EUROPEAN MEDICINES AGENCY
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Committee on Herbal Medicinal Products (HMPC)

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

Draft – Revision 1

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)		*Trigonella foenum-graecum* L., semen
Herbal preparation(s)		a) Comminuted herbal substance b) Powdered herbal substance c) Dry extract (DER 4:1), extraction solvent: ethanol 20% V/V d) Soft extract (DER 5-6:1), extraction solvent: ethanol 60% V/V
Pharmaceutical form(s)		Herbal substance or comminuted herbal substance as herbal tea for oral use. Herbal preparations in solid dosage forms for oral use. Herbal substance or powdered herbal substance for infusion for cutaneous use. The pharmaceutical form should be described by the European Pharmacopoeia full standard term.
First assessment	Rapporteur	A. Sawaya
	Peer-reviewer	P. Claeson
Revision	Rapporteur	C. Cavaleiro / A. Núñez / O. Palomino
	Peer-reviewer	E. Svedlund

Note: This draft assessment report is published to support the public consultation of the draft European Union herbal monograph on *Trigonella foenum-graecum* L., semen. It is a working document, not yet edited, and which shall be further developed after the release for consultation of the monograph.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on 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.

Table of contents

1. Introduction

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

- Herbal substance(s)

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). The nutritional 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 ,1999; 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).

- Herbal preparation(s)

Fenugreek seed is commonly used as herbal substance or grounded herbal substance. Additionally, some extracts are produced from the herbal substance by suitable procedures using ethanol 20% (v/v) or 60% (v/v).

- Combination(s)

A combination containing *Foenugraeci semen* is marketed in Poland since 1961: it consists on a paste for preparation of oral solution, containing, per 100g of product, 67.2g of combination herbal extract DER (1:1.3-1.6), extraction solvent ethanol 45% V/V of *Agropyri rhizoma* 12.5 parts, *Allii cepae squama* 5.0 parts, *Betulae folium* 10.0 parts, *Foenugraeci semen* 15.0 parts, *Petroselini radix* 17.5 parts, *Solidaginis herba* 5.0 parts, *Equiseti herba* 15.0 parts, *Levistici radix* 10.0 parts, *Polygoni avicularis herba* 15.0 parts. This combination will be not considered in current assessment report.

1.2. Search and assessment methodology

This Assessment Report resulted from a revision of that previously issued (EMA/HMPC/146220/2010) considering the decision of HMPC (July 24th 2018) on the need of revision following relevant updates from data published in the literature between 2011 and 2018.

Search web engines used: Google, Google Scholar

Scientific databases: Web of Science; PubMed; Science Direct; Clinical Key; Cochrane Database of Systematic Reviews

Medical or Toxicological databases: Toxline

Search terms: "Trigonella" or "fenugreek" (2011-2020)

Pharmacovigilance resources: Data from EU and non-EU regulatory authorities, European database for suspected adverse drug reaction reports.

Data from EU and non-EU regulatory authorities: Assessment Report On *Trigonella foenum-graecum* L. EMA/HMPC/146220/2010

No data was provided by the interested parties.

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Table 1 abridges information provided by the National Competent Authorities on medicinal products on the market containing Trigonellae foenugraeci semen or its preparations as single active substance. In Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, Ireland, Latvia, Lithuania, Malta, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, The Nederland's and United Kingdom there are no authorised or registered medicinal products on market.

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form	Regulatory Status
Trigonella foenum – graecum L., semen	a) internal use: loss of appetite b) external use: symptomatic treatment of localized inflammations	a) 2 g comminuted herbal substance is taken with liquid 3 times daily before meals b) as an decoction preparation for cutaneous use: 50 g powdered herbal substance is cooked with 250 ml water for 5 minutes and used as a cataplasm 1 time daily	Standard Marketing Authorisation, WEU (1996, DE)
Trigonella foenum – graecum L., semen	a) Internal use: covering agent in intestine (in different intestinal irritations) b) Internal use: loss of appetite	Decoction for oral use: 1 spoon (8 g) in a glass of water (200-250ml), boiled 15 min. 2 – 3 times a day 1-2 teaspoons (3-6g) before meals 1-6g daily 1.6g 3 times daily	Listed for use in pharmacies at least since 1962. Certified in 1994, 2000, 2005. TUR, 2008 (Poland).

Active substance	Indication	Pharmaceutical form	Regulatory Status
	c) External use: inflammation (rushes, furunculosis) as a healing aid.	Decoction (50g of seeds boiled for 5 min in 250 ml of water) for cutaneous use on a form of cataplasm (warm impregnated dressing). Applied on the affected skin, 2 – 3 times a day.	
Trigonella foenum – graecum L., semen	a) Internal use: appetite promotion	*Adults and the elderly:* Infusion for oral use: 1 tea spoon (2 g) in a glass of water (250ml), keep covered for 15 minutes. Drink the freshly prepared infusion, up to 3 times a day, before meals as an appetite stimulant.	Since 1992 (Poland)
	b) External use: inflammation (rushes, furunculosis) as a healing aid.	Decoction (50g of seeds boiled for 5 min in 250 ml of water) for cutaneous use on a form of cataplasm (warm impregnated dressing). Applied on the affected skin, 2 – 3 times a day.	
Trigonella foenum – graecum L., semen	a) Traditional herbal medicinal product used for temporary loss of appetite.	Herbal substance as a tea preparation: 1 to 6 g daily in divided doses.	1990, France Not in the market now, But included in the French Agency Instructions for Herbal Medicinal Products (Avis aux fabricants), 1986
	b) Traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the	Herbal substance as an infusion (50 g/250 ml of water) for cutaneous use: the still warm infusion is used in	

Active substance	Indication	Pharmaceutical form	Regulatory Status
	skin.	cataplasm.	
Herbal substance	Minor local skin inflammations	External use: up to 50 g a day for cutaneous use as an infusion	At least since 1992, Spain
Herbal substance	Used in loss of appetite	Oral use Up to 3 times a day (6 g day).	At least since 1992, Spain
Dry extract DER (4:1) extraction solvent: ethanol 20% v/v	Traditional herbal medicinal product used for temporary loss of appetite	295 mg, 2 times daily	1970, France 2003- 2016, France
Dry extract DER (4:1) extraction solvent: ethanol 40% v/v	Traditional herbal medicinal product used for temporary loss of appetite.	295 mg, 2 times daily	2016, France
Soft extract DER (5-6:1) extraction solvent: ethanol 60% v/v	Traditional herbal medicinal product used for temporary loss of appetite.	500 mg, 2 times daily	1970, France 2003-2016, France
Soft extract DER (3.3:1) extraction solvent: ethanol 60% v/v	Traditional herbal medicinal product used for temporary loss of appetite.	500 mg, 2 times daily	2016, France
Powder	Help weight gain	495 mg 3 to 5 times daily	Marketed in France 1990-2011
Powder	Used in loss of appetite	oral use 1100 mg 3 times a day.	1990, Spain
Powder	Used in loss of appetite	oral use 380 to 760 mg 3 times a day.	1992 – 2017 (since 2017 it is marketed as a Food supplement), Spain

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable

Information on other products marketed in the EU/EEA (where relevant)

Trigonella foenum–graecum L., semen is currently (2017) marketed in Poland as food supplement to be prepared as an herbal tea *"for securing digestive comfort", "help in controlling cholesterol and blood glucose level", "strengthen immunological system"* and *"valuable antioxidant".*

The marketing authorisation in 1992 in Spain was switched to a food supplement in 2017 and is still on the market *to promote appetite*.

This overview is not exhaustive.

2.1.2. Information on products on the market outside the EU/EEA

Not applicable

2.2. Information on documented medicinal use and historical data from literature

Fenugreek (*Trigonella foenum-graecum* L., Fabaceae) originates in India and Northern Africa. The medicinally used plant part of fenugreek is the seed.

An annual plant, fenugreek height is 20-60cm. 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.

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

The medicinal use of fenugreek seed to stimulate the appetite is documented at least since 1908 in the Indian Materia Medica: the powdered seeds are used to stimulate the appetite with the daily dosage of two tea-spoonfuls. The same indication is included in Paris and Moyse (1967), with no posology.

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.

According to the ESCOP monograph, available dosage recommendations for the powdered drug 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.

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; herbal tea: 0.5 g of cut seed macerated in 150 ml cold water for 3 hours, several cups.

Also the Commission E monograph (Blumenthal, 1998) includes the use of fenugreek preparations for the lack of appetite:

- cut/crushed seed with a daily dose of 6g
- macerate, prepared with 0.5g of herbal substance in 150ml of cold water for 3h and then, filtered. To be drunk several times daily

The use of the macerate, with the same indication and posology is also included in other text books such as the Wichtl "Herbal Drugs and Phytopharmaceuticals" (1994) and the PDR for Herbal Medicines (20064).

Table 2 includes a summary of the historical data on the use of fenugreek.

Table 2: Overview of historical data

Herbal preparation	**Documented Use / Traditional Use**	**Pharmaceutical form Strength (where relevant) Posology Duration of use**	**Reference**
Oral use			
Powder Liquid extract	Stimulate the appetite	0.25-0.50g powder 0.5-2g liquid extract (no complete data on posology, duration of use or liquid extract preparation)	Paris and Moyse, 1967.
Cut/crushed seed	Stimulate the appetite	6g per day	Blumenthal, 1990
Cut seed		Macerate: 0.5g cut seed macerated in 150ml cold water for 3h; strain. Drink several cups daily	
Cut seed	Stimulate the appetite	Macerate: 0.5g cut seed macerated in 150ml cold water for 3h; strain. Drink several cups daily	Blumenthal, 1990
Cut/crushed seed	Stimulate the appetite	6g per day	PDR, 2006
Cut/crushed seed	Stimulate the appetite	6g per day	Blumenthal, 1990
Cut seed		Macerate: 0.5g cut seed macerated in 150ml cold water for 3h; strain. Drink several cups daily	

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength (where relevant) Posology Duration of use	Reference
Oral use			
Seed	Stimulate the appetite	1-6g or equivalent, three times daily	Barnes et al., 2007
Powdered seeds	Adjuvant in diabetes or hypercholesterolemia	25g per day	Raghuram et al., 1994 Sharma et al., 1986, 1996a, 1996b
Powdered seeds	Lack of appetite	1-6g or equivalent, three times daily	Al-Habori et al., 1998
External use			
Powdered drug	Local inflammation	50g boiled for 5min with ¼ litre water and applied as a moist warm poultice	Blumenthal, 1990 Hagers Handbook, 1994 Wichtl 2002 PDR, 2006
Seeds	Emollient and vulnerary	No data	Barnes et al., 2007

