS). This study aimed to evaluate the effect of fenugreek seed aqueous (FSA) extract on serum E2 levels in a rat model of OHSS. Methods: A total of 34 female Sprague Dawley rats, aged 18 days old, weighing 40 to 45 g, were randomly divided into negative control, positive control, and treatment groups. A daily dose of 1500 mg/kg per body weight of FSA extract was administered orally to rats in the treatment group for 13 days. On day eight of the study, OHSS was induced in both positive control and treated groups by subcutaneous injection of pregnant mare's serum gonadotropin 50 IU for four consecutive days, followed by human chorionic gonadotropin 25 IU on the fifth day. The effect of FSA extract was evaluated by measuring the concentration of serum E2 using the enzyme-linked immunosorbent assay. Results: FSA extract reduced serum E2 level significantly in the treated OHSS model (p -value < 0.050) compared to the positive control group. Conclusions: The finding has important implications on the development of female infertility adjuvant drugs for safe assisted reproduction technology cycles in terms of OHSS prevention.” As taken from Hameid ASB et al. 2019. *Oman Med. J.* 34(3), 238–243. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31110632/>

“Background: Reports in the published literature indicate that fenugreek seeds possess significant medicinal effects. The present review showed that fenugreek (*Trigonella Foenum-graecum* L) seeds contain rich number of steroids and have an estrogen and progestogen like effect. Objective: Objective of this review was to evaluate the effects of fenugreek seeds on rat's reproductive system. Conclusion: This review revealed that that fenugreek seeds contain estrogen like action.” As taken from ElSawy NA. 2019. *Asian Journal of Research and Reports in Urology* 2(1), 1-11.

“The aim of this trial was to evaluate the effect of a standardised *Trigonella foenum-graecum* (Fenugreek) extract on the symptoms of benign prostate hyperplasia (BPH) using a double-blind randomised placebo controlled design. The study recruited 100 healthy males aged between 45 and 80 years with symptoms of BPH who recorded a minimum score of eight on the International Prostate Symptom Score. Participants were randomised to an oral dose of either 600mg *Trigonella foenum-graecum* per day or placebo for 12 weeks. The primary outcome measure was the International Prostate Symptom Score total and subdomain scores. The secondary outcomes were serum levels of the hormones (testosterone, free testosterone, and sex hormone binding globulin) prostate-specific antigen, and safety markers. The results indicated that *Trigonella foenum-graecum* did not have an effect on improving the symptoms of BPH. Hormone levels, safety markers, and prostate-specific antigen remained unchanged and within normal limits after 12 weeks, which adds to the safety profile of this specialised extract.” As taken from Rao A and Grant R. 2019. *Phytother. Res.* 34(3), 634-639. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31828857/>

“Aims: The present study was undertaken to assess the antispermatogenic and antifertility efficacy of ethanolic extract of *Trigonella foenum-graecum* seeds. Methodology: Aqueous solution of the extract (250 mg/kg b.wt/day) when administered orally for 45 days to adult male albino rat (Duckray strain). Results: Seeds extract caused inhibition of spermatogenesis as well as inability to mate with normal untreated female rats of proven fertility. There occurred a significant decrease in absolute and relative weights of testis and seminal vesicle and increase in epididymal weight, whereas of ventral prostate and coagulating gland remained unchanged. Sperm concentration and motility in the cauda epididymis was also decreased. Increased incidence of separation of head and tail pieces of spermatozoa was seen in the epididymal smear. Histologically, testis of experimental animals showed arrest of spermatogenesis at the secondary spermatocytes stage and there was deposition of cellular debris in seminiferous tubular lumen. The spermatids were not

properly developed and interstitial cells were very sparse and degenerated. However, there was no evidence of damage to the spermatogonia. Oral treatment of extract did not affect the body weight of animals. Quantitative estimation of marker testicular enzymes, e.g., sorbitol dehydrogenase showed a significant decrease whereas lactate dehydrogenase and gamma-glutamyl transpeptidase levels were significantly increased. In conclusion: These results suggest that *T. foenum-graecum*, may induce male infertility in rats, therefore, should be considered further as a potential male antifertility agent.” As taken from Singh M and Verma GN. 2019. *Journal of Complementary and Alternative Medical Research* 8(3), 1-8.

“Bisphenol A (BPA), an organic synthetic compound and endocrine disruptor, which majorly cause deleterious effects on male reproductive system. Fenugreek (*Trigonella foenum-graecum*), associated with Leguminosae family is used as a herbal medicine with potent antioxidant properties. The present study was aimed to scrutinise the preventative role of fenugreek seeds aqueous extract (FSEt) on BPA-induced testicular damage in mice. Study included four different groups of male Balb/c mice: control (C), fenugreek (FSEt), bisphenol A (BPA) and fenugreek + bisphenol A (FSEt + BPA). After two months of treatment, assessment of sperm parameters, antioxidant defence system, histopathological studies, germ cell count and gene expression of intrinsic apoptotic pathway were carried out. Administration of FSEt improved the damage caused by BPA as indicated by improved sperm parameters. FSEt-administered mice showed improvement in the histoarchitecture compared with BPA-administered animals. In addition, fenugreek treatment showed reduced levels of malondialdehyde and elevated levels of antioxidant enzymes. Expression studies of apoptotic markers revealed a significant decrease in the expression of Bcl-2 and significant increase in caspase-9 and caspase-3. However, FSEt restored the deleterious effects caused by BPA. The current findings plausibly might have promising protective role against BPA-induced testicular damage.” As taken from Kaur S and Sadwal S. 2019. *Andrologia* 52(2), e13492. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31793690/>

“Background: Neural tube defects (NTDs) are a group of birth defects that result from a partial or complete failure of the neural tube to close during embryogenesis. Their prevalence varies between 0.5 to 2 per 1000 births in countries without folic acid supplementation. The aim of our study is to assess the NTDs incidence and describe the risk factors within Moroccan newborn infants. Method: This is a descriptive study over a period of 5 and a half years including all births at "Les Orangers" Maternity and Reproductive Health Hospital of Rabat with notification of NTD cases, whether isolated or combined with other anomalies. Data were reported on pre-established sheets and on the teratovigilance registry. Statistical analysis was performed with SPSS version 18 statistical software. Results: During the study period, 43,923 births were recorded including 44 cases of neural tube defects, an incidence rate of 1 per 1000 births, with a female predominance; sex ratio = 0.8. These defects included anencephaly (50%), spina bifida (38.6%) and encephalocele (11.4%). The risk factors detected during this study include consanguinity (34%), consumption of fenugreek or other plants (36%), diabetes (4.5%) and medication (2.2%). A family history of malformation was reported in 6.8% of cases and among siblings in 4.5% of cases. The average maternal age was 30.38 ± 6.88 and the average gestational age was 36.80 ± 5.11 . A quarter of mothers did not benefit from any medical monitoring during pregnancy while 59% did not take folic acid supplementation during the first trimester of pregnancy and none of them took B9 vitamin during the periconceptional period. The antenatal diagnosis was performed in 63% of cases. The mortality rate was 3.8 per 10,000 and 16% of cases evolved positively. Conclusion: NTDs require high intensity and multidisciplinary care which stresses the importance, in our context, of strengthening and optimizing acid folic supplementation strategies during the periconceptional period.” As taken from Forci K et al. 2021. *BMC Pediatr.* 21(1), 124. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33722213/>

“The popularity of natural medicine is growing worldwide. Unlike conventional licensed medicines, herbal medicine practices are usually not supported by effectiveness, efficacy, or safety studies, which raise concerns about potential risks involved in their usage, particularly in high-risk patients such as pregnant women where teratogenicity is a concern. Despite a lack of science-based

evidence, the use of herbal products for the management of pregnancy-associated challenges is common, due to the common notion that they are free of toxic effects and adverse reactions because they are "natural." The lack of concern about utilizing herbal remedies during pregnancy is strengthened by the lack of regulation in most countries for their marketing. However, plant-based remedies are not free of adverse reactions. Medicinal plants and herbal remedies contain substances that can be toxic to the human body and the fetus. Potential effects of indiscriminate use of medicinal plants are embryotoxicity, teratogenic, and abortifacient effects. Some plant constituents can cross the placenta and reach the fetus. Phytochemicals and their metabolites are known to induce stimulation of uterine contraction and hormone imbalance that could result in abortion. The alterations to the hormonal profile can affect conception, induce teratogenic activity, and halt the pregnancy or produce a congenital malformation. Due to the wide range of modes of action of phytochemicals, some medicinal plants may be safe to use during certain trimesters of pregnancy and harmful at other stages. This manuscript reviews available scientific information concerning potential health hazards associated with the consumption of herbal medicines during pregnancy, highlighting those herbs that should be avoided due to their potential abortifacient and/or teratogenic activity. We focused on plants that were tested by preclinical studies, and studies of these plants are summarized. Common therapeutic use of these herbs, estimated effects, toxicological effects, and animal studies of these plants is summarized. The literature reviewed suggests that consumption of the following medicinal plants should be avoided during pregnancy: *Abrus precatorius*, *Achyranthes aspera*, *Ailanthus excelsa*, *Aloe vera*, *Aristolochia indica*, *Areca catechu*, *Bambusa vulgaris*, *Cassia occidentalis*, *Cicer arietinum*, *Cimicifuga racemosa*, *Dolichandrone falcata*, *Ginkgo biloba*, *Hydrastis canadensis*, *Indigofera trifoliata*, *Lavandula latifolia*, *Maytenus ilicifolia*, *Momordica cymbalaria*, *Moringa oleifera*, *Musa rosacea*, *Oxalis corniculata*, *Phytolacca dodecandra*, *Plumeria rubra*, *Ricinus communis*, *Ruta graveolens*, *Stachys lavandulifolia*, *Senna alata*, *Trigonella foenum-graecum*, *Vitis agnus-castus*, and *Valeriana officinalis*." As taken from Bernstein N et al. 2021. *Phytother. Res.* Epub ahead of print. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33164294/>

"The *Trigonella foenum-graecum* L. seeds, in a dormant or sprouted state, have been largely investigated for their therapeutic activities such as being antidiabetic, antioxidant, cholesterol-lowering, and as a digestive enhancer too. Nevertheless, there are no studies evaluating the potential developmental toxicity of germinated grains despite the availability of numerous research studies demonstrating the teratogenicity effect of ungerminated seeds. Therefore, this research work was conducted to assess the impact of fenugreek sprouts on maternal and neurobehavioral developmental toxicities on mice. The lyophilized aqueous extract of germinated seeds was administered via oral gavage on a daily basis to five groups of mated female mice throughout pregnancy at doses of 200, 500, 800, and 1000 mg/kg/day and the control group was administered distilled water. Maternal reproductive toxicity was evaluated, and the surviving pups were assessed for their physical development, malformation, and neurobehavioral toxicity by using a battery of tests from birth to the 25th postnatal day. Additionally, the aqueous extract of germinated and ungerminated seeds was analyzed by high-performance liquid chromatography (HPLC) for a comparison of their major compounds. For pregnant treated female mice, no death and no intoxication symptoms have been registered during the test. However, the sprouts' extract has provoked a significant decrease in fertility, spontaneous abortion, pup's mortality, and neurobehavioral disorder in offspring. HPLC analysis reveals an increase in total phenolic compound concentration by the process of sprouting." As taken from Oufquir S et al. 2020. *Evid. Based Complement. Alternat. Med.* 2020, 1615794. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32328121/>

Traditional use: There are no or limited data from use during pregnancy and lactation. Studies in animals have shown reproductive toxicity including male and female fertility (see section 5.3 'Preclinical safety data'). The use during pregnancy and lactation and in women of childbearing potential not using contraception is not recommended.

Adequate studies on reproductive toxicity have not been performed. However, testicular toxicity and decreased fertility have been reported in studies with high doses of fenugreek seed in mice, rats, and rabbits. In studies in rats and mice with high doses of fenugreek seed, embryo resorption, foetal death, growth retardation, malformations, and altered neurobehavioral performance have been reported.

As taken from EMA, 2021.

“Fenugreek seed exhibits antidiabetic, antineoplastic, hepatoprotective, antidepressant and immunomodulatory properties. Fenugreek also causes antifertility effects in rodents. However, the impact of fenugreek seed on male reproduction and the possible mode of its action are not properly evaluated. Herein, we examined the effect of aqueous seed extract of fenugreek (FSE) and the possible mechanism of its action on male reproductive health in mice. Parkes mice were orally administered FSE (600 mg/kg body weight/day) or distilled water for 28 and 56 days, respectively. Various sperm parameters, histopathology, serum testosterone level and fertility indices were assessed. Furthermore, steroidogenic enzymes activities, oxidative status and germ cell dynamics in the testis were evaluated. Toxicological endpoints were also assessed. Treatment with FSE caused degenerative changes in the testis histoarchitecture. The treatment also affected various sperm parameters and concentrations of sialic acid and fructose in the epididymis and seminal vesicle, respectively. Fenugreek treatment also had negative impact on oxidative status and germ cell dynamics in the testis; fertility indices were also affected in female mice impregnated by the extract-treated male mice, though libido of the treated male mice remained unaffected. Results show that treatment with FSE caused adverse effects on the male reproductive health and pregnancy outcome in Parkes mice.”

Singh, A., et al. (2022). Effect of *Trigonella foenum-graecum* L. seed extract on the reproductive system of male mice and possible mechanism of its action on spermatogenesis.

5.4. Mutagenicity

“Fenugreek seeds have been used in traditional medicines as a remedy for diabetes. Rich in protein, fenugreek seeds contain the unique major free amino acid 4-hydroxyisoleucine (4-OH-Ile), which has been characterized as one of the active ingredients for blood glucose control. Current use of fenugreek in foodstuff has been limited to its role as a flavoring agent, and not as an ingredient to help mitigate the blood glucose response for people with diabetes. As part of a safety evaluation of novel ingredients for use in blood glucose control, the potential genotoxicity of a fenugreek seed extract (THL), containing a minimum of 40% 4-OH-Ile, was evaluated using the standard battery of tests (reverse mutation assay; mouse lymphoma forward mutation assay; mouse micronucleus assay) recommended by US Food and Drug Administration (FDA) for food ingredients. 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. A wide safety margin is established, as anticipated doses are small compared to the doses administered in the assays.” As taken from Flammang AM et al. *Food Chem Toxicol.* 2004 Nov; 42(11):1769-75. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15350674&query_hl=34&itool=pubmed_DocSum

“Safety and anti-diabetic efficacy of a novel, proprietary *Trigonella foenum-graecum* seed extract [novel fenugreek extract (FE), Fenfuro™, CR0010810) enriched in furostanolic saponins (>60% w/w, HPLC) were assessed. Concerning safety, we undertook studies dealing with acute oral toxicity, 28-d sub-chronic toxicity and Ames' bacterial reverse mutation assay that revealed no toxicity. Concerning efficacy, we examined beneficial effects of the extract on rats with type 2 diabetes (T2D). Male Sprague-Dawley rats received a high-fat diet for 2 weeks followed by streptozotocin (STZ, 35 mg/kg i.p.) to produce T2D. Seven days post-STZ, rats showing ≥ 300 mg/dl fasting plasma glucose level (PGL) were included in the study. FE (150- or 450- mg/kg p.o.) and

glipizide (5 mg/kg p.o.) were administered once daily for 20 d and then twice daily for another 10 d (total 30 d). Blood samples were collected at 0, 10, 20 and 30 d of treatment and estimated for fasting plasma triglyceride (PTG), total cholesterol and insulin levels. After 30 d, FE and glipizide-treated diabetic animals were treated in combination with or without metformin (100 mg/kg) twice daily for another 10 d. FE did not influence body weight, feed and water intake. FE (150 mg/kg p.o.) reduced PTG levels in T2D rats by 22%, 24.6% and 29% at 10, 20 and 30 d of treatment, respectively, while glipizide (5 mg/kg p.o.) reduced the PTG levels by 57.4%, 46.2% and 39.4% at these time points. FE (450 mg/kg) treatment in STZ-induced diabetic rats produced significant hypoglycemic activity (approximately 31.5%) as compared to insulin (48.2% with 1 U/kg i.p.). FE (150 mg/kg p.o.) and metformin (100 mg/kg p.o.) combined produced significant reduction (20.7%) of PGL in T2D rats. No adverse effects were observed. We conclude after extensive in vitro and in vivo safety and efficacy studies that FE is safe and effective in treating T2D.” As taken from Swaroop A et al. 2014. Toxicol. Mech. Methods 24(7), 495-503. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25045923>

“BACKGROUND: The plant *Trigonella foenum-graecum* (TFG) is used as antidiabetic and diuretic. In order to ascertain antioxidant potential of leaf (early and mature) and seed of TFG, total phenolics, free radical scavenging assay, superoxide anion radical scavenging activity, reducing power, lipid peroxidation, ferric thiocyanate assay, hydroxyl radical scavenging activity and DNA damage protective activities were determined. The study was further carried out to assay the antimicrobial activity and HPLC analysis of plant parts. RESULTS: Ethanol extracts of leaf (early and mature) exhibited a high content of phenolics (54.79 and 41.28 g kg⁻¹) GAE) when it was compared with seed extract (23.85 g kg⁻¹) GAE). Results showed that mature TFG leaf extract had the lowest IC₅₀ for the free radical scavenging assay (IC₅₀ = 2.23 mg mL⁻¹), superoxide anion radical scavenging activity (IC₅₀ = 2.71 mg mL⁻¹), hydroxyl radical scavenging activity (IC₅₀ = 17.30 mg mL⁻¹) and highest reducing power (10.14 ascorbic acid equivalents mL⁻¹). However, the ethanol seed extract showed the maximum inhibition of lipid peroxidation and the ferric thiocyanate assay. Mature leaf also showed the maximum DNA damage protection activity and higher concentration of phytochemicals. CONCLUSION: The results showed that the mature TFG leaf had a higher antioxidant activity, which may be due to the presence of total phenolics. It may be used in herbal drugs or as a nutritional supplement.” As taken from Singh P et al. 2014. J. Sci. Food Agric. 94(12), 2497-504. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24464686>

In vivo					
Species	Test conditions	Endpoint	Result	Reference	
Mouse, strain Crl:CD-1(ICR)BR (5 males/group)	Mice were given fenugreek extract at 0, 500, 1000, or 2000 mg/kg bw/day for 3 days by gavage. Bone marrow was harvested 24 hr after the last dose and examined for micronuclei in polychromatic erythrocytes. [Good quality study carried out to ICH and FDA guidelines]	Chromosome damage	-ve	Flammang et al. 2004	
In vitro					
Test system	Test conditions	Endpoint	Activation	Result	References
Salmonella typhimurium strains TA98, TA100, and TA1535	Standard Ames test on fenugreek extract at up to 5 mg/plate. [Good quality study carried out	Mutation	With and without S9	-ve	Flammang et al. 2004

TA1537, and Escherichia coli, strain WP2uvrA	according to ICH and FDA guidelines]				
Salmonella typhimurium strains TA98 and TA100	Limited data presented but apparently aqueous and chloroform-methanol extracts of fenugreek were tested, at 10-100 mg herb/plate. Limited study, current protocols recommend testing in at least 4 strains.	Mutation	With S9	-ve	Rockwell & Raw, 1978
Mouse lymphoma, L5178Y cells	Cells exposed to fenugreek extract at concentrations of up to 4 mg/ml for 4 hr, before allowing expression time and selection of mutants. [Good quality study carried out according to ICH and FDA guidelines]	Mutation	With and without S9	-ve	Flammang et al. 2004
Bacillus subtilis strains H17(rec+) and M45(rec-)	Differential killing assays on fenugreek oil, using plate method (up to 30 ul/plate) or liquid method (up to 100 ul/ml)	DNA damage (indicative test)	Without	No result (toxicity was not obtained in either strain)	Kuroda et al. 1989
[+ve, positive; -ve, negative; ?, equivocal; with, with metabolic activation; without, without metabolic activation]					

Record for *Trigonella foenum-graecum* L. (Papilionaceae), seed, water extract (no CAS RN):

Type of Test	Route of Exposure or Administration	Species/ Test System	Dose Data	Reference
Cytogenetic analysis		Rodent - rat Liver	80.4 mg/L/3H	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 112,199,2007

As taken from RTECS, 2014.

“OBJECTIVE: To evaluate acute oral toxicity (AOT), subchronic (90-day repeated dose) toxicity, mutagenicity, and genotoxicity potential of IDM01, the botanical composition of 4-hydroxyisoleucine- and trigonelline-based standardized fenugreek (*Trigonella foenum-graecum* L) seed extract in laboratory rats. MATERIALS AND METHODS: The AOT and subchronic (90-day repeated dose) toxicity were evaluated using Sprague-Dawley rats as per the Organisation for Economic Co-operation and Development (OECD) guidelines No. 423 and No. 408, respectively. During the subchronic study, the effects on body weight, food and water consumption, organ weights with hematology, clinical biochemistry, and histology were studied. The mutagenicity and genotoxicity of IDM01 were evaluated by reverse mutation assay (Ames test, OECD guideline No.

471) and chromosome aberration test (OECD guideline No. 473), respectively. RESULTS: The IDM01 did not show mortality or treatment-related adverse signs during acute (limit dose of 2000 mg/kg) and subchronic (90-day repeated dose of 250, 500, and 1000 mg/kg with 28 days of recovery period) administration. The IDM01 showed oral median lethal dose (LD50) >2000 mg/kg during AOT study. The no-observed adverse effect level (NOAEL) of IDM01 was 500 mg/kg. IDM01 did not show mutagenicity up to a concentration of 5000 µg/plate during Ames test and did not induce structural chromosomal aberrations up to 50 mg/culture. CONCLUSIONS: IDM01 was found safe during preclinical acute and subchronic (90-day repeated dose) toxicity in rats without mutagenicity or genotoxicity. SUMMARY: Acute oral toxicity, subchronic (90-day) oral toxicity, mutagenicity and genotoxicity of IDM01 (4-hydroxyisoleucine- and trigonelline-based standardized fenugreek seed extract) was evaluated. The median lethal dose, LD50, of IDM01 was more than 2000 mg/kg of body weight in rats. No observed adverse effect level (NOAEL) of IDM01 was 500 mg/kg of body weight in rats. IDM01 was found safe during acute and subchronic oral toxicity studies in rats without mutagenicity or genotoxicity potential." As taken from Deshpande PO et al. 2017a. *Pharmacognosy Res.* 9(2), 138-150. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28539737>

"The present work is aimed at studying acute oral toxicity (AOT), subchronic oral toxicity, mutagenicity, and genotoxicity of furostanol glycosides-based standardized fenugreek seed extract (Fenu-FG) using the Organization for Economic Co-operation and Development (OECD) guidelines. The AOT and subchronic (90-day repeated dose) toxicity studies were performed on Wistar rats as per OECD 423 and OECD 408 guidelines, respectively. The mutagenicity (reverse mutation assay, Ames test) and genotoxicity (mammalian chromosome aberration test) were assessed in vitro using OECD 471 and OECD 473 guidelines, respectively. At an acute oral limit dose of 2,000 mg/kg, Fenu-FG did not show any mortality or treatment-related adverse signs. Ninety days of subchronic oral administration of Fenu-FG (250, 500, or 1,000 mg/kg) in rats did not induce any treatment-related significant changes with respect to body weight, hematology, blood biochemistry, urinalysis, gross pathology, or histopathology. The no-observed-adverse-effect-level of Fenu-FG was 1,000 mg/kg/day. Furthermore, Fenu-FG did not demonstrate mutagenic potential up to a concentration of 5,000 µg/plate (Ames test) and did not induce structural chromosome aberrations up to 2,000 µg/ml (in human lymphocyte cells in vitro). In conclusion, Fenu-FG was found safe during preclinical safety assessments." As taken from Deshpande P et al. 2017b. *J. Diet. Suppl.* 14(5), 521-541. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28156165>

"In our extensive review of the toxicological properties of fenugreek, we provided a lot of information about teratogenic, reproductive, neurodevelopmental, neurobehavioral and neuropathological abnormalities associated with fenugreek use in prospective and animal model studies [128]. In fact, many teratogenic effects of fenugreek, such as major malformations [129], growth alterations, functional developmental deficits [130], and significant reduction of total and live implants per pregnant female animal models [131], were largely reported. In addition, the developing nervous system appears to be particularly susceptible to fenugreek toxicity as reported by retrospective and animal model studies [132–134]. Otherwise, the anti-fertility effects of fenugreek in rats, mice, and rabbits, as well as an anti-implantation and abortifacient activity related to saponin compound of fenugreek, have been demonstrated in long-term daily use [128,135]. However, low to moderate doses of fenugreek have been reported to be safe for the nervous system [136] without any sign of toxicity over several weeks use in feeding studies [137]. Moreover, no evidence of mutagenicity or genotoxic activity of fenugreek was reported." As taken from El Bairi K et al. 2017. *Biomedicine and Pharmacotherapy.* 90: 479-491. Pubmed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/?term=28391170>

5.5. Cytotoxicity

"The protective effect of a polyphenolic extract of fenugreek seeds (FPEt) against ethanol (EtOH)-induced toxicity was investigated in human Chang liver cells. Cells were incubated with either 30 mM EtOH alone or together in the presence of seed extract for 24 h. Assays were performed in treated cells to evaluate the ability of seeds to prevent the toxic effects of EtOH. EtOH treatment suppressed the growth of Chang liver cells and induced cytotoxicity, oxygen radical formation and mitochondrial dysfunction. Reduced glutathione (GSH) concentration was decreased significantly ($P < 0.05$) while oxidized glutathione (GSSG) concentration was significantly elevated in EtOH-treated cells as compared with normal cells. Incubation of FPEt along with EtOH significantly increased cell viability in a dose-dependent manner, caused a reduction in lactate dehydrogenase leakage and normalized GSH/GSSG ratio. The extract dose-dependently reduced thiobarbituric acid reactive substances formation. Apoptosis was observed in EtOH-treated cells while FPEt reduced apoptosis by decreasing the accumulation of sub-G1 phase cells. The cytoprotective effects of FPEt were comparable with those of a positive control silymarin, a known hepatoprotective agent. The findings suggest that the polyphenolic compounds of fenugreek seeds can be considered cytoprotective during EtOH-induced liver damage." As taken from Kaviarasan S. et al. 2006. Alcohol Alcohol 41(3), 267-73. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/16574673>

Toxicity of Trigonella foenum graecum (Fenugreek) in bone marrow cell proliferation in rat (Abstract). Fenugreek has a wide range of medical applications and its medicinal use has been clear in several studies, however, few studies are available on effects on haematopoietic stem cell of bone marrow. The goal of the present study was to investigate the effect of Fenugreek on fetal macroscopic diameters and microscopic bone marrow cell histological changes in its teratogenic dosages. Fenugreek decoction was dissolved in 1.5 milliliter distilled water and injected intraperitoneumly in three dosages of 0.8 g/kg, 1.6 g/kg, and 3.2 g/kg for three groups of Wistar female rats mated by Wistar male. For another group (as control group) only 1.5 milliliter distilled water was injected. Bone marrow tissue was prepared from rat fetus and was cut using a microtome and stained with hematoxylin and eosin. Sections were evaluated for changes using light microscope. LD(50) for the measurement of teratogenic dosage of fenugreek was 4.1 and 3.5 g/kg in female and male rat, respectively. There was a positive relation between the injected drug dosage and fetal mortality rate. Among all fetal diameters, ear to ear diameter was decreased in groups received Fenugreek decoction. The severity of stem cell histological changes caused by 3.2 g/kg drug injection was lower than distilled water injection and in evaluation of other cells, differences in the severity of histological changes across three groups with different drug dosages and control group was detected. Fenugreek in teratogenic dosages can decrease the severity of bone marrow cell proliferation and increase fetal mortality rate. As taken from Araee et al.; Pak J Pharm Sci. 2009, Apr; 22(2):126-30. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/19339220> (CAS 68990-15-8)

Effects of aqueous extracts of medicinal plants on MNNG-treated rat hepatocytes in primary cultures (Abstract). Aqueous extracts of *Nigella sativa* (Ranunculaceae) (Ns), *Teucrium polium* (Labiatae) (Tp) and *Trigonella foenum-graecum* (Fabaceae) (Tf) have been traditionally used to treat inflammations, liver disorders, and arthritis. Experimentally, it has been demonstrated that these herbs possess antioxidant, anti-inflammatory and hepatoprotective properties. To evaluate their in vitro toxicological properties and potential antimutagenic effects aqueous extracts of the three plants were tested in primary rat hepatocyte cultures against N-methyl-N'-nitro-N-nitrosoguanidine. The extracts were applied before, during and after application of MNNG to discriminate between different mechanisms of action. Tp itself significantly increased apoptosis, but in the combined treatment with MNNG significantly reduced it. Post-treatment with Ns or combined treatment with Tf significantly reduced the percentages of necrotic cells. The three plant extracts themselves significantly increased the frequency of chromosomal aberrations. Summarizing, our results suggest that aqueous extracts of the three herbs have neither cytoprotective nor antimutagenic activity, instead there is evidence for a mutagenic potential. As taken from Khader M

et al.; J Ethnopharmacol. 2007, May 30; 112(1):199-202. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/17324542>(CAS 68990-15-8)

“Protodioscin (PD) was purified from fenugreek (*Trigonella foenumgraecum* L.) and identified by Mass, and ¹H- and ¹³C-NMR. The effects of PD on cell viability in human leukemia HL-60 and human stomach cancer KATO III cells were investigated. PD displayed strong growth inhibitory effect against HL-60 cells, but weak growth inhibitory effect on KATO III cells. Morphological change showing apoptotic bodies was observed in the HL-60 cells treated with PD, but not in KATO III cells treated with PD. Flow cytometric analysis showed that the hypodiploid nuclei of HL-60 cells were increased to 75.2, 96.3, and 100% after a 3-day treatment with 2.5, 5, and 10 microM PD, respectively. The fragmentation by PD of DNA to oligonucleosomal-sized fragments, that is a characteristic of apoptosis, was observed to be both concentration- and time-dependent in the HL-60 cells. These findings suggest that growth inhibition by PD of HL-60 cells results from the induction of apoptosis by this compound in HL-60 cells”. As taken from Habasami H et al. 2003. Int. J. Mol. Med. 11, 23-26. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/12469212>.

“The present work studied the effect of fenugreek seed extracts on cytotoxicity and testicular damage induced by adriamycin (ADR) in albino rats. Administering animals with ADR caused significant increase in the percentage of chromosomal aberrations, decreased the mitotic index, and induced DNA damage in bone marrow. Testes of ADR-treated rats showed many histopathological alterations and the number of sperm head abnormalities increased. Moreover, the concentration of malondialdehyde (MDA) increased and the activity of catalase (CAT) and superoxide dismutase (SOD) decreased in the testis. Treating animals with ADR and aqueous seed extracts of fenugreek led to an improvement in the cytogenetic effect and testicular alterations induced by ADR. Lipid peroxidation was reduced and the activities of CAT and SOD were increased. In conclusion, the results indicated that fenugreek seeds ameliorated the cytotoxicity and testicular alterations induced by ADR in albino rats and this may be mediated by its potent antioxidant effects”. As taken from Sakr SA et al. 2012b. Toxicol. Ind. Health. 28(3), 276-88. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/21949087?dopt=AbstractPlus>.

“*Trigonella foenum-graecum* generally known as fenugreek, has been normally cultivated in Asia and Africa for the edible and medicinal values of its seeds. Fenugreek leaves and seeds have been used widely for therapeutic purposes. Fenugreek seed is recognized to show anti-diabetic and anti-nociceptive properties and other things such as hypocholesterolaemic, and anti-cancer. Diosgenin is a steroidal saponin from therapeutic herbs, fenugreek (*T. foenum-graecum* L.), has been well-known to have anticancer properties. Telomerase activity is not identified in usual healthy cells, while in carcinogenic cell telomerase expression is reactivated. Therefore telomerase illustrates a promising cancer therapeutic target. We deliberate the inhibitory effect of pure diosgenin and fenugreek extract diosgenin on human telomerase reverse transcriptase gene (hTERT) expression which is critical for telomerase activity. MTT-assay and qRT-PCR analysis were achieved to discover cytotoxicity effects and hTERT gene expression inhibition properties, separately. MTT results exhibited that IC₅₀ for pure diosgenin were 47, 44 and 43 μM and for fenugreek extract diosgenin were 49, 48 and 47 μM for 24, 48 and 72 h after treatment. Culturing cells with pure diosgenin and fenugreek extract diosgenin treatment caused in down regulation of hTERT expression. These results indicate that pure and impure diosgenin prevents telomerase activity by down regulation of the hTERT gene expression in A549 lung cancer cell line, with the difference that pure compound is more effective than another.” As taken from Rahmati-Yamchi M et al. 2014. Mol. Biol. Rep. 41(9), 6247-52. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24973886>

“BACKGROUND: There are a number of dietary components that may prove useful in the prevention and treatment of cancer. In some cultures, fenugreek seeds are used to treat cancer. The current study focuses on the anticancer properties and proteomic profiles of seeds, and is prompted by the clinical profile of a case of primary CNS T cell lymphoma that responded to fenugreek treatment and resulted in tumor regression. METHOD: Various normal and cancer cell

lines were exposed to fenugreek extract at differing concentrations (100 µg/ml, 200 µg/ml and 300 µg/ml) and at different time points (0, 24, 48, 72 and 96 hrs). Protein fingerprints of fenugreek grain/seed types, obtained from four different geographical regions, were analyzed by proteomic expression profiles. RESULTS: We observed selective cytotoxic effects of fenugreek extract in vitro to a panel of cancer cell lines, including T-cell lymphoma. Additionally, the cluster analysis of proteomics data showed that the protein profile of the particular fenugreek used by the patient is significantly different from three other regional subtypes of fenugreek extract. CONCLUSION: The in vitro effect of fenugreek as a substance with significant cytotoxicity to cancer cells points to the potential usefulness of fenugreek in the prevention and treatment of cancer." As taken from Alsemari A et al. 2014. BMC Complement. Altern. Med. 14, 114. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24679057>

"Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and most current therapies are of limited efficacy. *Trigonella foenum* (Fenugreek) is a traditional herbal plant with antitumor activity, although the mechanisms of its activity remain unclear. Herein, a crude methanol extract was prepared from Fenugreek seeds (FCE) and its anticancer mechanism was evaluated, using HepG2 cell line. Growth-inhibitory effect and apoptosis induction of HepG2 cells were evidenced by MTT assay, cell morphology alteration, apoptosis enzyme-linked immunosorbent assay, flow cytometric analysis, caspase-3 activity, and expression of p53, proapoptotic protein, Bax, and proliferating cell nuclear antigen (PCNA) after (100~500 µg/mL) FCE treatment for 48 h. Furthermore, FCE was analyzed by Chromatography-Mass Spectrometry (GC/MS). Our results revealed that FCE treatment for 48 h showed a cytotoxic effect and apoptosis induction in a dose-dependent manner that was mediated by upregulation of p53, Bax, PCNA, and caspase-3 activation in HepG2 cells. GC-MS analysis of FCE showed the presence of fourteen bioactive compounds such as Terpenoids and Flavonoids, including two main constituents with anticancer activity, Squalene and Naringenin (27.71% and 24.05%), respectively. Our data introduced FCE as a promising nontoxic herbal with therapeutic potential to induce apoptosis in HepG2 cells through p53, Bax, and PCNA upregulation in caspase-3 dependent manner." As taken from Khalil MI et al. 2015. Biomed. Res. Int. 2015, 914645. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26557712>

"BACKGROUND:....The present study was designed to investigate the cytotoxicity of 65 crude extracts from 35 Sudanese medicinal plants towards various cancer cell lines expressing molecular mechanisms of resistance towards classical chemotherapeutics (two ATP-binding cassette transporters, ABCB1 (P-glycoprotein) and ABCB5, tumor suppressor p53, epidermal growth factors receptor (EGFR). And the aim was to identify plant extracts and isolated compounds thereof with activity towards otherwise drug-resistant tumor cells. METHODS: Cold maceration was performed to obtain crude extracts from the plants. The resazurin assay was used to determine cytotoxicity of the plant extracts. Microarray-based mRNA expression profiling, COMPARE, and hierarchical cluster analyses were applied to identify, which genes correlate with sensitivity or resistance to ambrosin, the main constituent of the most active extract *Ambrosia maritima*. RESULTS: The results of the resazurin assay on different tumors showed that *Lawsonia inermis*, *Trigonella foenum-graecum* and *Ambrosia maritima* were the most active crude extracts. Ambrosin was selected as one active principle of *A. maritima* for microarray-based expression profiling. Genes from various functional groups (transcriptional regulators, signal transduction, membrane transporters, cytoskeleton organization, chaperones, immune system development and DNA repair) were significantly correlated with response of tumor cell lines to ambrosin. CONCLUSION: The results revealed cytotoxicity and pharmacogenomics studies of Sudanese medicinal plants provide an attractive strategy for the development of novel cancer therapeutics with activity towards cell lines that resistance to established anticancer agents." As taken from Saeed ME et al. 2015. J. Ethnopharmacol. 174, 644-58. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26165828>

"A group of 11 medicinal plants, including *Lavandula pubescens*, *Trigonella foenugricium*, *Salsola schweinfurthii*, *Calligonum comosum*, *Silene succulenta*, *Silene villosa*, *Bogonvillea glabra*, *Cakile*

maritime, *Gomphrene celesoids*, *Mirabilis jalapa*, and *Silene nocturna* growing in Egypt, were extracted and examined for their immunomodulatory and antioxidant activities. RAW 264.7 cells were recruited to investigate the immunomodulatory effect through multiple parameters analysis. First, the proliferation index of macrophages cells was evaluated revealing that *Trigonella foenugricium*, *Silene succulenta* and *Silene villosa* have a significant cytotoxic effect on RAW cells. Interestingly, we observed enhancement of macrophages phagocytic function of by all extracts except *Cakile maritime*, *Gomphrena celosioides* and *Silene nocturna*. Afterwards, macrophages were challenged by incubation with LPS and the effect of various extracts on inflammatory responses was investigated; the generation of NO from activated macrophage was substantially suppressed by 7 extracts namely, *Trigonella foenugricium*, *Calligonum comosum*, *Silene succulenta*, *Bougainvillea glabra*, *Mirabilis jalapa*, *Gomphrena celosioides* and *Silene nocturna*. TNF- α was decreased by percentage range from 3.8 to 85.8% and *Trigonella foenugricium* extract showed the highest inhibition of TNF- α release. All extracts except *Trigonella foenugricium*, *Salsola schweinfurthii*, *Silene succulenta* and *Mirabilis jalapa* significantly inhibited COX-2 production from stimulated macrophage. Moreover, evaluating the potential antioxidant activity of these extracts showed that *Trigonella foenugricium*, *Salsola schweinfurthii*, *Calligonum comosum*, *Bougainvillea glabra* and *Mirabilis jalapa* exhibited some antioxidant activities. Taken together, our results suggest that some of these extracts may have a considerable antiinflammatory and antioxidant effects and may be a potential therapeutic choice in the treatment of inflammatory diseases.....” As taken from Ghonime M et al. 2015. *Immunol. Invest.* 44(3), 237-52. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25564700>

“Many cellular damages either in normal or cancerous tissues are the outcome of molecular events affected by ionizing radiation. T-cells are the most important among immune system agents and are used for biological radiation dose measurement in recommended standard methods. The herbs with immune modulating properties may be useful to reduce the risk of the damages and subsequently the diseases. The T-cells as the most important immune cells being targeted for biological dosimetry of radiation. This study proposes a flowcytometric-method based on fluorescein isothiocyanate- and propidium iodide (PI)-labeled annexin-V to assess apoptosis in blood T-cells after irradiation in both presence and absence of fenugreek extract. T-cells peripheral blood lymphocyte isolated from blood samples of healthy individuals with no irradiated job background. The media of cultured cells was irradiated 1-h after the fenugreek extract was added. The number of apoptotic cells was assessed by annexin-V protocol and multicolor flowcytometry. An obvious variation in apoptotic cells number was observed in presence of fenugreek extract (>80%). The results suggest that fenugreek extract can potentiate the radiation induced apoptosis or radiation toxicity in blood T-cells ($P < 0.05$).” As taken from Tavakoli MB et al. 2015. *J. Med. Signals Sci.* 5(3), 176-81. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26284174>

“/ENDOCRINE MODULATION/ Several studies support hypolipidemic effect of fenugreek in normal and diabetic subjects. However, very little is known about the possible direct action of fenugreek on adipose tissue. The present study was designed to investigate the effects of fenugreek seeds on adipogenesis and lipolysis. Preadipocytes were isolated from adipose tissue of normal rats and differentiated to adipocyte in the presence of ethanolic extract of fenugreek seeds. The effect of this extract on lipolysis was also evaluated in fat tissue isolated from diabetic rats. Fenugreek led to a significant reduction in lipid droplet accumulation as evaluated with Oil Red O staining. Incubation of preadipocytes with the extract for 24 hr resulted in significant decrease in cell viability. The extract, even at high concentrations (up to 1000 ug/mL), had virtually no significant effect on lipolysis. The present data demonstrated that fenugreek seed inhibits formation of new differentiated adipocytes from precursor cells through an anti-proliferative effect on preadipocytes. [Ghorbani A et al; *Pak J Biol Sci* 17 (4): 523-8 (2014)] **PEER REVIEWED**”

“/ALTERNATIVE and IN VITRO TESTS/ ... The current study focuses on the anticancer properties of fenugreek, an herb with proven anti-diabetic, antitumor and immune-stimulating functions. Jurkat cells were incubated with 30 to 1500 ug/mL concentrations of 50% ethanolic extract of dry

fenugreek seeds and were followed for changes in viability (trypan blue assay), morphology (microscopic examination) and autophagic marker LC3 transcript level (RT-PCR). Incubation of Jurkat cells with fenugreek extract at concentrations ranging from 30 to 1500 ug/mL for up to 3 days resulted in cell death in a dose- and time-dependent manner. Jurkat cell death was preceded by the appearance of multiple large vacuoles, which coincided with transcriptional up-regulation of LC3. GC-MS analysis of fenugreek extract indicated the presence of several compounds with anticancer properties, including gingerol (4.82%), cedrene (2.91%), zingerone (16.5%), vanillin (1.52%) and eugenol (1.25%). Distinct morphological changes involving appearance of large vacuoles, membrane disintegration and increased expression of LC3 transcripts indicated that fenugreek extract induced autophagy and autophagy-associated death of Jurkat cells. In addition to the already known apoptotic activation, induction of autophagy may be an additional mechanism underlying the anticancer properties of fenugreek. This is the first report showing fenugreek as an inducer of autophagy in human cells and further work is needed to define the various intermediates of the autophagic pathway. [Al-Daghri NM et al; BMC Complement Altern Med 12: 202 (2012)]
****PEER REVIEWED****

