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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*, aetheroleum

Draft – Revision 1

Based on Article 10a of Directive 2001/83/EC as amended (well-established use)

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)		Foeniculi amari fructus aetheroleum
Herbal preparation(s)		Not applicable
Pharmaceutical form(s)		Not applicable
First assessment	Rapporteur(s)	M Delbò
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Note: This draft assessment report is published to support the public consultation of the draft public statement on *Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*, aetheroleum. It is a working document, not yet edited, and which shall be further developed after the release for consultation of the public statement. 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 public statement.

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1. Introduction

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

This Assessment Report revises and updates the set of data used in the first HMPC assement report to support the establishment of individual European Union herbal monographs and/or European Union list entries on bitter fennel fruit oil.

- Herbal substance(s)

Foeniculum vulgare Mill. subsp. *vulgare* belongs to the Apiaceae (Umbelliferae) botanical family.

The European Pharmacopoeia describes two varieties: sweet (var. *dulce*) and bitter fennel fruit (var. *vulgare*)

Sweet fennel fruit consists of dry cremocarps and mericarps of *Foeniculum vulgare* Mill. subsp. *vulgare* var. *dulce* (Mill.) Batt. & Trab., and it is characterized by a content of essential oil not lower than 20 ml per kg anhydrous fruit with a 80.0% minimum content of anethole in its essential oil. Sweet fennel is pale green or pale yellowish-brown (Ph. Eur. 10th Edition 04/2011:0825 corrected 10.0).

Bitter fennel fruit consists of dry cremocarps and mericarps of *Foeniculum vulgare* Mill. ssp. *vulgare* var. *vulgare;* it contains not less than 40 ml per kg anhydrous fruit of essential oil that contains not less than 60.0% of anethole and not less than 15.0% of fenchone. Bitter fennel is greenish-brown, brown or green (Ph. Eur. 10th Edition 04/2013:0824 corrected 10.0).

The medicinal properties of fennel fruit are mainly attributed to its content of essential oil. This assessment focuses on the medicinal use of the essential oil obtained by steam distillation from the ripe fruits of bitter fennel.

Detailed information on fennel fruits is available in the Assessment report on *Foeniculum vulgare* Miller, fruit which supports the European Union monograph on bitter and sweet fennel fruits.

- Herbal preparation(s)

The European Pharmacopoeia describes the essential oils obtained from the bitter fennel.

Bitter-fennel fruit oil is the essential oil obtained by steam distillation from the ripe fruits of *Foeniculum vulgare* Miller, ssp. *vulgare* var. *vulgare*; it contains fenchone in the range 12.0% – 25.0% and *trans*-anethole in the range 55.0% - 75.0%. The bitter-fennel fruit oil appears as a clear, colourless or pale yellow liquid with a characteristic odour (Ph. Eur. 6th Edition 01/2008:1826).

The essential oil of bitter fennel fruits contains not less than 55.0% anethole and 12.0% fenchone and not more than 6.0% estragole (Ph. Eur. 10th Edition 01/2008:1826). Compounds identified in the essential oils of bitter based on the information reported in the European Pharmacopoeia monograph are reported below.

Table 1: Compounds present in the bitter fennel oil according to the monograph in the European Pharmacopoeia (01/2008:1826)

Compound	**Bitter fennel (+)**
Trans-anethole	55.0-75.0%
Fenchone	12.0-25.0%

Estragole	6.0 % (max)
Alpha-pinene	1.0-10.0%
Limonene	0.9-5.0%
Cis-anethole	0.5% (max)
Anisaldehyde	2.0 (max)
Ratio Alpha-pinene to Limonene	> 1.0

Other constituents of bitter fennel fruit oil are: camphene, *p*-cymene, *β*-pinene, *β*-myrcene, *α*-phellandrene, sabinene, *γ*-terpinene and terpinolene (ESCOP 2019).

The essential oil contents of 10 samples of dry, ripe fennel fruits of different origin, obtained by hydrodistillation, were analysed by gas chromatography-mass spectrometry (GC-MS); seven samples were commercial drugs from seven different firms, defined by the sellers as `sweet fennel', while the remaining three samples were ascribed to the bitter variety by the author, based on their smell and taste and the morphological characteristics of the original plants. None of the bitter fennel samples fulfilled the Pharmacopoeial requirements in terms of composition. The 16 main constituents of each sample were identified, *trans*-anethole, estragole, limonene and fenchone being the most abundant. The amounts of *trans*-anethole and estragole were inversely proportional, so that clear phytochemical differences within the investigated samples were observed (Miraldi, 1999).

The analysis by a capillary GC method of the composition of essential oils of commercial fennel fruits from pharmacies in different countries revealed that the samples of sweet fennel (obtained from Estonia and Moldova) contained more *trans*-anethole (80.9–82.0%) and less fenchone (1.6–9.7%) than the bitter fennel samples from Norway and Austria (63.7–64.6% *trans*-anethole and 21.2–22.8% fenchone). Estragole in all European samples was found below 5.0%. Conversely, the content of estragole in sample of fennel (not specified if bitter or sweet fennel) obtained from Turkey was 17.0% (Raal et al., 2012).

GC–MS analysis of the essential oil from dried fennel seed powder from Tunisian and French cultivars showed that although the same main compounds were found in Tunisian and French cultivars, some differences were present in their proportions allowing to classify them in two chemotypes. The first class was composed by *trans*-anethole (63.41%–78.26%) for Tunisian cultivars and the second one by estragole (44.72%–88.92%) for French cultivars (Kalleli et al., 2019).

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

Not applicable.

1.2. Search and assessment methodology

This assessment report reviews the available scientific data for bitter fennel oil (i.e. *Foeniculum vulgare* Miller sp. *vulgare* var. *vulgare*, *aetheroleum*) and particularly clinical data.

In preparing this report, medical databases have been reviewed. The results of a data search carried out in October 2020 in Embase, Medline, Pubmed covering the period from year 2011 to 2020 were taken into consideration in the revision process of the monographs.

Search engines used: Google Scholar

Toxicological databases: Toxnet, since 2019 PubMed.

Pharmacovigilance resources: The results of a data search on Eudravigilance carried out in May 2021.

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

Information on medicinal products marketed in the EU/EEA

Table 2: Overview of data obtained from marketed medicinal products: Foeniculi amari fructus aetheroleum (no evidence of use of sweet fennel fruit oil in Europe)

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
Foeniculi amari fructus aetheroleum	Children: expectorant in cough associated with cold	Syrup *children over 1 year of age:* SD: 3-3.25 mg DD: 6.5-9.75 mg If the symptoms do not improve after 5 days or worsen, a doctor should be consulted.	At least since 1976, Germany, WEU
Foeniculi amari fructus aetheroleum	Children: expectorant in cough associated with cold	Syrup *children over 1 year of age:* SD: 3.25 mg DD: 6.5-9.75 mg No longer than 2 weeks.	2003, Germany, WEU
Foeniculi amari fructus aetheroleum	Children: expectorant in cough associated with cold	Syrup *children over 1 year of age:* SD: 7 mg DD: 7 mg	1976 until 31/12/2019, DE, WEU *(MA expired because of written*

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
			renouncement from the company)

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)

Not applicable.

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

See section 2.2 on Traditional Chinese Medicine

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

Applications in the treatment of catarrh of the upper respiratory tract has been described in several handbooks and treaties (Brand, 1993; Czygan, 1989; Madaus, 1976; Parfitt, 1993; Merkes, 1980; Weiss, 1997; and Müller-Limmroth and Fröhlich, 1980).

Fennel fruit has also been reported as useful in the treatment of dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, bloating and flatulence (Brand *et al*., 1993; Czygan, 1989; Madaus, 1976; Schilcher, 1984 and 1986).

Fennel fruit has been reported to be in use in some areas for many years to relieve painful menstruation, symptoms of female climacteric and other purposes (Hare *et al*., 1916; Albert-Puleo, 1980; Zargari, 1991; Mills *et al*., 2000).

Fennel has been used as lactagogue since antiquity with no side effects reported (Keller, 1992).

Table 3: Overview of historical data **Herbal preparation**	**Documented use / Traditional use**	**Strength (where relevant) Posology Duration of use**	**Reference**
Foeniculi amari fructus aetheroleum	peptic discomforts, such as mild spastic disorders of the gastrointestinal tract, feeling of fullness, and flatulence; for catarrhs of the upper respiratory tract.	0.1-0.6 ml essential oil[1] or equivalent galenical preparations for internal use. Fennel honey syrup with 0.5 g fennel oil in kg (0.5 g in 1000 g) *Adults*: 10-20 g. *Children 4-10 years*: 6-1.0 g. *Children 14 years*: 3-6 g. Duration of use: Unless otherwise advised by a physician or pharmacist, one should not consume fennel oil for an extended period (several weeks)	Blumenthal and Goldberg, 2000
Foeniculi amari fructus aetheroleum	As a carminative and expectorant	0.05 to 0.2 ml	Keller 1992
Foeniculi amari fructus aetheroleum	To relief gastrointestinal complaints	1 to 10 drops on a sugar cube, equivalent to approximately 23-230 mg[2]	Leclerc 1983
Foeniculi amari fructus aetheroleum	abdominal bloating lack of appetite slow digestion aerophagia	1 to 5 drops in alcoholic solution 2 times daily	Valnet 1990

Table 3: Overview of historical data **Herbal preparation**	**Documented use / Traditional use**	**Strength (where relevant) Posology Duration of use**	**Reference**
	gastric pain, psychogenic vomiting, intestinal parasites, lung disturbances, prevention of flu, oliguria (small volume of urine) and bladder stones, gout, insufficient menstrual flow milk insufficiency in breastfeeding women		

[1]According to Ph. Eur. 8.0 Relative density (2.2.5): 0.961 to 0.975

[2]Assuming that 1 g of essential oil corresponds to 46 drops (Brand 1993)

2.3. Overall conclusions on medicinal use

Table 4: Overview of evidence on period of medicinal use: Foeniculi amari fructus aetheroleum

Herbal preparation Pharmaceutical form	**Indication**	**Strength Posology**	**Period of medicinal use**
Foeniculi amari fructus aetheroleum Syrup	Children: expectorant in cough associated with cold	Syrup *children over 1 year of age:* SD: 3-3.25 mg DD: 6.5-9.75 mg	Since 1976, Germany, WEU
Foeniculi amari fructus aetheroleum Syrup	Children: expectorant in cough associated with cold	Syrup *children over 1 year of age:* SD: 7 mg DD: 7 mg	1976 until 31/12/2019, DE, WEU *(MA expired because of written renouncement from the company)*
Foeniculi amari fructus aetheroleum	As a carminative and expectorant	0.05 to 0.2 ml	Keller 1992

Long-standing use for at least 30 years, 15 of them within the European community, is therefore demonstrated for bitter fennel oil as an expectorant in cough associated with cold.

Posology (based on long-standing use): d=0.961 to 0.975

Adults

200 microliters of essential oil, as a single dose per day or in multiple divided doses.

Children

The posology authorised in marketed herbal medicinal products in Germay since more than 30 years is: single dose 3-3.25 mg 2-3 times daily or 7 mg in one single dose; daily dose 6.5 – 9.75 mg.

Duration of use

Not to be taken for more than one week.

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

- *Expectorant effects*

Anethole and fenchone vapour were given by inhalation to urethanized rabbits as doses of 1 to 243 mg/kg b.w. added to the steam vaporizer (the amount actually absorbed by the animals being considerably less, estimated as not more than 1% of that added to the vaporizer). Inhalation of anethole did not affect the volume but produced a dose-dependent (1-9 mg/kg) decrease in the specific gravity of respiratory tract fluid. Inhalation of fenchone produced a dose-dependent (1-9 mg/kg) augmentation of the volume output of respiratory tract fluid and a dose-dependent (1-27 mg/kg) decline in its specific gravity (Boyd and Sheppard, 1971).

- *Anti-inflammatory effect*

F. vulgare essential oil inhibited 5-lipoxygenase in a similar way of the positive control Nordihydroguaiaretic acid (IC_{50}=67.7±2.3 µg/mL and IC_{50}=63.7±2.3 µg/mL, respectively) (Albano *et al*., 2012).

Ozbek et al. (2005) showed that *F. vulgare* essential oil had an anti-inflammatory effect matching to that of etodolac (50 mg/kg i.p.) at 0.050 and 0.200 ml/kg doses when administered intraperitoneally (i.p.) to SD rats with paw edema induced by carrageenan. The anti-inflammatory effects was significantly lower at all doses when compared to indomethacin (3 mg/kg i.p.).

The anti-inflammatory effects of fennel (herbal preparation not reported) was investigated in model of lipopolysaccharide (LPS)-induced acute lung injury. In five groups, the mice were intraperitoneally injected with 1% Tween 80-saline (vehicle), fennel (125, 250, 500 µl/kg), or dexamethasone (DEX) (1 mg/kg), followed 1 h later by intratracheal instillation of LPS (1.5 mg/kg). In the remaining two groups, the mice were intraperitoneally injected with 1% Tween 80-saline (vehicle) or fennel (250 µl/kg), followed 1 h later by intratracheal instillation of sterile saline. Fennel significantly and dose-dependently reduced lactate dehydrogenase (LDH) activity and immune cell numbers in LPS treated mice. In addition fennel effectively suppressed the LPS-induced increases in the production of the inflammatory cytokines interleukin-6 and tumor necrosis factor-alpha, with 500 µl/kg fennel showing maximal reduction. Fennel also significantly and dose-dependently reduced the activity of the proinflammatory mediator matrix metalloproteinase 9 and the immune modulator nitric oxide (Lee *et al*., 2015).

Several terpene derivatives including γ-terpinene and fenchone as well as phenylpropanoid, *trans*-anethole, showed considerable inhibitory action of 5-lipoxigenase. In particular, the IC_{50} of *trans*-anethole was 51.6 mM. In addition, *trans*-anethole (50 mg/kg and 200 mg/kg) showed significant inhibition by oral administration against arachidonic acid-induced ear edema in mice (Lee *et al*., 2012).

Trans-anethole was administrated (36.4, 72.8 or 145.6 mg/kg) as well as dexamethasone (5 mg/kg) orally once daily for 7 consecutive days in mice with acute lung injury induced by LPS (24 mg/kg). Trans-anethole, as well as dexamethasone, eliminated LPS-induced histopathological changes, decreased the number of inflammatory cells and resulted in a notable reduction in IL-17 mRNA expression. In addition, trans-anethole increased IL-10 mRNA expression in isolated lung tissues and resulted in a marked elevation in T regulatory cells and reduction in T helper 17 cells in spleen tissues (Zhang *et al*., 2018).