2.3. Overall conclusions on medicinal use

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	**Indication**	**Strength Posology**	**Period of medicinal use**
Dry extract (solvent: ethanol 20% v/v, DER 4:1)	Traditional herbal medicinal product used for temporary loss of appetite	295 mg 2 times daily	Marketed in France 1970-2016
Soft extract (solvent: ethanol 60% v/v, DER 5-6:1)	Traditional herbal medicinal product used for temporary loss of appetite	500 mg 2 times daily	Market in France 1970-2016
Herbal substance	Loss of appetite	Cut/crushed seed: 1-2g with liquid 3 times daily, before meals	Poland, at least since 1962 France, 1990 Spain, 1992 Germany, 1996 Hagers Handbook, 1994 Wichtl 2002
		Herbal substance as a tea preparation: 1 to 6 g daily in divided doses	France, 1990 Poland, 1992 included in the French Agency Instructions for Herbal Medicinal Products (Avis aux fabricants), 1986
		Macerate: 0.5g cut seed macerated in 150ml cold water for 3h; strain. Drink several cups daily	Blumenthal, 1990 Wichtl, 1994 WHO, vol 3, 2007
Herbal substance and powdered herbal substance	In skin inflammations, as emollient, coating and for skin healing	External use: 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	Poland, at least since 1962 France, 1990 Spain, 1992 Germany, 1996

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
			Blumenthal, 1990 Wichtl, 1994 ESCOP, 2nd ed. PDR, 2006
Powder	Loss of appetite	495mg, 3-5 times daily 1100mg 3 times a day 380-760mg 3 times a day	France, 1990 Spain, 1990 Spain, 1992

Historical data and documented period of use in the EU support the evidences of traditional use of:

- For oral administration in cases of temporary loss of appetite: the herbal substance or comminuted herbal substance: 2g with liquid 3 times daily, before meals; herbal substance as a tea preparation, 1 to 6 g daily before meals; herbal substance as a macerate: 0.5g cut seed macerated in 150ml cold water for 3h; strain and drink several cups daily; the soft extract (solvent: ethanol 60% v/v, DER 5-6:1), 500 mg, 2 times daily, the dry extract (solvent: ethanol 20% v/v, DER 4:1), 295 mg, 2 times daily; and the powder: 380-1100mg, three times daily,
- For cutaneous use for the symptomatic treatment of minor inflammations of the skin: the herbal substance as an infusion or decoction (50 g/250 ml of water), 2-3 times daily.

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

Table 4: Overview of the main non-clinical data/conclusions

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Comparable/similar preparations to preparations of the monograph				
Hydro-ethanolic extract, containing 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	10 and 100 mg/day/300 g bw Up to 14 days Oral route (diet)	In vivo (Rat) <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)	Petit et al, 1993	↑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
Trigonella foeno-graecum seeds´ dry extract, ethanol 70% (no details on D.E.R.)	Cutaneous application of dry hydroalcoholic extract as ointments at 5 or 10%.	In vivo albino rat: excision and incision wound models. 14 days	Muralidharan et al. 2016	Significant increase in cell proliferative activity and wound healing for the 10% preparation
Other preparations or substances				
Trigonella foeno-graecum seeds methanol (MeOH)		In vitro cultured cell	Kawabata et al, 2011	Supression of pro-inflammatory citokinines

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
extract and Isolated steroidal saponins	Isolated steroidal saponin glycosides: 0.1-100μM	lines (murine melanoma B16F1 cells)		Restrain of the intracellular synthesis of melanin
Diosgenin	Oral route: Low protein diet containing 0 (control), 0.05, 0.1 or 0.2% (w/w) of diosgenin. 8 weeks	In vivo Male hairless mice (Hos:HR-1) – low collagen skin mouse model	Haratake et al. 2017	Diosgenin led to a dose-dependent improvement in skin collagen content by shifting the dynamics of the fibroblasts.

Several non-clinical pharmacology studies have been performed with comparable preparations to the ones in the monograph or with isolated components for both oral and cutaneous use. Results are in line with the traditional uses, reporting effects that can be related to the increase of appetite dose-independent (Petit et al. 1993) and effects on mechanisms of skin inflammation and skin healing which are dose- or concentration dependent (Muralidharan et al. 2016, Haratake et al. 2017 and Kawabata et al., 2011).

3.1.2. Secondary pharmacodynamics

Hypoglycaemic effect

Most of the data found in the literature were performed in view of the use of fenugreek seeds in diabetes mellitus. Data are summarized in Table 5.

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 mice and 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 shown to be 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 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 an 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, acts 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). Mohammadi et al. (2016) reported positive effects on insulin resistance though a insulin-like effect and increase in adiponectin levels and PPARγ protein expression.

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. Naicker et al. (2016) demonstrated that antihyperglycemic effects were similar to via enhanced insulin signalling, gene expression, and increasing glucose uptake.

In addition, the compound(s) responsible for the hypoglycaemic effect is (are) not clearly identified. The main results found in the literature are summarized in table 5. Zia et al, (2001) concluded that the substance responsible for hypoglycaemic activity is probably polar in nature. Ribes et al, (1984, 1986, 1987) showed that the hypoglycaemic effect of fenugreek seeds in diabetic dogs 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).

Other authors (Korthikunta et al., 2015; Gautam et al., 2016) reported the enhanced insulin-stimulated glucose transport induced by 4-hydroxyisoleucine. Chou et al. (2017) reported the enhanced response of physiological levels of Glucagon-like peptide-1 by an unrevealed compound (N55) isolated from *Trigonella foenum-graecum* seeds.

Table 5: Overview of the main studies on hypoglycaemic effects

Herbal preparation tested	**Posology**	**Experimental model**	**Reference**	**Main non-clinical conclusions**
Comparable/similar preparations to preparations of the monograph				

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Aqueous extract from Seeds	Single dose, 1-5 mg/kg Intraperitroneal	*In vivo* Diabetic mice (AXN)	Vijayakumar et al., 2005	Hypoglycaemic effect in diabetic mice comparable to that of 1.5 U/kg insulin (at 15 mg/kg)
Aqueous extract from seeds	15 mg/kg/day Intraperitroneal 5 days	In vivo Diabetic mice (AXN)	Vijayakumar and Bhat 2008	Hypoglycaemic effect in diabetic mice
Aqueous extract from Seeds	Single dose, 15 mg/kg Intraperitroneal	In vivo Normal mice	Vijayakumar et al., 2005	Hypoglycaemic effect in normal mice
Aqueous extract from Seeds	Single dose, 15 mg/kg Intraperitroneal	In vivo Normal mice	Vijayakumar and Bhat, 2008	Hypoglycaemic effect in normal mice
Aqueous extract from Seeds	Single dose, 15 mg/kg Intraperitroneal	In vivo Diabetic	Vijayakumar and Bhat, 2008	Hypoglycaemic effect in diabetic mice comparable to that of 1.5 U/kg insulin; enhanced hepatic metabolism of glucose
Decoction Ethanol extract from seeds	Single dose Decoction: 0.5 ml; Extract: 200 mg/kg, oral administration	In vivo Normal and diabetic (AXN)	Ajabnoor and Tilmisany, 1988	Hypoglycaemic effect in normal and diabetic mice
Aqueous extract from Seeds	Single dose, 500 mg/kg oral administration	In vivo Normal mice	Zia et al, 2001	Hypoglycaemic effect in normal mice
Aqueous extract from seeds	Single dose, 50 mg/kg	In vivo Normal rat	Yadav et al, 2008	Hypoglycaemic effect in normal rats
Aqueous extract from seeds	440 mg/kg/day (gavage) 6 weeks	In vivo Diabetic rat (STZ)	Xue et al, 2007	Hypoglycaemic effect in diabetic rats

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Ethanol extract from seeds	2000 mg/kg/day Oral (gavage) 21 days	In vivo Diabetic rat (AXN)	Vats et al, 2002	Hypoglycaemic effect in diabetic rats
Ethanol extract from seeds	Single dose,1000 mg/kg/day Oral (gavage)	In vivo Normal rat	Vats et al, 2002	Hypoglycaemic effect in normal rats Lack of effect after oral glucose load in normal rats (suggests no effect on glucose absorption from the GI tract)
Ethanol (80%) extract from seeds	250 mg/kg/day, Oral (gavage) 14 days	In vivo Normal and diabetic (STZ)	Eidi et al, 2007	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
Powdered seeds	12.5 g/kg/day (5% in diet) Oral (diet) 21 days	In vivo Diabetic rat (AXN)	Raju et al, 2001	Hypoglycaemic effect in diabetic rats; modulation of key glucose metabolising enzymes
Powdered seeds	2000 mg/kg/day Oral (diet) 1 and 2 weeks	Normal and diabetic rats (AXN)	Khosla et al, 1995	Hypoglycaemic effect in normal and diabetic rats
Powdered of defatted seeds	1250 mg/kg/day 9 days	Normal and diabetic rats (STZ)	Mondal et al, 2004	Hypoglycaemic effect in diabetic rats
Powdered of defatted seeds. Preparation containing 3.9%	1860 mg/kg/day Oral (diet) 8 days	Normal and diabetic dogs (AXN)	Ribes et al, 1984 Valette et al, 1984	Hypoglycaemic effect in normal and diabetic dogs – attributed in part to the high percentage

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
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				of dietetary fibers of the preparation.
Dry extract, (D.E.R. not given) solvent water	1000 mg/kg/day intragastric Injection 4 weeks	In vivo, Wistar rats insulin resistant 60% fructose diet	Mohammadi et al., 2016	Positive effects on insulin resistance though a insulin-like effect, increase in adiponectin levels and PPARγ protein expression.
Aqueous extract (details not accessed)	100 ng/mL	In vitro HepG2 cells	Naicker et al. 2016	Antihyperglycemic effects similar to via enhanced insulin signalling, gene expression, and increasing glucose uptake.
Other preparations				
Methanol extract from seeds	1000 mg/kg Single dose, oral administration	In vivo Normal mice	Zia et al, 2001	Hypoglycaemic effect in normal mice
Aqueous, ethanol, methanol, hexane and chloroform extracts from seeds	200 mg/kg Single dose Oral	In vivo normal rats	Yadav et al, 2008	Hypoglycaemic effect reported for aqueous ethanol and methanol extracts in normal rats
Defatted fractions from seeds. testa + endosperm: preparation containing 10.0% moisture, 3.0%	1145 mg/kg Oral (diet) 21 days	Diabetic dogs (AXN)	Ribes et al, 1986 Ribes et al, 1987	Hypoglycaemic effect in diabetic dogs - dietetary fibers may play a role.