As taken from HSDB, 2016

Record for *Trigonella foenum-graecum* Linn., seed, alcohol extract (no CAS RN given):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo - Inhibitor Concentration Low	In vitro	Human gastrointestinal tumor	100 mg/L/4H	Biochemical Metabolism (Intermediary) effect on inflammation mediation inflammation	-JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) or V.1- 1979- Volume(issue)/page/year: 141,403,2012
ICLo - Inhibitor Concentration Low	In vitro	Mouse - other normal tissue	10 mg/L/72H	Biochemical Metabolism (Intermediary) effect on inflammation mediation inflammation	-JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) or V.1- 1979- Volume(issue)/page/year: 144,514,2012
IC50 - Inhibitor Concentration 50	In vitro	Mouse - other normal tissue	15.4 mg/L/72H	Biochemical Metabolism (Intermediary) effect on inflammation mediation inflammation	-JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) or V.1- 1979- Volume(issue)/page/year: 144,514,2012
TDLo - Lowest published toxic dose	Oral	Rodent mouse	750 mg/kg/3D (intermittent)	Biochemical Metabolism (Intermediary) effect on inflammation mediation inflammation	-JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) or V.1- 1979- Volume(issue)/page/year: 144,514,2012

As taken from RTECS, 2018b

“Plant defense stimulators (PDSs) rely on the activation of plant innate immunity in order to protect crops against various pests. These molecules are thought to be a safer alternative to classical plant protection products. Given that innate immune systems share common features in plants and vertebrates, PDS can potentially cross-react with innate immunity of non-target organisms. To test this hypothesis, we studied effects of the commercial PDS Stifenia (FEN560), which is composed of crushed fenugreek seeds. We tested various concentrations of Stifenia (0.03-1 mg mL⁻¹) on human peripheral blood mononuclear cells and checked, 20 h later, cell metabolic activity (MA) using XTT assay, cell death by flow cytometry analysis, and IL-1 β inflammatory cytokine released in the culture medium using ELISA. Stifenia induced a general decrease of the cell MA, which was concomitant with a dose-dependent release of IL-1 β . Our results highlight the activation of human immune cells. The inflammatory effect of Stifenia was partially inhibited by pan-caspase inhibitor. Accordingly, Stifenia induced the release of p20 caspase-1 fragment into the culture medium suggesting the involvement of the NLRP3 inflammasome. Furthermore, we observed that Stifenia can induce cell death. We also tested the effect of Stifenia on Zebrafish larvae. After 24 h of exposure, Stifenia induced a dose-dependent IL-1 β and TNF α gene expression. The human-cell-based approach developed in this work revealed a high sensitivity concerning inflammatory properties of a plant protection product. These tests could be routinely used to screen the potential adverse effects of this type of compounds. Finally, our results suggest a potential danger of using extensively certain PDS for crop protection.” As taken from Teysier L et al. 2017. *Front. Public Health* 5, 74. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28484691>

“The need of antibiotics obviate in treated cancer patients when suppression of immune system leads to secondary infections development. The objective of the present study was to evaluate the antibacterial activity and biochemical profiling of various medicinal plants *Trigonella foenum-graecum*, *Ocimum basilicum*, *Olea europaea*, *Mentha longifolia* and *Boswellia sacra* against clinical isolates of blood cancer cases. Crude plant extracts in ethanol and methanol were used to test antimicrobial activity through disc diffusion method. Biochemical profiling identified the presence of Gallic acid, parahydroxy benzoic acid, vanillic acid, syringic acid and ferulic acid by high performance liquid chromatography (HPLC). *Boswellia sacra* showed the maximum antibacterial activity against *Streptococcus viridian* with 12.4 mm inhibition zone. *Trigonella foenum-graecum* showed the maximum antibacterial activity against *Salmonella* Group B 11.8 mm with crude extracts in methanol. The antibacterial activity showed that *Streptococcus viridian* and *Corynebacterium* were more inhibited bacteria but *Klebsiella pneumonia* was found more resistant. Total phenolics analysis by HPLC revealed that parahydroxy benzoic acid was the major phenolic acid found in *Olea europaea* with 797.8 ng/g. The highest concentration of Gallic acid was found in *Ocimum basilicum* with 547.02 ng/g. These results indicated that these medicinal plants may serve as antimicrobial agents against clinical bacterial isolates from cancer patient successfully.” As taken from Farhan A AJ et al. 2017. *Biosci. Biotech. Res. Asia* 14(4), 1277-1284. Available at <http://www.biotech-asia.org/vol14no4/antimicrobial-activity-and-biochemical-profiling-of-selected-medicinal-plants-against-blood-cancer-clinical-isolates/>

“Objective: This study was focussed on an evaluation of antibacterial activities of aqueous and alcoholic extracts of commonly consumed spices, namely, Ajwain (*Trachyspermum ammi*), Coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*), fennel (*Foeniculum vulgare*), and Fenugreek (*Trigonella foenum-graecum*). Methods: This study includes the antibacterial effects of spices against six bacterial strains, namely, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* to compare their antibacterial effects by the paper disc agar diffusion method with three antibiotics such as amikacin, chloramphenicol, and vancomycin. Results: According to findings, it is determined that inhibitory activity was detected on aqueous and alcoholic extracts of Ajwain, aqueous extract of cumin and on alcoholic mixed spice sample. Conclusions: Among the five spices tested, only aqueous extracts of Ajwain and cumin exhibited antibacterial activity against one organism (*S. aureus*). Comparatively the alcoholic extracts gave a better response than the aqueous extracts. The effectiveness of the antibacterial activity was recorded better for the mixed spice samples when

compared to that of the individual spices. This clearly emphasizes that the combined effect of the spices exhibited better antibacterial activity and the kill rate of the bacterial strains is higher relatively." As taken from Salma S et al. 2018. Asian J. Pharm. Clin. Res. 11(2), 252-254. Available at <https://innovareacademics.in/journals/index.php/ajpcr/article/view/22652>

"Aim: To evaluate antibacterial efficacy of Chlorhexidine gluconate (2%), Fenugreek (*Trigonella foenum*) and Fennel (*Foeniculum vulgare*) as intracanal irrigant on isolated bacteria from infected primary tooth. Materials and methods: Thirty patients were selected based on inclusion and exclusion criteria. After rubber dam isolation access opening was done and collection of sample using absorbent paper point was done. Samples were processed for microbiological procedure and isolation of different species of bacteria was done. All the individual species were subjected to antibacterial sensitivity for three irrigants. Results: Different species of obligatory and facultative anaerobes were isolated mainly *Peptostreptococcus* colonies of obligatory anaerobic gram positive cocci followed by facultative anaerobe *E. faecalis*, followed by gram negative Bacilli such as *P. intermedia*, *Porphyromonas* species, *Bacteroides* species, and *Fusobacterium* species. Facultative Gram-positive anaerobic cocci such as *Streptococcus pyogenes*, *S. sobrinus*, and *Staphylococcus aureus* were also found but were comparatively less in number. These were subjected to antibacterial sensitivity against three irrigants. The results statistically analysed using Pearson's Chi-square test for two non-parametric data and proportional comparisons were done using Z test for two sample proportion. Chlorhexidine was found most sensitive, followed by Fennel extract and least sensitive is Fenugreek Extract for facultative as well as obligatory anaerobes. Conclusion: The bacterial profile in infected primary teeth consists of mainly obligatory anaerobes *Peptostreptococcus* colonies, followed by *E. Faecalis* and black pigmented colonies. Amongst two herbal irrigant, fennel can act a potent herbal substitute for chlorhexidine as intracanal irrigant in infected primary teeth." As taken from Gupta P et al. 2018. IJAR 4(2), 162-165. Available at <http://www.allresearchjournal.com/archives/2018/vol4issue2/PartC/4-2-5-588.pdf>

"The role of angiogenesis in tumor progression and metastasis formation has been well recognized. Recent studies have reported that *Trigonella foenum-graecum* L. (fenugreek) seed extracts have potential anticancer properties. The current study was planned to investigate the anti-angiogenic activity of hydroalcoholic extract of fenugreek (HAEF) in vitro and in vivo. Effect of HAEF (50-3000 µg/mL) and thalidomide (200-3000 µmol/L), as a positive control, on the viability of human umbilical vein endothelial cells (HUVECs) and 3T3 fibroblast cells was assessed by thiazolyl blue tetrazolium bromide (MTT) assay. Effect of HAEF on vessel-like tube formation by HUVECs was examined in the matrigel-based assay. Furthermore, the chick chorioallantoic membrane (CAM) was used as in vivo model to study the anti-angiogenic effect of HAEF. HAEF, similar to thalidomide, significantly inhibited the viability of HUVECs and 3T3 cells dose-dependently after 24 h. Moreover, both HAEF and thalidomide significantly reduced tube formation by HUVECs in cell culture condition. In CAM model, HAEF and thalidomide caused a significant decline in the number of neovascular points and in the amount of grades 1 and 2 vessels. These findings revealed that fenugreek has cytotoxic and anti-angiogenic effects in vitro and in vivo. Therefore, this medicinal plant can be subjected to further investigations as antitumor agents." As taken from Iranmanesh M et al. 2018. Res. Pharm. Sci. 13(4), 343-352. PubMed, 2019 available at <https://www.ncbi.nlm.nih.gov/pubmed/30065767>

"Background: Cancer has been recognized as a major cause of death globally. Traditional medicines have been used to treat cancer for several periods, and herbal medicines are currently being used for the treatment of cancer worldwide. Fenugreek is an annual plant (family: Fabaceae) and is documented as a medicinal herb since olden days. Objective: The present study was aimed to investigate the anticancer activity of Fenugreek seed oil (FSO) against human hepatocellular carcinoma (HepG2) cell line. Materials and Methods: The HepG2 cells were exposed to 10–1000 µg/ml of FSO for 24 h to assess the cytotoxicity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide test, neutral red uptake assay, and morphological changes. The cytotoxic concentrations of FSO were used to study the induction in reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) levels. Further, the expression of pro-and antiapoptotic genes (p53, caspase-3, caspase-9, Bax and Bcl-2) were studied. Results: A concentration-

dependent decrease in cell viability of HepG2 cells was observed after FSO exposure. Our results showed that FSO decreased cell viability of HepG2 cells in a concentration-dependent manner. FSO was also found to increase the ROS generation and decrease the MMP level in HepG2 cells. Our gene expression study indicates upregulation in apoptotic marker genes and downregulation in antiapoptotic gene. Conclusion: The results showed that FSO is capable to induce mitochondrial-mediated apoptosis through ROS generation in HepG2 cells and could be a useful resource in developing effective remedies for the treatment of hepatocellular carcinoma.” As taken from Al-Sheddi ES et al. 2019. *Pharmacognosy Magazine* 15(60):12-17.

“This work is about the utilization of fenugreek seed as an antibacterial and anticancer agents. The antibacterial activity of fenugreek seed extract on six pathological bacteria strains were specified through conventional biochemical tests using the Vitek2 automated system and diffusion agar method. The anticancer activities of fenugreek seed extract, on MCF-7 breast cancer cells, liver cancer HCAM cells and the non-cancerous Vero cell lines, were investigated using colorimetric MTT assay. Results showed that the highest activity of the extract of the seed was found on *Staphylococcus aureus* and *Pseudomonas aeruginosa* (22 mm and 17 mm diameter of inhibition zones respectively). The seed extract showed proliferative inhibition on MCF-7 cell line at a concentration of 400 µg/ml and 72 h of the incubation period. This was accompanied by insignificant apoptosis or necrosis. The seed extract showed no anticancer effect on liver and Vero cell lines. This work emphasizes that fenugreek seed extract is a potential source of antibacterial and anticancer agents.” As taken from Al-Timimi LAN. 2019. *Asian Pac. J. Cancer Prev.* 20(12), 3771-3776. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31870120/>

“Food preservation and safety is drawing more attention globally due to the increasing prevalence of food-borne diseases. The natural methods of food preservation are considered safer compared to methods using synthetic preservatives. The essential oils with natural preservative properties could be useful for food safety and preservation. The objective of this study was to analyze the chemical composition of commercially available *Allium sativum* and *Trigonella foenum-graecum* essential oils by gas chromatography-mass spectroscopy (GC-MS). The antimicrobial activities of *Allium sativum* and *Trigonella foenum-graecum* essential oils were determined by agar well diffusion technique. The GC-MS analysis of Garlic essential oil (GEO) revealed that, Allyl methyl trisulfide (13.10%), Di-allyl sulfide (9.47%) and Di-allyl tetrasulfide (4.38%) were the major components, while methanolic extract of Fenugreek essential oil (FEO) showed limonene (12.92%), Silane trimethylphenyl (10.71%), carvone (4.57%) and Trigolline (0.38%) as major components. The results of our study showed a significant antimicrobial activity of GEO and FEO against the tested microbial strains, which indicates the presence of broadspectrum antimicrobial constituents in GEO and FEO. However, further studies are needed for individual bioactive components and safety aspects for their application in food preservation.” As taken from Ashraf SA et al. 2019. *J. Pure Appl. Microbiol.* 13(4), 2545-2552. Available at <https://pdfs.semanticscholar.org/a2b8/ffdf3570431a3d2f58ccd1e9e6a267e58bed.pdf>

“Plants are a rich source of secondary metabolites that have been found to have medicinal properties. The present study was conducted to evaluate the phytochemical screening, antibacterial activities and heavy metal analysis of seven medicinal plants i.e., *Nigella sativa* (seeds), *Trigonella foenum-graecum* (seeds), *Brassica campestris* (seeds), *Pistacia integerrima* (galls), *Linum usitatissimum* (seeds), *Hyssopus officinalis* (flowers), *Ephedra vulgaris* (dry branches) and its two recipes which are used by hakims (Practitioners of local herbal medicines), against different diseases particularly respiratory tract infections. The obtained results revealed that alkaloids (30%) and flavonoids (41%) were in maximum quantity in *P. integerrima* (galls) while saponins (10.9%) were in maximum quantity in Recipe 1. The antibacterial activity was determined by the agar well disc diffusion method using methanol, ethanol, chloroform and deionized water extracts. Each plant extract was tested against one Gram-positive (*Streptococcus pneumonia*) and two Gram-negative (*Pseudomonas aeruginosa* and *Klebsiella pneumonia*) bacteria. Maximum zones of inhibition in methanol, ethanol, chloroform and aqueous extract were seen in *T. foenum-graecum* against *S. pneumonia* (20.06 ± 0.16 mm), *B. campestris* against *S. pneumonia* (22.40 ±

0.24 mm), Recipe 2 against *K. pneumonia* (20.06 ± 0.16 mm) and *N. sativa* against *S. pneumonia* (20.23 ± 0.16 mm), respectively. The concentrations of heavy metals were determined by atomic absorption spectrophotometer and showed the existence of high concentration of Iron (Fe), Lead (Pb) and Chromium (Cr). Thus, it has been found that medicinal plants individually as well as their recipes are potentially active against various diseases particularly respiratory tract infections.” As taken from Mahmood N et al. 2019. *Plants (Basel)* 8(11), 454. PubMed 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31717808/>

5.6. Carcinogenicity

“*Trigonella foenum graecum* (fenugreek) is traditionally used to treat disorders such as diabetes, high cholesterol, wounds, inflammation, and gastrointestinal ailments. Recent studies suggest that fenugreek and its active constituents may possess anticarcinogenic potential. We evaluated the preventive efficacy of dietary fenugreek seed and its major steroidal saponin constituent, diosgenin, on azoxymethane-induced rat colon carcinogenesis during initiation and promotion stages. Preneoplastic colonic lesions or aberrant crypt foci (ACF) were chosen as end points. In addition, we assessed the mechanism of tumor growth inhibition of diosgenin in HT-29 human colon cancer cells. To evaluate the effect of the test agent during the initiation and postinitiation stages, 7-week-old male F344 rats were fed experimental diets containing 0% or 1% fenugreek seed powder (FSP) or 0.05% or 0.1% diosgenin for 1 week and were injected with azoxymethane (15 mg/kg body weight). Effects during the promotional stage were studied by feeding 1% FSP or 0.1% diosgenin 4 weeks after the azoxymethane injections. Rats were sacrificed 8 weeks after azoxymethane injection, and their colons were evaluated for ACF. We found that, by comparison with control, continuous feeding of 1% FSP and 0.05% and 0.1% diosgenin suppressed total colonic ACF up to 32%, 24%, and 42%, respectively ($P < \text{or} = 0.001$ to 0.0001). Dietary FSP at 1% and diosgenin at 0.1% fed only during the promotional stage also inhibited total ACF up to 33% ($P < \text{or} = 0.001$) and 39% ($P < \text{or} = 0.0001$), respectively. Importantly, continuous feeding of 1% FSP or 0.05% or 0.1% diosgenin reduced the number of multicrypt foci by 38%, 20%, and 36% by comparison with the control assay ($P < \text{or} = 0.001$). In addition, 1% FSP or 0.1% diosgenin fed during the promotional stage caused a significant reduction ($P < \text{or} = 0.001$) of multicrypt foci compared with control. Dietary diosgenin at 0.1% and 0.05% inhibited total colonic ACF and multicrypt foci formation in a dose-dependent manner. Results from the in vitro experiments indicated that diosgenin inhibits cell growth and induces apoptosis in the HT-29 human colon cancer cell line in a dose-dependent manner. Furthermore, diosgenin induced apoptosis in HT-29 cells at least in part by inhibition of bcl-2 and by induction of caspase-3 protein expression. On the basis of these findings, the fenugreek constituent diosgenin seems to have potential as a novel colon cancer preventive agent.” As taken from Khader M et al. *Cancer Epidemiol Biomarkers Prev.* 2004 Aug; 13(8):1392-8. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15298963&query_hl=49&itool=pubmed_docsum

“Cancer is the second leading cause of death worldwide. Conventional therapies cause serious side effects and, at best, merely extend the patient's lifespan by a few years. Cancer control may therefore benefit from the potential that resides in alternative therapies. There is thus an increasing demand to utilize alternative concepts or approaches to the prevention of cancer. In this report, we show a potential protective effect of Fenugreek seeds against 7,12-dimethylbenz(alpha)anthracene (DMBA)-induced breast cancer in rats. At 200 mg/kg b.wt., Fenugreek seeds' extract significantly inhibited the DMBA-induced mammary hyperplasia and decreased its incidence. Epidemiological studies also implicate apoptosis as a mechanism that might mediate the Fenugreek's anti-breast cancer protective effects. To our knowledge, this is the first study that suggests significant chemopreventive effects of Fenugreek seeds against breast cancer.” As taken from Amin A et al. *Cell Biol Int.* 2005 Aug; 29(8):687-94. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15936223&query_hl=48&itool=pubmed_docsum

"The antineoplastic effect of *Trigonella foenum graecum* seed extract has been evaluated in the Ehrlich ascites carcinoma (EAC) model in Balb-C mice. Intra-peritoneal administration of the alcohol extract of the seed both before and after inoculation of EAC cell in mice produced more than 70% inhibition of tumour cell growth with respect to the control. Treatment with the extract was found to enhance both the peritoneal exudate cell and macrophage cell counts. The extract also produced a significant antiinflammatory effect. We report here the antiinflammatory and antineoplastic effects, of *Trigonella foenum graecum* seed extract." As taken from Sur P et al. *Phytother Res.* 2001 May; 15(3):257-9. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11351364&query_hl=50&itool=pubmed_docsum

"The effect of fenugreek seeds on the activities of beta-glucuronidase and mucinase during 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in rats was studied. Rats were given a weekly subcutaneous injection of DMH at a dose of 20 mg/kg body weight, for 15 weeks. Fenugreek seed powder was weighed depending upon the weight of individual rats and incorporated in the powdered pellet diet at a dose of 2 g/kg body weight. After an experimental period of 30 weeks the activity of beta-glucuronidase significantly increased in the colon, intestine, liver and colon contents in DMH administered rats when compared to an untreated control group. Increase in beta-glucuronidase may increase the hydrolysis of carcinogen-glucuronide conjugate, liberating carcinogen and/or co-carcinogen within the colonic lumen. Inclusion of fenugreek seed powder in the diet significantly decreased the activity of beta-glucuronidase in all the tissues studied. This may prevent the free carcinogens from acting on colonocytes. Mucinase helps in hydrolysing the protective mucin. Mucinase activity was increased in the colon content and fecal content of animals given DMH when compared to control, while the activity was significantly reduced in animals given DMH + fenugreek when compared to animals given DMH only. Our study shows that supplementation of fenugreek seeds in the diet inhibits colon carcinogenesis, by modulating the activities of beta-glucuronidase and mucinase. The beneficial effect may be attributed to the presence of fibre, flavonoids and/or saponins." As taken from Devasena T and Menon VP. *Phytother Res.* 2003 Nov; 17(9):1088-91. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=14595593&query_hl=34&itool=pubmed_DocSum

"Cancer is not a single disease but a group of complex genetic diseases of aged cells. Chemoprevention of cancer is the attempt to use natural and synthetic compounds to intervene in the early stages of cancer, before invasive disease begins. Consuming a diet rich in plant foods can provide a milieu of phytochemicals and non-nutritive plant substances that possess health-protective effects. Some phytochemicals derived in spices and herbs as well as other plants possess substantial cancer preventive properties. Thus the cancer chemo preventive potential of naturally occurring phytochemicals is of great interest because of their preventive role and as they are not perceived as "medicine". During the course of present study *Trigonella foenum graecum* (L.) seed- TFGS (commonly called fenugreek) extract was given at pre-initiation, post-initiation, promotional and throughout the experiment along with 7,12-dimethylbenz [a] anthracene DMBA and 12-O-tetradecanoylphorbol-13-acetate TPA treatment in Swiss albino mice. A significant reduction of papillomas in DMBA + TPA + TFGS (400 mg/kg. body wt.) treated group was found to be effective in decreasing the rate of tumor incidence in comparison to control. Furthermore, cumulative number of papillomas, tumor yield and tumor burden were also found to be reduced. The TFGS extract treatment before DMBA and TPA application (i.e. Pre initiation) were more effective than that of treatment during, and /or after DMBA treatment, however TFGS extract treatment was most effective when treated throughout all the stages of tumorigenesis. The TFGS treatment also showed a modulatory influence on mouse hepatic antioxidant defense system (GSH and LPO level)". As taken from Chatterjee S et al. 2012. *Toxicol. Int.* 19(3), 287-94. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23293468?dopt=AbstractPlus>.

"The main bioactive compounds of *Trigonella foenum graecum* L. (fenugreek) seeds are protodioscin, trigoneoside, diosgenin and yamogenin, which have anticarcinogenic potency through

inhibition of cell proliferation and inhibition of prostaglandin synthesis. The effect of fenugreek on ALOX and COX genes was examined in AKR/J H-2(k) mice exposed to dimethylbenz[*a*]anthracene (DMBA), a potent carcinogen. The expression pattern of these genes was determined by detecting the mRNA expression in various tissues (the lungs, liver, spleen and the kidneys) in four groups of mice. Two groups were fed with normal and two of them with fenugreek containing nutriment. Each group divided into DMBA treated and control groups. Mice were autopsied on day 7 after DMBA treatment for mRNA isolation. Fenugreek consumption itself did not change gene expression compared with the control group. DMBA could increase the expression of ALOX12, ALOX15, ALOX5 genes mainly in all organs. Fenugreek consumption was generally protective in each organ in a different manner. DMBA treatment increased COX2 gene expression, but fenugreek was protective in all tissues examined. In COX1 gene, the fenugreek diet could suppress the expression, except for spleen, independently from carcinogen exposure. Therefore by inhibiting the arachidonic acid metabolism fenugreek may prevent tumorigenesis". As taken from Vargas T et al. 2011. *Phytotherap. Res.* 25, 221-227. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/20641053?dopt=AbstractPlus>.

Diosgenin targets Akt-mediated prosurvival signaling in human breast cancer cells (Abstract). In recent years, Akt signaling has gained recognition for its functional role in more aggressive, therapy-resistant malignancies. As it is frequently constitutively active in cancer cells, several drugs are being investigated for their ability to inhibit Akt signaling. The purpose of this study is to determine effect of diosgenin (fenugreek), a dietary compound on Akt signaling and its downstream targets on estrogen receptor positive (ER(+)) and estrogen receptor negative (ER(-)) breast cancer (BCa) cells. Diosgenin inhibits pAkt expression and Akt kinase activity without affecting PI3 kinase levels, resulting in the inhibition of its downstream targets, NF-kappaB, Bcl-2, survivin and XIAP. The Raf/MEK/ERK pathway, another functional downstream target of Akt, was inhibited by diosgenin in ER(+) but not in ER(-) BCa cells. Additionally, we found that diosgenin caused G1 cell cycle arrest by downregulating cyclin D1, cdk-2 and cdk-4 expression in both ER(+) and ER(-) BCa cells resulting in the inhibition of cell proliferation and induction of apoptosis. Interestingly, no significant toxicity was seen in the normal breast epithelial cells (MCF-10A) following treatment with diosgenin. Additionally, in vivo tumor studies indicate diosgenin significantly inhibits tumor growth in both MCF-7 and MDA-231 xenografts in nude mice. Thus, these results suggest that diosgenin might prove to be a potential chemotherapeutic agent for the treatment of BCa. As taken from Srinivasan et al. *Int J Cancer.* 2009, Aug 15; 125(4):961-7. PubMed, 2010 available at [http://www.ncbi.nlm.nih.gov/pubmed/19384950\(CAS 68990-15-8\)](http://www.ncbi.nlm.nih.gov/pubmed/19384950(CAS 68990-15-8))

"BACKGROUND: There are a number of dietary components that may prove useful in the prevention and treatment of cancer. In some cultures, fenugreek seeds are used to treat cancer. The current study focuses on the anticancer properties and proteomic profiles of fenugreek seeds, and is prompted by the clinical profile of a case of primary CNS T cell lymphoma that responded to fenugreek treatment and resulted in tumor regression. METHOD: Various normal and cancer cell lines were exposed to fenugreek extract at differing concentrations (100 µg/ml, 200 µg/ml and 300 µg/ml) and at different time points (0, 24, 48, 72 and 96 hrs). Protein fingerprints of fenugreek grain/seed types, obtained from four different geographical regions, were analyzed by proteomic expression profiles. RESULTS: We observed selective cytotoxic effects of fenugreek extract in vitro to a panel of cancer cell lines, including T-cell lymphoma. Additionally, the cluster analysis of proteomics data showed that the protein profile of the particular fenugreek used by the patient is significantly different from three other regional subtypes of fenugreek extract. CONCLUSION: The in vitro effect of fenugreek as a substance with significant cytotoxicity to cancer cells points to the potential usefulness of fenugreek in the prevention and treatment of cancer." As taken from Alsemari A et al. 2014. *BMC Complement. Altern. Med.* 14, 114. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24679057>

"...the information available in the literature on the health benefits and pharmaceutical effects of trigonella accounts for its known medicinal properties and adds new therapeutic effects in newer indications. besides its known medicinal properties such as carminative, gastric stimulant,

antidiabetic and galactogogue (lactation-inducer) effects, newer research has identified hypocholesterolemic, antilipidemia, antioxidant, hepatoprotective, anti-inflammatory, antibacterial, antifungal, antiulcer, antilithogenic, anticarcinogenic and other miscellaneous medicinal effects of fenugreek. Although most of these studies have used whole seed powder or different forms of extracts, some have identified active constituents from seeds and attributed them medicinal values for different indications. Conclusion: The research on trigonella exhibits its health benefits and potential medicinal properties in various indications and has little or no side effects, suggesting its pharmaceutical, therapeutic and nutritional potential." As taken from YADAV UC & BAQUER NZ. 2014. PHARM. BIOL. 52(2), 243-54. PUBMED, 2014 AVAILABLE AT <http://www.ncbi.nlm.nih.gov/pubmed/24102093>

"Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and most current therapies are of limited efficacy. *Trigonella foenum* (Fenugreek) is a traditional herbal plant with antitumor activity, although the mechanisms of its activity remain unclear. Herein, a crude methanol extract was prepared from Fenugreek seeds (FCE) and its anticancer mechanism was evaluated, using HepG2 cell line. Growth-inhibitory effect and apoptosis induction of HepG2 cells were evidenced by MTT assay, cell morphology alteration, apoptosis enzyme-linked immunosorbent assay, flow cytometric analysis, caspase-3 activity, and expression of p53, proapoptotic protein, Bax, and proliferating cell nuclear antigen (PCNA) after (100~500 µg/mL) FCE treatment for 48 h. Furthermore, FCE was analyzed by Chromatography-Mass Spectrometry (GC/MS). Our results revealed that FCE treatment for 48 h showed a cytotoxic effect and apoptosis induction in a dose-dependent manner that was mediated by upregulation of p53, Bax, PCNA, and caspase-3 activation in HepG2 cells. GC-MS analysis of FCE showed the presence of fourteen bioactive compounds such as Terpenoids and Flavonoids, including two main constituents with anticancer activity, Squalene and Naringenin (27.71% and 24.05%), respectively. Our data introduced FCE as a promising nontoxic herbal with therapeutic potential to induce apoptosis in HepG2 cells through p53, Bax, and PCNA upregulation in caspase-3 dependent manner." As taken from Khalil MI et al. 2015. Biomed. Res. Int. 2015, 914645. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26557712>

"BACKGROUND:....The present study was designed to investigate the cytotoxicity of 65 crude extracts from 35 Sudanese medicinal plants towards various cancer cell lines expressing molecular mechanisms of resistance towards classical chemotherapeutics (two ATP-binding cassette transporters, ABCB1 (P-glycoprotein) and ABCB5, tumor suppressor p53, epidermal growth factors receptor (EGFR). And the aim was to identify plant extracts and isolated compounds thereof with activity towards otherwise drug-resistant tumor cells. METHODS: Cold maceration was performed to obtain crude extracts from the plants. The resazurin assay was used to determine cytotoxicity of the plant extracts. Microarray-based mRNA expression profiling, COMPARE, and hierarchical cluster analyses were applied to identify, which genes correlate with sensitivity or resistance to ambrosin, the main constituent of the most active extract *Ambrosia maritima*. RESULTS: The results of the resazurin assay on different tumors showed that *Lawsonia inermis*, *Trigonella foenum-graecum* and *Ambrosia maritima* were the most active crude extracts. Ambrosin was selected as one active principle of *A. maritima* for microarray-based expression profiling. Genes from various functional groups (transcriptional regulators, signal transduction, membrane transporters, cytoskeleton organization, chaperones, immune system development and DNA repair) were significantly correlated with response of tumor cell lines to ambrosin. CONCLUSION: The results revealed cytotoxicity and pharmacogenomics studies of Sudanese medicinal plants provide an attractive strategy for the development of novel cancer therapeutics with activity towards cell lines that resistance to established anticancer agents." As taken from Saeed ME et al. 2015. J. Ethnopharmacol. 174, 644-58. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26165828>

"Bentrad et al. [71] have conducted an in vivo study to evaluate the molecular mechanisms of anticancer activity of fenugreek using Wistar rats with intracranial grafted C6 glioma, rats with subcutaneously grafted Guerin carcinoma and Guerin carcinoma substrains resistant to doxorubicin

and cisplatin, as well as C57Bl/6 mice with grafted Lewis lung carcinoma and grafted Ca755 mammary carcinoma. Administration of the fenugreek powder (250 mg/kg of bw) increased the lifetime of animals by 15–50%. Also, a decrease of the average volume of metastases by 18–86% was observed. A mechanistic analysis of these results found that fenugreek increased the level of global DNA methylation, reduced the NF- κ B expression in cells nuclei and decreased the NF κ B-dependent genes expression (C-myc, Bcl-xl and Cox-2) [71]. This study suggests that the mechanisms of the antitumor action of fenugreek maybe mediated by NF- κ B-dependent signaling pathways and their influence on DNA methylation. More recently, an anti-proliferative effect of water extract of fenugreek seeds on the growth of Leukemia L1210 cells induced ascities in experimental mice has been shown to reduce ascites development and cancer transformation to a solid mass [70]. Interestingly, the anticancer activities of fenugreek seed oil (10–1000 mg/mL for 24 h) against cancer cell lines, including human epidermoid cancer cells (HEp2), human breast adenocarcinoma cells (MCF-7), human amniotic epithelial cells (WISH), and a normal cell line African green monkey kidney cells (Vero) have shown that fenugreek seeds oil significantly reduced the cell viability, and altered the cellular morphology in a dose-dependent manner [84]." As taken from El Bairy K et al. 2017. *Biomedicine and Pharmacotherapy*. 90: 479-491. Pubmed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/?term=28391170>

"Using the azoxymethane-induced rat colon carcinogenesis model (HT-29 human colon cancer model), Raju et al. [99] showed that dietary fenugreek seed and diosgenin reduced and retarded the appearance of colonic aberrant crypt foci (ACF) during the initiation/progression stages of colon carcinogenesis and even when given only during the promotional stage. In addition, diosgenin inhibits cancer cell proliferation and induces apoptosis by suppressing the expression of the antiapoptotic Bcl-2 while increasing the expression of the proapoptotic enzymes such as caspase-3 [99]." As taken from El Bairy K et al. 2017. *Biomedicine and Pharmacotherapy*. 90: 479-491. Pubmed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/?term=28391170>

"In a double-blind study designed to assess the potential chemopreventive properties of diosgenin on azoxymethane (AOM)-induced rat colon carcinogenesis, Malisetty et al. [88] showed that 0.1% of diosgenin suppressed significantly the incidence of both invasive and non-invasive colon adenocarcinomas by up to 60% and suppressed colon tumor multiplicity (adenocarcinomas/rat) by up to 68%. By contrast in mice, diosgenin at doses of 20, 100 and 500 mg/kg (bw) in the diet did not alter the incidence of colon tumors (adenoma + adenocarcinoma) induced by AOM/dextran sodium sulfate, but reduced the tumor multiplicity significantly at all the three tested doses [94]. It has been reported that 10 mg/kg (bw) of diosgenin, administered intratumorally, significantly inhibited the growth of human breast cancer MCF-7 and MDA 231 tumor xenografts in mice [90]. Diosgenin induced p53 tumor suppressor protein in ER positive MCF-7 human breast cancer cells, and the activation of caspase-3 and down-regulation of Bcl-2 in ER-negative MDA human breast carcinoma cells [90]. In addition, oral administration of diosgenin significantly inhibited the growth of mouse LA795 lung adenocarcinoma tumors by 33.94% in T739 inbred mice [91]. Similarly, Jagadeesan et al. investigated the therapeutic potential of diosgenin against DMBA-induced hamster buccal pouch carcinogenesis and found that diosgenin administered orally (80 mg/kg bw) significantly reduced the formation of oral tumors [121]. As taken from El Bairy K et al. 2017. *Biomedicine and Pharmacotherapy*. 90: 479-491. Pubmed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/?term=28391170>

5.7. Irritation/immunotoxicity

A patient with atopic dermatitis developed systemic itching, diarrhoea and bronchial asthma after eating a curry. On oral "challenge" with the individual spices in water, cardamom and fenugreek both elicited these symptoms, and the patient was shown to have high levels of allergen-specific IgE antibodies to both spices (Ohnuma et al. 1998).

Inhalation of fenugreek seed powder by a patient with food allergy resulted in runny nose, wheezing and fainting. Another patient with chronic asthma and food allergy developed "numbness

of the head”, facial swelling and wheezing after using fenugreek paste on her scalp. Skin-prick tests showed that both subjects were sensitive to fenugreek extract (Patil et al. 1997).

No skin sensitization reactions were seen when a maximization test was carried out on 27 volunteers at a concentration of 2% fenugreek absolute in petrolatum (Epstein, 1976).

The French literature contains an early paper reporting a case of occupational asthma to fenugreek grain (Dugue et al. 1993).

Record for fenugreek absolute (CAS RN 68990-15-8):

Type of Test	Route of Exposure	Species Observed	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration onto the skin	Rodent rabbit	500 mg/24H	Moderate	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year: 16,755,1978

As taken from RTECS, 2018c.

“Allergic reactions after consumption of spices are well-known. In Asia, fenugreek seeds are consumed as spices and also as medicines. Literature survey carried out does not reveal reports of allergic reactions to fenugreek. In our survey carried out on patients with food allergy, we found two cases of severe allergy to fenugreek. METHODS: We report here two cases of immediate allergy following ingestion, inhalation, and external application of fenugreek seed powder. In the first case, inhalation of the fenugreek seed powder resulted in rhinorrhea, wheezing, and fainting. The second case was of a patient with chronic asthma who developed numbness of head, facial angioedema, and wheezing after application of fenugreek paste to her scalp as a treatment for dandruff. Skin scratch test was performed with fenugreek and other members of the Leguminosae family as fenugreek also belongs to Leguminosae. Objective evidence of the reaction was obtained by conducting double-blind placebo-controlled challenges (DBPCFC). For detecting IgE binding by immunoblotting method, the proteins of the fenugreek extract were resolved using sodium dodecyl sulphate polyacrylamide gel electrophoresis. RESULTS: Skin scratch tests for the patients revealed strong sensitivity to fenugreek and chickpeas. None of the controls showed such response with fenugreek extract. During DBPCFC, both patients showed > 20% drop in peak flow rate after consumption of fenugreek and chickpea. Immunoblots demonstrated binding of specific IgE from the patients' sera with the protein from extracts between 20 kD to 70 kD bands. CONCLUSION: This case report has enlarged the list of food allergens with the addition of fenugreek.” As taken from Patil SP et al. Ann Allergy Asthma Immunol. 1997 Mar; 78(3):297-300. PubMed, 2010 available at

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=9087156&query_hl=34&itool=pubmed_DocSum

“The seeds of fenugreek (*Trigonella foenum-graecum* L.) have medicinal uses as hypoglycemic, antinociceptive and anti-inflammatory agents. We aimed to evaluate the antinociceptive and anti-inflammatory effects of the major fractions of fenugreek seeds. The methanolic extract of the plant seeds was partitioned using a liquid-liquid extraction procedure to give six major fractions. Following phytochemical screening of isolated fractions, the total extract and each fraction were evaluated for their antinociception and anti-inflammatory effects using formalin and carrageenan-induced paw edema tests respectively. The methanolic extract exhibited both antinociceptive and anti-inflammatory effects at a dose of 100mg/kg. Among the tested fractions, alkaline chloroform fraction (AKC), which was alkaloid positive in screening tests, showed the most anti-nociceptive effect in a dose-dependent manner. AKC fraction was as effective as morphine (5mg/kg) in this regard. Both aqueous and acidified chloroform fractions (ACC) could significantly inhibit paw edema at a different dose. The latter fraction dose-dependently inhibited carrageenan-induced paw edema. The results of phytochemical screening tests confirmed the presence of flavonoids in both

ACC and aqueous fractions. It can be concluded that the alkaloid and flavonoid content of fenugreek seeds can be responsible for antinociception and anti-inflammatory effects of the plant respectively". As taken from Mandegary A et al. 2012. *Fd Chem. Toxic.* 50, 2503-2507. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22542922?dopt=AbstractPlus>.

"Several legumes may induce allergy, and there is extensive serological cross-reactivity among legumes. This cross-reactivity has traditionally been regarded to have limited clinical relevance. However, the introduction of novel legumes to Western countries may have changed this pattern, and in some studies cross-allergy to lupin has been reported in more than 60% of peanut-allergic patients. We wanted to explore cross-reactions among legumes using two newly established mouse models of food allergy. Mice were immunized perorally with fenugreek or lupin with cholera toxin as adjuvant. The mice were challenged with high doses of fenugreek, lupin, peanut or soy, and signs of anaphylactic reactions were observed. Cross-allergic mechanisms were investigated using serum mouse mast cell protease-1 (MMCP-1), antibody responses, immunoblotting and ex vivo production of cytokines by spleen cells. Signs of cross-allergy were observed for all the tested legumes in both models. The cross-allergic symptoms were milder and affected fewer mice than the primary allergic responses. The cross-allergy was reflected to a certain extent in the antibody and T-cell responses, but not in serum MMCP-1 levels. Cross-allergy to peanut, soy, fenugreek and lupin was observed in lupin-sensitized and fenugreek-sensitized mice. Differences in serological responses between primary allergy and cross-allergy might be due to mediation through different immune mechanisms or reflect different epitope affinity to IgE. These differences need to be further investigated". As taken from Vinje NE et al. 2012. *Scand. J. Immunol.* 76, 387-397. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22803695?dopt=AbstractPlus>.

"Preliminary trials have suggested possible hypoglycaemic, hypolipidaemic and immunomodulatory properties of the fenugreek plant. Here, we evaluated and compared the efficacy of Egyptian fenugreek seed powder (FSP, 0.5 and 1.0 g/kg body weight) in alleviating the experimentally induced metabolic syndrome (in type 1 diabetic and obese rat models) and experimentally induced immunosuppression and delay in burn-healing (in cyclophosphamide (CP)-treated rats). FSP significantly alleviated ($P < 0.05-0.001$) most signs of the metabolic syndrome resulting from experimentally induced type 1 diabetes and obesity by 40-76 and 56-78 %, respectively, including hyperglycaemia, hyperlipidaemia, elevation in atherogenic indices, impairment of liver functions, severe changes in body weight and oxidative stress. Besides, FSP (especially the high dose) completely modulated the immunosuppressive activity of CP including leucopenia (resulting from neutropenia and lymphopenia), decrease in weights and cellularity of lymphoid organs, serum γ -globulin level, delayed type of hypersensitivity response and delay in the skin-burning healing process. FSP decreased the immunosuppressive activity of CP by 57-108 %. These beneficial effects of FSP were dose dependent in most cases, and FSP doses used here were considered safe in general. FSP was more efficient in alleviating the signs of the metabolic syndrome in the obese animals (over 9 %) than in the type 1 diabetic animals. Moreover, the immunostimulant activity of fenugreek seeds exceeded their anti-metabolic syndrome activity by 15-24 %. In conclusion, fenugreek seeds may be useful not only as a dietary adjunct for the control of the metabolic syndrome in diabetic/obese patients, but also as an immunostimulant in immunocompromised patients such as those under chemotherapeutic interventions". As taken from Ramadan G et al 2011. *Br. J. Nutr.* 105, 995-1004. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/21205429?dopt=AbstractPlus>.

"Allergic reactions, exacerbation of asthma, and a 14% decrease in serum potassium have been reported."

As taken from LactMed, 2021. Record for CAS RN 68990-15-8.

"A report has indicated that fenugreek absolute is nonirritating, nonsensitizing, and nonphototoxic to human skin."

As taken from Khan IA and Abourashed EA, 2010.