Table 5: Overview of the main non-clinical data

Herbal preparation tested	**Strength** **Dosage** **Route of administration**	**Experimental model** ***In vivo/*** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
Expectorant effects				
Anethole vapour	1 to 243 mg/kg b.w. added to the steam vaporizer by inhalation (the amount actually absorbed by the animals being considerably less, estimated as not more than 1% of that added to the vaporizer)	*In vivo* urethanized rabbits	Boyd and Sheppard, 1971	Inhalation of anethole did not affect the volume but produced a dose-dependent (1-9 mg/kg) decrease in the specific gravity of respiratory tract fluid.
Fenchone vapour	1 to 243 mg/kg b.w. added to the steam vaporizer by inhalation (the amount actually absorbed by the animals being considerably less, estimated as not more than 1% of that added to the vaporizer)	*In vivo* urethanized rabbits	Boyd and Sheppard, 1971	Inhalation of fenchone produced a dose-dependent (1-9 mg/kg) augmentation of the volume output of respiratory tract fluid and a dose-dependent (1-27 mg/kg) decline in its specific gravity
Anti-inflammatory effects				
trans-anethole	*Trans*-anethole orally at 50 and 200 mg/kg	*In vivo* mice	Lee *et al*., 2012	Significant inhibition against arachidonic acid-induced ear edema in

Herbal preparation tested	**Strength** **Dosage** **Route of administration**	**Experimental model** ***In vivo*/** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
				mice
Fennel essential oil	12.5 µL in DMSO	*In vitro*	Albano *et al*. 2012	Inhibition of 5-lipoxygenase similar to Nordihydroguaiaretic acid (IC_{50}=67.7±2.3 µg/mL and IC_{50}=63.7±2.3 µg/mL, respectively)
Fennel essential oil	Intaperitoneally at 50, 100 and 200 µl/kg	*In vivo* Rats: model of paw edema induced by carrageenan	Ozbek 2005	Anti-inflammatory effect significantly lower than indomethacin (3 mg/kg i.p.), but comparable to etodolac (50 mg/kg i.p.)
Fennel (details on herbal preparation not reported)	Intraperitoneally at 125, 250, 500 µl/kg	*In vivo* Mice: model of LPS-induced acute lung injury	Lee *et al*. 2015	significant and dose-dependent reduction of LDH activity and immune cell numbers in LPS treated mice; suppression of the LPS-induced increases in the production of IL-6 and TNF-α; significant and

Herbal preparation tested	**Strength** **Dosage** **Route of administration**	**Experimental model** ***In vivo*/** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
				dose-dependent reduction of the activity of MMP-9 and nitric oxide
Trans-anethole	Orally at 36.4, 72.8 or 145.6 mg/kg for 7 days	*In vivo* Mice: model of LPS-induced acute lung injury	Zhang *et al*., 2018	Eliminated LPS-induced histopathological changes, decreased the number of inflammatory cells, reduced IL-17 mRNA expression, increased IL-10 mRNA expression, increase T regulatory cells and reduced T helper 17 cells in spleen tissues

3.1.2. Secondary pharmacodynamics

- *Antimicrobial effects*

Fennel fruit oil as well as some oil components, exhibited *in vitro* strong inhibitory activities against the growth of a wide spectrum of bacteria and fungi known to be pathogenic for man and other species.

Fennel oil inhibited the growth of *Escherichia coli* (Minimal inhibitory concentration (MIC): 0.5% V/V), *Staphylococcus aureus* (MIC: 0.25%), *Salmonella typhimurium* (MIC: 1.0%) and *Candida albicans* (MIC: 0.5%) using the agar dilution method (Hammer *et al*, 1999). Significant antibacterial activity of the oil (10 µl of undiluted oil added to wells in the agar plates) was demonstrated against *Brevibacterium linens, Clostridium perfringens, Leuconostoc cremoris* and *Staphylococcus aureus* (Ruberto *et al*., 2000). Earlier studies also demonstrated the antibacterial activity of the oil (Afzal and Akhtar, 1981, Ramadan *et al*., 1972).

In vitro growth of *Candida albicans* strain ATCC 10261 was inhibited by fennel essential oil (Ezzat, 2001).

Bactericidal activities of a number of plant essential oils, including fennel fruit oil, and of their isolated constituents were tested against *Campylobacter jejuni, Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica* (Friedman *et al*., 2002). Fennel fruit oil was shown to reduce bacterial activity of all tested bacteria (*C. jejuni* > *S. enterica* > *E. coli* > *L. monocytogene*s). As far as the antibacterial activity of isolated compounds is concerned, estragole had an inhibitory pattern close to that of the fennel oil; limonene showed an inhibitory activity only on *C. jejuni* and *L. monocytogenes* and *trans*-anethole only inhibited *C. jejuni.*

An essential oil, extracted by hydro-distillation of bitter fennel crushed fruits and containing as major components 59% *trans*-anethole,15% limonene and 12.6% fenchone, was tested *in vitro* for antibacterial activity against 27 different phytopathogenic bacterial species and 2 mycopathogenic ones (Lo Cantore, 2004). An antibacterial activity of the oil was detected against several gram-negative bacteria (i.e. *Pseudomonas syringae pv. atrofaciens and pv. glycinae, P. tolaasil, Erwinia carotovora subsp. carotovora and subsp, atroseptica, Agrobacterium tumefaciens, Burkholderia gladioli pv. agaricicola, Xanthomonas campestris pv. phaseoli, pv. phaseoli var. fuscans pv. vesicatoria and pv. campestris)* as well as against a few gram-positive bacteria (i.e. *Clavibacter michiganensis subsp. michiganensis and subsp. sepedonicus and Rhodococcus fascians*). The antibacterial activity of the fennel oil against the tested strains was much lower (generally below 1/1,000) than that of purified rifampicin.

Essential oil, extracted by steam distillation from fennel fruits and containing 83% *trans*-anethole, 2.6% p-anisaldehyde, 0.94% carvone, 5.12% estragole, 1.23% alpha-tujone, 3.77% limonene and 0.27% alpha-pinene, showed, when applied *in vitro* at concentrations between 5 and 80 µl/ml, significant inhibitory effects on several foodborne bacteria (*Salmonella typhymurium* > *Escherichia coli O157:H7* > *Listeria monocytogenes* > *Staphylococcus aureus)* (Dadalioglu and Evrendilek, 2004).

Growth of 4 *Streptococcus* strains (i.e. KCTC 3065, NHS 1DD, UBF GTFC, and GS-5) was inhibited completely by an *in vitro* concentration of 80 ppm of fennel oil containing about 78% *trans*-anethole and minor amounts of limonene, estragole and fenchone. As a similar inhibition of the growth of the above mentioned *Streptococcus* strains by 70 ppm of *trans*-anethole was observed, it was concluded that *trans*-anethole was responsible for the antibacterial activity of fennel oil (Park *et al*., 2004).

The essential oils of anise (500 ppm), fennel (2,000 ppm) and other plants showed a dose-dependent inhibitory effect on the growth of tested fungi including *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliformis* (Farag *et al*., 1989; Hasan, 1994; Soher, 1999; Soliman and Badeaa, 2002).

The aniseed and fennel oils were found to have a high antibacterial activity against *Staphylococcus aureus* (responsible for bases, sepses and skin infections), *Streptococcus haemolyticus* (causing infection of the throat and nose)*, Bacillus subtilis* (infection in immunecompromised patients)*, Pseudomonas aeruginosa* (causing hospital acquired infection)*, Escherichia coli* (responsible for urogenital tract infections and diarrhoea)*, Klebsella species* and *Proteus vulgaris* (Singh *et al*., 2002).

Essential oils of the fruits of three organically grown cultivars of Egyptian fennel (*Foeniculum vulgare* var. *azoricum*, *Foeniculum vulgare* var. *dulce* and *Foeniculum vulgare* var. *vulgare*) were examined by disc diffusion assay for their antimicrobial activities, against two species of fungi, two species of Gram negative and two species of Gram positive bacteria. All essential oil samples have antibacterial activity against Gram negative and Gram positive bacteria. The most effective oil against Gram negative bacteria was *Foeniculum vulgare azoricum*, which is less effective than ampicillin by 25% and 7% in the *Escherichia coli* and *Psedumonas aeruginosa* bioassays, respectively, while the most effective essential oil against Gram positive bacteria was *Foeniculum vulgare vulgare* which gave a larger inhibition zone than ampicillin by 58.3% and 114% in the *Staphylococcus aureus* and *Bacillus subtilis* tests, respectively. The results also showed that *Foeniculum vulgare azoricum* was more effective antifungal than that of the reference commercial fungicidal clotrimazole. It produced 46% more inhibition (inhibition zone in mm) compared to the standard drug for *Aspergillus niger,* and it also has the same high activity against yeast, forming an inhibition zone larger than that of the standard drug by 40% (Shahat et al., 2011).

The antibacterial effects of the essential oil from the fennel seeds were evaluated by disk diffusion method against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 12228, and *Candida albicans*. The results of the disk diffusion method clarified that *E. coli* and *S. aureus* were the most sensitive microorganisms tested, displaying the inhibition zones of 20 and 18 mm, respectively, with a concentration of 50 µg/ml. The lowest activity was exhibited against *C. albicans* with the lowest inhibition zones of 10 mm at the concentration of 100 µl/disk as compared to gentamicin (4 µg/cup) and nystatin (100 IU/cup) positive controls. Furthermore, the lowest MIC and MBC of essential oil were against *S. aureus* including 64 µg/ml and 128 µg/ml, respectively, followed by 128 µg/ml and 256 µg/ml, respectively, against the both *E. coli* and *S. epidermidis* strains (Ghasemian et al., 2019).

- *Estrogenic effects*

Trans-anethole administered orally to immature female rats at 80 mg/kg b.w. for 3 days significantly increased uterine weight to 2 g/kg compared to 0.5 g/kg in controls and 3 g/kg in animals given estradiol valerate subcutaneously at 0.1 µg/rat/day ($p<0.001$). The results confirmed that *trans*-anethole has estrogenic activity; other experiments showed that it has no anti-estrogenic, progestational, anti-progestational, androgenic or anti-androgenic activity (Dhar, 1995).

Estrogenic activity of *trans*-anethole at high concentrations has been determined by a sensitive and specific bioassay using recombinant yeast cells expressing the human estrogen receptor (Howes *et al*., 2002).

- *Antioxidant activity of fennel extracts*

Antioxidant activity of the essential oil obtained by the fresh aerial part of *Foeniculum vulgare* against lipid peroxidation was demonstrated in the thiobarbituric acid reactive species assay and in a micellar model system (Ruberto *et al*., 2000).

Antioxidant activity of the essential oils of *Foeniculum vulgare* var. *azoricum*, *Foeniculum vulgare* var. *dulce* and *Foeniculum vulgare* var. *vulgare* was evaluated by several complementary tests consisting of the DPPH free radical scavenging, the ferric reducing power (FRAP) assay, thiobarbituric acid reactive species assay (TBARS) and the ferrous ion-chelating (FIC) assay, using butylated hydroxytoluene (BHT) and ascorbic acid as references or positive controls. All of the assays were carried out at concentrations of 25, 50 and 100 mg/mL. *Foeniculum vulgare* var. *azoricum* showed the highest activity in quenching of DPPH radical (IC50 0.33 mg/mL), even higher than either ascorbic acid (IC50 0.40 mg/mL) or BHT (0.44 mg/mL). *Foeniculum vulgare* var. *dulce* showed compatible scavenging activity (IC50 0.41 mg/mL) to ascorbic acid or BHT. The cultivar *Foeniculum vulgare* var. *vulgare* was the least effective radical scavenger, with an IC50 of 15.33 mg/mL which is about 44 and 37 times higher than the *azoricum* or *dulce* var. respectively. Similar results were also obtained from the FRAP assay. In the TBARS assay the essential oil of *Foeniculum vulgare* var. *azoricum, dulce and vulgar* showed inhibitory activity against lipid peroxidation with IC50 values of 0.08, 0.03 and 30.51 mg/mL, respectively. The metal chelating scavenging effect of fennel oils and standards decreased in the order of *azoricum* > *dulce* > BHA > ascorbic acid > *vulgare*, with IC50 of 2.23 mg/mL, 2.51 mg/mL, 45.15 mg/mL, 100.43 mg/mL, and 117.11 mg/mL, respectively (Shahat et al., 2011).

Essential oils (EOs) and aqueous extracts of aerial parts of four aromatic species, *Calamintha nepeta*, *Foeniculum vulgare*, *Mentha spicata* and *Thymus mastichina*, from southwest of Portugal were analysed in order to evaluate their antioxidant potential activities. EO of *F. vulgare* showed the highest activity by β-carotene/linoleic acid method (IC_{50} 0.160 ± 0.008 mg/ml vs acscorbic acid IC_{50} 1.116 ± 0.003). Aqueous extracts showed higher antioxidant potential than EOs with ability to protect the lipid substrate, free radical scavenging and iron reducing power. Extract of *C. nepeta* showed the highest activity by β-carotene/linoleic acid and free radical DPPH methods, whereas fennel extract had the highest iron reducing power (Arantes et al., 2017).

- *Effects of fennel on gastro-intestinal tract* and *spasmolytic effect on contracted smooth muscles*

Fennel essential oil cause primarily a relaxation (lasting from 5 to 30 minutes) of the walls and decrease in the peristaltic contractions of the stomach in unanesthetized dogs (5 to 25 ml of distillate administered to dogs by means of a catheter inserted into stomach or an intestinal fistula). In about half of the observations the relaxation and decrease in peristalsis was followed by some increase in tone or in amplitude of the contractions or both. In less than half of the tests where it could be observed, there was some increase in the activity of a loop of intestine when the fennel essential oil was placed in the stomach. When introduced into the colon, dilute solutions of fennel essential oil increase the tone and contractions, just as they do in the small intestine, but the effect lasts longer in the colon than it does in the ileum (Plant and Miller, 1926).

Fennel fruit oil exerted a relaxing effect on in vitro pre-contracted smooth muscles from different organs (jejunum, stomach and uterus) by antagonizing several contraction-inducing agents. Addition of anethole to 10 to 25 ml/l of physiological solution in which an isolated mouse intestinal jejunum is plunged induced intestinal motility at low concentrations, but an intestinal relaxation was observed at concentrations higher than 50 mg/l (Imaseki et al., 1962).

Fennel administered orally at 24 mg/kg b.w. increased spontaneous movement of the stomach in unanaesthetized rabbits and reduced the inhibition of stomach movement induced by sodium pentobarbitone (Niiho et al., 1977).

Anethole was reported to have a contractile effect on smooth muscle (Reiter and Brandt, 1985).

Fennel essential oil had a spasmogenic effect on smooth muscle of isolated guinea-pig ileum at a concentration of 8 x 10-5 g/ml. On a skeletal muscle preparation of isolated rat phrenic nerve diaphragm, it caused contracture and inhibition of the twitch response to nerve stimulation at a concentration of 2 x 10-4 g/ml (Lis-Balchin and Hart, 1997).

Fennel essential oil was reported, at a concentration of 10 mg/ml, to antagonize the action of acethylcoline, pilocarpine, physostigmine or of barium chloride on intestinal jejunum isolated from different animals (quoted by Teuscher at al., 2005).

Oral administration of anethole (0.3 mg/kg and 3 mg/kg) significantly improved clonidine-induced delayed gastric emptying examined by the phenol red method. Anethole was administered to mice (fasted for 18 hours) and clonidine 30 mg/kg was given by subcutaneous administration 50 minutes later. Anethole also stimulated gastric accommodation in rats (Asano et al., 2016).

Dexamethasone (2 mg/kg) and fennel essential oil at 200, 400 mg/kg, administered to rats by oral gavage for 5 consecutive days significantly reduced the macroscopic and microscopic lesions in acetic acid-induced colitis ($p<0.01$, $p<0.001$). In addition, these agents decreased the activity of MPO and the expression of TNF-α positive cells in the colon tissue compared to acetic acid group. Furthermore, they inhibited acetic acid-induced expression of p-NF-kB p65 protein (Rezayat *et al*., 2017).

Fennel essential oil significantly and dose-dependently reduced the intensity of oxytocin-induced contractions ($p<0.01$ at 50 μg/ml) and PGE_2-induced contractions ($p<0.01$ at 10 and 20 μg/ml) of the isolated rat uterus. The oil also reduced the frequency of contractions induced by PGE_2 (but not by oxytocin) (Ostad *et al*., 2001).

- *Hepatoprotective effect*

The hepatoprotective activity of steam distilled essential oil from fennel fruit was studied by Ozbek *et al.* (2003a) by using the carbon tetrachloride induced acute liver injury model in rats. When simultaneously administered with carbon tetrachloride, the fennel oil significantly reduced hepatotoxicity as shown by the decreased levels ($p<0.01$) of serum aspartate amino-transferase, alanine aminotransferase, alkaline phosphatase and bilirubin.

These results were confirmed by Ozbek *et al.* (2004) with a steam distilled essential oil from fennel fruit (main components: *trans*-anethole 74.8%; limonene 11.1%; eugenol 4.7%; fenchone 2.5%; alpha-pinene 1.3% and beta-ocimene 1.2%) administered to rats a few times a week for seven weeks, evaluating the above-mentioned biochemical markers as well as rat body weight and liver histopathology.

No such activity was detected by Ozbek *et al.* (2003b) in the diethyl ether extract obtained by maceration of fennel fruit for two hours, separation of the liquid phase and evaporation of the solvent (so-called 'fixed fennel oil').

