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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Oenothera biennis* L. or *Oenothera lamarckiana* L., oleum

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	n/a
Herbal preparation(s)	Fatty oil from the seeds of *Oenothera biennis* L. or *Oenothera lamarckiana* L.
Pharmaceutical form(s)	Herbal preparations in solid dosage forms for oral use
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1. Introduction

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

- Herbal substance(s)

The HMPC has established a Community herbal monograph on the oil obtained from the seeds of *Oenothera biennis* L. or *Oenothera lamarckiana* L. The monograph does not cover the herbal substance itself, i.e. the seeds from the two species.

- Herbal preparation(s)

The fatty oil is obtained from seeds of *Oenothera biennis* L. or *Oenothera lamarckiana* L. by extraction and/or expression. It contains 65-85% linoleic acid, 7-14% gamma-linolenic acid (γ-linolenic acid) and a maximum of 0.5% is alpha-linolenic acid. Other substances are 5-12% oleic acid, 1-4% stearic acid, 4-10% palmitic acid and a maximum of 0.3% saturated fatty acids of chain length less than C_{16} (European Pharmacopoeia, 2010).

The oil of *Oenothera biennis* and *Oenothera lamarckiana* is more reactive and less stable than most other fatty oils. Besides oxidation after exposure to air and light, the oil is also sensitive to heat and humidity. Consequently, it should be stored in a cool, dark place (Price & Price, 2007).

- 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

Basic references were taken from the first edition of the assessment report dated 16 December 2011.

This revised assessment report was completed with and additional literature search using the following sources (September 2017):

Eudravigilance: screened on 30 January 2018: *Oenothera, primrose, evening primrose.* No records.

PubMed: *Oenothera biennis*:

- 141 references in total of which 49 between 2012-2017
- 19 references selected on title.

PubMed: *Oenothera biennis oil*:
- 49 references of which 14 between 2012-2017
- 10 references selected on title.

PubMed: *Oenothera safety*:
- 11 references of which 2 between 2012-2017
- 1 reference selected on title

PubMed: *Primrose oil*:
- 525 references of which 54 between 2012-2017
- 49 references selected on title

Embase: *Primrose oil* (in title):
- 228 references of which 33 between 2012-2017
- 24 references on title

Toxline: *Primrose oil*:
- 220 references of which 15 between 2012-2017
- 10 references on title

A total number of references: 113
After elimination of the doubles: 57 referencesof which 28 clinical studies.

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

In the previous assessment report it is reported that there were authorised products containing *Oenothera biennis* fatty oil on the market in the following countries:

- Germany: soft capsules (several products since 1990) and cream (1999)

- Hungary: capsules (1992)

- Ireland: cream (no date specified)

- United Kingdom: cream (no date specified)

The fatty oil of *Oenothera biennis* was accepted for traditional use, only in the oral form.

An inquiry was sent out in July 2017 to the National Competent Authorities (NCAs).

The following NCAs communicated that there are no authorised products on the market:

Austria; Belgium; Bulgaria; Cyprus; Finland; France; Greece; Italy; Latvia; Lithuania; Malta; Portugal; Romania; Slovakia; Slovenia; Spain.

There are authorised products on the market in Germany:

- Soft capsules containing *Oenothera biennis* oleum (fatty oil): since 1990

- Cream containing *Oenothera biennis* oleum (fatty oil): since 1999.

There are several products on the German Market. Some of them are identical concerning active substance, indication, pharmaceutical form, and/or posology and only differ in the date of marketing authorisation. For reasons of clarity only the oldest of such a product group is listed in the table underneath.

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
Oenothera biennis oleum (fatty oil)	For the treatment and symptomatic relief of neurodermatitis, especially of the associated pruritus.	Capsule, soft children 1-12 years: Single dose (SD): 1000-2000 mg Daily dose (DD): 2000-4000 mg adolescents and adults: SD: 2000-3000 mg DD: 4000-6000 mg It is recommended to start the treatment with the highest indicated dose. It is possible, that in some patients only after 8-12 weeks of use an amelioration of symptoms can be observed. As soon as a therapeutic success appears the treatment can be continued with a lower dose or can be stopped.	Germany since 1990 WEU
Oenothera biennis oleum (fatty oil) *	For the symptomatic relief of acute and chronic dry skin	Cream 100 g cream contain 20 g *Oenotherae biennis*	Germany since 1999 WEU

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status (date, Member State)
	conditions, to restore moisture content of the skin and improve skin smoothness.	oleum Apply evenly on the affected areas of the skin 2-3 times daily	

*This product was mentioned in the previous market overview from 01/2009 is not any longer on the German market. The marketing authorization was valid until 04/2012 (sunset clause).

Preparation 1: **side-effects oral use**: Uncommon: nausea, dyspeptic complaints, headache. Rare: hypersensitive reactions like exanthema, abdominal pain and very rare rise in temperature.

Preparation 1: **interactions oral use**: Onset of seizures-promoted by intake of /…/ cannot be excluded. Patients with epilepsy or schizophrenia should consult a doctor before the use of /…/.

In patients with a pathologically increased bleeding tendency (haemorrhagic diathesis) and concomtant anticoagulant and antiplatelet treatment, the medical product should only be used after consultation with a doctor (for the oral medication only).

Use in children and adolescents.

Preparation 1 (oral use): the use in children under 1 year of age is not recommended.

Preparation 2 (cutaneous use): there is no use restriction for children.

Special Warnings and precautions for use

Preparation 1 (oral use): the use in children under 1 year of age has not been established due to lack of adequate data.

Preparation 2 (cutaneous use): there is no restriction of use for children.

These preparations should not be used:

- near the eyes;

- in case of broken skin as it may cause burning;

- simultaneously with latex products (e.g. condom, diaphragm), reduced performance and comprised safety could result.

Preparations 1 and 2: intense UV-exposure should be avoided.

- in patients with epilepsy, a doctor should be consulted

- during pregnancy and breast feeding

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

Not applicable

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

Oenothera biennis was first grown by North American Indians. They used the plant as treatment for swelling in the body and other health problems.

Before the arrival of the Pilgrim Fathers, *Oenothera* species oil was used by the Indians in poultices to relieve skin disorders. In 1614, botanists from Virginia brought it to Europe, in order to study it. English herbalist Nicholas Culpeper wrote in 1650: "It opens obstructions of the liver and spleen, provokes urine, is good for dropsy (oedema) if infused in common drink." It was introduced to Europe by the name 'king's cure-all', which made it popular in Britain. At this time it was a popular folk remedy. Since then it was ignored for centuries, even though old herbal text books described it as astringent and sedative, with its oil being 'helpful in treating gastro-intestinal disorders, whooping cough, asthma, female complaints and wound healing'. The German scientist Unger discovered that the oil can be extracted from the seeds, which contain 15% oil, using light petroleum. In 1919, an unusual linolenic acid was found by Heiduschka and Lüft when they analysed the seed oil; they named it gamma-linolenic acid (GLA). British scientists started to examine the effects of the oil in the 1960s with medical experiments carried out in rats. These experiments demonstrated that the human body metabolised this GLA far more effectively than linoleic acid (LA). Other trials found that GLA could also control cholesterol levels (Cottier, 1996; Senapati *et al.*, 2008).Interest in *Oenothera biennis* oil increased in the late 1970s when the oil was proposed to treat various ailments. *Oenothera biennis* oil products were marketed as a supplement for the treatment of PMS (premenstrual syndrome), alcoholism, pregnancy-induced hypertension, atopic eczema, elevated cholesterol levels, hypertension, scleroderma, multiple sclerosis, rheumatoid arthritis, mastalgia and other problems. Products containing *Oenothera biennis* oil were investigated in clinical trials during the following decades. Initially, results looked promising, however, subsequent reviews called into question the standards of efficacy seen in the clinical studies.

In 2002, the UK Medicines Agency withdrew all marketing authorisations for oral evening primrose oil capsules. This followed a review by the UK Medicines Agency of all the relevant information, including new studies and statistical analyses. The UK Medicines Agency concluded that the data did not support the current standards of efficacy required for authorisation of these products as medicines for the treatment of eczema and mastalgia (Anonymous, 2002).

