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ASSESSMENT REPORT ON *FOENICULUM VULGARE* MILLER

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Assessment Report

***FOENICULUM VULGARE* MILLER (bitter fennel, sweet fennel)**

Botanical species and variety	***Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare* (bitter fennel)** ***Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* (Miller) Thellung (sweet fennel)**
Botanical family	**Apiaceae (Umbelliferae)**
Botanical synonyms	***Anethum foeniculum* L.** ***Foeniculum capillaceum* Gilib.** ***Foeniculum officinale* All.**
Common names	**Fennel (English)** **Venkel (Dutch)** **Fenkoli (Finnish)** **Fenouil (French)** **Fenchel (German)** **Finocchio amaro (o selvatico)** **dolce (o romano) (Italian)** **Fennikel (Norwegian)** **Hinojo (Spanish)** **Fankal (Swedish)**
Part of the plant	**Fruit (whole cremocarp and mericarp)**
Pharmaceutical preparations	**Herbal substance or herbal preparations in solid or liquid dosage forms or as a herbal tea for oral use**
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I. Introduction

This assessment report reviews the available scientific data for bitter fennel and sweet fennel (i.e. *Foeniculum vulgare* Miller sp. *vulgare* var. *vulgare* and *Foeniculum vulgare* Miller sp. *vulgare* var. *dulce (*Miller*)* Thellung, respectively) and particularly clinical data. Bitter and sweet fennels belong to the *Apiaceae* botanical family and to the *capillaceum* botanical subspecies. The material of interest for medicinal use is the fruit (i.e. whole cremocarp and mericarp), sweet fennel being more broadly used. This herbal substance is administered after crushing in solid or liquid dosages (Niesel, 1992). The essential oil obtained by steam distillation from the dry ripe fruits is also used.

In preparing this report, a number of data sources were reviewed. The main ones are as follows:

- The ESCOP monographs published in 2003;
- The results of a literature search carried out in mid 2005 by the Italian National Institute of Health in Pubmed;
- The results of a data search carried out in mid 2005 by the Italian National Institute of Health in several electronic archives (i.e. Napralert, Caplus, Dart, Toxcenter, Embase and Medline);
- The bibliographic references made available by the Association of the European Self-Medication Industry (AESGP) at the end of 2005;
- The monographs of "*Foeniculi amari fructus*", of "*Foeniculi dulcis fructus*" and of "*Bitter-fennel fruit oil*" present in the current (5th edition) of the European Pharmacopoeia and of sweet-fennel fruit oil ("*Foeniculi aetheroleum*") in the Italian Pharmacopoeia (XI Ed.);
- The Council of Europe monograph on *Foeniculum vulgare* as a cosmetic ingredient (2002);
- The monograph on *Foeniculum vulgare* Mill. published in Teuscher et al. (2005);
- The results of a data search carried out at the end of 2005 on Micromedex (including Martindale, Drugdex, Posindex, Altmedex, Reprotox, Herbal Medicines: A Guide for Health-Care Professionals);
- The results of a data search carried out at the end of 2005 on phytovigilance data banks available on internet (i.e. www/farmacovigilanza.org; www.epicentro.iss.it/focus/erbe/sorv_piante_officinali.htm);
- The result of the update in literature search carried out in Pubmed until the end of June 2007 by the Department of Clinical and Experimental Medicine and Pharmacology of Messina University.

Fennel crushed fruits as infusions, tincture, syrups and honey are traditionally used (see section III.5 Traditional use) for the treatment of a variety of symptoms including:

- Dyspeptic complaints, a broad range of adverse symptoms including, among others, spasmodic ailments involving altered functional motility of local smooth muscles induced by anomalous hormonal secretions, *Helicobacter* infections, stress and psychological disturbances and other idiopathic causes;

- Bloating and flatulence, symptoms associated with an altered composition of intestinal flora mainly caused by food borne infections or physiological alterations causing a slowing down of the intestinal content transit;

- Infantile colic, a self-limiting condition caused by immaturity of gastro-intestinal tract resulting in excessive gas formation and intestinal cramping due to contractions of smooth muscular layers;

- Primary dysmenorrhea, a condition associated with ovulatory cycles, caused by myometrial contractions induced by prostaglandins originating in secretory endometrium, which results in uterine ischemia and pain; and

- Catarrh, an excessive secretion of epithelial cells due to respiratory tract infections generally also inducing prostaglandin-mediated bronchoconstriction; this secretion, cleared by pneumocyte cilia, consists mainly of flaked away epithelial cells, micro-organisms and mononuclear cells.

These uses are substantiated mainly by empirical data deriving from investigations carried out into the constituents and their pharmacology, while only poor clinical data are available.

II. Clinical Pharmacology

II.1.Phyto-chemical characterization

Bitter fennel is characterized by a content of essential oil not lower than 40 ml per kg anhydrous fruit, whereas sweet fennel only contains not less than 20 ml of essential oil per kg anhydrous fruit.

The oil in bitter fennel fruits has been reported to contain not less than 60% anethole and 15% fenchone and not more than 6% estragole, whereas in sweet fennel fruits the oil contains not less than 80% anethole (as determined with reference to *anethol R* that consists of at least 99% *trans*-anethole) and not more than 7.5% fenchone and 10% estragole (European Pharmacopoeia, 1/2005:824; European Pharmacopoeia, 1/2005:825; Brand, 1993; Tóth, 1967; Trenkle K 1969, 1971 and 1972). The essential oils of bitter and sweet fennels also contain relatively small amounts of alpha-pinene, limonene, p-cymene, beta-pinene, beta-myrcene and of a variety of other compounds (for some examples, see Table 1).