"A group of 11 medicinal plants, including *Lavandula pubescens*, *Trigonella foenugricium*, *Salsola schweinfurthii*, *Calligonum comosum*, *Silene succulenta*, *Silene villosa*, *Bogonvillea glabra*, *Cakile maritime*, *Gomphrena celosoids*, *Mirabilis jalapa*, and *Silene nocturna* growing in Egypt, were extracted and examined for their immunomodulatory and antioxidant activities. RAW 264.7 cells were recruited to investigate the immunomodulatory effect through multiple parameters analysis. First, the proliferation index of macrophages cells was evaluated revealing that *Trigonella foenugricium*, *Silene succulenta* and *Silene villosa* have a significant cytotoxic effect on RAW cells. Interestingly, we observed enhancement of macrophages phagocytic function of by all extracts except *Cakile maritime*, *Gomphrena celosoids* and *Silene nocturna*. Afterwards, macrophages were challenged by incubation with LPS and the effect of various extracts on inflammatory responses was investigated; the generation of NO from activated macrophage was substantially suppressed by 7 extracts namely, *Trigonella foenugricium*, *Calligonum comosum*, *Silene succulenta*, *Bougainvillea glabra*, *Mirabilis jalapa*, *Gomphrena celosoids* and *Silene nocturna*. TNF- α was decreased by percentage range from 3.8 to 85.8% and *Trigonella foenugricium* extract showed the highest inhibition of TNF- α release. All extracts except *Trigonella foenugricium*, *Salsola schweinfurthii*, *Silene succulenta* and *Mirabilis jalapa* significantly inhibited COX-2 production from stimulated macrophage. Moreover, evaluating the potential antioxidant activity of these extracts showed that *Trigonella foenugricium*, *Salsola schweinfurthii*, *Calligonum comosum*, *Bogonvillea glabra* and *Mirabilis jalapa* exhibited some antioxidant activities. Taken together, our results suggest that some of these extracts may have a considerable antiinflammatory and antioxidant effects and may be a potential therapeutic choice in the treatment of inflammatory diseases." As taken from Ghonime M et al. 2015. *Immunol. Invest.* 44(3), 237-52. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25564700>

"BACKGROUND: Several experimental and clinical studies support beneficial effects of *Trigonella foenum-graecum* (fenugreek) in the management of metabolic diseases and inflammatory disorders. OBJECTIVES: The purpose of this study was to examine the effect of *T. foenum-graecum* seed extract in reducing the metabolic and inflammatory alternations associated with menopause. MATERIALS AND METHODS: In this experimental study, 49 rats were divided into seven groups: (I) sham-control, (II) ovariectomized-control, (III and IV) ovariectomized treated with 50 and 150 mg/kg of *T. foenum-graecum* seed ethanolic extract, (V and VI) ovariectomized treated with 50 and 150 mg/kg of *T. foenum-graecum* hexanic extract, (VII) ovariectomized-positive control treated with 10 μ g/kg of estradiol. The extracts were injected intraperitoneally one day after ovariectomy and the treatments were lasted for 42 days. RESULTS: Fasting blood glucose and body weight gain increased significantly in the ovariectomized-control group compared with that in the sham animals ($P < 0.05$). Administration of estradiol and *T. foenum-graecum* (50 and 150 mg/dL of hexanic extract and 150 mg/kg of ethanolic extract) significantly diminished the increase in glucose and body weight ($P < 0.05$). The serum level of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) in the ovariectomized control group was significantly higher than those in the sham animals ($P < 0.05$). Both hexanic and ethanolic extracts as well as estradiol were able to decrease level of these cytokines in the serum of ovariectomized rats ($P < 0.05$). CONCLUSIONS: The results of the present study show that administration of *T. foenum-graecum* corrects metabolic and inflammatory alterations associated with ovariectomy and has a potential for the management of menopause." As taken from Abedinzade M et al. 2015. *Iran. Red Crescent Med. J.* 17(11), e26685. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26732240>

"Many cellular damages either in normal or cancerous tissues are the outcome of molecular events affected by ionizing radiation. T-cells are the most important among immune system agents and are used for biological radiation dose measurement in recommended standard methods. The herbs with immune modulating properties may be useful to reduce the risk of the damages and subsequently the diseases. The T-cells as the most important immune cells being targeted for biological dosimetry of radiation. This study proposes a flowcytometric-method based on fluorescein isothiocyanate- and propidium iodide (PI)-labeled annexin-V to assess apoptosis in blood T-cells after irradiation in both presence and absence of fenugreek extract. T-cells peripheral

blood lymphocyte isolated from blood samples of healthy individuals with no irradiated job background. The media of cultured cells was irradiated 1-h after the fenugreek extract was added. The number of apoptotic cells was assessed by annexin-V protocol and multicolor flowcytometry. An obvious variation in apoptotic cells number was observed in presence of fenugreek extract (>80%). The results suggest that fenugreek extract can potentiate the radiation induced apoptosis or radiation toxicity in blood T-cells ($P < 0.05$)." As taken from Tavakoli MB et al. 2015. J. Med. Signals Sci. 5(3), 176-81. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26284174>

"A group of 11 medicinal plants, including *Lavandula pubescens*, *Trigonella foenugricium*, *Salsola schweinfurthii*, *Calligonum comosum*, *Silene succulenta*, *Silene villosa*, *Bogonvillea glabra*, *Cakile maritime*, *Gomphrena celosoids*, *Mirabilis jalapa*, and *Silene nocturna* growing in Egypt, were extracted and examined for their immunomodulatory and antioxidant activities. RAW 264.7 cells were recruited to investigate the immunomodulatory effect through multiple parameters analysis. First, the proliferation index of macrophages cells was evaluated revealing that *Trigonella foenugricium*, *Silene succulenta* and *Silene villosa* have a significant cytotoxic effect on RAW cells. Interestingly, we observed enhancement of macrophages phagocytic function of by all extracts except *Cakile maritime*, *Gomphrena celosoides* and *Silene nocturna*. Afterwards, macrophages were challenged by incubation with LPS and the effect of various extracts on inflammatory responses was investigated; the generation of NO from activated macrophage was substantially suppressed by 7 extracts namely, *Trigonella foenugricium*, *Calligonum comosum*, *Silene succulenta*, *Bougainvillea glabra*, *Mirabilis jalapa*, *Gomphrena celosoides* and *Silene nocturna*. TNF- α was decreased by percentage range from 3.8 to 85.8% and *Trigonella foenugricium* extract showed the highest inhibition of TNF- α release. All extracts except *Trigonella foenugricium*, *Salsola schweinfurthii*, *Silene succulenta* and *Mirabilis jalapa* significantly inhibited COX-2 production from stimulated macrophage. Moreover, evaluating the potential antioxidant activity of these extracts showed that *Trigonella foenugricium*, *Salsola schweinfurthii*, *Calligonum comosum*, *Bogonvillea glabra* and *Mirabilis jalapa* exhibited some antioxidant activities. Taken together, our results suggest that some of these extracts may have a considerable antiinflammatory and antioxidant effects and may be a potential therapeutic choice in the treatment of inflammatory diseases....." As taken from Ghonime M et al. 2015. Immunol. Invest. 44(3), 237-52. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25564700>

"/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Immunomodulatory activity of aqueous extract of *Trigonella foenum graecum* L., a widely used medicinal and dietary herb, was evaluated in male Swiss albino mice. Mice were treated with three doses of extract (50, 100 and 250 mg/kg body weight per os) for 10 days. Body weight, relative organ weight, cellularity of lymphoid organs, delayed type of hypersensitivity (DTH) response, plaque-forming cell (PFC) assay, hemagglutination titer (HT), quantitative hemolysis of SRBC (QHS) assay, phagocytosis, and lymphoproliferation were studied in various groups of animals. At doses of 50 and 100 mg/kg, a significant increase ($p < 0.05$) in relative organ weight of thymus was observed but there was no effect on kidney and spleen weights. Liver weight also increased significantly at doses of 100 and 250 mg/kg. However, no elevation in the levels of liver function test (LFT) enzymes was observed. As regards lymphoid organ cellularity, spleen recorded no significant increase at any dose, whereas cellularities of thymus and bone marrow were significantly increased. *T. foenum graecum* extract elicited a significant ($p < 0.001$) increase in the DTH response at doses of 50 and 100 mg/kg, but the change at higher dose of 250 mg/kg was not statistically significant. Humoral immunity as measured by PFC showed an elevated response at a dose of 100 mg/kg, but at 50 and 250 mg/kg, no significant effect was observed. In the HT test, plant extract also showed modulatory effect at all the doses. Plant extract elicited a significant increase in phagocytic index and phagocytic capacity of macrophages. Stimulatory response of plant extract was also observed in lymphoproliferation assay but the response was weak. Overall, *T. foenum graecum* 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 Ayurvedic and Unani drugs.[Bin-Hafeez B et al; Int Immunopharmacol 3 (2): 257-65 (2003)] **PEER REVIEWED**

As taken from HSDB, 2016

"This review focuses on contact dermatitis as an adverse effect of a selection of topically used herbal medicinal products for which the European Medicines Agency has completed an evaluation up to the end of November 2013 and for which a Community herbal monograph - now (since 2014) called a 'European Union herbal monograph' - has been produced. The present part 4 addresses species from *Solidago virgaurea* L. to *Vitis vinifera* L." As taken from Minciullo PL et al. 2017. Contact Dermatitis 77(2), 67-87. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28543097>

"Dermatological testing of herbal preparations and extracts of fenugreek for use in medicinal or cosmetic products appears not to have been reported. However, the fragrance raw material Fenugreek Absolute, when applied undiluted to the backs of hairless mice and swine, was found not to be irritating. When it was applied full strength to intact or abraded rabbit skin for 24 h under occlusion, it was moderately irritating. Tested at 2% pet., it produced no irritation after a 48-h closed-patch test on human subjects. A maximization test carried out on 27 volunteers at a concentration of 2% pet. produced no sensitization reactions. No phototoxic effects were reported for undiluted fenugreek absolute on hairless mice and swine (233)." As taken from Minciullo PL et al. (2017) Contact Dermatitis, 77: 67-87. Pubmed, 2017 available at: <https://www.ncbi.nlm.nih.gov/pubmed/?term=28543097>

"Adverse dermatological reactions following the use of fenugreek-containing products are only rarely reported. These are usually IgE-mediated reactions (219, 234, 235) that occur after ingestion or inhalation. Cross-reactions between fenugreek and other plants from the legume family, such as peanut, soy, lupin, lentil, pea, bean, and chickpea (219, 234), have been shown, as has sensitization to both fenugreek and coriander (*Elettaria cardamomum* Maton, family Zingiberaceae) (235, 236). Sensitization to fenugreek has also occurred after skin application of a fenugreek-containing product. The patient, who was affected by chronic asthma, developed numbness of the head, facial angioedema and wheezing after application of a fenugreek paste to her scalp as a treatment for dandruff (234). A 32-year-old female who was taking a herbal preparation made from fenugreek seeds to improve lactation developed toxic epidermal necrolysis (237). She had taken fenugreek for a similar purpose after the birth of her previous child, without incident." As taken from Minciullo PL et al. (2017) Contact Dermatitis, 77: 67-87. Pubmed, 2017 available at: <https://www.ncbi.nlm.nih.gov/pubmed/?term=28543097>

"Fenugreek (*Trigonella foenum-graecum*) is a food product that belongs to the Leguminosae family along with other legumes. It has been used in India, Greece, and Egypt for culinary and medical purposes since ancient times, and today, fenugreek is used for flavoring foods, dyes, and drugs throughout the world. Many members of the Leguminosae family have been associated with allergies including soybean, green pea, and peanut. Fenugreek is also included in this family and may result in allergic reactions. Two cases of anaphylaxis have been described in children after ingestion of curry and pastes that contain fenugreek, although the true nature of the causative agent was unclear. We report the first case of fenugreek anaphylaxis in a pediatric patient defined by skin testing, immunoglobulin E ImmunoCAP assays, and clear ingestion." As taken from Joseph NI et al. 2018. Allergy & Rhinology 9, 1-3. Available at <http://journals.sagepub.com/doi/pdf/10.1177/2152656718764134>

"Plant defense stimulators (PDSs) rely on the activation of plant innate immunity in order to protect crops against various pests. These molecules are thought to be a safer alternative to classical plant protection products. Given that innate immune systems share common features in plants and vertebrates, PDS can potentially cross-react with innate immunity of non-target organisms. To test this hypothesis, we studied effects of the commercial PDS Stifenia (FEN560), which is composed of crushed fenugreek seeds. We tested various concentrations of Stifenia (0.03-1 mg mL⁻¹) on human

peripheral blood mononuclear cells and checked, 20 h later, cell metabolic activity (MA) using XTT assay, cell death by flow cytometry analysis, and IL-1 β inflammatory cytokine released in the culture medium using ELISA. Stifenia induced a general decrease of the cell MA, which was concomitant with a dose-dependent release of IL-1 β . Our results highlight the activation of human immune cells. The inflammatory effect of Stifenia was partially inhibited by pan-caspase inhibitor. Accordingly, Stifenia induced the release of p20 caspase-1 fragment into the culture medium suggesting the involvement of the NLRP3 inflammasome. Furthermore, we observed that Stifenia can induce cell death. We also tested the effect of Stifenia on Zebrafish larvae. After 24 h of exposure, Stifenia induced a dose-dependent IL-1 β and TNF α gene expression. The human-cell-based approach developed in this work revealed a high sensitivity concerning inflammatory properties of a plant protection product. These tests could be routinely used to screen the potential adverse effects of this type of compounds. Finally, our results suggest a potential danger of using extensively certain PDS for crop protection.” As taken from Teyssier L et al. 2017. *Front. Public Health* 5, 74. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28484691>

CAS-Nr.	Substance	Owner	Endpoint	Reference
84625-40-1	Fenugreek Absolute	Robertet S.A	In vitro eye irritation	OECD 492, ROBERTET, 2018
84625-40-1	Fenugreek Absolute	Robertet S.A	Ames Test (Bacterial Reverse Mutation Test)	OECD 471, ROBERTET, 2018

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Fenugreek extract (84625-40-1) was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of Fenugreek extract (84625-40-1) when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	52	JTI KB Study Report(s)
In vitro cytotoxicity	52	JTI KB Study Report(s)

“Fenugreek and Balanites are two plants commonly used in Egyptian folk medicine as hypoglycemic agents. In the present study, the effects of 21 days oral administration of Fenugreek seed and Balanites fruit extracts (1.5 g/kg bw) on the liver and kidney glycogen content and on some key liver enzymes of carbohydrate metabolism in STZ-diabetic rats were studied. In addition, the effects of these two plant extracts on the intestinal alpha-amylase activity in vitro and starch digestion and absorption in vivo were also examined. Results indicated that single injection of STZ (50 mg/kg bw) caused 5-folds increase in the blood glucose level, 80% reduction in serum insulin level, 58% decrease in liver glycogen and 7-folds increase in kidney glycogen content as compared to the normal levels. The activity of glucose-6-phosphatase was markedly increased, whereas, the activities of both glucose-6-phosphate dehydrogenase and phospho-fructokinase were significantly decreased in the diabetic rat liver. Administration of Fenugreek extract to STZ-diabetic rats reduced blood glucose level by 58%, restored liver glycogen content and significantly decreased kidney glycogen as well as liver glucose-6-phosphatase activity. [...] On the other hand, our results

demonstrated that both the Fenugreek and Balanites extracts were able to in vitro inhibit alpha-amylase activity in dose-dependent manner. Fenugreek was more potent inhibitor than Balanites. This inhibition was reversed by increasing substrate concentration in a pattern which complies well with the effect of competitive inhibitors. Furthermore, this in vitro inhibition was confirmed by in vivo suppression of starch digestion and absorption induced by both plant extracts in normal rats. These findings suggest that the hypoglycemic effect of Fenugreek and Balanites is mediated through insulinomimetic effect as well as inhibition of intestinal alpha-amylase activity.” As taken from Gad MZ et al. Mol Cell Biochem. 2006 Jan; 281(1-2):173-83. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16328970&query_hl=34&itool=pubmed_DocSum

“The in vivo hypoglycaemic activity of a dialysed fenugreek seed extract (FSE) was studied in alloxan (AXN)-induced diabetic mice and found to be comparable to that of insulin (1.5 U kg(-1)). FSE also improved intraperitoneal glucose tolerance in normal mice. The mechanism by which FSE attenuated hyperglycaemia was investigated in vitro. FSE stimulated glucose uptake in CHO-HIRc-mycGLUT4eGFP cells in a dose-dependent manner. This effect was shown to be mediated by the translocation of glucose transporter 4 (GLUT4) from the intracellular space to the plasma membrane. These effects of FSE on GLUT4 translocation and glucose uptake were inhibited by wortmannin, a phosphatidylinositol 3-kinase (PI3-K) inhibitor, and bisindolylmaleimide 1, a protein kinase C (PKC)-specific inhibitor. In vitro phosphorylation analysis revealed that, like insulin, FSE also induces tyrosine phosphorylation of a number of proteins including the insulin receptor, insulin receptor substrate 1 and p85 subunit of PI3-K, in both 3T3-L1 adipocytes and human hepatoma cells, HepG2. However, unlike insulin, FSE had no effect on protein kinase B (Akt) activation. These results suggest that in vivo the hypoglycaemic effect of FSE is mediated, at least in part, by the activation of an insulin signalling pathway in adipocytes and liver cells.” As taken from Vijayakumar MV et al. Br J Pharmacol. 2005 Sep; 146(1):41-8. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15980869&query_hl=34&itool=pubmed_DocSum

“Trigonelline and fenugreek infusion have been shown to have hypoglycemic effects in animals. When fed both before and after experimental diabetes induction, fenugreek has antidiabetic activity in rats.”

“Its medicinal uses include fever reducing and treating mouth ulcers, bronchitis, chronic coughs, and chapped lips, for milk promotion, as digestive aid, for cancers, and others.”

As taken from Khan IA and Abourashed EA, 2010.

Aqueous extract of *Trigonella foenum graecum* (fenugreek) prevents cypermethrin-induced hepatotoxicity and nephrotoxicity (Abstract). Cypermethrin (CM) is an important type II pyrethroid pesticide used extensively in pest control and is reported to cause hepatic and renal toxicity. Oxidative stress and lipid peroxidation (LPO) has been implicated in the toxicology of pyrethroids. Fenugreek is known for its antitoxic and antioxidant potential. We have investigated the protective effect of aqueous extract of germinated fenugreek seeds in CM-induced hepatic and renal toxicity. Male Wistar rats were treated with 1/10 LD(50) (25 mg/kg body weight) of 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. There was 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. Phytochemicals present in fenugreek could play an important role in ameliorating the pesticide-

induced toxicity. As taken from Sushma & Devasena; Hum Exp Toxicol. 2010, Apr; 29(4):311-9. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/20147568>(CAS 68990-15-8)

Efficacy of dietary supplementation with botanicals on carbohydrate metabolism in humans

(Abstract). Botanical products are widely used in nutritional supplementation for promotion of health or prevention of diseases. With the high prevalence of obesity and type 2 diabetes, abnormalities in carbohydrate metabolism are common in the general population and obtaining glycemic control is important in reducing the complications of diabetes. If shown to be effective, botanical products have a unique position in potentially aiding the general public in regard to obesity and diabetes. They can be obtained "over-the-counter" and may have less side effects compared to many synthetic drugs. Although most of the popular botanicals have a long history in folk medicine, there is paucity of data regarding their efficacy and safety, particularly as it relates to human studies. In this review, we discuss the data that was available in the literature for nine botanicals that are frequently promoted to help manage blood glucose. They are Bitter Melon (*Momordica charantia*), Fenugreek (*trigonella foenum graecum*), Gymnema Sylvestre, Ivy Gourd (*Coccinia indica*), Nopal or Prickly Pear Cactus (*Opuntia streptacantha*), Ginseng, Aloe Vera, Russian Tarragon (*Artemisia dracunculus*), and Garlic (*Allium sativum*). The discussion is emphasized on the clinical aspect of these botanicals. Due to the lack of sufficient evidence from clinical studies for any of the botanicals reviewed, it is premature to actively recommend use of any particular herb to treat either glucose or other risk factors. Thus, well defined randomized clinical trials are warranted in this area. As taken from Cefalu et al.; Efficacy of dietary supplementation with botanicals on carbohydrate metabolism in humans; Endocr Metab Immune Disord Drug Targets. 2008, Jun; 8(2):78-81. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/18537692> (CAS 68990-15-8)

"Preliminary trials have suggested possible hypoglycaemic, hypolipidaemic and immunomodulatory properties of the fenugreek plant. Here, we evaluated and compared the efficacy of Egyptian fenugreek seed powder (FSP, 0.5 and 1.0 g/kg body weight) in alleviating the experimentally induced metabolic syndrome (in type 1 diabetic and obese rat models) and experimentally induced immunosuppression and delay in burn-healing (in cyclophosphamide (CP)-treated rats). FSP significantly alleviated ($P < 0.05-0.001$) most signs of the metabolic syndrome resulting from experimentally induced type 1 diabetes and obesity by 40-76 and 56-78 %, respectively, including hyperglycaemia, hyperlipidaemia, elevation in atherogenic indices, impairment of liver functions, severe changes in body weight and oxidative stress. Besides, FSP (especially the high dose) completely modulated the immunosuppressive activity of CP including leucopenia (resulting from neutropenia and lymphopenia), decrease in weights and cellularity of lymphoid organs, serum γ -globulin level, delayed type of hypersensitivity response and delay in the skin-burning healing process. FSP decreased the immunosuppressive activity of CP by 57-108 %. These beneficial effects of FSP were dose dependent in most cases, and FSP doses used here were considered safe in general. FSP was more efficient in alleviating the signs of the metabolic syndrome in the obese animals (over 9 %) than in the type 1 diabetic animals. Moreover, the immunostimulant activity of fenugreek seeds exceeded their anti-metabolic syndrome activity by 15-24 %. In conclusion, fenugreek seeds may be useful not only as a dietary adjunct for the control of the metabolic syndrome in diabetic/obese patients, but also as an immunostimulant in immunocompromised patients such as those under chemotherapeutic interventions". As taken from Ramadan G et al 2011. Br. J. Nutr. 105, 995-1004. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/21205429?dopt=AbstractPlus>.

"CONTEXT AND OBJECTIVE: The herb fenugreek, *Trigonella foenum-graecum* Linn (Fabaceae), seeds have been traditionally used in the treatment of diabetes but its effect on oxidative stress and pro-inflammatory cytokines in the improvement of exocrine function of diabetes has not been studied. The effect of hydroalcoholic extract of *Trigonella foenum-graecum* seeds (HEF) on alloxan-induced type-II diabetic rat model was investigated. MATERIALS AND METHODS: Effect of HEF (500, 1000, and 2000 mg/kg), glimepiride (4 mg/kg), and combination of HEF (500 mg/kg) + glimepiride (2 mg/kg), on alloxan-induced diabetic rats was evaluated by assaying

(blood glucose, serum protein, glycosylated hemoglobin, muscle and liver glycogen, glucose uptake by diaphragm, liver glucose transport, serum pancreatic enzymes (α -amylase, lipase), pro-inflammatory cytokines (TNF- α , IL-6), antioxidant enzymes [glutathione (GSH), superoxide dismutase (SOD)], lipid peroxides (liver and pancreas), and histoarchitecture (liver, pancreas). RESULTS: Treatment with HEF (at different doses), glimepiride, and HEF + glimepiride increased body weight and glucose uptake, reduced plasma glucose, glycosylated hemoglobin, liver glucose transport, pro-inflammatory cytokines, pancreatic enzymes and restored depleted glycogen (muscle, liver) and total protein significantly ($p < 0.01$) and dose dependently, including prevention of lipid peroxidation and restoration of GSH and SOD (liver and pancreas). Treatment with HEF + glimepiride potentiated hypoglycemic activity of glimepiride. Histoarchitecture of liver and pancreas showed marked improvement. CONCLUSION: Present experimental findings suggest that HEF possesses promising hypoglycemic activity, presumably by amelioration of oxidative stress and pro-inflammatory cytokines. HEF may be useful as an adjuvant with clinically effective antidiabetic drugs in the management of type-II diabetes." As taken from Joshi DV et al. 2015. Pharm Biol. 53(2), 201-11. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25339548>

"AIM: Combined use of herbs and drugs may result in clinically important herb-drug interactions. The majorities of these interactions are thought to be metabolism-based and involve induction or inhibition of cytochrome P450 (CYP). The current study was designed to investigate the effect of some commonly used herbs on rat CYP2C11 gene expression and metabolic activity. METHODS: Wistar rats were treated for 7 days with increasing doses of 3 herbs; Nigella sativa, Trigonella foenum-graecum, and Ferula asafoetida. Thereafter, CYP2C11 mRNA and protein levels were determined by real-time polymerase chain reaction (RT-PCR) and western blot analyses, respectively. In vitro metabolic activity of CYP2C11 was performed on rat hepatic microsomes using tolbutamide as specific substrate. RESULTS: Our results showed that all the 3 herbs significantly inhibited the mRNA and protein expression levels of CYP2C11 in a dose-dependent manner. Furthermore, the in vitro enzyme metabolic activity study showed a significant decrease in the formation of 4-hydroxy-tolbutamide, a tolbutamide metabolite, at the higher doses. The inhibitory effects of the investigated herbs on rat CYP2C11 was in the order: Nigella Sativa > Trigonella foenum-graecum > Ferula asafoetida. CONCLUSIONS: The 3 herbs are strong inhibitor of CYP2C11 expression, which can lead to an undesirable pharmacological effect of clinically used CYP2C11 substrate drugs with a low therapeutic index." As taken from Korashy HM et al. 2015. Drug Res. (Stuttg.) 65(7), 366-72. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25099385>

"Trigonella foenum-graecum generally known as fenugreek, has been normally cultivated in Asia and Africa for the edible and medicinal values of its seeds. Fenugreek leaves and seeds have been used widely for therapeutic purposes. Fenugreek seed is recognized to show anti-diabetic and anti-nociceptive properties and other things such as hypocholesterolaemic, and anti-cancer. Diosgenin is a steroidal saponin from therapeutic herbs, fenugreek (*T. foenum-graecum* L.), has been well-known to have anticancer properties. Telomerase activity is not identified in usual healthy cells, while in carcinogenic cell telomerase expression is reactivated. Therefore telomerase illustrates a promising cancer therapeutic target. We deliberate the inhibitory effect of pure diosgenin and fenugreek extract diosgenin on human telomerase reverse transcriptase gene (hTERT) expression which is critical for telomerase activity. MTT-assay and qRT-PCR analysis were achieved to discover cytotoxicity effects and hTERT gene expression inhibition properties, separately. MTT results exhibited that IC₅₀ for pure diosgenin were 47, 44 and 43 μ M and for fenugreek extract diosgenin were 49, 48 and 47 μ M for 24, 48 and 72 h after treatment. Culturing cells with pure diosgenin and fenugreek extract diosgenin treatment caused in down regulation of hTERT expression. These results indicate that pure and impure diosgenin prevents telomerase activity by down regulation of the hTERT gene expression in A549 lung cancer cell line, with the difference that pure compound is more effective than another." As taken from Rahmati-Yamchi M et al. 2014.

Mol. Biol. Rep. 41(9), 6247-52. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24973886>

“BACKGROUND: The plant *Trigonella foenum-graecum* (TFG) is used as antidiabetic and diuretic. In order to ascertain antioxidant potential of leaf (early and mature) and seed of TFG, total phenolics, free radical scavenging assay, superoxide anion radical scavenging activity, reducing power, lipid peroxidation, ferric thiocyanate assay, hydroxyl radical scavenging activity and DNA damage protective activities were determined. The study was further carried out to assay the antimicrobial activity and HPLC analysis of plant parts. RESULTS: Ethanol extracts of leaf (early and mature) exhibited a high content of phenolics (54.79 and 41.28 g kg⁻¹) GAE when it was compared with seed extract (23.85 g kg⁻¹) GAE. Results showed that mature TFG leaf extract had the lowest IC₅₀ for the free radical scavenging assay (IC₅₀ = 2.23 mg mL⁻¹), superoxide anion radical scavenging activity (IC₅₀ = 2.71 mg mL⁻¹), hydroxyl radical scavenging activity (IC₅₀ = 17.30 mg mL⁻¹) and highest reducing power (10.14 ascorbic acid equivalents mL⁻¹). However, the ethanol seed extract showed the maximum inhibition of lipid peroxidation and the ferric thiocyanate assay. Mature leaf also showed the maximum DNA damage protection activity and higher concentration of phytochemicals. CONCLUSION: The results showed that the mature TFG leaf had a higher antioxidant activity, which may be due to the presence of total phenolics. It may be used in herbal drugs or as a nutritional supplement.” As taken from Singh P et al. 2014. J. Sci. Food Agric. 94(12), 2497-504. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24464686>

“One nursing mother developed toxic epidermal necrolysis thought to be cause [sic] by her intake of fenugreek as a galactagogue.”

“Caution should be used in giving high dosages to women with diabetes mellitus or those taking warfarin.”

“Perhaps its most unusual side effect is the imparting an odor of maple syrup to the urine, sweat, feces, and possibly breastmilk by the sotonon in fenugreek.”

“/EPIDEMIOLOGY STUDIES/ ... The aim of our study was to investigate the effects of a repeated administration of a fenugreek seed extract on the eating behavior of overweight subjects. Thirty-nine healthy overweight male volunteers completed a 6-week double-blind randomized placebo-controlled parallel trial of a fixed dose of a fenugreek seed extract. Main endpoints were energy intake (dietary records and meal test), weight, fasting and post-absorptive glucose and insulin, appetite/satiety scores and oxidative parameters. Daily fat consumption, expressed as the ratio fat reported energy intake/total energy expenditure (fat-REI/TEE), was significantly decreased in our overweight subjects administered the fenugreek seed extract relative to those receiving the placebo (fat-REI/TEE 0.26 +/- 0.02 vs. 0.30 +/- 0.01, respectively; P = 0.032). We also observed a significant decrease in the insulin/glucose ratio in subjects treated with fenugreek seed extract relative to the placebo group (0.89 +/- 0.09 vs. 1.06 +/- 0.10 mUI/mmol, respectively; P = 0.044). No significant effect was observed on weight, appetite/satiety scores or oxidative parameters. The repeated administration of a fenugreek seed extract slightly but significantly decreased dietary fat consumption in healthy overweight subjects in this short-term study.[Chevassus H et al; Eur J Clin Pharmacol 66 (5): 449-55 (2010)] **PEER REVIEWED**

As taken from HSDB, 2016

“To assess the metabolically beneficial effects of fenugreek (*Trigonella foenum-graecum*), C57BL/6J mice were fed a low- or high-fat diet for 16 weeks with or without 2% (w/w) fenugreek supplementation. Body weight, body composition, energy expenditure, food intake, and insulin/glucose tolerance were measured regularly, and tissues were collected for histological and biochemical analysis after 16 weeks of diet exposure. Fenugreek did not alter body weight, fat mass, or food intake in either group, but did transiently improve glucose tolerance in high fat-fed mice. Fenugreek also significantly improved high-density lipoprotein to low-density lipoprotein ratios in high fat-fed mice without affecting circulating total cholesterol, triglycerides, or glycerol levels.

Fenugreek decreased hepatic expression of fatty acid-binding protein 4 and increased subcutaneous inguinal adipose tissue expression of adiponectin, but did not prevent hepatic steatosis. Notably, fenugreek was not as effective at improving glucose tolerance as was four days of voluntary wheel running. Overall, our results demonstrate that fenugreek promotes metabolic resiliency via significant and selected effects on glucose regulation, hyperlipidemia, and adipose pathology; but may not be as effective as behavioral modifications at preventing the adverse metabolic consequences of a high fat diet.” As taken from Knott EJ et al. 2017. *Sci. Rep.* 7(1), 12770. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28986580>

“BACKGROUND: The objective of this systematic review was to assess available scientific data on the efficacy and safety of medicinal food plants for the treatment of impaired glucose tolerance. METHODS: We included randomized controlled trials (RCTs) with a minimum follow-up period of 6 weeks. The diagnosis was determined by fasting plasma glucose values after two-hour oral glucose tolerance testing (OGTT). Two authors independently extracted data and evaluated bias. The Cochrane tool of risk of Bias Tool was used. RESULTS: This review included ten trials. Most studies were highly biased as data were incomplete or reporting was selective. The two-hour fasting plasma glucose after the curcumin extract intervention showed statistical significance after 3, 6 and 9months: $p < 0.01$. Also, glycosylated haemoglobin levels A1c (HbA1c) values after curcumin extract intervention showed statistical significance after 3, 6 and 9months: $p < 0.01$. Insulin resistance (HOMA-IR) after curcumin extract intervention showed statistical significance after 6months and after 9months: $p < 0.05$ and $p < 0.01$. CONCLUSIONS: Curcumin has shown the confident results to be effective for the treatment of impaired glucose tolerance. Fenugreek and flaxseed may also be effective, but due to low quality of these studies the results must be interpreted with caution.” As taken from Demmers A et al. 2017. *Diabetes Res. Clin. Pract.* 131, 91-106. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28750220>

“The need of antibiotics obviate in treated cancer patients when suppression of immune system leads to secondary infections development. The objective of the present study was to evaluate the antibacterial activity and biochemical profiling of various medicinal plants *Trigonella foenum-graecum*, *Ocimum basilicum*, *Olea europaea*, *Mentha longifolia* and *Boswellia sacra* against clinical isolates of blood cancer cases. Crude plant extracts in ethanol and methanol were used to test antimicrobial activity through disc diffusion method. Biochemical profiling identified the presence of Gallic acid, parahydroxy benzoic acid, vanillic acid, syringic acid and ferulic acid by high performance liquid chromatography (HPLC). *Boswellia sacra* showed the maximum antibacterial activity against *Streptococcus viridian* with 12.4 mm inhibition zone. *Trigonella foenum-graecum* showed the maximum antibacterial activity against *Salmonella* Group B 11.8 mm with crude extracts in methanol. The antibacterial activity showed that *Streptococcus viridian* and *Corynebacterium* were more inhibited bacteria but *Klebsiella pneumonia* was found more resistant. Total phenolics analysis by HPLC revealed that parahydroxy benzoic acid was the major phenolic acid found in *Olea europaea* with 797.8 ng/g. The highest concentration of Gallic acid was found in *Ocimum basilicum* with 547.02 ng/g. These results indicated that these medicinal plants may serve as antimicrobial agents against clinical bacterial isolates from cancer patient successfully.” As taken from Farhan A AJ et al. 2017. *Biosci. Biotech. Res. Asia* 14(4), 1277-1284. Available at <http://www.biotech-asia.org/vol14no4/antimicrobial-activity-and-biochemical-profiling-of-selected-medicinal-plants-against-blood-cancer-clinical-isolates/>

“Objective: This study was focussed on an evaluation of antibacterial activities of aqueous and alcoholic extracts of commonly consumed spices, namely, Ajwain (*Trachyspermum ammi*), Coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*), fennel (*Foeniculum vulgare*), and Fenugreek (*Trigonella foenum-graecum*). Methods: This study includes the antibacterial effects of spices against six bacterial strains, namely, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* to compare their antibacterial effects by the paper disc agar diffusion method with three antibiotics such as amikacin, chloramphenicol, and vancomycin. Results: According to findings, it is determined that inhibitory activity was detected on aqueous and alcoholic extracts of Ajwain, aqueous extract of cumin and

on alcoholic mixed spice sample. Conclusions: Among the five spices tested, only aqueous extracts of Ajwain and cumin exhibited antibacterial activity against one organism (*S. aureus*). Comparatively the alcoholic extracts gave a better response than the aqueous extracts. The effectiveness of the antibacterial activity was recorded better for the mixed spice samples when compared to that of the individual spices. This clearly emphasizes that the combined effect of the spices exhibited better antibacterial activity and the kill rate of the bacterial strains is higher relatively.” As taken from Salma S et al. 2018. Asian J. Pharm. Clin. Res. 11(2), 252-254. Available at <https://innovareacademics.in/journals/index.php/ajpcr/article/view/22652>

“Aim: To evaluate antibacterial efficacy of Chlorhexidine gluconate (2%), Fenugreek (*Trigonella foenum*) and Fennel (*Foeniculum vulgare*) as intracanal irrigant on isolated bacteria from infected primary tooth. Materials and methods: Thirty patients were selected based on inclusion and exclusion criteria. After rubber dam isolation access opening was done and collection of sample using absorbent paper point was done. Samples were processed for microbiological procedure and isolation of different species of bacteria was done. All the individual species were subjected to antibacterial sensitivity for three irrigants. Results: Different species of obligatory and facultative anaerobes were isolated mainly *Peptostreptococcus* colonies of obligatory anaerobic gram positive cocci followed by facultative anaerobe *E. faecalis*, followed by gram negative Bacilli such as *P. intermedia*, *Porphyromonas* species, *Bacteroides* species, and *Fusobacterium* species. Facultative Gram-positive anaerobic cocci such as *Streptococcus pyogenes*, *S. sobrinus*, and *Staphylococcus aureus* were also found but were comparatively less in number. These were subjected to antibacterial sensitivity against three irrigants. The results statistically analysed using Pearson’s Chi-square test for two non-parametric data and proportional comparisons were done using Z test for two sample proportion. Chlorhexidine was found most sensitive, followed by Fennel extract and least sensitive is Fenugreek Extract for facultative as well as obligatory anaerobes. Conclusion: The bacterial profile in infected primary teeth consists of mainly obligatory anaerobes *Peptostreptococcus* colonies, followed by *E. Faecalis* and black pigmented colonies. Amongst two herbal irrigant, fennel can act a potent herbal substitute for chlorhexidine as intracanal irrigant in infected primary teeth.” As taken from Gupta P et al. 2018. IJAR 4(2), 162-165. Available at <http://www.allresearchjournal.com/archives/2018/vol4issue2/PartC/4-2-5-588.pdf>

Record for *Trigonella foenum-graecum* L., seed, methanol extract (no CAS RN):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human leukemia cells	6.1 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 174,644,2015
IC50 - Inhibitor Concentration 50	In vitro	Human kidney	5.6 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 174,644,2015

IC50 - Inhibitor Concentration 50	In vitro	Human colon tumor	- 32.7 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 174,644,2015
IC50 - Inhibitor Concentration 50	In vitro	Human glioma	- 16.1 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 174,644,2015
ICLo - Inhibitor Concentration Low	In vitro	Human leukemia cells	- 10 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 174,644,2015

As taken from RTECS, 2017a

Record for *Trigonella foenum-graecum* L., seed, dichloromethane extract (no CAS RN):

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo - Inhibitor Concentration Low	In vitro	Human leukemia cells	- 10 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 174,644,2015

As taken from RTECS, 2017b

“Fenugreek is known since ancient times as a traditional herbal medicine of its multiple beneficial effects. Fenugreek's most studied and employed effect is its hypoglycemic property, but it can also be useful for the treatment of certain thyroid disorders or for the treatment of anorexia. The regulation of glucose homeostasis is a complex mechanism, dependent on the interaction of different types of hormones and neurotransmitters or other compounds. For the study of how diosgenin and fenugreek seeds modify insulin sensitivity, we used a rat insulin resistance model induced by high-fat diet. Diosgenin in three different doses (1mg/bwkg, 10mg/bwkg, and 50 mg/bwkg, respectively) and fenugreek seed (0.2 g/bwkg) were administered orally for 6 weeks. Insulin sensitivity was determined by hyperinsulinemic euglycemic glucose clamp method. Our research group found that although glucose infusion rate was not significantly modified in either group, the increased insulin sensitivity index and high metabolic clearance rate of insulin found in

the 1 mg/kg diosgenin and the fenugreek seed treated group suggested an improved peripheral insulin sensitivity. Results from the 10 mg/kg diosgenin group, however, suggest a marked insulin resistance. Fenugreek seed therapy results on the investigated anabolic hormones support the theory that, besides insulin and gastrointestinal peptides, the hypothalamic-hypopituitary axis regulated hormones synchronized action with IGF-1 also play an important role in the maintaining of normal glucose levels. Both diosgenin and fenugreek seeds are capable of interacting with substrates of the above-mentioned regulatory mechanisms, inducing serious hormonal disorders. Moreover, fenugreek seeds showed the ability to reduce the thyroid hormone levels at the periphery and to modify the T4/T3 ratio. It means that in healthy people this effect could be considered a severe side effect; however, in hypothyroidism this effect represents a possibility of alternative natural therapy." As taken from Kiss R et al. 2019. Biomed. Res. Int. 2019, 7213913. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31080828/>

"Fenugreek (*Trigonella foenum graecum*) seed extract is a bioactive ingredient of many food supplements. Hence, there is a need for systematic assessment of the quality of published toxicological studies for its use in human health, hazard consideration, and risk assessment. The aim of the present investigation was to determine the reliability of published toxicological studies of fenugreek seed by using ToxRTool (Toxicological data reliability assessment tool). A comprehensive systematic literature search was conducted in PubMed, EMBASE, Cochrane Library, CPCI-S, ICTRP, Ovid, and Google Scholar till October 2018. Each identified study was evaluated for its quality using the ToxRTool with outcomes such as combined score, weighted score, and reliability category by three independent raters. Correlations of various criteria groups with the combined score were evaluated by Pearson correlation and Kendall rank correlation coefficient. Inter-rater consistency was measured by Cronbach's alpha coefficient. The database searches initially yielded 436 results, of which 391 (89.67%) studies were "not assignable". The remaining 45 studies were included for quantitative analysis by ToxRTool. Based on the weighted score, 17 in-vivo, and 3 in-vitro studies were determined to be "Reliable Without Restriction" which were conducted according to international guidelines such as GLP. These studies have a significant difference ($p < 0.05$) for the combined and weighted score as compared to non-GLP studies. Remaining 28 in-vivo and 2 in-vitro studies were determined to be "Not Reliable." The GLP studies conducted with "identified study material" have a significant difference ($p < 0.0001$) between combined and weighted score as compared to studies which used "non-identified study material". For criteria group of ToxRTool I, III and V, the Pearson correlation with the combined score was found to be 0.875, 0.734 and 0.905, respectively and Kendall rank correlation coefficient was found to be 0.764, 0.551 and 0.752, respectively. Cronbach's alpha coefficient for combined score and weighted score were 0.920 and 0.887, respectively. In conclusion, the ToxRTool was found useful to identify seventeen toxicity studies of fenugreek seeds as "Reliable without Restrictions". These studies showed a broad margin of safety for the standardized extract of fenugreek seeds and can form a basis for toxicological risk assessment with reasonable certainty." As taken from Kandhare AD et al. 2019. Heliyon 5(4), e01536. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31049444/>

"Testosterone concentrations in males tend to decline with advancing age. Low testosterone, also known as androgen deficiency (AD), is associated with an increased risk of morbidity and mortality. Currently, the primary treatment for AD is testosterone replacement therapy (TRT), which may exacerbate pre-existing medical conditions. Therefore, the use of alternative options, such as herbs, spices, plants, or their extracts, has been explored as a potential treatment option for AD. The aim of this systematic review was to summarize and critically evaluate randomized controlled trials published on the efficacy of single herbal ingredients on testosterone concentrations, in addition to its fractions or binding proteins, in men (≥ 18 y). From the 4 databases searched, there were 13 herbs identified in 32 studies, published between 2001 and 2019. The main findings of this review indicate that 2 herbal extracts, fenugreek seed extracts and ashwagandha root and root/leaf extracts, have positive effects on testosterone concentrations in men. Also, some evidence exists for another herb and herbal extract, Asian red ginseng and forskohlii root extract. Overall, 9 out of

32 studies demonstrated statistically significant increases in testosterone concentrations. Moreover, 6 studies out of 32 were judged as having a low risk of bias. Current evidence is largely based on young, nonclinical populations, with 16 out of 32 studies using men <40 y of age. Conclusions are moderated by the paucity of research for many herbs, the variation in dosages and extracts used, small sample sizes, and the heterogeneity of study characteristics. Also, further research is required before definitive conclusions on efficacy and safety can be made.” As taken from Smith SJ et al. 2021. Adv. Nutr. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33150931/>

High doses (between 25 g and 100 g daily of powder of fenugreek seeds divided into two equal doses) have been reported to cause minor gastrointestinal symptoms such as diarrhoea and flatulence in 4 out of 10 cases.