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
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				
Lipid extract from seeds	>105 mg/kg/day Oral (diet) 8 days	In-vivo Normal dogs	Ribes et al, 1984 Valette et al, 1984	None
Powdered leaf	>12.5% BW in food 15 days	In vivo Diabetic rat (AXN)	Jelodar et al, 2005	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)
Powdered Leaf	500 mg/kg/day in food 45 days	In vivo Diabetic rat (STZ)	Devi et al, 2003	Hypoglycaemic effect in diabetic rats + stimulation of insulin secretion
Isolated compounds				
4-hydroxyisoleucine (isolated from fenugreek seeds) Synthetic 4-hydroxyisoleucine derivatives.	10µM	In vitro L6 rat skeletal muscle cells expressing ratGLUT4	Korthikunta et al., 2015	Glucose uptake activity 4-hydroxyisoleucine (10µM) enhanced the glucose uptake by 38.67% Some synthetic derivatives are more active.
4-Hydroxyisoleucine (4-HIL) isolated	10 µM for 24 h	In vitro C2C12 myoblasts	Gautam et al., 2016	4-hydroxyisoleucine enhanced insulin-stimulated glucose

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
from the seeds of *Trigonella foenum-graecum*		cultures		transport rate in a dose-dependent manner.
N55- Compound isolated from *Trigonella foenum-graecum*	Intraperitoneal injection (dose not given)	In vivo Fasted ans non-fasted mice Monitoring insulin and GLP-1 plasma levels	Chou et al 2017	N55 lowers plasma glucose according to prandial status by enhancing the response of physiological levels of GLP-1

Table 6. 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 Korthikunta et al., 2015 Gautam et al., 2016	Insulinotropic property *in vitro* Stimulation of intestinal secretion *in vivo* Improvement of glucose tolerance in diabetic rats and dogs Enhancement of glucose uptake and insulin-stimulated glucose transport rate
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
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,	Inhibition of glucose uptake *in vitro*

Compound	Ref.	Claimed mechanism of action or effect
	2007	
	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

Hypolipidaemic effect

Investigations were conducted on the ability of fenugreek seed to lower blood lipids levels. The data are summarized in Table 7.

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) and Chaturvedi et al, (2013). Belguith-Hadriche et al. (2013) reported the same effect regarding an ethyl acetate fraction. Kumar et al. (2014) reported the reduction of fat accumulation and of dyslipidemia in Wistar rats following 28 days of administration of water extract (0.5 and 1.0 g/kg by day)

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).

Moriwaki et al. (2014) suggested that the hypolipidemic effect of fenugreek can be mediated by yamogenin since this saponine suppress the triglycerides accumulation and mRNA expression of fatty acid synthesis related genes in hepatocytes.

Table 7: Overview of the main studies on hypolipidemic effects

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Comparable/similar preparations to preparations of the monograph				
Hydro-ethanolic extract, containing 12.5% steroid saponins, 4.8% free amino acids, 0.002% 3-hydroxy-4,5-	10 and 100 mg/day/300 g bw Up to 14 days	In vivo (Rat) Metabolic studies (blood glucose, plasma insulin, plasma glucagon, triglycerides and total + free	Petit et al, 1993	Reduction of plasma total cholesterol, HDL free cholesterol and VLDL-LDL total cholesterol

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
dimethyl-2(5H)-furanone (HDMF) – no protein and lipids. Obtained from Monal Laboratories, Palaiseau, France	Oral route (diet)	cholesterol levels)		
Aqueous extract from seeds	440 mg/kg/day (gavage) 6 weeks	In vivo Diabetic rat (STZ)	Xue et al, 2007	Hypolipidaemic effects in diabetic rats with favourable impact on HDL-cholesterol
Powdered of defatted seeds. 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	1860 mg/kg/day Oral (diet) 8 days	Normal and diabetic dogs (AXN)	Ribes et al, 1984 Valette et al, 1984	Hypocholesterolaemic effect in normal and AXN-induced hypercholesterolaemic dogs
Dry extract (D.E.R. not given) solvent : water	0.5 and 1.0 g/kg (b.w.), oral	In vivo Wistar rats under fat diet 28 days	Kumar et al, 2014	Reduction of fat accumulation and dyslipidemia.
Dry extract (D.E.R. 66:1) ethanol absolute.	50, 100, and 200 mg/kg Bw, during 7 day 30 days	In vivo Charles Foster rats, triton or high-fat diet–induced hyperlipidemia	Chaturvedi et al, 2013	Dose of 200 mg/kg/day lowered cholesterol (26.19%) and triglycerides (36.6%)
Other preparations				

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Water, methanol, hexane, dichloro-methane and ethyl-acetate (sequentially) extracts. No further details given.	2.5% of seeds in diet Or 0.125% of extract 16 weeks	In vivo Wistar rats, diet supplemented with 1% cholesterol and 0.1% cholic acid.	Belguith-Hadriche et al. 2013	ethyl acetate extract decreased serum total cholesterol, triglycerides and LDL- cholesterol, increasing HDL-cholesterol.
Defatted fractions from seeds. 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	>1126 mg/kg/day (glycaemia) 1126 mg/kg/day (lipids) Oral (diet) 21 days	Diabetic dogs (AXN)	Ribes et al, 1986 Ribes et al, 1987	Hypolipidaemic effect (decreased cholesterol and/or triglycerides).
Defatted fractions from seeds. 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	1145 mg/kg Oral (diet) 21 days	Diabetic dogs (AXN)	Ribes et al, 1986 Ribes et al, 1987	Hypolipidaemic effect (decreased cholesterol and/or triglycerides); saponins may play a role but not amino acids

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Isolated compounds				
Yamogenin, isolated from fenugreek seeds	5-10 µM	In vitro Mouse hepatocytes	Moriwaki et al., 2014	Yamogenin suppressed the triglycerides accumulation and mRNA expression of fatty acid synthesis- related genes in hepatocytes, suggesting that contributes for the hypolipidemic effect

Effects of fenugreek on conditions associated to diabetes:

- Jin et al. (2014) described the reduction of symptoms of diabetic nephropathy in Sprague-Dawley diabetic rats when treated with a dry water extract (DER not given) from fenugreek seeds.
- Haghani et al. (2016) assessed the hypoglycaemic effect of the dry extract, (DER 5:1), solvent water, in diabetic male Wistar rats when administered orally (1.74g/kg bw) during 6 weeks. Results showed a beneficial effect by reducing the cardiovascular complications of type 1 diabetes.
- Joshi et al. (2015) showed the beneficial effect of an hydroalcoholic extract from fenugreek seeds (quantified to 53.89% of 4-hydroxyisoleucine (other relevant details are not given) on oxidative stress and pro-inflammatory cytokines in the improvement of exocrine function of diabetes induced by alloxan in diabetic and normal Sprague–Dawley rats.

Anti-Inflammatory activity

- Ahmadiani et al, (2001) showed 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.
- Parvizpur et al. (2006) - lack of inhibitory effect on COX enzyme.
- Pundarikakshudu et al. (2016) reported the reduction in inflammation of the paw in carrageenan and formaldehyde-induced paw edema by fenugreek seed soft extract, solvent petroleum ether.
- Sharma et al. (2017) reported that fenugreek seed extract provides cytoprotection to fibroblasts (LPS inflamed) via a reactive oxygen species independent pathway.
- Piao et al (2017) - *Trigonella foenum-graecum* has a significant anti-inflammatory effect on allergic asthma, reducing the collagen deposition and goblet cells, decreasing the high expression of Th2 cytokines and increasing Th1cytokines in BALF and lung homogenates.

- Ahmadiani et al. (2001) described the anti-pyretic effect in hyperthermic rats (injected brewer's yeast) for the same extract administered at 1000 mg/kg by both oral and intraperitonial routes.
- Murugesan et al. (2011) described the cardioprotective effect related to the decrease of TBARS and enhanced the activities of enzymatic and non-enzymatic antioxidants (SOD, CAT, GPx and GSH) in myocardial infarcted rats.
- Assad and Khan (2017). Methanol extract (200mg/Kg) induced sedation and skeletal muscle relaxant effects suggesting antianxiety activity on albino mice

Assessor's comment:

Several studies conducted with fenugreek seeds, different preparations from the seeds or isolated compounds 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. A lower number of studies also showed that fenugreek seeds exerted 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.

Nonetheless, several weaknesses in these secondary pharmacological studies make their interpretation difficult, mainly due to the lack of control groups, the administration of only one dose of the preparation, or doses much higher than those which could be administered for human use or the uncertainty of the real composition of the tested preparation, among others.

Effects on glycaemia, in humans, should not be neglected and the monograph on Trigonella foeno-graecum semen should include the appropriate warnings in regard potential interferences with glycaemia and interactions with treatments for diabetes mellitus.

3.1.3. Safety pharmacology

Few publications dealing with preclinical safety of fenugreek preparations were found in the scientific literature. A summary is provided in Table 8.

Table 8: 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	Water extract Slight stimulating effect on intestinal motility Ethanol extract Inhibition of intestinal motility, similar to that observed with ethanol control

Ref.	Part	Formulation	System	Test system (species, route, dose, duration, parameters)	Noteworthy findings
				lever on a smoked drum paper moving at slow speed	
			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	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
			Cardiovascular	Isolated and perfused guinea pig heart Test solution (2 ml from water or ethanol extract)	Water extract Acceleration of heart beats Ethanol extract 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 sphymograph 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

Ref.	Part	Formulation	System	Test system (species, route, dose, duration, parameters)	Noteworthy findings
					aggregation)

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. Nonetheless, this study was conducted with fenugreek leaves, which are not covered by *T. foenum-graecum* monograph (Parvizpur et al., 2006).
- The uterine stimulant properties reported on pieces of guinea pig uterus (Abdo and Al-Kafawi, 1969) 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).

3.1.4. Pharmacodynamic interactions

No data available

3.1.5. Conclusions

Pharmacological results are not contradictory to the indications of the traditional use of fenugreek seeds, particularly those related to the increase of appetite and skin healing. Furthermore, hypoglycaemic effects were shown in various animal models of diabetes following the administration of fenugreek seeds. 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 was the defatted fraction. Nonetheless, there exist several weaknesses in these secondary pharmacological studies which make their interpretation difficult, mainly due to the lack of control groups, the administration of only one dose of the preparation, or doses much higher than those which could be administered for human use or the uncertainty of the real composition of the assayed preparation, among others (see also section 4.2 and 5.5.3).