Table 1- Compounds identified in essential oils obtained by steam distillation from ripe fruits of bitter and sweet fennels

Compound	Bitter fennel (+)	Sweet fennel (++)
Trans-anethole	55.0-75.0%	79.8-83.1%
Fenchone	12.0-25.0%	4.6%
Estragole	6.0 % (max)	3.9-5.1%
Alpha-pinene	1.0-10.0%	3.6-0.3%
Limonene	0.9-5.0%	2.2-3.8%
Alpha-pinene/Limonene	> 1.0	
Cis-anethole	0.5% (max)	
Anisaldehyde	2.0 (max)	
Beta-myrcene		1.4%

(+) Monograph on bitter fennel fruit oil (European Pharmacopeia-5th Ed)
(++) Monograph on sweet fennel fruit oil (Italian Pharmacopoeia-XI Ed); Dadalioglu and Evrendilek (2004)

Crushed or powdered fennel fruits gradually lose their volatile constituents upon aging (Czygan, 1989). Teabags examined 30 days after opening showed in general a loss of essential oil ranging from 4 to 10%; moreover, in these samples a decrease of anethole content and an increase of anisaldehyde content (considered as the degradation product of the former) was also evident (Bilia et al., 2002).

Some chemotypes are known for their lower content of *trans*-anethole (less than 50%), higher content of fenchone (more than 30%) and higher content of estragole (more than 30%) (Teuscher et al., 2005).

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); 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).

A considerable variability among relative proportions of different compounds in fennel fruits has been observed in relation to the methodology used to extract the essential oil. Table 2 shows the differences observed among relative amounts of the main components extracted from the same sample (unknown variety) of fennel fruits by simultaneous distillation–extraction (SDE) or supercritical fluid extraction (SFE), as analysed by GC-MS.

Table 2 - Relative amounts of main components extracted from fennel fruit by simultaneous distillation–extraction (SDE) or supercritical fluid extraction (SFE) (+)

Compound	SDE	SFE
Trans-anethole	49.21%	63.80%
Fenchone	19.33%	12.71%
Estragole	25.84%	20.33%
Alpha-pinene	0.62%	0.31%
Limonene + 1,8-cineolo	1.01%	0.87%
Cis-anethole	0.12%	0.10%
Beta-myrcene	0.17%	0.13%
p-Anisaldehyde	1.90%	0.99%

(Diaz-Maroto et al., 2005).

Chemical compositions of volatiles in infusions or microwave decoctions prepared from crushed fruits or in teas from pre-packaged tea bags or in instant teas may be very different among themselves and from volatiles obtained by hydro-distillation of crushed fruits (Forster, 1983; Bilia et al., 2002). Anethole (30-90%) and/or anisaldehyde (0.7-51.0%) were detected in all the samples; estragole (0.8-4.1%), eugenol (1.5-11.3%) and fenchone (0.5-47.0%) were detected in most samples (Bilia et al., 2002).

The fennel fruits also contain water-soluble glycosides of monoterpenoid, alkyl and aromatic compounds (Kitajima et al., 1998) as well as, among other substances, proteins, cellulose, lignin, pectins, triglycerides containing mainly petrosilinic, oleic and linoleic acids, wax esters, phospholipids, phytosterols (e.g. *beta*-sitosterol and stigmasterol), flavonoids, hydroxycoumarins, furanocoumarins and vitamins (tocopherol and tocotrienol) (Kunzemann and Herrmann, 1977; Zlatanov, 1994; Ivanov and Aitzetmuller, 1995; Reiter and Brandt, 1985; Council of Europe, 2002).

This AR supports the establishment of individual Community herbal monographs and/or Community list entries on bitter fennel fruit, sweet fennel fruit and bitter fennel fruit oil that comply with the European Pharmacopoeia monographs 5th Ed.

II.2 Absorption, metabolism and excretion

No data available for fennel 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).

II.3. Pharmacodynamics

II.3.1 Mode of action

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

- *Spasmolytic effect on contracted smooth muscles*

Fennel fruit alcoholic extracts and oil exerted a relaxing effect on *in vitro* pre-contracted smooth muscles from different organs (tracheal, ileal and uterine) by antagonizing several contraction-inducing agents.

The relaxant effect of fennel aqueous extract, ethanolic extract and essential oil on methacholine pre-contracted isolated tracheal chains of guinea pig was studied by Boskabady and Kathami (2003) and by Boskabady et al. (2004). A bronchodilatory effect of the fennel ethanolic extract and essential oil was detected, possibly partly due to a potassium channel opening effect, whereas no relaxant effect was detected in the fennel aqueous extract.

Fennel 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).

A 30%-ethanolic extract from bitter fennel produced a concentration-dependent decrease in acetylcholine-and histamine-induced contractility of isolated guinea pig ileum at concentrations of 2.5-10 ml/litre; however, taking into account the effect of ethanol, only the results with histamine were significant ($p<0.005$ at 10 ml/litre) (Forster et al., 1980). In the same test system, the extract at 2.5 and 10 ml/litre also concentration-dependently reduced carbachol-induced contractility (Forster, 1983). Fennel essential oil was also 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).

Fennel essential oil (5 to 25 ml of distillate administered to dogs by means of a catheter inserted into an intestinal fistula) (Plant and Miller, 1926) and anethole (10 to 25 ml/l of physiological solution in which an isolated mouse intestinal jejunum is plunged) (Imaseki et al., 1962) induced intestinal motility at low concentrations, but an intestinal relaxation was observed at concentrations higher than 50 mg/l.