Decreased thyroid hormone levels (T3, triiodothyronine) were reported in rodents treated with hydro-ethanolic extracts at 110 mg/kg per day and above; a NOAEL was not determined.

As taken from EMA,2021.

6. Functional effects on

6.1. Broncho/pulmonary system

“Allergic reactions, exacerbation of asthma, and a 14% decrease in serum potassium have been reported.”

As taken from LactMed, 2021. Record for CAS RN 68990-15-8.

“Decreased testosterone levels in men are often a normal sign of aging. Testosterone replacement therapy (TRT) is a well-established option for those with symptomatic hypogonadism related to low testosterone levels. Conversely, designer herbal supplements in the context of testosterone supplementation are poorly studied, yet remain popular among aging men who seek the well-known, often enhancing, effects of testosterone that involve muscle mass and sexual function/drive. In 2014, the Food and Drug Administration (FDA) issued a warning about the significant risk of venous clots secondary to testosterone product use. Testosterone-induced polycythemia is one of the proposed mechanisms for this increased clotting propensity. Increased thromboxane A2 receptor density on platelets and increased platelet aggregation have also been linked to testosterone treatment in men. Fenugreek extract is a common active ingredient in commercially available herbal supplements that are often marketed as testosterone enhancers. It is thought that certain fenugreek compounds inhibit aromatase and 5-alpha-reductase activity, leading to diminished testosterone breakdown. However, the efficacy and safety profile of this agent in its use for boosting testosterone levels are unclear. In this case report, we present a patient with new-onset, bilateral pulmonary embolism possibly associated with the daily use of fenugreek-containing testosterone supplements.” As taken from Nguyen SM et al. 2017. Cureus 9(8), e1545. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29018642>

6.2. Cardiovascular system

“The water extract has also been reported to have accelerating effects on the heartbeats of the isolated mammalian heart.”

As taken from Khan IA and Abourashed EA, 2010.

Effect of ginger (*Zingiber officinale* Rosc.) and fenugreek (*Trigonella foenumgraecum* L.) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease (Abstract). In a placebo-controlled study the effect of ginger and fenugreek was examined on blood lipids, blood sugar, platelet aggregation, fibrinogen and fibrinolytic activity. The subjects included in this study were healthy individuals, patients with coronary artery disease (CAD), and patients with non-insulin-dependent diabetes mellitus (NIDDM) who either had CAD or were without

CAD. [...] Fenugreek given in a dose of 2.5 g twice daily for 3 months to healthy individuals did not affect the blood lipids and blood sugar (fasting and post prandial). However, administered in the same daily dose for the same duration to CAD patients also with NIDDM, fenugreek decreased significantly the blood lipids (total cholesterol and triglycerides) without affecting the HDL-c. When administered in the same daily dose to NIDDM (non-CAD) patients (mild cases), fenugreek reduced significantly the blood sugar (fasting and post prandial). In severe NIDDM cases, blood sugar (both fasting and post prandial) was only slightly reduced. The changes were not significant. Fenugreek administration did not affect platelet aggregation, fibrinolytic activity and fibrinogen (Bordia A et al., 1997).

Fenugreek seeds reduce atherogenic diet-induced cholesterol gallstone formation in experimental mice (Abstract). Dietary hypocholesterolemic adjuncts may have a beneficial role in the prevention and treatment of cholesterol gallstones (CGS). In this investigation, fenugreek (*Trigonella foenum-graecum*) seed was evaluated for this potential on the experimental induction of CGS in laboratory mice. CGS was induced by maintaining mice on a lithogenic diet (0.5% cholesterol) for 10 weeks. Fenugreek seed powder was included at 5%, 10%, and 15% of this lithogenic diet. Dietary fenugreek significantly lowered the incidence of CGS in these mice; the incidence was 63%, 40%, and 10% in the 5%, 10%, and 15% fenugreek groups, respectively, compared with 100% in the lithogenic control. The antilithogenic influence of fenugreek is attributable to its hypocholesterolemic effect. Serum cholesterol level was decreased by 26%-31% by dietary fenugreek, while hepatic cholesterol was lowered by 47%-64% in these high cholesterol-fed animals. Biliary cholesterol was 8.73-11.2 mmol/L as a result of dietary fenugreek, compared with 33.6 mmol/L in high-cholesterol feeding without fenugreek. Cholesterol saturation index in bile was reduced to 0.77-0.99 in fenugreek treatments compared with 2.57 in the high-cholesterol group. Thus, fenugreek seed offers health-beneficial antilithogenic potential by virtue of its favourable influence on cholesterol metabolism. As taken from Reddy and Srinivasan. *Can J Physiol Pharmacol.* 2009, Nov; 87(11):933-43. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/19935901>(CAS 68990-15-8)

Antihyperglycemic effect of *Trigonella foenum-graecum* (fenugreek) seed extract in alloxan-induced diabetic rats and its use in diabetes mellitus: a brief qualitative phytochemical and acute toxicity test on the extract (Abstract). The effects of ethanol extract of *Trigonella foenum-graecum* (Fenugreek) seeds on the blood glucose levels in alloxan-induced diabetic rats at different doses (2 g/kg, 1 g/kg, 0.5 g/kg and 0.1 g/kg) were studied. The hypoglycemic effect of extract was compared with that of the standard antidiabetic drug (glibenclamide, 4 mg/kg) single dose. The extract showed significant activity against the diabetic state induced by alloxan but the intensity of hypoglycemic effect varied from dose to dose. The most effective dose recognized was 1 g/kg but that is still lower than the standard antidiabetic drug. No acute toxicity was observed for ethanol extract of *T. foenum-graecum* seed when it was administered orally at high dose level (3 g/kg body weight), which is higher than effective antihyperglycemic dose, and closely observed for 24 hrs for any mortality and next 10 days for any delayed toxic effects on gross behavioral activities. Phytochemical group tests were also accomplished and presence of alkaloids, steroids and carbohydrates were recognized in the extract. As taken from Mowla et al. *Afr J Tradit Complement Altern Med.* 2009; 6(3):255-61. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2816457/> (CAS 68990-15-8)

“OBJECTIVES: Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia. *Trigonella foenum-graecum* (fenugreek) and swimming training have previously been reported to have hypoglycemic and antioxidant effects. We aimed to evaluate the effects of swimming training and fenugreek aqueous extract, alone and in combination, on plasma glucose and cardiac antioxidant enzymes activity of streptozotocin-induced diabetes in rats. METHODS: We divided 70 male Wistar rats equally into 7 groups: diabetic control (DC), healthy control (HC), swimming (S), fenugreek seed extract (1.74 g/kg) (F1), fenugreek seed extract (0.87 g/kg) (F2), swimming + fenugreek seed extract (1.74 g/kg) (SF1), and swimming + fenugreek seed extract (0.87 g/kg) (SF2). We used streptozotocin for the induction of diabetes. Statistical analyses were

performed using the statistical program SPSS. RESULTS: We did not detect any significant differences in body weight in the F1, F2, S, SF1 and SF2 groups compared with the DC group ($p > 0.05$). The results also revealed that the hypoglycemic effect of combined swimming and fenugreek was significantly stronger ($p < 0.05$) than either of those alone. The F1, S, SF1 and SF2 groups showed improved superoxide dismutase activity with respect to the DC group ($p < 0.05$). Catalase activity in the F1, S, SF1 and SF2 groups were significantly higher than those of the DC group ($p < 0.05$). Glutathione peroxidase activity in the S, SF1 and SF2 groups were significantly increased compared with the DC group ($p < 0.05$). CONCLUSIONS: Our findings suggest that the combination of fenugreek seed extract and swimming could be useful for the treatment of hyperglycemia and cardiac oxidative stress induced by type 1 diabetes mellitus.” As taken from Haghani K et al. 2016. Can. J. Diabetes 40(2), 135-142. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26778682>

“OBJECTIVE: Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia condition resulting from defective insulin secretion or resistance insulin action, or both. The purpose of this study was to evaluate the effect of 6 weeks swimming training and Trigonella foenum-graecum seed (fenugreek) extract, alone and in combination, on plasma glucose and cardiac antioxidant enzyme activity of streptozotocin-induced diabetic rats. METHODS: Fifty male Wistar rats were divided into five groups: diabetic control (DC, $n = 8$); healthy control (HC, $n = 11$); swimming training (S, $n = 11$); swimming training + fenugreek seed extract (1.74 g/kg body weight; SF1, $n = 11$); and swimming training + fenugreek seed extract (0.87 g/kg body weight; SF2, $n = 9$). Streptozotocin was used for the induction of diabetes. Results were analyzed using one-way analysis of variance followed by Tukey test. RESULTS: In comparison with the DC group, all groups exhibited a significant decrease in body weight ($p < 0.05$), except for the HC group. SF1 and HC groups showed significant decreases in plasma glucose levels compared with the DC group ($p < 0.05$). S, SF1, SF2, and HC groups showed significant elevations in cardiac antioxidant enzymes activity in comparison with the DC group. CONCLUSION: The results indicated that the combination of endurance swimming training and fenugreek seed extract can significantly reduce the plasma glucose levels and increase cardiac antioxidant enzymes activity in diabetic rats. Our findings suggest that this combination could be useful for the treatment of hyperglycemia and cardiac oxidative stress induced by diabetes mellitus.” As taken from Arshadi S et al. 2015b. Osong Public Health Res. Perspect. 6(2), 87-93. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25938017>

“Allergic reactions, exacerbation of asthma, and a 14% decrease in serum potassium have been reported.”

“Dosages typically used to increase milk supply are 1 to 6 grams daily; in dosages of about 25 grams or more daily, fenugreek may cause lowering of cholesterol and blood sugar. It can also interact with warfarin to cause bleeding.”

As taken from LactMed, 2021. Record for CAS RN 68990-15-8.

Record for Trigonella foenum-graecum Linn., extract (no CAS RN 68990-15-8):

Type of Test	Route of Exposure Administration	Species/ or Test System	Dose Data	Toxic Effects	Reference
LD50 Lethal dose, 50 percent kill	Intraperitoneal	Rodent rat	500 mg/kg	Vascular - BP lowering characterized in autonomic section	IJEB 6 Indian Journal of Experimental Biology. (Publications & Information Directorate, CSIR, Hillside Rd., New Delhi 110 012, India) V.1- 1963- Volume(issue)/page/year: 16,228,1978

As taken from RTECS, 2010a.

“EXPL THER/ ... The Trigonella foenum-graecum extract has now been investigated for its effects on general properties, blood glucose and blood lipid, and hemorheological parameters in experimental diabetic rats. Streptozotocin-induced diabetic rats were administrated by oral intragastric intubation separately with low dose (0.44 g/kg.d), middle dose (0.87 g/kg.d), high dose (1.74 g/kg.d) of Trigonella foenum-graecum extract, and Metformin HCl (0.175 g/kg.d) for 6 weeks. Compared with diabetic group, rats treated with Trigonella foenum-graecum extract had an increase in body weight and a decrease in kidney /body weight ratio ($p < 0.05$). Compared with diabetic group, rats treated Trigonella foenum-graecum extract had lower blood glucose, glycated hemoglobin, triglycerides, total cholesterol and higher higher-density-lipoprotein-cholesterol in a dose-dependent manner ($p < 0.05$). The plasma viscosity, whole blood viscosity of high shear rate (200 s⁻¹) and low shear rate (40 s⁻¹), erythrocyte sedimentation rate, whole blood reduction viscosity and platelet conglutination were significantly reduced in diabetic rats treated with high and middle doses of Trigonella foenum-graecum extract, but not in those treated with low dose of Trigonella foenum-graecum extract. It may be concluded that Trigonella foenum-graecum extract can lower kidney /body weight ratio, blood glucose, blood lipid levels and improve hemorheological properties in experimental diabetic rats following repeated treatment for 6 weeks. [Xue WL et al; Asia Pac J Clin Nutr 16 Suppl 1: 422-6 (2007)] **PEER REVIEWED**”

As taken from HSDB, 2016

“Objectives: This study was designed to determine the effects of fenugreek seed (FS) on serum irisin levels, blood pressure, and liver and kidney function in patients with type 2 diabetes mellitus (T2DM). Methods: In an 8-week randomized controlled clinical trial, T2DM patients (n = 50) were assigned to the intervention (5 g FS powder, 3 times a day) or control group. Both groups received anti-diabetic drugs and nutritional consults. Serum samples were collected and blood pressure was measured at baseline and end of the trial. Data on dietary intake and physical activity was determined using the questionnaires. Results: Compared to the control group, FS consumption resulted in a significant decrease in fasting plasma glucose (FPG) ($p = 0.024$), as well as a significant change in serum alanine aminotransferase (ALT) ($p = 0.02$) and alkaline phosphatase (ALP) ($p = 0.001$). Within-group analysis showed a significant decrease in aspartate aminotransferase (AST) ($p = 0.014$), systolic blood pressure (SBP) ($p = 0.001$), and irisin ($p = 0.001$) in the FS group, and a significant increase in creatinine (Cr) ($p = 0.001$) and decrease in estimated glomerular filtration rate (eGFR) ($p = 0.001$) in the control group. FS consumption did not have any significant effect on diastolic blood pressure (DBP) and blood urea nitrogen (BUN). Conclusion: FS intake has some beneficial effects on FPG, SBP, and some liver and kidney function tests in patients with T2DM. Further studies are required to investigate the effect of FS on irisin levels.” As taken from Hadi A et al. 2020. Complement. Ther. Med. 49, 102315. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/32147060/>

6.3. Nervous system

“In our previous work, we demonstrated that Trigonella foenum (TFG) leaves extract can exert analgesic effects in both formalin (F.T.) and tail flick (T.F.) tests. Spinal serotonergic system, but not endogenous opioid system, was involved in TFG induced analgesia (in the second phase of formalin test). Some reports concern the similarity between NSAIDs and TFG extract in many pharmacological effects or the interaction between NSAIDs and purinergic system; so the present study was designed to investigate the relationship between TFG extract and purinergic system or the inhibition of cyclo-oxygenase (COX). We examined the effect of TFG extract on: (1) the

response of rabbit platelets to ADP induced aggregation, (2) the contraction of mouse vas deferens induced by alpha,beta-Me-ATP (a P(2) receptor agonist; this receptor mediates the rapid phase of ADP- and ATP-evoked influx of Ca(2+) through a non-specific cation channel in platelets), (3) alpha,beta-Me-ATP induced hyperalgesia in tail flick test in male rats and (4) the specific inhibition of COX-1 and COX-2. Our results showed that TFG extract (0.5, 1, 1.5, 3 mg/ml) inhibited ADP (10(-5) mol) induced platelet aggregation (IC(50)=1.28 mg/ml). alpha,beta-Me-ATP (30 microM) induced isometric contraction in vas deferens while suramin (a P(2) receptor antagonist, 50, 150, 300 microM) or TFG extract (0.5, 1, 2, 3 mg/ml) inhibited this effect significantly (IC(50) were 91.07 microM and 1.57 mg/ml, respectively). Moreover, alpha,beta-Me-ATP (3 microg/rat, i.t.) induced hyperalgesia in tail flick test, but it was prevented by co-injection of alpha,beta-Me-ATP with suramin (120 microg/rat, i.t.) or TFG extract (1mg/rat, i.t.). Effective concentrations of TFG extract in the above mentioned experiments did not inhibit COX enzymes in EIA tests. In conclusion, these results indicate that the blocking of spinal purinoceptors may contribute in the analgesic effect of TFG leaves extract." As taken from Parvizpur A et al. J Ethnopharmacol. 2006 Mar 8; 104(1-2):108-12. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16298092&query_hl=34&itool=pubmed_DocSum

"Fenugreek (*Trigonella foenum graecum* (L.)), is a medicinal plant whose seeds and leaves are widely used in Moroccan traditional medicine. Consumption of fenugreek seeds during pregnancy has been associated with a range of congenital malformations, including hydrocephalus, anencephaly and spina bifida. In previous work we have shown that exposure of pregnant mice to aqueous extract of fenugreek seeds (AEFS) leads to reduced litter size, intrauterine growth retardation, and malformations. However, there have been no studies to date of its longer-term neurobehavioral effects. We investigated these effects in prenatally exposed mice. MATERIALS AND METHODS: Pregnant females were exposed to 0, 500 or 1000 mg/kg/day AEFS, by gavage, for the whole period of gestation. Pups body weight was measured at 1, 7, 14, 21 and 28 day of age. Behavior of progeny was evaluated three weeks after birth using the open field, the rotarod test and the continuous alternation task by the T-maze. At 28 postnatal day age, brain of progeny was removed and cut for histological evaluation. RESULTS: The progeny of exposed mice displayed reduced body weight at birth (1000 mg/kg group: 27%; 500 mg/kg group: 32%) and reduced brain weight (10% in both treated groups). Both males and females mice prenatally exposed to AEFS displayed a significant decrease in the locomotor activity, in the boli deposits during the open field test and in motor coordination. These results seem to show that exposure to AEFS induces a depressive effect in the offspring. Assessment on a continuous alternation T-maze test showed a significant reduction in successful spontaneous alternations in males and females but only in the 1000 mg/kg group. CONCLUSION: These results suggest that prenatal exposure of mice to high dose of fenugreek seeds causes growth retardation and altered neurobehavioral performance in the post-weaning period in both male and female". As taken from Khalki L et al 2012. J. Ethnopharmacol. 139, 672-677 PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22178172?dopt=AbstractPlus>.

"*Trigonella foenum-graecum* Linn (Fabaceae) is an annual aromatic herb and no wit is cultivated globally like in Pakistan, Egypt, India, Middle East etc. Traditionally it was used in anorexia, as febrifuge, to soothe gastritis and gastric ulcers, as a galactagogue and as condiment, hypoglycemic agent and employed in various as nervous disorders. The study aimed to investigate the antidepressant effect of ethanolic extract of seeds of *Trigonella foenum graecum* and underlying mechanism of action. For assessment of antidepressant activity Forced Swimming Test (FST), Tail Suspension Test (TST), Monoamine (MAO) Assay and Locomotor Activity Test were studied. Acute toxicity, Rota Rod and Grip Strength Tests were also performed. The significant declining in immobility time as compared to control was shown in Forced swimming test as compared to tail suspension test. Considerable change was not found in open field test (OFT). EtOH extract of seeds of fenugreek represent maximum significant reduction which was 30 and 24.65% in MAO- A and B activity respectively in the rat's whole brain as compared to control animals in Monoamine

oxidase (MAO) assay. All tested doses were found ineffective in impairment of muscle coordination in Rota rod and in grip strength related to muscle relaxant property. According to experimental findings it is revealed that ethanolic extract of seeds of *Trigonella foenum graecum* showed antidepressant effect by inhibiting the activity of MAO-A and B." As taken from Khursheed R et al. 2014. *Pak. J. Pharm. Sci.* 27(5 Spec. No.), 1419-25. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25176235>

"BACKGROUND: Primary dysmenorrhea is a prevalent disorder and its unfavorable effects deteriorates the quality of life in many people across the world. Based on some evidence on the characteristics of fenugreek as a medical plant with anti-inflammatory and analgesic properties, this double-blind, randomized, placebo controlled trial was conducted. The main purpose of the study was to evaluate the effects of fenugreek seeds on the severity of primary dysmenorrhea among students. METHODS: Unmarried Students were randomly assigned to two groups who received fenugreek (n = 51) or placebo (n = 50). For the first 3 days of menstruation, 2-3 capsules containing fenugreek seed powder (900 mg) were given to the subjects three times daily for two consecutive menstrual cycles. Pain severity was evaluated using a visual analog scale and systemic symptoms were assessed using a multidimensional verbal scale. RESULTS: Pain severity at baseline did not differ significantly between the two groups. Pain severity was significantly reduced in both groups after the intervention; however, the fenugreek group experienced significantly larger pain reduction ($P < 0.001$). With respect to the duration of pain, there was no meaningful difference between the two cycles in the placebo group ($p = 0.07$) but in the fenugreek group, the duration of pain decreased between the two cycles ($P < 0.001$). Systemic symptoms of dysmenorrhea (fatigue, headache, nausea, vomiting, lack of energy, syncope) decreased in the fenugreek seed group ($P < 0.05$). No side effects were reported in the fenugreek group. CONCLUSION: These data suggest that prescription of fenugreek seed powder during menstruation can reduce the severity of dysmenorrhea." As taken from Younesy S et al. 2014. *J. Reprod. Infertil.* 15(1), 41-8. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24695380>

"The alcoholic (TA), aqueous (TQ), petroleum ether (PE), alkaloidal (TK) and glycosidal (TG) extracts of fenugreek; fenugreek oil (FO); diosgenin (DI) and trigonelline (TR) were evaluated for their neuropharmacological activities in albino Wistar rats. All the extracts and active principles except TQ showed significant CNS stimulant activities, while TQ alone showed a significant CNS depressant activity. The results indicated that the active compounds present in fenugreek seed extracts had significant CNS stimulant and depressant activities (Toxnet)."

As taken from SCENIHR, 2016

"Currently available anxiolytics cause numerous adverse effects and show craving and tolerance during long term treatment. Currently traditional medicines have been re-evaluated widely through work on various plant species. Numerous plants in traditional system show pharmacological activity with unlimited prospective for therapeutic use. Hence we planned to evaluate the effect of methanol extract of *T. foenum-graecum* L. seeds on anxiety, sedation and motor coordination in mice at different doses following 15 days of oral feeding. Effect on anxiety was assessed by Hole board test and Light and Dark transition models. Phenobarbitone induced sleeping time and Rota rod test were performed to assess effect on sedation and motor coordination. In Hole board test, *T. foenum-graecum* L. seeds decreased the number of head dips in mice at all the three doses. In Light and Dark transition model, *T. foenum-graecum* L. seeds increased the period spent in the light box and the number of moves among the two compartments at 100 and 200 mg/kg as compared to control animals. In phenobarbitone induced sleeping time, *T. foenum-graecum* L. seeds did not reveal any sedative effect. In Rota rod test, extract exhibited significant skeletal muscle relaxant effect at 200 mg/kg (at 90 min) as compared to the control animals. Results of our study shows significant anti-anxiety effects of *T. foenum-graecum* L. seeds and may also recommend improved adverse effect profile as compared to diazepam." As taken from Assad T and Khan RA. 2017. *Metab. Brain Dis.* 32(2), 343-349. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/27639708>

6.4. Other organ systems, dependent on the properties of the substance

“Both water and alcoholic extracts have been reported to have a stimulating effect on the isolated guinea pig uterus, especially during the last period of pregnancy, indicating that these extracts may have a highly oxytocic activity; they were suggested as possible replacements for oxytocin....Fenugreek was first introduced into Chinese medicine in the Sung Dynasty (ca. 1057) and has since been used as a nutrient and in treating kidney ailments, beriberi, hernia, impotence, other male problems, and others.” As taken from Khan IA and Abourashed EA, 2010.

“Fenugreek (*Trigonella foenum-graecum*) is used as a spice, vegetable and a medicinal plant. Since antioxidant properties have been linked to health benefits of natural products, such properties were studied in germinated fenugreek seeds which are considered to be more beneficial than dried seeds. Different fractions of the germinated seeds were used to determine their antioxidant potential at different levels. The assays employed were ferric reducing antioxidant power, radical scavenging by 1,1-diphenyl-2-picrylhydrazyl, ferrylmyoglobin/2,2'-azobis-3-ethylbenzthiazoline-6-sulfonic acid, pulse radiolysis, oxygen radical absorbance capacity and inhibition of lipid peroxidation in mitochondrial preparations from rat liver. An aqueous fraction of fenugreek exhibited the highest antioxidant activity compared with other fractions. As the quantity of phenolic and flavonoid compounds can be related to antioxidant activity, the contents from these extracts were measured. HPLC analysis was carried out to detect polyphenols, flavonoids and other components. This study reveals significant antioxidant activity in germinated fenugreek seeds which may be due partly to the presence of flavonoids and polyphenols.” As taken from Dixit P et al. *Phytother Res.* 2005 Nov; 19(11):977-83. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=16317656&query_hl=34&itool=pubmed_DocSum

“The effects of fenugreek seed extract on the alterations in serum thyroid hormone concentrations were studied in adult male mice and rats. Simultaneously, hepatic lipid peroxidation (LPO) and the activities of the antioxidant enzymes, viz superoxide dismutase (SOD) and catalase (CAT) were examined. Administration of methi seed extract (0.11 g kg body wt.(-1) day(-1) for 15 days) to both mice and rats significantly decreases serum triiodothyronine (T(3)) concentration and T(3)/T(4) ratio, but increases thyroxine (T(4)) levels and body weight. While hepatic LPO and CAT activities were not altered, a significant decrease in SOD activity was observed in both the animal models. These findings suggest that fenugreek seed extract induced inhibition in T(4)to T(3) conversion is not peroxidation-mediated and the inhibition in SOD activity could be the result of a decrease in the protein anabolic hormone, T3.” As taken from Panda S et al. *Pharmacol Res.* 1999 Nov; 40(5):405-9. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=10527654&query_hl=34&itool=pubmed_DocSum

“Results from 3 experiments in rodents showed that a hydro-ethanolic extract of fenugreek seeds induced a decrease in T3 levels. In 2 experiments, there were concomitant increase in T4 levels and decrease in T3/T4 ratio. These results suggest decreased conversion of T4 to T3. Unfortunately, TSH levels were not monitored. The decrease in T3/T4 ratio reveals decreased 5'-deiodinase activity since the majority of circulating serum T3 is produced by peripheral conversion of T4 to T3. A NOAEL was not determined” (EMA, 2011).

A patient with chronic asthma and food allergy reported “numbness of the head” after using fenugreek “paste” on her scalp, and it is possible that this reflects an anaesthetic effect (Patil et al. 1997).

Antioxidant and hepatoprotective activities of *Ocimum basilicum* Linn. and *Trigonella foenum-graecum* Linn. against H₂O₂ and CCL₄ induced hepatotoxicity in goat liver (Abstract). Significant hepatoprotective effects were obtained by ethanolic extract of leaves of *O. basilicum* and *T. foenum-graecum* against liver damage induced by H₂O₂ and CCl₄ as evidenced by decreased levels of antioxidant enzymes (enzymatic and non enzymatic). The extract also

showed significant anti lipid peroxidation effects in vitro, besides exhibiting significant activity in superoxide radical and nitric oxide radical scavenging, indicating their potent antioxidant effects. As taken from Meera et al. Indian J Exp Biol. 2009, Jul; 47(7):584-90. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/19761043>(CAS 68990-15-8)

Protective action of fenugreek (*Trigonella foenum graecum*) seed polyphenols against alcohol-induced protein and lipid damage in rat liver (Abstract). The study investigates the effect of fenugreek seed polyphenol extract (FPEt) on ethanol-induced damage in rat liver. Chronic ethanol administration (6 g kg⁻¹ day⁻¹) x 60 days) caused liver damage that was manifested by excessive formation of thiobarbituric-acid-reactive substances, lipid hydroperoxides, and conjugated dienes, the end products of lipid peroxidation, and significant elevation of protein carbonyl groups and diminution of sulfhydryl groups, a marker of protein oxidation. Decreased activities of enzymic and non-enzymic antioxidant levels and decreased levels of thiol groups (both non-protein and protein) were observed in ethanol-treated rats. Further, ethanol significantly increased the accumulation of 4-hydroxynonenal protein adducts, nitrated and oxidized proteins in liver which was evidenced by immunohistochemistry. Administration of FPEt to ethanol-fed rats (200 mg kg⁻¹ day⁻¹) significantly reduced the levels of lipid peroxidation products and protein carbonyl content, increased the activities of antioxidant enzymes, and restored the levels of thiol groups. The effects of FPEt were comparable with those of a positive control, silymarin. These findings show that FPEt ameliorates the pathological liver changes induced by chronic ethanol feeding. As taken from Kaviarasan et al. Cell Biol Toxicol. 2008, Oct; 24(5):391-400. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/18240000> (CAS 68990-15-8)

Clinical observation on trigonella foenum-graecum L. total saponins in combination with sulfonylureas in the treatment of type 2 diabetes mellitus (Abstract).

OBJECTIVE: To evaluate the efficacy and safety of trigonella foenum-graecum L. total saponins (TFGs) in combination with sulfonylureas (SU) in the treatment of patients with type 2 diabetes mellitus (T2DM) not well controlled by SU alone. **METHODS:** Sixty-nine T2DM patients whose blood glucose levels were not well controlled by oral sulfonylureas hypoglycemic drug were randomly assigned to the treated group (46 cases) and the control group (23 cases), and were given TFGs or placebo three times per day, 6 pills each time for 12 weeks, respectively. Meanwhile, the patients continued taking their original hypoglycemic drugs. The following indexes, including effects on traditional Chinese medicine (TCM) symptoms, fast blood glucose (FBG), 2-h post-prandial blood glucose (2h PBG), glycosylated hemoglobin (HbA1c), clinical symptomatic quantitative scores (CSQS), body mass index (BMI), as well as hepatic and renal functions, were observed and compared before and after treatment. **RESULTS:** The efficacy on TCM symptoms was obviously better in the treated group than that in the control group ($P < 0.01$), and there were statistically remarkable decreases in aspect of FBG, 2h PBG, HbA1c and CSQS in the treated group as compared to those in the control group ($P < 0.05$ or $P < 0.01$), while no significant difference was found in BMI, hepatic and renal functions between the two groups ($P > 0.05$). **CONCLUSION:** The combined therapy of TFGs with sulfonylureas hypoglycemic drug could lower the blood glucose level and ameliorate clinical symptoms in the treatment of T2DM, and the therapy was relatively safe. As taken from Lu et al.; Chin J Integr Med. 2008, Mar; 14(1):56-60. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/18219452> (CAS 68990-15-8)

Fenugreek seed (*Trigonella foenum graecum*) polyphenols inhibit ethanol-induced collagen and lipid accumulation in rat liver (Abstract). Chronic alcoholism is associated with fatty liver and fibrosis characterized by collagen accumulation. Seeds of fenugreek, an annual herb, are reported to possess hepatoprotective activity. The study aims to investigate the effects of fenugreek seed polyphenol extract (FPEt) on liver lipids and collagen in experimental hepatotoxic rats. Hepatotoxicity was induced in male albino Wistar rats by administering ethanol (6 g/kg per day) for 30 days. Control rats were given isocaloric glucose solution. FPEt was co-administered with ethanol at a dose of 200 mg/kg per day for the next 30 days. Silymarin was used as a positive control. Ethanol treatment caused increase in plasma and liver lipids, together with alterations in

collagen content and properties. Administration of FPEt to alcohol-fed rats significantly improved lipid profile and reduced collagen content, crosslinking, aldehyde content and peroxidation. The effects were comparable with that of silymarin. FPEt administration had a positive influence on both lipid profile and on the quantitative and qualitative properties of collagen in alcoholic liver disease. The protective effect is presumably due to the bioactive phytochemicals in fenugreek seeds. As taken from Kaviarasan et al.; Cell Biol Toxicol. 2007, Nov; 23(6):373-83. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/17453353> (CAS 68990-15-8)

Fenugreek seeds modulate 1,2-dimethylhydrazine-induced hepatic oxidative stress during colon carcinogenesis (Abstract). 1,2-dimethylhydrazine (DMH) is a colon carcinogen which undergoes oxidative metabolism in the liver. We have investigated the modulatory effect of fenugreek seeds (a spice) on colon tumor incidence as well as hepatic lipid peroxidation (LPO) and antioxidant status during DMH-induced colon carcinogenesis in male Wistar rats. In DMH treated rats, 100% colon tumor incidence was accompanied by enhanced LPO and a decrease in reduced glutathione (GSH) content as well as a fall in glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) activities. Inclusion of fenugreek seed powder in the diet of DMH treated rats reduced the colon tumor incidence to 16.6%, decreased the LPO and increased the activities of GPx, GST, SOD and CAT in the liver. We report that fenugreek modulates DMH-induced hepatic oxidative stress during colon cancer. As taken from Devasena and Venugopal Menon. Ital J Biochem. 2007, Mar; 56(1):28-34. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/17511351> (CAS 68990-15-8)

Fenugreek (*Trigonella foenum graecum*) seed polyphenols protect liver from alcohol toxicity: a role on hepatic detoxification system and apoptosis (Abstract). The present study investigates the hepatoprotective effect of fenugreek seed polyphenolic extract (FPEt) against ethanol-induced hepatic injury and apoptosis in rats. Chronic ethanol administration (6 g/kg/day x 60 days) caused liver damage that was manifested by the elevation of markers of liver dysfunction - aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin and gamma-glutamyl transferase (GGT) in plasma and reduction in liver glycogen. The effects on alcohol metabolizing enzymes such as alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) were studied and found to be altered in the alcohol-treated group. Ethanol administration resulted in adaptive induction of the activities of cytochrome p450 (cyt-p-450) and cytochrome-b5 (cyt-b5) and reduction in cytochrome-c-reductase (cyt-c-red) and glutathione-S-transferase (GST), a phase II enzyme. Further, ethanol reduced the viability of isolated hepatocytes (ex vivo) as assessed by the trypan blue exclusion test and increased hepatocyte apoptosis as assessed by propidium iodide staining (PI). Treatment with FPEt restored the levels of markers of liver injury and mitigated the alterations in alcohol metabolizing and detoxification enzymes and the electron transport component cytochrome-c reductase. Increased hepatocyte viability and reduced apoptotic nuclei were observed in FPEt-treated rats. These findings demonstrate that FPEt acts as a protective agent against ethanol-induced abnormalities in the liver. The effects of FPEt are comparable with those of a known hepatoprotective agent, silymarin. As taken from Kaviarasan & Anuradha; Fenugreek (*Trigonella foenum graecum*) seed polyphenols protect liver from alcohol toxicity: a role on hepatic detoxification system and apoptosis; Pharmazie. 2007, Apr; 62(4):299-304. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/17484288> (CAS 68990-15-8)

“Oxidative stress is involved in the development and progression of diabetic nephropathy (DN). Because *Trigonella foenum graecum* has been reported to have antidiabetic and antioxidative effects, we hypothesized that *T. foenum graecum* seed aqueous extract (TE) restores the kidney function of diabetic rats via its antioxidant activity. Rats were fed diets enriched with sucrose (50%, wt/wt), lard (30%, wt/wt), and cholesterol (2.5%, wt/wt) for 8 weeks to induce insulin resistance. After a DN model was induced by streptozotocin, the rats were administered a low (440 mg/kg), medium (870 mg/kg), or high (1740 mg/kg) dose of TE by oral intragastric intubation for 6 weeks. In TE-treated DN rats, blood glucose, kidney/body weight ratio, serum creatinine, blood urea nitrogen, 24-hour content of urinary protein, and creatinine clearance were significantly decreased compared

with nontreated DN rats. Diabetic rats showed decreased activities of superoxide dismutase and catalase, increased concentrations of malondialdehyde in the serum and kidney, and increased levels of 8-hydroxy-2'-deoxyguanosine in urine and renal cortex DNA. Treatment with TE restored the altered parameters in a dose-dependent manner. Furthermore, all of the ultramorphologic abnormalities in the kidney of diabetic rats, including the uneven thickening of the glomerular base membrane, were markedly ameliorated by TE treatment. We conclude that TE confers protection against functional and morphologic injuries in the kidneys of diabetic rats by increasing activities of antioxidants and inhibiting accumulation of oxidized DNA in the kidney, suggesting a potential drug for the prevention and therapy of DN". As taken from Xue W et al. 2011. *Nutr. Res.* 31, 555-562. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/21840472?dopt=AbstractPlus>.

"The purpose of this study was to evaluate the protective effects of fenugreek (*Trigonella foenum graecum* L.) upon dieldrin-induced perturbations of haematological parameters and damages to liver and kidney of male Wistar rats. Under our experimental conditions, dieldrin poisoning resulted in 1) an alteration of several haematological parameters, 2) an oxidative stress evidenced by an increase of lipids peroxidation level associated with an increase of superoxide dismutase activity and a decrease of glutathione peroxidase and catalase activities in hepatic and renal tissues, 3) increased levels of glucose, total cholesterol, triglycerides, creatinine, urea, uric acid and proteins in blood, 4) increased activities of lactate dehydrogenase, alkaline phosphatase and transaminases in blood. Previous administration of fenugreek was found to hinder these dieldrin-induced damages: all hematological, renal and hepatic biomarkers, level of lipids peroxidation and activities of catalase and glutathione-peroxidase in liver and kidney were kept close to control values. This protective effect is mainly attributed to antioxidant properties of fenugreek". As taken from Hfaiedh N et al. 2012. *Gen. Physiol. Biophys.* 31(4), 423-30. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/23255669?dopt=AbstractPlus>.

"Long term alcohol consumption is one of the important causes for liver failure and death. To complicate the existing problem there are no dependable hepatoprotective drugs and a large number of patients prefer using complementary and alternative medicines for treating and managing hepatic complications. Almost 25 centuries ago, Hippocrates, the father of medicine, proclaimed "Let food be thy medicine and medicine be thy food." Exploring the association between diet and health continues even today. Preclinical studies carried out in the recent past have shown that the commonly used dietary agents like, *Trigonella foenum-graecum* (fenugreek) protect against ethanol-induced hepatotoxicity. Mechanistic studies have shown that the beneficial effects of these phytochemicals in preventing the ethanol-induced hepatotoxicity are mediated by the antioxidant, free radical scavenging, anti-inflammatory and anti-fibrotic effects. The present review for the first time collates the hepatoprotective effects of these agents and also emphasizes on aspects that need future research to establish their utility in humans". As taken from Shivasankhara AR et al. 2012. *Food Function* 3, 101-109. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22119904?dopt=AbstractPlus>.