No specific safety pharmacology studies are available according to current guidelines. However, the uterine stimulant properties reported in guinea pigs with water and ethanolic extracts should be taken into consideration (see also section 3.3.5 and 5.5.5).

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

In a rabbit study by Zhao et al (2003), aimed at studying the pharmacokinetics of trigonelline determined by HPLC, after post-intragastric administration 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.

A standardized hydroalcoholic extract of fenugreek seeds (further details not given) and trigonelline were assessed regarding the potential inhibition of CYP3A4 and CYP2D6 (expressed in terms of IC_{50} values) on rat liver microsome (RLM) and cytochrome P450 isozymes using CYP450-carbon monoxide (CYP450-CO) complex assay and fluorescence assay, respectively (Ahmmed et al. 2015).

The grounded fenugreek seeds were extracted with 70% methanol by cold maceration method and then evaporated to dryness in rotary vacuum evaporator at 45°C to produce a semi-solid residue which was then lyophilized. The extract was solubilized in both ethanol and dimethyl sulphoxide (DMSO) solvent separately to make a concentration of 1 mg/ml, while trigonelline (0.1mg/ml) was solubilized in both DMSO and ethanol solvent. Ketoconazole and quinidine served as positive control.

Authors found that both the extract and trigonelline showed a concentration-dependent inhibition on microsome, significantly lower than ketoconazole. The fenugreek extract dissolved in DMSO showed the highest percentage of inhibition (24.32 ± 1.46%), while trigonelline showed the lowest inhibition (13.25 ± 0.76%) in ethanol. Thus, the interaction of the extract with pooled microsome was higher than isolated trigonelline.

The IC_{50} values obtained for the extract (102.65 ± 2.63 and 142.23 ± 2.61 µg/ml) and trigonelline (168.73 ± 4.03 and 180.90 ± 2.49 µg/ml) showed that the inhibition potential of both preparations with cytochrome isozymes was very low. The slightly superior IC_{50} values for the isolated trigonelline when compared to the extract may be due to other components present in the extract. IC_{50} values of the extract were much higher than positive controls ketoconazole and quinidine, this meaning a lower interaction.

Results from this study suggest that fenugreek extract has a low inhibitory potential with the DMEs (CYP450 enzymes), and no pharmacokinetic concern arose regarding the use of fenugreek (Ahmmed et al. 2015).

Another study conducted to assess the potential inhibitory effect of fenugreek on CYP 450 enzymes was conducted by using dextromethorphan (DEX) based on its CYP2D6- and CYP3A4-mediated metabolism to dextrorphan (DOR) and 3-methoxymorphinan (3-MM), respectively. First of all, the *in vitro* assay incubated DEX (25µM) with human liver microsomes and NADPH and tested with and without the fenugreek extract at different concentrations. For the *in vivo* study, phase I, 6 subjects received a single dose of DEX (30 mg); in phase II, after washout period, the fenugreek seeds powder was administered for 1 week and DEX was administered with its last dose. Results of the *in vitro* study showed that fenugreek extract at 50 and 100µg/ml inhibited CYP2D6 and CYP3A4 activity. *In vivo* results didn't show any significant inhibition of CYP2D6 and CYP3A4 metabolic activity. Thus, results from this publication suggested that fenugreek may not have substantial effect on the metabolic activity of CYP2D6 and CYP3A4 (Al-Jenoobi et al., 2015). Nonetheless, when comparing the concentrations tested and the doses given to the volunteers with the pharmacokinetics study conducted by Zhao et al (2003), the former doses seem to be much lower than the later ones, and thus the results given by the authors shouldn't be considered as conclusive.

In the study by Al-Jenoobi et al. (2014), rabbits received an 8-days treatment with fenugreek seed powder (300 mg/kg *p.o.*) and then, on 8th day they received a single dose of cyclosporine (30 mg/kg, p.o.) and carbamazepi2ne (40 mg/kg, p.o.). Pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$, Kel, $MRT_{0-\infty}$, $V_{z/F}$, and Cl/F) for cyclosporine and carbamazepine were measured in blood samples at several time points. Results showed no statistically significant difference between pre- and post-treated pharmacokinetic parameters in rabbits. According to the authors, the conclusion should not be extended to the frequent use of fenugreek with narrow therapeutic index drugs (CYP3A and P-glycoprotein substrates) in humans.

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 9.

Table 9: 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	*Ip* 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	LD_{50}=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
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	LD_{50}=7000 mg/kg CNS effects Mild CNS stimulation at low and intermediate doses Tachypnea, twitches, strabtail, tremors, generalized convulsions at higher doses
Deshpande et al. 2016	seed extract	Standardized fenugreek seed extract with 96.21 % water-soluble low molecular weight galactomannan containing oligosaccharides	Sprague-Dawley rats	Oral 2000 mg/kg	After 14 days: Death Weight loss Pathological changes (necropsy)	LD_{50} > 2000 mg/kg

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

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

3.3.2. Repeat dose toxicity

The available data are summarized in Table 10.

Table 10: 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	90 -95 days Oral route 0, 1, 5, 10% in	Mortality and clinical signs Body weight, food intake	None

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
			days	diet	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	
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

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
						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%)
Deshpande et al. 2016	seed extract	standardized fenugreek seed extract with 96.21 % water-soluble low molecular weight galactomannan containing oligosaccharides	Sprague-Dawley rats	Oral, 250, 500 and 1000 mg/kg, 90-days repeated dose followed by a recovery period of 28 days	Mortality Ophtalmological control Body weight Functional observations Grip strength Motor activity Urinalysis, haematological and coagulation analysis, clinical chemistry Pathological examination after	No mortality No signs of toxicity in any parameter. NOAEL: 1000mg/Kg

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
					euthanasy	

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

Assessor's comment

The effects of the sub-chronic (90 days) administration of fenugreek (153, 305 and 610 mg/kg/day) on the reproductive, cytological and biochemical toxicity were assessed in mice. The highest dose caused significant changes in the percent motility, sperm count, spermatozoa morphology, chromosomal aberrations, rate of pregnancy and pre-implantation loss. Male fertility was decreased in higher treated doses (Al-Yahya, 2013).

Three 90-day rat studies were found in the literature. The experimental protocols of Muralidhara et al, (1999) and Udayasekhara Rao P et al, (1996) 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. In the study by Deshpande et al, (2016), a standardized fenugreek seed extract with 96.21 % water-soluble low molecular weight galactomannan containing oligosaccharides was administrated in oral doses up to 1000 mg/kg.

No toxic effects were observed in the studies by Muralidhara et al, (1999) and Deshpande et al, (2016). 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 the studies by Muralidhara et al, (1999) and Udayasekhara Rao P et al, (1996), 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 reported hypoglycaemic effect of fenugreek seeds (see pharmacology).

3.3.3. Genotoxicity

The available data are summarized in Table 11.

Table 11: 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	Gene mutation in bacteria	*Salmonella typhimurium* strains TA98, YG1024 and	Concentration range not detailed but 4 different concentrations	Potent mutagenic activity with and without detected in this model mimicking

Ref.	Part Formulation		Type of test	Test system	Concentration metabolising system	Results
	temperature			YG1029	were used to establish a dose-response curve +/- S9 (chlorophene-induced rat liver)	coffee roasting The authors report that pure trigonelline is not mutagenic when not heated (Fung et al, Mutat Res, 1988)
Flamm ang 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
Flamm ang 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 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

Ref.	Part Formulation		Type of test	Test system	Concentration metabolising system	Results
						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
Deshpande et al. 2016	seed extract	standardized fenugreek seed extract with 96.21 % water-soluble low molecular weight galactomannan containing oligosaccharides		OECD Guidelines. *Salmonella typhimurium* (TA97a, TA98, TA100, TA1535 and TA102)	Doses (5000.00, 1666.67, 555.55, 185.18 and 61.72 µg/plate) Rat liver activated (+/-S9)	No mutagenic effects were evidenced

* 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

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 undefined extracts of fenugreek seeds.

Neither the mode of extraction, nor the full composition of the extracts (qualitative and quantitative) were described.

The data reported in the WHO monograph were obtained with irrelevant extracts and the number of strains used was not sufficient. The extract used in the study by Deshpande et al, (2016) does not correspond to the herbal substance or herbal preparations included in the monograph.

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.

3.3.5. Reproductive and developmental toxicity

In-vivo studies have been published since the late fifties, reporting effects related to reproductive toxicity. New data on embryo-foetal and peri- and postnatal toxicity should also be considered regarding Section 5.3. in the monograph. The available data are summarized in Table 12.

The first studies regard to the uterine stimulation by seeds preparations [Goto et al (1957), Abdo and Al-Kafawi (1969)], to the emmenagogue effects of seed extracts and of the whole plant [Casey (1960); Malhi and Trivedi (1972), Saha e*t al*., (1961)] and to the abortion (Casey, 1960).

Latter several other studies were performed exploring the reproductive and developmental toxicity and their mechanisms. 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 foetuses 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.

More recent studies confirm the reproductive and developmental toxicity:

The administration of a lyophilized extract of fenugreek seeds (DER 5:1, solvent water) to pregnant mice during the gestational period (500 and 1000mg/kg/day) produced developmental toxicity in the offspring: increase in the foetal death rate, decrease in the litter size, reduction in the foetal body weight, reduction of the brain weight. The dose of 1000mg/kg/day induced significant delay in sensorimotor and coordination functions in the prenatally treated mice and altered locomotor coordination at advanced age. Based on these studies authors concluded that fenugreek seeds extract may have deleterious toxic effects on reproductive performance, potential teratogenic effects in foetuses, growth retardation and altered neurobehavioral performance in the post-weaning (Khalki *et al*., 2010, 2012, 2013).

Ouzir et al. (2016) abridged the toxicological properties of fenugreek reported in literature (including data on seeds´ preparations, isolated compounds and combinations) and advised that reproductive, teratogenic, neurophysiological, behavioural and other effects of fenugreek seeds are to take seriously into account. As relevant for the assessment of the safety of fenugreek herbal preparations use as traditional medicinal products the following conclusions should be taken in account:

- fenugreek is a teratogen capable of producing a variety of birth defects; testicular toxicity and anti-fertility effects in laboratory in vivo models (males) are related to components that induce oxidative stress and DNA damage; anti-fertility effects on females, especially during late pregnancy, are related to saponins stimulating uterine contractility and restricting the development of embryo. It was suggested that anti-fertility effects can be due to a long term use of at least 100 mg/kg bw of animal.