Posology

Based on the posology published in handbooks, the dosages for atopic eczema for adults and children are 6-8 g daily and 2-4 g daily, respectively. The dosage used for mastalgia is 3-4 g a day (Barnes *et al.,* 2007). The doses for PMS and menopausal complaints are 3-6 g a day (Capasso *et al.,* 2003). Based on clinical trials, the dosages for atopic eczema for children and adults are 2-4.5 g a day and 4-8 g a day, respectively. One gram of *Oenothera* oil contains approximately 80-116 mg GLA. The dosages used for mastalgia, menopause and PMS are 3 g, 4 g and 4-6 g, respectively. The daily dosage in diabetic patients with diabetic neuropathy is 4 to 8 g (Halat & Dennehy 2003). Patients with rheumatoid arthritis received a daily dosage of 6 g Evening Primrose Oil, (EPO) which contains 540 mg GLA (Brzeski *et al.*, 1991; Belch *et al.,* 1988). Subjects with Raynaud's phenomenon and Sjögren syndrome received 6 g Evening Primerose Oil (EPO) (540 mg GLA) and 1.5–3 g EPO (9% GLA), respectively (Belch & Hill 2000; Oxholm *et al.,* 1986). In children with attention deficit hyperactivity disorder (ADHD), a daily dosage of 3 g is administered (Aman *et al.,* 1987).

Duration of use

Three months of therapy is usually required before a full therapeutic effect is noticed (Bédard 2003). Results of clinical studies on atopic eczema demonstrated a clinical effect after 3-5 months of oral use. In trials reporting positive results, a treatment duration of 6-12 months was usually required for diabetic neuropathy patients (Halat & Dennehy 2003).

Table 2: Overview of historical data

Herbal preparation	**Documented use / Traditional use**	**Pharmaceutical form Posology Duration of use**	**Reference**
Fatty oil	Atopic eczema	Adults: 6-8 g/day Children: 2-4 g/day	Barnes *et al.,* 2007
Fatty oil	Mastalgia	Adults: 3-4 g/day	Barnes *et al.,* 2007
Fatty oil	Menopausal complaints	Adults: 3-6 g/day	Capasso *et al.,* 2003
Fatty oil	Diabetic neuropathy	Patient's age not specified: 4-8 g/day	Halat & Dennehy 2003
Fatty oil	Rheumatoid arthritis	Patient's age not specified: 6 g/day	Brzeski *et al.,* 1991; Belch *et al.,* 1988
Fatty oil	Raynaud's phenomenon (RP); Sjögren syndrome (SS)	Patient's age not specified: 6 g/day (RP); 1.5-3 g/day (SS)	Belch & Hill 2000; Oxholm *et al.,* 1986
Fatty oil	Attention Deficit Hyperactivity Disorder	Children: 3 g/day	Aman *et al.,* 1987

2.3. Overall conclusions on medicinal use

the new market overview did not reveal any new developments. Whereas in the previous assessment report Germany, Hungary, Ireland and United Kingdom had preparations on the market, only Germany kept soft capsules with fatty *Oenothera* oil as an authorised product. Germany has also a cream with *Oenothera* oil on the market, but the 30 years requirement is not fulfilled for this preparation. Apart from epileptic seizures in patients at risk, there are no serious safety concerns related to the use of *Oenothera* fatty oil.

Historically, *Oenothera* species seem to be rooted in a North-American tradition, going back centuries ago. The oil was already known at the beginning of the 17th century as a kind of panacea. It was in the early 20th century that unsaturated fatty acids were isolated from the oil. More particularly the metabolism and possible therapeutic properties of gamma-linolenic acid (GLA) were studied extensively. In the late 1970s, *Oenothera biennis* oil was investigated clinically, but most of the expectancies were not fulfilled.

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	**Indication**	**Posology, Strength**	**Period of medicinal use**
Oenothera biennis oleum (fatty oil)	For the treatment and symptomatic relief of neurodermatitis, especially of the associated pruritus.	capsule, soft adolescents and adults: SD: 2000-3000 mg DD: 4000-6000 mg	Germany since 1990

From the market overview it seems like the conditions for traditional use are not fulfilled according to Directory 2001/83/EC, i.e. 30 years of use. However, in the previous edition of the monograph

traditional use of the fatty oil was accepted because clinical trials have been conducted with the oil since 1981 (see table 6). In the publications of these studies the therapeutic use of *Oenothera* fatty oil is mentioned as a therapeutic practice at that time.

The capsules marketed in Germany can also be used in children between 1 and 12 years. However, because there are only limited data on the specific use in children the use of the oil was finally restricted to adolescents and adults (see also sections 5 and 6).

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

No studies found on primary pharmacology.

3.1.2. Secondary pharmacodynamics

Tumor suppressing activity

Abou El-Ela (1987) conducted his research on 40-day old female Sprague-Dawley rats. They could eat and drink *ad libitum*. A single intragastric dose of 5 or 10 mg DMBA (dimethoxybenzaldehyde) in 0.5 ml corn oil was given to induce mammary tumour development. In the first study, the rats received 10 mg DMBA and were divided in two groups of each 18 animals. Twenty-one days after DMBA-administration, 18 animals received a diet with 20% corn oil, the others with 20% *Oenothera* fatty oil (EPO). Three rats, who had received a corn oil diet, died before the end of the experiment, while only one died who had received an EPO diet. In study 2, no rats died previously. In the second study, the rats received 5 mg DMBA and were divided in two groups of each 30 animals. Fourteen days after administration of DMBA, the same is done as in study 1 with 30 rats instead of 18. At 16 or 13 weeks post-DMBA, the surviving rats were killed. Blood was drawn from the heart for plasma lipid analysis and the tumours were analysed. There was no significant difference in weight gain between the two diets. The average number of tumours and tumour-burden per tumour-bearing rat in study 1 was significantly greater than in study 2. The average latency period was significantly longer in study 1 than in study 2. When rats received an *Oenothera* feeding, there is a significantly decreased of malignant tumours in comparison with feeding corn oil. The lineolate level in the plasma is similar in both feedings. GLA and arachidonic acid (AA) levels were significantly higher, and oleic acid levels were significantly lower in rats who received an *Oenothera* feeding compared with a corn oil feeding. DMBA–induced mammary tumorigenesis was dose-dependent in rats fed both *Oenothera* and corn oil diets. The mammary tumour promoting effect of a diet containing 20% fat can be diminished by substituting *Oenothera* EPO for corn oil. The promoting effect on mammary cancer by a high-fat diet could be depressed by feeding a source of GLA.

Abou El-Ela (1988) used female Sprague-Dawley rats, which were forty days old. At the age of 50 days, all the rats received a single intragastric dose of 10 mg DMBA (DiMethoxyBenzAldehyde as cancerogenic agent) in 0.5 ml corn oil. Twenty-one days after the DMBA administration, the rats were divided in 3 groups of 26 and received a fed diet containing either 20% corn oil (CO), *Oenothera* fatty oil or menhaden oil (MO). The controls rats were divided into 3 groups of 10 rats each and received the same diets. The 20% CO and *Oenothera* diet contained respectively 12% linolenic acid and 16.8% linolenic acid + GLA. The 20% MO diet contained 0.8% essential fatty acid (EFA). Weekly, the rats were weighed and palpated for the presence of the tumours. The size and location of each tumour was noted. One rat who received the CO-diet and one who received the MO-diet, died previously. After 16

weeks, every rat was killed and analysed. Although tumour incidence was similar in rats fed the MO, *Oenothera* and CO diets, the number of malignant tumours was reduced by 24% and 21% in the MO- and *Oenothera*-fed rats, respectively, compared with malignant tumours recovered from rats fed the 20%-CO diet. Moreover, the frequency of mitosis and the extent of necrosis and inflammation within tumours were higher in CO-fed rats compared with MO- or *Oenothera*-fed rats. These changes denote a more rapid tumour growth and a greater, although altered, immunologic reactivity. Feeding a 20%-*Oenothera* diet significantly extended tumour latency and reduced tumour burden compared with feeding the 20%-CO diet. Although tumour incidence was unchanged with any of the three diets, rats who received an *Oenothera* diet had 21% fewer malignant tumours, longer latency and reduced tumour burden. This can be mediated by an increased synthesis of prostaglandin E1 (PGE1) that may, in turn, alter the immune responses by opposing leukotriene B4 (LTB4). The finding that a MO diet favours the immunologic generation of LTB5, which has attenuated biological activity compared with LTB4, suggests that eicosapentaenoic acid (EPA)-enriched tissues may exhibit less proinflammatory activity than EPA-poor tissues. *Oenothera* fatty acid diet may decrease malignancy by altering eicosanoid profiles.

Pellegrina *et al.* (2005): A phenolic fraction from *Oenothera biennis* showed potent and selective cytotoxic effects against bone marrow-derived tumour cells *in vitro* and *in vivo*, where it delayed the growth of established tumours.