"This study determined the effects of fenugreek on postprandial plasma glucose (PPG) and satiety among overweight and obese individuals. Fourteen subjects were studied in the morning after overnight fasts on four separate occasions. Glycaemic responses elicited by 50 g carbohydrate portions of white bread and jam with or without 5.5 g of fenugreek and fried rice with or without 5.5 g fenugreek were determined over 2 h. The primary endpoint was the incremental area under the plasma glucose response curve (IAUC). Adding fenugreek to both foods significantly reduced the IAUC compared to the food alone: white bread and jam, 180 ± 22 versus 271 ± 23 mmol \times min/L ($P = 0.001$); fried rice, 176 ± 20 versus 249 ± 25 mmol \times min/L ($P = 0.001$). Fenugreek also significantly reduced the area under the satiety curve for white bread with jam (134 ± 27 versus 232 ± 33 mm \times hr, $P = 0.01$) and fried rice (280 ± 37 versus 379 ± 36 mm \times hr, $P = 0.01$). It is concluded that fenugreek significantly decreased the PPG response and increased satiety among overweight and obese individuals." As taken from Robert SD et al. 2014. *J. Nutr. Metab.* 2014, 964873. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25276421>

“OBJECTIVE: To determine the hypolipidemic effect of fenugreek (Methi) seeds and its comparison with atorvastatin on experimentally induced hyperlipidemia in rabbits. **STUDY DESIGN:** Experimental interventional study. **PLACE AND DURATION OF STUDY:** Department of Physiology, Dr. S. N. Medical College, Jodhpur, Rajasthan, India, from April 2012 to March 2013. **METHODOLOGY:** Twenty, 1 - 2 years old albino rabbits of European Strains were randomly divided into two groups of 10 rabbits each. All were fed pure cholesterol (0.5 g/kg body weight/day) for 4 weeks to induce hyperlipidemia. Group-I comprised of 10 hyperlipidemic rabbits which were fed normal (regular) diet supplemented with 2 ml aqueous emulsified fenugreek seeds powder (500 mg/kg body weight/day) for 4 weeks. Group-II comprised of 10 hyperlipidemic rabbits, which were fed normal (regular) diet supplemented with 2 ml aqueous emulsion of atorvastatin (0.5 mg/kg body weight/day) for 4 weeks. Fasting blood samples were collected two times during experimental period at weeks (4 and 8) and analyzed for serum total cholesterol and triglycerides, using semi-automated chemistry analyzer. HDL-C was determined by precipitation method and LDL-C and VLDL-C were estimated by Friedewalds formula. LDL/HDL ratio and TG/HDL ratios were also calculated. The significance of difference in mean values of both groups (lipid profile) was assessed by independent student's t-test. **RESULTS:** Atorvastatin showed a more potent hypolipidemic activity. It reduced serum total cholesterol, TG, LDL and VLDLcholesterol, and the atherogenic index (LDL-C/HDL-C; $p < 0.001$) highly significantly as compared to fenugreek. There was a significant increase of HDL-C ($p < 0.01$) in group-I as compared to group-II. **CONCLUSION:** Fenugreek and atorvastatin both have hypolipidemic activity in rabbits but atorvastatin is more potent than fenugreek seeds powder.” As taken from Sharma MS & Choudhary PR. 2014. J. Coll. Physicians Surg. Pak. 24(8), 539-42. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25149829>

“BACKGROUND AND AIM: Fenugreek is a herb that is widely used in cooking and as a traditional medicine for diabetes in Asia. It has been shown to acutely lower postprandial glucose levels, but the long-term effect on glycemia remains uncertain. We systematically reviewed clinical trials of the effect of fenugreek intake on markers of glucose homeostasis. **METHODS:** PubMed, SCOPUS, the Cochrane Trials Registry, Web of Science, and BIOSIS were searched up to 29 Nov 2013 for trials of at least 1 week duration comparing intake of fenugreek seeds with a control intervention. Data on change in fasting blood glucose, 2 hour postload glucose, and HbA1c were pooled using random-effects models. **RESULTS:** A total of 10 trials were identified. Fenugreek significantly changed fasting blood glucose by -0.96 mmol/l (95% CI: -1.52, -0.40; $I^2 = 80\%$; 10 trials), 2 hour postload glucose by -2.19 mmol/l (95% CI: -3.19, -1.19; $I^2 = 71\%$; 7 trials) and HbA1c by -0.85% (95% CI: -1.49%, -0.22%; $I^2 = 0\%$; 3 trials) as compared with control interventions. The considerable heterogeneity in study results was partly explained by diabetes status and dose: significant effects on fasting and 2 hr glucose were only found for studies that administered medium or high doses of fenugreek in persons with diabetes. Most of the trials were of low methodological quality. **CONCLUSIONS:** Results from clinical trials support beneficial effects of fenugreek seeds on glycemic control in persons with diabetes. However, trials with higher methodology quality using a well characterized fenugreek preparation of sufficient dose are needed to provide more conclusive evidence.” As taken from Neelakantan N et al. 2014. Nutr. J. 13, 7. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24438170>

“...the information available in the literature on the health benefits and pharmaceutical effects of trigonellaaccounts for its known medicinal properties and adds new therapeutic effects in newer indications. besides its known medicinal properties such as carminative, gastric stimulant, antidiabetic and galactogogue (lactation-inducer) effects, newer research has identified hypocholesterolemic, antilipidemia, antioxidant, hepatoprotective, anti-inflammatory, antibacterial, antifungal, antiulcer, antilithigenic, anticarcinogenic and other miscellaneous medicinal effects offenugreek. although most of these studies have used whole seed powder or different forms of extracts, some have identified active constituents from seeds and attributed them medicinal values for different indications. conclusion: the resarch ontrigonellaexhibits its health benefits and potential medicinal properties in various indications and has little or no side effects, suggesting its

pharmaceutical, therapeutic and nutritional potential.” As taken from YADAV UC & BAQUER NZ. 2014. PHARM. BIOL. 52(2), 243-54. PUBMED, 2014 AVAILABLE AT <http://www.ncbi.nlm.nih.gov/pubmed/24102093>

“BACKGROUND AND AIM: An example of a medicinal plant with numerous potential activities is fenugreek (*Trigonella foenum-graecum* L.). The aim of the present study was to investigate the effects of fenugreek seed on bone mechanical properties in rats with normal and decreased estrogen level (developing osteoporosis). MATERIALS AND METHODS: The experiments were carried out on 3-month-old non-ovariectomized (NOVX) and ovariectomized (OVX) Wistar rats, divided into control rats, rats receiving pulverized fenugreek seed (1% in the diet) and rats receiving fenugreek seed extract standardized for 4-hydroxy-L-isoleucine (50 mg of 4-hydroxy-L-isoleucine/kg p.o. daily) for 4 weeks. Serum bone turnover markers, bone mineralization and mechanical properties were examined. RESULTS: Fenugreek seed added to food did not significantly affect bone mineralization and serum turnover markers, independently of the estrogen status. It tended to increase the strength of the tibial metaphysis (cancellous bone) in NOVX rats, and increased the strength of the femoral diaphysis (compact bone) in OVX rats. The fenugreek seed extract did not affect the skeletal system of NOVX rats, and significantly worsened mineralization of the vertebra in OVX rats, decreased due to estrogen deficiency. CONCLUSIONS: Low dietary intake of fenugreek seed may exert slight favorable skeletal effects, whereas at high doses it may damage the skeletal system.” As taken from Folwarczna J et al. 2014. Eur. Rev. Med. Pharmacol. Sci. 18(13), 1937-47. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/25010626>

“Fenugreek with the scientific name of *Trigonella foenum-graecum* L and with leaves consisting of 3 small obovate to oblong leaflets is an annual herbaceous plant of the Fabaceae family. It is native to the eastern Mediterranean but is cultivated worldwide. This plant has medicinal alkaloids, steroid compounds, and saponin and many uses have been mentioned for this plant in traditional medicine. This plant has been used to ease childbirth, to aid digestion, and as a general tonic to improve metabolism. Trigonelline is considered as the most important metabolite of fenugreek, which is very effective in treating diabetes and decreasing blood cholesterol. Diaszhenin is another important compound in seeds of this plant, which is used in producing medicinal steroids like contraceptive pills. Many studies have been performed on the therapeutic effects and identification of chemical compounds of this plant. In this article, the most important biological effects and reported compounds about fenugreek seed are reviewed and its therapeutic applications are investigated.” As taken from Bahmani M et al. 2016. J. Evid. Based Complementary Altern. Med. 21(1), 53-62. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25922446>

“Fenugreek has been used in a number of geographical regions worldwide as a galactagogue to increase milk supply, and is included in numerous proprietary mixtures promoted to increase milk supply. The galactagogue effect of fenugreek may be primarily psychological in humans; however, animal studies indicate that fenugreek might work primarily by increasing insulin and oxytocin secretion. Evidence for a galactagogue effect is mostly anecdotal. A limited number of published studies of low to moderate quality have found mixed results for a galactagogue effect for fenugreek. A meta-analysis of controlled studies found fenugreek to have a mild galactagogue effect and unknown safety profile. Some evidence indicates that fenugreek might be more effective in the first few days postpartum than after 2 weeks postpartum. Some of these studies used a multi-ingredient combination products in which fenugreek was only one component, so the results might be different from studies in which fenugreek was used alone.”

“When used as a medicinal, it is generally well tolerated in adults, but gastrointestinal side effects such as diarrhea and flatulence may occur.”

“A study of healthy women who delivered a fullterm infant and desired to breastfeed for at least 4 months compared fenugreek, torbangun (*Coleus amboinicus*) and a product containing placental extract and vitamin B12 (Molocco+B12) for their effects on breastmilk volume. No mention was made of any breastfeeding support provided to the women. Participants were randomly assigned to

receive one of the products for 30 days and followed for another 30 days. Capsules containing powdered fenugreek seeds 600 mg (Bullivants Natural Health, Auckland, New Zealand) were given 3 times daily. Infants were weighed before and after each nursing at 2-week intervals during the study to measure 24-hour milk volume. At no time point during the study was milk volume in the fenugreek group (n = 22) statistically different from the reference group (n = 22) who received Molocco+B12, although the torbangun group did have a statistically significant increase. The daily volume of milk actually decreased in the fenugreek group over time, although the change was not statistically significant. The dosage of fenugreek used in this study was lower than that typically recommended by most sources.”

“In a survey of 188 nursing women from 27 states (52% from Louisiana), 85 (46%) had used fenugreek as a galactagogue. Of those who used it, 54% felt that it increased their milk supply and 45% reported side effects, including a maple syrup smell emitted from the mother's body, gassiness in the baby, or breastmilk oversupply.”

“A nonrandomized, nonblinded case-control study compared healthy age-matched postpartum mothers of preterm newborns admitted to the NICU who either received (n = 30) or did not receive 200 mL of fenugreek tea (n = 30), containing 50 grams of fenugreek seed 3 times daily. The allocation method was not stated, but the groups were well matched. Each group pumped milk with a breast pump 8 times daily. Milk volume was measured on days 3, 8 and 15 of the study; maternal serum prolactin was measured on days 3 and 15. Milk volumes were significantly different only on day 3 postpartum, favoring the fenugreek group (275 vs 246 mL). The fenugreek group also had higher serum prolactin values only on day 3 (153 vs 135 mcg/L). The authors concluded that fenugreek affects only the early stage of lactogenesis, and not production of mature milk.”

As taken from LactMed, 2021. Record for CAS RN 68990-15-8.

Record for *Trigonella foenum-graecum* L. (Papilionaceae), seed, water extract (no CAS RN):

Type of Test	Route of Exposure or Administration	Species or Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent rat	15 mL/kg	Gastrointestinal - alteration in gastric secretion Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - catalases Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - other oxidoreductases	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 81,393,2002

As taken from RTECS, 2014.

“BACKGROUND: Fenugreek (*Trigonella foenum-graecum*) is globally recognized for its medicinal properties and hypoglycemic effects. The seed extract as well as its active compound, 4-hydroxyisoleucine (4-OH-Ile), have been shown to reduce hyperglycemic insulin resistance. The mechanism by which this occurs has not been investigated in human liver cells (HepG2) in comparison to the antihyperglycemic drug, metformin. METHODS: We investigated the effects of an aqueous fenugreek seed extract (FSE), 4-OH-Ile, and metformin in HepG2 cells relative to insulin as a positive control. Cells were treated with FSE and 4-OH-Ile at 100 ng/mL under normoglycemic (5 mM glucose) and hyperglycemic (30 mM glucose) conditions for 72 hr. Tyrosine phosphorylation of insulin receptor-β (IR-β), protein kinase B (Akt), glycogen synthase kinase-3α/β

(GSK-3 α/β), and glucose transporter 2 (GLUT2) was determined by western blotting. Gene expression of sterol regulatory element-binding protein 1c (SREBP1c), GLUT2, glycogen synthase (GS), and glucokinase (GK) was evaluated by quantitative polymerase chain reaction, and supernatant glucose levels were measured using the Piccolo biochemistry analyzer. RESULTS: Under normo- and hyperglycemic conditions, FSE, 4-OH-Ile, insulin (100 ng/mL), and metformin (2 mM) caused a significant increase in phosphorylation of IR- β , Akt, GSK-3 α/β , and GLUT2. Glucose uptake, however, was most significantly increased in FSE-treated cells during both conditions. FSE induced the most significant changes in downstream insulin signaling, GS, GK, SREBP1c, and GLUT2 expression compared to 4-OH-Ile, metformin, and insulin. In addition, FSE significantly increased glucose uptake. CONCLUSIONS: Collectively, these findings provide a mechanism by which FSE exerts antihyperglycemic effects similar to metformin and insulin that occurs via enhanced insulin signaling, gene expression, and increasing glucose uptake." As taken from Naicker N et al. 2016. *Metab. Syndr. Relat. Disord.* 14(2), 114-120. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26835874>

"Fenugreek is a plant of the Fabaceae family that is native to India, China, and North Africa (Prabhakar and Doble, 2011). The most studied bioactive compounds from fenugreek with reported hypoglycemic actions are diosgenin (3 β -hydroxy-5-spirostene), 4-hydroxyisoleucine, and the soluble dietary fiber fraction of fenugreek seeds (Fuller and Stephens, 2015). Diosgenin in fenugreek is a major aglycone of saponin, and the reported hypoglycemic mechanisms of its action include renewal of pancreatic β -cells and stimulation of insulin secretion (Kalailingam et al., 2014), antioxidative effects (Son et al., 2007), and promotion of adipocyte differentiation and enhancement of insulin-dependent glucose uptake (Uemura et al., 2010). 4-Hydroxyisoleucine is a branched-chain amino-acid derivative that is only found in plants, and it represents the majority of the total content of free amino acids in fenugreek seeds (Fuller and Stephens, 2015). It has been shown that the insulinotropic and antidiabetic properties of 4-hydroxyisoleucine act through stimulation of glucose-dependent insulin secretion and reduction of insulin resistance in muscle and/or liver (Jetté et al., 2009). Fenugreek seeds are also a rich source of fiber (50–65 g fiber/100 g seeds). The soluble dietary fiber fraction of fenugreek (i.e., galactomannan) has been shown to enhance glycemic control. This effect has been attributed to inhibition of lipid-hydrolyzing and carbohydrate-hydrolyzing enzymes in the digestive system (Hannan et al., 2007). Galactomannan also reduces the rate of glucose uptake through its actions as a significant physical barrier to glucose diffusion (Srichamroen et al., 2009). Clinical studies reported that intake of fenugreek seeds significantly changed fasting blood glucose, 2 h postload glucose and hemoglobin A1c (HbA1c; Neelakantan et al., 2014). Results of a recent study conducted on men and women with prediabetes strongly suggests that the enhancement of insulin levels is due to insulinotropic effect of fenugreek and suggest that the mode of action is a result of alkaloids present (Gaddam et al., 2015). Although results from clinical trials support beneficial effects of fenugreek seeds on glycemic control in persons with diabetes, trials with better methodology quality and well characterized preparation of sufficient dose are needed to provide more conclusive evidence. With its hypoglycemic and antidyslipidemic effects, fenugreek represents an attractive new candidate for treatment of type 2 diabetes, obesity, and dyslipidemia, the key components of metabolic syndrome." As taken from Ota A and Ulrich NP (2017) *Front. Pharmacol.* 8:436, doi: 10.3389/fphar.2017.00436. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5499308/>

"BACKGROUND/OBJECTIVE: The purpose of the present study was to evaluate the effect of fenugreek seed extract in combination with swimming exercise compared to glibenclamide consumption on type 2 diabetic rats. DESIGN: The acute toxicity test was carried out to choose the safe doses and identify the toxicity effects of the fenugreek seed extract. To investigate the hypoglycemic effect of the extract and its effect in combination with swimming training, 80 Wistar Kyoto male streptozotocin-induced diabetic rats were divided randomly into eight groups: diabetic control (C); fenugreek seed extract 0.8 g/kg (F1); fenugreek extract 1.6 g/kg (F2); swimming training (S); swimming training plus fenugreek extract 0.8 g/kg (SF1); swimming training plus fenugreek extract 1.6 g/kg (SF2); glibenclamide (G) and swimming training plus glibenclamide

(SG). The rats were orally administered with the treatments once a day with the respective treatment, and the training groups were subjected to swimming training every day for 60 min. Fasting blood samples were collected to measure fasting blood glucose, lipid profile, adiponectin, leptin, and insulin concentrations. RESULTS: The results obtained from acute toxicity study showed no toxicity effect of fenugreek seed extract on the tested dose. Biochemical analysis showed significant improvements in all of the groups compared to the control group ($p < 0.05$). Plasma insulin concentration and insulin resistance (HOMA-IR) was significantly reduced in treated groups compared with the diabetic control group. Plasma leptin were significantly decreased in treated groups compared with the control group; while adiponectin had markedly increased ($p < 0.05$). CONCLUSION: The findings suggest that fenugreek seed consuming, alongside swimming exercise, has a strong therapeutic effect on the improvement of diabetic parameters.” As taken from Arshadi S et al. 2015a. Food Nutr. Res. 59, 29717. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26699937>

“A group of 11 medicinal plants, including *Lavandula pubescens*, *Trigonella foenugricium*, *Salsola schweinfurthii*, *Calligonum comosum*, *Silene succulenta*, *Silene villosa*, *Bogonvillea glabra*, *Cakile maritime*, *Gomphrena celosoids*, *Mirabilis jalapa*, and *Silene nocturna* growing in Egypt, were extracted and examined for their immunomodulatory and antioxidant activities. RAW 264.7 cells were recruited to investigate the immunomodulatory effect through multiple parameters analysis. First, the proliferation index of macrophages cells was evaluated revealing that *Trigonella foenugricium*, *Silene succulenta* and *Silene villosa* have a significant cytotoxic effect on RAW cells. Interestingly, we observed enhancement of macrophages phagocytic function of by all extracts except *Cakile maritime*, *Gomphrena celosoides* and *Silene nocturna*. Afterwards, macrophages were challenged by incubation with LPS and the effect of various extracts on inflammatory responses was investigated; the generation of NO from activated macrophage was substantially suppressed by 7 extracts namely, *Trigonella foenugricium*, *Calligonum comosum*, *Silene succulenta*, *Bougainvillea glabra*, *Mirabilis jalapa*, *Gomphrena celosoides* and *Silene nocturna*. TNF- α was decreased by percentage range from 3.8 to 85.8% and *Trigonella foenugricium* extract showed the highest inhibition of TNF- α release. All extracts except *Trigonella foenugricium*, *Salsola schweinfurthii*, *Silene succulenta* and *Mirabilis jalapa* significantly inhibited COX-2 production from stimulated macrophage. Moreover, evaluating the potential antioxidant activity of these extracts showed that *Trigonella foenugricium*, *Salsola schweinfurthii*, *Calligonum comosum*, *Bogonvillea glabra* and *Mirabilis jalapa* exhibited some antioxidant activities. Taken together, our results suggest that some of these extracts may have a considerable antiinflammatory and antioxidant effects and may be a potential therapeutic choice in the treatment of inflammatory diseases.” As taken from Ghonime M et al. 2015. Immunol. Invest. 44(3), 237-52. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25564700>

“BACKGROUND: Several experimental and clinical studies support beneficial effects of *Trigonella foenum-graecum* (fenugreek) in the management of metabolic diseases and inflammatory disorders. OBJECTIVES: The purpose of this study was to examine the effect of *T. foenum-graecum* seed extract in reducing the metabolic and inflammatory alternations associated with menopause. MATERIALS AND METHODS: In this experimental study, 49 rats were divided into seven groups: (I) sham-control, (II) ovariectomized-control, (III and IV) ovariectomized treated with 50 and 150 mg/kg of *T. foenum-graecum* seed ethanolic extract, (V and VI) ovariectomized treated with 50 and 150 mg/kg of *T. foenum-graecum* hexanic extract, (VII) ovariectomized-positive control treated with 10 μ g/kg of estradiol. The extracts were injected intraperitoneally one day after ovariectomy and the treatments were lasted for 42 days. RESULTS: Fasting blood glucose and body weight gain increased significantly in the ovariectomized-control group compared with that in the sham animals ($P < 0.05$). Administration of estradiol and *T. foenum-graecum* (50 and 150 mg/dL of hexanic extract and 150 mg/kg of ethanolic extract) significantly diminished the increase in glucose and body weight ($P < 0.05$). The serum level of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) in the ovariectomized control group was significantly higher than those in the sham animals ($P < 0.05$). Both hexanic and ethanolic extracts as well as estradiol

were able to decrease level of these cytokines in the serum of ovariectomized rats ($P < 0.05$). CONCLUSIONS: The results of the present study show that administration of *T. foenum-graecum* corrects metabolic and inflammatory alterations associated with ovariectomy and has a potential for the management of menopause." As taken from Abedinzade M et al. 2015. Iran. Red Crescent Med. J. 17(11), e26685. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26732240>

"OBJECTIVES: Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia. *Trigonella foenum-graecum* (fenugreek) and swimming training have previously been reported to have hypoglycemic and antioxidant effects. We aimed to evaluate the effects of swimming training and fenugreek aqueous extract, alone and in combination, on plasma glucose and cardiac antioxidant enzymes activity of streptozotocin-induced diabetes in rats. METHODS: We divided 70 male Wistar rats equally into 7 groups: diabetic control (DC), healthy control (HC), swimming (S), fenugreek seed extract (1.74 g/kg) (F1), fenugreek seed extract (0.87 g/kg) (F2), swimming + fenugreek seed extract (1.74 g/kg) (SF1), and swimming + fenugreek seed extract (0.87 g/kg) (SF2). We used streptozotocin for the induction of diabetes. Statistical analyses were performed using the statistical program SPSS. RESULTS: We did not detect any significant differences in body weight in the F1, F2, S, SF1 and SF2 groups compared with the DC group ($p > 0.05$). The results also revealed that the hypoglycemic effect of combined swimming and fenugreek was significantly stronger ($p < 0.05$) than either of those alone. The F1, S, SF1 and SF2 groups showed improved superoxide dismutase activity with respect to the DC group ($p < 0.05$). Catalase activity in the F1, S, SF1 and SF2 groups were significantly higher than those of the DC group ($p < 0.05$). Glutathione peroxidase activity in the S, SF1 and SF2 groups were significantly increased compared with the DC group ($p < 0.05$). CONCLUSIONS: Our findings suggest that the combination of fenugreek seed extract and swimming could be useful for the treatment of hyperglycemia and cardiac oxidative stress induced by type 1 diabetes mellitus." As taken from Haghani K et al. 2016. Can. J. Diabetes 40(2), 135-142. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26778682>

"OBJECTIVE: Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia condition resulting from defective insulin secretion or resistance insulin action, or both. The purpose of this study was to evaluate the effect of 6 weeks swimming training and *Trigonella foenum-graecum* seed (fenugreek) extract, alone and in combination, on plasma glucose and cardiac antioxidant enzyme activity of streptozotocin-induced diabetic rats. METHODS: Fifty male Wistar rats were divided into five groups: diabetic control (DC, $n = 8$); healthy control (HC, $n = 11$); swimming training (S, $n = 11$); swimming training + fenugreek seed extract (1.74 g/kg body weight; SF1, $n = 11$); and swimming training + fenugreek seed extract (0.87 g/kg body weight; SF2, $n = 9$). Streptozotocin was used for the induction of diabetes. Results were analyzed using one-way analysis of variance followed by Tukey test. RESULTS: In comparison with the DC group, all groups exhibited a significant decrease in body weight ($p < 0.05$), except for the HC group. SF1 and HC groups showed significant decreases in plasma glucose levels compared with the DC group ($p < 0.05$). S, SF1, SF2, and HC groups showed significant elevations in cardiac antioxidant enzymes activity in comparison with the DC group. CONCLUSION: The results indicated that the combination of endurance swimming training and fenugreek seed extract can significantly reduce the plasma glucose levels and increase cardiac antioxidant enzymes activity in diabetic rats. Our findings suggest that this combination could be useful for the treatment of hyperglycemia and cardiac oxidative stress induced by diabetes mellitus." As taken from Arshadi S et al. 2015b. Osong Public Health Res. Perspect. 6(2), 87-93. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25938017>

The aim of the study was to evaluate the effect of *Trigonella foenum-graecum* (fenugreek) seed extract on sex hormones and sexual function in healthy menstruating women who reported low sexual drive. This short term, single site, double blind, randomised, placebo-controlled study was conducted on 80 women, aged 20 to 49 years. Participants were randomised to either an oral dose of a standardised *T. foenum-graecum* seed extract (libifem) at a dose of 600 mg/day or placebo over two menstrual cycles. Dehydroepiandrosterone sulfate, progesterone, androstenedione, total

and free testosterone, estradiol (E2), luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin and cholesterol were measured at baseline and 8 weeks. The individual aspects of sexual function were measured using the Derogatis interview for sexual functioning and female sexual function index self-administered questionnaires. Stress, fatigue and quality of the relationship with partner were also measured using the PSS (Perceived Stress Scale), MFI-20 (Multidimensional Fatigue Inventory) and DAS (Dyadic Adjustment Scale) quality of life measures, respectively. There was a significant increase in free testosterone and E2 in the active group as well as sexual desire and arousal compared with the placebo group. The results indicate that this extract of *T. foenum-graecum* may be a useful treatment for increasing sexual arousal and desire in women.” As taken from Rao A et al. 2015. *Phytother. Res.* 29(8), 1123-30. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25914334>

“This study examined the effect of Testofen, a specialised *Trigonella foenum-graecum* seed extract on the symptoms of possible androgen deficiency, sexual function and serum androgen concentrations in healthy aging males. This was a double-blind, randomised, placebo-controlled trial involving 120 healthy men aged between 43 and 70 years of age. The active treatment was standardised *Trigonella foenum-graecum* seed extract at a dose of 600 mg/day for 12 weeks. The primary outcome measure was the change in the Aging Male Symptom questionnaire (AMS), a measure of possible androgen deficiency symptoms; secondary outcome measures were sexual function and serum testosterone. There was a significant decrease in AMS score over time and between the active and placebo groups. Sexual function improved, including number of morning erections and frequency of sexual activity. Both total serum testosterone and free testosterone increased compared to placebo after 12 weeks of active treatment. *Trigonella foenum-graecum* seed extract is a safe and effective treatment for reducing symptoms of possible androgen deficiency, improves sexual function and increases serum testosterone in healthy middle-aged and older men.” As taken from Rao A et al. 2016. *Aging Male* 19(2), 134-142. PubMed, 2017 available at <http://www.ncbi.nlm.nih.gov/pubmed/26791805>

“Candidiasis is one of the most common opportunistic infections caused by *Candida albicans*. Fluconazole is the drug of choice for prevention and management of this condition. However, the emergence of fluconazole resistant candidal strains has become a major concern. Many herbs like fenugreek, cinnamon, papaya, oregano, garlic are rich in phytochemical constituents known to express antimycotic activity. With the available information, the present research study was carried out to assess the in vitro anti-mycotic activity of hydro alcoholic extracts of *Trigonella foenum-graecum* seeds, *Cinnamomum verum* bark and *Carica papaya* leaves and seeds against fluconazole resistant *Candida albicans*. MATERIALS AND METHODS: Hydro alcoholic extracts of *Trigonella foenum-graecum* (seeds), *Cinnamomum verum* (bark), *Carica papaya* CO.2 strain (male and female leaves) and *Carica papaya* CO.2 strain (seeds) were prepared by maceration. The anti-mycotic activity of the prepared extracts against *Candida albicans* was assessed by agar well diffusion method. Three independent experiments were performed in triplicates and the mean and standard deviation were calculated. Minimum inhibitory concentration was determined. RESULTS: The results of the present study revealed that all the extracts exhibited anti-mycotic activity in a dose dependent manner and minimum inhibitory concentration of all the extracts was found to be 15.62 µg/ml. CONCLUSION: The results of the present study shed light on the fact that plant extracts could be used not only as an alternate drug for management of fluconazole resistant candidiasis but also explored further for oral cancer prevention as a therapeutic adjunct.” As taken from Varadarajan S et al. 2015. *J. Clin. Diagn. Res.* 9(8), ZC07-10. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26436036>

“INTRODUCTION: The aim of the study was to explore the in vitro antibacterial activity of seven ethanolic extracts of spices against high level gentamicin resistant (HLGR) enterococci isolated from human clinical samples. MATERIAL AND METHODS: Two hundred and fifteen enterococcal strains were isolated from clinical samples. High level gentamicin resistance in ethanolic extracts of cumin (*Cuminum cyminum*), cinnamon (*Cinnamomum zeylanicum*), ginger (*Zingiber officinale*), fenugreek (*Trigonella foenum-graecum*), cloves (*Syzygium aromaticum*), cardamom (*Elettaria*

cardamomum Maton) and black pepper (*Piper nigrum*) were prepared using Soxhlet apparatus. The antibacterial effect of the extracts was studied using the well diffusion method. Statistical analysis was carried out by χ^2 test using SPSS 17 software. RESULTS:....Fenugreek, black pepper and cardamom did not show any effect on the isolates....” As taken from Revati S et al. 2015. Arch. Med. Sci. 11(4), 863-8. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26322099>

“*Trigonella foenum-graecum* L. (Fenugreek) is an important plant of the Leguminosae family known to have medicinal properties. However, fraction based antiquorum sensing and antibiofilm activities have not been reported from this plant. In the present study *T. foenum-graecum* seed extract was sequentially fractionated and sub-MICs were tested for above activities. The methanol fraction of the extract demonstrated significant inhibition of AHL regulated virulence factors: protease, LasB elastase, pyocyanin production, chitinase, EPS, and swarming motility in *Pseudomonas aeruginosa* PAO1 and PAF79. Further, QS dependent virulence factor in the aquatic pathogen *Aeromonas hydrophila* WAF38 was also reduced. Application of *T. foenum-graecum* seed extract to PAO1, PAF79, and WAF38 decreased the biofilm forming abilities of the pathogens by significant levels. The extract also exhibited reduced AHL levels and subsequent downregulation of *lasB* gene. In vivo study showed an enhanced survival of PAO1-preinfected *C. elegans* after treatment with extract at 1 mg/mL. Further, the major compound detected by GC-MS, caffeine, reduced the production of QS regulated virulence factors and biofilm at 200 μ g/mL concentration indicating its role in the activity of the methanol extract. The results of the present study reveal the potential anti-QS and antibiofilm property of *T. foenum-graecum* extract and caffeine.” As taken from Husain FM et al. 2015. Evid. Based Complement. Alternat. Med. 2015, 879540. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26000026>

“Diabetes is a life-threatening metabolic disorder. This study was undertaken to evaluate the antihyperglycemic and antioxidative potential of seed powder of *Trigonella foenum-graecum* L in alloxan (55 mg/kg) induced diabetic rats. The results obtained showed that extensive oxidative stress is generated in tissues of diabetic rats as evidenced by increased production of hydrogen peroxide, increased accumulation of malondialdehyde (MDA) and 4-hydroxynonanal (4HNE) and decreased activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) in tissues of diabetic rats. It was observed that the transcription of genes of SOD, GPx, and CAT was also significantly decreased when compared with control. Treatment of *Trigonella* for 15 days to diabetic rats showed hypoglycemic effect and improved the altered levels of H₂O₂, MDA, and 4HNE, the activities of SOD, GPx, and CAT as well as transcription of these genes in the liver and the brain of diabetic rats.” As taken from Sharma S et al. 2015. J. Evid. Based Complementary Altern. Med. 20(3), 203-11. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25854675>

“Following an application from Avesthagen Limited, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of France, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Teestar™ and a reduction of post-prandial glycaemic responses. The Panel considers that the food, Teestar™, a fenugreek seed extract standardised by its content of galactomannan, is sufficiently characterised. A reduction of post-prandial glycaemic responses might be a beneficial physiological effect. The applicant submitted one unpublished and eight published human studies as being pertinent to the health claim. No conclusions can be drawn from the eight published studies, as they were not carried out with Teestar™ or any other fenugreek seed extract which complied with the specifications of the food which is the subject of the claim. In one unpublished study, the consumption of Teestar™ did not lead to a reduction in mean peak post-prandial blood glucose concentrations, which was the primary endpoint of the study. The Panel concludes that a cause and effect relationship has not been established between the consumption of Teestar™, a fenugreek seed extract standardised by its content of galactomannan, and a reduction of post-prandial glycaemic responses.” As taken from EFSA, 2015.

"Fenugreek (*Trigonella foenum graecum*), a medicinal herb with potent antihyperglycaemic and hypoglycaemic effects, is used to treat diabetes. This study is aimed to explore the interaction of fenugreek seed extract (FSE) and HPT (hypothalamic-pituitary-thyroid) axis in context of leptin secretion which have important role in normal and type-1 diabetic subjects. FSE (confirmed to contain trigonelline, diosgenin, 4 hydroxyisoleucine) was gavaged (0.25 gm/kg body weight/day) to normal and alloxan-induced type-1 diabetic rats for 4 weeks. Expression of hypothalamic prepro-TRH (Thyrotropin releasing hormone) mRNA, serum levels of TRH, TSH (Thyroid stimulating hormone), fT3, fT4, insulin, leptin, glucose; thyroperoxidase activity and growth of thyroid gland, food intake, adiposity index were also studied FSE significantly down regulated prepro-TRH mRNA expression; decreased serum TRH, TSH, fT3, fT4 levels, and regressed thyroid gland in FSE-fed normal and diabetic rats than those observed in normal diet-fed control and diabetic rats. FSE decreased ($p < 0.005-0.001$) adiposity index and leptin secretion, increased food intake and body weight in all FSE-fed rats. FSE improved insulin secretion, decreased glucose level but impaired HPT axis in diabetic rats, indicating insulin-independent central hypothyroidism. Results suggested that the dominant signal to hypothalamus suppressing HPT axis is the fall in leptin level which resulted from decreased adiposity index following FSE feeding. Fenugreek simultaneously having hypoglycaemic and hypothyroidal actions raises questions whether it can be safely used to treat diabetes and/or hyperthyroidism as was suggested by many workers." As taken from Majumdar J et al. 2017. *Exp. Clin. Endocrinol. Diabetes* 125(7), 441-448. PubMed, 2018 available at: <https://www.ncbi.nlm.nih.gov/pubmed/28407664>

"/ENDOCRINE MODULATION/ Several studies support hypolipidemic effect of fenugreek in normal and diabetic subjects. However, very little is known about the possible direct action of fenugreek on adipose tissue. The present study was designed to investigate the effects of fenugreek seeds on adipogenesis and lipolysis. Preadipocytes were isolated from adipose tissue of normal rats and differentiated to adipocyte in the presence of ethanolic extract of fenugreek seeds. The effect of this extract on lipolysis was also evaluated in fat tissue isolated from diabetic rats. Fenugreek led to a significant reduction in lipid droplet accumulation as evaluated with Oil Red O staining. Incubation of preadipocytes with the extract for 24 hr resulted in significant decrease in cell viability. The extract, even at high concentrations (up to 1000 ug/mL), had virtually no significant effect on lipolysis. The present data demonstrated that fenugreek seed inhibits formation of new differentiated adipocytes from precursor cells through an anti-proliferative effect on preadipocytes. [Ghorbani A et al; *Pak J Biol Sci* 17 (4): 523-8 (2014)] **PEER REVIEWED**"

"The combined effects of *Trigonella foenum-graecum* and *Allium sativum* extracts were evaluated for their ameliorative potential in the L-thyroxine-induced hyperthyroidic rat model to contribute to an understanding of interaction between the two extracts. The investigation was carried out using two different doses. A comparison was made with the response of individual plant extracts at the previously studied effective dose in adult Wistar rats rendered hyperthyroidic by daily injections of L-thyroxine (300 ug/kg body wt., s.c.). Propylthiouracil (PTU), an antithyroid drug, was used as a reference compound. Alterations in serum triiodothyronine (T3), thyroxine (T4), glucose, hepatic glucose-6-phosphatase (G-6-Pase) and oxygen consumption were studied as end parameters. Superoxide dismutase (SOD), catalase (CAT) activities, lipid peroxidation (LPO) and reduced glutathione (GSH) were examined to reveal any toxic effects of the drugs. The combined effects of *Trigonella* and *Allium* at 200 and 500 mg/kg body wt. respectively, were equipotent as compared to the individual extracts in lowering the serum concentrations of T3 and T4 in hyperthyroidic rats. Our findings reveal that some plant extracts in combination may not always prove to be synergistic. It is therefore suggested that *Trigonella foenum-graecum* and *Allium sativum* extracts may be used individually and not together in the regulation of hyperthyroidism. [Tahiliani P, Kar A; *Phytomedicine* 10 (8): 665-8 (2003)] **PEER REVIEWED**"

"/EXPL THER/ ... The *Trigonella foenum-graecum* extract has now been investigated for its effects on general properties, blood glucose and blood lipid, and hemorheological parameters in experimental diabetic rats. Streptozotocin-induced diabetic rats were administered by oral intragastric intubation separately with low dose (0.44 g/kg.d), middle dose (0.87 g/kg.d), high dose

(1.74 g/kg.d) of *Trigonella foenum-graecum* extract, and Metformin HCl (0.175 g/kg.d) for 6 weeks. Compared with diabetic group, rats treated with *Trigonella foenum-graecum* extract had an increase in body weight and a decrease in kidney /body weight ratio ($p < 0.05$). Compared with diabetic group, rats treated *Trigonella foenum-graecum* extract had lower blood glucose, glycated hemoglobin, triglycerides, total cholesterol and higher higher-density-lipoprotein-cholesterol in a dose-dependent manner ($p < 0.05$). The plasma viscosity, whole blood viscosity of high shear rate (200 s⁻¹) and low shear rate (40 s⁻¹), erythrocyte sedimentation rate, whole blood reduction viscosity and platelet conglutination were significantly reduced in diabetic rats treated with high and middle doses of *Trigonella foenum-graecum* extract, but not in those treated with low dose of *Trigonella foenum-graecum* extract. It may be concluded that *Trigonella foenum-graecum* extract can lower kidney /body weight ratio, blood glucose, blood lipid levels and improve hemorheological properties in experimental diabetic rats following repeated treatment for 6 weeks. [Xue WL et al; Asia Pac J Clin Nutr 16 Suppl 1: 422-6 (2007)] **PEER REVIEWED**

As taken from HSDB, 2016

“This study was undertaken in the animal house belong to Animal Production Department, Faculty of Agriculture, Al-Azhar University. Twenty-four male albino rats weighed 100-130 g were divided into 4 equal groups G1, control group, G2, G3 and G4 groups fed diet contained 2.5, 5 and 7.5 % fenugreek, respectively. Rats provided feed and water ad libitum. All animals were healthy and clinically free from any diseases. Blood samples were collected after 4 and 8 weeks from the start of the experiment. At the end of the experiment, rats were sacrificed to obtain the kidneys. The aim of the study was to investigate the effect of using different levels of fenugreek on kidney structure and some physiological parameters in albino rats. Results indicated that the kidney histopathological sections of the control and 2.5% fenugreek showed normal glomeruli, tubules and cortical blood vessels. On the other hand, renal section of groups treated with 5% or 7.5% fenugreek, were observed mild ischemic changes for glomeruli (small sizes). Treatment of rats with 5% or 7.5% fenugreek significantly increased serum urea and creatinine levels, while serum glucose, cholesterol and triglycerides significantly decreased. Treatment of rats with 2.5% fenugreek for 8 weeks did not show any significant effect on serum urea, creatinine and glucose levels, while serum cholesterol and triglycerides significantly decreased. It could be concluded that 2.5% fenugreek is safe to be used as a hypocholesterolemic agent without any side effect for better kidney structure and function.” As taken from Badr MI et al. 2017. Middle East Journal of Applied Sciences 7(4), 967-973. Available at https://www.researchgate.net/publication/322152737_Effect_of_fenugreek_seeds_on_kidney_structure_and_some_physiological_parameters_in_rats

“Liver toxicity has been reported, both taken alone and in herbal combinations that included fenugreek.”

As taken from LactMed, 2021. Record for CAS RN 68990-15-8.

“Dimethoate is a widely used organophosphate insecticide known to be toxic to the pancreas. The aim of this study is to detect the possible protective effects of the fenugreek seed ethanolic extract on the biochemical, histological, and ultra-structural abnormalities induced by dimethoate chronic exposure in the pancreas of adult male rats. The study was conducted on 50 adult male albino rats that were divided equally into 5 groups: (group I) negative control, (group II) vehicle control group, (group III) fenugreek-treated group that was given 400 mg/kg ethanolic fenugreek seed extract once daily, (group IV) dimethoate group received 20 mg/kg/day dimethoate, and (group V) dimethoate- + fenugreek-treated group received a combination of dimethoate and fenugreek in the same previous doses. Dimethoate treatment caused a significant increase in serum glucose, amylase, and lipase levels and a significant decrease in serum insulin. A significant increase in lipid peroxidation and pro-fibrotic cytokine (TGF- β 1) together with a significant reduction of the antioxidant {reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD)} activities and the anti-inflammatory (IL-4) in pancreatic tissues was also recorded. There was a histological and ultra-structural evidence of pancreatic acinar and islet cell injury. The recorded abnormalities

were reversed in dimethoate+fenugreek treated group indicating that fenugreek ethanolic extract can serve as an antidote for dimethoate-induced pancreatic insult.” As taken from Mesallam DIA et al. 2018. Environ. Sci. Pollut. Res. Int. 25(4), 3894-3904. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29177779>

“The human body on exposure to high-altitude, undergoes many physiological challenges. The cardiopulmonary reserves are favoured against the digestive system. Hence, the efficiency of digestion is compromised to a great extent, which leads to anorexia, hypophagia, epigastralgia, dyspepsia, nausea, and peptic ulcers. The present study was focused on in vitro digestive influence of selected food ingredients viz. cardamom, carom, cumin, coriander, fennel, fenugreek, ginger, pepper, star anise, turmeric, papaya, orange, pineapple, liquorice, valerian, and tarragon on the activities of digestive enzymes of rat pancreas, duodenum, and small intestine. In-vitro antioxidant activities of the above food ingredients were also carried out with respect to their radical scavenging activity against DPPH·, NO·, and ferrous reducing antioxidant power. All the studied food ingredients showed a comparative range of free radical scavenging activity. Further, pineapple has shown enhanced enzymatic activity of pancreatic amylase, trypsin and chymotrypsin among the tested samples with 432, 252, and 86%, respectively. However, all food ingredients showed inhibitory effect towards maltase activity, while the sucrose activity was enhanced in tarragon compared to control. Almost all the selected food ingredients have been observed to have low glycemic index and low protein efficiency ratio except pineapple. The results suggested that ample merit in the use of pineapple extract can be carried forward for the formulation of highly digestible foods for extreme environmental conditions.” As taken from Anusha MB et al. 2018. J. Food Sci. Technol. 55(5), 1913-1921. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29666544>

“In a survey of nursing mothers in the United States, 85 had used fenugreek as a galactagogue and 45% reported having experienced an adverse reaction from the supplement”.

As taken from LactMed, 2021. Record for CAS RN 68990-15-8.