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, degeneration 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 oestrogen 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 one, 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) and ex vivo data on guinea-pig uterus (Abdo and

Al-Kafawi, 1969). 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. Thus, fenugreek was classified among plants having abortifacient and emmenagogue (which induces or hastens menstrual flow) applications.

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.

Additional data have shown that fenugreek seeds extracts cause increased foetal death rate, decreased litter size, decreased foetal body weight, reduction of brain weight and altered neurobehavioral performance in the post-weaning (Khalki 2010, 2012 and 2013), together with a decrease in the rate of pregnancy, an increase in implantation loss, and a decrease in male fertility through significant changes in the percent motility, sperm count, spermatozoa morphology and chromosomal aberrations (Al-Yahya, 2013). However, there are no reproductive and development toxicity studies performed in accordance with current guidelines and further studies are needed. In conclusion, studies in animals have shown reproductive and development toxicity including male and female fertility. The use during pregnancy and lactation is not recommended. Furthermore, the use is not recommended when pregnancy is planned since male and female fertility may be affected by the treatment (see also section 5.5.5).

Table 12: 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	Organ weight ↓ weight of epididymis, ventral prostate, seminal vesicles Sperm parameters ↓ motility ↓ density in cauda epididymis and testis Fertility 100% negative results in treated animals in spite of successful matings (confirmed by vaginal plug) Tissue biochemistry Testis: ↓ protein, ↑ cholesterol, ↓glycogen, ↓fructose Seminal vesicle: ↓ protein, ↓ sialic acid, ↓fructose Epididymides: ↓ protein, ↓ sialic acid Ventral prostate: ↓ protein, ↓ sialic acid Serum biochemistry

Ref.	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
						↑ cholesterol, ↓ protein, ↓ phospholipids, ↓ triglycerides Histopathology 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	Parental Animals Hormone assessment ↓ testosterone (-66%) ↓ estrogen (-18%) ↓ progesterone (pre-breeding -14%) ↑ progesterone (GD10 and GD20, +78% and +111%) Sperm parameters ↓ sperm count (-47%) Organ weight ↓ testicular weight (-25%) Histopathology: 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	Dams Number of implantations Number of resorptions Foetuses 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	Dams Body weights, food consumption Number of implantations Number of resorptions Placenta weight Foetuses Body weight	None

Ref	Part	Formulation	Species	Duration, route, dose	Parameters	Noteworthy findings
Al-Yahya, 2013	Seed	Capsules containing 1.22 g of fenugreek seed	Mice	90 days Oral route 153, 305 and 610 mg/Kg/day	Reproductive, cytological and biochemical parameters	High doses caused significant changes in the motiliy, sperm count, spermatozoa morphology; chromosomal aberrations, rate of pregnancy and pre-implantation loss. Decreased male fertility
Khalki et al., 2010	Seed	Lyophilized aqueous extract (DER 5:1)	Pregnant mice	Gestational period Oral route 500 and 1000mg/kg/day	Foetal parameters: litter size, foetal viability, body weight and weight gain; malformations and abnormal morphological changes	Developmental toxicity: increased foetal death rate, decreased litter size, decreased foetal body weight. Delay in sensorimotor and coordination functions in prenatally treated mice and altered locomotor coordination at advanced age

Khalki et al., 2012	Seed	Lyophilized aqueous extract (DER 5:1)	Pregnant mice	Gestational period Oral route 500 and 1000mg/kg/day	Behaviour 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 was removed and cut for histological evaluation	Decreased foetal body weight, reduction of brain weight Delay in sensorimotor and coordination functions. Significant reduction in successful spontaneous alternations
Khalki et al., 2013	Seed	Lyophilized aqueous extract (DER 5:1)	Pregnant mice	Gestational period Oral route 1000mg/kg/day	Evaluation of the effects of prenatal treatment of fenugreek seeds on the development of sensorimotor functions from birth to young adults: righting reflex and cliff avoidance during the first postnatal week. geotaxis response at P10 and P12. Long-term effects (5 weeks of age):	Maternal exposure to fenugreek altered the excitability of the spinal locomotor network early during development. These alterations are likely associated with significant changes in motor performance and gait in adults. .

					Motor ability and coordination were evaluated using a Rotarod apparatus	

3.3.6. Local tolerance

No data available

3.3.7. Other special 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 13.

Table 13: Studies 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. The results of these studies can't substantiate the impact of fenugreek preparations in thyroid function due to the lack of complete experimental data.

3.3.8. Conclusions

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.

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.

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 embryolethality in rats. This conclusion is coherent with the reported historical/theoretical use of fenugreek as an abortifacient and for labour induction.

Additional data have shown that fenugreek seeds extracts may have toxic effects on reproductive performance, potential teratogenic effects in foetuses, growth retardation and altered neurobehavioral performance in the post-weaning (Khalki 2010, 2012 and 2013), together with a decrease in the rate of pregnancy, an increase in implantation loss, and a decrease in male fertility through significant changes in the percent motility, sperm count, spermatozoa morphology and chromosomal aberrations (Al-Yahya, 2013).

Overall, there are no reproductive and development toxicity studies performed in accordance with current guidelines and further studies are needed. However, studies in animals have shown reproductive toxicity including male and female toxicity which mechanism of action remains unknown. Reports on teratogenicity and anti-fertility effects make it necessary to not recommend the use of fenugreek seed when pregnancy is planned or during pregnancy and lactation and in women of childbearing potential not using contraception. In accordance with the EMA 'Guideline on risk assessment of medicinal products on human reproduction and lactation: From data to labelling' the following information will be added to section 4.6 of the monograph:

"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."

Information on effects on male and female fertility and embryo-foetal effects and associated risks should be reported in the monograph in section 5.3.

The wording proposed for section 5.3 in the monograph is:

"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.

Tests on genotoxicity and carcinogenicity have not been performed."

3.4. Overall conclusions on non-clinical data

Results from relevant experimental studies on *Trigonella foenum-graecum* L., semen to support the proposed indications are very limited. However, the reported pharmacological effects are not contradictory to the traditional uses.

Specific data on pharmacokinetics and interactions are not available.

Considering potential hypoglycaemic effects, warnings regarding the use of the herbal substance or preparations of fenugreek by patients treated for diabetes mellitus should be included in the monograph.

Considering the information on reproductive and developmental toxicity and the reports on teratogenicity and other anti-fertility effects, the use of fenugreek when pregnancy is planned or during pregnancy and lactation and in women of childbearing potential not using contraception is not recommended.

Tests on genotoxicity and carcinogenicity have not been performed with preparations of fenugreek covered by the monograph.

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 documented in humans.

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 (Kandhare et al., 2015).

4.2. Clinical efficacy

4.2.1. Dose response studies

No dose-response studies conducted with fenugreek have been found.

4.2.2. Clinical studies (case studies and clinical trials)

Appetite stimulant effect

The French approved: "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 Union.

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). 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.

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 (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).
- 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

Table 14: 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/m²)	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.

Table 15: 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.

Hypoglycaemic and antihyperlipidemic properties

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 hypoglycaemic/hypolipidemic effects which are described hereafter.

For the assessment on clinical efficacy of fenugreek seed preparations in hyperlipidemic treatment, the EMA document 'Guideline on clinical investigation of medicinal products in the treatment of lipid disorders' (EMA/CHMP/748108/2013) is considered appropriate to use.

For the assessment on clinical efficacy of fenugreek seed preparations in hypoglycemic treatment, the EMA document 'Guideline on clinical investigation of medicinal products in the treatment or prevention of diabetes mellitus' (CPMP/EWP/1080/00) is considered appropriate to use.

Several clinical studies were performed in order to evaluate the safety and efficacy of fenugreek in the reduction of hyperlipidaemia and hyperglycemia. The quality of these studies, however, did not always correspond to the current scientific and regulatory requirements. Therefore, their results, as well as those of some older studies, are only briefly discussed in this AR.

In the study by Rafraf et al. (2014), the influence of powdered fenugreek seeds on glucose metabolism and adiponectin levels in Type 2 Diabetic Patients (n=88, men and women) was assessed through a triple-blind randomized controlled trial. Test group (n = 44) received powdered whole fenugreek seeds (10 g/day) while placebo group (n = 44) received 5 g/d of wheat starch for 8 weeks. The authors report that fenugreek seeds improved glucose metabolism, serum lipid profile and adiponectin levels.

Robert et al. (2016) conducted a randomized, controlled crossover trial to assess the effects of foodstuff products containing 10% fenugreek seed powder on postprandial blood glucose. 10 healthy individuals aged between 21 and 48 years (5 men + 5 woman) without histories of cardiac disease, diabetes mellitus, thyroid problems, wheat allergy, smokers, alcohol or drugs, received 50 g glucose and buns and flatbreads prepared with (10%) or without fenugreek seed powder. The authors concluded that glycaemic response and the glycemic index value after consuming food added with fenugreek were reduced in healthy individuals.

Also a 3-years randomized, controlled, parallel study for the evaluation of the efficacy of a "debitterized, defatted and deodorized fenugreek fiber with vitamins, minerals and amino acids" on the reduction of the incidence rate of type 2 diabetes on pre-diabetic individuals was published (Gaddam et al., 2015). 140 men and women aged 30–70 years with criteria of prediabetes (test, n = 66; control, n = 74) were monitored at baseline and every 3 months for 3 years. Test group received fenugreek powder, 5 g twice a day before meals. The authors report that fasting plasma glucose, postprandial plasma glucose and low density lipoprotein cholesterol were reduced statistically significant whereas serum insulin increased. Cumulative incidence rate of diabetes was reduced.

The study by Yousefi (2017) showed the results of a randomized, double-blind, placebo-controlled clinical trial on the effects of Fenugreek seeds supplementation on serum biochemical parameters of patients with borderline hyperlipidemia. Patients (n=56) received 8 g fenugreek seeds powder or placebo daily for 8 weeks. The authors reported that triglycerides, total cholesterol, low-density lipoproteins and fasting blood glucose decreased statistically significant versus baseline, although the differences between the treated group and the control group before and after the study in HDL, LDL/HDL, TC/HDL and BMI were not significant

Three interesting reviews on the clinical studies with fenugreek, mainly focused on its effect on glycemic and lipidic levels have been published. In the review by Demmers et al., 2017, a systematic review assessing scientific data on the efficacy and safety of medicinal food plants for the treatment of impaired glucose tolerance was done. Controlled trials (RCTs) with a minimum follow-up period of 6 weeks were included. Only one study on fenugreek was included in the review (i.e. Gaddam et a., 2015). The authors of the review state that the risk of bias in the study was high and should be interpreted with caution.