Lewandowska *et al.* (2014) evaluated the influence of an evening primrose extract (EPE) from defatted seeds on viability and invasiveness of three human cell lines: PNT1A (normal prostate cells), DU145 (prostate cancer cells) and MDA-MB-231 (breast cancer cells). The results revealed that after 72 h of incubation the tested extract reduced the viability of DU 145 and MDA-MB-231 with IC_{50} equal to 14.5 µg/mL for both cell lines. In contrast, EPE did not inhibit the viability of normal prostate cells. Furthermore, EPE reduced PNT1A and MDA-MB-231 cell invasiveness; at the concentration of 21.75 µg/mL the suppression of invasion reached 92% and 47%, respectively (versus control). Additionally, zymographic analysis revealed that after 48 h of incubation EPE inhibited metalloproteinase-2 (MMP-2) and metalloproteinase-9 (MMP-9) activities in a dose-dependent manner. For PNT1A the activities of MMP-2 and MMP-9 decreased 4- and 2-fold, respectively, at EPE concentration of 29 µg/mL. In the case of MDA-MB-231 and DU 145 the decrease in MMP-9 activity at EPE concentration of 29 µg/mL was 5.5-fold and almost 1.9-fold, respectively. In conclusion, this study suggests that EPE may exhibit antimigratory, anti-invasive and antimetastatic potential towards prostate and breast cancer cell lines.

Singh *et al.* (2017) investigated the antiproliferative and antimicrobial activity of compounds/mixture (1-8) isolated and characterized from the roots of *O. biennis* L. A possible mechanism of antiproliferative activity was also studied by targeting ornithine decarboxylase (ODC) and cathepsin D (CATD). Antiproliferative efficacy of the compounds/mixture was examined in selected cancer cell lines along with their probable mechanism of action. The antimicrobial activity was also studied against selected microorganisms (bacteria and fungi). Antiproliferative potential was evaluated by MTT assay against selected cell lines. The mechanism of action was studied spectrophotometrically by targeting ODC and CATD using both an in-vitro and an in-silico approach. The antimicrobial efficiency was analysed using the disc diffusion and broth dilution methods. *Oenothera* lanosterol B (3) and the mixture of *Oenothera* lanosterol A and *Oenothera* lanosterol B (4) exhibited antiproliferative activity against breast, hepatic, prostate and leukaemia cancer cell lines as well as in mouse macrophages (IC_{50} 8.35-49.69 µg/ml). *Oenothera* lanosterol B (3) and the mixture of *Oenothera* lanosterol A and *Oenothera* lanosterol B (4) displayed a strong molecular interaction with succinate dehydrogenase (binding energy (BE) -6.23 and -6.84 kcal/mol and Ki 27.03 and 9.6 µm, respectively). *Oenothera* lanosterol A (1), *Oenothera* lanosterol B (3) and mixture of *Oenothera* lanosterol A and *Oenothera* lanosterol B (4) potently inhibited the ODC activity with IC_{50} ranging from 4.65 ± 0.35 to 19.06 ± 4.16 µg/ml and also showed a strong interaction with ODC (BE -4.17 to -4.46 kcal/mol).

Oenothera lanosterol A (1), cetoleilyl diglucoside (2), *Oenothera* lanosterol B (3), dihydroxyprenylxanthone acetylated (6) and dihydroxyprenylxanthone (7) inhibited CATD activity (IC_{50} 3.95 ± 0.49 to 24.35 ± 2.89 µg/ml). The in-silico molecular interaction analysis of compounds with CATD revealed the non-specific interaction. A moderate antimicrobial activity was observed against selected microbes with a growth inhibition ranging from 6 to 14 mm and minimum inhibitory concentration between 125 and 500 µg/ml. *Oenothera* lanosterol B (3) and dihydroxyprenylxanthone acetylated (6) exhibited better antimicrobial activity with an MIC range from 62.50 to 500 µg/ml. *Oenothera* lanosterol B (3) exhibited stronger antiproliferative and antimicrobial potential with respect to the other compounds tested, whereas *Oenothera* lanosterol A (1) was a potent inhibitor of ODC and CATD. *Oenothera* phenoxylactone (5) only altered the proliferation of cervical cancer cells (IC_{50} 50.90 µg/ml), while cetoleilyl diglucoside (2), dihydroxyprenylxanthone (7) and *Oenothera* phytyllactone (8) significantly decreased prostate cancer cell proliferation with an IC_{50} range from 5.34 to 16.61 lg/ml, without affecting the growth of other tested cell lines. Hence, it is suggested that these in-vitro findings could be studied further *in vivo* for biological activity, safety evaluation and derivatization to enhance potency and efficacy.

Zeng *et al.* (2013) isolated and purified a water-soluble polysaccharide named as evening primrose polysaccharide (EPP) from evening primrose by hot water extraction, alcohol-precipitation, anion exchange and gel permeation chromatography. EPP had a weight-average molecular weight of about 9.8 kDa and was composed of glucose (Glc), galactose (Gal), mannose (Man), arabinose (Ara) and rhamnose (Rha) in a molar ratio of 2.4:3.1:1.3:1.9:1.0. After 10 days of administration with EPP to H22 tumor-bearing mice, a significant increase in tumor growth inhibitory rate, body weight, relative spleen weight, lymphocytes proliferation, natural killer (NK) cell activity, macrophage phagocytosis, delayed type hypersensitivity (DTH), white blood cell and lymphocyte counts were observed at the dose of 25, 50 and 100 mg/kg. Meanwhile EPP treatment decreased the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkalines phosphatases (ALP), creatinine and urea of tumor-bearing mice to normal levels. These data proved that EPP might be employed as effective therapeutic agents in the regulation of diverse immune reactions implicated in cancer.

Lipid lowering activity

De La Cruz *et al.* (1997) performed a study of 6 weeks. Animals used were forty male white New Zealand rabbits of 2 months and had a body weight of 2498 ±36 g. Four groups were made with each ten rabbits. The first group received a normal diet and was seen as controls. The second group received an atherogenic diet. The following group had to eat a normal diet with 15% *Oenothera* fatty oil. The last group of rabbits received an atherogenic diet with 15% *Oenothera* fatty oil. The rabbits received their meal periodically. Serum lipid profile, platelet aggregation in whole blood, tromboxane B2 production and platelet lipid peroxide were measured. *Oenothera* fatty oil reduced platelet production. The oil also inhibited the lipid peroxide production. The cholesterol was reduced by 25%, the triglyceride value by 51% and the HDL-cholesterol was raised by 64%. *Oenothera* fatty oil reduced platelet hyperaggregability in rabbits fed an atherogenic diet.

Gastro-intestinal activity

Al–Shabanah (1997) used Wistar albino rats of both sexes, approximately the same age and weighing 150-200 g. They were feeding on a standard rat chow. The rats in the control groups received orally 10 ml corn oil/kg body weight. The non-control groups received orally 5 or 10 ml *Oenothera* oil. The ulcerogenic drugs used were aspirin and indomethacin. In the first study, pylorus ligation-induced ulcers were tested. The rats were deprived of food for 36 hours with free access to water until the morning of the experiment. Under anaesthetic a small midline abdominal incision was made. Immediately after pylorus ligation, the *Oenothera* oil or the corn oil were given intragastrically. Six hours after pylorus ligation, the rats were killed and their stomachs were analysed. Pylorus ligation

for 6 hours in fasted rats caused an increase in gastric acid secretion and acidity output. *Oenothera* reduced the volume of gastric secretion, free acid and total acid output significantly, compared with controls. The second study proceeded NSAID-induced gastric ulcers. The *Oenothera* or corn oil were administered intragastrically, 30 minutes before the ulcerogenic drugs. Aspirin and indomethacin were administered orally after fasted 40 hours. The dose of aspirin was 200 mg/kg (0.5 ml/kg) body weight and the rats were killed after 4 hours administration of aspirin. The dose of indomethacin was 30 mg/kg body weight (0.5 ml/100 g). The rats were killed after 6 hours administration of indomethacin. Each stomach was examined. Phenylbutazone induced gastric lesions. Two hundred mg/kg phenylbutazone was given intraperitoneal to rats who had fasted 24 hours. After 6 hours of administration, the rats were killed. The results are: pre-treatment with *Oenothera* produced a dose-dependent decrease in the gastric ulceration induced by aspirin and phenylbutazone whereas, in the indomethacin-treated group of rats, *Oenothera* significantly inhibited gastric ulceration at the dose of 10 ml/kg only. The inhibition of gastric erosions was in the range of 32 to 95%. The maximum inhibition of gastric erosion following *Oenothera* administration was in the phenylbutazone group at the dose of 10 ml /kg *Oenothera*. The third study proceeded hypothermic restraint stress-induced ulcers. The rats were fasted for 36 hours with access to water *ad libitum*. One hour after receiving the *Oenothera* or corn oil treatment, the rats were immobilised in restraint cages and placed inside a ventilated refrigerator at a temperature of 2-4°C for 2 hours and further they were killed. The stomachs were analysed. The results of the treatment with *Oenothera* oil was a dose-dependently decreased intraluminal bleeding in comparison with the control rats, whereas *Oenothera* (10 ml/kg body weight), produced also a significant reduction in gastric ulceration with an inhibition of ulceration index by 85%, relative to the control values. The last study consisted of gastric ulcers induced by necrotising agents (cytoprotective studies). After fasting 36 hours with access to water *ad libitum*, 1 ml of necrotising agent (0.6 M HCl, 0.2 M NaOH, 25% NaCl or 80% (V/V) aqueous ethanol) was given intragastrically. *Oenothera* or corn oil was administered 30 minutes before the necrotising agents. One hour after the administration of necrotising agents, the rats were killed and their stomachs were examined. The inhibitory action exerted by *Oenothera* oil on the ulcers induced by HCl and ethanol was dose-dependent and highly significant, the inhibition of ulceration being in the range of 36-84%. Those ulcers induced by HCl and NaOH were prevented by *Oenothera* oil at a dose of 10 ml/kg only, with the percentage inhibition in the range of 33-84%.