“We previously demonstrated galactagogue effect of fenugreek in a rat model of lactation challenge, foreshadowing its use in women's breastfeeding management. To assess longitudinal molecular mechanisms involved in milk synthesis/secretion in dams submitted to fenugreek supplementation, inguinal mammary, pituitary glands and plasma were isolated in forty-three rats nursing large 12 pups-litters and assigned to either a control (CTL) or a fenugreek-supplemented (FEN) diet during lactation. RT-PCR were performed at days 12 and 18 of lactation (L12 and L18) and the first day of involution (Inv1) to measure the relative expression of genes related to both milk synthesis and its regulation in the mammary gland and lactogenic hormones in the pituitary gland. Plasma hormone concentrations were measured by ELISA. FEN diet induced 2- to 3-times higher fold change in relative expression of several genes related to macronutrient synthesis (Fasn, Acaca, Fabp3, B4galt1, Lalba and Csn2) and energy metabolism (Cpt1a, Acads) and in IGF-1 receptor in mammary gland, mainly at L12. Pituitary oxytocin expression and plasma insulin concentration (+77.1%) were also significantly increased. Altogether, these findings suggest fenugreek might extend duration of peak milk synthesis through modulation of the insulin/GH/IGF-1 axis and increase milk ejection by activation of oxytocin secretion.” As taken from Sevrin T et al. 2020. Genes (Basel) 11(10), 1208. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33081164/>

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing fenugreek extract and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of fenugreek extract. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	311 (68990-15-8)	Carmines, 2002 & Rustemeier et al., 2002
	23 (extract, 84625-40-1) 12 (oil and oleoresin, 84625-40-1) 585 (tincture, 84625-40-1)	Baker et al., 2004a
	300(No CAS) 1,500 (68990-15-8)	JTI KB Study Report(s)
	-	Gaworski et al., 2011
	132 (CAS 84625-40-1) 798(CAS 68990-15-8)	Roemer et al., 2014
	300 (CAS 84625-40-1)	Stabbert et al. 2019
In vitro genotoxicity	311 (68990-15-8)	Carmines, 2002 & Röemer et al., 2002
	200 (extract, 84625-40-1) 12 (oil and oleoresin, 84625-40-1) 585 (tincture, 84625-40-1)	Baker et al., 2004c
	300 (No CAS) 1,500 (68990-15-8)	JTI KB Study Report(s)
	4,630 (84625-40-1)	fGLH Study Report (2010)
	-	Gaworski et al., 2011
	132 (CAS 84625-40-1) 798(CAS 68990-15-8)	Roemer et al., 2014
	300 (CAS 84625-40-1)	Stabbert et al. 2019
In vitro cytotoxicity	311 (68990-15-8)	Carmines, 2002 & Röemer et al., 2002
	200 (extract, 84625-40-1) 12 (oil and oleoresin, 84625-40-1) 585 (tincture, 84625-40-1)	Baker et al., 2004c
	300 (No CAS) 1,500 (68990-15-8)	JTI KB Study Report(s)
	4,630 (84625-40-1)	fGLH Study Report (2010)
	-	Gaworski et al., 2011
	132 (CAS 84625-40-1) 798(CAS 68990-15-8)	Roemer et al., 2014

	300 (CAS 84625-40-1)	Stabbert et al. 2019
Inhalation study	2.5 (68990-15-8)	Gaworski et al., 1998
	311 (68990-15-8)	Carmines, 2002 & Vanscheeuwijck et al., 2002
	200 (extract, 84625-40-1) 12 (oil and oleoresin, 84625-40-1) 585 (tincture, 84625-40-1)	Baker et al., 2004c
	300 (No CAS) 1,500 (68990-15-8)	JTI KB Study Report(s)
	-	Gaworski et al., 2011
	132 (CAS 84625-40-1) 798(CAS 68990-15-8)	Schramke et al., 2014
Skin painting	25 (68990-15-8)	Gaworski et al., 1999
	300 (No CAS) 1,500 (68990-15-8)	JTI KB Study Report(s)
In vivo genotoxicity	132 (CAS 84625-40-1) 798(CAS 68990-15-8)	Schramke et al., 2014

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Fenugreek extract (84625-40-1) was tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the TPM was not increased by the addition of Fenugreek extract (84625-40-1) when compared to TPM from 3R4F cigarettes. The table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
In vitro genotoxicity	52	JTI KB Study Report(s)
In vitro cytotoxicity	52	JTI KB Study Report(s)

Aerosol from heated tobacco stick(s) containing Fenugreek extract was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	0.00691	Labstat International Inc. (2021a) Labstat International Inc. (2023a) JTI Heated Tobacco Stick Study Report(s)
In vitro genotoxicity	0.00691	Labstat International Inc. (2021b) Labstat International Inc. (2023b) JTI Heated Tobacco Stick Study Report(s)
In vitro cytotoxicity	0.00691	Labstat International Inc. (2021b) Labstat International Inc. (2023b) JTI Heated Tobacco Stick Study Report(s)

10. Ecotoxicity

10.1. Environmental fate

EPISuite provides the following data for CAS 68990-15-8:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	1.24E-004 atm-m3/mole (1.26E+001 Pa-m3/mole)
Group Method:	Incomplete
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.453E-010 atm-m3/mole (1.472E-005 Pa-m3/mole) VP: 0.000192 mm Hg (source: MPBPVP) WS: 2.23E+005 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Log Kow used:	-0.44 (KowWin est)
Log Kaw used:	-2.295 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	1.855
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	1.0195
Biowin2 (Non-Linear Model) :	0.9983
Biowin3 (Ultimate Survey Model):	3.2162 (weeks)
Biowin4 (Primary Survey Model) :	4.0213 (days)
Biowin5 (MITI Linear Model) :	0.8491
Biowin6 (MITI Non-Linear Model):	0.8990
Biowin7 (Anaerobic Linear Model):	1.0210
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:

Vapor pressure (liquid/subcooled):	0.0373 Pa (0.00028 mm Hg)
Log Koa (Koawin est):	1.855
Kp (particle/gas partition coef. (m ³ /ug)):	8.04E-005
Mackay model:	1.76E-011
Octanol/air (Koa) model:	

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.00289
Mackay model:	0.00639
Octanol/air (Koa) model:	1.41E-009

Atmospheric Oxidation (25 deg C) [AopWin v1.92]: Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	40.9298 E-12 cm ³ /molecule-sec
Half-Life =	0.261 Days (12-hr day; 1.5E6 OH/cm ³)
Half-Life =	3.136 Hrs

Ozone Reaction:

OVERALL Ozone Rate Constant =	7.393750 E-17 cm ³ /molecule-sec
Half-Life =	0.155 Days (at 7E11 mol/cm ³)
Half-Life =	3.720 Hrs
Fraction sorbed to airborne particulates (phi): 0.00464 (Junge-Pankow, Mackay avg) 1.41E-009 (Koa method)	
Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	1.367 L/kg (MCI method)
Log Koc:	0.136 (MCI method)
Koc :	1.602 L/kg (Kow method)
Log Koc:	0.205 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]: Rate constants can NOT be estimated for this structure!

Volatilization from Water: Henry LC: 0.000124 atm-m³/mole (estimated by Bond SAR Method)

Half-Life from Model River:	6.5 hours
Half-Life from Model Lake:	165.8 hours (6.909 days)

Removal In Wastewater Treatment:

Total removal:	7.65 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.67 percent
Total to Air:	5.90 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.916	2.33	1000
Water	51.8	360	1000
Soil	47.2	720	1000
Sediment	0.0977	3.24e+003	0

Persistence Time: 209 hr

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, fenugreek (CAS RN 68990-15-8) are of uncertain persistence in the environment.

Data accessed April 2017 on the OECD website.

“ARTIFICIAL POLLUTION SOURCES:

Fenugreek extract's production and use as a flavoring agent in foods, curry powders, chutney and imitation maple syrup(1,2), as an additive ingredient in cigarettes(3) and as an emollient(2) may result in its release to the environment through various waste streams(SRC).[(1) Grenis AT; Spices. Kirk-Othmer Encyclopedia of Chemical Technology. (1999-2016). New York, NY: John Wiley & Sons. Online Posting Date: Sep 15, 2006. (2) O'Neil MJ, ed; The Merck Index. 16th ed., Cambridge, UK: Royal Society of Chemistry, p. 736 (2013) (3) RJ Reynolds; List of Ingredients (cigarettes), RJ Reynolds Tobacco Company (2014). Available from, as of June 9, 2016: <http://www.rjrt.com/commercial-integrity/ingredients/cigarette-ingredients/> **PEER REVIEWED**”

As taken from HSDB, 2016

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, fenugreek (CAS RN 68990-15-8) are not inherently toxic to aquatic organisms and are of low ecotoxicological concern.

Data accessed April 2017 on the OECD website.

ECOSAR version 1.11 reports the following aquatic toxicity data for CAS RN 68990-15-8:

Values	used	to	Generate	ECOSAR	Profile:		
Log	Kow:	-0.442	(EPISuite	Kowwin	v1.68	Estimate)	
Wat	Sol:	2.228E+005	(mg/L,	EPISuite	WSKowwin	v1.43	Estimate)

ECOSAR	v1.11	Class-specific	Estimations
Esters			
Vinyl/Allyl			Alcohols
Vinyl/Allyl Esters			

ECOSAR Class Organism Duration End Pt Predicted mg/L (ppm)

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Esters : Fish 96-hr LC50 456.649
 Esters : Daphnid 48-hr LC50 1232.857
 Esters : Green Algae 96-hr EC50 772.481
 Esters : Fish ChV 57.352
 Esters : Daphnid ChV 1701.202
 Esters : Green Algae ChV 89.626
 Esters : Fish (SW) 96-hr LC50 806.727
 Esters : Mysid 96-hr LC50 2642.455
 Esters : Fish (SW) ChV 74.508
 Esters : Mysid (SW) ChV 2.82e+007 *
 Vinyl/Allyl Alcohols : Fish 96-hr LC50 5.753
 Vinyl/Allyl Alcohols : Daphnid 48-hr LC50 0.655
 Vinyl/Allyl Alcohols : Green Algae 96-hr EC50 505.472
 Vinyl/Allyl Alcohols : Fish ChV 0.799 !
 Vinyl/Allyl Alcohols : Daphnid ChV 0.089 !
 Vinyl/Allyl Alcohols : Green Algae ChV 42.845
 Vinyl/Allyl Esters : Fish 96-hr LC50 2.426
 Vinyl/Allyl Esters : Daphnid 48-hr LC50 54.637
 Vinyl/Allyl Esters : Green Algae 96-hr EC50 18.190
 Vinyl/Allyl Esters : Fish ChV 0.318 !
 Vinyl/Allyl Esters : Daphnid ChV 6.597 !
 Vinyl/Allyl Esters : Green Algae ChV 2.732

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Neutral Organic SAR : Fish 96-hr LC50 16404.311

(Baseline Toxicity) : Daphnid 48-hr LC50 7434.533

: Green Algae 96-hr EC50 2180.485

: Fish ChV 1229.065

: Daphnid ChV 387.153

: Green Algae ChV 345.642

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

NOTE: ! = exclamation designates: The toxicity value was estimated through application of acute-to-chronic ratios per methods outlined in the ECOSAR Methodology Document provided in the ECOSAR Help Menu.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Uptake and translocation of metals in fenugreek grown on soil amended with tannery sludge: involvement of antioxidants (Abstract). Agricultural and industrial activities cause heavy metal pollution in the soil, which adversely affect the plant growing therein. The plants of fenugreek (*Trigonella foenum-graecum* L.) were grown in soil amended with different percent of tannery sludge (TS) (10%, 25%, 35%, 50%, and 100% TS) in order to study the effect on antioxidant levels due to translocation of metals (Fe, Zn, Mn, Cu, Cr, Pb). The accumulation of the metals was found more in shoots than roots, except Fe and Cr. The level of metals in seeds of the plant increased with increase in sludge amendments ratio except Mn, which decreased in roots, shoots, and seeds of the plant. Chromium was found below detection limits in the seeds at 10% and 25% TS. Correlation coefficient (r) between total metal accumulation and extractable metals showed that Zn ($P < 0.01$), Cr ($P < 0.01$), and Cu ($P < 0.05$) are significantly correlated, whereas, correlation with pH showed significant positive relation with all the studied metals except Mn. Significant positive correlation was recorded between metal accumulation (Fe, Zn, Cu) and electrical conductivity, cation exchange capacity, and organic matter, however, Zn, Cr, and Cu showed significant positive correlation with bulk density, nitrate, ammonia, and available phosphorus. The analysis of the results showed that total chlorophyll content showed significant ($P < 0.5$) increase in lower amendment of sludge (up to 35% TS at 30 d and 25% TS at 60 d) as over their controls. In roots, malondialdehyde, cysteine, non-protein thiol, proline, protein, ascorbic acid contents increased up to 35% TS at 30 d. Principal component analysis also showed that strong association exists among malondialdehyde, nonprotein thiol, protein, and cysteine contents in the plants grown on different amendments of TS. The level of antioxidants increased which enabled the plant to cope up the stress induced in the plants grown on lower amendments of TS, however, toxicity was observed at higher amendments. As taken from Sinha et al. *Ecotoxicol Environ Saf.* 2007 Jun; 67(2):267-77. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/1704937>(CAS 68990-15-8)

“The insecticidal efficacy of *Trigonella foenum-graecum* (fenugreek) on the 3rd stage larvae of *Musca domestica* and adult fecundity was evaluated under controlled laboratory conditions. The concentrations from 25% to 100% completely killed the larvae. 5%, 2% and 1% caused mortality percent of 44.4, 33.3 and 22.2 respectively. Less concentration of fenugreek was not tried. On the other hand, the fecundity of the emerged adults was 20%, Zero% and 28.6%. On the other hand,

only one control larva died and the nine emerged adults were fertile. So, fenugreek at low concentration not only has a larvicidal action against house fly larvae but also affected the adult fecundity". As taken from Abdel Halim AS & Morsy TA. 2006. J. Egypt. Soc. Parasitol. 36, 329-334 PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/16605122?dopt=AbstractPlus>.

Potential of biologically active plant oils to control mosquito larvae (*Culex pipiens*, Diptera: Culicidae) from an Egyptian locality (Abstract). The insecticidal effect of six commercially available plant oils was tested against 4th larval instars of *Culex pipiens*. Larvae were originally collected from Meit El-Attar, Qalyubia Governorate, Egypt, and then reared in the laboratory until F1 generation. The LC50 values were 32.42, 47.17, 71.37, 83.36, 86.06, and 152.94 ppm for fenugreek (*Trigonella foenum-grecum*), earth almond (*Cyperus esculentus*), mustard (*Brassica compestris*), olibanum (*Boswellia serrata*), rocket (*Eruca sativa*), and parsley (*Carum ptroselinum*), respectively. The tested oils altered some biological aspects of *C. pipiens*, for instance, developmental periods, pupation rates, and adult emergences. The lowest concentrations of olibanum and fenugreek oils caused remarkable prolongation of larval and pupal durations. Data also showed that the increase of concentrations was directly proportional to reduction in pupation rates and adult emergences. Remarkable decrease in pupation rate was achieved by mustard oil at 1000 ppm. Adult emergence was suppressed by earth almond and fenugreek oils at 25 ppm. In addition, the tested plant oils exhibited various morphological abnormalities on larvae, pupae, and adult stages. Consequently, fenugreek was the most potent oil and the major cause of malformation of both larval and pupal stages. Potency of the applied plant oils provided an excellent potential for controlling *C. pipiens*. As taken from Khater and Shalaby. Rev Inst Med Trop Sao Paulo. 2008 Mar-Apr; 50(2):107-12. PubMed, 2010 available at <http://www.ncbi.nlm.nih.gov/pubmed/18488090>(CAS 68990-15-8)

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 68990-15-8:

Values used	to	Generate	ECOSAR	Profile:
Log Kow:	-0.442	(EPISuite Kowwin	v1.68	Estimate)
Wat Sol:	2.228E+005	(mg/L, EPISuite WSKowwin	v1.43	Estimate)
ECOSAR Esters	v1.11	Class-specific		Estimations
Vinyl/Allyl				Alcohols
Vinyl/Allyl Esters				

ECOSAR Class Organism Duration End Pt Predicted mg/L (ppm)

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Esters : Earthworm 14-day LC50 10232.608

10.5. All other relevant types of ecotoxicity

EPISuite provides the following data for CAS 68990-15-8:

Bioaccumulation Estimates (BCFBAF v3.01): Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-2.3206 days (HL = 0.00478 days)

Log BCF Arnot-Gobas method (upper trophic):	-0.046 (BCF = 0.9)
Log BAF Arnot-Gobas method (upper trophic):	-0.046 (BAF = 0.9)
log Kow used:	-0.44 (estimated)

The Ecological Categorization Results from the Canadian Domestic Substances List simply state that oils, fenugreek (CAS RN 68990-15-8) are of uncertain bioaccumulative potential in the environment.

Data accessed April 2017 on the OECD website: <http://webnet.oecd.org/CCRWeb/Search.aspx>

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13. Last audited

February 2025

Fenugreek (*Trigonella foenum-graecum*)



Synonyms / Common Names / Related Terms

4-hydroxyisoleucine (4-OH-Ile), abish, alholva, bird's foot, bockhornsklover, bockshornsamen, bockshornklee, cemen, chilbe, diosgenin, diosgenin fenegriek, fenogregó, fenugree, fenugreek flour, fenugreek gums, fenugreek leaves, fenugreek seed, fenugreek spouts, fenogreco, fenigreko, fenu-thyme, foenugraeci semen, gorogszena, graine de fenugrec, gray hay, Greek hay seed, griechische Heusamen (German), fieno greco, halba, hilbeh, hulba, hu lu ba, kasoori methi, kozieradka pospolita, kreeka lambalaats, mente, mentikura, mentula, methi, methika, methini, methri, methro, mithiguti, N,N'-dicarbazyl, pazhitnik grecheskiy, penantazi, phenolic acids, protodioscin, sag methi, sambala, sarviapila, shabaliidag, shambelile, star fenugreek, trigonella, *Trigonella balansae*, *Trigonella caerulea*, *Trigonella foenum-graecum*, *Trigonella semen*, *Trigonella stellata*, *Trigonella*, trigonelline, uluhaal, uwatu, vendayam, venthiam, wheat-fenugreek.

Mechanism of Action

Pharmacology:

- **Constituents:** In laboratory tests, fenugreek has been found to contain 4-hydroxyisoleucine (4-OH-Ile), fat, diosgenin, iron, phenolic acids, protein, and protodioscin.^{27,28,30,7,31,29}
- **Analgesic effects:** In a rat study, *Trigonella foenum-graecum* extract showed analgesic activity, which may be similar to nonsteroidal anti-inflammatory drugs (NSAIDs) via the spinal 5-HT system or purinoceptors.^{1,12}
- **Antiadhesive properties:** Bactericidal and anti-adhesive properties against *Helicobacter pylori* have been studied.⁵ The bactericidal activity of the extract was assessed by a standard kill-curve with seven strains of *H. pylori*. The anti-adhesive property was assessed by the inhibition of binding of four strains of FITC-labeled *H. pylori* to stomach sections. Fenugreek was found to have no bactericidal effect on any of the isolates.
- **Anticarcinogenesis effects:** In rats, dietary fenugreek seeds inhibited colon carcinogenesis³ and diosgenin from fenugreek suppressed total colonic aberrant crypt foci formation⁴. In the seeds, the effect may be due to the fiber, flavonoids, or saponins that modulate beta-glucuronidase and mucinase activities. However, the diosgenin study indicated that the extract inhibited bcl-2 and induced caspase-3 protein expression, thereby inducing apoptosis and inhibiting cell growth. Another study of diosgenin indicates that its activity may be due to inhibition of NF-kappaB-regulated gene expression.²⁷ Another chemical extracted from fenugreek, protodioscin, strongly inhibited growth of HL-60 cells, but had little effect on KATO III cells *in vitro*.⁷ Apoptosis in the HL-60 cells seems to have been due to the concentration- and time-dependent fragmentation of DNA by protodiosgenin.
- In a review article, authors presented evidence that numerous agents such as diosgenin (fenugreek) identified from fruits and vegetables can interfere with several cell-signaling pathways.³² The results of several studies indicate that a diet rich in fresh vegetables protects against several common epithelial neoplasms.³³ This probable effect has been related to specific micronutrients contained in vegetables. A case-control study and systematic assessment of the relationship between vegetable intake and the risk of gallbladder cancer was conducted in 153 patients with gallbladder cancer and 153 controls with gallstone disease. Each patient's consumption of vegetables was assessed by using a food frequency questionnaire. The frequency

of vegetable consumption was divided into three levels: ≥ 3 days/week, 1-2 days/week and no or rare consumption. Participants were divided into three groups according to the level of vegetable intake. Odds ratios and 95% confidence intervals were computed for subsequent levels of vegetable consumption compared with the high level of consumption. A low consumption of vegetables showed an increase in odds ratio for gallbladder cancer for almost all the vegetables studied. A significant inverse trend was observed for green leafy vegetables and gallbladder cancer. An inverse association was observed for amaranth with an OR of 3.45 for the low vs. high level of consumption. Corresponding values were 2.14 for spinach, 1.86 for bathua, 1.02 for bengalgram leaves, 2.26 for cabbage, 3.06 for fenugreek leaves, 1.95 for mustard leaves and 1.44 for radish leaves. An inverse relationship between the risk of gallbladder cancer and the level of vegetable consumption was observed.

- **Antioxidant activity:** In an ethanol toxicity rat study, an aqueous extract of fenugreek seeds prevented the rise in lipid peroxidation and enhanced antioxidant potential.⁹ These results are supported by *in vitro* evidence in diabetic human erythrocytes, that polyphenol acids from fenugreek seeds showed a concentration-dependent inhibition of lipid peroxidation.²
- **Antiplatelet activity:** In a rat study, a fenugreek extract inhibited ADP (10^{-5} M) induced platelet aggregation ($IC_{50}=1.28$ mg/mL).¹²
- **Exercise recovery effects:** In trained male cyclists, a glucose beverage and 4-hydroxyisoleucine isolated from fenugreek seeds significantly increased muscle glycogen concentration 63% from immediately post exercise to four hours after exercise compared to the control.²⁸
- **Hepatoprotective activity:** In an *in vitro* study using Chang liver cells treated with ethanol, a polyphenolic extract of fenugreek seeds significantly and dose-dependently increased cell viability by reducing oxidation and apoptosis.⁸
- **Hypoglycemic effects:** Hypoglycemic effects of fenugreek observed in animal studies have been associated with a fraction that contains the testa and endosperm of the defatted seeds, called the "A" subfraction. These effects have not been observed with lipid extracts.^{34,35} Hypoglycemic effects have been attributed to several mechanisms: Sauvaire et al. demonstrated that the amino acid 4-hydroxyisoleucine in fenugreek seeds increases glucose-induced insulin release *in vitro* in human and rat pancreatic islet cells.¹⁶ This amino acid appeared to act only on pancreatic beta cells, since somatostatin and glucagon were not altered in the study. However, another *in vitro* study indicates that fenugreek seed extract phosphorylates a number of proteins, including the insulin receptor, insulin receptor substrate 1 and p85 subunit of PI3-K, in both 3T3-L1 adipocytes and human hepatoma cells, HepG2.¹⁷ These results suggest that fenugreek's effects may be due to activation of the insulin-signaling pathway in adipocytes and liver cells. In human studies, fenugreek reduced the area under the plasma glucose curve (AUC) and increased the number of insulin receptors via an unclear mechanism.¹⁰ Also, a combination of bittergourd, jamun seeds, and fenugreek seeds significantly reduced fasting and postprandial glucose level of the diabetic patients¹⁵. Fenugreek seeds have also been postulated to exert hypoglycemic effects by stimulating glucose-dependent insulin release by beta cells¹³, or via inhibition of α -amylase and sucrase activity¹⁴. A unique major free amino acid, 4-hydroxyisoleucine (4-OH Ile), has also been characterized as one of the active ingredients for blood glucose control.¹¹

- **Insulin sensitization effects:** When administered to type 2 diabetic rats, the amino acid 4-hydroxyisoleucine extracted from fenugreek seeds increased peripheral glucose utilization and decreased hepatic glucose production, thereby improving insulin resistance.⁶ Chronic ingestion of 4-hydroxyisoleucine significantly reduced insulinemia.
- **Lipid-lowering effects:** In animal studies, fenugreek has been found to lower triglycerides, total cholesterol, and low density lipoprotein (LDL) levels.^{18,19,20,21,22} These effects may be due to saponins, a class of molecule present in fenugreek that is transformed in the gastrointestinal tract to sapogenins. Sapogenins increase biliary cholesterol secretion, potentially leading to lower serum cholesterol levels.^{23,18,24,25} Based on another *in vitro* study, fenugreek may increase intraluminal binding of cholesterol, which results in increased fecal excretion of bile acids and neutral sterols.²⁶

Pharmacodynamics/Kinetics:

- Pharmacokinetic data are not available for all components of fenugreek, or for the compound as a whole. Saponins present in fenugreek are believed to be primarily absorbed in the terminal ileum.³⁶
- In a rabbit study by Zhao et al., after post-intragastric injection of fenugreek extract, the pharmacokinetic parameters of one compartment model were half-life, $t_{1/2}$ (Ka) = 0.9 hr, $t_{1/2}$ (Ke) = 2.2hr, volume of distribution = 0.64L/kg and AUC = 1.93mg*min/L.³⁷ After intravenous injection, the two compartment open model parameters were $t_{1/2}$ (Ka) = 10.8 min, $t_{1/2}$ (Ke) = 44 min, $K_{2,1}$ = 0.044/min, $K_{1,0}$ = 0.026 min, $K_{1,2}$ = 0.017/min, and the AUC = 931mg*min/L.

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EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

27 January 2011
EMA/HMPC/146220/2010
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Trigonella foenum-graecum* L., semen

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	<i>Trigonella foenum-graecum</i> L., semen
Herbal preparation(s)	Dry extract (solvent ethanol 20% v/v, DER 4:1) Soft extract (solvent ethanol 60% v/v, DER 5-6:1)
Pharmaceutical forms	Herbal substance or herbal preparations in solid dosage forms or as herbal tea for oral use. Herbal substance for infusion for cutaneous use
Rapporteur	Antoine SAWAYA
Assessor(s)	Pharmaceutical: Jacqueline VIGUET POUPELLOZ Non-clinical: Fabien LAVERGNE Clinical: Roxane FORNACCIARI



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1. Introduction

The aim of this report is to assess the non-clinical and clinical data available on *Trigonellae foenugraeci* semen for preparing a Community herbal monograph. This report is based on the documentation published in the literature.

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Fenugreek seed

- Herbal preparation(s)

Powder, dry extract, soft extract

Fenugreek seed is rich in mucilage polysaccharides (consisting mainly of galactomannans 25–45%) and contains a small amount of essential oil (0.015%) and a variety of secondary metabolites, including protoalkaloids, trigonelline (up to 0.37%), choline (0.05%); saponins (0.6–1.7%) derived from diosgenin, yamogenin, tigogenin and other compounds; sterols including β -sitosterol; and flavonoids, among which are orientin, isoorientin and isovitexin (WHO, 2007). Furthermore, the nutrition composition of fenugreek seeds is : moisture 2.4%, protein 30%, lipids 7%, saponins 4.8%, total dietary fibre 48% (insoluble 28%, soluble 20%), and ash 3.9% (WHO, 2007; ESCOP, 2003; Muralidhara et al, 1999; BRUNETON, 1998; Udayasekhara Rao et al, 1996; PARIS AND MOYSE, 1967).

The European Pharmacopoeia does not prescribe any assay (monograph ref. 01/2008:1323 corrected 6.6).

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

1.2. Information about products on the market in the Member States

Fenugreek as single active substance is authorised in France, Poland and Spain.

The active substance is present on the market as herbal substance for herbal tea and for infusion for external use (Poland, over 30 years; Spain), powder (France 1990; Spain 1990, 1992), dry extract (solvent: ethanol 20% v/v, DER 4:1) (France 1970, 2003), soft extract (solvent: ethanol 60% v/v, DER 5-6:1) (France 1970, 2003).

Regulatory status overview

Member State	Regulatory Status				Comments (not mandatory field)
Austria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
Belgium	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only as food supplement
Bulgaria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Cyprus	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
Czech Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
Denmark	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
Estonia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Finland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
France	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Germany	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only one standard marketing authorisation
Greece	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Hungary	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Iceland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Ireland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
Italy	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only as food supplement
Latvia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only in combinations
Liechtenstein	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Lithuania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Luxemburg	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Malta	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
The Netherlands	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
Norway	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Poland	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Portugal	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
Romania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Slovak Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
Slovenia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
Spain	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Sweden	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products
United Kingdom	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No herbal medicinal products

MA: Marketing Authorisation

TRAD: Traditional Use Registration

Other TRAD: Other national Traditional systems of registration

Other: If known, it should be specified or otherwise add 'Not Known'

This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

Non-clinical and clinical strategies

Online databases were used to research available non-clinical and clinical data on fenugreek preparations. No data was provided by the interested parties.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Based on the feedback obtained from Member States, a use is reported for a long period in the EU. Moreover, publications also report a use in non EU countries, in line with the fact that this plant is cultivated in the Indian continent, in the Mediterranean region and in North Africa.

2.2. Information on traditional/current indications and specified substances/preparations

Fenugreek (*Trigonella foenum-graecum* L., *Fabaceae*) is one of the oldest medicinal plants, originating in India and Northern Africa.

An annual plant, fenugreek grows to an average height of two feet. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use. Applications of fenugreek were documented in ancient Egypt, where it was used in incense and to embalm mummies. In modern Egypt, fenugreek is still used as a supplement in wheat and maize flour for bread-making. In ancient Rome, fenugreek was purportedly used to aid labour and delivery. In traditional Chinese medicine, fenugreek seeds are used as a tonic, as well as a treatment for weakness and oedema of the legs. In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant. There are numerous other folkloric uses of fenugreek, including the treatment of indigestion and baldness. The possible hypoglycaemic and antihyperlipidemic properties of oral fenugreek seed powder have been suggested by the results of preliminary animal and human trials.

The medicinally used plant part of fenugreek is the seed. It was already mentioned in the French Pharmacopoeia published in 1908. Herbal preparations like powder or liquid extract have been used in the past to stimulate the appetite (Paris and Moyse, 1967).

An internal use as adjuvant therapy in diabetes mellitus, anorexia, as an adjunct to a low fat diet in the treatment of mild to moderate hypercholesterolemia and an external use in case of furunculosis, ulcers and eczema are mentioned in the ESCOP Monograph.

Fenugreek is also a part of the ayurvedic pharmacopoeia and used in arthritis and spondylosis, adjunct in diabetes mellitus and hyperlipidaemia (Selected medicinal Plants of India, 1992).

Fenugreek has been used as herbal substance since 1970 in France and over 30 years in Poland.

In France, Poland and Spain, fenugreek is a traditional herbal medicinal product. The current therapeutic indications in these European countries are:

For **oral use**:

In France: traditionally used to help weight gain

In Poland:

- as appetite stimulant
- in lack of appetite
- orally as gastrointestinal emollient

In Spain: loss of appetite

For **external use**:

In Poland:

- topically in a form of cataplasms in skin inflammations, as emollient, coating and for skin healing
- topically in skin inflammations, topically in wounds, rashes, furunculosis
- traditionally used externally in a form of cataplasms in skin inflammations (eruptions, furunculosis) as healing promotion
- externally in skin inflammation conditions

In Spain: in minor local skin inflammations

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

In France: 2 herbal medicinal products (extracts) have been on the market since 1970 and 1 (powder) since 1990 for oral use:

1. Dry extract (solvent: ethanol 20% v/v, DER 4:1):295 mg 2 times daily.
2. Soft extract (solvent: ethanol 60% v/v, DER 5-6:1):500 mg 2 times daily.
3. 495 mg 3 to 5 times daily traditionally used to help weight gain.

In Poland: 5 herbal medicinal products for oral use (herbal tea) and external use (cataplasm) have been on the market for over 30 years:

1. Externally in a form of cataplasms in skin inflammations, as emollient, coating and for skin healing: 50 g of seeds, bring to the boil 5 min in 250 ml of water, use the obtained warm pulp as cataplasm 2 to 3 times daily. Orally as appetite stimulant: 1 teaspoon (2 g) of grained seeds, use before meals.
2. Orally in lack of appetite: 1-2 teaspoons (3-6 g) taken before meals. Topically in skin inflammations, mix ground seeds with water (25 g of seeds to 100 ml of water), bring to the boil in 5 minutes. Use the obtained warm paste as a warm cataplasm 2-3 times daily.
3. Orally in lack of appetite: 1-6 g of ground seeds before meals. Topically in wounds, rashes and furunculosis: mix 20 g of seeds with 100 ml of water (1/2 of glass), heat 5 minutes. Use as warm cataplasms 2-3 times daily.
4. Orally: use a decoction, 8 g seeds in a glass of water; bring to the boil 15 minutes. Drink 2-3 times daily, before meals. Externally in a form of cataplasms in skin inflammations (eruptions,

furunculosis) 50 g of seeds in 250 ml of water, bring to the boil. Use the warm pulp as a cataplasm 2–3 times daily.

5. Orally in lack of appetite. 1.6 g of ground seeds (1/4 of teaspoon), 3 times daily. Externally in skin inflammation conditions, mix 50 g of ground seeds with 250 ml (1 glass) of water, heat and use such a warm cataplasm several times a day.

In Spain: 1 herbal medicinal product on the market for external use and 3 herbal medicinal products on the market for oral use (1 herbal tea and 2 powders (1990 and 1992)).

1. Up to 50 g a day for external use, minor local skin inflammations.
2. Up to 3 times a day (6 g of herbal substance a day).
3. 1100 mg 3 times a day.
4. 380 to 760 mg 3 times a day. Used in loss of appetite.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

The WHO described the medicinal uses of fenugreek seeds, either, supported by clinical data, described in pharmacopoeias and well-established documents, or described in traditional medicine (WHO, 2007).

3.1.1. Primary pharmacodynamics

Only one study dealing with the effect of fenugreek seeds on appetite was located in the literature. Petit et al (1993) showed in rats that oral administration of a hydro-ethanolic seed extract increased food intake and motivation to eat. However, treatment had no preventative effect on drug-induced anorexia/decreased motivation to eat (see **Table 1**).

Assessor's comment

Only sparse non-clinical pharmacology study is available to support the use of fenugreek seeds for loss of appetite.

3.1.2. Secondary pharmacodynamics

Hypoglycaemic effect

Most of the data found in the literature were performed to support the use of fenugreek seeds in diabetes mellitus. They are summarized in **Table 2**.

Fenugreek seeds as well as some water and ethanol extracts were shown to have a hypoglycaemic effect in normal as well as in diabetic models of rats. The seed powder was not tested in normal and diabetic mice, however aqueous and ethanol extracts induced the same effect. The hypoglycaemic effect of fenugreek seeds was also tested in a non-rodent species, namely the dog. The lipid extract was shown to have no effect on blood glucose levels. The remaining part termed defatted fraction, and more precisely the testa and endosperm, was the active fraction of the seed on glycaemia.

The mechanism underlying this effect is not clearly established. A widely found hypothesis is that fenugreek interferes with intestinal glucose absorption as a result of local effects at the gastrointestinal level mainly due to dietary fibres contained in fenugreek seeds and/or viscosity of the preparation. However, Abajnoor and Tilmisany (1988) excluded the involvement of gastrointestinal

action of fibre to explain the hypoglycaemic effect they reported in mice because i) they used fasting mice and ii) they administered extract instead of the whole seed. Instead, they suggested that the mechanism of antidiabetic action of fenugreek seeds may be similar to that of tolbutamide, i.e. stimulation of pancreatic insulin secretion, but did not exclude other pathways. Yadav et al, (2008) also suggested that fenugreek seeds, more precisely the water extract, act as an insulin secretor but unfortunately, they did not monitor insulin levels in their experiments. Interestingly, increased insulin secretion was observed in the experiments conducted by Petit et al, (1993); Devi et al, (2003); Eidi et al, (2007). Further, Vijayakumar and Bhat (2008) also report that the hypoglycaemic effect of fenugreek seeds, at least in part, is contributed by its action on the modulation of insulin secretion.

Other authors suggested that fenugreek inhibits intestinal glycosidase or digestive enzymes (Riyad et al, 1988 cited by Eidi et al, 2007, Wong et al, 1985 and Edwards et al, 1985 both cited by Zia et al, 2001). However, Vijayakumar and Bhat (2008) mention that this mechanism could not explain the hypoglycaemic effect they observed in mice because they used the intraperitoneal route of administration. The ability of fenugreek seeds to modulate key glucose metabolising enzymes such as hexokinase (glycolysis), glucose-6-phosphatase or fructose-1,6-bisphosphatase (gluconeogenesis) was also considered as a possible mechanism (Devi et al, 2003; Raju et al, 2001; Vijayakumar and Bhat, 2008).

In vitro investigations conducted by Vijayakumar et al, (2005) showed that fenugreek seed extract stimulates the insulin signalling pathway resulting in enhanced glucose transporter GLUT4 translocation to the cell surface in CHO cells and so enhanced mediated glucose uptake. It was notably shown in HepG2 cells that tyrosine phosphorylation of IR- β (insulin-receptor β) is activated, thus subsequently enhancing tyrosine phosphorylation of IRS-1 and p85 subunit of PI3-kinase.

In addition, the compound(s) responsible for the hypoglycaemic effect is (are) not clearly identified. The main hypotheses found in the literature are summarized in **Table 3**. Zia et al, (2001) concluded that the substance responsible for hypoglycaemic activity is probably polar in nature. Ribes et al, (1984, 1986, 1987) showed in diabetic dogs that the hypoglycaemic effect of fenugreek seeds is due to the defatted fraction, and more precisely the defatted fraction containing testa and endosperm. The lipid extract had no such effect (Ribes et al, 1984; Valette et al, 1984).

Table 1: summary of primary pharmacodynamic studies

Ref.	Test-article		Test system (species, route, dose, duration, parameters...)	Noteworthy findings
	Plant part	Formulation		
Petit et al, 1993	Seed	Hydro-ethanolic extract*	Rat Oral route (diet) 10 and 100 mg/day/300 g bw Up to 14 days <u>Parameters monitored</u> Food intake, weight gain Motivation to eat (food-rewarded runway behaviour) Preventing effect on d-fenfluramine-induced anorexia Metabolic studies (blood glucose, plasma insulin, plasma glucagon, triglycerides and total + free cholesterol levels)	↑food intake; the intensity of the effect was similar between treated groups. Reversible 3-5 days after treatment cessation. ↑body weight gain; the intensity of the effect was similar between treated groups ↑motivation to eat ↑plasma insulin ↓plasma total cholesterol, ↓ HDL free cholesterol, ↓ VLDL-LDL total cholesterol No preventative effect on d-fenfluramine-induced anorexia

* 12.5% steroid saponins, 4.8% free amino acids, 0.002% 3-hydroxy-4,5-dimethyl-2(5H)-furanone (HDMF) – no protein and lipids. Obtained from Monal Laboratories, Palaiseau, France

Table 2: summary of secondary pharmacodynamic studies dealing with potential activity in diabetes and/or hyperlipidaemia

Ref.	Part	Formulation	Model	Route	Duration	Minimal effective dose	Conclusion
Studies performed in mice							
Vijayakumar et al, 2005	Seed	Aqueous extract	Diabetic (AXN)	Intraperitoneal	Single dose	1-5 mg/kg	Hypoglycaemic effect in diabetic mice comparable to that of 1.5 U/kg insulin (at 15 mg/kg)
Vijayakumar and Bhat 2008	Seed	Aqueous extract	Diabetic (AXN)	Intraperitoneal	5 days	15 mg/kg/day	Hypoglycaemic effect in diabetic mice
Vijayakumar et al, 2005	Seed	Aqueous extract	Normal	Intraperitoneal	Single dose	15 mg/kg	Hypoglycaemic effect in normal mice
Vijayakumar and Bhat 2008	Seed	Aqueous extract	Normal	Intraperitoneal	Single dose	15 mg/kg	Hypoglycaemic effect in normal mice
Vijayakumar and Bhat 2008	Seed	Aqueous extract	Diabetic (STZ)	Intraperitoneal	Single dose	15 mg/kg	Hypoglycaemic effect in diabetic mice comparable to that of 1.5 U/kg insulin; enhanced hepatic metabolism of glucose
Ajabnoor and Tilmisany 1988	Seed	Decoction Ethanol extract	Normal and diabetic (AXN)	Oral	Single dose	Decoction: 0.5 ml Extract: 200 mg/kg	Hypoglycaemic effect in normal and diabetic mice
Zia et al, 2001	Seed	Aqueous extract	Normal	Oral	Single dose	500 mg/kg	Hypoglycaemic effect in normal mice
Zia et al, 2001	Seed	Methanol extract	Normal	Oral	Single dose	1000 mg/kg	Hypoglycaemic effect in normal mice
Studies performed in rats							
Jelodar et al,	Leaf	Powder	Diabetic	Oral (diet)	15 days	>12.5% BW in	No effect of treatment on

Ref.	Part	Formulation	Model	Route	Duration	Minimal effective dose	Conclusion
2005			(AXN)			<i>food</i>	the parameters monitored; the authors explain that this may be due to the plant part used (leaf instead of seed)
Devi et al, 2003	Leaf	Powder	Diabetic (STZ)	Oral (diet)	45 days	500 mg/kg/day	Hypoglycaemic effect in diabetic rats + stimulation of insulin secretion
Yadav et al, 2008	Seed	Aqueous extract	Normal	Oral	Single dose	50 mg/kg	Hypoglycaemic effect in normal rats
Xue et al, 2007	Seed	Aqueous extract	Diabetic (STZ)	Oral (gavage)	6 weeks	440 mg/kg/day	Hypoglycaemic effect in diabetic rats Hypolipidaemic effects in diabetic rats with favourable impact on HDL-cholesterol
Yadav et al, 2008	Seed	Aqueous, ethanol, methanol, hexane and chloroform extracts	Normal	Oral	Single dose	200 mg/kg	Hypoglycaemic effect reported for aqueous ethanol and methanol extracts in normal rats
Vats et al, 2002	Seed	Ethanol extract	Diabetic (AXN)	Oral (gavage)	21 days	2000 mg/kg/day	Hypoglycaemic effect in diabetic rats
Vats et al, 2002	Seed	Ethanol extract	Normal	Oral (gavage)	Single dose	1000 mg/kg	Hypoglycaemic effect in normal rats Lack of effect after an oral glucose load in normal rats (suggests that the

Ref.	Part	Formulation	Model	Route	Duration	Minimal effective dose	Conclusion
							extract failed in affecting glucose absorption from the GI tract)
Eidi et al, 2007	Seed	Hydro-ethanolic extract (80%)	Normal and diabetic (STZ)	Oral (gavage)	14 days	250 mg/kg/day	Hypoglycaemic effect + stimulation of insulin secretion in diabetic rats, <u>but not in normal rats</u> Favourable effect on cholesterol and triacylglycerol and on hepatic transaminases in diabetic rats
Raju et al, 2001	Seed	Powder	Diabetic (AXN)	Oral (diet)	21 days	12.5 g/kg/day (5% in diet)	Hypoglycaemic effect in diabetic rats; modulation of key glucose metabolising enzymes
Khosla et al, 1995	Seed	Powder	Normal and diabetic (AXN)	Oral (diet)	1 and 2 weeks	2000 mg/kg/day	Hypoglycaemic effect in normal and diabetic rats
Mondal et al, 2004	Seed	Powder (defatted)	Normal and diabetic (STZ)	Oral (<i>assessor's hypothesis</i>)	9 days	1250 mg/kg/day	Hypoglycaemic effect in diabetic rats
Studies performed in dogs							
Ribes et al, 1984 Valette et al, 1984	Seed	Defatted fraction ^a	Normal and diabetic (AXN)	Oral (diet)	8 days	1860 mg/kg/day	Hypoglycaemic effect in normal and diabetic dogs – attributed in part to the high percentage of dietary fibers of the preparation Hypocholesterolaemic

Ref.	Part	Formulation	Model	Route	Duration	Minimal effective dose	Conclusion
							effect in normal and AXN-induced hypercholesterolaemic dogs
Ribes et al, 1986 Ribes et al, 1987	Seed	Defatted fractions C+A ^c	Diabetic (AXN)	Oral (diet)	21 days	>1126 mg/kg/day (glycaemia) 1126 mg/kg/day (lipids)	No effect on blood glucose level Hypolipidaemic effect (decreased cholesterol and/or triglycerides); saponins may play a role, but not amino acids.
Ribes et al, 1986 Ribes et al, 1987	Seed	Defatted fractions T+E ^b	Diabetic (AXN)	Oral (diet)	21 days	1145 mg/kg	Hypoglycaemic effect in diabetic dogs - dietary fibers may play a role Hypolipidaemic effect (decreased cholesterol and/or triglycerides); saponins may play a role but not amino acids
Ribes et al, 1984 Valette et al, 1984	Seed	Lipid extract	Normal	Oral (diet)	8 days	>105 mg/kg/day	None

^a preparation containing 3.9% ash, 30.3% crude proteins, 53.9% dietary fibres (19.0% gum, 23.6% hemicelluloses, 8.9% cellulose, 2.4% lignin), 4.8% steroid saponins

^b testa + endosperm: preparation containing 10.0% moisture, 3.0% ash, 6.8% crude proteins, 79.4% dietary fibres (32.4% gum, 28.6% hemicelluloses, 14.6% cellulose, 3.8% lignin), 0.6% steroid saponins

^c cotyledons + axes: preparation containing 9.6% moisture, 4.9% ash, 52.8% crude proteins, 6.7% dietary fibres (traces of gum, 4.0% hemicelluloses, 2.1% cellulose, 0.6% lignin), 7.2% steroid saponins

Hypolipidaemic effect

The data are summarized in **Table 2**.

