6 May 2010
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)

Draft

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Trigonella foenum-graecum* 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

Note: This Draft Assessment Report is published to support the release for public consultation of the draft Community herbal monograph on *Trigonella foenum-graecum* L., semen. It should be noted that this document is a working document, not yet fully edited, and which shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which the Rapporteur and the MLWP will take into consideration but no 'overview of comments received during the public consultation' will be prepared in relation to the comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7523 7051
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

Table of contents

1. Introduction

The aim of this report is to assess the non-clinical and clinical available data 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 polysaccharide (consisting mainly in 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 dietetary fibre 48.% (insoluble 28.%, soluble 20.%), and ash 3.9 % (WHO, 2003; ESCOP 2003; MURALIDHARA et al, 1999; BRUNETON 1998; 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	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only as food supplement

Member State	Regulatory Status				Comments (not mandatory field)
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only one standard marketing authorisation
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only as food supplement
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only in combinations
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Poland	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Spain	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ 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 medicinal 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 furonculosis, 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): 500mg 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 30years:
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 – 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) take before meals. Topically in skin inflammations, mix grinded seeds with water (25 g of seeds to 100 ml of water), bring to the boil 5 min. Use the obtained warm paste such a warm cataplasm 2 – 3 times daily.
3. Orally in lack of appetite: 1 – 6 g of grinded seeds before meals.
   Topically in wounds, rashes, furunculosis: mix 20 g of seeds with 100 ml of water (1/2 of glass), heat 5 min. 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 min. 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 grinded seeds (1/4 of teaspoon), 3 times daily.
   Externally in skin inflammation conditions, mix 50 g of grinded 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/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, 2003).

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 preventing effect on drug-induced anorexia / decreased motivation to eat (see Table 2).

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 3.

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 gastro-intestinal level mainly due to dietary fibers 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 hypoglycaemic effect of fenugreek seeds, at least in part, is contributed by its action on the modulation of insulin secretion.
Others author 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 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 1. 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 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 : 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 *in vitro* Stimulation of intestinal secretion *in vivo* Improvement of glucose tolerance in diabetic rats and dogs
Alkaloids	Eidi et al, 2007	Inhibition of glucose uptake *in vitro*
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 *in vitro*
	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
Tanins	Yadav et al, 2008	The highest hypoglycaemic activity observed with the water extract may be related to higher content of tanins which are water soluble and previously reported for hypoglycaemic potential
Trigonelline	Eidi et al, 2007	Inhibition of glucose uptake *in vitro*
	Shani et al,	Hypoglycaemic betain

Compound	Ref.	Claimed mechanism of action or effect
	1974[a,b]	
Tryptophan	Eidi et al, 2007	Antidiabetic and hypoglycaemic effect

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

Hypolipidaemic effect

The data are summarized in Table 3.

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 an hydro-ethanolic extract. No significant change was reported for levels of HDL-cholesterol. In diabetic rats, 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, hypocholesterolaemic effect was reported for the defatted fraction of fenugreek seeds. Further work in diabetic dogs showed 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 hypolipidaemic effect in this experimental model, whereas it did not induce 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).

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 of for 7 days. The effective dose amounted to 1000 mg/kg/day. Further work performed by Parvizpur et al (2006) showed a lack of inhibitory effect on COX enzyme. Ahmadiani et al (2001) also reported anti-pyretic effect in hyperthermic rats (injected brewer's yeast) for the same extract administered at 1000 mg/kg by both oral and ip 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 an 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 that may be available in humans for effects 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 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 platelet in a concentration-dependent way, that is related to some antagonistic effect on ADP.

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 platelet 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).