The same injury model was used by Rabeh & Aboraya (2014) to assess the hepatoprotective activity of dill (*Anethum graveolens* L.) and fennel oil seeds in rats. Dill oil (1 mL/kg), fennel oil (1 mL/kg) and a mixture of both oils (0.5 mL/kg each) were given orally for 4 weeks. The hepatotoxicity induced by CCl_4 administration was found to be inhibited by either dill or fennel oil or by the mixture with evidence of significant ($p<0.05$) decrease levels of serum AST and ALT and significantly ($p<0.05$) increase the level of serum total protein, but with no effect on serum albumin. Moreover, dill and fennel oil supplementation induced suppression of the increased ALP activity with the concurrent depletion of raised bilirubins; treatment with either dill or fennel oil and their mixture significantly ($p<0.05$)

reversed the increase in MDA level and the decrease activity of SOD enzymes (Rabeh & Aboraya, 2014).

- *Hypotensive effect*

The angiotensin converting enzyme (ACE) inhibition and antioxidant activity of the oils obtained by hydrodistillation from powdered seeds of *Foeniculum vulgare* Mill and *Coriandrum sativum* L. was assessed *in vitro* by an UV method using hippuryl-L-histidyl-L-leucine (HHL) as substrate. GC-MS analysis of the essential oils showed that linalool (83.6%) was the major constituent of coriander oil and anethole (85.6%) of fennel oil. Coriander oil showed the higher ACE inhibition with an IC50 value of 34.8 ± 2.3 µg/mL, than fennel oil with an IC50 value of 40.7 ± 3.5 µg/mL. Both oils showed strong DPPH radical scavenging activity (Chaudhary et al., 2013).

- *Hypoglycemic effect*

A hypoglycemic effect was observed in alloxan-induced diabetic mice treated with steam distilled fennel essential oil, but not with fixed fennel oil (Ozbek, 2002; Ozbek *et al*., 2003b).

Nanoemulsions (NEs) formulated to contain 2% fennel essential oil (FEO), 5.6% oleic acid, 68% surfactant and co-surfactant mixed together Smix (1:1) and distilled water were tested at doses of 60 and 120 mg/kg together with FEO at the same doses in male Wistar rats with diabetes induced through intraperitoneal injection of STZ. The administration was done by topical application on an area of 6 cm^2 of rat dorsal area. The results indicated a non-significantly different antidiabetic effect between the four treatment groups throughout the first 4 h. They all showed a significant decrease in plasma glucose levels compared to diabetic control groups. A proof of the prolonged antidiabetic effect exhibited by NEs could be revealed where a significant difference is observed between NEs, at the two tested doses, and FEO up to 7 days. Plasma glucose of rats treated with NEs in a dose of 120 mg/kg was significantly lower than that of NEs with a dose of 60 mg/kg, with a glucose level of 80.8 mg/dl; this value was still significantly higher than the normal control rats in the present study (61.7 mg/dl after 7 days) (Mostafa et al., 2015).

- *Nephroprotective effect*

Mazaheri et al. (2013) studied the protective effect of fennel essential oil (FEO) on Cisplatin (CDDP)-induced nephrotoxicity in ovariectomized rats. Ovariectomized Wistar rats were divided into seven groups. Groups 1-3 received different intraperitoneal doses of FEO (250, 500, and 1000 mg/kg/day, respectively) for 10 days. Group 4 received saline for 10 days plus single dose of CDDP (7 mg/kg, intraperitoneally) at day 3. Groups 5-7 received FEO similar to groups 1-3, respectively; plus a single dose of CDDP (7 mg/kg, ip) on day 3. The serum levels of blood urea nitrogen (BUN) and creatinine (Cr), kidney tissue damage score (KTDS), and kidney weight (KW) and body weight changes in CDDP-treated groups increased significantly ($P < 0.05$). FEO did not reduce the levels of BUN and Cr, KTDS, and KW and body weight changes. Also, the serum and tissue levels of nitrite were not altered significantly by FEO. Therefore, FEO did not induce kidney damage. In addition, FEO similar to estrogen was not a nephroprotectant agent against CDDP-induced nephrotoxicity.

- *Cytotoxic effect*

The cytotoxicity of the fennel essential oil was tested against HeLa, Caco-2 (human colorectal adenocarcinoma), MCF-7 (human breast adenocarcinoma), CCRF-CEM (human T lymphoblast leukaemia) and CEM/ADR5000 (adriamycin resistant leukaemia) cancer cell lines by MTT assay. IC_{50} values were 207 mg/L for HeLa, 75 mg/L for Caco-2, 59 mg/Lfor MCF-7, 32 mg/Lfor CCRF-CEM, and 165 mg/L for CEM/ADR5000 cell lines. As compared to the positive control doxorubicin, the essential oil exhibits low

cytotoxicity (Sharopov et al., 2017).

The IC_{50} of fennel fruit essential oil against human breast cancer (MDA-Mb) and cervical epithelioid carcinoma (HeLa) cell lines determined by MTT assay was 0.68 and 1.26 µg/mL, respectively (Akhbari et al., 2018).

In addition, fennel fruit essential oil collected from the three types of different regions of Iran, including Kerman, Golestan, and East Azerbaijan Provinces, conferred inhibitory effect on MCF-7 cells (determined by MTT assay) at the concentrations of 50 µg/ml and 100 µg/ml ($P < 0.001$) increasing in a dose-dependent manner. Furthermore, the IC_{50} of F. vulgare essential oil was calculated to be 14.93, 11.34, and 15.93 mg/ml, respectively. The essential oil increased the expression of Bax, but decreased Bcl2 gene expression significantly ($P < 0.001$). (Ghasemian et al., 2020).

- *Other effects*

Fennel oil increased the pentobarbital-induced sleeping time in mice following simultaneous intra-peritoneal (i.p.) administration (Marcus and Liechtenstein, 1982).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

Animal studies have shown that key constistuents of bitter fennel fruit oil, such as fenchone and trans-anethole could be responsible for the expectorant activity of the essential oil. Indeed, fenchone augmented volume output of respiratory tract fluids whereas fenchone and anethole decreased its specific gravity in in vivo experiments on rabbits.

These effects are coherent with the long-standing use of fennel as expectorant in cough associated with cold.

In addition, fennel essential oil and *trans*-anethole have anti-inflammatory effects in several *in vivo* and *in vitro* studies acting on different inflammatory mediators such as by inhibiting 5-LPO, decreasing the level of LDH, IL-6 and TNF-α or reducing IL-6 and IL-10 mRNA expression.

Fennel fruit oil also exhibited *in vitro* strong inhibitory activities against the growth of a wide spectrum of bacteria and fungi known to be pathogenic for man and other species.

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

No data are available on absorption, metabolism and excretion of fennel oil in human beings or animals.

After oral administration of radioactively-labelled *trans*-anethole (as the methoxy-^{14}C compound) to 5 healthy volunteers at dose levels of 1, 50 and 250 mg on separate occasions, it was rapidly absorbed. 54-69% of the dose (detected as ^{14}C) was eliminated in the urine and 13-17% in exhaled carbon dioxide; none was detected in the faeces. The bulk of elimination occurred within 8 hours and, irrespective of the dose level, the principal metabolite (more than 90% of urinary ^{14}C) was 4-

methoxyhippuric acid (Caldwell and Sutton, 1988). *Trans*-anethole is metabolized in part to the inactive metabolite 4-methoxybenzoic acid (Schulz *et al*., 1998). An earlier study with 2 healthy subjects taking 1 mg of trans-anethole gave similar results (Sangster *et al*., 1987).

In mice and rats *trans*-anethole is reported to be metabolized by O-demethylation and by oxidative transformation of the C3-side chain. After low doses (0.05 and 5 mg/kg body weight (b.w.)) O-demethylation occurred predominantly, whereas higher doses (up to 1,500 mg/kg b.w.) gave rise to higher yields of oxygenated metabolites (Sangster *et al*., 1984a and 1984b).

Experimental studies on rats and mice showed that the anethole is completely absorbed but slowly after oral administration. The major metabolic pathways involve O-demethylation, oxidation of the C3-side chain, and conjugation with glucuronic acid, glycine, sulfate, and glutathione. The main metabolites of anethole are 4-methoxy-hippuric acid, 4-methoxy-benzoic acid, 4-hydroxypropenylbenzene, 2-hydroxy-1-methylthio-1-(4'-methoxyphenyl)-propane, 4-methoxy derivatives of acetophenone, cinnamic alcohol, and cinnamic acid. The elimination of anethole occurs within 48–72 h, and the major routes are renal, pulmonary, and fecal excretion. The metabolism and excretion of anethole are dose-dependent in animals. Low doses of anethole are mainly metabolized via O-demethylation and eliminated via exhalation as CO_2. With increasing doses, the metabolism of anethole involves side-chain oxidation and epoxidation, and the renal excretion predominates (Aprotosaie et al. 2016).

The metabolism of estragole was thoroughly studied in order to clarify the source of carcinogenicity. Four common main metabolites detected in the urine of rats and mice treated with [^{14}C]estragole were 1'-hydroxyestragole, 4-methoxycinnamyl alcohol, 4-methoxyphenyllactic acid, and 4-methoxyhippuric acid. In rats, most of the radioactivity (mean 54%) was exhaled as [^{14}C]CO_2, resulting from demethylation of the [^{14}C]estragole with the radioactive label at the methoxy group. Thus, p-allylphenol was probably one of the most abundant intermediate metabolites. Among multiple pathways resulting in reactive metabolites, the hydroxylation at the 1'-carbon to 1'-hydroxyestragole and subsequent sulfotransferase (SULT)-catalyzed conversion to 1'-sulfoxyestragole was the most important step. Recently, N-acetyl-S-[3'-(4-methoxyphenyl)allyl]-L-Cys (AMPAC) has been observed in human urine samples after consumption of fennel tea. The formation of this further metabolite has been supposed to derive by the detoxification of 1'-sulfoxyestragole by glutathione (Monien et al., 2019).

Investigations showing liver enzymes-inducing effects of compounds present in fennel oil strongly raise the possibility for interactions of fennel with other medicinal products to take place. Fennel was tested for its *in vitro* CYP1A2, 2D6, and 3A4 inhibitory potential. An aqueous extract of fennel was made from commercially available herbal products, and incubations were performed with recombinant cDNA-expressed human CYP enzymes in the presence of positive inhibitory controls. Metabolite formation was determined by validated LCMS/MS or HPLC methodologies. IC_{50} inhibition constants were estimated from CYP activity inhibition plots using non-linear regression. Inhibition was shown for fennel towards CYP2D6 and 3A4 with respective IC50 constants of 23 ± 2 and 40 ± 4 µg/ml. Based on the recommended dosing of the commercial herbal products investigated, clinically relevant systemic CYP inhibitions could be possible for fennel. In addition, fennel might cause a clinically relevant inhibition of intestinal CYP3A4 (Langhammer & Nilsen, 2013). However this *in-vitro* findings does not justify a specific mention in the monograph.

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

3.3.1. Single dose toxicity

Values of the oral LD_{50} corresponding to 3.8 g/kg b.w. (Opdyke, 1974) and 3.12 g/kg b.w. (von Skramlik, 1959) had been reported for fennel oil in rats, but in a more recent study the oral LD_{50} was estimated to be 1.326 g/kg b.w. (Ostad *et al*., 2001). In this last study, animals treated with the highest dose showed prostration, sedation, respiratory distress, movement disorders, unresponsiveness to external stimulation, hind limb weakness, tremor and fasciculation in dorsal muscles during the first 24 h from single dose ingestion. In treated animal groups with lower doses the most evident adverse effect was sedation. In all groups, the amount of 24h urine increased parallel to the amount of fennel oil administered.

The dermal LD_{50} in rabbits was estimated to be higher than 5,000 mg/kg (Opdyke, 1974). The dermal LD_{50} in rats was reported as > 2.5 g/kg and as 5 ml/kg (Opdyke, 1976).

Oral LD_{50} values per kg b.w. were determined for *trans*-anethole as 1.8-5 g in mice, 2.1-3.2 g in rats, and 2.16 g in guinea pigs; i.p. LD_{50} values for *trans*-anethole were determined as 0.65-1.41 g/kg b.w. in mice and 0.9-2.67 g/kg b.w. in rats (Lin, 1991).

Anethole activates the central nervous system and its excessive use may lead to convulsions (Zargari, 1991).

3.3.2. Repeat dose toxicity

Repeated dose toxicity studies with fennel oil were not available.

Male rats receiving 0.25% of anethole in their diet for 1 year showed no toxic effects, while other receiving 1% for 15 weeks had slight oedematous changes in liver cells (Hagan *et al*., 1967).

In 90-day experiments in rats, 0.1% of *trans*-anethole in their diet caused no toxic effects. However, dose-related oedema of the liver was reported at higher levels of 0.3%, 1% and 3%, which have no therapeutic value (Lin, 1991).

Rats given *trans*-anethole as 0.2, 0.5, 1 or 2% of their diet for 12-22 months showed no effects at any level on clinical chemistry, haematology, histopathology or mortality. Slower weight gain and reduced fat storage were noted at the 1% and 2% levels (Lin, 1991).

3.3.3. Genotoxicity

- *Mutagenicity*

Sweet fennel oil was found to be mutagenic in the *Bacillus subtilis* DNA-repair test, although it did not induce a significant increase in revertant numbers in the Ames test with *Salmonella thyphimurium* with and without metabolic activation; in addition, mutagenicity assay with *E. coli WP2 uvrA trp* was negative.(Sekizawa and Shibamoto, 1982). Fennel oil did not show any activity in the chromosomal aberration test using a Chinese hamster fibroblast cell line (Ishidate *et al*., 1984).

Essential oil of *F. vulgare* fruit showed antimutagenic activity in mouse models of experimentally cyclophosphamide (CP)-induced genotoxicity. Animals were administered two different doses of FEO (1 and 2 mL/kg) continuously for 3 days at intervals of 24 hours by the oral route before tissue sampling. The results showed that pre-treatments with FEO significantly inhibited the frequencies of aberrant

metaphases, chromosomal aberrations, micronuclei formation, and cytotoxicity in mouse bone marrow cells induced by CP and also produced a significant reduction of abnormal sperm and antagonized the reduction of CP-induced SOD, CAT, and GSH activities and inhibited increased MDA content in the liver. FEO inhibits genotoxicity and oxidative stress induced by CP (Tripathi et al., 2013).

The genetic damage and cytostatic effects of fennel powdered seeds (FSPw) and fennel seeds essential oil (FSEO) was assessed in HepG2 cells. According to the data obtained from the comet assay, after 4 h exposure, none of the tested concentrations of FSPw (10, 20 and 40 µg/ml) and FSEO (0.5, 1.0 and 2.0 µl/ml) induced a significant increase in percentage of DNA in the comet tail (*i.e.* tail intensity, %), as compared to control (untreated) cells. The induction of chromosomal aberrations was examined using the CBMN test; after 24 h of exposure to the tested compounds, the frequencies of MN in treated cells did not vary significantly from those of the respective controls, whereas positive control cells showed significantly increased micronucleated cell frequencies (MN ‰ = 11.67 ± 1.09; $p < 0.05$). In addition, FSPw failed to induce apoptosis and cell cycle perturbation, confirming the absence of DNA damage, whereas FSEO showed a dose-dependent increase in the proportion of early ($p = 0.001$) and late ($p = 0.001$) apoptotic cells. FSEO caused a dose-dependent decrease of the proportion of G0/G1 cells ($p = 0.001$) with a concurrent accumulation of cells at G2/M phase ($p = 0.002$) (Levorato et al., 2018).

- *Anethole*

Tested in *Salmonella* mutagenesis assay and also in mouse lymphoma L5178Y $TK^{+/-}$cell mutagenesis assay, anethole was inactive in *Salmonella thyphimurium* tester strains TA1535, TA1537, TA15358, TA98 and TA 100 and was active in the mouse lymphoma assay only with Aroclor 1254-induced rat liver S9 activation (Heck *et al.*, 1989).