Investigations were conducted on the ability of fenugreek seed to lower blood lipids levels. In normal rats, Petit et al, (1993) observed decreased levels of total cholesterol and VLDL-LDL total cholesterol in normal rats given a hydro-ethanolic extract. No significant change was reported for levels of HDL-cholesterol. In diabetic rats, a hypolipidaemic effect with favourable impact on HDL-cholesterol was shown by Xue et al, (2007). Similar results were obtained by Eidi et al, (2007).

In normal and diabetic dogs, a hypocholesterolaemic effect was reported for the defatted fraction of fenugreek seeds. Further work in diabetic dogs showed a hypolipidaemic effect (decreased cholesterol and/or triglycerides) for the defatted fraction containing testa and endosperm shown to induce also hypoglycaemic effects. However, the defatted fraction containing cotyledon and axes also showed a hypolipidaemic effect in this experimental model, whereas it did not induce a hypoglycaemic effect. The authors conclude that saponins may play a role, but exclude any effect of amino acids on lipidaemia (Ribes et al, 1984, 1986, 1987; Valette et al, 1984).

Table 3: compounds claimed to be involved in the hypoglycaemic activity of fenugreek seeds

Compound	Ref.	Claimed mechanism of action or effect
4-hydroxyisoleucine	Eidi et al, 2007	Insulinotropic property <i>in vitro</i> Stimulation of intestinal secretion <i>in vivo</i> Improvement of glucose tolerance in diabetic rats and dogs
Alkaloids	Eidi et al, 2007	Inhibition of glucose uptake <i>in vitro</i>
Arginine	Eidi et al, 2007	Antidiabetic and hypoglycaemic effect
Coumarin	Shani et al, 1974 ^{a,b}	Main hypoglycaemic constituent of fenugreek seeds (from Shani et al, 1974)
Nicotinic acid	Shani et al, 1974 ^a	Main hypoglycaemic constituent of fenugreek seeds (from Shani et al, 1974)
Steroid saponins	Eidi et al, 2007	Inhibition of glucose uptake <i>in vitro</i>
	Yadav et al, 2008	The highest hypoglycaemic activity observed with the water extract may be related to higher content of saponins which are water soluble and previously reported for hypoglycaemic potential
Tannins	Yadav et al, 2008	The highest hypoglycaemic activity observed with the water extract may be related to higher content of tannins which are water soluble and previously reported for hypoglycaemic potential
Trigonelline	Eidi et al, 2007	Inhibition of glucose uptake <i>in vitro</i>
	Shani et al, 1974 ^{a,b}	Hypoglycaemic betain
Tryptophan	Eidi et al, 2007	Antidiabetic and hypoglycaemic effect

^a cited by Abajnoor and Tilmisany, 1988; ^b cited by Ali et al, 1995

Other effects

Ahmadiani et al, (2001) reported an anti-inflammatory effect in the formalin induced rat paw oedema model for a water extract of fenugreek leaves administered orally once for 7 days. The effective dose amounted to 1000 mg/kg a day. Further work performed by Parvizpur et al, (2006) showed a lack of inhibitory effect on COX enzyme. Ahmadiani et al, (2001) also reported an anti-pyretic effect in hyperthermic rats (injected brewer's yeast) for the same extract administered at 1000 mg/kg by both oral and intraperitoneal routes.

Assessor's comments

Fenugreek seeds were shown to induce hypoglycaemic effects in various animal models of diabetes. The mechanism underlying the hypoglycaemic effect remains unestablished but a number of hypotheses were found in the literature: local action at the gastro-intestinal level to lower the absorption of glucose, enhancement of insulin secretion, modulation of glucose metabolism, stimulation of insulin signalling pathway at the cellular level. Similarly, the compound(s) responsible for this effect are currently not identified. However, it was established in diabetic dogs that the active part of fenugreek seeds is the defatted fraction.

A lower number of studies also showed that fenugreek seeds have a hypolipidaemic effect in diabetic rats, and in both normal and diabetic dogs. It was also shown in dogs that the active part is the defatted fraction.

According to the results, an action that may be relevant in humans is the effect on glycaemia. Warnings could be included in the monograph regarding potential interactions with treatments for diabetes mellitus.

3.1.3. Safety pharmacology

Two publications describing the results of experimental studies dealing with the potential undesirable effect of fenugreek preparations on some of the main physiological functions were found in the scientific literature. A summary is provided in **Table 4**.

Abdo and Al-Kafawi (1969) investigated the effects of water and ethanol seed extracts on various systems:

- Either a slight effect or an effect similar to that reported for the control vehicle was reported on the motility of isolated guinea pig intestine pieces.
- A positive chronotropic effect was observed in isolated perfused guinea pig hearts with the water extract; a negative chronotropic effect was reported for the ethanol extract and ethanol control vehicle. However, no effect on blood pressure or respiratory movements was reported in anaesthetized dogs treated with each extract.
- Both extracts showed stimulating effect on uterine contractility, particularly in tissues obtained from pregnant guinea pigs.

Parvizpur et al, (2006) showed that a water extract of fenugreek leaves inhibits the aggregation of rabbit platelets in a concentration-dependent way that is related to some antagonistic effect on ADP.

Table 4: summary of safety pharmacology studies

Ref.	Part	Formulation	System	Test system (species, route, dose, duration, parameters)	Noteworthy findings
Abdo and Al-Kafawi 1969	Seed	Water and ethanol (liquid) extracts	Gastro-intestinal tract	Isolated guinea pig intestine pieces (5 cm) Test solution (2 ml from water or ethanol extract) or control (either water or ethanol) added to a bath containing duodenum pieces in oxygenated Tyrode's solution Intestinal motility was recorded by means of a light lever on a smoked drum paper moving at slow speed	<u>Water extract</u> Slight stimulating effect on intestinal motility <u>Ethanol extract</u> Inhibition of intestinal motility, similar to that observed with ethanol control
			Female reproductive tract	Isolated uterus pieces (4 cm) from pregnant and non-pregnant guinea pig Test solution (2 ml from water or ethanol extract) or control (either water or ethanol) added to a bath containing duodenum pieces in oxygenated Dale's solution Uterine motility was recorded by means of a light lever on a smoked drum paper moving at slow speed	<u>Water extract</u> Stimulating effect on uterine contractility; the effect is markedly increased on tissues obtained from pregnant animals <u>Ethanol extract</u> Same results as those obtained with water extract

Ref.	Part	Formulation	System	Test system (species, route, dose, duration, parameters)	Noteworthy findings
			Cardiovascular	Isolated and perfused guinea pig heart Test solution (2 ml from water or ethanol extract)	<u>Water extract</u> Acceleration of heart beats <u>Ethanol extract</u> Decrease in heart beats, similar to that observed with ethanol control
			Cardiovascular and respiratory	Anaesthetized dogs Blood pressure recorded from carotid artery (manometer) Respiratory movements recorded by using a sphygmograph fitted around the chest of animals and connected with a tambour	No effect reported for both extracts
Parvizpur et al, 2006	Leaf	Water extract	Blood	Rabbit platelet-rich plasma Effect of extract (0.5, 1, 1.5 and 3 mg/ml) on ADP-induced platelet aggregation	Dose-dependent inhibition of aggregation response to ADP ⇒ some antagonistic effect on ADP (in rabbit platelet, COX and arachidonic pathways are not involved in aggregation)

Assessor's comment

From the studies detailed above, two results may deserve a particular attention:

- *A water extract of fenugreek leaves was shown to inhibit the aggregation of rabbit platelets in a concentration-dependent way that is related to some antagonistic effect on ADP.*
- *The uterine stimulant properties reported on pieces of guinea pig uterus should be viewed in the context of its historical use as an abortifacient or for labour induction that is mentioned by Ulbricht et al, (2007).*

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

No data were found in the literature.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

3.3.1. Single-dose toxicity

The available data are summarized in **Table 5**.

Table 5: summary of single-dose toxicity studies

Ref.	Part	Formulation	Species	Route, dose	Parameters	Noteworthy findings
Muralidhara et al, 1999	–	Debitterized powder ^a	Mouse (CFT Swiss)	Oral gavage 0, 250, 500, 1000, 2000 mg/kg	Mortality and clinical signs for up to 14 days postdose Body weight, food intake Weight and microscopic examination of liver, lungs, kidneys and spleen	None
Muralidhara et al, 1999	–	Debitterized powder ^a	Rat (CFT Wistar)	Oral gavage 0, 1000, 2000, 4000 ^b , 5000 ^b mg/kg	Mortality and clinical signs for up to 14 days postdose Body weight, food intake Weight and microscopic examination of liver, lungs, kidneys and spleen	None
Abdel-Barry and Al-Hakiem, 2000	Leaf	Glycosidic extract	Mouse (Wistar) 10/group	Intraperitoneal 0, 200, 400, 500, 800, 1000 mg/kg	Mortality and clinical signs for up to 7 days postdose Body weight, food intake Histopathological examination of liver, kidney, stomach and large intestine	LD50=650 mg/kg CNS effects Mild CNS stimulation at low and intermediate doses Tachypnea, twitches, strabtail, tremors, generalized convulsions at higher doses Early liver degeneration and mild hepatitis observed only in animals which died before the end of the study

Ref.	Part	Formulation	Species	Route, dose	Parameters	Noteworthy findings
Abdel-Barry and Al-Hakim 2000	Leaf	Glycosidic extract	Mouse (Wistar) 10/group	Oral gavage 0, 1000, 2000, 4000, 6000, 8000, 10000 mg/kg (oral)	Mortality and clinical signs for up to 7 days postdose Body weight, food intake Histopathological examination of liver, kidney, stomach and large intestine	LD50=7000 mg/kg CNS effects Mild CNS stimulation at low and intermediate doses Tachypnea, twitches, strabtail, tremors, generalized convulsions at higher doses

^a supplied by M/s Sterling Home Products (Chennai, India)

^b divided into two equal doses and dosed at 2-hourly intervals

Assessor's comment

Studies performed by Abdel-Barry and Al-Hakiem (2000) suggest a low acute toxic potential by oral route (LD50 = 7 g/kg). However, the preparation administered to mice is a glycosidic extract obtained from fenugreek leaves and is not used traditionally.

Muralidhara et al, (1999) also showed a low acute toxic potential in rodents with a debitterized powder obtained from an unknown part of fenugreek.

3.3.2. Repeat-dose toxicity

The available data are summarized in **Table 6**.

Table 6: summary of repeat-dose toxicity studies

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
Muralidhara et al, 1999	–	Debitterized powder ^a	Rat (CFT Wistar) aged 28 days	90 -95 days Oral route 0, 1, 5, 10% in diet	Mortality and clinical signs Body weight, food intake Haematological examination Biochemistry: serum ALP, AST, ALT, cholesterol, creatinine and urea Weight and microscopic examination of adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen and testes	None
Udayasekhara Rao P et al, 1996	Seed	Powder	Rat (Wistar/NIN) 12/sex/group	90 days Oral route 0, 5, 10, 20% in diet	Mortality and clinical signs Body weight, food intake Haematological examination Biochemistry: serum ALP, AST, ALT, cholesterol, and fatty acid profile Weight and microscopic examination of liver, kidney, lung, spleen, gastrointestinal tract, pancreas, testis, ovary	<u>Body weights, Food intake</u> Transient decrease in food intake during the first few days (≥ 5%) <u>Biochemistry</u> ↑ (dose-related) serum ALP (M, significant at 20% only) ↓ cholesterol level (M, 10 and 20%) <u>Organ weights</u> ↑ relative liver weight (F, +15% at 10% and +28% at 20% compared to controls) <u>Histopathological</u>

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
						<u>examination</u> Lungs: mild to moderate chronic interstitial pneumonitis: 17/24, 18/24, 16/24, 18/24 (at 0, 5, 10, 20%, higher frequency in males) Lungs: severe chronic interstitial pneumonitis: 3/24, 0/24, 1/24, 0/24 (at 0, 5, 10, 20%)

^a supplied by M/s Sterling Home Products (Chennai, India)

Assessor's comment

Two 90-day rat studies were found in the literature. The experimental protocols were similar. Muralidhara et al, (1999) administered the debitterized powder prepared from an unknown part of fenugreek, at up to 10% in the diet. Udayasekhara Rao P et al, (1996) administered a fenugreek seed powder at up to 20% in the diet.

No toxic effect was observed in the first study. Udayasekhara Rao P et al, (1996) reported increased liver weight in females receiving 10 and 20% of seed powder with increased ALP levels. However, this did not correlate with any hepatic finding at histopathological examination. Chronic interstitial pneumonitis was observed at a similar incidence in all groups including controls (~70-85%) of which described to be due to murine respiratory mycoplasmosis, the main causative agent is *Mycoplasma pulmonis*. An inbred colony of rats was used in this study, and the results suggest that it was infected by *Mycoplasma pulmonis*. Therefore, some doubts remain regarding the sanitary conditions of the animals.

In both studies, the list of organs selected for histopathological examination was quite limited. Contrary to results obtained in rats and rabbits which are further detailed in the reproduction toxicity section, no testicular finding was reported. In addition, no decrease in blood glucose levels (or corroborating finding) was noted in both studies, although this was expected due to the hypoglycaemic effect of fenugreek seeds (see pharmacology).

3.3.3. Genotoxicity

The available data are summarized in **Table 7**.

In addition, the WHO monograph on Semen Trigonellae Foenugraeci reports that an aqueous and a chloroform/methanol extract of the seeds were not mutagenic in the *Salmonella* microsome assay using *S. typhimurium* strains TA98 and TA100 (Rockwell and Raw, 1979 and Mahmoud et al, 1992 / cited by WHO 2007).

Table 7: summary of genotoxicity studies

Ref.	Part Formulation		Type of test	Test system	Concentration metabolising system	Results
Wu et al, 1997	Trigonelline, heated for 20 minutes at 250°C then let cool down at room temperature		Gene mutation in bacteria	<i>Salmonella typhimurium</i> strains TA98, YG1024 and YG1029	Concentration range not detailed but 4 different concentrations were used to establish a dose-response curve +/- S9 (chlorophene-induced rat liver)	Potent mutagenic activity with and without detected in this model mimicking coffee roasting The authors report that pure trigonelline is not mutagenic when not heated (Fung et al, Mutat Res, 1988)
Flammang et al, 2004	Seed	Extract (THL)*	Gene mutations in bacteria	<i>Salmonella typhimurium</i> strains TA1535, TA1537, TA98, TA100 <i>Escherichia coli</i> strain WP2uvrA	33.3 to 5000 µg/plate +/- S9 (aroclor-induced rat liver)	Negative
Flammang et al, 2004	Seed	Extract (THL)*	Gene mutations in mammalian cells	L5178Y mouse lymphoma cells (TK locus)	+S9: 500 to 5000 µg/ml -S9: 150 to 4000 µg/ml	Negative The authors indicate that THL caused dose-related increase in cytotoxicity as measured by the reduction in relative total growth <u>Comment:</u> According to OECD guideline no.476**, RTG should range from 10 to 20% if the maximum

Ref.	Part Formulation		Type of test	Test system	Concentration metabolising system	Results
						<p>concentration is based on cytotoxicity</p> <p>In this experiment, RTG reached 19.4% at 4000 µg/ml without S9, and 29.1% at 5000 µg/ml with S9</p> <p>Therefore, the level of cytotoxicity is acceptable</p> <p>It is also noted that the maximal concentrations used are in line with the OECD guideline no.476 (5 mg/mL for relatively non-cytotoxic compounds)</p>
Flammang et al, 2004	Seed	Extract (THL)*	Chromosomal aberrations in vivo	Mouse, micronuclei in bone marrow	500, 1000, 2000 mg/kg/day for 3 days by oral gavage	Negative

* containing ≥40% 4-hydroxyisoleucine, mode of extraction not detailed

** OECD guidelines for the testing of chemicals, Test n°476: *in vitro* mammalian cell gene mutation test, 1997

Assessor's comment

Flammang et al (2004) performed an ICH-compliant battery of 3 genotoxicity tests which yielded negative results. However, the tests were performed with an extract of fenugreek seeds called "THL". Neither the mode of extraction, nor the composition (qualitative and quantitative) is described, it is just mentioned that THL contains a minimum of 40% of 4-hydroxyisoleucine.

The data reported in the WHO monograph were obtained with irrelevant extracts and the number of strains used is not sufficient.

*Overall, it is considered that conventional genotoxicity data obtained with a clinically relevant herbal preparation is lacking, thus precluding the inclusion of *Trigonella foenum-graecum* in the list of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products.*

3.3.4. Carcinogenicity

No conventional carcinogenicity study is available.

Assessor's comment

From a non-clinical perspective, the duration of treatments with fenugreek seed preparations should not exceed 6 months due to the lack of conventional carcinogenicity study.

3.3.5. Reproduction toxicity

The available data are summarized in **Table 8**.

Kamal et al, (1993) treated male rats with the steroidal fraction of fenugreek seed extract for 2 months. The sperm count and motility of treated animals were decreased. In addition, the weight of reproductive tissues and androgen-dependent parameters (protein, sialic acid and fructose) were lower, thus indicating reduced levels of circulating androgens. These findings were shown to have histological correlates (arrest of spermatogenesis, degeneration of seminiferous tubules and epididymis). Cholesterol levels were higher in treated vs. control animals in serum and testis so that the authors concluded that it may be co-related with its non-utilisation thus leading to decreased circulating androgen and altered testicular histoarchitecture. The functional consequence was a loss of fertility for 20/20 treated males. They conclude that the test-article exerts both anti-fertility and antiandrogenic activities.

Kassem et al, (2006) showed that administration of fenugreek seed powder in feed (30%) for 3 months induced testicular toxicity in rabbits, as shown by marked decreases in testosterone levels, testes weight and sperm count. This correlated histologically with a decreased number of seminiferous tubules and disruption of spermatogenesis (mild hypoplasia). According to the authors, these results are coherent with those of Kamal et al, (1993). However, they indicate that fenugreek may induce testicular toxicity rather than anti-fertility effects based on the lack of difference in the litter size when treated males were mated with untreated females.

In female rabbits treated the same way as their male counterparts, pre-breeding estrogen and progesterone levels were decreased, whereas gestational progesterone levels were markedly increased. Histopathological examination reported increased ovulation (increased number of corpus luteum), and proliferative changes of endometrial glands. The development of fetuses obtained after mating of treated males and females is reported as abnormal, due to marked decreases in "foetal + placental" weight (-80% on gestation day 20 (GD20)) and litter size (-75%).

Sethi et al, (1990) administered fenugreek seed powder to rats during the first ten days of gestation at 175 mg/kg a day. The number of resorptions was increased. This is coherent with the results published

by Elbetieha et al, (1996) and Adhikary (1990) with fenugreek seed extracts administered from the beginning up to the 6th or 10th day of pregnancy, respectively. In addition, some gross and visceral anomalies were reported in the study published by Sethi et al, (1990).

The only negative study was conducted by Mital and Gopaldas (1986) by administration of up to 20% fenugreek seed powder in the diet of rats for the whole gestation period.

Table 8: summary of reproduction toxicity studies

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
Kamal et al, 1993	Seed	Steroidal fraction of extract obtained via extraction with toluene and n-hexane ^a	Rat (Holtzman) 20M/group	60 days Oral route 0, 100 mg/day/rat, i.e. approx. 450 mg/kg/day ^b	Body weight Fertility test (mating with untreated females on Day 61 and check for implantation sites 7 days thereafter) Biochemistry (serum and reproductive tissues) Sperm parameters (count, motility) Organ weight: liver, heart, kidney, adrenal, reproductive tissues Histopathology: testis, epididymides, vas deferens, seminal vesicles	<u>Organ weight</u> ↓ weight of epididymis, ventral prostate, seminal vesicles <u>Sperm parameters</u> ↓ motility ↓ density in cauda epididymis and testis <u>Fertility</u> 100% negative results in treated animals in spite of successful matings (confirmed by vaginal plug) <u>Tissue biochemistry</u> Testis: ↓ protein, ↑ cholesterol, ↓glycogen, ↓fructose Seminal vesicle: ↓ protein, ↓ sialic acid, ↓fructose Epididymides: ↓ protein, ↓ sialic acid Ventral prostate: ↓ protein, ↓ sialic acid <u>Serum biochemistry</u>

^a containing 0.6% total steroidal sapogenin

^b assuming a body weight value of 225 g as mentioned in the article

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
						↑ cholesterol, ↓ protein, ↓ phospholipids, ↓ triglycerides <u>Histopathology</u> Testis: arrest of spermatogenesis, degenerating seminiferous tubules Cauda epididymis: severe degenerative changes Vas deferens: ↓ lumen diameter, ↑ thickness of lamina propria
Kassem et al, 2006	Seed	Powder	Rabbit (NZW) 4M+12F/group	3 months; sacrifice on GD10, GD20, or after parturition Oral route 0, 30% in diet	Body weight Hormonal assessment: determination of plasma progesterone, estrogen and testosterone Mating parameters Implantations, corporea lutea, resorptions Foetal weight, litter size, newborn weight Sperm count Histopathology: ovaries, uterus, testes	<u>Parental Animals</u> <u>Hormone assessment</u> ↓ testosterone (-66%) ↓ estrogen (-18%) ↓ progesterone (pre- breeding -14%) ↑ progesterone (GD10 and GD20, +78% and +111%) <u>Sperm parameters</u> ↓ sperm count (-47%) <u>Organ weight</u> ↓ testicular weight (- 25%) <u>Histopathology:</u> Testis: ↓ number of

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
						seminiferous tubules Testis: mild spermatogenesis hypoplasia Ovary: higher development of the secondary and tertiary follicles in the cortex area Ovary: ↑ number of corpus luteum → ↑ ovulation activity Uterus: proliferative changes of some endometrial glands Uterus: ↑ proliferation of the endometrial glands with hyperplastic changes <u>Embryo-foetal development</u> ↓ foetal + placenta weight on GD20 (-80%) <u>Newborns</u> ↓ litter size (-75%) ↑ newborn weight (+26%)

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
Elbetieha et al, 1996	Seed	Aqueous extract	Rat (SD) 9F/group	GD1-GD6 (C-section on GD20) Oral route (gavage) 0, 800 mg/kg/day	Number of implantations Number of resorptions Number of live foetuses	↑ number of total resorptions ↑ number of dams with resorptions
Adhikary 1990	-	Petroleum extract (60-80%)	Rat	GD1-GD10 Oral route 500-1250 mg/kg/day	Screening for anti-fertility activity	60-66% anti-fertility activity
Sethi 1990	Seed	Powder	Rat (Charles Foster) 5F/group	GD1-GD10 (C-section on GD20) Oral route 0, 175 mg/kg/day	<u>Dams</u> Number of implantations Number of resorptions <u>Foetuses</u> Number of live births Number of still births Malformations (gross, skeletal and visceral)	↑ number of resorptions Treated: 54 corporea lutea, 54 implantations, 44 live births, 0 still births, 10 resorptions ⇒ 10/54 = 18% abortifacient activity Controls: 47 corporea lutea, 47 implantations, 46 live births, 0 still births, 1 resorptions ⇒ 1/47 = 2% abortifacient activity ↓ foetal body weight and foetal crown-rump length (-41% and -22% compared to controls) Various gross anomalies including notably inverted/averted claw (18% and 21% vs. 0% and 0% in controls),

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
						shoulder joint defect (18% vs. 0%), tail kinking (18% vs. 0%) and clubbing of hind limb (9% vs. 0%) Visceral anomalies: neuralpore (18% vs. 0%), enlarged neural canal (6% vs. 0%) Skeletal effects: nonossified skull bones (18% vs. 0%)
Mital and Gopaldas 1986	Seed	Powder	Rat (Charles Foster) 5-8F/group	GD1-GD21 (C-section on GD22) Oral route 0, 5, 20% in diet	<u>Dams</u> Body weights, food consumption Number of implantations Number of resorptions Placenta weight <u>Foetuses</u> Body weight	None

Assessor's comment

Studies published by Kamal et al, (1993) and Kassem et al, (2006) were designed to evaluate the effect of fenugreek seeds preparation on fertility. Both studies report testicular toxicity shown by decreased testosterone levels, altered sperm parameters, decreased testis weight, lowered/arrest of spermatogenesis, degenerating seminiferous tubules. This toxicity is attributed to the treatment-related decrease in testosterone, which seems consistent. A NOAEL was not determined. A potential impact on fertility cannot be excluded.

In female rabbits, changes in estrogen and progesterone levels were reported by Kassem et al, (2006).

Three studies showed that fenugreek seeds preparations (extract or powder) could increase the number of resorptions when given to rats from the first day up to the 10th day of gestation. In two studies, the number of implantations was not reported to be affected, in the third the authors did not indicate whether this parameter has been monitored. In the study performed by Kassem et al, (2006) in rabbits, the number of implantations was also not affected by administration of seed powder but the litter size was decreased by 75% compared to controls. In the study performed by Kamal et al, (1993), successful mating occurred but there is no data provided regarding the number of implantations. Therefore, it seems reasonable to conclude that fenugreek seed induces embryoletality in rats. This conclusion is also supported by the reported historical/theoretical use of fenugreek as an abortifacient and labour inducer (Ulbricht et al, 2007). Other supportive data were summarized by Farnsworth et al, (1975) who performed an extensive review of published articles dealing with the effects of various plants on fertility and the underlying mechanism. Fenugreek was classified among plants having abortifacient and emmenagogue (which induces or hastens menstrual flow) applications based on the following data:

	Type of activity	Plant part	Other details
Casey RC, 298 alleged anti-fertility plants of India. Indian J Med Sci, 1960	Abortifacient		
Saha JC et al, 1961	Emmenagogue	Whole plant, seed	
Malhi BS and Trivedi VP, Vegetable Antifertility drugs of India. Q J Crude Drug Res, 1972	Emmenagogue	Seed	
Goto M. Takeda Kenkyusho Nempo, 1957	Uterine stimulant	Seed	
Abdo MS and Al-Kafawi AA, Experimental studies on the effect of Trigonella foenum-graecum. Planta Medica, 1969	Uterine stimulant	Seed	Formulation: water and alcoholic extract Species: guinea pig (in vitro study)

Regarding the impact of fenugreek seed on embryo-foetal development, contradictory results were obtained in rats. Sethi et al, (1990) reported gross and visceral malformations in rats at non maternotoxic doses, whereas Mital and Gopaldas (1986) did not observe any effect on reproduction in the same species.

The design of both studies is not in line with current recommendations for evaluation of embryo-foetal toxicity. Indeed, the number of animals and dose levels were insufficient and the duration of treatment was not optimal – the test-article should have been administered for the whole period of organogenesis, i.e. from GD 6-7 to GD 15-18.

Therefore, the information on embryo-foetal toxicity is considered limited and the malformations reported in rats by Sethi et al, (1990) have to be considered as a safety signal. In the future, conventional embryo-foetal toxicity studies in 2 species should be performed to clarify this point. No information is available regarding potential effects on pre-post-natal development.

Other studies

Some studies focused on the impact of fenugreek seeds on thyroid function because thyroid hormones are involved in carbohydrate metabolism. The data are summarized in **Table 9**.

Table 9: summary of studies focused on effects on thyroid

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
Tahiliani and Kar 2003	Seed	Hydro-ethanolic extract (20%)	Rat	15 days Oral route (gavage) 0, 220 mg/kg/day	Serum levels of: T3, T4, glucose, cholesterol, AST, ALT	↓ T3 levels (-40%) No other effect (notably on glucose and T4 levels)
Panda et al, 1999	Seed	Hydro-ethanolic extract (20%)	Mouse (7M/group)	15 days Oral route (gavage) 0, 110 mg/kg/day	Body weight Serum T3 and T4 levels Hepatic biochemistry: protein, hepatic lipid peroxidation, superoxide dismutase (SOD) and catalase (CAT) activities	↑ body weight Thyroid hormones: ↓ T3, ↑ T4, ↓ T3/T4 ratio ↓ SOD activity
			Rat (7M/group)	15 days Oral route (gavage) 0, 110 mg/kg/day	Body weight Serum T3 and T4 levels Hepatic biochemistry: protein, hepatic lipid peroxidation, superoxide dismutase (SOD) and catalase (CAT) activities	↑ body weight (statistical significance not reached) Thyroid hormones: ↓ T3, ↑ T4, ↓ T3/T4 ratio ↓ SOD activity

Assessor's comment

Results from 3 experiments in rodents showed that a hydro-ethanolic extract of fenugreek seeds induced a decrease in T3 levels. In 2 experiments, there were concomitant increase in T4 levels and decrease in T3/T4 ratio. These results suggest decreased conversion of T4 to T3. Unfortunately, TSH levels were not monitored. The decrease in T3/T4 ratio reveals decreased 5'-deiodinase activity since the majority of circulating serum T3 is produced by peripheral conversion of T4 to T3. A NOAEL was not determined.

3.4. Overall conclusions on non-clinical data

Pharmacology

Fenugreek seeds were shown to induce hypoglycaemic effects in various animal models of diabetes. The mechanism underlying the hypoglycaemic effect remains unestablished but a number of hypotheses were found in the literature: local action at the gastro-intestinal level to lower the absorption of glucose, enhancement of insulin secretion, modulation of glucose metabolism, stimulation of insulin signalling pathway at the cellular level. Similarly, the compound(s) responsible for this effect are currently not identified. However, it was established in diabetic dogs that the active part of fenugreek seeds is the defatted fraction.

A lower number of studies also showed that fenugreek seeds have a hypolipidaemic effect in diabetic rats and in both normal and diabetic dogs. It was also shown in dogs that the active part is the defatted fraction.

No specific safety pharmacology study is available which is acceptable according to current guidelines. However, the inhibition of rabbit platelet aggregation with a water extract, and uterine stimulant properties reported in guinea pigs with a water and ethanolic extracts could be taken into consideration.

Toxicology

Two 90-day repeat-dose toxicity studies in rats did not identify any target organ but some doubts remain regarding the sanitary conditions of the animals in one study due to the occurrence of murine respiratory mycoplasmosis. In addition, the lack of effects on testes is rather surprising in view of the testicular toxicity consistently reported in reproduction toxicity studies.

Specific studies conducted in rats with a hydro-ethanolic extract of fenugreek seeds reported a decrease in T3 levels with concomitant increase in T4 levels and decrease in T3/T4 ratio. These results suggest decreased conversion of T4 to T3 – unfortunately, TSH levels were not monitored. The decrease in T3/T4 ratio suggests a decrease in 5'-deiodinase activity.

An ICH-compliant battery of tests did not report any genotoxic effect for a proprietary extract of fenugreek seeds. However, the characteristics of this extract have not been published so that these results cannot be taken into account. Overall, it is considered that relevant information on genotoxicity is lacking. In addition, conventional carcinogenicity studies are lacking.

Testicular toxicity was reported in rats treated for 2 or 3 months with either seed powder or the steroidal fraction of seeds. It was characterized by altered sperm parameters, decreased testis weight, lowered/arrest of spermatogenesis and degenerating seminiferous tubules. These effects are attributed to the treatment-related decrease in testosterone. Therefore, a potential impact on fertility cannot be excluded.

Three studies showed that fenugreek seeds preparations (extract or powder) could increase the number of resorptions when given to rats from the first day up to the 10th day of gestation. From the available data, it seems reasonable to conclude that fenugreek seed induces embryoletality in rats. This conclusion is coherent with the reported historical/theoretical use of fenugreek as an abortifacient and for labour induction.

The information on embryo-foetal toxicity is rather limited. Available studies showed conflicting results but were not designed adequately. In this context, the malformations reported in rats by Sethi et al, (1990) have to be considered as a safety signal. In the future, conventional embryo-foetal toxicity studies in 2 species should be performed to clarify this point.

No information is available regarding potential effects on pre- and post-natal development.

Overall, the administration of fenugreek seeds impacted on various components of the endocrine system: pancreas (effect on insulin levels), thyroid (effect on T3 and T4 levels) and gonads (effects on testosterone, estrogen and progesterone levels).

Monograph

- Some warnings could be included for patients treated for diabetes mellitus and thyroid disorders.
- Treatment-related testicular toxicity due to decrease in testosterone levels as well as interference with thyroid hormone levels were reported in animals. In addition, female hormone levels were affected in one study in rabbits. In view of the paramount importance of gonads and thyroid during development, these points should be considered for administration in patients under the age of 18 years.
- Embryo-lethal effects could be reported in the monograph. Regarding embryo-foetal toxicity, it should be indicated in section 5.3 that only limited data are available.
- The wording proposed for section 5.3 is:

“Tests on genotoxicity have not been performed with preparations of fenugreek covered by this monograph.

Decreased thyroid hormone levels (T3, triiodothyronine) were reported in rodents treated with hydro-ethanolic extracts at 110 mg/kg a day and above; a NOAEL was not determined.

Testicular toxicity (altered sperm parameters, decreased testis weight, lowered/arrest of spermatogenesis, and degenerating seminiferous tubules) was reported in rats treated for 2 to 3 months with either fenugreek seed powder or the steroidal fraction of seeds. These effects are attributed to the treatment-related decrease in testosterone and a NOAEL was not determined.

Conventional embryo-foetal and peri- and post-natal toxicity studies were not performed. Limited studies showed conflicting results regarding the occurrence of malformations in rats.”

4. Clinical Data

4.1. Clinical Pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Clinical pharmacology on fenugreek is not well documented in humans. The majority of pharmacological effects have been studied in animals, mainly in rats and dogs and to a lesser extent in

rabbits, either through in vitro or in vivo experiments to search or reveal the hypocholesterolaemic and hypoglycaemic effects of fenugreek (see data previously detailed in the non-clinical section).

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Pharmacokinetic data are not available for all components of fenugreek or for the compound as a whole. In humans, it has been shown that saponins present in fenugreek are believed to be primarily absorbed in the terminal ileum as a potential mechanism assessed for its hypocholesterolemic activity.

In a rabbit study by Zhao et al, aimed at studying the pharmacokinetics of trigonelline determined by HPLC, after post-intragastric injection of fenugreek extract, the pharmacokinetic parameters of one compartment model were half-life, $t_{1/2} = 0.9$ hour, $t_{1/2} = 2.2$ hours, volume of distribution = 0.64 l/kg and AUC = 1.93 mg/min/l.

4.2. Clinical Efficacy

4.2.1. Dose response studies

According to the provided literature, no dose-finding studies have been conducted with fenugreek.

According to the WHO monograph, available dosage recommendations are the following:

- for internal use, average daily dose, cut or crushed seed, 6 g or equivalent of preparations; infusions, 0.5 g of cut seed macerated in 150 ml cold water for 3 hours, several cups.

According to the ESCOP monograph, available dosage recommendations are the following:

- for internal use, in adults, as adjuvant therapy in diabetes or for hypercholesterolaemia, 25 g of powdered seeds or equivalent preparations daily; for lack of appetite, 1-6 g of powdered drug up to three times daily with water before meals.
- for external use, in adults, as an emollient 50 g of powdered seeds boiled in 250 ml of water for 5 minutes then applied as a warm moist poultice.

4.2.2. Clinical studies (case studies and clinical trials)

Appetite stimulant effect

The French approved indication stated as follows: "traditionally used to gain weight in adults" is granted for more than 30 years in France. The traditional use of fenugreek is based on the experience and historical use of this herbal product in the European Community.

When searching reference to substantiate the efficacy/safety of fenugreek in the literature in this indication only one reference has been found: **M. Rguibi and R. Belahsen (2006). Fattening practices among Moroccan Saharawi Women. Eastern Mediterranean Health Journal, Vol.12, No.5, 2006.**

This reference reports a survey of Moroccan Saharawi women as regards their fattening practices for gaining weight as a socio-cultural willingness of increasing their physical attractiveness.

Use of fenugreek is reported as an appetite enhancer in this survey.

Methodology of the survey

All participants were interviewed face-to-face by an interviewer who belonged to this Saharawi ethnic group. A discussion guide was developed including questions on socio-demographic characteristics, satisfaction with their body size, dietary history and practical behaviours used to lose or to gain weight.

To determine the perceptions of body weight, participants were invited to answer the following questions: Have you wanted to gain weight in the past? Do you want to gain weight now? Do you want to lose weight now? Participants were asked to describe any actions that they have taken to lose or gain weight. All fattening practices used by the women were recorded, as well as other details such as portion size, frequency of eating, food composition and food preparation techniques.

This survey is conducted between October 2001 and April 2002 on a sample of 249 urban non-pregnant women aged 15 to 70 years old, without any previous systemic disease.

Demographic characteristics

- Women belonging to the Saharawi ethnic group: communication skills in Hassaniyya dialects, traditional clothing, history of their family's residence. Informed consent obtained verbally before they took part to the survey.
- Body Mass Index (BMI) was calculated as weight (kg) and height (m²) The World Health Organization (WHO) definitions were used for underweight (BMI < 18.5 kg/m²), normal weight (18.5 ≤ BMI < 25 kg/m²), overweight (25 ≤ BMI < 30 kg/m²) and obesity (BMI ≥ 30 kg/m²).
- Socio-demographic characteristics were recorded: marital status, educational level.
- Investigations regarding their perceptions of body weight have been recorded as well as their potential actions that they have taken to lose or gain weight.

Clinical Results

Socio-demographic characteristics of the study sample (n = 249 women)		
Variable	Value	
	Mean (SD)	Range
Age (years)	36.8 (11.8)	15.0-70.0
BMI (kg/m ²)	29.6 (5.3)	17.3-41.4
	Number	Percentage
Marital status		
Single	50	20.1
Married	166	66.7
Divorced	19	7.6
Widow	14	5.6
Education		
Never attended school	155	62.2
Primary school	47	18.9
Secondary school	47	18.9

The mean BMI was 29.6 kg/m² and 30% of women were overweight and 49% were obese.

A large majority of women (79.9%) described their weight as appropriate and only 50 described it as inappropriate (8 desired to lose weight and 42 desired to gain it). The desire to gain weight was in

most cases accompanied by practising certain behaviours, for example using drugs, overfeeding and restriction of physical activity. The fattening practices changed between the past and currently as shown in the following table.

Fattening practices used by Saharawi women desiring to gain weight		
Practice	In the past (n=175)	Currently (n=42)
Appetite stimulant	71 (40.6)	3 (7.1)
Overeating	56 (32.0)	30 (71.4)
Corticosteroids (drugs intentionally used for their promotion of weight gain as a side effect)	41 (23.4)	4 (9.5)
Other	7 (4.0)	5 (11.9)

In addition to the therapeutic medication, the women reported that some seeds such as fenugreek (halba) consumed directly or added to dishes have been used to stimulate hunger.

Assessor's comment:

This study is the main "clinical support" of the use of fenugreek as an appetite stimulant besides the animal data. It is an observational survey where fenugreek is "mentioned" as being used by women desiring to gain weight. However, the study description does not enable to quantify the use of fenugreek among the appetite enhancers and ultimately to appreciate the potential contribution of fenugreek in the weight gain.

Therefore per nature, this observational study is of no relevance to substantiate the efficacy and safety of fenugreek as an appetite enhancer.

To complete the data above, there is some information in the WHO monographs on selected medicinal plants which are also substantiated to some extent by the literature data, in particular hypoglycemic/hypolipemiant effects which are described hereafter.

Hypoglycaemic and antihyperlipidemic properties

The possible hypoglycaemic and antihyperlipidemic properties of oral fenugreek seed powder are suggested in the literature. However, the references suffer from critical methodological limitations (most available studies are case series lacking proper controls, randomization or blinding) precluding any formal conclusion on these properties. They could only be regarded as exploratory.

As an illustration, the Rapporteur will particularly describe one recent study (2009) from Chevassus et al, and a long-term study from Sharma et al, 1996.

- A fenugreek seed extract selectively reduces spontaneous fat consumption in healthy volunteers. Chevassus H, Molinier N, Costa F, Galtier F, Renard E, Petit P. Eur J Clin Pharmacol. 2009 Dec; 65(12):1175-8. Epub 2009 Oct 7.

Aim

The aim of the study was to investigate the effects of a repeated administration of a fenugreek seed extract on the eating behaviour of overweight subjects.

Study design

The study was designed as a 6-week double-blind randomized placebo-controlled parallel trial.

Data analysis and statistics

The sample size (40 subjects to be enrolled in two groups of 20) was determined using data obtained in a previous study, with an expected mean difference for energy consumption (main outcome) between the fenugreek seed extract and placebo of 216 kcal per day, a common standard deviation (SD) of 238 kcal per day, a two-sided alpha of 0.05 and statistical power of 80%.

Test compound

The test compound was a marketed dry hydro-alcoholic fenugreek seed extract administered three times daily as oral coated tablets. The total daily dose of 1176 mg (approximately 14 mg kg⁻¹) is double the daily dose of extract commonly prescribed for human consumption.

Investigations

The diet and physical activity of the patients were assessed under free-living conditions before and at the end of the ambulatory treatment period, using a 7-day record that was reviewed by a trained dietician and a physician for its accuracy. The main **endpoints** were **energy intake**, assessed in volunteers under normal ambulatory and free-living conditions by a 3-day detailed dietary record and during a meal test, **weight, fasting glucose level, insulin and lipid profile, visual analogue scale scores of appetite/satiety and blood glucose and insulin levels** measured repeatedly after a standardized breakfast.

Reported energy intake (REI) was determined with Enkal-Pro software and total energy expenditure (TEE) was calculated as basal metabolic rate (BMR) multiplied by physical activity level (PAL). Energy intake was defined as a ratio REI/BMR < 1.1.

Subjects

Thirty-nine healthy overweight male volunteers, aged 18-59 years (mean 38 years) completed this study. All were of stable weight (mean weight 85.4 kg, range 75.2-105.5; mean body mass index 27.3 kg m⁻²; range 24.9-29.4). One subject among the 40 initially enrolled was withdrawn from the study before the first administration of the drug due to partaking in a non-authorized treatment.

Assessor's comment:

Referring to a hydro-alcoholic fenugreek containing medicinal product in France the dose received would represent around twice the dose recommended in the posology section.

Results

Daily fat consumption was significantly decreased by the higher dose of fenugreek seed extract [3.73 vs. 4.51 MJ day⁻¹], -17.3% vs. placebo, 95% confidence interval (CI) -1.51 to -0.05, n = 12, P = 0.038]. This specific reduction tended to lower the **total energy intake** (9.97 vs. 11.29 MJ day⁻¹), -11.7% vs. placebo, 95% CI -2.91 to 0.26, n = 12, **P = 0.094**).