- *Anti-inflammatory effect*

Oral pre-treatment of rats with a dry 80%-ethanolic extract from sweet fennel at 100 mg/kg b.w. inhibited carrageenan-induced paw oedema by 36% ($p<0.01$) compared to 45% inhibition by indometacin at 5 mg/kg (Mascolo et al., 1987).

- *Secretolytic and 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).

An increase of about 12% in mucociliary transport velocity was observed in isolated ciliated epithelium from the frog oesophagus 90 seconds after application of 200 µl of an infusion from bitter fennel (4.6 g per 100 ml of water) (Müller-Limmroth and Fröhlich, 1980).

Fennel aqueous extracts, in a concentration of 10% weight/volume, increased gastric acid secretion in the stomach of anesthetized rats from the basal level of 0.12 to 0.42 ml ($p<0.02$). It has been shown that fennel increases gastric acid secretion. An experiment showed that following administration of an fennel aqueous extract, the increase in acid production in aspirin-injured stomachs compared to that in healthy stomachs was markedly reduced, leading to the conclusion that gastric stimulation requires a healthy, intact gastric mucosa (Vasudevan et al., 2000).

- *Estrogenic effects*

Thirty-eight women affected by idiopathic hirsutism were treated, in a double-blind placebo controlled study, for 12 weeks, twice a day, on their face with a cream containing 2%, 1% or 0% of fennel fruit extract obtained with ethanol in a Soxhlet apparatus for 5 hours. In both treatment groups (and more in the 2% fennel fruit extract), a significant reduction in facial hair growth and diameter was observed (Javidnia et al., 2003).

Subacute oral administration of an acetonic extract from fennel to adult female ovarectomized rats at 0.5-2.5 mg/kg b.w./day caused dose-dependent estrogenic effects: induction of the estrus phase (after 10 days, in 40% of rats at 0.5 mg/kg, in 100% at 2.5 mg/kg), increase in mammary gland weight ($p<0.05$ at 0.5 mg/kg, $p<0.01$ at 2.5 mg/kg) and increase in weights of endometrium, cervix and vagina ($p<0.01$ to $p<0.001$ at 2.5 mg/kg). Estrogenic effects were also evident in mature male rats after treatment with the extract at 1.5 or 2.5 mg/kg b.w./day for 15 days: no significant change in body or organ weights but, particularly at the higher dose, significant changes in protein and acid and alkaline phosphatase in the testes, vas deferens, seminal vesicles and prostate (Malini et al., 1985). An increased weight of mammal glands was also observed following administration of the fennel extract to non-ovarectomized rats (quoted by Teuscher al., 2005).

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).

- *Antimicrobial effect*

Fennel fruit extracts and 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.

Acetone, n-butanol, ethanol and ether extracts of fennel inhibited the growth of a range of bacteria including *Escherichia coli* and *Staphylococcus aureus*, and also exhibited antifungal activity against *Candida albicans* and other organisms (Maruzzella et al., 1959).

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 olants 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 haemoliticus* (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).

II.3.2 Other studies

- *Anti-tumour effect*

See section IV.1.1. Preclinical data

- *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').

- *Hypotensive effect*

A lyophilized aqueous extract of fennel administered orally at 190 mg/kg b.w. (equivalent to crude herbal substance at 1,000 mg/kg) for 5 days significantly lowered the systolic blood pressure of spontaneously hypertensive (SH) rats ($p<0.05$), but had no effect on normotensive rats. The extract also significantly increased the urine output of SH rats, by 80% at day 3 ($p<0.05$), and increased renal excretion of sodium and potassium ($p<0.05$), suggesting that fennel acts mainly as a diuretic and natriuretic in the SH rats (El Bardai et al., 2001).

- *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).

- *Local anaesthetic activity*

Trans-anethole concentration-dependently reduced electrically-evoked contractions of rat phrenic nerve-hemidiaphragm, by 10.3% at 10^{-3} µg/ml, by 43.9% at 10^{-2} µg/ml, by 79.7% at 10^{-1} µg/ml and by 100% at 1 µg/ml (Ghelardini et al., 2001).

In the rabbit conjunctival reflex test, solutions of *trans*-anethole admistered into the conjunctival sac increased concentration-dependently the number of stimuli required to evoke the conjunctival reflex ($p<0.001$); the effect was comparable to that of procaine (Ghelardini et al., 2001).

- *Other effects*

Fennel essential oil had a spasmogenic effect on smooth muscle of isolated guinea-pig ileum at a concentration of 8×10^{-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 2×10^{-4} g/ml (Lis-Balchin and Hart, 1997).

Anethole was reported to have a contractile effect on smooth muscle (Reiter and Brandt, 1985).
It was also reported to increase the pentobarbital-induced sleeping time in mice (Marcus and Lichtenstein, 1982).

Addition of 0.5% of fennel to the diet of rats for 6 weeks shortened food transit time by 12% ($p<0.05$) (Platel and Srinivasan, 2001). Fennel oil increased the pentobarbital-induced sleeping time in mice following simultaneous intra-peritoneal (i.p.) administration (Marcus and Liechtenstein, 1982).

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).

An aqueous extract of fennel (10% w/v), perfused through the stomach of anaesthetized rats at 0.15 ml/minute and collected over periods of 20 minutes, significantly increased gastric acid secretion ($p<0.02$) to more than 3-fold compared to the basal secretion determined from perfusion of saline solution (Vasudevan et al., 2000).