In the *Salmonella*/microsome mutagenicity assay with Aroclor 1254-induced rat liver S9 activation performed with *Salmonella thyphimurium* tester strains TA1535, TA1537, TA15358, TA98 and TA 100 showed that anethole may have a very weak activity for strain TA100; however, no obvious dose-related response can be found (Hsia *et al.*, 1979).

Mortelmans *et al.* (1986) reported negative results of mutagenicity testing of anethole performed in the *Salmonella* pre-incubation assay, which is a modification of the standard plate incorporation assay, using four *Salmonella* strains (TA1535, TA1537, TA98 and TA100) in the presence and absence of rat and hamster Aroclor 1254-induced liver S9 activation.

The mutagenic activities of anethole and its metabolite 3'-hydroxyanethole were studied using three tester strains of *Salmonella thyphimurium (*TA1535, TA00, TA98). Addition of an NADPH-generating system and liver S13 fraction from Aroclor-treated rats (6.8 mg liver/protein plate) to the incubation mixture of TA100 tester strain increased mutagenic activities. Approximately 45 revertants were obtained per µmole of anethole. Under the same conditions, 3'-hydroxyanethole showed no significant mutagenic activity with less than 7 µmoles/plate. Above this concentration the S13-mediated mutagenicity increased linearly with increased doses up to 15 µmoles/plate (about 1000 revertants with 15 µmoles/plate) (Swanson *et al.*, 1979).

Five strains of *Salmonella thyphimurium* (TA1535, TA1537, TA1538, TA98 and TA100) with and without S9 fractions from Aroclor 1254-induced rats were used to study potential mutagenic effects of *trans*-anethole. The lowest overtly toxic concentration for *trans*-anethole was 1 mg/plate. No mutagenic activity was observed at concentration of up to 50 µg *trans*-anethole/plate with or without metabolic activation. However the addition of 3'phosphoadenosine- 5' phosphosulphate (PAPS) to the microsomal assay markedly increase the mutagenicity of trans-anethole in TA1535 tester stain, The

mutation rate observed was approximately 4, 5, 10, 11, 9 and 3 times that of the background rate at *trans*-anethole concentrations off 0.05, 0.20, 1.0, 5.0, 15.0 and 50.0 µg/plate respectively (To *et al.*, 1982).

Gorelick in 1995 reviewed nine previously conducted gene mutation studies (Heck *et al.*, 1989; Hsia *et al.*, 1979; Marcus & Liechtenstein, 1982; Mortelmans *et al.* 1986; Nestmann *et al.*, 1980; Sekizawa & Shibamoto, 1982; Swanson *et al.*, 1979; To *et al.*, 1982) and repeated the *Salmonella* /microsome test as well as the L5178Y mouse lymphoma TK +/-assay to ascertain their reproducibility and relevance. In the nine studies reviewed, anethole was uniformly negative in the *Salmonella* tests to detect base-pair substitutions or frameshift mutations without metabolic activation and this was also the case in four studies with metabolic activation after careful consideration of all experimental conditions. The studies which suggested a weak mutagenic potential of anethole (Marcus & Liechtenstein, 1982; Swanson *et al.*, 1979; Mortelmans *et al.*, 1986; Sekizawa & Shibamoto, 1982) were the result of the use of non-standard protocols (using longer pre-incubation times, excessive quantities of S-9 protein and/or the addition of co-factors) and have also been found to be irreproducible (Gorelick, 1995).

Gorelik (1995) reported dose-dependent response of *trans*-anethole only in the mouse lymphoma assay with metabolic activation. Anethole was found to be mutagenic in the mouse lymphoma assay which is known for its extreme sensitivity and poor selectivity for genotoxicity also by other authors (Heck *et al.*, 1989; Caldwell, 1993).

Other results showing the absence of mutagenic potential of anethole include assays in *Escherichia coli* (Sekizawa & Shibamoto, 1982) and in *Saccharomyces cerevisiae* (Nestmann & Lee, 1983).

A mouse micronucleus assay was negative, with no micronuclei found at 6 and 30 hours after anethole i.p. administration to groups of 5 male and 5 female mice in two doses of 0.25 or 0.5 g/kg b.w. (Marzin, 1979 in Lin, 1991). Similarly no significant increase in genotoxicity was observed in the mouse bone marrow micronucleus test after the oral pre-treatment of mice with trans-anethole at 40-400 mg/kg b.w. 2 and 20 hours before i.p. injection of genotoxins; a moderate, dose-dependent protective effects against known genotoxins such as cyclophosphamide, pro-carbazine, N-methyl-N'-nitrosoguanidine, urethane and ethyl methane sulfonate was observed ($p<0.05$ to $p<0.01$ at various dose levels) (Abraham, 2001).

Very low levels of DNA adducts (<1.4 pmol/mg DNA) were observed after administration of anethole to mice, whereas 150 and 220 times as many adducts were detected following administration of safrole and estragole, respectively (Phillips *et al.*, 1984).

Unscheduled DNA synthesis (UDS) assays in rat hepatocytes did not indicate any mutagenic potential of anethole (Howes *et al.*, 1990; Muller *et al.*, 1994).

Anethole has three primary metabolites in the rat and the pathway of toxicological concern is that of epoxidation of the 1,2 double bond at the side chain; in fact, 3'-hydroxylation does not result in genotoxicity or marked cytotoxicity and O-demethylation is a detoxication reaction (Sangster *et al.*, 1984a and 1984b; Bounds & Caldwell, 1996). Cytotoxicity of anethole is enhanced when the cellular epoxide defence mechanisms of conjugation with reduced glutathione and hydration by cytosolic epoxide hydrolase are severely compromised. However, modulation of epoxide metabolism by the same mechanism in cultured cells failed to induce UDS by anethole nor was there a UDS response in hepatocytes of female rats dosed with anethole *in vivo* (Marshall & Caldwell, 1996). The synthetic epoxide of anethole was also tested and found to be cytotoxic, but not genotoxic. The lack of induction of UDS by anethole epoxide provided a further support to the hypothesis that marginal

hepatocarcinogenesis observed in female rats given 1% anethole in the diet for 121 weeks was not initiated by a genotoxic event (Marshall & Caldwell, 1996).

In the 51st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) a document on safety evaluation of *trans*-anethole was prepared; the conclusions were that *trans*-anethole and its metabolites are unlikely to be genotoxic *in vivo*; the cytotoxic metabolite, anethole epoxide, was suggested to be the possible causative agent of the hepatotoxic effect observed in pre-clinical studies in rats. The report of JECFA allocated the acceptable daily intake (ADI) at the dose of 0-2 mg/kg b.w. on the basis of scientific pre-clinical data published on *trans-anethole* (JECFA, 1999).

In 1999 the USA Expert Panel of FEMA (Flavour and Extract Manufacturers' Association) released a review of scientific data relevant to the safety evaluation of *trans-anethole* as a flavouring substance. The review concluded that trans-anethole does not represent a carcinogenic risk to humans and can be "generally recognized as safe" (GRAS) at low level of intake (54 μg/kg b.w./day) (Newberne *et al.*, 1999).

- *Estragole*

Estragole usually is a minor constituent of fennel oil but sometimes may be contained in important amounts in wild bitter fennel essential oils from the Mediterranean region (Kalleli, 2019). European Pharmacopoeia limits the content of estragole in bitter-fennel fruit oil monograph to max 6%.

The genotoxicity of estragole has been assessed in the Public Statement on the use of herbal medicinal products containing estragole (EMA/HMPC/137212/2005 Rev 1)

The genotoxic mechanisms of estragole contained in the fennel oil might be counteracted by detoxification mechanisms (see above Monien et al., 2019) and antimutagenic abilities of other fennel oil components (see above Tripathi et al., 2013), but further evidence is needed to confirm these findings.

3.3.4. Carcinogenicity

Carcinogenicity studies with fennel oil were not available.

Anethole

From a series of studies investigating the effect of anethole when added to female CD-1 mice diet or given orally or by i.p. injection to male pre-weaning B6C3F1 mice, Miller et al. (1983) concluded that anethole was not a hepato-carcinogen; although these studies were not carried out for test animal lifetimes. Safrole and estragole were found to be highly active as liver carcinogens in both these tests.

In another bioassay carried out in Sprague–Dawley (SD) rats, 0.25, 0.5, or 1.0% anethole was administered in the diet for 121 weeks. Results showed the occurrence of a small, but statistically significant, incidence of hepatocellular carcinomas in female rats receiving 1% anethole (Truhaut et al., 1989). These hepatocellular carcinomas were associated with other changes to the liver (increase in relative liver weight) similar to those observed after enzyme induction (Newberne et al., 1989) and were considered not to be caused by a direct genotoxic effect of *trans-anethole* (Lin, 1991). Reed & Caldwell (1992) also showed that i.p. administration of anethole to SD rats increased liver weight, microsomal protein and cytochrome P-450 content.

In Swiss albino mice with Ehrlich ascites tumour (EAT) in the paw, anethole administered orally at 500 or 1000 mg/kg on alternate days for 60 days significantly and dose-dependently reduced tumour weight ($p<0.05$ at 500 mg/kg, $p<0.01$ at 1000 mg/kg), tumour volume ($p<0.01$ at 500 mg/kg, $p<0.001$ at 1000 mg/kg) and body weight ($p<0.05$ to 0.01) compared to EAT-bearing controls. Mean survival time increased from 54.6 days to 62.2 days (500 mg/kg) and 71.2 days (1,000 mg/kg). Histopathological changes were comparable to those after treatment with cyclophosphamide (a standard cytotoxic drug). These and other results demonstrated the anti-carcinogenic, cytotoxic and non-clastogenic nature of anethole (Al-Harbi *et al*., 1995).

Anethole at a concentration below 1 mM has been shown to be *in vitro* a potent inhibitor of tumour necrosis factor (TNF)-induced cellular responses, such as activation of nuclear factor-kappa B (NF-κB) and other transcription factors, and also to block TNF-induced activation of the apoptotic pathway. This might explain the role of anethole in suppression of inflammation and carcinogenesis (Chainy *et al*., 2000).

- *Estragole*

The carcinogenicity of estragole has been assessed in the Public Statement on the use of herbal medicinal products containing estragole (EMA/HMPC/137212/2005 Rev 1).

For estragole, metabolic activation pathway and DNA adduct formation are amply demonstrated in animals (mice and rats) and the same pathway is operative in human *in vitro* systems. There is general consensus that adduct formation is causally related to tumorigenesis, unless there are specific and biologically persuasive reasons to the contrary. Consequently, the mode of action for tumour formation is relevant for humans (EMA/HMPC/137212/2005 Rev 1).

The major metabolic pathways of estragole have been well characterised in rats and mice *in vitro* and *in vivo* and studies have been published on *in vitro* metabolism of estragole in human hepatic preparations. Three major metabolic pathways have been established:

1) O-demethylation resulting 4-allylphenol and more distal metabolites (identified as a detoxification pathway);

2) 1'-hydroxylation, which is a proximal active metabolite undergoing sulfoconjugation to 1'-sulfooxyestragole capable of binding to DNA and protein;

3) epoxidation of the allyl side chain leading to estragole-2',3'-epoxide, which is rapidly metabolised 145 by epoxide hydrolase and glutathione transferase to detoxified metabolites (also identified as a detoxification pathway)

Although toxicokinetics and metabolism of estragole have not been thoroughly studied in humans, there is evidence that under *in vivo* administration of estragole to humans, the liver is exposed to the compound and the first step in metabolic activation, the formation of 1'-hydroxyestragole, takes place. In the view of Puns *et al*. (2009), in spite of significant differences in the relative extent of different metabolic pathways between human and male rat, it is probable that there is a minor differences on the ultimate overall bioactivation of estragole to 1'-sulfooxyestragole. As the processess observed *in vitro* on humans liver microsomes, have some similarities to those in rodents (Jeurissen *et al*. 2007) in which carcinogenicity has been observed, the extrapolation can be regarded as plausible (EMA/HMPC/137212/2005 Rev 1).

3.3.5. Reproductive and developmental toxicity

Trans-anethole exerted a dose-dependent, anti-implantation activity after oral administration to adult female rats on days 1-10 of pregnancy. When compared with control animals (all of which delivered normal offspring on completion of term), *trans*-anethole administered at 50, 70 and 80 mg/kg b.w. inhibited implantation by 33%, 66% and 100% respectively. Further experiments were conducted with the 80 mg/kg dose at different stages of pregnancy. When rats were administered *trans*-anethole on days 1-2 of pregnancy, normal implantation and delivery occurred; however rats administered anethole on days 3-5 of pregnancy, implantation was completely inhibited; and in those given *trans*-anethole on days 6-10 of pregnancy three out of five rats failed to deliver at term. No gross malformations of offspring were observed in any of the groups. The results demonstrated that *trans*-anethole has antifertility activity. From comparison with the days 1-2 group (lack of antizygotic activity), the lower level of delivery in the days 6-10 group was interpreted as a sign of early abortifacient activity (Dhar, 1995).

Ostad *et al*. (2004) exposed cultivated limb bud cells obtained from day 13 rat embryo were cultivated and exposed to concentrations of fennel essential oil between 0.0186 and 9.3 mg/ml for 5 days at 37°C. A significant reduction in the number of stained differentiated foci due to cell loss rather than decreased cell differentiation was observed in the presence of fennel essential oil. These findings were interpreted as a toxic effect of fennel essential oil on foetal cells with no evidence of teratogenicity.

A well-established feto-placental co-culture system has been used to study *in vitro* the potential interference of fennel essential oil with feto-placental steroidogenesis. The fetal compartment (adrenal zone and liver) was represented by H295R human adrenocortical carcinoma cells and BeWo human placental choriocarcinoma cells were used to represent the placental trophoblast compartment. After a 24 hours exposure to the cells, the fennel seed oil at concentrations ranging from 0.0005% to 0.005%, significantly increased hormone concentrations of estradiol, estrone, dehydroepiandrosterone (DHEA), androstenedione, progesterone, and estriol. Fennel oil significantly altered the expression of steroidogenic enzymes involved in cholesterol transport and steroid hormone biosynthesis, including *StAR, CYP11A1, 3β-HSD1/2, SULT2A1, and HSD17β1, -4,* and *-5*. Also, fennel seed oil stimulated placental-specific promoter I.1 and pII-derived CYP19 mRNA in BeWo and H295R cells, respectively, as well as, increased CYP19 enzyme activity (Yancu & Sanderson, 2019).

The same authors, used the same feto-placental co-culture system to investigate *in vitro* the potential effects of *trans*-anethole and estradiole on steroidogenesis. After a 24 h exposure of the co-culture to 2.5, 5.2 and 25 μM estragole or *trans*-anethole, hormone concentrations of estradiol, estrone, dehydroepiandrosterone, androstenedione, progesterone and estriol were significantly increased. Using RT-qPCR, estragole and *trans*-anethole were shown to significantly alter the expression of several key steroidogenic enzymes, such as those involved in cholesterol transport and steroid hormone biosynthesis, including *StAR, CYP11A1, HSD3B1/2, SULT2A1, and HSD17B1, -4,* and *-5* (Yancu et al., 2019).

To uncover the fennel-derived essential oil (FVEO)-induced effects on male reproductive potential, 24 mature male albino mice (average weight 30-35 g) were divided into, control, 0.37, 0.75, and 1.5 mg/kg FVEO-received groups. Following 35 days, the animals were euthanized and the testicular tissue and sperm samples were collected. FVEO, dose dependently, increased histological damages, resulted in germ cells dissociation, depletion, nuclear shrinkage and significantly ($P<0.05$) decreased tubular differentiation and spermiogenesis ratios. Moreover, the FVEO-received animals (more significantly in 1.5 mg/kg-received group) exhibited decreased sperm count, viability, and motility and represented enhanced percentage of sperms with decondensed chromatin and DNA fragmentation. Finally, the

animals in FVEO-received group showed diminished zygote formation and represented decreased pre-implantation embryo development compared to control animals (Minas et al., 2018).

3.3.6. Local tolerance

Not applicable

3.3.7. Other special studies

No data available

3.3.8. Conclusions

Very limited and non-conclusive information on fennel oil toxicity derives from single and repeated-dose toxicity studies due to the limited number of studies and the small number of tested animals.