Table 10

Comparison of fasting data of plasma glucose, serum insulin and lipid profile between healthy overweight subjects receiving fenugreek seed extract 1176 mg/day and those receiving placebo

Main metabolic parameters	Fenugreek	Placebo	P
Fasting plasma glucose (mmol l ⁻¹)			
-Baseline	4.61±0.21	4.87±0.19	0.355
-Post-treatment	5.38±0.10	5.26±0.16	0.545
Fasting serum insulin (mU l ⁻¹)			

Main metabolic parameters	Fenugreek	Placebo	P
-Baseline	5.10±0.41	5.02±0.31	0.887
-Post-treatment	4.73±0.43	5.38±0.36	0.057
Fasting insulin/glucose ratio (mU mmol ⁻¹)			
-Baseline	1.17±0.13	1.07±0.08	0.708
-Post-treatment	0.89±0.09	1.06±0.10	0.044
Total cholesterol (mmol l ⁻¹)			
-Baseline	4.82±0.26	5.19±0.19	0.254
-Post-treatment	4.88±0.25	5.06±0.20	0.207
HDL-cholesterol (mmol l ⁻¹)			
-Baseline	1.27±0.05	1.22±0.08	0.555
-Post-treatment	1.30±0.05	1.17±0.07	0.067
Triglycerides (mmol l ⁻¹)			
-Baseline	1.25±0.15	1.15±0.11	0.732
-Post-treatment	1.27±0.16	1.41±0.14	0.148

Values are given at the mean ± standard error of the mean (SEM)

The ratio of fasting serum insulin/plasma glucose was significantly decreased in subjects treated with fenugreek seed extract relative to the placebo group [0.89±0.09 (n=19) vs 1.06±0.10 (n=19) mUI mmol⁻¹, respectively. No effect on plasma lipid profile, antioxidant parameters and oxidative stress markers were observed.

Authors' conclusions: The repeated administration of a fenugreek seed extract slightly but significantly decreased dietary fat consumption in human volunteers in this short-term study.

Assessor's comments:

According to the authors, the lower ratio of fasting serum insulin/plasma glucose may reflect an improved insulin sensitivity. However, still as underlined by the authors, this property cannot be regarded as established and specific investigations would be required. This is all the more disputable that in this study FPG even increases from baseline to post-treatment (4.61 mmol/L to 5.38 mmol/L). As regards the lipid parameters, the trend is rather towards an increase of total cholesterol and triglycerides, the only "positive" trend being a slight and non-significant increase of HDL.

- Sharma RD et al. Use of fenugreek seed powder in the management of non-insulin dependent diabetes mellitus. *Nutrition Research*, 1996, 16: 1331-1339

Study population

Sixty patients with mild (22), moderate (35) and severe (7) non insulin dependent diabetes mellitus (NIDDM) were registered for the study. These patients were drawn from the outpatients' diabetic clinics, OPD and the Postgraduate Department of Medicine, S. N. Medical College, Agra (India). Of these 45 were male patients and 15 female. Their ages ranged between 30 to 70 years.

According to body mass 21 were obese and 39 non-obese. Twenty six patients had multiple complications. Twenty one patients had hypertension, 18 patients suffered from diabetic neuropathy, 2 were suffering from diabetic nephropathy, 2 showed retinopathy, 8 had angina and 8 developed myocardial infarction.

All patients registered had uncontrolled blood glucose levels. They were not taking adequate medicine due to either poverty or ignorance. Poor patients who could not afford adequate food were given suboptimal doses of drugs. Initially, 18 patients took a product with 4.72 mg glibenclamide

daily. Eight patients took a product with 1.06 mg of metform daily. A combination of both drugs was taken by 5 severe diabetic patients, 17 mg of glibenclamide + 1.7 mg metform daily. Nine patients took other drugs of homeopathy treatment. Twenty patients did not take any medication for diabetes.

Long-term data up to 24 weeks are available in this study.

A control group comprised of 10 subjects was also run simultaneously. This group was drawn from staff of S. N. Medical College, Agra. of these, 7 were male and 3 were females. Their ages, like study group patients, ranged between 30 to 70 years.

In the beginning of the study, both control and diabetic subjects were put on a prescribed diet comprising of 300 g carbohydrate for seven days of the control period. For the estimation of basal parameters, glucose tolerance test with 75 g glucose load was initially performed for each subject. **For the long-term follow up study, diabetic patients were asked to continue to consume the prescribed diet in addition to 25 g fenugreek seed powder divided into two doses at lunch and dinner.**

Glucose tolerance test (GTT) was performed for each subject at an interval of 4, 8, 12 and 24 weeks.

Results

Both control and experimental diets provided similar calories and had similar nutrient composition except fibre content which was higher in the fenugreek seed powder diet.

The food and mean energy intake of diabetic subjects during control and experimental periods were almost similar and constant. The mean energy intake being 2056±289 kilo calories, of which 63±5.1% was derived from carbohydrates, 18±2.8% from fat and 19.1±2.7% from protein.

There was no significant change in the body mass for these two groups.

A significant fall in serum levels were observed at ½ h, 1 h and 2 h during GTT. Although fasting levels of insulin remained unchanged, the mean insulin area was reduced significantly ($p < 0.05$).

Table 11

Blood glucose and insulin levels and the area under the curve for diabetic subjects before and after administration of fenugreek seed powder

Time (h)	Blood glucose (mmol/L)		Serum insulin (mU/L)	
	Initial	24 th week	Initial	24 th week
0 h	8.4±0.3@	6.2±0.3**	16.2±8.9	17.3±1.3
1/2 h	11.9±0.4	6.9±0.9**	31.2±5.2	22.9±2.1*
1 h	13.6±0.5	10.9±0.6**	40.3±3.9	33.4±2.8*
1.5 h	14.6±0.5	10.7±0.6**	-	
2 h	14.6±0.6	9.5±0.6**	38.2±2.5	25.8±2.2*
	Mmol/L/min		mU/L/min	
Area under the curve	593.8±31.1	351±32.9**	2892.8±510.0	1786.4±188.5*

@ = mean ± S.E.

Level of significance for comparison of initial versus 24th week values

In 10 diabetic patients, 24 h urinary sugar was estimated at the beginning and at the 8th week, after fenugreek seed powder administration. A fall of 13% in urinary sugar was observed which was found to be statistically significant ($p < 0.001$) (table 12). **Glucosylated haemoglobin was also determined**

initially and at the end of 8th week. A highly significant reduction was observed with a percentage decrease of 12.5% as compared to initial values (table 12).

Table 12

Urinary sugar and glycosylated haemoglobin levels in diabetic subject before and after administration of fenugreek seed powder

Weeks	Urinary (mmol/24 h)	Serum glycosylated haemoglobin (%)
Initial	76.7±1.7 [@]	9.6±1.9
8 weeks	43.3±4.3**	8.4±1.4**

@ = mean ± S.E

Level of significance initial versus 8th week (** p<0.001)

The degree of glycaemic control was assessed by measuring 2 h post-prandial blood sugar levels initially and at the 24th week of fenugreek seed powder ingestion. At the end of this study, 46.7% of these patients showed full glycaemic control, 33.3% showed moderate glycaemic control and 20% exhibited minimal glycaemic control (table 13).

Table 13

Percent distribution of patients according to glycaemic control at the initial and 24th week of fenugreek seed powder administration to NIDDM patients

Postprandial blood sugar (mg/ml)	Initial study (%)	24 th week study (%)
>140 (full glycaemic control)	5	46.7
140-180 (moderate glycaemic control)	21.7	33.3
< 180 (minimal glycaemic control)		20.0

Assessor's comments:

As compared to other literature data, this study presents the interest of providing long-term data as well as data on glycosylated haemoglobin. Unfortunately, there are critical limitations precluding any reliable interpretation. The sample size is limited and the population is heterogeneous. It is unclear to what extent the control group can be regarded as a valid control group for adequately estimating the true contribution of fenugreek in the patient's diet in terms of glycemic control. It is very unlikely that the significant (1.2%!) change in glycosylated haemoglobin could be put at the credit of fenugreek. Indeed, these patients were badly controlled for their diabetes before the study entry and appear to have benefitted from standard treatments after inclusion. It is unclear whether both the experimental and control groups have received superimposable therapeutic, biological and clinical monitorings to enable an adequate assessment of the fenugreek effect.

4.2.3. Clinical studies in special populations (e.g. elderly and children)

No clinical data are available in children.

4.3. Overall conclusions on clinical pharmacology and efficacy

When scrutinizing the published literature to substantiate the clinical efficacy of fenugreek in the adopted indications, it has to be acknowledged that the data are scarce and of poor relevance.

Subsequently, the effect of fenugreek relies more on a traditional use than on a well-established use.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

- The safety and efficacy of *Trigonella foenum-graecum* extract was investigated by Abdel-Barry et al, in 20 male volunteers aged 20-30 years. They were randomly treated with either 40 mg/kg aqueous extract powder in 10 ml distilled water or 10 ml distilled water in which coffee simulated the extract. A significant reduction of 14.1% was observed in potassium levels. No significant alteration in serum cholesterol, total serum protein and blood urea occurred. Approximately one-third experienced feelings of hunger, increased micturition frequency or dizziness during the 24 hours after ingestion. The authors concluded that the hypokalaemic effect of fenugreek merits further investigation.
- Adverse events including transient diarrhoea and flatulence have been reported in studies evaluating the effects of fenugreek on blood glucose (Sharma RD et al, 1996).
- Some patients developed dyspepsia and mild abdominal distension after fenugreek seed intake in one double blind placebo controlled study (Gupta et al, 2001) evaluating the effects of *Trigonella foenum-graecum* seeds on glycaemic control and insulin resistance. Twenty-five patients were enrolled and 12 received 1 mg/d hydro-alcoholic extract of fenugreek seeds. The other 13 patients received usual care (dietary control, exercise) and placebo. Duration of the study was 2 months.

Assessor's comments:

It should be pointed out that the number of patients included in these studies is very limited. However, the administration of fenugreek seems to be potentially associated with digestive disorders, dizziness and increase in micturition frequency. We agree that no conclusions can be drawn with regard to the reduction of potassium levels observed in the first study.

5.2. Patient exposure

Not applicable.

5.3. Adverse events and serious adverse events and deaths

Three publications highlight the risk of allergy after fenugreek ingestion, inhalation or external application:

- The first publication (Patil SP et al, 1997) reports two cases of immediate allergy following inhalation and external application of fenugreek seed powder. In the first case, inhalation of the fenugreek seed powder resulted in rhinorrhoea, wheezing, and fainting. The second case was of a patient with chronic asthma who developed numbness of head, facial angioedema and wheezing after application of fenugreek paste to her scalp as a treatment for dandruff. Skin scratch test was performed with fenugreek and revealed strong sensitivity to fenugreek and chickpeas. Immunoblots demonstrated binding of specific IgE from the patients' sera with the protein from extracts between 20 kD to 70 kD bands.
- The second one, reports one case of bronchospasm after inhalation of curry powder (Ohuma et al, 1998).

- The last case report involves one patient having used fenugreek powder orally as an appetite stimulant and topically as a healing agent (Bessot et al, 1996). He experienced asthma and rhinitis. The prick test performed with fenugreek powder was strongly positive.

Three publications report a false diagnosis of maple syrup urine disease owing to ingestion of herbal tea:

- The first case (Sewell et al, 1999) involved a five-week old Egyptian infant, who had a 10-minute episode of unconsciousness while drinking bottled tea. He recovered spontaneously but the parents nevertheless sought medical attention. On admission, the child was in good clinical condition and alert, and the physical examination was unremarkable. The child exuded a specific aroma and a spontaneously voided urine sample had a similar aroma. This observation initiated emergency evaluations of metabolic amino acids and organic acids to rule out maple syrup urine disease; the results of all tests were normal. The parents mentioned that they had given their child a herbal tea (Helba tea) to reduce flatulence and prevent fever. This tea contains seeds of fenugreek (*Trigonella foenum-graecum L.*). Analysis of the infant's urine by enantioselective multidimensional gas chromatography and mass spectrometry revealed the presence of sotolone, the compound responsible for the aroma in maple syrup urine disease. The tea prepared from fenugreek seeds was found to contain sotolone.
- Two similar reports were published earlier in 1981 (Bartley et al) and 2001 (Korman et al).

A publication reports one case of aplastic anaemia in a 51 year-old woman, having taken 3 dietary supplements (during a 30-day herbal program). The product packaging listed a total of 39 plant-based products, including fenugreek. The woman received transfusions of red blood cells and platelets, and was later discharged feeling well (Smereck et al, 2009).

Assessor's comments:

According to these data the local application, as well as inhalation or ingestion of fenugreek have been associated with allergic reactions that are sometimes serious. Positive skin scratch test and prick test in two of the three case-reports demonstrate the responsibility of fenugreek. The risk of false diagnosis of maple syrup urine disease and potential unnecessary investigations in young children will not be introduced in the monograph as fenugreek is not recommended for children and adolescents under 18 years of age because of incomplete safety data.

5.4. Laboratory findings

N/A

5.5. Safety in special populations and situations

Drug interactions

An interaction between fenugreek and warfarin has also been retrieved in 2 publications, including one case report (Heck et al, 2000 and Lambert et al, 2001). The case report involved a patient who was treated with warfarin for atrial fibrillation. During treatment, an increase in international normalized ratio (INR) and the patient's admission that she was taking a variety of natural products which included boldo and fenugreek, led the authors to suspect that some of these natural products could alter the effect of warfarin. When the patient stopped the herbal products, the INR returned to normal after 1 week. The herb-drug interaction was observed a second time, after both products were reintroduced a few days later. The imputability of this interaction to both natural products, as

determined by the Naranjo algorithm, suggests a probable association between boldo-fenugreek and increased bleeding time in patients treated with warfarin. No undesirable reaction was reported during telephone discussions with the patient. Nevertheless, the authors recommend that clinicians, treating patients with anticoagulant therapy, be vigilant when patients also take herbal agents.

Assessor's comments:

The data regarding a possible interaction with oral anticoagulants are definitely too sparse and the evidence is very weak.

Only one clinical case is available (Lambert JP, Cormier A in Pharmacotherapy, 2001:21:509-12). The patient showed slight increases of her INR values, usually comprised between 2 and 3, and which increased to 3.1 after one week of the combination and to 3.4 after two weeks.

Firstly, these increases are to be considered slight. Moreover, the patient seemed to present with memory disorders ("It was difficult to make a precise list of OTC and natural products consumed because the patient has some memory confusion"). Moreover the authors themselves appear disbelieving ("we did experience some difficulty in obtaining the exact name of the various OTC products the woman consumed. It is not impossible that she may have omitted or forgotten to mention some change in nutrition such as decreased consumption of food rich in vitamin K or excessive consumption of alcohol").

The French Pharmacovigilance Database with 22 reports of patients receiving fenugreek as an active substance did not reveal any case sustaining this hypothesis.

Taking into account all these elements, it is considered not suitable to add a specific warning in the 4.5 section of the monograph as regards this putative, poorly documented, far not proven interaction.

Use in pregnancy and lactation

In one study, both water and alcoholic extracts of fenugreek exerted a stimulating effect on the isolated guinea pig uterus, especially during late pregnancy. As a result, the authors concluded that **fenugreek may possess abortifacient effects** and is not recommended for use, in doses higher than those found in foods, during pregnancy (Abdo et al, 1969)

Assessor's comments:

No data are available in humans regarding pregnancy and lactation. As a precautionary measure, the use of fenugreek should not be recommended in this population.

Overdose, drug abuse

No data available.

Withdrawal and rebound

No data available.

Effects on ability to drive or operate machinery or impairment of mental ability

No data available.

5.6. Overall conclusions on clinical safety

Data from the literature have enabled to identify mainly two kinds of adverse effects after fenugreek intake: **digestive disorders and allergic reactions**. However, other adverse effects have been reported in some studies: dizziness and increase of micturition frequency.

The risk of false diagnosis of maple syrup urine disease and potential unnecessary investigations underlined in young children will not be introduced in the monograph as fenugreek is not recommended for children and adolescents under 18 years of age because of incomplete safety data.

Taking into account all the elements mentioned above regarding the potential risk of interaction between fenugreek and anticoagulants (page 48/49), it is considered not suitable to add a specific warning in the 4.5 section as regards this putative, poorly documented, so far not proven interaction.

6. Overall conclusions

Fenugreek containing preparations are reported as being on the EU market for more than 30 years in products for oral use in lack of appetite (Poland and France) and in products for external use for skin inflammation treatment (Poland). Only the preparations which have been used for at least 30 years are described in the monograph.

Nevertheless, when scrutinizing the published literature to substantiate the clinical efficacy of fenugreek in the first indication, it has to be acknowledged that the data are scarce and of poor relevance in adults.

The clinical data are of poor relevance in adolescents. In children, no efficacy data are available, clinical experience is sparse and mainly through case reports of adverse events.

The literature data appear to be relatively more abundant concerning the hypoglycaemic and hypolipidaemic properties of fenugreek, however, due to significant methodological deficiencies and inconsistencies, still without providing adequate demonstration of their clinical impact.

Finally, the cutaneous clinical use of fenugreek for skin inflammation is not substantiated in the literature data.

Consequently, the effect of fenugreek is plausible and relies on a traditional use (data cannot substantiate a well-established use).

As regards the safety profile of fenugreek, it is mainly characterized by **digestive disorders and allergic reactions**.

The use of fenugreek in pregnant women should be avoided, in view of the uncertainties surrounding a beneficial effect of this plant on one hand, and the uterine stimulant properties reported in animal studies (even justifying an historical use as an abortifacient) on the other.

The use in children and adolescents under 18 years of age is not recommended because of incomplete data on safety.

Available genotoxicity data do not allow the inclusion of *Trigonella foenum-graecum* L., semen in the list of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products.

Annex

List of references

SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to Teestar™, a fenugreek seed extract standardised by its content of galactomannan, and a reduction of post-prandial glycaemic responses pursuant to Article 13(5) of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Avesthagen Limited, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of France, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Teestar™ and a reduction of post-prandial glycaemic responses. The Panel considers that the food, Teestar™, a fenugreek seed extract standardised by its content of galactomannan, is sufficiently characterised. A reduction of post-prandial glycaemic responses might be a beneficial physiological effect. The applicant submitted one unpublished and eight published human studies as being pertinent to the health claim. No conclusions can be drawn from the eight published studies, as they were not carried out with Teestar™ or any other fenugreek seed extract which complied with the specifications of the food which is the subject of the claim. In one unpublished study, the consumption of Teestar™ did not lead to a reduction in mean peak post-prandial blood glucose concentrations, which was the primary endpoint of the study. The Panel concludes that a cause and effect relationship has not been established between the consumption of Teestar™, a fenugreek seed extract standardised by its content of galactomannan, and a reduction of post-prandial glycaemic responses.

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KEY WORDS

Teestar™, *Trigonella foenum-graecum*, fenugreek, post-prandial glycaemic responses, health claims

¹ On request from the Competent Authority of France following an application by Avesthagen Limited, Question No EFSA-Q-2014-00153, adopted on 11 December 2014.

² Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Correspondence: nda@efsa.europa.eu

³ Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Marina Heinonen, Ambroise Martin, Hildegard Przyrembel, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Sean (J.J.) Strain, Inge Tetens, Hendrik Van Loveren, Hans Verhagen and Peter Willatts for the preparatory work on this scientific opinion.

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SUMMARY

Following an application from Avesthagen Limited, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of France, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Teestar™ and a reduction of post-prandial glycaemic responses.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.

The food that is the subject of the health claim is Teestar™, which is a fenugreek (*Trigonella foenum-graecum* L.) seed extract standardised by its content (i.e. $70 \pm 5\%$) of galactomannan. The Panel considers that the food, Teestar™, a fenugreek seed extract standardised by its content of galactomannan, is sufficiently characterised.

The claimed effect proposed by the applicant is “reduction of post-prandial blood glucose levels”. The target population proposed by the applicant is “healthy adults with or without impaired glycaemic pre-obese and obese conditions”. The Panel considers that a reduction of post-prandial glycaemic responses (as long as post-prandial insulinaemic responses are not disproportionately increased) might be a beneficial physiological effect.

The applicant submitted one unpublished and eight published human studies as being pertinent to the health claim.

The eight published human studies were not carried out with Teestar™ or any other fenugreek seed extract which complied with the specifications of the food which is the subject of the claim. The Panel considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

In one unpublished, open-label, randomised, placebo-controlled, three-arm parallel study, 27 subjects consumed, for seven consecutive days, Teestar™ capsules, Teestar™ crackers or a placebo prior to breakfast and dinner. The primary endpoint was a reduction in the mean peak post-prandial blood glucose concentrations. Compared with the placebo, consumption of Teestar™ did not lead to a reduction in mean peak post-prandial blood glucose concentrations after breakfast or dinner. As secondary endpoints of the study, mean blood glucose concentrations at the time points 60, 90 and 120 minutes after the start of breakfast and dinner were compared between the Teestar™ groups and the placebo group. The time points were treated as independent in the analysis, and no correction for multiple comparisons was carried out. The Panel considers that no conclusions can be drawn from the secondary analysis of the study. The Panel considers that this study does not provide evidence for an effect of the consumption of Teestar™ on a reduction of post-prandial glycaemic responses.

The Panel notes that in the absence of evidence for an effect of Teestar™ on post-prandial glycaemic responses in humans, animal studies on potential mechanisms do not provide support for the scientific substantiation of the claim.

The Panel concludes that a cause and effect relationship has not been established between the consumption of Teestar™, a fenugreek seed extract standardised by its content of galactomannan, and a reduction of post-prandial glycaemic responses.

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BACKGROUND

Regulation (EC) No 1924/2006⁴ harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 04/03/2014.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.
- On 19/05/2014, during the validation process of the application, EFSA sent a request to the applicant to provide missing information.
- On 19/06/2014, EFSA received the missing information as submitted by the applicant.
- The scientific evaluation procedure started on 19/06/2014.
- On 18/09/2014, the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application and the clock was stopped on 29/09/2014, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- On 14/10/2014, EFSA received the applicant's reply (which was made available to EFSA in electronic format on 13/10/2014) and the clock was restarted, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- During its meeting on 11/12/2014, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to Teestar™, a fenugreek seed extract standardised by its content of galactomannan, and a reduction of post-prandial glycaemic responses.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: Teestar™, a fenugreek seed extract standardised by its content of galactomannan, and a reduction of post-prandial glycaemic responses.

⁴ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of Teestar™, a positive assessment of its safety, nor a decision on whether Teestar™ is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Avesthagen Limited, Avesthagen One, No 7/6 Brunton Road, Bangalore 560025, Karanataka, India.

The application includes a request for the protection of proprietary data, in accordance with Article 21 of Regulation (EC) No 1924/2006.

Food/constituent as stated by the applicant

According to the applicant, the food that is the subject of the health claim is Teestar™, which is a standardised fenugreek (*Trigonella foenum-graecum* L.) fibre extract containing 70 ± 5 % galactomannan.

Health relationship as claimed by the applicant

According to the applicant, consumption of Teestar™ prior to meals leads to a reduction of post-prandial blood glucose levels.

The proposed mechanism is that in the presence of water the galactomannan in Teestar™ forms a colloidal-type suspension in the stomach and small intestine, thereby increasing the viscosity in the gastrointestinal tract and slowing down gastrointestinal transit of food and glucose absorption through intestinal microvilli.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: “Teestar™ lowers blood glucose levels”.

Specific conditions of use as proposed by the applicant

The applicant has proposed an intake of 1 g Teestar™ twice a day 20 minutes prior to consumption of a meal. Teestar™ can be consumed in the form of capsules, crackers or as a powder (to be dissolved in water or milk).

The target population proposed by the applicant are healthy adults with or without impaired glycaemic pre-obese and obese conditions.

According to the applicant, children and teenagers up to 18 years of age, adults with malnutrition, underweight adults and pregnant women should not consume Teestar™, in order “to avoid any imbalance in calorie input and calorie requirement”.

ASSESSMENT

1. Characterisation of the food/constituent

The food that is the subject of the health claim is Teestar™, which is a standardised fenugreek (*Trigonella foenum-graecum* L.) seed extract containing 70 ± 5 % galactomannan.

Teestar™ is extracted from the seeds of fenugreek (*Trigonella foenum-graecum* L.) by water and alcohol extraction and is standardised by its content of galactomannan (i.e. 70 ± 5 %). Teestar™ contains 19-21 % protein and 3-5 % moisture.

Galactomannan is a water-soluble type of fibre. It is a polysaccharide which is composed of a backbone of D-mannose units (linked by β -1,4-glycosidic bonds) with D-galactose moieties linked (by α -1,6-glycosidic bonds) to the mannose units. The ratio of mannose to galactose in galactomannan from fenugreek is 1:1. The average molecular weight of the galactomannan is about 217 kDa. Galactomannan can be measured in foods by established methods.

An overview of the manufacturing process, batch-to-batch variability and stability data were provided. Teestar™ is manufactured in the form of capsules, crackers and as a powder.

The Panel considers that the food, Teestar™, a fenugreek seed extract standardised by its content of galactomannan, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is “reduction of post-prandial blood glucose levels”. The target population proposed by the applicant is “healthy adults with or without impaired glycaemic pre-obese and obese conditions”.

The elevation of blood glucose concentrations after consumption of a food and/or meal, i.e. post-prandial glycaemia, is a normal physiological response which varies in magnitude and duration, and which may be influenced by the chemical and physical nature of the food or meal consumed, as well as by individual factors (Venn and Green, 2007). Decreasing post-prandial glycaemic responses, as long as post-prandial insulinaemic responses are not disproportionately increased, may, for example, be beneficial to individuals with impaired glucose tolerance. Impaired glucose tolerance is common in the general population of adults.

The Panel considers that a reduction of post-prandial glycaemic responses (as long as post-prandial insulinaemic responses are not disproportionately increased) might be a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed, Science Direct, the WIPO (World Intellectual Property Organization) and Google, using the search terms “fenugreek”, “*Trigonella foenum graecum*”, “diabetes”, “efficacy”, “safety”, “preclinical”, “clinical”, “glucose”, “galactomannan” and “glycaemic control”. In addition, references of key articles were hand searched. The search was limited to studies published from 1980-2011 in the English language. Studies were excluded if they were primarily concerned with diabetic complications such as neuropathy, nephropathy or retinopathy, or if they were available only as abstracts.

The applicant submitted one unpublished and eight published human studies as being pertinent to the health claim.

The eight published human studies (Sharma, 1986; Sharma and Raghuram, 1990; Raghuram et al., 1994; Sharma et al., 1996a, b; Bordia et al., 1997; Abdel-Barry et al., 2000; Mitra and Bhattacharya, 2006) were not carried out with Teestar™ or any other fenugreek seed extract which complied with the specifications indicated in section 1 but rather with whole or powdered fenugreek seeds, fenugreek leaves, an extract from fenugreek leaves, fenugreek gum isolate or fenugreek composites. The Panel considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

One study (Avesthagen Limited, 2008, unpublished, claimed as proprietary by the applicant) was carried out with Teestar™ (in the form of capsules and crackers). The study was an open-label, randomised, placebo-controlled, three-arm parallel study in 27 healthy male subjects who consumed Teestar™ capsules (n = 9), Teestar™ crackers (n = 9) (both providing 1 g galactomannan per portion)

or a placebo (1 g milk casein in capsules; n = 9) 20 minutes prior to breakfast and dinner for seven consecutive days. The Panel notes that the intervention with the crackers did not have an appropriate control matched for the macronutrient content of the crackers. Power calculations indicated that eight subjects per study group would yield a power of 95 % to detect a difference in peak blood glucose concentrations of 0.5 mmol/L, at a significance level (two-sided) of 5 % and assuming a standard deviation of 0.15 mmol/L. In order to assess blood glucose concentrations, blood samples were taken every day before (i.e. at baseline) and at 15, 30, 45, 60, 90 and 120 minutes after the start of breakfast and dinner (which contained 100 g carbohydrate per meal). In addition, serum insulin concentrations were measured in nine subjects (randomly selected among the 27 study subjects) on day 2 and day 7. The primary endpoint of the study was a reduction in the mean peak post-prandial blood glucose concentrations between the time points -20 minutes and 120 minutes in the Teestar™ groups compared with the placebo group after breakfast and dinner. A t-test was used for the statistical analysis. Compared with the placebo, consumption of Teestar™ did not lead to a reduction in mean peak post-prandial blood glucose concentrations after breakfast or dinner. The applicant was requested to provide a statistical analysis of the incremental areas under the blood glucose and blood insulin curves (0-120 minutes), applying standard methodology, following consumption of Teestar™ in comparison with placebo. No such analysis was provided. As secondary endpoints of the study, mean blood glucose concentrations at the time points 60, 90 and 120 minutes after the start of breakfast and dinner were compared between the Teestar™ groups and the placebo group, using a t-test and choosing a significance level of $p \leq 0.1$. The time points were treated as independent in the analysis, and no correction for multiple comparisons was carried out. The Panel considers that no conclusions can be drawn from the secondary analysis of the study. The Panel considers that this study does not provide evidence for an effect of the consumption of Teestar™ on a reduction of post-prandial glycaemic responses.

The Panel notes that in the absence of evidence for an effect of Teestar™ on post-prandial glycaemic responses in humans, animal studies on potential mechanisms do not provide support for the scientific substantiation of the claim.

The Panel concludes that a cause and effect relationship has not been established between the consumption of Teestar™, a fenugreek seed extract standardised by its content of galactomannan, and a reduction of post-prandial glycaemic responses.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food, Teestar™, a fenugreek seed extract standardised by its content of galactomannan, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is “reduction of post-prandial blood glucose levels”. The target population proposed by the applicant is “healthy adults with or without impaired glycaemic pre-obese and obese conditions”. A reduction of post-prandial glycaemic responses might be a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of Teestar™, a fenugreek seed extract standardised by its content of galactomannan, and a reduction of post-prandial glycaemic responses.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on Teestar™ and a reduction of post-prandial glycaemic responses pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (EFSA-Q-2014-00153, Claim serial No: 0414_FR). March 2014. Submitted by Avesthagen Limited.

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* <i>Fine Chemicals</i>	* <i>Herbal Extracts</i>	20 Lake Drive	750 Innovation Circle
* <i>Cosmeceuticals</i>	* <i>Speciality Chemicals</i>	East Windsor, NJ 08520	Payson, UT 84651
		Tel: (732) 777-1111	Tel: (801) 465-8400
		Fax: (732) 777-1443	Fax: (801) 465-8600

SAFETY DATA SHEET (SDS)

FENUSTEROLS[®] (FENUGREEK STEROLS)

Section 1: Chemical Product and Company Identification

Product Name:	FENUSTEROLS [®] (FENUGREEK STEROLS)
Catalog Codes:	0566
CAS#:	68990-15-8
RTECS:	Not available
TSCA:	Not available
CI#:	Not available
Synonym:	Fenugreek extract - Fenusterols
Chemical Name:	Not available
Chemical Formula:	Not available
Contact Information:	Sabinsa Corporation. 20 Lake Drive, East Windsor, NJ. USA - 08520

Section 2: Hazards Identification

Potential Acute Health Effects:	Not known
Potential Chronic Health Effects:	Not known
CARCINOGENIC EFFECTS:	Non carcinogenic
MUTAGENIC EFFECTS:	Not mutagenic
TERATOGENIC EFFECTS:	Not known
DEVELOPMENTAL TOXICITY:	Not known

Section 3: Composition and Information on Ingredients

Composition:	Fenugreek extract: 65% - 75%; Dextrin: 25% - 35%
Name:	Fenusterols [®] (Fenugreek sterols)
CAS #:	68990-15-8
% by Weight:	Fenugreek extract: 65% - 75%
Toxicological Data on Ingredients:	Safe at recommended dosage
Ascorbic acid:	Absent

Section 4: First Aid Measures

Eye Contact:	Important, immediately rinse with water for at least 15 minutes. Hold eyelids apart. Get medical attention if any discomfort continues.
Skin Contact:	Remove contaminated clothing. Wash skin thoroughly with soap & water. Get medical attention promptly if any symptoms occur after washing.

Inhalation: Move the exposed person to fresh air at once. Get medical attention if any discomfort continues.

Ingestion: Rinse mouth thoroughly. Never give liquid to an unconscious person. Get medical attention if any discomfort continues.

Section 5: Fire-fighting Measures

Flammability of the Product: Non flammable

Auto-Ignition Temperature: Not applicable

Flash Points: Not available

Flammable Limits: Not available

Products of Combustion: Carbon dioxide, Water, Carbon monoxide

Fire Hazards in Presence of Various Substances: Not known

Explosion Hazards in Presence of Various Substances Not known

Fire Fighting Media and Instructions: Water spray, Dry powder or Carbon dioxide.
Water spray should be used to cool containers.
Wear protective equipment by fire fighters.
Self contained breathing apparatus and full protective clothing must be worn in case of fire by fire fighters.

Special Remarks on Fire Hazards: Material may emits toxic fumes on combustion.

Special Remarks on Explosion Hazards: Not known.

Section 6: Accidental Release Measures

Small Spill: Wear self-contained breathing apparatus, rubber boots and rubber gloves. Cover with absorbents and complete pick up material. Absorbents: Sand, saw dust, dry lime, soda ash. Keep in a closed container for waste disposal.

Large Spill: Evacuate the area. Remove sources of Ignition. Ensure protection (Including respiratory protection) during removal of spillages in a confined area. Avoid discharge into drains, water courses or on to the ground. Ventilate well. Avoid dust formation. Collect powder using special dust vacuum cleaner with particle filter or carefully sweep into closed container. Wash contaminated area with water & detergent.

Section 7: Handling and Storage

Precautions:

Provide good ventilation. Avoid handling which leads to dust formation. Avoid inhalation of dust and contact with skin and eyes. Do not eat, drink or smoke when using the product. Wash hands after handling. Wash contaminated clothing before reuse.

Storage:

Store the material in tightly closed original container. Protect against direct sunlight. Avoid heat, flames & other sources of ignition. Avoid contact with strong oxidizers.

Section 8: Exposure Controls/Personal Protection

Engineering Controls:

Provide adequate ventilation.

Personal Protection:

Respiratory equipment

In case of inadequate ventilation use suitable respiratory equipment with particle filter, type P1.

Hand protection

Wear protective gloves.

Eye protection

Wear approved safety goggles.

Other protection

Wear suitable protective clothing.

Hygiene measures

When using do not eat, drink or smoke. Wash hands after handling.

Wash contaminated clothing before reuse.

Environmental exposure controls

Avoid discharge into drains, water courses or on to the ground.

Personal Protection in Case of a Large Spill:

Exposure Limits:

No exposure limits noted for this ingredient.

Section 9: Physical and Chemical Properties

Physical state and appearance:

Yellow and hygroscopic powder

Odor:

Characteristic

Taste:

Not available

Molecular Weight:

Not available

Color:

Yellow

pH (1% saline /water):

Not available

Boiling Point:

Not applicable

Melting Point:	
Flash Point:	Not available
Critical Temperature:	Not available
Specific Gravity:	Not available
Vapor Pressure:	Not available
Vapor Density:	Not available
Volatility:	Not available
Odor Threshold:	Not available
Water/Oil Dist. Coeff.:	Not available
Ionicity (in Water):	Not available
Dispersion Properties:	Not available
Flammability:	Not available
Solubility:	Partially soluble in water and organic solvents

Section 10: Stability and Reactivity Data

Stability:	Product is stable from safety point of view.
Instability Temperature:	Not known
Conditions of Instability:	Not known
Incompatibility with various substances:	Not known
Corrosivity:	Non- corrosive
Special Remarks on Reactivity:	None
Special Remarks on Corrosivity:	None
Polymerization:	Not known

Section 11: Toxicological Information

Toxicity to Animals:	
Acute Toxicity (Oral LD ₅₀):	No information available
Acute Toxicity (Dermal LD ₅₀):	No information available
Acute Toxicity (Inhalation LC ₅₀):	No information available
Respiratory Sensitisation:	No information available
Skin Sensitisation:	No information available
General Information:	No information available
Subchronic Oral Toxicity:	No information available
Chronic Effects on Humans:	Not known
Other Toxic Effects on Humans:	Not known
Special Remarks on Toxicity to Animals:	Not known
Special Remarks on Chronic Effects on Humans:	Not known
Special Remarks on other Toxic Effects on Humans:	Not known

Section 12: Ecological Information

Ecotoxicity:	There are no data on the ecotoxicity of this product.
Acute Toxicity (Fish)	No information available
Acute Toxicity (Aquatic Invertebrates)	No information available
Acute Toxicity (Aquatic Plants)	No information available
BOD5 and COD:	No information available
Products of Biodegradation:	There are no data on the degradability of this product.
Toxicity of the Products of Biodegradation:	Not known
Special Remarks on the Products of Biodegradation:	Not known

Section 13: Disposal Considerations

Waste Disposal:	Can be eliminated as a non-hazardous industrial waste according to and Federal Law concerning waste elimination. Dispose of waste & residues in accordance with local authority requirements.
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Section 14: Transport Information

General Information

	The product is not covered by International Regulation on the transport of dangerous goods (IMDG, IATA, ADR/RID).
UN No	Not applicable
UN proper shipping name	Not dangerous goods
Transport hazard class(es)	Material is Non-Hazardous. Transport warning sign is not required.
Packing group	Not applicable
Environmental hazards	Not recorded
Marine Pollutant	Not known
Special precaution for user	To be handled and used by qualified personnel
DOT Classification:	Not known
Special Provisions for Transport:	Consult applicable regulations for transport information

Section 15: Other Regulatory Information

Federal and State Regulations:	No data available
Other Regulations:	No data available
Other Classifications:	No data available
WHMIS (Canada):	No data available
DSCL (EEC):	No data available
HMIS (U.S.A.):	No data available
National Fire Protection Association (U.S.A.):	No data available
Protective Equipment:	No data available
European Information:	No data available

Section 16: Other Information

This is a natural product, extracted from seed of *Trigonella foenum graecum*. This product belongs to class of Non-Hazardous substances and can be used as Botanical supplements.

Issue Date	26/12/2015
Next Review Date	25/12/2018
Revision No.	01
Revision Indication	New SDS
Risk Phrases In Full (R-Phrases)	Not classified

National Fire Protection Association (NFPA) Rating:

Health	2
Flammability	1
Reactivity	0

Disclaimer:

This information relates only to the specific material designated & may not be valid for such material used in combination with any other materials or in any process. Such information is, to the best of the company's knowledge & belief, accurate & reliable as of the date indicated. However, Sabinsa Corporation makes no warranty guarantee or representation to its accuracy, reliability or completeness. It is the user's responsibility to satisfy himself as to the suitability of such information for his own particular use.

End of Safety Data Sheet

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.5

Revision Date 26.05.2025

Print Date 03.12.2025

GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking**1.1 Product identifiers**

Product name : Fenugreek absolute

Product Number : W248606

Brand : Aldrich

REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration or the annual tonnage does not require a registration.

CAS-No. : 84625-40-1

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Merck Life Science Sp. z o.o
Pastelowa 8
PL-60-198 POZNAN

Telephone : +48 61 8290-100

Fax : +48 61 8290-120

E-mail address : TechnicalService@merckgroup.com

1.4 Emergency telephone number

Emergency Phone # : +(48)-223988029 (CHEMTREC) 112
(numer alarmowy)

SECTION 2: Hazards identification**2.1 Classification of the substance or mixture**

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.2 Label elements

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.3 Other hazards - none

SECTION 3: Composition/information on ingredients

3.1 Substances

CAS-No. : 84625-40-1
EC-No. : 283-415-1

No components need to be disclosed according to the applicable regulations.

SECTION 4: First aid measures

4.1 Description of first aid measures

If inhaled

After inhalation: fresh air.

In case of skin contact

In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact

After eye contact: rinse out with plenty of water. Remove contact lenses.

If swallowed

After swallowing: make victim drink water (two glasses at most). Consult doctor if feeling unwell.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Unsuitable extinguishing media

For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Nature of decomposition products not known.

Vapours are heavier than air and may spread along floors.

Forms explosive mixtures with air on intense heating.

5.3 Advice for firefighters

In the event of fire, wear self-contained breathing apparatus.

5.4 Further information

Remove container from danger zone and cool with water. Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Do not breathe vapours, aerosols. Keep away from heat and sources of ignition. Evacuate the danger area, observe emergency procedures, consult an expert.

For personal protection see section 8.



6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent material (e.g. Chemisorb®). Dispose of properly. Clean up affected area.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on protection against fire and explosion

Keep away from open flames, hot surfaces and sources of ignition. Take precautionary measures against static discharge.

Hygiene measures

Change contaminated clothing. Wash hands after working with substance. For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Tightly closed.

Storage class

Storage class (TRGS 510): 10: Combustible liquids

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

8.2 Exposure controls

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

The selected protective gloves have to satisfy the specifications of Regulation (EU) 2016/425 and the standard EN 374 derived from it.

Full contact

Material: Nitrile rubber



Minimum layer thickness: 0,11 mm
Break through time: 480 min
Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0,11 mm
Break through time: 480 min
Material tested: Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374

If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Respiratory protection

Not required; except in case of aerosol formation.

Control of environmental exposure

Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

- | | |
|---|--|
| a) Physical state | liquid |
| b) Color | dark orange |
| c) Odor | No data available |
| d) Melting point/freezing point | No data available |
| e) Initial boiling point and boiling range | No data available |
| f) Flammability (solid, gas) | No data available |
| g) Upper/lower flammability or explosive limits | No data available |
| h) Flash point | 74 °C - closed cup |
| i) Autoignition temperature | No data available |
| j) Decomposition temperature | No data available |
| k) pH | No data available |
| l) Viscosity | Viscosity, kinematic: No data available
Viscosity, dynamic: No data available |
| m) Water solubility | No data available |



- n) Partition coefficient: No data available
n-octanol/water
- o) Vapor pressure No data available
- p) Density 0,982 g/mL at 25 °C - lit.
Relative density No data available
- q) Relative vapour density No data available
density
- r) Particle No data available
characteristics
- s) Explosive properties No data available
- t) Oxidizing properties No data available

9.2 Other safety information

No data available

SECTION 10: Stability and reactivity

10.1 Reactivity

Forms explosive mixtures with air on intense heating.
A range from approx. 15 Kelvin below the flash point is to be rated as critical.

10.2 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

Strong heating.

10.5 Incompatible materials

Strong oxidizing agents

10.6 Hazardous decomposition products

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

Oral: No data available
Inhalation: No data available
Dermal: No data available

Skin corrosion/irritation

No data available

Serious eye damage/eye irritation

No data available

Respiratory or skin sensitization

No data available

Germ cell mutagenicity



No data available

Carcinogenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

11.2 Additional Information

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity

No data available

12.2 Persistence and degradability

No data available

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Endocrine disrupting properties

No data available

12.7 Other adverse effects

No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

No data available



SECTION 16: Other information

Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

The information is believed to be correct but is not exhaustive and will be used solely as a guideline, which is based on current knowledge of the chemical substance or mixture and is applicable to appropriate safety precautions for the product. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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