III. Clinical efficacy

III.1 Preparations marketed in Europe

Herbal teas

Fennel fruit, sweet (**Fructus *Foeniculi***, Species) is marketed in **Latvia** since 1970 with the following indications: Orally as a carminative, for gastrointestinal disorders and spasms, as galactagogue increases breast-milk production, may be taken as a mild expectorant. For children as an infusion can be given for colic and as carminative (ATC code: V03 AX).
Daily dosage: 5.0-7.0 g crude drug or equivalent preparations as an infusion. Infusion: pour 180 ml of hot water over a tablespoon (~ 5.0 g) of the fruits, allow them to stand for 20 minutes, then remove the fruits with a strainer. ½ cup of the freshly prepared infusion is drunk 2-3 times daily before eating. For children aged up to 4 years daily dose is 1 teaspoon, for children aged 4 to 10 years – 1 dessertspoon of the fruits.
Sweet fennel herbal tea is authorised in **France** since February 1990, traditionally used in the symptomatic treatment of digestive upsets such as epigastric distension, slow digestion, eructation, flatulence, with the following posology: 1.8 g 2 to 3 times daily.
In **Germany** the authorised indications since 1996 for bitter fennel herbal tea are: symptomatic treatment of dyspeptic complaints such as minor gastrointestinal spasms, repletion and flatulence.
For the relief of symptoms in coughs and colds with viscous mucilage. Posology: 2.5 g herbal substance/150 ml water 2-3 daily.
The indications authorised in **Austria** for the herbal tea are the following:
Dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, anorexia, bloating and flatulence. Effect on hormones such as stimulating milk production. Catarrh of the upper respiratory tract, cough (expectorant)
Posology: Adult daily dose: 5-7 g of crushed fruits as an infusion or similar preparation.
Children, average daily dose: 0-1 year of age: 1-2 g of crushed fruits as an infusion; 1-4 years of age: 1.5 - 3 g; 4-10 years of age: 3-5 g; 10-16 years of age: the adult dose.
Herbal tea is also authorised in the **Czech Republic** as an adjuvant in mild gastrointestinal complaints such as bloating, flatulence and minor spasms and in catarrhs of the upper respiratory tract; posology: 1.5 g poured with 0.25 l of boiling water (extracted for 15 minutes) three times daily (no restrictions).
In **Poland** herbal tea has been traditionally used over 30 years in digestive disorders, spastic complaints, feeling of fullness and flatulence, as spasmolytic and cholagogic. It is also used as expectorant in inflammations of upper respiratory tract and in lactagogue mixtures. Posology: Infusion made of 1 teaspoon of fruits in a glass of boiling water. Drink 1/3 – 1/2 of portion 3 – 2 times a day. For children in single doses of one teaspoon. Not abuse during pregnancy and lactation.

Fennel powder

Hard capsules containing sweet fennel powder are authorised in **France** since November 1990 with the following indications:

- Traditionally used in the symptomatic treatment of digestive upsets such as: epigastric distension, slow digestion, eructation, flatulence.
- Traditionally used as an adjuvant treatment for the painful component of functional digestive disorders.

Posology: 390 mg 3 times a day (if necessary: until 1,950 mg daily)

Bitter Fennel fruit oil

Bitter fennel fruit oil is authorised as a syrup in Germany since 1978 for the relief of symptoms in coughs and colds with viscous mucilage in children over one year. Posology: 3 times daily 1 cup, equivalent 0.003 g fennel oil

No authorised/registered products are on the market in the following European countries: Belgium, Ireland, Italy, The Netherlands, Portugal, Finland and Norway.

Various fixed combinations containing fennel are authorised/registered in different European countries.

Food supplements containing fennel are on the market.

III.2 Posology, duration of use, method of administration

Posology

There are no dose-finding studies available.

The recommended dosage for adults and children over 12 years of age is supported by clinical experience and expert opinions (Brand, 1993; Czygan and Hiller, 2002; Dorsch et al., 2002; Leclerc, 1983; Valnet, 1990).

Fennel fruit (Blumenthal et al., 1998)**:**

Adult and children over 12 years:

*5-7 g of crushed fennel fruits as single **dose** per day or in **multiple divided** doses as a herbal tea or similar preparations.*

Fennel tincture (Blumenthal et al., 2000): 5 to 7.5 ml two to three times daily

Fennel oil (Blumenthal et al., 1998): 0.2 ml essential oil, *as single dose per day or in multiple divided doses*.

The use in the paediatric age (see section III.4.1 Use in children) is not recommended for the presence of estragole, whose exposure should be minimised in young children.

The posology recommended by the HMPC per age category and in the different indications can be found in the corresponding Community herbal monographs/Community list entries.

Duration of use

Unless otherwise advised by a physician or pharmacist, one should not consume fennel oil for an extended period (several weeks) (Blumenthal et al., 1998).

Because of the poor evidence of clinical trials, considering the lack of available safety data on long-term use of fennel preparations and due to the potential toxicity of *trans*-anethole and estragole, a limit of two weeks is consistent with a self-medication indication, which is the case for traditional herbal medicinal products.