Fennel oil did not show any activity in the chromosomal aberration test using a Chinese hamster fibroblast cell line. On the other hand, more recent studies have shown that fennel oil had antimutagenic activity in mouse models of experimentally cyclophosphamide (CP)-induced genotoxicity and it did not show to be genotoxic in Comet assay after 4 h of treatment in HepG2 cells at 0.5, 1.0 and 2.0 µl/ml.

In vitro and *in vivo* studies showed a weak mutagenic potential of anethole. However, taking into consideration the more recent results of the *Salmonella* tests repeated with the updated protocols as well as the results from the other genotoxicity studies it is considered that the positive response of anethole observed in the mouse lymphoma assay is most likely to be via a non-DNA mechanism. In addition, *trans*-anethole is reported as "generally recognised as safe" at the intake of 54 µg/kg b.w./day) and the ADI is about 0-2 mg/kg b.w. based on JECFA assessment (JECFA, 1999).

There is no evidence of carcinogenicity for fennel oil. The genotoxicity and the carcinogenicity of estragole have been evaluated by HMPC and presented in the Public Statement on the use of herbal medicinal products containing estragole (EMA/HMPC/137212/2005 Rev 1). Estragole is carcinogenic via DNA adduct formation; this mode of action for tumour formation is relevant for humans. There is also evidence that under *in vivo* administration to humans, estragole can be metabolically activated to 1'-hydroxyestragole in liver, probably in a similar way to rodents in which carcinogenicity has been observed; therefore, the extrapolation can be regarded as plausible. (EMA/HMPC/137212/2005 Rev 1)

Trans-anethole, which is known to be a phytoestrogen, plays a relevant role in the anti-fertility effects of fennel as confirmed by studies carried out with fennel oil.

Adequate reproductive toxicity studies with fennel oil have not been carried out.

In vitro studies have shown the potential of fennel oil to disrupt steroidogenic enzyme activity and expression in a co-culture model composed of fetal-like adrenocortical (H295R) and placental trophoblast-like (BeWo) cells, but adequate *in vivo* studies to confirm these effects have not been carried out. In a small *in vivo* study on male mice, fennel oil at HED of approximately 6 mg, adversely impacted the fertilization potential and decreased pre-implantation embryo development. The findings of this study can be considered as preliminary and should be further investigated.

3.4. Overall conclusions on non-clinical data

The non-clinical data make plausible the traditional use of bitter fennel essential oil as an expectorant in cough associated with cold.

The medicinal use of fennel is largely due to antispasmodic, secretolytic, secretomotor and inflammatory effects of its essential oil.

This indication is also made plausible by the expectorant effects exhibited by anethole, a main component of bitter fennel essential oil.

Fennel oil was found to be mutagenic *in vitro* in only one test although more recently it showed antimutagenic activity in mouse models of experimentally cyclophosphamide (CP)-induced genotoxicity and it did not show to be genotoxic in Comet assay after 4 h of treatment in HepG2 cells at 0.5, 1.0 and 2.0 µl/ml.

Results from studies carried out in the laboratory animals showed a weak mutagenic potential of anethole. *Trans*-anethole is reported as "generally recognised as safe" at the intake of 54 µg/kg b.w./day) and the ADI is about 0-2 mg/kg b.w. (JECFA, 1999). In the case of bitter fennel oil the daily intake of anethole is above the ADI established by JECFA when taken according the posology reported in literature (200 microliters/day), but no specific concern is expected taking into account the short duration of use (not more than one week).

Though there is no evidence of carcinogenicity for fennel oil, studies have shown the carcinogenic effects of estragole and 1'-hydroxyestragole in mice and rats (liver tumours). These evidences are considered relevant also for humans. Therefore, the EMEA/HMPC assessment in the 'Public statement on the use of herbal medicinal products containing estragole' (EMA/HMPC/137212/2005 Rev 1) concluded that the intake of estragole from (T)HMPs in the general population should be as low as possible, which includes a short-time duration of use (maximum 14 days) and a discussion about the single / daily doses necessary according to the risk assessment relevant for the concerned (T)HMP. For example, to reach or come as close as possible to the guidance value of 0.05 mg/person per day, the lowest dose should be consistently selected if ranges of single and daily doses are available from traditional use. Furthermore, 'low estragole plant varieties' should be recommended or a calculated adequate limitation of the estragole content in the specification of the herbal medicinal products should be made.

For children, the EMEA/HMPC assessment in the 'Public statement on the use of herbal medicinal products containing estragole' (EMA/HMPC/137212/2005 Rev 1) concluded that the use of estragole containing (T)HMPs in children is not recommended if the daily intake of estragole exceeds the guidance value of 1.0 µg/kg bw, unless otherwise justified by a risk assessment based on adequate safety data.

According to the posology reported in literature for bitter fennel essential oil in adults, and based on its density, the maximum amount of estragole is 11.5-11.7 mg/day (for an adult of 50 kg), calculated taking into account a 6% content of estragole in the essential oil approved by the European Pharmacopoeia. This daily intake of estragole (see EMA/HMPC/137212/2005 Rev 1) would expose patients to safety concerns which are not balanced by the beneficial effects in the therapeutic indications supported by the evidence of traditional use, taking into account that other safer therapeutic options, including herbal preparations from several plants, are available on the European market.

With the use of the minimum dose of 6.5 mg/day of fennel essential oil a very low content of estragole would be required to esure a daily intake below the guidance value of 1.0 µg/kg bw set by the HMPC public statement. Therefore, the use of fennel essential oil in children between 1-12 years is not recommended.

The use of bitter fennel oil is contraindicated in children under 1 year of age because of the lack of data and because of the presence of estragole.

In conclusion, taking into account the conclusions and the recommendations of the revised "Public Statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1), and taking into account the high intake of estragole when bitter fennel oil is taken as an expectorant in cough associated with cold according to the posologies supported by evidence of traditional use, the monograph for bitter fennel oil has not been upheld; therefore, a Public statement is published (see Conclusions).

The body of the data indicates that reproductive system is a target for the action of fennel oil, and its principal constituent *trans*-anethole, which may cause changes in male and female organs and tissues involved directly or indirectly in the reproductive mechanisms. Consequences of these changes are not easily predictable or detectable in humans. In addition, most of the reproductive toxicity studies were carried out on a limited number of animals tested. NOAELs were not determined in these studies.

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

No data available

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

No data available for fennel oil in humans. Few information is only available for *trans*-anethole.

To date very little is known about the metabolism of *trans*-anethole by humans. Caldwell's research group published two articles on metabolism of trans-anethole in humans, both including essentially the same experiments (Sangster *et al.*, 1987; Caldwell & Sutton, 1988). The fundamental conclusion of the authors regarding these experiments is only that "the pattern of urinary metabolites of trans-anethole is unaffected by dose size". Any consideration on the risk influence is lacking. These Caldwell's experiments show essentially the difference in anethole metabolism between rodents and humans.

After oral administration of radioactively-labelled trans-anethole (as the methoxy-^{14}C compound) to 5 healthy volunteers at dose levels of 1, 50 and 250 mg on separate occasions, it was rapidly absorbed. 54-69% of the dose (detected as ^{14}C) was eliminated in the urine and 13-17% in exhaled carbon dioxide; it was not detected in the faeces. The bulk of elimination occurred within 8 hours and, irrespective of the dose level, the principal metabolite (more than 90% of urinary ^{14}C) was 4-methoxyhippuric acid (Caldwell & Sutton, 1988). Trans-anethole is metabolized in part to the inactive metabolite 4-methoxybenzoic acid (Schulz *et al.*, 1998). An earlier study with 2 healthy subjects taking 1 mg of trans-anethole gave similar results (Sangster *et al.* 1987).

In humans, anethole is mainly metabolized to anisic acid, p-hydroxybenzoic acid, and 4-methoxy-hippuric acid. Anethole metabolites are eliminated within 8 h after anethole administration, and the major routes include renal excretion (54–69 %) and exhalation (13–17 %) (Aprotosaie et al. 2016).

4.2. Clinical efficacy

4.2.1. Dose response studies

No data available

4.2.2. Clinical studies (case studies and clinical trials)

- *Primary dysmenorrhoea*

A randomized parallel-group clinical trial was conducted to evaluate the effects of oral fennel drops (containing 2% fennel essential oil) for treating primary dysmenorrhea. Sixty college students suffering from primary dysmenorrhea were randomly assigned to two groups and followed up for two cycles. $P < 0.05$ was considered to be statistically significant. Parametric and non-parametric tests were adopted. Comparison of pain intensity in the two groups showed that there was no significant difference in pain relief between the two groups. Comparison of bleeding severity in the study group before and after intervention was demonstrated from the first day to the fifth day (PV on first day, second day, third day, fourth day, and fifth day 0.948, 0.330, 0.508, 0.583, 0.890, respectively): only on the fifth day of menstruation, the severity of menstruation bleeding significantly increased in the study and control groups (PV = 0.001) and only one case had severe menstruation after taking fennel drops. Also, a comparison of bleeding severity in the study group before and after intervention showed that 45% of the subjects who had taken fennel had severe bleeding in their cycle, which is probably due to the effect of muscular relaxation of fennel. The study confirms that fennel can be effective in reducing the severity of dysmenorrhea. It was signalled that fennel an unpleasant taste in view of most of the volunteers, that suggests the use of capsules or tablets rather than drops (Bokaie *et al*., 2013).

Pattanittum et al. (2016) carried out a systematic review to determine the efficacy and safety of dietary supplements for treating dysmenorrhoea. The authors included parallel group or crossover randomised controlled trials (RCTs) of dietary supplements for moderate or severe primary or secondary dysmenorrhoea. They excluded studies of women with an intrauterine device. The effectiveness was evaluated using pain scores (all on a visual analogue scale (VAS) 0 to 10 point scale) or rates of pain relief, or both, at the first post-treatment follow-up. Four RCTs with fennel were included: fennel extract (46 mg) versus placebo every six hours (Moslemi 2012), fennel capsule 30 mg every four hours versus no treatment (Ghodsi 2014), fennel oil 1% or 2% (0.3 to 1 mL) versus placebo, as required no more than four-hourly (Khorshidi 2003), and fennel 20 to 30 drops every four to eight hours versus placebo (Nazarpour 2007). Also, clinical trials comparing fennel versus NSAIDs were identified: fennel 20 to 30 drops every four to eight hours versus mefenamic acid 250 mg every six hours (Nazarpour 2007) and fennel 2% versus mefenamic acid 250 mg (Bokaie 2013). Finally, two clinical studies which compared fennel to Vitamin E were included: fennel extract (46 mg) versus vitamin E (100 units) every six hours (Moslemi 2012) and fennel extract (60 mg) versus vitamin E (150 IU), four times a day (Nasehi 2013). For treating primary dysmenorrhoea, there was no consistent evidence of effectiveness for fennel. When fennel was compared to NSAIDs, there was no evidence of a difference. In addtion to the lack of high methodological quality of the studies included (e.g. small sample sizes, failure to report study methods) the authors pointed out that most included trials of primary dysmenorrhoea recruited university students and all included studies were conducted

in low and middle-income countries, predominantly in Iran. The applicability of the evidence to women in other contexts is uncertain (Pattanittum et al. 2016).

Recently a further systematic review supported by a meta-analysis included four clinical trials which assessed the effect of fennel on the amount of menstrual bleeding: 46 mg of hydroalcoholic extract of fennel seed, or placebo, daily 5 capsules during the first 3 days of menstruation (Akhavan Amjadi 2010), 25 drops of oral fennel drop 2 % or no treatment (Bokaie 2014), fennel oil 1% or 2% (0.3 to 1 mL) versus placebo, as required no more than four-hourly (Khorshidi 2003), and 30 drops of fennel every 4 hours, 40 drops of *Vitex agnus-castus* every 4 hours, 250 mg of mefenamic acid or placebo (Shobeiri 2014). Meta-analysis results showed that using fennel significantly increases mean menstrual bleeding in the first cycle after treatment in the intervention group compared to the control (Std. mean difference: 0.46; 95 % CI: 0.18–0.73; p = 0.001). Of these four articles, only those conducted by Akhavan Amjadi et al. and Shobeiri et al. had assessed the amount of bleeding in the second cycle after treatment, and therefore, only these two articles were included meta-analysis for assessing the effect of fennel on menstrual bleeding in the second cycle after treatment. Fennel has no significant effect on the amount of menstrual bleeding in the second cycle after treatment in the intervention group compared to the control (Mean difference: 1.44; 95 % CI: −5.09 to 7.96; p = 0.67). The authors admitted the poor quality of articles and concluded that further clinical trials are necessary to determine the effect of fennel on menstrual bleeding (Abdollahi et al. 2018).

- *Amenorrhoea*

The efficacy of fennel and low-dose combined oral contraceptive (LD-COC) on inducing menstrual bleeding and method continuation was assessed in Iranian women using depot medroxyprogesterone acetate (DMPA) who had no menstrual bleeding within the previous 45 to 140 days. In this double-blind double-dummy trial, 78 married women referred to public health centers in Hamadan, Iran, who complained of menstrual cessation induced by DMPA were randomly assigned into fennel esssential oil, LD-COC or placebo groups with an allocation ratio of 1:1:1. All participants received two fennel or placebo capsules and one placebo or LD-COC pill (each containing 30 µg ethinylestradiol and 150 µg levonorgestrel) daily for 21 days. The essential oil was derived from fennel seeds using steam distillation. The % wt/wt formulation of fennel soft capsule 100 mg was 30% FEO (containing 71–90 mg anethole), 0.02% butylated hydroxy toluene combined with q.s. to 100% wt/wt sunflower oil. The placebo capsules were of a similar shape and size, but fully filled with sunflower oil. The primary outcome was experience of menstrual bleeding. The menstrual bleeding was evaluated using the Higham pictorial chart within 40 days following initiating intervention. Occurrence of menstrual bleeding was 73% among women receiving fennel and 81% among women receiving LD-COC, both of which were significantly higher compared to the placebo group (19%): relative risk (RR) 3.1 [95% confidence interval (CI) 1.6 to 6.2] and RR 4.2 (95% CI 1.9 to 9.4), respectively. However, there was no significant difference between the fennel and LD-COC groups (RR 0.8, 95% CI 0.4 to 1.4). Mean amount of menstrual bleeding among those who experienced menstruation was significantly higher in the fennel group (21 cc) than both the LD-COC (14 cc) and placebo (12 cc) groups. Also, women using fennel (73%) and LD-COC (65%) were significantly more likely than those using placebo (31%) to have subsequent DMPA injection [RR 2.5 (95% CI 1.3 to 4.9) and RR 2.0 (95% CI 1.1 to 3.7), respectively] (Mohebbi-kian et al. 2014).

- *Clinical studies in post-menopausal women*

In a triple-blind, placebo-controlled trial, 90 postmenopausal women aged 45 to 60 years in Teheran were randomly assigned to soft capsules containing 100 mg fennel (n=45) or placebo (n=45) groups (2 per day for each group) for 8 weeks. The participants were followed for 2 weeks post-intervention to assess the

continuance of the effect of intervention. Each 100 mg soft capsule contained 30% fennel essential oil (71-90 mg anethole) and 0.02% butylated hydroxytoluene, combined with qs to 100% wt/wt with sunflower oil. The inclusion criteria were married women in the first 1 to 5 years of the postmenopausal period (a woman was defined as postmenopausal beginning 1 year after her last menstrual period). They had to achieve a minimum score of 9 on the Menopause Rating Scale (MRS) questionnaire (medium severity of menopausal symptoms) and also had to have a negative history of physical or psychological disease, HT, CAM for menopausal symptoms, allergies to herbal medicine, sedative or antidepressant drug use, addiction, and smoking. Exclusion criteria were allergies to fennel/placebo, worsening of symptoms during the intervention, poor cooperation, failure to use fennel/placebo for a total of 6 days, and use of other remedies for menopausal symptoms during the study.The MRS questionnaire was used to assess changes in menopausal symptoms at baseline and at 4, 8, and 10 weeks after onset of intervention. In the intervention group, the mean MRS score was 20.02±6.18 at baseline, which decreased to 11.20±4.92 at week 4, 9.35±4.54 at week 8, and 13.05±4.94 at week 10. There was a significant statistical difference between the scores at baseline and weeks 4, 8, and 10 ($P<0.001$). In the control group, the mean MRS score was 20.37±5.51 at baseline, which decreased to 19.23±5.42 at week 4, 18.58±6.11 at week 8, and 19.20±5.97 at week 10. There were no significant differences between scores at baseline and at weeks 4, 8, and 10 in the control group. When the fennel and the placebo groups were compared, the independent t test showed significant differences in mean scores between groups at 4, 8, and 10 weeks ($P<0.001$) (Rahimikian et al. 2017).