Gong et al., 2016 published a meta-analysis covering randomized clinical trials in relation to the efficacy and safety of fenugreek on prediabetes or diabetes mellitus. A total of 10 articles (12 studies) were included in the analysis i.e. Bordia et al., 1997; Gaddam et al., 2015; Ghattas et al., 2008; Guo et al., 2012; Gupta et al., 2001; Moosa et al., 2006; Rafraf et al., 2014; Shen et al., 2013; Suchitra and Parthasarathy, 2015; Xiao, 2008 (dissertation, not published in journal). Based on Jadad scores, three studies that scored four or more were deemed as high-quality i.e. Gaddam et al., 2015, Rafraf et al., 2014 and Xiao, 2008. These studies reported the random method, allocation concealment, blindness or dropouts. The other seven studies were of low quality. The average score 2.7 indicated the risk of bias. However, in the study by Bordia A, the patients with severe Type 2 DM (T2DM) also took nitrates, in Gupta A's study, the patients took a combination of sulfonylurea and biguanides as well as fenugreek, Rafraf M's study made use of metformin and glibenclamide, Xiao J combined fenugreek with metformin or sulfonylurea. The confounding effect of these co-medications is not known and therefore, the meta-analysis by Gong et al. is considered not relevant for the monograph on fenugreek seed.

Also Neelakantan et al. (2014) published a meta-analysis covering trials of at least 1 week duration comparing the intake of fenugreek seeds with a control intervention, measuring changes in fasting blood glucose, 2 hour post-load glucose and HbA1c. Nine articles reporting 10 studies were included and pooled using random-effects models i.e. Bordia et al., 1997, Chevassus et al., 2010, Gupta et al., 2001, Lu et al, 2008, Alamdari et al., 2009, Chevassus et al., 2009, Raghuram et al., 1994, and two articles by Sharma et al, 1990. The authors report that only the studies by Chevassus et al., 2010, Gupta et al., 2001, Lu et al, 2008, and Chevassus et al., 2009, were double-blind and randomised. The authors report substantial heterogeneity in study results. Differences in the diabetes status of the participants (most trials included participants with type 2 diabetes treated with diet or oral anti-diabetic medication) and a large variation in dose of fenugreek preparation and type of preparation appeared to be contributors to variation in study results. Therefore, the meta-analysis by Neelakantan et al. is considered not relevant for the monograph on fenugreek seed.

Other studies are also available: Sharma et al. (1996b), Prasanna (2000), ,; Raghuram et al.(1994), Abdel-Barry et al. (2000), Memon et al. (2010), Bawadi et al. (2009), Losso et al. (2009), Madar et al. (1988), Mathern et al. (2009)

Assessor's comment:

Data are insufficient or not relevant to propose an indication related to hypoglycaemic/hypolipidemic effects. There is no information available that fenugreek has been in medicinal use within the EU for at least ten years for the treatment of diabetes or other metabolic disorders. The reported clinical studies are of limited value due to several quality issues, mainly the short duration and the sample size. Thus, none of the new clinical studies introduce the possibility for the establishment of a well-establish use monograph

However, the possible interference with glycaemia should be considered in the monograph, under section 4.4. - Special warnings and precautions for use – with the following wording: "Due to a possible hypoglycaemic effect of fenugreek, close monitoring of glycaemic control should be considered in patients treated for diabetes mellitus."

Table 16: Clinical studies on humans, in eating behaviour, lipidic and glycemic levels

Type (aim) and objective(s) of Study Reference	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment**	**Number of Subjects (including age, sex, drop out)**	**Healthy Subjects or Diagnosis of Patients (inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score**	**Comments on clinical relevance of results**
Weigt gain Rguibi and Belahsen, 2006	Observational study	Fenugreek seeds "mentioned" as used by women desiring to gain weight	249 urban non-pregnant women Aged 15 to 70 years old willing to gain weight	Healthy subjects: Mean BMI 29.6 kg/m^2; 30% overweight and 49% obese Without any previous systemic disease	Weight gain	No	Fenugreek consumption not quantified among the appetite enhancers and impossible to appreciate the potential contribution of fenugreek in the weight gain Observational survey no relevant to substantiate the efficacy and safety of fenugreek as an appetite enhancer

Effects of repeated administration of a fenugreek seed extract on the eating behaviour of overweight subjects Chevassus et al, 2009	6-week double-blind randomized placebo-controlled parallel trial Main endpoints: energy intake, weight, fasting glucose level, insulin and lipid profile, visual analogue scale scores of appetite/satiety and blood glucose and insulin levels	Dry hydro-alcoholic fenugreek seed extract Three times daily as oral coated tablets with a total daily dose of 1176 mg (approximately 14 mg/kg)	40 subjects in two groups of 20 Thirty-nine male, aged 18-59 years (mean 38 years) completed this study	Healthy overweight male volunteers	The main end point i.e. reduced total energy intake was not statistically significant vs placebo. Ratio of fasting serum insulin/plasma glucose decreased statistically significant vs placebo, but no effect on fasting plasma glucose, fasting serum insulin, or plasma lipid profile.markers	For the energy intake, 95% CI -2.91 to 0.26, n = 12, p = 0.094)	The main end point i.e. reduced total energy intake was not statistically significant vs placebo in human volunteers in this small, short-term study. No effect on fasting plasma glucose, fasting serum insulin, or plasma lipid profile.

Use of fenugreek seed powder in the management of non-insulin dependent diabetes mellitus Sharma et al, 1996b	24 weeks Control group (10 patients)	Diet plus 25 g fenugreek seed powder Divided into two doses at lunch and dinner	60 patients 45 male, 15 female Ages 30 - 70 years 21 obese and 39 non-obese	Mild (n=22), moderate (n=35) and severe (n=7) NIDDM All patients with uncontrolled blood glucose levels, not taking adequate medicine	46.7% of patients showed full glycaemic control, 33.3% showed moderate glycaemic control 20% exhibited minimal glycaemic control Glycosylated haemoglobin showed a significant reduction (12.5% percentage decrease) compared to initial values	Not available	The main points of this study are the long-term use and data on glycosylated haemoglobin. Critical limitations are the sample size, heterogeneous population, validity of the control group, lack of reliability on the role of fenugreek in the slight reduction in glycosylated haemoglobin. Patients badly controlled for their diabetes before the study entry, having standard treatments after inclusion.

Influence of powdered fenugreek seeds on glucose methabolism and adiponectin levels in Type 2 Diabetic Patients Rafraf et al., 2014	Triple-blind randomized controlled trial Duration: 8 weeks	Test group (n = 44) received powdered whole fenugreek seeds (10 g/day) Placebo (n = 44) received 5 g/d of wheat starch	n=88, men and women	Type 2 diabetic patients	Fenugreek seeds improved glucose metabolism, serum lipid profile and adiponectin levels	Significant decrease in fasting blood glucose (p=0.007) and HbA1c (p?0.0001), serum levels of insulin (p=0.03), total cholesterol (p=0.005) and triglycerides (p=0.00001) and increased serum adiponenctin (p=0.001) versus placebo	Study limitations are the sample size.
Effects of foodstuff products containing 10% fenugreek seed powder on postprandial blood glucose Robert et al., 2016	Randomised, controlled crossover trial	50 g glucose and buns and flatbreads prepared with (10%) or without fenugreek seed powder	10 individuals Age 21 - 48 years 5 men, 5 woman	Healthy subjects without histories of cardiac disease, diabetes mellitus, thyroid problems, wheat allergy, smokers, alcohol or drugs,	The glycaemic response and the glycemic index value after consuming food added with fenugreek were reduced	Values were subjected to repeated measures ANOVA, and significant differences were obtained by Bonferroni multiple comparisons. P < 0.05 was significant.	Fenugreek seed powder was added to buns and flatbreads as part of the daily food. Only 10 healthy individuals were included.

Effects of Fenugreek seeds supplementation on serum biochemical parameters of patients with borderline hyperlipidemia Yousefi, 2017	Randomised, double-blind, placebo-controlled clinical trial	8 g fenugreek seeds powder or placebo, daily 8 weeks	Patients (N=56)	Borderline hyperlipidemia	Triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and fasting blood glucose (FBG) significantly decreased compared with the baseline.	Kolmogorov–Smirnov test for determining quantitative data distribution Paired samples t-test to identify differences between conditions before and after measurements Two independent samples t-test for analysis of differences between the groups. P-values < 0.05	Differences between two groups (fenugreek group and placebo group) before and after the study in HDL, LDL/HDL, TC/HDL and BMI were not significant. Low number of patients

Clinical studies on humans, in menopause and sexual health.

Several studies have been conducted with some fenugreek preparations in order to evaluate their efficacy in patients with oestrogens or androgens deficiency (Table 19).

Steels et al. (2017) performed a double-blind, randomized, placebo-controlled trial to evaluate the efficacy of T. foenum- graecum seed dry extract 33:1 (equivalent to 9.9-g dry herb, standardized for a minimum of 50% of furostanol saponins), 300 mg oral daily (12 weeks), in reducing menopausal symptoms. 115 women aged 40 to 65 were included, 54 completed test group, 50 completed the placebo group. Specific Quality of Life (MENQOL) questionnaire, estradiol levels and biochemical blood parameters were measured. Authors suggested that the preparation was effective for reducing vasomotor symptoms and associated menopausal symptoms.

A randomized, double-blinded, placebo-controlled trial to assess the effect of an hydroethanolic standardized extract of fenugreek seed husks DER 18:1 (w/w), rich in protodioscin, trigonellin and 4-hydroxyiosleucin, on plasma oestrogens and postmenopausal discomforts (scores of Greene Climacteric Scale, short form SF-36® and structured medical interview) was published by Begum et al. (2016). 88 Women included, 32 completed test group, receiving 500mg - 1000 mg/day during 90 days, 36 completed the placebo group. A significant increase in plasma estradiol ($p<0.01$) (120%) and improvements on various postmenopausal discomforts and quality of life were observed. All subjects in the extract group reported reduction of hot flashes, while 32% reported no hot flashes. No adverse events were reported.

Assessor's comment:

There is no information available that fenugreek has been in medicinal use within the EU for at least ten years for the treatment of menopausal symptoms. The reported clinical studies included only a few number of patients and a limited duration of treatment according to the guideline for Hormone Replacement Therapy of Oestrogen Deficiency Symptoms in Postmenopausal Women (EMEA/CHMP/021/97) for the evaluation of symptom efficacy Thus, the results can not be considered as relevant for the establishment of a well-establish use monograph.