The results show that *Oenothera* oil prevents an increase in acid secretion in pylorus-ligated rats, and inhibits formation of gastric ulcers induced by different ulcerogenic drugs, by cytodestructive agents and by stress caused by hypothermic restraint. *Oenothera* exerts a dose-dependent inhibitory action on gastric mucosal lesions caused by various necrotising agents. In this study, *Oenothera* has a significant anti-ulcer and cytoprotective effect on various experimentally induced gastric lesions.

Data suggest that γ-linolenic acid, administered as *Oenothera* oil, can prevent or reverse diabetic neuropathy in animal models.

Anticoagulating activity

Riaz *et al.* (2009) assessed the effect on coagulation parameters in healthy rabbits. *Oenothera* fatty oil contained 73% linoleic acid, 9% γ-linolenic acid, 8.6% oleic acid, 6.3% palmitic acid, 1.9% stearic acid and 10 IU vitamin E. The study was carried out on 50 healthy white rabbits of either sex weighing 1 to 15 kg. The rabbits were divided in 5 groups of each 10 animals. Three groups were administered normal (90 µl/kg daily), moderate (180 µl/kg daily) and high doses (360 µl/kg daily) of *Oenothera*. As a standard, 0.54 mg/kg warfarin sodium was used. The control animals were administered with water equivalent to the corresponding dose of *Oenothera*. Blood samples were collected once at 30 days and once at 60 days on the end of the study. Haematological parameters, red blood cell (RBC), white blood cell (WBC), platelet (PLT) and haemoglobin (Hb) were measured. Thrombin time (TT), prothrombin time (PT), activated prothromboplastin time (aPTT) and fibrinogen time (Fg) were measured to monitor

the influence of *Oenothera* on the blood coagulation process. After 30 days of treatment a significant increase was found in PT and TT at normal, moderate and high doses and at standard drug warfarin. Also a significant increase in aPTT was observed at normal, moderate and high dose of *Oenothera* oil and standard drug warfarin. After 60 days significant increase in TT and in PT were found at all doses. Fibrinogen time was not significantly affected at any dose. This study concluded that *Oenothera* oil has anticoagulant properties and its anticoagulant activity is supported by its anti-inflammatory effect. These effects along with antiplatelet activity suggest that *Oenothera* oil may be of value in cardiovascular diseases.

Abo-Gresha NM *et al.* (2014) investigated the effects of evening primrose oil (EPO) in an experimental rat model for myocardial infarction (MI) in the presence of diet-induced hyperaggregability. This study was designed to examine its cholesterol lowering, antithrombotic and anti-inflammatory effects. High fat diet was administered for 4 weeks then MI was induced by isoproterenol (85 mg/kg/s.c./24 h). Treatment with EPO (5 or 10 gm/kg/day) for 6 weeks improved the electrocardiographic pattern, serum lipid profile, cardiac biomarkers as well as the platelet aggregation *ex vivo*. The authors reported decreased serum level of TNF-alpha (Tumor Necrosis Factor-alpha), IL-6 (Interleukine-6) and COX-2 (Cyclo-oxygnease 2) with attenuation of TNF-alpha and TGF-beta in the cardiac homogenate. Moreover, histopathology revealed marked amelioration. Finally, it was demonstrated that EPO improved cardiac recovery in hypercholesterolemic myocardial infarct rats. These effects were attributed by the authors to a direct hypocholesterolemic effect and an indirect effect on the synthesis of eicosanoids (prostaglandins, cytokines).

Mosaad *et al.* (2017) explored the effect of evening primrose oil (EPO), a source of prostaglandin E1, and forskolin (a cyclic adenosine monophosphate stimulator) against the prothrombotic effect of celecoxib in mice. Lipopolysaccharide mouse model of endotoxemia was used to induce an upregulation of TF (Tissue Factor) activity. Male mice received celecoxib (25 mg/kg), celecoxib (25 mg/kg) plus EPO (5 g/kg), or celecoxib plus forskolin for 4 weeks and then subjected to a prothrombotic challenge in the form of an intraperitoneal injection of lipopolysaccharide. Results showed an increase in plasma TF activity, endothelial TF expression, and thrombin-antithrombin (TAT) but lower antithrombin III (ATIII) level in mice that received celecoxib in comparison to those that received the vehicle. Adding EPO or forskolin to celecoxib regimen significantly decreased the prothrombotic effect of celecoxib. A positive correlation (r = 0.8501) was found between TF activity and TAT. Co-administration of EPO or forskolin decreased the activity of TF and mitigated the prothrombotic effect of celecoxib. The authors concluded that these combinations may have the utility to abrogate the prothrombotic adverse effect of celecoxib in clinical setting.

Neuroprotective activity

Badri *et al.* (2017) investigated the effects of electrical stimulation (ES), combined with evening primrose oil (EPO), on sciatic nerve function after a crush injury in rats. In anesthetized rats, the sciatic nerve was crushed using small haemostatic forceps followed by ES and/or EPO treatment (450 mg/kg intragastrically) for 4 weeks. Functional recovery of the sciatic nerve was assessed using the sciatic functional index. Histopathological changes of gastrocnemius muscle atrophy were investigated by light microscopy. Electrophysiological changes were assessed by the nerve conduction velocity of sciatic nerves. Immunohistochemistry was used to determine the remyelination of the sciatic nerve following the interventions. EPO + ES, EPO, and ES obviously improved sciatic nerve function assessed by the sciatic functional index and nerve conduction velocity of the sciatic nerve at 28 days after operation. Expression of the peripheral nerve remyelination marker, protein zero (P0), was increased in the treatment groups at 28 days after operation. Muscle atrophy severity was decreased significantly less while the nerve conduction velocity was increased significantly more in the injury + EPO + ES group as compared to the EPO or ES group. The combined use of EPO and ES may improve sciatic nerve function after injury. The increased expression of P0 may have contributed to improvement by

combined therapy with EPO and ES as well as the electrophysiological and histopathological features of the injured peripheral nerve.

Ramli *et al.* (2017) classified 72 healthy adult Sprague-Dawley rats into three groups: normal group, control group, and experimental group. The result indicates that there was significant difference in toe-spreading reflex between the normal and the control groups (1.9 ± 0.031, $p<0.05$) and the normal and the EPO groups (6 g/day) (0.4 ± 0.031, $p<0.05$) and significant difference between EPO and the control groups (1.5 ± 0.031, $p<0.05$). Regeneration of axons and myelin in nerve fibre in the EPO-treated group developed better and faster than in the control group. In the control group, the shape of the axon was irregular with a thinner myelin sheath. In the experimental group, the shape of the axons, the thickness of the myelin sheath, and the diameter of the axons were almost the same as in the normal group. In The authors concluded that EPO supplementation may be beneficial as a therapeutic option for disturbances of nerve interaction.