Table 2: 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 preventing 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 3: 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)	Intraperitroneal	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)	Intraperitroneal	5 days	15 mg/kg/day	Hypoglycaemic effect in diabetic mice
Vijayakumar et al, 2005	Seed	Aqueous extract	Normal	Intraperitroneal	Single dose	15 mg/kg	Hypoglycaemic effect in normal mice
Vijayakumar and Bhat, 2008	Seed	Aqueous extract	Normal	Intraperitroneal	Single dose	15 mg/kg	Hypoglycaemic effect in normal mice
Vijayakumar and Bhat, 2008	Seed	Aqueous extract	Diabetic (STZ)	Intraperitroneal	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	Hypoglycaemic effect in normal mice
Zia et al, 2001	Seed	Methanol extract	Normal	Oral	Single dose	1000	Hypoglycaemic effect in normal mice

Studies performed in rats							
Jelodar et al, 2005	Leaf	Powder	Diabetic (AXN)	Oral (diet)	15 days	*>12.5% BW in food*	No effect of treatment on 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 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, but not in normal rats 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 *(assessor's hypothesis)*	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 dietetary fibers of the preparation Hypocholesterolaemic 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 - dietetary 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 fibers (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 fibers (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 fibers (traces of gum, 4.0% hemicelluloses, 2.1% cellulose, 0.6% lignin), 7.2% steroid saponins

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
			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)	No effect reported for both extracts

Ref.	Part	Formulation	System	Test system (species, route, dose, duration, parameters,…)	Noteworthy findings
				• Respiratory movements recorded by using a sphymograph fitted around the chest of animals and connected with a tambour	
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)

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.

Assessor's comment
Studies performed by Abdel-Barry and Al-Hakiem (2000) suggest a low acute toxic potential by oral route (LD_{50} = 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.

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 correlated with any hepatic finding at histopathological examination. Chronic interstitial pneumonitis was observed at similar incidence in all groups including controls (≈70-85%). This is described to be due to murine respiratory mycoplasmosis, whose 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).

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 seeds 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 antifertility 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 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 antifertility effects based on the lack of difference in the number of litter size when treated males were mated with untreated females.
In female rabbits treated the same way as their male counterparts, prebreeding 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 foetuses obtained after mating of treated males and females is reported as abnormal, due to marked decreases in "fetal + placental" weight (-80% on 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/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 6^{th} or 10^{th} 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.

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 embryolethality 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-fetal 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-fetal toxicity. Indeed, the number of animals and that of 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-fetal 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-fetal toxicity studies in 2 species should be performed to clarify this point. No information is available regarding potential effects on pre-post-natal development.

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

3.3.6. 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.

Assessor's comment
Results from 3 experiments in rodents showed that an 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.

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	• LD50=650 mg/kg • CNS effects Mild CNS stimulation at low and intermediate doses Tachypnea, twitches,

Ref.	Part	Formulation	Species	Route, dose	Parameters	Noteworthy findings
					large intestine	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
Abdel-Barry and Al-Hakiem, 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

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 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)

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 min at 250°C then let cool down at room's temperature		Gene mutation in bacteria	*Salmonella typhimurium* 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	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 *Escherichia coli* strain WP2*uvr*A	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

Ref.	Part Formulation		Type of test	Test system	Concentration metabolising system	Results
						reduction in relative total growth Comment: According to OECD guideline no.476**, RTG should range from 10 to 20% if the maximum concentration is based on cytotoxicity. In this experiment, RTG reached 19.4% at 4000 µg/mL without S9, and 29.1% at 5000 µg/mL with S9. Therefore, the level of cytotoxicity is acceptable. It is also noted that the maximal concentrations used are in line with the OECD guideline no.476 (5 mg/mL for relatively non-cytotxic compounds)
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.

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	Organ weight ↓ weight of epididymis, ventral prostate, seminal vesicles Sperm parameters ↓ motility ↓ density in cauda epididymis and testis Fertility

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
					(serum and reproductive tissues) Sperm parameters (count, motility) Organ weight: liver, heart, kidney, adrenal, reproductive tissues Histopathology: testis, epididymides, vas deferens, seminal vesicles	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> ↑ 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	Body weight Hormonal assessment: determination of plasma progesterone, estrogen and	**<u>Parental Animals</u>** <u>Hormone assessment</u> ↓ testosterone (-66%) ↓ estrogen (-18%) ↓ progesterone

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
				Oral route 0, 30 % in diet	testosterone Mating parameters Implantations, corporea lutea, resorptions Fetal weight, litter size, newborn weight Sperm count Histopathology: ovaries, uterus, testes	(prebreeding, -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 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-fetal development</u>** ↓ fetal + placenta weight on GD20 (-80%) **<u>Newborns</u>** ↓ litter size (-75%) ↑ newborn weight