In the study by Maheshwari et al., 2017, an one-arm, open-labelled, multi-center study was designed in order to evaluate the effects of the oral administration of 500 mg /day (12 weeks) of T*. foenum-graecum* seed extract enriched in 20% protodioscin (patented - details not given) on testosterone levels, sperm profile, sperm morphology, libido and sexual health of 50 male volunteers (age: 35 to 65 years) diagnosed with symptomatic hypogonadism. The authors reported that free testosterone level was increased by 1.47-fold with a significant decrease in abnormal sperm morphology.

Assessor's comment:

There is no information available that fenugreek has been in medicinal use within the EU for at least ten years for the treatment of symptomatic hypogonadism. The study was designed with only one-arm, open-labelled, in only 50 patients receiving a fenugreek extract enriched in 20% protodioscin and thus, it does not introduce the possibility for the establishment of a well-establish use monograph.

Also Rao et al. (2016) published a double-blind, randomized, placebo-controlled trial to evaluate the effect of a standardized *Trigonella foenum-graecum* seed extract on the symptoms of androgen deficiency, sexual function and serum androgen concentrations. 120 healthy men aged between 43 and 70 years of age (test group received 600 mg/day for 12 weeks) were studied for the change in the Aging Male Symptom questionnaire (AMS), sexual function and serum testosterone. The authors reported that over time and between the active and placebo groups, significant decrease in AMS score and improvement of sexual function were reported. Serum testosterone and free testosterone levels also increased.

Assessor's comment:

.The study by Rao et al (2016) included only healthy subjects for a short period of 12 weeks and There is no information available that fenugreek has been in medicinal use within the EU for at least ten years for treatment of the symptoms of androgen deficiency and sexual function. Thus, it does not introduce the possibility for the establishment of a well-establish use monograph.

Another open label, one arm, non-randomized, post-marketing surveillance study to access the efficacy of fenugreek seed water/ethanol extract (No details on DER) enriched in furostanolic saponins on ovarian cyst reduction was published by Swaroop et al. (2015). 50 Premenopausal women (age=18-45 years), diagnosed with Polycystic Ovarian Syndrome. The authors reported that administration for 3 consecutive months led to reduction of ovarian volume number of ovarian cysts and other related parameters, significant increase in LH and FSH. 47 Subjects demonstrated reduced cyst size. Of these, 36 subjects exhibited no cyst at the completion of the study. Over the 90 days of treatment no biochemical evidences of hepatotoxicity, nephrotoxicity or cardiotoxicity. No significant change in hemogram.

Assessor's comment:

The quality of his clinical study is low (open study, one-arm, non-randomized, only 50 patients included) and does not introduce the possibility for the establishment of a well-establish use monograph for ovarian cyst reduction.

Table 17: Overview of the clinical studies on humans, in menopause and sexual health.

Type (aim) and objective(s) of Study Reference	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment**	**Number of Subjects (including age, sex, drop out)**	**Healthy Subjects or Diagnosis of Patients (inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score**	**Comments on clinical relevance of results**
Efficacy in reducing menopausal symptoms **Steels et al. (2017)**	Double-blind, randomized, placebo-controlled trial	Capsule containing: 300 mg of a *T. foenum-graecum* de husked seed dry extract 33:1, equivalent to 9.9-g dry herb, standardized for a minimum of 50% of furostanol saponins] Duration: 12 weeks:	115 women aged 40 to 65 59 were allocated to test group (n=54 completed) 56 allocated to placebo group (n=50 completed)	Healthy women experiencing menopausal symptoms scoring greater than mild on the Menopause-Specific Quality of Life (MENQOL) Included both peri - menopausal and menopausal women.	4,8 and 12 weeks: Primary endpoint: Menopause-Specific Quality of Life (MENQOL) questionnaire; Secondary endpoint: Oestradiol levels and biochemical blood parameters	MENQOL score ($p < 0.001$);	Not relevant due to the lack of qualification of peri- -menopausal and menopausal status and the sample size.
Effect on plasma oestrogens and	Randomized, double-blinded,	500mg - 1000 mg/day of hydroethanolic	Eighty-eight women extract-treated group - n	moderate to severe postmenopaus	Significant increase in plasma estradiol	Mean ± SD.	No adverse events reported

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score	Comments on clinical relevance of results
postmenopausal discomforts. **Begum et al. (2016)**	placebo-controlled	standardized extract of fenugreek seed husks, rich in protodioscin, trigonellin and 4-hydroxyiosleucin ('FenuSMART™') DER 18:1 (w/w) with respect to fenugreek husks (1kg FHE was equivalent to 24kg of dried fenugreek seeds) 90 days	= 44; drop out =12 placebo group - n=44 ; drop out=6	al discomforts and poor quality of life (evidenced from the scores of Greene Climacteric Scale, short form SF-36® and structured medical interview)	(p<0.01) (120%) and improvements on various postmenopausal discomforts and quality of life. All subjects in the extract group reported reduction of hot flashes, 32% reported no hot flashes. Analysis of haematological and biochemical parameters revealed safety and beneficial effects on lipid	One-way analysis of variance followed by Tukey's multiple comparison test. Values of p<0.05 were considered significant.	Not relevant due to the small number of participants with a high drop-out rate in both groups (not follow ups and lack of adherence to treatment regime) The study design does not fulfil the recommendations of the current guidelines for Hormone Replacement Therapy of Oestrogen Deficiency Symptoms

Type (aim) and objective(s) of Study Reference	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment**	**Number of Subjects (including age, sex, drop out)**	**Healthy Subjects or Diagnosis of Patients (inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score**	**Comments on clinical relevance of results**
					profile Significant enhancement of the estradiol levels		Tested preparation is obtained from husks, not representative of the whole seed
Effects on testosterone levels, sperm profile, sperm morphology, libido and sexual health, **Maheshwari et al. (2017)**	One-arm, open-labelled, multi-center study	500 mg each/day after breakfast *T. foenum-graecum* seed extract enriched in 20% protodioscin (patented - details not given) Duration: 12	50 male volunteers (age: 35 to 65 years)	Diagnosed with Symptomatic hypogonadism	Free testosterone level: increased by 1.47-fold (p value=0.000**) Sperm count and sperm motility: significant decrease in abnormal sperm morphology. Secondary	Testosterone levels and sperm count: Mean; St dev. Other parameters : Wilcoxon signed-rank test	Not relevant as the product in test doesn´t correspond to an herbal preparation since it is enriched in 20% of protodioscin, the clinical relevance concerning the effects of *T. foenum-graecum* herbal preparations

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score	Comments on clinical relevance of results
		weeks			endpoints: mental alertness, mood alleviation, reflex erection and overall performance; Biochemiocal parameters		cannot be considered. Other shortcomings were observed (N=15), duration, open study
Effect of T*rigonella foenum-graecum* seed extract on the symptoms androgen deficiency, sexual function and serum androgen concentrations.	Double-blind, randomised, placebo-controlled trial	Standardised *Trigonella foenum-graecum* seed extract at a dose of 600 mg/day for 12 weeks	120 men aged between 43 and 70 years of age.	Healthy subjects Without erectile dysfunction, not receiving or recently received any treatment therapy for sexual disorders	Primary outcome: the change in the Aging Male Symptom questionnaire (AMS), a measure of possible androgen deficiency symptoms;	Two-way ANOVA for treatment and time DISF-SR within groups ANOVA for change in pathology data	Not relevant as it was a small sample size and only included healthy subjects during a short period. Thus, results can not be extrapolated to erectile dysfunction

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score	Comments on clinical relevance of results
Rao et al. (2016)				No prostate cancer, BPH, chronic alcohol or drug abuse.	Secondary outcome: measure of sexual function and serum testosterone. Others: sleep quality Significant decrease in AMS score over time and between the active and placebo groups. Sexual function improved Both total serum testosterone and free testosterone increased	Pearson correlation Effect sizes reported as Eta squared	

Type (aim) and objective(s) of Study Reference	**Study Design and Type of Control Study duration (if available)**	**Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment**	**Number of Subjects (including age, sex, drop out)**	**Healthy Subjects or Diagnosis of Patients (inclusion criteria)**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score**	**Comments on clinical relevance of results**
Efficacy of fenugreek seed water/ethanol extract enriched in furostanolic saponins (Furocyst) on ovarian cyst reduction **Swaroop et al. (2015)**	Open label, one arm, non-randomized, post-marketing surveillance study	3 consecutive months No details on DER	50 premenopausal women (age=18-45 years BMI=23.88 ± 4.72 kg/m2, diagnosed with PCOS)	Polycystic Ovary Syndrome (PCOS) women	Reduction of ovarian volume number of ovarian cysts and other related parameters. Significant increase in LH (p = 0.045). Significant increases in FSH (p = 0.010) 47 subjects demonstrated reduced cyst size. Of these, 36 subjects exhibited no cyst at the completion	Comparison of data expressed as mean ± SDwith parametric and non-parametric assessments	Open label, one-arm, non-randomized study including only 50 patients

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score	Comments on clinical relevance of results
					of the study No increases in the parameters of hepatotoxicity, nephrotoxicity or cardiotoxicity over the 90 days of treatment. No significant change in hemogram.		

Other clinical studies

Nathan et al., 2014 -Double-blind placebo-controlled proof of concept clinical study to assess the effects of a defatted ethanol/water standardized extract (30% trigonelline) of fenugreek as adjuvant to L-Dopa in the Management of Parkinson disease. The extract administered orally, 300 mg, twice daily, for 6 months to patients stabilized on L-Dopa therapy (men and women, aged 18-70; test group: N=25; placebo group: N=25). Efficacy outcome, based on the scores of Unified Parkinson's Disease Rating Scale and Hoehn and Yahr monitoring, showed slower rise as opposed to steep rise shown by placebo. Trigonella extract was found to be safe and tolerable.

Assessor's comment:

There is no information available that fenugreek has been in medicinal use within the EU for at least ten years as an adjuvant to L-Dopa in the management of Parkinson disease. The study by Nathan et al. (2014) assayed a standardized extract of fenugreek in only 50 patients and didn't show any significant improvement according to the applied scale. Thus, this study does not support the establishment of a well-establish use monograph for the treatment of Parkinson's disease.

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

No clinical data in special populations are available.