Anti-inflammatory activity

El-Sayed *et al.* (2014) evaluated the anti-angiogenic, anti-inflammatory, and anti-oxidant effects of evening primrose oil (EPO), rich in gamma linolenic acid (GLA), either alone or in combination with aspirin or celecoxib, on adjuvant-induced arthritis. Arthritis was induced by subcutaneous injection of complete Freund's adjuvant (CFA) in the right hind paw of male albino rats. All treatments were administered orally from day 0 (EPO, 5 g/kg b.w.) or day 4 (celecoxib, 5 mg/kg; aspirin, 150 mg/kg) till day 27 after CFA injection. In the arthritic group, the results revealed significant decrease in the body weight and increase in ankle circumference, plasma angiopoietin-1 (ANG-1) and tumor necrosis factor-alpha (TNFalpha) levels. Anti-oxidant status was suppressed as manifested by significant decline in reduced glutathione content along with decreased enzymatic activity of superoxide dismutase and increased lipid peroxidation. Oral administration of EPO exerted normalization of body weight, ANG-1, and TNFalpha levels with restoration of activity as shown by reduced malondialdehyde levels. Moreover, histopathological examination demonstrated that EPO significantly reduced the synovial hyperplasia and inflammatory cells invasion in joint tissues, an effect that was enhanced by combination with aspirin or celecoxib. The joint use of GLA-rich natural oils, which possess anti-angiogenic, anti-inflammatory, and anti-oxidant activities, with traditional analgesics represents a promising strategy to restrain the progression of rheumatoid arthritis.

Granica *et al.* (2013) investigated the chemical composition of extracts prepared from aerial parts of *Oenothera paradoxa* Hudziok and *Oenothera biennis* L. and their antioxidative and anti-inflammatory activities. Ultra high pressure liquid chromatography (UHPLC)-DAD-MS/MS studies showed that both extracts contained a wide variety of polyphenols (39 identified constituents) among which macrocyclic ellagitannin turned out to be the main constituent. During the *in vitro* studies, using noncellular models, both extracts scavenged reactive oxygen species (ROS) in a concentration-dependent manner, and the lowest IC_{50} values (between 1 µg/ml and 16,8 µg/ml in different experimental models) were obtained for O_2^- and H_2O_2. Both extracts inhibited ROS production by stimulated human neutrophils. The stronger activity in the case of formyl-met-leu-phenylalanine stimulation suggests that both extracts may act through the receptor-dependent pathway. *O. paradoxa* extract and *O. biennis* extract exhibited anti-inflammatory activity by the inhibition of hyaluronidase and lipoxygenase in a concentration-dependent manner. The stronger activity of *O.biennis* extract toward lipoxygenase may be explained by its higher oenothein B content.

Montserrat-de la Paz *et al.* (2012) isolated sterols from the unsaponifiable matter of evening primrose oil, and the composition was identified and quantified by GC and GC-MS. The major components of sterols fraction were β-Sitosterol and campesterol. The authors investigated the ability of sterols from evening primrose oil to inhibit the release of different proinflammatory mediators *in vitro* by murine peritoneal macrophages stimulated with lipopolysaccharide. Sterols significantly and

dose-dependently decreased nitric oxide production (concentra tions between 25-100 µg/ml). Western blot analysis showed that nitric oxide reduction was a consequence of the inhibition of inducible nitric oxide synthetase expression. Sterols also reduced tumor necrosis factor-α, interleukine 1β and tromboxane B_2. However, sterols did not reduce prostaglandin E_2. The reduction of eicosanoid release was related to the inhibition of cyclooxygenase-2 expression. The authors concluded that these results showed that sterols may have a protective effect on some mediators involved in inflammatory damage development, suggesting its potential value as a putative functional component of Evening Primrose oil.

Montserrat-de la Paz *et al.* (2014) demonstrated the *in vitro* anti-inflammatory effect of long-chain fatty alcohols, minor compounds isolated from Evening primrose oil (EPO). A mixture of long chain fatty alcohols (LCFAs) was isolated from the non-triacylglycerol fraction of the EPO. Hexacosanol (38.65%), tetracosanol (31.59%), docosanol (11.36%) and octocosanol (7.64%), were the major constituents, identified and quantified by GC and GC-MS. LCFA was tested with LPS (lipopolysaccharide) stimulated murine peritoneal macrophage. This fraction, significantly and dose-dependently decreased nitric oxide production induced by LPS ($p<0.001$) and the inhibitory effect seems to be consequence of an action at the level of the inducible nitric-oxide synthethase (iNOS) gene enzyme expression rather than to a direct inhibitory action on enzyme activity. The release of PLA2 and TXB2 also was significantly inhibited by LCFAs ($p<0.001$) although LCFAs did not affect to PGE2 generation, however the western blot assay showed that LCFAs reduced cyclooxygenase-2 enzyme gene expression at all doses assayed. In the same way, the secretion of inflammatory cytokines interleukin 1beta (IL-1beta) and tumour necrosis factor alpha (TNF-alpha) from LPS-stimulated murine macrophage, were also significantly reduced ($p<0.001$). These results demonstrates the anti-inflammatory activity of LCFAs, providing an additional value about the role of bioactive minor compounds in the beneficial effect of EPO.

Singh *et al.* (2012) *Oenothera* lanosterol A and B from *Oenothera biennis* roots and their suppression of IL-6 and TNF-α expression in mouse macrophages was investigated *in vitro*. *Oenothera* lanosterol A and B (Oen-A & Oen-B) along with gallic acid (GA) were isolated and characterized using column chromatography and NMR. The compounds were tested with LPS stimulated peritoneal mouse macrophages assaying for suppression of IL-6, TNF-α and NO synthesis. Significant inhibition of TNF-α and IL-6 by GA, Oen-A and Oen-B (1 µg/ml) was observed ($p<0.05$). Inhibition was concentration dependent and no synergistic or antagonistic effect on pro-inflammatory cytokines was found when used in combination (1:1) ($p>0.05$). The study demonstrates the anti-inflammatory activity of *Oenothera biennis* root compounds.

Diabetes

Omran (2012) investigate the possible beneficial effects of evening primrose oil (EPO) on histopathological changes of sciatic nerves in streptozotocin-induced diabetic rats. The rats were randomly allotted into three experimental groups: A (control), B (diabetic untreated), and C (diabetic treated with EPO: 1.25 g/day); each group contained 10 animals. Groups B and C received streptozotocin (STZ) to induce diabetes. The rats in group C were given EPO for 2 weeks after 6 weeks of STZ injection. Blood and tissue samples were obtained for biochemical and histopathological investigation. STZ-treated diabetic rats showed reduction of the size of islets of Langerhans, fatty degeneration in the pancreatic acini with dilation, irregularity, and increased thickness of blood vessels. Electron micrography of sciatic nerves of diabetic rats showed multiple vaculations and partial separation of myelinated nerve fibers with axonal atrophy, endoneural edema, and increased collagen fibers. Compared with diabetic rats, EPO induced partial recovery from diabetes-induced pancreatic and nerve damage. Histologic evaluation of the tissues in diabetic animals treated with EPO showed fewer morphologic alterations with significant decrease of myelin breakdown. Furthermore, the ultrastructural features of axons showed partial improvement. Further preclinical research into the

utility of EPO may indicate its usefulness as a potential treatment on peripheral neuropathy in STZ-induced diabetic rats.

Elkoussi and Abdellah (2014) studied the potential therapeutic usefulness of single and concurrent administration of evening primrose oil and alpha lipoic acid in diabetic neuropathy. They evaluated the pharmacological activity of the single and combined administration of EPO, Alpha-Lipoic acid (ALPA) and insulin in treating STZ-induced diabetic neuropathy in rats. The effects of the tested drugs on the biochemical changes, manifestations associated with diabetic neuropathy and histopathological changes in sciatic nerve and pancreas were investigated. EPO and ALPA attenuated the hyperglycemia induced by STZ in rats. Insulin alone or in combination with EPO corrected the blood glucose to near normal level. EPO, ALPA and insulin were also effective in treatment of dyslipedemia associating STZ-induced diabetes in rats and corrected the deficient NO serum level in these animals. The tested drugs decreased lipid peroxidation, increased total antioxidant status, decreased thermal and mechanical hyperalgesia, improved the defect in motor nerve conduction velocity and the sensory ataxia in diabetic rats. EPO and ALPA treatment exerted some protective effect on the beta-cells of diabetic rat's pancreas and improved the histopathological changes on the sciatic nerve of these animals. This study reveals that EPO improves glycemic control, lipid abnormalities and antioxidant capacity; thus restores the impaired functional properties of peripheral nerves. The beneficial effects of EPO seemed to be augmented after its combined administration with ALPA or insulin.