If the symptoms persist during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

***Method of administration*:** Oral use.

III.3 Clinical studies

- *Primary dysmenorrhoea*

Primary dysmenorrhoea, a condition associated with ovulatory cycle, is due to myometrial contractions induced by prostaglandins originating in secretory endometrium, which results in pain. Intensity of pain of thirty patients with moderate to severe dysmenorrhoea was recorded by using a linear analog technique for 5 days in three consecutive cycles (first cycle: no treatment; second cycle: treatment with mefenamic acid-250 mg q6h, orally; third cycle: treatment with a 2% fennel oil obtained by steam distillation of dried fruit-25 drops q4h, orally) (Jahromi et al., 2003). Although the study was not blinded and the sample tested was small, the results obtained suggest that both treatments effectively ($p<0.001$) reduced menstrual pain as compared to the control cycle, mefenamic acid being more active than fennel oil. However, five cases, representing 16.6% of all treated cases, withdrew from the study due to fennel's odour and one subject reported a mild increase in the amount of menstrual flow during the fennel treated cycle.

Conclusion

Investigations available in human beings on the role of fennel in reducing pain in primary dysmenorrhoeal are very preliminary. However, the likelihood of the postulated effect is considerably increased by several *in vitro* studies showing that fennel fruit alcoholic extracts and oil exerted a relaxing effect on *in vitro* pre-contracted smooth muscles from tracheal, ileal and uterine tissues by antagonizing several contraction- inducing agents.

III.4 Clinical studies in special populations

III.4.1 Use in 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 F et al., 2005).

Iten and Saller in 2004 published a comment to the statement of the German 'Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin' (BgVV, May 2001), where consumers are advised to reduce their intake of foods or fruits containing estragole for reasons of health. The crucial points of criticism concerned the transfer of data obtained in animal models to the human situation as well as the high doses of the applied monosubstance and difference in estragole metabolism between mice and men. The authors concluded admitting the lack of epidemiological and clinical studies on use of fennel tea but, at the same time, declared that in their opinion the probability of a serious cancerogenic potential risk connected with the consumption of fennel tea seems to be negligibly small.

Conclusion

Investigations available in human beings on the role of fennel in reducing pain in infantile colic are very preliminary. However, the likelihood of the postulated effect is considerably increased by several *in vitro* studies showing that fennel fruit alcoholic extracts and oil exerted a relaxing effect on *in vitro* pre-contracted smooth muscles from tracheal, ileal and uterine tissues by antagonizing several contraction-inducing agents.
The missing data cannot be extrapolated from the use in adults and available data cannot be accepted for supporting the use in children for safety reasons, on the basis of Eur. Ph requirements for the content of estragole (no more than 5.0% in bitter fennel and no more than 10.0 % in sweet fennel). Considering that even authors, who are in favour of the use of fennel without limits in children admit the lack of epidemiological studies (the type of studies that could give more information on safety), according to the recommendations of the HMPC 'Public statement on the use of herbal medicinal products containing estragole', "the exposure of estragole to sensitive groups such young children, pregnant and breastfeeding women should be minimised."

The use of fennel oil in children and adolescents under 18 years of age is therefore contraindicated (lack of data and presence of estragole).

The prescription of fennel tea in infants and children under 4 years of age should be restricted to the paediatrician and no general recommendation for the use without any medical advice should be given.

The indicative average daily dose for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating, and flatulence coming from the traditional use in Austria may be the following:
3 months-1 year of age: 1-2 g of comminuted fruits as an infusion
1-4 years of age: 1.5-3 g of comminuted fruits as an infusion.

In children between 4 and 12 years of age, with the aim to minimise the exposure to estragole, a short-term use (less than one week) of fennel tea in mild transitory symptoms according to the traditional Austrian posology is considered acceptable: 3-5 g of comminuted fruits, in three divided doses.

III.4.2 Use during pregnancy and lactation

There are no clinical studies available.

According to Madaus (1938), fennel oil produces an excitation of the gravid uterus and can lead to abortion. An estrogenic activity (see section II.3.1) and anti-fertility and foetal cell toxicity effects (see section IV.2) have been shown for fennel oil and *trans*-anethole in rats.

Moreover, 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).

In view of the above-mentioned data, as a precautionary measure, the use of fennel oil and alcoholic extracts is not recommended during pregnancy and lactation.

Despite the fact that the European Scientific Cooperative on Phytotherapy reports that the herbal substance and preparations of fennel at the recommended dosage may be used during pregnancy and lactation (ESCOP, 2003), no data are available in relation to the use of fennel during pregnancy and lactation at the recommended dosages. According to the recommendations of the HMPC 'Public statement on the use of herbal medicinal products containing estragole', "the exposure of estragole

to sensitive groups such young children, pregnant and breastfeeding women should be minimised."

It is unknown if fennel constituents are excreted in human breast milk.

III.5 Traditional use

Anti-asthma and dyspnea effects have been described for fennel in Iranian ancient medical books (Avesina, 1985). Applications in the treatment of catarrh of the upper respiratory tract has been described in several handbooks and treaties (Brand et al., 1993; Czygan and Hiller, 2002; Madaus, 1976; Sweetman, 2002; 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 and Hiller, 2002; Madaus, 1976; Schilcher, 1984 and 1986).

Fennel fruit has also 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; Jahromi et al., 2003).

Fennel fruit (Xiaohuixiang) has been in use for many centuries in Chinese Medicine, generally as decoction with other herbs (L'Italia Agricola, 1989, Chinese Herbal Medicine 1999, p.203-204) and the Chinese Herbal Medicine (1999, p.502-503) classifies fennel fruit as being able "to control gastrointestinal smooth muscle" and to treat "stomach ache". Fennel fruit (Xiaohuixiang) is described in the Pharmacopoeia of the People's Republic of China (English edition, 2005, Vol. I;) with the action: "to dispel *cold* and relieve pain, to regulate the stomach function". The indications reported in the Chinese Pharmacopoeia are as follows: "it is used for lower abdominal pain with cold sensation, dysmenorrhoea; distending pain in the epigastrium with reduced appetite, vomiting and diarrhoea; hydrocele testis".