The effect of fennel in anxiety and depression symptoms was evaluated in a double-blind, randomised, placebo-controlled trial in post-menopausal Iranian women. Inclusion criteria were: (1) post-menopausal status, which was defined as an age of over 40 years with no vaginal bleeding for 1 year and (2) conduction of a normal mammogram in the last year. The exclusion criteria were: (1) a history of endometrial or breast cancer, (2) allergy to fennel and (3) regular ingestion of phytoestrogen or soy-based products. Participants (n=60) were randomly assigned to take orally fennel capsules three times a day (every eight hour) or placebo. Each 100-mg fennel soft capsule contained 30% fennel (Standardised to 21–27 mg anethole) combined with sunflower oil. Then, symptoms of anxiety and depression were measured using Hospital Anxiety and Depression Scale (HADS) and Zung's Self Rating Depression Scale (SDS). After a three-month treatment, the score of HADS (depression and anxiety subgroups) and SDS did not show any significant decrease in the sample under study. Only the scores of anxiety domain obtained using the HADS questionnaire after the treatment showed a significant decrease of the mean score in the intervention group compared to placebo group ($p<0.001$) (Ghazanfarpour et al. 2018).

The same group of authors conducted a randomised clinical trial on 60 post-menopausal Iranian woman to evaluate the effects of orally administered fennel on vaginal atrophy. The study inclusion criteria were: (1) Postmenopausal status, defined as age 45 to 65 and the absence of vaginal bleeding for one year and (2) a normal mammogram obtained in the last year. The exclusion criteria were: (1) vaginal infection, (2) smoking, (3) the use of estrogen (systemic or vaginal) over the past 12 months or vaginal moisturizers or lubricants over the past six weeks, (4) history of endometrial or breast cancer, (5) allergy to fennel and (6) regular ingestion of phytoestrogen or soy-based products. The study interventions were the same as the study above-reported. The Maturation Vaginal Index and maturation values were measured once at baseline and again upon a three-month follow-up. The paired t-test showed statistically significant changes in the Maturation Vaginal Index (i.e. a decline in the parabasal cells and an increase in the intermediate and superficial cells) and maturation values in both the fennel and placebo groups at the end of the trial compared to at baseline. Nonetheless, no significant differences were observed in the percentages of the parabasal, intermediate and superficial cells, which was also the case for the maturation values (Ghazanfarpour et al. 2017a).

Finally, the same group of authors determined the effect of short-term treatment with fennel on bone density and lipid profile on 60 post-menopausal Iranian woman. Inclusion criteria were: 1) postmenopausal status, which was defined as an age of over 40 years with no vaginal bleeding for one year; 2) no regular ingestion of phytoestrogen or soy-based products (which was defined as consumption more than once a week) and 3) a normal mammogram in the last year. Exclusion criteria were: 1) use of any fluoride or bisphosphonates; 2) diseases or medications affecting bone metabolism; 3) current (or over past 6 months) use of estrogen or calcitonin; 4) history of endometrial or breast cancer; 5) any fracture, and 6) regular physical exercise or allergy to fennel. The study interventions were the same as the study above-reported. The dual energy X-ray absorptiometry was utilized to measure bone mineral density (BMD) and bone mineral content (BMC) of the spine, femoral neck, intertrochanter, and trochanter at the baseline and after three-month follow-up. The mean BMD and BMC at lumbar spine (P = 0.14, P = 0.504), total hip femoral (P = 0.427, P = 0.471), trochanter (P = 0.075, P = 0.07), intertrochanter, (P = 0.864, P = 0.932) and femoral neck (P = 0.439, P = 0.641) was not significantly different between the fennel and placebo groups. Total blood cholesterol, cholesterol fractions, and triglycerides were tested at the baseline, and after three-month follow-up. There was no significant difference in triglyceride (P = 0.679), total cholesterol (P = 0.103), low-density lipoprotein cholesterol (LDL-C; P = 0.146) and high-density lipoprotein cholesterol (HDL-C; P = 0.266) levels between the two groups. In addition, in both groups, a paired t–test showed no significant difference in all mentioned parameters, except for HDL-C, indicating significant borderline improvement (P = 0.052) in the fennel group (Ghazanfarpour et al. 2017b; Afiat et al. 2018a).

A double–blinded and placebo-controlled trial examined the fennel effect on Pittsburgh Sleep Quality Index (PSQI). Total score and relevant 7 components, including sleep duration, sleep latency, use of sleeping medication, subjective sleep quality, sleep disturbances, daytime dysfunction and habitual sleep efficiency among 50 menopausal Iranian women compared to control group within a 12-week follow-up. Each subscale is ranged from 0 to 3, and total PSQI score from 0 to 21, higher score indicating worse sleep quality. The study inclusion criteria were healthy post-menopausal women (women aged range of 45-65 years with no vaginal bleeding at least for a year), no history of systemic or topical estrogen taking during the last 6 months and a normal mammogram in the last year. Participants randomly assigned to soft capsules containing 100 mg fennel (n=25) or placebo (n=25) groups (3 times daily for each group) for three months. Each 100 mg soft capsule contained 30% fennel (standardized to 21-27 mg anethole) combined with sunflower oil. The mean actual sleep duration was 5 hours and 66 minutes. Intergroup comparison revealed no statistically significant differences in the mean total PSQI score (P = 0.439), subjective sleep quality (P = 0.826), habitual sleep efficiency (P = 0.127), sleep disturbances (P = 0.130), use of sleeping medication (P = 0.52) and daytime dysfunction (P = 0.439). A tendency toward significant between 2 groups was seen concerning the sleep duration (P = 0.059). Intergroup comparison showed significantly borderline levels (P = 0.059) (Afiat et al. 2018b).

- *Polycystic ovarian syndrome (PCOS)*

The effects of fennel essential oil capsules on PCOS symptoms was investigated in a double-blinded, randomized placebo-controlled study in 30 Iranian female students who were selected from the age range of 20–35 and met the Rotterdam diagnostic criteria for PCOS. The essential oil was produced by distilling the fennel seeds with water vapour. The manufacturer produced 15 fennel boxes that contained 168 capsules (46 mg) and 15 placebo boxes. The subjects were requested to use that drug for 12 weeks, twice a day (each 12 h) after meals. The ultrasonography assessments, body mass index, biochemical, and hirsutism variables were measured individually before and after three months usage of the fennel and placebo as our main outcome measurements. The comparison of menstruation cycle, hirsutism, BMI, biochemical, and ultrasonography measurements revealed that the interventions did not cause significant

differences in the two groups, except in the dehydroepiandrosterone sulphate (DHEAS) and both ovarian follicles number ($p < 0.05$) (Ghavi et al. 2019).

Table 6: Clinical studies on humans, in primary dismenorrhoea

Type	**Study**	**Test Product(s):**	**Number of Subjects**	**Healthy Subjects**	**Outcomes (primary and secondary endpoints)**	**Statistical analysis**	**Comments**
Treatment of primary dysmenorrhoea Comparative study vs. mefenamic acid Bokaie *et al*., 2013	Randomized parallel-group clinical trial Duration: 2 menstrual cycles Primary outcome: Intensity of pain reported by using a 10 - point linear Visual Analog Scale (VAS),(10-cm horizontal line) (Time point: before menstruation and the first day until the fourth day of menstruation) Secondary outcome:	Study: 25 drops of fennel preparation (containing 2% fennel essential oil) orally every 6 hour starting from the beginning of pain. If pain not reduced within 2 hours, 250 mg mefenamic acid every 6 hours if necessary Control group: 250 mg mefenamic acid every 6 hours if necessary.	60 female students, randomly (by use of random number table) divided in 2 groups of 30 students (study and control) Drop outs: 1 in the study group due to the odour and but taste of fennel drops.	18-25 years of age, living in a dormitory, nonsmoker, no systemic disease, not taking OCP and other hormonal and herbal drugs prior to and during menstrual cycle, a regular menstruation condition, and suffering from moderate to severe primary dysmenorrhea (exclusion criteria intolerance to fennel drop, no desire to take any of the treatments, and taking other NSAID (Non anti inflammatory drugs) through study)	Study group was compared with the control group and by themselves before and after the intervention. Primary outcome (pain relief): no significant difference in pain relief between the two groups: mean analgesic consumption in the study group before intervention was 6 and after intervention was 1 (significantly lower: P < 0.001); in the control groups was 5; only four subjects in the study group took mefenamic acid cap 250 mg. Number of mefenamic acid caps 250 mg was significantly lower in the study group compared to the control group (P	Statistical Analysis Software (SAS) version 16. *P*<0.05 considered statistically significant. Parametric and non-parametric tests such as Friedman, Wilcoxon, and Mann–Whitney were adopted. Mean differences of continuous parametric data analyzed	Limitation: not blinded, small sample size. The results support the traditional use in primary dysmenorrhoea.

Type	Study	Test Product(s):	Number of Subjects	Healthy Subjects	Outcomes (primary and secondary endpoints)	Statistical analysis	Comments
	menstruation bleeding. The time point was the first day to the fifth day of menstruation, and method of measurement was pad count				< 0.05) Secondary outcome (menstruation bleeding): Only 1 case had severe menstruation after taking fennel drops. 45% of the subjects who had taken fennel had severe bleeding in their cycle.	with Student's *t*-test and paired *t*-test	

Table 7: Clinical studies on humans, in amenorrhea

Type	**Study**	**Test Product(s):**	**Number of Subjects**	**Healthy Subjects or**	**Outcomes**	**Statistical analysis**	**Comments**
Induction of menstrual bleeding in depot medroxyprogesterone acetate-induced amenorrhea Mohebbi-kian *et al.* 2014	Double-blind double-dummy randomized, placebo-controlled, parallel clinical trial. The primary outcome was experience of menstrual bleeding. Secondary outcomes included receiving subsequent DMPA injection and amount of bleeding among those who had menstrual bleeding during the study.	Fennel soft capsule 100 mg was 30% FEO (containing 71–90 mg anethole), LD-COC pills (each containing 30 mcg ethinylestradiol and 150 mcg levonorgestrel) or placebo capsules/pills. All participants received two fennel or placebo capsules and one placebo or LD-COC pill daily for 21 days.	78 married women who complained of menstrual cessation induced by DMPA	Mean age of the participants was 28.5 (SD 4.0) years. The mean length of having no experience of menstrual bleeding was 94 (SD 20) days.	Occurrence of menstrual bleeding higher among women receiving fennel or LD-COC, compared to placebo (73% vs 81% vs 19%): RR 3.1 [95% CI 1.6 to 6.2] and RR 4.2 (95% CI 1.9 to 9.4), respectively. However, there was no significant difference between the fennel and LD-COC groups (RR 0.8, 95% CI 0.4 to 1.4). Total amount of menstrual bleeding among women who experienced menstruation in the fennel group (21 cc) higher than the placebo [mean difference 9, 95% CI 7 to 11] and LD-COC (MD 6, 95% CI 5 to 8) groups	Study power 80%. Analysis was done using the SPSS statistical package version 16. Chi-square test was used for the primary endpoint.	Overall, good methodological quality, although the effects of FEO should be confirmed in larger clinical trials. Population included in this study is highly selected (women who complained of DMPA-induced amenorrhea, thus is not possible estrapolation to all women with amenorrhoea

Table 8: Clinical studies on humans, in post-menopausal women

Type	**Study**	**Test Product(s):**	**Number of Subjects**	**Healthy Subjects or**	**Outcomes**	**Statistical analysis**	**Comments**
Management of menopausal symptoms in postmenopausal women. Rahimikian *et al.* 2017	Randomized, triple-blind, placebo-controlled clinical trial. Primary endpoint: reduction in MRS score.	100 mg soft capsule containing 30% FEO (71-90 mg anethole) or placebo capsule for 8 weeks 5 drop-outs in fennel group and 6 drop-outs in placebo group	90 women aged 45 to 60 years who were married and in the first 1 to 5 years of the postmenopausal period (at least score 9 on MRS).	Inclusion criteria: negative history of physical or psychological disease, HT, CAM for menopausal symptoms, allergies to herbal medicine, sedative or antidepressant drug use, addiction, and smoking. Main exlusion criteria: allergies to fennel/placebo during the intervention, failure to use fennel/placebo for a total of 6 days, and use of other remedies for menopausal symptoms during the study.	Significant reduction of MRS score in fennel group compared to baseline at weeks 4, 8 and 10 (11.20±4.92 at week 4, 9.35±4.54 at week 8, and 13.05±4.94 at week 10 vs 20.02±6.18 at baseline; P<0.001). Significant differences in the scores between fennel and placebo groups at weeks 4, 8, and 10, and scores in the fennel group were lower than the placebo group (11.20±4.92 vs 19.23±5.42 at week 4, 9.35±4.54 vs 18.58±6.11 at week 8, and 13.05±4.94 vs 19.20±5.97 at week 10; P<0.001).	Study power 80%. The data were analyzed PP using SPSS software (version 22). The independent t test was used to compare the means between groups, and the Friedman test was used to compare the means of total scores before and after treatment.	Main limitations of this study were its short duration and small sample size.

To evaluate the effect of oral fennel on anxiety and depression symptoms in post-menopausal women Ghazanfarpour *et al*. 2018	Double-blind, randomised, placebo-controlled trial Primary endpoints: reduction in SDS and HADS scores	100-mg fennel soft capsule contained 30% fennel (Standardised to 21–27 mg anethole) or placebo three times a day (every eight hour) for three months.	60 postmenopausal women randomly assigned to fennel (n=30) and placebo groups (n=30). The mean age of the patients in the intervention and control groups was respectively 57.04 ± 4.67 and 54.79 ± 4.22 years. By the end of the study data from 49 patients, 25 patients in the intervention group and 24 cases in the placebo group were analysed	Inclusion criteria were: (1) post-menopausal status, which was defined as an age of over 40 years with no vaginal bleeding for 1 year and (2) conduction of a normal mammogram in the last year. The exclusion criteria were: (1) a history of endometrial or breast cancer, (2) allergy to fennel and (3) regular ingestion of phytoestrogen or soy-based products.	After the intervention, the score of the two questionnaires did not show a significant decrease. After the treatment, only a significant decrease of the mean score in the anxiety domain of HADS was observed in the intervention group but not in placebo group ($p<.001$).	Study power 80%. Data analysis was performed by SPSS 11. The differences between the two groups assessed by Chi-square for categorical variables and independent t-test for continuous variables. The paired sample t-test used to compare the differences between the baseline and the three-month follow-up.	The results of this study did not reveal any positive effect of fennel on alleviating depression and anxiety in post-menopausal women.