Kim *et al.* (2012) studied the anti-inflammatory and anti-fibrotic effects of gamma linolenic acid in diabetic nephropathy. Sprague-Dawley rats were intraperitoneally injected with either a diluent [n=16, control (C)] or streptozotocin [n=16, diabetes (DM)], and eight rats each from the control and diabetic groups were treated with evening primrose oil by gavage for three months. Rat mesangial cells and NRK-52E cells were exposed to medium containing 5.6 mM glucose and 30 mM glucose (HG), with or without GLA (10 or 100 μM). Intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP-1), and fibronectin (FN) mRNA and protein expression levels were evaluated. Twenty-four-hour urinary albumin excretion was significantly increased in DM compared to C rats, and GLA treatment significantly reduced albuminuria in DM rats. ICAM-1, MCP-1, FN mRNA and protein expression levels were significantly higher in DM than in C kidneys, and these increases were significantly abrogated by GLA treatment. *In vitro*, GLA significantly inhibited increases in MCP-1 mRNA expression and protein levels under high glucose conditions in HG-stimulated mesangial and tubular epithelial cells ($p<0.05$, respectively). ICAM-1 and FN expression showed a similar pattern to the expression of MCP-1. The authors conclude that GLA not only attenuates inflammation by inhibiting enhanced MCP-1 and ICAM-1 expression, but also ECM accumulation in diabetic nephropathy.

Antiasthmatic properties

Salama *et al.* (2015) evaluated the effects of evening primrose oil (EPO) alone or combined with dexamethasone (DEX) on a rat model of allergic asthma. Rats were sensitized to OVA (1 mg/kg; i.p.) for 3 consecutive days, rats were pretreated orally with DEX (1 mg/kg), EPO alone in three doses (1, 2 and 3 g/kg), EPO (1.5 g/kg) combined with DEX (0.5 mg/kg) and saline 1h before exposure to 1% OVA aerosol challenge (1 day/week for 3 weeks). The results were compared with positive control rats (OVA rats). Lung function tests were assessed after the last challenge and 24 h thereafter, blood films were prepared for assessment of eosinophil count and blood samples were collected for assessment of serum total protein as well as immunoglobulin E (Ig-E) levels. Lungs were isolated for histopathological study and also for determination of tumor necrosis factor-alpha (TNF-alpha) content in lung tissue . Additionally, the effects of test agents were evaluated in acetyl choline (ACh)-induced airway constriction. PO alone and combined with DEX modulate Ig-E, TNF-alpha, eosinophil recruitment, airway constriction and remodeling. The authors concluded that EPO alone or combined with a lower dose of DEX had antiasthmatic effects.

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

There were no preclinical studies related with primary pharmacological outcomes. On the contrary quite a lot of secondary preclinical studies were found in the literature.

Evening primrose preparations has tumor suppressing activity *in vitro* and *in vivo*. Tumor cell lines studied *in vitro* included bone marrow derived tumor cells, prostate and breast tumor cells. Some studies used a biochemical approach. In most studies evening primrose oil (EPO) was used. In one study the effects of evening primrose polysaccharide (EPP) were investigated.

Other activities studied *in vivo* included: lipid lowering effects (in rabbits), gastroprotective activity (in rats), anticoagulation in rabbits, protection agaist myocard infarct in rats and antagonising the prothrombotic effect of celecoxib in mice.

Evening primrose oil (EPO) and the sterole fraction of it, had also anti-inflammatory activity in adjuvant arthritic rats *in vivo*. In several *in vitro* models it exerted radical scavenging activity and it inhibited the release of inflammatory mediators. In one study aerial parts of evening primrose were used. EPO protected streptozotocin-induced diabetic rats against neuropathy. It had also a protective activity against allergic asthma in ovalbumin pretreated rats.

In general it is difficult to relate all these effects to the therapeutic indication for EPO in the monograph, although anti-inflammatory and antiasthmatic activity and the influence on the release of inflammatory mediators may play a role for the development of symptoms in atopic patients.

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

No data available

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

3.3.1. Single dose toxicity

No data available.

3.3.2. Repeat dose toxicity

Everett *et al.* (1988a) studied the toxic effects of *Oenothera* oil in a long-term study carried out on dogs (52 weeks) and rats (53 weeks). Twenty male and 20 female beagle dogs received a daily dose of 5 ml/kg oil by oral gavage. A natural vitamin E was added to the oils. There were 4 groups with a different dose of evening primrose oil (EPO) (0, 1, 3, 5 ml/kg) and corn oil. Food consumption was measured every week, body weight every 4 weeks and ophtalmoscopic examination was performed at 0, 25 and 52 weeks. Haematology analysis, clinical chemistry analysis and urinalysis were carried out at 0, 12, 26 and 50 weeks. After 52 weeks, the animals were killed and a *post mortem* examination

was carried out. One dog died during the study, after administration of an intermediate dose ofEPO , was also examined. A histopathologic examination was performed on internal and external body parts. These data were analysed and the EPO treatment was compared with the control group. No significant differences were found in food consumption, clinical signs or body weight changes neither in haematology, urinalysis nor clinical chemistry. No differences were found in the necroscopical or histopathological examination.

Male and female Sprague-Dawley rats (n=100 in each group), 5-6 weeks old, received a daily dose of 2.5 ml/kg oil by oral gavage, which contained 0, 0.3, 1 or 2.5 ml/kg evening primrose oil. Examinations were the same for all rats. Only the ophtalmoscopic examination was carried out at 0 and 50 weeks and the *post mortem* was carried out at 53 weeks. Eleven rats died, 5 in the control and 6 in the EPO group. A significant increase in potassium level was found in female high dose rats. No other significant differences were found. No important adverse effects were found in comparison with corn oil.

3.3.3. Genotoxicity

No data available.

3.3.4. Carcinogenicity

Everett *et al.* (1988b) performed a long-term study on a total number of 500 rats randomly assigned to 4 treatment groups and 1 placebo group. Two hundred male and 200 female Sprague-Dawley rats were administered with 2.5 ml/kg/day oil during 5-6 weeks, 50 animals from each gender received a daily dose of 0.3 ml/kg, 1 ml/kg or 2.5 ml/kg *Oenothera* oil. The lower doses were diluted to 2.5 ml with corn germ oil. The remaining 50 males and females were given 2.5 ml corn germ oil as a control. Fifty other animals from each gender received a normal laboratory diet. After 104 weeks, the surviving and deceased animals were subjected to a *post mortem* histopathologic examination. An identical experiment with CD-1 mice, where the *post mortem* histopathologic examination was conducted after 78 weeks because of the short life expectation of the animals, showed the same results. These experiments did not find any significant difference in the nature and the frequency of the tumours between the animals with a different dose of *Oenothera* and the control animals (Hänsel *et al.,* 1993).

3.3.5. Reproductive and developmental toxicity

In animal studies, *Oenothera* oil was found not to be teratogenic (Barnes *et al.,* 2007).

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

No specific data available.

3.3.8. Conclusions

There was no repeated-dose, carcinogenic or teratogenic effect observed in the studies.

3.4. Overall conclusions on non-clinical data

There are no preclinical studies related with primary pharmacological outcomes. Secondary pharmacological studies with evening primrose oil concerned possible antitumoral activity. Furthermore lipid lowering effects, gastroprotective activity, anticoagulation, protection agaist myocard infarct and antagonising the prothrombotic effect of celecoxib were demonstrated. Also anti-inflammatory, anti scavenging, neuroprotective and anti-asthmatic activity was shown. All these studies were performed *in vitro* and *in vivo*. As compared to the previous edition of the European Union herbal monograph (2011), more experimental pharmacological perspective is created, although clinical studies must confirm therapeutic applications.

Anti-inflammatory and antiasthmatic activity and the influence on the release of inflamapmatory mediators may play a role in the development of symptoms in atopic patients. These activities can be linked indirectly with the monograph.

Specific data on pharmacokinetics and interactions are not available.

Non-clinical information on the safety of evening primrose oil is scarce.

There was no repeated-dose, carcinogenic or teratogenic effect observed in a limited number of studies. As there is only limited information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

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

Linoleic acid (LA) is an essential fatty acid that is metabolised by delta-6-desaturase (D6D) and elongase, which forms dihomo-gamma-linolenic acid (DGLA). The D6D step is time and rate-limiting. Through delta-5-desaturase, AA is formed (Figure 1). Cyclooxygenase (COX) transforms DGLA and AA into serie 1 and serie 2 prostaglandines and thromboxanes, respectively. Leukotrienes are formed by the metabolisation of AA by lipoxygenase (Martens-Lobenhoffer & Meyer, 1998).