For oral use in Traditional Chinese Medicine (TCM) the dosage is 3-6 g per day as a herbal tea in single or divided doses.

According to the TCM, fennel fruit can be 'hotted' and wrapped in a bag (10 grams) for ironing the lower abdomen to treat abdominal pain of cold type (Liu Gan Zhong et al., 2003).

Fructus Foeniculi processed with salt acts to dispel "cold" from the interior and relieve pain. It is used for lower abdominal pain with cold sensation, dysmenorrhoea. This magisterial preparation described in appendix II D of the Chinese Pharmacopoeia, is traditionally prescribed by physicians when kidneys or renal function are involved.

Otherwise, according to the TCM criteria, fennel fruit should not be taken in the following conditions:

Internal "Heat" due to Yin Deficit (SATCM 2002)

- Febrile disease with persistent high fever (internal "Heat" in the Qi System) (*Chinese Materia Medica* , TianJin. 2001)
- Late stage of febrile disease ("Heat" syndrome due to Yin deficiency) (SATCM 2002)
- Acute infections of the gastro – enteric tract (internal "Fire" and toxins) (SATCM 2002)
- Acute inflammation of the gastro – enteric tract such as gastritis, gastric or duodenal ulcer, irritable bowels disease, etc. (Endogenous "Heat" in Stomach and Intestine) (SATCM 2002)
- Constipation due to dryness (Deficit of body fluid due to Yin Deficiency) (SATCM 2002)

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

The Treaty "Farmacologia Teorica e Pratica", also named "Farmacopea Italiana" of Giuseppe Orosi (1851- Vincenzo Mansi Ed.-Livorno) lists fennel fruit in the Materia Medica Botanica Chapter (Orosi, 1851).

IV. Safety

IV.1 Genotoxic and carcinogenic risk

IV.1.1 Preclinical data

- *Mutagenicity and carcinogenicity*

Aqueous and methanolic extracts of fennel did not show any mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA 98 and TA 100, with or without metabolic activation (Morimoto et al., 1982; Yamamoto et al., 1982), whereas fennel oil (2.5 mg/plate) was mutagenic (Marcus and Lichtenstein, 1982). The two extracts showed no activity also in the *Bacillus subtilis rec*-assay (Morimoto et al., 1982).

Sweet fennel oil was found to be mutagenic in the *Bacillus subtilis* DNA-repair test (Sekizawa and Shibamoto, 1982), but fennel oil did not show any activity in the chromosomal aberration test using a Chinese hamster fibroblast cell line (Ishidate et al., 2000).

- *Anethole*

From a series of studies in mice, Miller et al. (1983) concluded that anethole fed to female CD-1 mice in the diet or given orally or by i.p. injection to male pre-weaning B6C3F1 mice 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.

Another bioassay carried out in Sprague–Dawley (SD) rats, administered 0.25, 0.5, or 1% anethole in the diet for 121 weeks, 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 histological changes to the liver as those observed after enzymes inducers (Newberne et al., 1989) and were considered as not caused by a direct genotoxic effect of *trans*-anethole (Lin, 1991). Reed et al. (1992) also showed that i.p. administration of anethole to SD rats increased liver weight, microsomal protein and cytochrome P-450 content.

In nine Salmonella studies to detect base-pair substitutions or frameshift mutations without metabolic activation, anethole was uniformly negative and this was also the case in four studies with activation after careful consideration of all experimental conditions (Heck et al., 1989; Hsia et al., 1979; Marcus and Liechtenstein, 1982; Mortelmans et al., 1986; Nestmann et al., 1980; Sekizawa and Shibamoto, 1982; Swanson et al., 1979; To et al., 1982). The four findings suggesting a weak mutagenic potential of anethole (Marcus and Liechtenstein, 1982; Swanson et al., 1979; Mortelmans et al., 1986; Sekizawa and Shibamoto, 1982; Lin, 1991) 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 and Marshall and Caldwell, 1996).

Anethole was found to be mutagenic in the mouse lymphoma assay which is known for its extreme sensitivity and poor selectivity for genotoxicity (Gorelik, 1995; Heck et al., 1989; Caldwell, 1993; Casciano et al., 1992).

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

A mouse micronucleus assay was negative, with no micronuclei found at 6 and 30 hours after anethole administration (Marzin, 1979). Similarly, in the mouse bone marrow micronucleus test, oral pre-treatment of mice with *trans*-anethole at 40-400 mg/kg b.w. 2 and 20 hours before the administradion of the genotoxins cyclophosphamide, procarbazine, N-methyl-N^7-nitro-N-nitrosoguanidine, urethane, ethyl methane sulfonate, no significant increase in genotoxicity was observed (Abraham, 2001).

Very low levels of DNA adducts were evidenced 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 and 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 and 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 and Caldwell, 1996).

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, Caldwell et al., 1987; Caldwell and 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.

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 can be "generally recognised as safe" (GRAS) at low level of intake (54 μg/kg b.w./day) (Newberne et al., 1999).

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 recommended that the acceptable daily intake (ADI) should not exceed the dose 2 mg/kg b.w. on the basis of scientific pre-clinical data published on *trans*-anethole (JECFA, 1999).