To evaluate the effects of oral fennel on vaginal atrophy in post-menopausal women Ghazanfarpour *et al*. 2017a	Double-blind, randomised, placebo-controlled trial Primary outcome: Maturation Vaginal Index (MVI) which provides the percentages of parabasal, intermediate and superficial epithelial cells and maturation value (MV).	100-mg fennel soft capsule contained 30% fennel (Standardised to 21–27 mg anethole) or placebo three times a day (in the morning, in the midday and at night) for three months.	60 postmenopausal women randomly assigned to fennel (n=30) and placebo groups (n=30). The mean age of the patients in the intervention and control groups was respectively 55.6 ± 5.2 and 54.9 ± 6.4 years. Eight subjects dropped out. Three in the placebo group withdrew in the follow-up and five in fennel group were excluded because they reported side-effects.	Inclusion criteria were: (1) Postmenopausal status, defined as age 45 to 65 and the absence of vaginal bleeding for one year and (2) a normal mammogram obtained in the last year. The exclusion criteria were: (1) vaginal infection, (2) smoking, (3) the use of estrogen (systemic or vaginal) over the past 12 months or vaginal moisturizers or lubricants over the past six weeks, (4) history of endometrial or breast cancer, (5) allergy to fennel and (6) regular ingestion of phytoestrogen or soy-based products.	There were no significant differences in the percentages of the parabasal (P=0.191), intermediate (P=0.219) and superficial (P=0.82) cells, which was also the case for the MV (P=0.64). The vaginal pH remained unchanged in all the participants. The paired t-test showed that the changes induced in the MVI (i.e. the decline in parabasal and the increase in intermediate and superficial cells), and the MVs were all statistically significant at the end of the trial compared to at baseline in both the fennel and placebo groups.	Study power 80%. Data analysis was performed by SPSS 11. Differences between the two groups were evaluated using the Chi-square test and the independent t-test. The paired sample t-test was used to draw a comparison between the baseline and the three-month follow-up results. All the statistical tests were two-sided.	The results of the study showed no significant positive effects for fennel treatment on vaginal atrophy over the three-month period examined.

To assess the effect of oral fennel on bone density in post-menopausal women Ghazanfarpour *et al*. 2017b	Double-blind, randomised, placebo-controlled trial Primary endpoint: measurement of bone mineral density (BMD) and bone mineral content (BMC) of the spine, femoral neck, intertrochanteric, and trochanter at the baseline and after three months	100-mg fennel soft capsule contained 30% fennel (Standardised to 21–27 mg anethole) or placebo three times a day (in the morning, in the midday and at night) for three months.	60 postmenopausal women randomly assigned to fennel and placebo groups. The mean age of the patients in the intervention and control groups was respectively 56.1 ± 6.5 and 56.2 ± 4.7 years.	Inclusion criteria were: 1) postmenopausal status, which was defined as an age of over 40 years with no vaginal bleeding for one year; 2) no regular ingestion of phytoestrogen or soy-based products and 3) a normal mammogram in the last year. Exclusion criteria were: 1) use of any fluoride or bisphosphonates; 2) diseases or medications affecting bone metabolism; 3) current (or recent past use of estrogen or calcitonin; 4) history of endometrial or breast cancer; 5) any fracture, and 6) regular physical exercise or allergy to fennel	The fennel was different from placebo in term of the mean BMD and BMC at lumbar spine (P = 0.14, P = 0.504), total hip femoral (P = 0.427, P = 0.471), trochanter (P = 0.075, P = 0.07), intertrochanter (P = 0.864, P = 0.932) and femoral neck (P = 0.439, P = 0.641). Also, no significant difference was observed in both groups at the baseline and three-month follow-up in terms of mean BMD and BMC at lumbar spine, total hip femoral, trochanter and intertrochanter (data not shown).	Paired t-tests (intra group) was used to draw a comparison between the baseline and three-month follow-up, and Student's t-test (inter groups) was used to compare the two treatment groups. Statistical tests were two-sided.	Fennel did not have any significant positive effect on BMD and BMC over a three-month period

To investigate the soporific effect of fennel among menopausal women Afiat *et al*. 2018b	Double-blind, randomised, placebo-controlled trial Primary outcome measure: Pittsburgh sleep quality index (PSQI) score	100 mg of soft capsules containing 30% fennel (standardized to 21-27 mg anethole) or placebo 3 times a day, morning, noon and night, for three months.	50 post-menopausal women randomly assigned to fennel and placebo groups. The mean age of the patients in the intervention and control groups was respectively 56 ± 4.2 and 55 ± 4.7 years. All subjects completed the study	Inclusion criteria were healthy postmenopausal women (women aged range of 45-65 years with no vaginal bleeding at least for a year), no history of systemic or tropical estrogen taking during the last 6 months and a normal mammogram in the last year.	Intergroup comparison at the end of treatment period, showed no statistically significant difference in terms of the mean total PSQI score (P = 0.596), subjective sleep quality (P = 0.826), sleep latency (P = 0.417), sleep efficiency (P = 0.127), sleep disturbance (P = 0.130), use of sleeping medication (P = 0.50) and daytime dysfunction (P = 0.439); a tendency toward significant between the 2 groups was seen concerning the sleep duration (P = 0.059).	Study power 80%. Data were analyzed in SPSS version 11. Differences between the 2 groups were evaluated by $\chi 2$ and Student's t-test. Mann-Whitney U test was used for non- normal data. Paired t-test was applied for normal data and Wilcoxon signed rank test for non-normal data to compare pre- and post-treatment results.	Fennel failed to reveal significant beneficial effect on all components of PSQI.

To assess the effects of fennel on lipid profiles in post-menopausal women. Afiat *et al*. 2018a	Double-blind, randomised, placebo-controlled trial Primary endpoint: determination of levels of plasma total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol (LDL-C) and high-density lipoprotein (HDL) cholesterol (HDL-C)	100 mg of soft capsules containing 30% fennel (standardized to 21-27 mg anethole) or placebo 3 times a day, morning, noon and night, for three months.	60 postmenopausal women randomly assigned to fennel and placebo groups. The mean age of the patients in the intervention and control groups was respectively 56.1 ± 6.5 and 56.2 ± 4.7 years.	Inclusion criteria were: 1) postmenopausal status, which was defined as an age of over 40 years with no vaginal bleeding for one year; 2) no regular ingestion of phytoestrogen or soy-based products and 3) a normal mammogram in the last year. Exclusion criteria were: 1) use of any fluoride or bisphosphonates; 2) diseases or medications affecting bone metabolism; 3) current (or recent past use of estrogen or calcitonin; 4) history of endometrial or breast cancer; 5) any fracture, and 6) regular physical exercise or allergy to fennel	There was no significant difference in profiles lipids triglyceride (P = 0.679), total cholesterol (P = 0.103), LDL concentrations (P = 0.104) and HDL (P = 0.266) between groups. Also, in both group, Paired t-test showed no significant difference in all mentioned parameters except HDL-C that showed a significant borderline improvement (P = 0.052) in the fennel group.	Paired t-tests (within group) was used to compare between the baseline and three-month period, and Student's t-test was used to compare between treatment groups. Statistical tests were two-sided.	No significant difference was found between the two groups on the lipid parameters.

Table 9: Clinical studies on humans, in PCOS

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** e.g. Jadad score	**Comments on clinical relevance of results**
To evaluate the effects of fennel essential oil capsules on PCOS symptoms Ghavi *et al*., 2019	Double-blinded, randomized controlled study. Primary outcome measures: 1) degree of hirsutism assessed by using the modified Ferriman-Gallwey method; 2) Mean ± SD of the BMI; 3) duration of the menstruation cycle; 4) TSH,	capsules containing 46 mg of essential oil from fennel seeds or placebo (100 mg soya) for 12 weeks, twice a day (each 12 h) after meals	30 women aged 18-25 years randomly assigned to each group (n=15) One drop-out in placebo group due to unsatisfactory results	Students aged 20–35 fulfilling Rotterdam diagnostic criteria for PCOS. Exclusion criteria: 1) use of any medications to treat PCOS, such as oral hormonal contraceptive during treatment; 2) thyroid dysfunction, pituitary dysfunction and adrenal dysfunction; 3) history of renal or liver disease; 4) any	The rate of hair growth was not reduced significantly in the two groups after the intervention (P > 0.05). Differences in BMI in both groups were not statistically significant after treatment (p=0.172). The duration of the menstruation cycle when compared before (p=0.359) and after (p=0.588) the intervention in the two groups was not significantly different.	The data obtained was analyzed using SPSS version 21. Mann-Whitney and the Wilcoxon tests used for the comparison of quantitative data between the two studied groups.	Limitation: too small sample size.

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** e.g. Jadad score	**Comments on clinical relevance of results**
	FSH, LH, DHEAS, prolactin, and free testosterone; 5) endometrial thickness and ovarian volume by ultrasonography before and after three months of treatment			allergy to herbal or suspected contraindication to herbal remedies; 5) history of major pelvic surgery, seizures, stress factors in the last 6 months, severe gastrointestinal disorders, or any diseases that might interfere with the conduct of the study or the interpretation of the results	The comparison of the biochemical blood test after the intervention showed that there was no significant difference between the two groups ($p > 0.05$). Endometrial thickness and ovarian volume was not different between the placebo and fennel groups; however, the left ovarian follicular number ($p=0.05$) and right ovarian follicular number ($p=0.00$) were significantly different between the two groups.		

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

- *Anti-colic effect*

The efficacy of fennel tea for treating infantile colic was addressed by Weizman *et al*. (1993). A randomised, placebo-controlled study, carried out on 121 (62 in the treated group and 59 in the control group) infants between 2 to 12 weeks of age, suggested that an oral administration for 7 days of a 0.1% fennel seed oil emulsion in water (corresponding to about 12 mg/kg b.w. and day) is significantly superior ($p<0.01$) to placebo in decreasing intensity of infantile colic.

Relief of colic symptoms was assessed as a decrease of cumulative crying to less than 9 hours per week. No side effects were noted in the treated infants with colic (Alexandrovich *et al*., 2003). As the postulated mechanism in the pathogenesis of colic is a spasm of the intestinal smooth muscles, the therapeutic effect of fennel fruit oil was interpreted as possibly secondary to a spasmolytic action.

A randomised, double-blind, placebo-controlled trial was carried out to investigate the effectiveness and side effects of a phytotherapeutic agent based on enpowdered extracts of *Matricariae recutita* L., *Foeniculum vulgare* M. var. *dulce* and *Melissa officinalis* L. in the treatment of 93 breastfed colicky infants. The results showed that colicky infants treated with the extract improved within 1 week of treatment (Savino *et al*., 2005).

A Cochrane systematic revision to assess the eIectiveness and safety of pain-relieving agents for reducing colic in infants younger than four months of age included also clinical studies carried out with fennel (Weizmann 1993, Aleksandrovich 2003, Arikan 2008 and Savino 2005). Herbal agents (i.e. extract of *Matricaria recutita*, *Foeniculum vulgare* and *Melissa officinalis*; fennel seed emulsion; Fumaria extract; and herbal tea preparation) were associated with reductions in crying duration compared with placebo or no treatment, and with improvement in symptoms, compared with placebo. However, the quality of the evidence is low or moderate (Biagioli et al. 2016).

4.4. Overall conclusions on clinical pharmacology and efficacy

There are no clinical studies relevant to the indications summarised in Table 4; available clinical data concern medical uses other than of herbal medicinal products authorised in the European Union countries.

In a clinical study (Bokaie et al. 2013) fennel showed no significant difference compared to mefenamic acid in pain relief in women with primary dysmenorrhoea, which is consistent with the findings of an *in vitro* study showing that fennel essential oil decreased the intensity of contraction induced by oxytocin and PGE_2 in isolated rat uterus (Ostad *et al*., 2001). These findings may explain the traditional use of fennel preparations for symptomatic treatment of minor spasm associated with menstrual periods. However, the clinical relevance of this study is strongly limited by the small sample size, the lack of a placebo group and the absence of blinding and therefore cannot be sufficient to support a well-established use.

Several clinical studies have been carried out to study the effect of fennel on post-menopausal symtoms. Most of these studies failed to show a significant effect of fennel compared to placebo. In addition, even when positive results were observed, the small sample size and the short duration of treatment were the shortcomings that limited the clinical relevance. Finally, in most cases the herbal substance/preparation used was not reported.

A clinical study was carried out using fennel in women with amenorrhoea which suggested that the essential oil might promote and increase menstrual bleeding, but due to the small sample size, larger trials are needed to confirm the positive results obtained to support a therapeutic indication based on well-established use.

Finally, there is only one double-blinded, randomized placebo-controlled study which investigates the effect of capules containing fennel oil on PCOS symptoms. The intervention failed to show significant differences compared to placebo in the primary outcome measures.

In conclusion, the medicinal use of fennel essential oil is not supported by clinical evidence. On the basis of the long standing use reported in scientific literature (see section 2.1) traditional medicinal use only could be supported.

5. Clinical Safety/Pharmacovigilance

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

No significant side effect have been reported in the few studies performed with fennel essential oil (orally). People taking oral essential oil experienced heartburn. In a few clinical studies, Iranian post-menopausal women treated with oral capsules containing 30% fennel standardised to 21–27 mg anethole experienced mainly frequent urination and spotting; notably, there were two cases of vaginal bleeding.

A retrospective observational study to investigate the proportion, prevalence of use, attitude and knowledge base in a sample of Italian pregnant women in the South of Italy was conducted during the period November 2010 – September 2013. Six hundred and thirty expectant mothers (31-40 years of age) were interviewed within three days after childbirth to explore the possible influence and risks of herbal consumption on pregnancy and neonatal outcomes. Fennel was among the most commonly used herbal products, taken by oral route and for the entire period of pregnancy by 94 women (15.7%). The following pregnancy and neonatal outcomes were considered in the present study: the course of pregnancy (physiological or pathological course), abnormalities in foetal growth, type of labor (spontaneous or induced labor), gestational age, birth weight, small for gestational age, Apgar score, circumference of the skull and newborn's length. No side effects were reported after fennel consumption. A regular consumption of fennel throughout the pregnancy resulted in shorter gestational age compared to non-users (38.8± 2.2 weeks versus 39.1±1.6 weeks; $P<0.05$). Moreover, the frequency of lower length of the newborn resulted relatively higher in users, although this did not reach statistical significance (49.5± 2.6 cm versus 49.9±1.2 cm; $P=0.06$). None of the remaining evaluated outcome variables were significantly influenced by the mother's consumption of fennel (Trabace et al. 2015).

5.2. Patient exposure

No data available.

5.3. Adverse events, serious adverse events and deaths

An epileptic seizure was reported in a 38-year-old woman, known to be an epileptic patient. Although she was under antiepileptic treatment (lamotrigine 300 mg/day) and had well-controlled epilepsy, she developed a typical generalised tonic-clonic seizure and remained unconscious for 45 minutes following ingestion of five or six cakes containing an unknown quantity of fennel essential oil. Involuntary

diarrhoea accompanied her epileptic seizure. The patient's last seizure had been three years before and she was under clinical follow-up (Skalli & Bencheik, 2011).

Allergic reactions to fennel oil, affecting the skin or the respiratory system, occur rarely (Levy, 1948; Schwartz *et al*., 1997; Blumenthal and Golberg, 2000).

Enzyme immunoassay inhibition studies with one patient's serum revealed cross-reactivity among the IgE components deriving from aniseed, fennel, caraway, coriander and dill extracts (Garcia Gonzalez *et al*., 2002).

A "mugwort-celeryspice-syndrome", a pollen-food allergy that occurs in a minority of mugwort pollen-allergic was first reported more than 25 years ago. Reported offending foods include celery root, anise, fennel, coriander, cumin, pepper, and paprika patients. Borghesan et al. (2013) reported two cases of cross-reactivity between mugwort pollen and fennel. The first case was a 20 year old man with a history of mild grass pollen allergy who experienced anaphylaxis a few minutes after the ingestion of a small portion of raw fennel (generalized urticaria, dysphonia, lips angioedema, palm-plantar itch). The allergic reaction subsided at home after the administration of systemic steroids and oral cetirizine. On allergological assessment the patient showed strong skin reactivity to fresh raw fennel (mean wheal diameter 20 mm) and a moderate reactivity to fresh cooked fennel. Skin prick tests with a series of commercial food extracts (ALK-Abellò) scored positive for peanut, hazelnut, and peach, as did a SPT with fresh apple. SPT scored frankly positive for grass and mugwort pollen. The second case was a 41 year old man with a history of mild perennial rhinitis with seasonal worsening who experienced two episodes of oral allergic syndrome and dyspnoea few minutes after ingestion of raw and cooked fennel, respectively. In both occurrences the allergic reaction subsides in two hours without therapy. On the allergological assessment, which was performed three months after the last adverse reaction, the patient showed strong skin reactivity to grass, mugwort, cypress, mites, Alternaria, cat dander, and raw fennel. The authors suspected a 60 kDa allergen, highly homologous to Api g 5, recognized in fennel by patient's IgE, to be responsible for the mugwort-celery-spice syndrome (Borghesan et al. 2013).