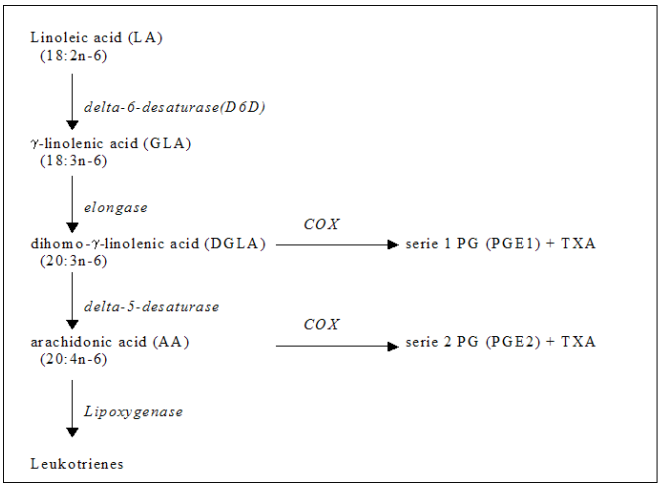

Figure 1: Metabolic pathway of linoleic acid.

Only the cis-configuration of linoleic acid and its metabolites are biologically active. The D6D activity depends on different factors: ATP, insulin and protein-rich diet are activating; factors with inhibitory effect include c-AMP, glucagons, glucocorticoids and thyroxine (Hänsel *et al.,* 1993).

There is a deficit of D6D enzyme in atopic eczema and premenstrual syndrome (PMS). This causes an increase of LA and a decrease of GLA and DGLA. An increased sensitivity for prolactin and other hormones during the luteal phase in PMS might be caused by the abnormal fatty acid metabolism (Collins *et al.,* 1993). Because of this D6D deficit in atopic eczema, there is a lower concentration of PGE1 and PGE2. The decrease of PGE1 causes an increased IgE concentration which leads to a release of mediators including histamine from leukocytes, mast cells and basophils. A decrease of PGE2 results in less activation of T-suppressor lymphocytes (= T-regulatory cells). These cells discriminate self from non-self antigens. Because of a reduction in T-suppressor lymphocytes, self antigens will be recognised as non-self antigens and will activate helper T cells. These cells will produce interleukin-2 (IL-2), which leads to stimulation of cytotoxic T cells. Helper T cells will also activate B cells, which produce IgE, which will once more lead to more release of histamine and other mediators. The shortage of EFA also plays a role in the skin disorder because it is necessary for the maintenance of the epithelial barrier which causes an increased permeability (Kerscher & Korting, 1992).

Patients with atopic eczema and premenstrual syndrome have a deficit in D6D, the enzyme that converts linoleic acid in γ-linolenic acid. *Oenothera* oil contains γ-linolenic acid. Based on this biochemistry, its therapeutic use can be hypothesised in patients with atopic eczema and PMS with a D6D deficit.

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

Martens-Lobenhoffer & Meyer (1998) investigated the pharmacokinetics of GLA with and without (control) the administration of *Oenothera* oil. Three male and female volunteers between 21-25 years old participated in the study. From 6 volunteers, serum concentration time curves of fatty acids were profiled 24 hours with and without the administration of evening primrose oil (EPO). The volunteers took 6 capsules of EPO at 7 am and 7 pm. Six capsules contain a total amount of 240 mg GLA, resulting in a daily amount of 480 mg GLA. The volunteers were on a low fat diet during the experiment. On the days without EPO , blood samples were taken every 2 hours. Blood samples were taken before and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 9 and 11.5 hours after the beginning of the first treatment at 7 am and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 9 and 12 hours after the second treatment at 7 pm. A significantly higher concentration of GLA was noticed compared to the baseline levels. Non-significant elevations in AA, DGLA and LA were detected. In the average of all administrations, C_{max} is about 4.5 times higher than the baseline in the related volunteer. The study also observed a much slower uptake of GLA in the morning. In the morning, the mean time to reach the maximum concentration of gamma-linolenic acid takes 4.4 $\pm$ 1.9 hours, in the evening this takes only 2.7 $\pm$ 1.2 hours (Table 4). Bioavailability was not calculated.

Table 4: Pharmacokinetic parameters of gamma-linolenic acid (Martens-Lobenhoffer & Meyer, 1998).

Volunteer	t_{max} AM (h)	C_{max} AM ($\mu g \times ml^{-1}$)	t_{max} PM (h)	C_{max} PM ($\mu g \times ml^{-1}$)	AUC_{12h} AM ($\mu g \times ml^{-1} \times h$)	AUC_{12h} PM ($\mu g \times ml^{-1} \times h$)	AUC_{24h} ($\mu g \times ml^{-1} \times h$)
Mean (SD)	4.4 (1.9)[a]	22.6 (16.9)	2.7 (1.2)	20.7 (12.2)	119.0 (103.1)	155.1 (131.3)	274.1 (232.8)[b]

[a] Significantly higher than t_{max} PM ($p<0.05$).

[b] Significantly higher than AUC_{24h} of the baseline concentration of gamma-linolenic acid (114.5 $\pm$ 87.6 µg x ml^{-1} x h, $p<0.05$).

Assessor's overall conclusions on pharmacokinetics

GLA, administered as *Oenothera* oil, is absorbed from the gastro-intestinal tract, without significant elevations in AA, DGLA and LA when compared to baseline levels.

4.2. Clinical efficacy

4.2.1. Dose response studies

There are no dose response studies available.

4.2.2. Clinical studies (case studies and clinical trials)

Clinical studies related to the therapeutic indication of the monograph, i.e. atopic eczema

'Atopic eczema/dermatitis syndrome' is the summarised term for 'atopic dermatitis', 'atopic eczema' and 'prurigo Besnier'. This disease is a chronic inflammatory of the skin, which can appear everywhere on the body. Mostly it begins in childhood. Frequently, it is related to other atopic diseases such as asthma. Both genetic and socio-economic factors play an important role. A deficiency of the immune system can provoke atopic eczema. A shortage of structural lipids like ceramid, which can hold water in the stratum corneum, and a shortage of EFA can also be the cause of atopic dermatitis. The diagnosis is clinically established and based on a variety of sensitive and specific symptoms and signs (Bamford *et al.,* 2013).

Cutaneous application

Anstey *et al.* (1990) performed a randomised, double-blind, placebo-controlled trial over a period of 2 weeks. Twelve patients between 4 and 46 years old, 6 women and 6 men, with mild or moderate atopic eczema were included in this pilot study. During a period of 14 days, the patients applied an *Oenothera* cream (water-in-oil -w/o- emulsion) or the placebo cream. The subjects could only treat themselves with the given cream during the entire trial. One patient left the study. Self-assessment scores from the patients indicated significant better results for the *Oenothera* cream compared to the placebo cream. However, the physician's scores did not reveal any significant difference between the *Oenothera* cream or the placebo cream.

Assessor's comment: This study may be considered as of low quality, because of the limited number of patients, the inconsistency between the findings by patients and physicans and the way of assessing.

Gehring *et al.* (1999) conducted a vehicle-controlled, randomised, double-blind trial with a two within-person right/left forearm parallel design. Transepidermal water loss (TEWL) and stratum corneum hydration were the epidermal barrier function parameters investigated. For the barrier function test 0.1N sodium lauryl sulphate (SLS), as hydrophilic irritant, and nicotinic acid, as lipophilic irritant, were used. Two studies were performed during 5 weeks, based on 4 weeks treatment following by a 1-week treatment-free period. Each study included 20 study subjects (age = 19-42 years) with an atopy score of 10 or more. In the first study, an amphiphilic oil-in-water emulsion of 20% *Oenothera* fatty oil was used. The placebo was a vehicle with 20 % miglyol. This population consisted of 14 females and 6 males with an average age of 25.1 years (range: 19 to 42 years; median of 24 years). After four weeks a reduction of TEWL and an improvement in stratum corneum hydratation was noticed. The curves were parallel as well as in the vehicle with and without *Oenothera* fatty oil. The barrier function tests of these 2 formulations were indistinguishable. The barrier function assessed in various ways was improved equally in both groups. In the second study, a stable w/o emulsion of 20% *Oenothera* fatty oil was used. The vehicle of the comparator was liquid paraffin. Eighteen females and

2 males of a mean age of 22.9 years (range: 18 to 42 years; median of 23.5 years) took part in this study. Only one female dropped out because of an acute exacerbation of atopic eczema. The effect of TEWL was significantly improved through the application of the vehicle with *Oenothera* fatty oil. The barrier function test with nicotinic acid ester noticed a statistic significant difference between vehicle with or without *Oenothera* fatty oil. While there was no difference found in the barrier function test with SLS. The latter study confirms the positive effect of *Oenothera* on the stability of the stratum corneum barrier. Generally, this vehicle controlled trial highlighted that the efficacy of *Oenothera* fatty oil in a cream depends on the choice of the vehicle. The study also proved that the onset of a prolonged interaction with the epidermal barrier lipids, above and beyond the physical properties of *Oenothera* fatty oil, is slow (Hoare *et al.,* 2000).