- *Estragole*

Estragole, a minor constituent of fennel oil, has shown its ability to produce genotoxic effects in bacteria, yeasts and mammalian cells, while no mutagenic activity was observed in *Salmonella typhimurium* probably because of the absence of the complex metabolism needed for bioactivation (EMEA/HMPC, 2005).

It has been shown that estragole and its 1'-hydroxy metabolite caused significant increases in the incidences of hepatocellular carcinomas in male CD-1 mice that received the compounds by subcutaneous injection at 1-22 days of age (Drinkwater et al., 1976).

Estragole or its metabolite, 1'-hydroxyestragole, administered to mice binds readily to DNA and several DNA adducts have been characterized. Several studies showed the carcinogenic effects of estragole in mice (mainly malignant liver tumours); moreover, 1'-hydroxyestragole and other metabolites and synthetic derivatives were shown to be potent carcinogens in mice (Wiseman et al., 1987; EMEA/HMPC, 2005).

The EMEA/HMPC (2005) assessment is that the profiles of metabolism, metabolic activation and covalent binding of estragole are dose-dependent and tend to markedly decrease at low levels of exposure (less than linear decrease with respect to dose); according to this assessment, rodent studies indicate that these events are probably minimal in the dose range 1-10 mg estragole/kg b.w., which is approximately 100-1,000 times the anticipated human exposure to this substance from traditional diet and as added flavouring substance.

The major metabolic pathway of low doses of estragole as established in rats and mice is O-demethylation with carbon dioxide being the terminal metabolite, but as the dose increases the proportion of O-demethylation decreases and other pathways, notably 1'-hydroxylation, come into prominence.

- *Conclusion*

In conclusion, fennel oil extracts were found to be mutagenic *in vitro* and studies carried out in laboratory animals showed a weak mutagenic potential of anethole. However, *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.

Several studies have shown the carcinogenic effects of estragole and some of its metabolites in mice (mainly malignant liver tumours). The EMEA/HMPC (2005) assessment is that the profiles of metabolism, metabolic activation and covalent binding of estragole are dose-dependent and tend markedly to decrease at low levels of exposure. The genotoxic risk related to estragole is not considered to be relevant for adults in the recommended dosage due to the small amount present in fennel oil but the risk cannot be calculated with high doses or prolonged use or in young children.

IV.1.2 Clinical data

No data available.

IV.1.3 Conclusion

- *Anti-tumor activity of anethole*

In Swiss albino mice with Ehrlich ascites tumour (EAT) in the paw, anethole administered orally at 500 or 1,000 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 1,000 mg/kg), tumour volume ($p<0.01$ at 500 mg/kg, $p<0.001$ at 1,000 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).

In the mouse bone marrow micronucleus test, 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 led to moderate, dose-dependent protective effects against known genotoxins such as cyclophosphamide, pro-carbazine, N-methyl-N'-nitrosoguanidine, urethane and ethyl methane sulfonate ($p<0.05$ to $p<0.01$ at various dose levels). No significant increase in genotoxicity was observed when *trans*-anethole (40-400 mg/kg b.w.) was administered alone (Abraham, 2001).

- *Antioxidant activity of fennel extracts*

The inhibitory effects of some constituents isolated from a methanolic extract of fennel fruit were investigated on the oxidation of linoleic acid and on the activation of inactive hyaluronidase. Among the test compounds, six stilbene trimers, miyabenol C, *cis*-miyabenol C, foeniculoside I, foeniculoside II, foeniculoside III and foeniculoside IV, exhibited greater antioxidative activities than butylated hydroxyanisole (BHA). Furthermore, miyabenol C and *cis*-miyabenol C showed strong hyaluronidase inhibitory effects (Ono et al., 1997).

Antioxidant activity of fennel oil against lipid peroxidation was demonstrated in the thiobarbituric acid reactive species assay and in a micellar model system (Ruberto et al., 2000).

The antioxidant activities of fennel fruit aqueous and ethanolic extracts were compared *in vitro* to those of standard antioxidants such as BHA, butylated hydroxutoluene and tocopherols in various antioxidant assays including total antioxidant, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activities and reducing power (Oktay et al., 2003). Both fennel extracts showed effective reducing power, free radical scavenging, hydrogen peroxide scavenging and metal chelating activities.

Antioxidant activity of an aqueous extract from fennel fruit (as evaluated *in vitro* from amounts needed for 50% scavenging of superoxide radicals or for 50% inhibition of lipid peroxide or for 50% inhibition of hydroxyl radicals) was found to be higher than that of ascorbic acid and comparable to those of 4 other umbelliferous fruits (Satyanarayana et al., 2004).
Considering the above-mentioned data and all uses of fennel fruit, it is further concluded that human exposure resulting from short-term use of fennel fruit-based medicinal products, complying with the above-mentioned specifications, is unlikely to pose any significant cancer risk.

IV.2. Toxicity

IV.2.1 Acute toxicity

Oral administration of an ethanolic extract of fennel to mice at 0.5, 1 and 3 g/kg b.w. caused no mortality and no significant difference in body and vital organ weights or in external morphological, haematological or spermatogenic parameters in comparison with the control group over a period of 24 hours (Shah et al., 1991).

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).

Fennel extracts in high dosages resulted in abnormal movements, tremor, fasciculation and drowsiness in experimental animals (Ostad et al., 2000).

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 (Zagari, 1991).

IV.2.2 Subchronic toxicity

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).

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).

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; Le Bourhis, 1973).