4.4. Overall conclusions on clinical pharmacology and efficacy

Several clinical trials have been conducted with fenugreek preparations to substantiate the clinical efficacy and safety in different indications. For most of the preparations included in the reported studies, there are no medicinal products in the market, so it is not possible to justify a well-established use in the EU.

The only clinical data on the use of fenugreek as appetite enhancer is an observational survey in which no data are given in relation to the doses, test product and many other items. Thus, this study can not be considered enough to support the well-established use of fenugreek for the cited indication and subsequently, the use of fenugreek relies more on a traditional use than on a well-established use.

Some studies assayed the effect of different fenugreek preparations on lipidic and glycemic levels, menopause and sexual health, and Parkinson's disease. There is no information available that fenugreek has been in medicinal use within the EU for at least ten years for any of such conditions. Moreover, the reported clinical studies are of limited value due to several quality issues, mainly the short duration, the sample size and conditions and the study design. Thus, none of the new clinical studies introduce the possibility for the establishment of a well-establish use monograph for any of the above-cited indications.

5. Clinical Safety/Pharmacovigilance

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

Safety data on the use of fenugreek preparations is reported as part of the clinical trials conducted to assess their efficacy in different indications (see also section 5.3).

Table 18: Clinical safety data from clinical trials

Study	Herbal preparation	Number of subjects	Treatment duration	Adverse reactions
Abdel-Barry et al., 2000	**40 mg/kg aqueous extract powder in 10 ml distilled water**	**20 male volunteers aged 20-30 years**	**One single dose**	**Significant reduction of 14.1% in potassium levels. Increased micturition frequency or dizziness during the 24h after ingestion**
Sharma et al, 1996a	**25 g fenugreek seed powder divided into two doses at lunch and dinner**	60 patients, 45 male, 15 female, 30 - 70 years	**24 weeks**	**Transient diarrhoea and flatulence**
Gupta et al, 2001	**1 mg/d hydroalcoholic extract of fenugreek seeds**	**12**	**2 months**	**Dyspepsia and mild abdominal distension**

5.2. Patient exposure

Aside from market presence and data from studies, there are no concrete data concerning patient exposure.

Pharmacovigilance data obtained from EudraVigilance database (October 2020) gave no new safety issues related to *Trigonella foenum-graecum*, *semen*, preparations consumption.

5.3. Adverse events, serious adverse events and deaths

The safety and efficacy of *Trigonella foenum-graecum* extract was investigated by Abdel-Barry et al. (2000) 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 et al, 1996a).

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.

Adverse dermatological reactions following the use of fenugreek have been reported. These are usually IgE-mediated reactions that occur after ingestion or inhalation (Minciullo et al., 2017) or cross-reactions involving other plants from the legume family, such as peanut, soy, lupin, lentil, pea, bean, and chickpea (Vinje et al. 2012; Che et al. (2017)

Allergenicity and antigenicity of fenugreek proteins and a high rate of cross-reactivity to peanut were evidenced by Faeste *et al.* (2009). Potential allergens, characterized by MS-based proteomic analysis (Faeste *et al*., 2010) showed homologies to major peanut allergens supporting a molecular explanation for clinical cross-reactivity.

Patil et al, 1997, reported 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.

One case of bronchospasm after inhalation of curry powder has been reported (Ohnuma et al, 1998).

One 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.

The case reported by 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 comment:

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.

Possibility of occurrence of allergic reactions after local application or ingestion was already considered in the first version of the monograph, section 4.8.: "Allergic reactions have been reported after local application (facial angioedema, wheezing) or ingestion (asthma, allergic rhinitis). The frequency is not known."

However, new data strengthen the concerns regarding the occurrence of allergic reactions and cross-reactivity supporting the contraindication of the use of fenugreek in case of hypersensitivity to peanut,

soya and to other plants of the Fabaceae family. In consequence, the risk of the consumption of fenugreek for persons with peanut allergy should be contraindicated in the monograph (section 4.3) in accordance with the 'Public statement on the allergenic potency of herbal medicinal products containing soya or peanut protein' (EMA/HMPC/138139/2005).

5.4. Laboratory findings

No relevant data in relation to the safety of fenugreek preparations are available.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

The use in children and adolescents under 18 years of age has not been established due to lack of adequate data.

5.5.2 Contraindications

Hypersensitivity to the active substance, peanut, soya, and to other plants of the Fabaceae (legume) family (Public statement on the allergenic potency of herbal medicinal products containing soya or peanut protein - EMA/HMPC/138139/2005).

5.5.3 Special Warnings and precautions for use

For oral use, due to a possible hypoglycaemic effect of fenugreek, close monitoring of glycaemic control should be considered in patients treated for diabetes mellitus.

5.5.4 Drug interactions and other forms of interaction

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 comment:

Thus, 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 et al., 2001). 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").

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.

5.5.5 Fertility, pregnancy and lactation

Sabiri et al. (2013) report a prospective study conducted at the maternity Souissi at Rabat, Morroco in which data from the obstetrical and perinatal records were analysed aiming to disclose the main risk factors associated to the occurrence of congenital malformations. From the multivariate analysis of the data (N=1000 births: 960 healthy babies and 40 carriers of malformations) the consumption, during pregnancy, of medicines (antiepileptic) and/or plants, particularly fenugreek, are given, together with chronic pathology in the mother (diabetes), the family background of congenital malformation and the twin pregnancy, as the major risk factors.

The main factors involved in the occurrence of premature births in Morocco were latter assessed (Sabiri et al. 2015) throughout a descriptive and analytical study conducted at the maternity Souissi in Rabat. Analysis of data from interviews with postpartum women and from obstetric and perinatal records (N= 1015 births; 954 full term and 61 preterms babies) lead authors to conclude that the consumption during pregnancy of medicines (antiepileptic) and/or plants, particularly fenugreek, plays a significant role in increasing prematurity.

The bulletin issued by the Morocco Center for Pharmacovigilance (Skalli, 2016) reports the signalization of 8 cases (in a period of six months) of congenital malformations coincident with the consumption of fenugreek seeds during pregnancy. Considering the severity of these events this Center for Pharmacovigilance delivered recommendations to professionals to discourage the consumption of fenugreek seeds during pregnancy and lactation.

Assessor's comment:

Non-clinical reproduction toxicity was already reported in the first assessment report and considered in the first version of the monograph, Section 4.6.: "the use is not recommended during pregnancy and lactation". Additional clinical data together with additional non-clinical studies on embryo-foetal and peri- and postnatal toxicity fenugreek have been found in the literature since the first version of the monograph was adopted.

As explained in section 3.3.5, studies in animals have shown reproductive and development toxicity including male and female fertility. However, there are no reproductive and development toxicity studies performed in accordance with current guidelines and further studies are needed. In conclusion, The use during pregnancy and lactation is not recommended. Furthermore, the use is not recommended when pregnancy is planned since male and female fertility may be affected by the treatment.

Therefore, in accordance with the EMA 'Guideline on risk assessment of medicinal products on human reproduction and lactation: From data to labelling' the following information will be added to section 4.6 of the monograph:

"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."

5.5.6 Overdose

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 (Sharma et al, 1996a.)

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

No data available.

5.5.8 Safety in other special situations

No information 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. The following undesirable effects have been included in section 4.8. of the monograph:

Oral use
Gastrointestinal disorders: flatulence, diarrhoea may occur.
Nervous system disorders: dizziness may occur.
The frequency is not known.

Oral use and cutaneous use
Allergic reactions have been reported after local application (facial angioedema, wheezing) or ingestion (asthma, allergic rhinitis). The frequency is not known.

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.

Patients with known allergy to peanut protein carry an enhanced risk for severe reactions to fenugreek preparations. The risk of the consumption of fenugreek for persons with peanut allergy should be contraindicated in the monograph (section 4.3) in accordance with the 'Public statement on the allergenic potency of herbal medicinal products containing soya or peanut protein' (EMA/HMPC/138139/2005).

Taking into account all the elements mentioned above regarding the potential risk of interaction between fenugreek and anticoagulants, 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.

Non-clinical reproduction toxicity was already reported in the first assessment report and considered in the first version of the monograph. Additional clinical data together with additional non-clinical studies on embryo-foetal and peri- and postnatal toxicity fenugreek have been found in the literature since the first version of the monograph was adopted. Therefore, in accordance with the EMA 'Guideline on risk

assessment of medicinal products on human reproduction and lactation: From data to labelling' the following information will be added to section 4.6 of the monograph:

"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."

6. Overall conclusions (benefit-risk assessment)

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

Several clinical studies have been performed with different fenugreek preparations. Nevertheless, when scrutinizing the published literature results do not substantiate the clinical efficacy of fenugreek to stimulate appetite as in the first indication, it has to be acknowledged that the data are limited, studies are not performed according the current guidelines and of show 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 use in children and adolescents under 18 years of age is not recommended because of incomplete data on safety.

Consequently, on the basis of the pharmacological and clinical data, HMPC concluded that a well-established use indication cannot be established for the European Union herbal monograph on *Trigonella foenum-graecum* L. *semen*. Based on the data on its long-standing use in the European Union and on the available bibliographic references, the use for more than 30 years and acceptable safety are documented for *Trigonella foenum-graecum* L. *semen* for the following preparations a) *for oral use*: herbal substance, comminuted herbal substance and powdered herbal substance; dry extract (DER 4:1), extraction solvent: ethanol 20% v/v; soft extract (DER 5-6:1), extraction solvent: ethanol 60% v/v; *for cutaneous use*: herbal substance.

In conclusion, the above-cited fenugreek preparations can be accepted as traditional herbal medicinal products for the following indication:

Indication 1): Traditional herbal medicinal product used for temporary loss of appetite (oral route)

The herbal substance and the powdered herbal substance can be accepted as traditional herbal medicinal products for the following indication:

Indication 2): Traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the skin (cutaneous use)

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

Studies have shown reproductive toxicity including male and female fertility. The use during pregnancy and lactation and in women of childbearing potential not using contraception is not recommended.

Warnings regarding the use of the herbal substance or preparations of fenugreek by patients treated for diabetes mellitus should be included in the monograph.

No constituent with known therapeutic activity or active marker can be recognised by the HMPC.

Tests on genotoxicity have been performed only with undefined fenugreek extracts; these data cannot be extrapolated to the herbal substance or to the preparations considered in the monograph (dry

extract, DER 4:1, extraction solvent ethanol 20% v/v and soft extract DER 5-6:1, extraction solvent ethanol 60% v/v). Therefore, a European Union list entry cannot be supported due to lack of adequate data on genotoxicity.

Annexes

List of references