Nico et al. (2014) analyzed a recent series of 189 well diagnosed cases of food allergy, with the purpose of estimating the occurrence of fennel allergy, in a population with a typically Mediterranean Diet, from Apulia – Southern Italy. For fennel, the investigation was carried out by quantitative skin prick tests with a commercial extract, quantitative prick by prick procedure with the fresh vegetable and CAP RAST for fennel. Allergy to fennel was clearly diagnosed in 57 patients (30% of all food allergy patients), 11 (19%) of whom were positive only for fennel. Many of these patients exhibited also multiple sensitisations to food allergens of the Apiaceae family. Thus 45 patients (79%) had positive skin tests for celery, 22 (39%) for parsley (Petroselinum crispum), and 21 (37%) for carrot (Daucus carota). Notably, all of these patients had lip angioedema and oral itching after fennel's ingestion. However, 17 (30%) had also Quincke's edema, 11 (19%) urticaria and finally, one patient experienced severe anaphylaxis, after eating row fennel.

A recent case of an 11-year-old boy, presented to the specialist pediatric allergy clinic with a history of recurrent, immediate hypersensitivity reactions to a variety of toothpastes, in addition to curry, mint, licorice, cauliflower, and broccoli over the last few years, has been recently published. The most troublesome reaction was to Kingfisher Fennel Natural Toothpaste, which the patient now avoids. The patient had no eczema or asthma, but he did have seasonal allergic rhinitis. He was not taking any medication. The findings from the examination of his skin, cardiovascular system, and lungs were normal. He had signs of nasal congestion but no nasal polyps. Serum specific IgE test result was positive to fennel (fresh fennel 10.5 kUA/L). Skin prick to prick testing to Kingfisher fennel-flavored

toothpaste had a positive result with a wheal of 12 mm. To confirm the clinical allergy to the toothpaste, a physician-supervised open challenge was performed. After being challenged with 200 mg of toothpaste, the patient immediately developed an itchy mouth and rhinorrhea, spat out the toothpaste and rinsed his mouth. Within a minute, he started coughing but did not wheeze. His oxygen saturation and other vital signs remained within the reference range. He had no flushing, urticaria, angioedema, vomiting, or other gastrointestinal symptoms. All symptoms resolved within 10 minutes without any treatment. On a separate occasion, an oral challenge to 100 ground fennel seeds was performed, to which patient showed no reaction. However, immediately after chewing fresh fennel root, the patient started to cough and his voice became hoarse, indicating that the allergen was present in the fennel root but not in the seeds (Denaxa & Arkwright, 2020). Interestingly, two cases of cheilitis and perioral dermatitis secondary to allergic contact dermatitis to limonene contained in toothpaste (Trokoudes & McFadden, 2016); limonene is a natural constituent in fennel essential oil.

Rare cases of contact dermatitis to anethole containing preparations (Andersen, 1978; Franks, 1998) have been reported.

It has been observed that fennel contains coumarin-derivatives, which competitively can inhibit vitamin K and may interfere with blood clotting (Shlosberg and Egyed, 1985). No further data are available.

Fennel contains small amounts of bergapten, a linear furocoumarin that might be responsible for phototoxicity (Kwon *et al*., 2002).

EudraVigilane database

In total, 91 reports were found in EudraVigilance database (search date: 04th of May 2021) using "Fennel" and "Foeniculum" as search terms, including also combinations. Out of these, 31 cases were serious whose 10 resulted in hospitalisation and 1 was life-threatening. Most of the reports referred to polyherbal preparations, with fennel combined with at least other two plants; in particular, Salviathymol N® a product containing levomenthol, cinnamom oil, clove oil, eucalyptus oil, thymol, fennel oil, sage oil, star anise oil) accounts for 23% of the total reports. Adverse events more frequently reported concerns allergic reactions and epidermal/dermal conditions (hypersensitivity, angioedema, puritus, rash, swelling of lips, tongue and face); two cases of Steven-Johnson syndrome (one life-threatening) were also reported. In a vast majority of reports, people recovered from these adverse events also when they were serious. Gastrointestinal disturbances were also frequently observed (abdominal pain, nausea, diarrhoea) and in all cases they were not serious. Three serious cases of renal adverse events (acute or chronic kidney injury and blood creatinine increased) which involved polyherbal preparations (including at least 5 plants apart fennel) were found; one case of acute hepatitis and one case of hepatic cytolysis with teas were reported with tablets containing Cassia angustifolia, Althaea Officinalis, Foeniculum Vulgare and with a herbal tea made of chamomile and fennel extracts, respectively were also reported. Causality assessment was not possible due to poor narrative, use of concomitant mediciation and absence of challenge/rechallenge.

Products containing fennel as monoingredient were involved in only 9 reports. Four cases of premature telarche were reported in children assuming fennel tea for flatulence (Türkyilmaz et al. 2008). One case of post-menopausal haemorrhage was observed in a female 65 year old. One case of alleric dermatitis which caused hospitalisation was also reported. All remaining reports were not serious.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

5.5.1. Use in children and adolescents

There is evidence of traditional use of bitter fennel oil as an expectorant in cough associated with cold in children over 1 year of age when taken as a syrup in single doses of 3-3.25 mg 2-3 times daily or 7 mg in one single dose (daily dose 6.5 – 9.75 mg).

No information is available on the use of bitter fennel oil in adolescents.

The use of fennel oil in children between 1-12 years of age raises safety concerns because of the presence of estragole; indeed estragole content in 6.5 mg of the essential oil would be easily above than the guidance value of 1.0 µg/kg bw reported in the HMPC "Public statement on the use of herbal medicinal products containing estragole". Therefore, the use of fennel oil in children under 12 years of age is not recommended.

The use of bitter fennel oil is contraindicated in children under 1 year of age because of the lack of data and because of the presence of estragole.

5.5.2. Contraindications

Hypersensitivity to the active substance or to Apiaceae (Umbelliferae) (aniseed, caraway, celery, coriander and dill) or to anethole. A cross-allergenicity between fennel and celery has been reported (Stager *et al*., 1991). A common allergen called Bet v 1 possibly accounting for the observed cross-sensitivity was found in subjects showing allergic symptoms as rhinitis, angioedema, asthma, wheezing, urticaria, eczema, abdominal pain, vomiting, and diarrhoea (Jensen-Jarolim *et al.*, 1997; Garcia-Gonzalez *et al.*, 2002).

Cross-reactivity with mugwort pollen has been also reported (Borghesan et al. 2013), therefore the use of fennel should be contraindicated in case of known hypersensitivity to mugwort pollen.

5.5.3. Special Warnings and precautions for use

The use of bitter fennel oil in adolescents is not recommended due to tha lack of data and to the presence of estragole.

The use of bitter fennel oil is not recommended in children over 1 year of age due to the high risk of estragole daily intake above the guidance value of 1.0 µg/kg bw reported in the HMPC "Public statement on the use of herbal medicinal products containing estragole".

Allergic reactions to fennel or to fennel oil, affecting the skin or the respiratory system, may occur. The frequency is not known.

5.5.4. Drug interactions and other forms of interaction

No case has been reported.

Fennel contains a high amount of minerals, mainly calcium, magnesium, iron, zinc, manganese, and copper. It has been shown in the rat that co-administration of fennel and ciprofloxacin may lead to decreased bioavailability of ciprofloxacin in rats due to formation of a ciprofloxacin-cation complex with possible decrease of ciprofloxacin efficacy. Formation of a ciprofloxacin-cation complex resulted in reduced ciprofloxacin absorption. Co-administration of ciprofloxacin with fennel led to a 83% reduction

in ciprofloxacin C_{max} while T_{max} remained virtually unaffected resulting in a significant reduction in area under the curve. This interaction has not been observed in humans (Zhu *et al*., 1999).

Experiments in which rats were injected intra-peritoneally with a mixture of trans-anethole (100 mg/kg b.w.) and [^{14}C]parathion (1.5 mg/kg) showed no significant effect of *trans*-anethole on metabolism and excretion of the insecticide. However, when rats were fed a diet containing 1% of *trans*-anethole for 7 days and subsequently cell fractions from the livers of these rats were incubated for 2 hours with [^{14}C]parathion, significantly less unchanged parathion (1.6%) was recovered compared to controls (12.5%). The data were interpreted as suggesting that feeding trans-anethole to rats for 7 days induced the synthesis of parathion-degrading liver enzymes (Marcus and Lichtenstein, 1982).

Limonene was found to increase levels of reduced glutathione in mouse liver (Reicks and Crankshaw, 1993) and beta-myrcene was found to increase levels of specific subtypes of cytochrome P450 in rat liver (De-Oliveira *et al*., 1997).

In case of prolonged use or if excessive doses are ingested, the estrogenic activity of preparations containing fennel oil may affect hormone therapy or oral contraception (see section 3.1.2 Secondary pharmacodynamics - Estrogenic effects and Section 4.2 Clinical studies: Induction of menstrual bleeding in depot medroxyprogesterone acetate-induced amenorrhea). If the patient is on other medications, he/she should seek medical advice.

5.5.5. Fertility, pregnancy and lactation

According to Madaus (1938), fennel oil produces an excitation of the gravid uterus and can lead to abortion. An estrogenic activity (section 3.1.2 Secondary pharmacodynamics - Estrogenic effects) and anti-fertility and foetal cell toxicity effects (see section 3.3.5 Reproductive and developmental toxicity) have been shown for fennel oil and *trans*-anethole (the major constituent of fennel essential oil) in rats.

Pre-clinical studies showed anti-fertility effects of fennel oil, mainly due to the presence of *trans*-anethole, which is known to be oestrogenic. Only one study was carried out *in vivo*, but it is considered to have limited relevance for human due to the limited number of animals tested and to the longer treatment period of animals (35 days) compared to the short-term treatment reported in the monograph.

No fertility data in humans are available.

A descriptive retrospective survey on 86 consultations due to ingestion of herbal infusion with abortive intent received at the Toxicological Information and Advisory Centre of Montevideo between 1986 and 1999 (Ciganda and Laborde, 2001) indicated that multi-systemic failure was found in those patients that had taken Ruta only and Ruta together with parsley and fennel. Death occurred in four patients, who had ingested Ruta (two cases Ruta alone and two cases Ruta with parsley and fennel).

There is evidence that *trans*-anethole is excreted in human breast milk (Hausner *et al*. 2008). Fennel has been traditionally used as galactogue. Foong et al. (2020) carried out a systematic revision of natural oral galactagogues, including fennel. Results were judged as uncertain about the magnitude of this effect because of substantial heterogeneity of the studies, imprecision of measurement methods and incomplete reporting; therefore no definitive conclusion could be drawn on galactogue effects of fennel.

Fennel essential oil significantly and dose-dependently reduced the intensity of oxytocin-induced contractions and PGE_2-induced contractions of the isolated rat uterus (see section 3.1.1 Primary pharmacodynamics - *Spasmolytic effect on contracted smooth muscles*).

In a cohort study involving 630 pregnant women, collected data from mothers revealed that regular consumption of fennel during pregnancy (n=94 using fennel) can lead to shorter gestational age in women compared to non-users (Trabace *et al*. 2015).

In conclusion, safety during pregnancy and lactation has not been established; in the absence of sufficient data, the use of bitter fennel oil is not recommended during lactation and pregnancy.

Finally, based on the HMPC 'Public statement on the use of herbal medicinal products containing estragole' that the usage of estragole containing (T)HMPs in pregnant and breast-feeding women is not recommended if the daily intake of estragole exceeds the guidance value of 0.05 µg/kg bw.

5.5.6. Overdose

None reported.

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

No studies on the effect on the ability to drive and use machines have been performed.

5.5.8. Safety in other special situations

No data available.

5.6. Overall conclusions on clinical safety

No significant safety concern was identified during clinical trials and from post-marketing surveillance with bitter fennel oil. A retrospective observational study involving 630 pregnant women (n=94 using fennel) revealed that regular consumption of fennel during pregnancy can lead to shorter gestational age in women compared to non-users, however no information on the amount of fennel has been reported; in addition, the duration of fennel consumption was longer than that reported in the monograph.

6. Overall conclusions (benefit-risk assessment)

Available clinical data cannot support the establishment of a well-established use monograph for bitter fennel fruit oil.

Based on the information available from literature, there is evidence of traditional use for bitter fennel fruit oil as an expectorant in cough associated with cold when taken orally in adults as a single dose of 200 microliters per day or in multiple divided doses.

An estrogenic activity, anti-fertility and foetal cell toxicity effects have been shown for fennel oil and *trans*-anethole both *in vitro* and *in vivo* pre-clinical studies. However, *in vivo* studies with *trans*-anethole were carried out using high dosages, whilst study with fennel oil had a long treatment duration (one month), therefore are not considered relevant to human exposure given the short-term use (maximum two weeks) reported in the market overview (Table 5).

Results from studies carried out in the laboratory animals showed a weak mutagenic potential of anethole. However *trans*-anethole is reported as "generally recognized as safe" (GRAS) at the intake of 54 μg/kg b.w./day) and the acceptable daily intake is 0-2 mg/kg b.w..

An anti-tumour activity of anethole has also been reported (see section 3.3.4. Carcinogenicity).

Several studies have shown the carcinogenic effects of estragole in mice and rats (liver tumours) through a pathway including metabolic activation and DNA adduct formation; the same pathway is operative in human *in vitro* systems. There is general consensus that adduct formation is causally related to tumorigenesis, unless there are specific and biologically persuasive reasons to the contrary. Consequently, the mode of action for tumour formation is relevant for humans and the extrapolation of carcinogenicity to humans can be regarded as plausible (EMA/HMPC/137212/2005 Rev 1). As a consequence, recently the HMPC has revised the "Public statement on the use of herbal medicinal products containing estragole", concluding that "…the intake of estragole from HMPs in the general population should be as low as possible, which includes a short-time duration of use (maximum 14 days) and a discussion about the single/daily doses necessary according to the risk assessment relevant for the concerned HMP. For example, to reach or come as close as possible to the guidance value of 0.05 mg/person per day, the lowest dose should be consistently selected if ranges of single and daily doses are available from traditional use.".

There is a risk to have high daily intakes of estragole when bitter fennel oil is taken according to the posologies supported by evidence of traditional use. Taking into account the maximum amount 6% of estragole permitted in the bitter fennel oil according to the relevant Ph. Eur. monograph, these intakes may raises safety concerns due to risks of genotoxicity and carcinogenicity in humans (see "Public statement on the use of herbal medicinal products containing estragole" (EMA/HMPC/137212/2005 Rev 1).

The daily intake of estragole with bitter fennel oil would expose patients to safety concerns, which are not balanced by the beneficial effects in the therapeutic indication supported by the evidence of traditional use, taking into account that other safer therapeutic options, including herbal preparations from several plants, are available on the European market. Therefore the benefit-risk balance on *Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*, aetheroleum is considered unfavorable with respect to the establishment of a European Union herbal monograph.

The use of bitter fennel oil in adolescents is not recommended due to lack of data and the presence of estragole.

Based on the products on the market in Germany, there is evidence of traditional use of bitter fennel oil as an expectorant in cough associated with cold in children over 1 year of age when taken as a syrup in single doses of 3-3.25 mg 2-3 times daily or 7 mg in one single dose (daily dose 6.5 – 9.75 mg).

The use of bitter fennel oil is contraindicated in children under 1 year of age because of the lack of data and because of the presence of estragole.

The HMPC/MLWP concluded that the following requirements for the establishment of a European Union herbal monograph on traditional or well-established herbal medicinal products containing *Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*, aetheroleum are not fulfilled:

- the requirement laid down in Article 16a(1)(e) of Directive 2001/83/EC that "the data on the traditional use of the medicinal product are sufficient; in particular the product proves not to be

harmful in the specified conditions of use and the pharmacological effects or efficacy of the medicinal product are plausible on the basis of long-standing use and experience”

- the requirement laid down in Article 10a of Directive 2001/83/EC that the active substance has a recognised efficacy and an acceptable level of safety and that the period of well-established medicinal use has elapsed.

Annexes

List of references