Oral administration in drinking water of an ethanolic extract of fennel to mice at 100 mg/kg b.w. and day for 3 months caused significant body weight gain in male mice and weight loss in females. Alopecia was observed in 3/10 males, swollen testes in 1/10 males and penile erection in 2/10 males. No toxic symptoms were observed in females. It caused no significant differences in mortality or in haematological and spermatogenic parameters in comparison with the control group (Shah et al., 1991).

IV.2.3 Reproductive toxicity

Malini et al. (1985) showed estrogenic activity of the seed extract in both male and female rats. The protein concentration was found to be significantly decreased in testes and vas deferens and increased in seminal vesicles and prostate gland, following oral administration of an acetone extract of Foeniculum vulgare seeds at the doses of 150 μg/kg and 250 μg/kg for 15 days in male rats. Moreover, the activity of acid and alkaline phosphatase decreased in all these tissues. Only the alkaline phosphatase was unchanged in vasa. The same doses of the extract, after oral administration for 10 days in female rats, caused vaginal cornification and estrus cycle. The lower doses caused increase in weight of mammary glands and the higher doses increased the weight of oviduct, endometrium, myometrium, cervix and vagina.

Trans-anethole exerted dose-dependent anti-implantation activity after oral administration to adult female rats on days 1-10 of pregnancy. Compared to 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 33.3%, 66.6% and 100% respectively. Further experiments at the 80 mg/kg dose level showed that in rats given *trans*-anethole only on days 1-2 of pregnancy normal implantation and delivery occurred; in those given anethole only on days 3-5, implantation was completely inhibited; and in those given *trans*-anethole only on days 6-10, 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 anti-fertility activity. From comparison with the days 1-2 group (lack of anti-zygotic 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 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.

The body of the data indicates that reproductive system is a target for the action of fennel extracts 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. However they cannot definitely be excluded.

IV.3 Contraindications

Persons with hypersensitivity to the active substance or to Apiaceae (Umbelliferae) (aniseed, caraway, celery, coriander and dill) or to anethole should avoid the use of fennel preparations and fennel oil. A cross-allergenicity between fennel and celery has been reported (Stager et al., 1991).

The use of fennel oil in children and adolescents under 18 years of age is contraindicated because of the lack of data and because of the presence of estragole.

IV.4 Special warnings and precautions for use

The use of fennel tea is not recommended in children under 4 years of age due to the lack of adequate data and paediatrician advice should be sought.

Preparation with high fennel content (> 7 g of herbal substance) should not be taken for more than two weeks without medical advice.

If excessive doses are ingested, the estrogenic activity of fennel oil may affect hormone therapy, including the oral contraception and hormone replacement therapy (see sections IV.6 Interactions and II.3.1. Mode of action - Estrogenic effects).

Patients should seek medical advice if symptoms persist for more two weeks or worsen upon administration of the medicinal product.

IV.5 Undesirable effects

Allergic reactions to fennel, affecting the skin or the respiratory system, occur rarely (Levy, 1948; Schwartz et al., 1997; Blumenthal et al., 1998).

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).

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).

IV.6 Interactions

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).

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.
In fact, 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).

Excessive doses of preparations containing fennel oil may affect hormone therapy or oral contraception (see section IV.4 Special warnings and precautions for use). If the patient is on other medications, he/she should seek medical advice.

IV.7 Overdose

No data available.

V. Overall Conclusion

The traditional uses of fennel for *"dyspeptic complaints such as mild, spasmodic gastro-intestinal ailments, bloating and flatulence" and "catarrh of the upper respiratory tract"* are supported not only by expert's opinion and clinical experience, but also by available data.

Clinical trials showing the efficacy of fennel as a smooth muscles spasmolytic remedy are limited and preliminary, but pharmacological data show a **significant relaxing** effect of fennel alcoholic extracts and essential oil on tracheal, ileal and uterine smooth muscles contracted by several contraction-inducing agents (i.e. metacholine, oxytocin, PGE_2, acetylcholine, carbachol and histamine).
Moreover, the ability to counteract prostaglandins actions may also explain the anti-inflammatory effect observed following an oral administration to rats.

The above-mentioned effects are also likely to play a beneficial role in the treatment of inflammation of mucous membranes of upper respiratory tract with an excessive secretion of epithelial cells induced by respiratory tract infections, generally also inducing prostaglandin-mediated bronchoconstriction. Moreover, this indication is also made plausible by the secretolytic and expectorant effects exhibited by anethole and fenchone, two main components of fennel oil, when administered by inhalation to urethanized rabbits.
It has been shown that both anethole and estragole given intravenously (10 mg/kg) elicit in the laboratory animal vagal effects causing a transitory bradycardic and depressor effect (de Siqueira, 2006).

Lastly, when considering the plausibility of the above indications, particularly with reference to inflammation of mucous membranes of upper respiratory tract, bloating and flatulence, the likely role of a number of compounds detected in fennel fruit and very active in inhibiting growth of pathogenic bacteria and fungi should not be underestimated.

On the basis of long-standing use and experience, the HMPC recommends the following indications for bitter fennel fruit and sweet fennel fruit: "*Traditional herbal medicinal product*

i) for symptomatic treatment of mild, spasmodic gastro-intestinal complaints including bloating and flatulence;
ii) for symptomatic treatment of minor spasm associated with menstrual periods;
iii) used as an expectorant in cough associated with cold."

No other traditional medicinal uses of fennel are supported by adequate data.

For bitter fennel oil, traditional use is known only as "*an expectorant in cough associated with cold*" and is recommended by the HMPC.

For fennel fruit, long-standing use in TCM is also documented with indication i) and ii) when the symptoms are triggered or worsen by "cold" and improve by "heat" application.