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
						(+26%)
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 fetuses	↑ 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 antifertility activity	60-66% antifertility 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>Fetuses</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 ↓ fetal body weight and fetal crown-rump length (-41% and -22% compared to controls) Various gross anomalies including notably inverted/everted claw (18% and 21% vs 0% and 0% in

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
						controls), 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>Fetuses</u> Body weight	None

[a] containing 0.6% total steroidal sapogenin; [b] assuming a body weight value of 225 g as mentioned in the article

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	Body weight Serum T3 and T4 levels Hepatic biochemistry: protein, hepatic lipid	↑ body weight Thyroid hormones: ↓ T3, ↑ T4, ↓ T3/T4 ratio

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
				mg/kg/day	peroxidation, superoxide dismutase (SOD)and catalase (CAT) activities	↓ 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

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 an 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. 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 however 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 an 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, this extract is not characterized 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 embryolethality 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-fetal 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-fetal toxicity studies in 2 species should be performed to clarify this point.
No information is available regarding potential effects on pre-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 18 years of age.
- Embryolethal effects could be reported in the monograph. Regarding embryo-fetal 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/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-fetal and peri-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 hypocholestaerolemic 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, t1/2 = 0.9 hour, t1/2 = 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. 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 sociodemographic 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 portions 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 (m^2) The World Health Organization (WHO) definitions were used for underweight (BMI < 18.5 kg/m^2), normal weight (18.5 ≤ BMI < 25 kg/m^2), overweight (25 ≤ BMI < 30 kg/m^2) and obesity (BMI ≥ 30 kg/m^2).
- Sociodemographic 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

Sociodemographic 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

Sociodemographic characteristics of the study sample (n = 249 women)		
Primary school	47	18.9
Secondary school	47	18.9

The mean BMI was 29.6 kg/m^2 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 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 appetite enhancer.

To complete the data above, there are 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 an 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 1
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 1^{-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 1^{-1})			
-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 1^{-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 1^{-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 1^{-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 Dionil, 4.72 mg Glibenclamide daily. Eight patients took Glycophage, 1.06 mg of Metform daily. A combination of both drugs i.e. Dionil + Glycophage 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 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 subject 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 hr, 1 hr and 2 hr during GTT. Although fasting levels of insulin remained unchanged, mean insulin area was reduced significantly ($p<0.05$).

Table1
Blood glucose and insulin levels and the area under the curve for diabetic subjects before and after administration of fenugreek seed powder.

Time (hr)	**Blood glucose (mmol/L)**		**Serum insulin (Mu/L)**	
	Initial	24th week	Initial	24th week
0 hr	8.4±0.3@	6.2±0.3**	16.2±8.9	17.3±1.3
1/2 hr	11.9±0.4	6.9±0.9**	31.2±5.2	22.9±2.1*
1 hr	13.6±0.5	10.9±0.6**	40.3±3.9	33.4±2.8*
1.5 hr	14.6±0.5	10.7±0.6**	-	
2 hr	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 hr 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 2). **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 2)

Table 2
Urinary sugar and glycosylated haemoglobin levels in diabetic subject before and after administration of fenugreek seed powder

Weeks	Urinary (mmol/24 hr)	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 hr 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 3).

Table 3
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 (%)	24th 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 badly controlled for their diabetes before the study entry, appear to have benefit from standard treatments after inclusion. It is unclear whether both the experimental and control groups have received superimposable therapeutic, biological and clinical monitorings for enabling 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**.**

The effect of fenugreek then more relies 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 seeds 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 13 other 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 regards 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 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 an 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).

One publication reports one case of aplastic anaemia in one 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, 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, to include 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**. Other adverse effects have however 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 40/41), it is considered not suitable to add a specific warning in the 4.5 section as regards this putative, poorly documented, 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 as concern the hypoglycaemic and hypolipemiant *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 hand.

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 Trigonellae foenugraeci semen in the list of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products.

Annex

List of references