Assessor's comment: This study is of low quality because of the limited number of patients and the indirect way of evaluating the outcome.

Oral intake

Lovell *et al.* (1981) carried out a randomised, double-blind, placebo-controlled cross-over study during 6 weeks with 32 patients. There were 17 children (18 months to 13 years) and 15 adults (14 to 32 years). The subjects had **atopic eczema** for at least 6 months. Adults and children received twice a day 4 and 2 evening primrose oil (EPO) capsules, respectively for 3 weeks per treatment period. These capsules contained 500 mg evening primrose oil, including 45 mg γ-linolenic acid. The placebo was liquid paraffin. All patients were allowed to continue the use of a mild topical steroid preparation. Patients or their relatives and a doctor were asked to score the severity of the eczema on a continuous 10 cm linear scale, at the beginning and the end of the treatment periods. The authors concluded that the patients receiving evening primrose oil showed a modest but significant improvement on both the doctor's ($p<0.01$) and their own assessment ($p<0.05$).

Assessor's comment: This study is of low quality by the limited number of patients and the way of assessing the outcome.

Wright & Burton (1982: cited by Berth-Jones & Graham-Brown 1993; Hoare *et al.,* 2000) studied 99 patients, among them adults (n=60; 15-58 years) and children (n=39; 8 months to 14 years) with moderate to severe atopic dermatitis. The researchers used a cross-over design during 12 weeks in a blocked randomisation with approximately equal numbers. Capsules containing 500 mg evening primrose oil (= 360 mg LA, 45 mg GLA) was administered in different doses. Adult patients received 3 different doses: 2 capsules 2 times daily, 4 capsules 2 times daily and 6 capsules 2 times daily. Children received 2 different doses: 1 capsule 2 times daily and 2 capsules 2 times daily. The placebo was 500 mg liquid paraffin. The use of mild topical steroids, emollients, oral antihistamines was allowed. Sixteen adults and 3 children dropped out early.

In the lowest dose groups for children and adults, itch was the only symptom which improved more with *Oenothera* as compared to placebo ($p<0.05$). In the higher dose groups, a significant clinical improvement was found on a 10 cm linear scale. The patient assessment is significantly superior to the placebo in itch, scaling and general impression of severity ($p<0.01$ to 0.002). There was also a beneficial effect shown in the doctor's assessments ($p<0.002$). There was a mean improvement of about 30% of the overall severity of the eczema.

Assessor's comment: This study can be considered as being of moderate quality, although the value of the evaluation tool can be discussed.

Bamford *et al.* (1985) conducted a double-blind, blocked cross-over trial with random controlled assignment to treatment groups. The study lasted 6 months. One hundred fifty four individuals started the study and 123 remained, of which 49 children (2-16 years) and 74 adults (16-66 years). These people had atopic eczema and used a prescribed topical steroid but they did not use systemic steroids

or chemotherapy. One group started with *Oenothera* for 3 months, the other group with placebo. After these 3 months, they were treated 3 months with respectively placebo and *Oenothera*. Some of the individuals received a high dose, the others a lower dose. Thirty-three children were administered the lowest dose of *Oenothera* (2 capsules twice daily). The other 16 children swallowed 4 capsules twice a day. Forty adults took 6 capsules twice daily, the 34 others took 8 capsules twice a day. Each capsule contained 500 mg *Oenothera* (9% = 45 mg GLA and 72% = 360 mg LA) and 10 IU d-alpha tocopheryl acetate. The placebo capsules contained 500 mg liquid paraffin oil and 10 IU d-alpha tocopheryl acetate. During the study, the patients could use emollients, topical steroids or oral antihistamines. Of the 31 persons who dropped out, 14 were taking *Oenothera* and 17, placebo. Twenty-nine dropped out for personal reasons, 3 had an allergic reaction to the capsules, 1 adult discontinued because of increased dermatitis. One child (placebo) discontinued because of a developing hyperactivity. Side effects were minor and temporary. The complaints were equal in both groups: nausea and bloating (5 subjects using *Oenothera* versus 1 subject using placebo), hyperactivity (3 children placebo versus 1 *Oenothera*). Eighty patients achieved a compliance of 50%, 56 patients achieved 75%. Both patients (or parents of child patients) and physicians had a similar rating of the average appearance of lesions. The patients had 3 evaluation visits, namely before the trial, and 3 and 6 months after the beginning of the study. No changes in weight, triceps, skin-fold thickness, blood pressure, appetite and stress were observed. Erythema, scale, excoriation, lichenification or overall severity had marked no significant effect. There were no advantages found in the *Oenothera* treatment with atopic eczema patients, although a 50% and 33% higher dose was respectively used in adults and children compared to the trial of Wright and Burton.

Assessor's comment: The study may be considered as being of moderate quality. The limited compliance is a hampering factor.

Schalin-Karrila *et al.* (1987) investigated 25 young adults (9 men, 16 women, 19-31 years) with **moderate to severe atopic dermatitis** in a 12 weeks continuous parallel double-blind, randomised, placebo-controlled trial. These persons had a family history of atopy or had atopic respiratory symptoms. An *Oenothera* capsule contained 360 mg LA, 50 mg oleic acid and 45 mg GLA. A placebo-capsule consisted of 500 mg liquid paraffin. Fourteen subjects received four capsules of *Oenothera* twice a day and 11 subjects received four capsules of placebo. One person of the *Oenothera* group dropped out because of an allergic reaction due to topical dequaline chloride. Patients did not change diets. Two weeks before the start of the trial, topical or systemic treatments were changed to an emollient cream of which they could use as much as needed. If not sufficient, a topical cream or oral antihistamines could exceptionally be employed. Potent steroids or systemic steroid therapy were not used. Before the start of the trial and every 3 weeks the clinical parameters were evaluated. At the beginning of the study, at 6 and at 12 weeks a blood sample was taken. All the clinical parameters (overall severity and grade of inflammation, dryness and itch, reduction surface area) significantly improved in patients treated with *Oenothera*. These patients used less topical steroids than the placebo groups. Patients treated with *Oenothera* had a more significant reduction in inflammation than the placebo group. The DGLA concentration increased significantly after 6 weeks of treatment with *Oenothera*. However, PGE1, a metabolite of DGLA, did not rise. The ratio of PGE1 to PGE2 was unaltered. A clinical improvement in the patients suggested that the increased DGLA level may play a role in the effects of *Oenothera*. The use of topical steroid during *Oenothera* treatment could be reduced to about 30%.

Assessor's comment: Because the number of patients is limited, this study must be considered as being of moderate quality.

Morse *et al.* (1989) reported on a meta-analysis of 9 controlled trials, which were carried out in 8 centres. Four had a parallel design and 5 a cross-over design. Approximately 200 persons with atopic dermatitis were included during 8 to 12 weeks. They received a daily dose between 2 and 6 g, which is

equal to 160–480 mg GLA. In the parallel trials, patient's and doctor's score had a highly significant improvement of the symptoms like inflammation grade, dryness, scaliness, itching and overall skin involvement. Furthermore, the authors noticed a dose-dependent treatment response and a positive relation between improvement of the clinical symptoms and the plasma concentration of DGLA and AA. The effect on itch was particularly striking. A similar conclusion was made in the cross-over trials, although there was no significant improvement in the doctor score.

A significant after-effect was noticed in cross-over treated *Oenothera* patients and was interpreted as an evidence of the therapeutic effect (Schulz *et al.,* 2004). The use of *Oenothera* fatty oil in atopic dermatitis patients was concluded as having a modest beneficial effect (Hoare *et al.,* 2000). Patients with the greatest increase in blood levels of GLA, DGLA and AA had the greatest progress in their skin condition.

Assessor's comment: A forest plot and a funnel plot are necessary to exclude publication bias. This study can be considered as being of moderate quality.

Berth-Jones & Graham-Brown (1993) included 123 patients with atopic eczema in a double blind placebo randomised controlled trial with a parallel design. The study took 16 weeks, followed by a wash out period of 8 weeks. Twenty-one persons dropped out during the trial and another 6 during the wash out period. The patients were older than 12 years and were divided in 3 groups based on gender, age and disease severity. One group received evening primrose oil capsules, which contained 500 mg *Oenothera*, which consisted of 321 mg LA and 40 mg GLA. Another group received evening primrose oil capsules, which contained 430 mg *Oenothera* and 107 mg marine fish oil. The latter group was the placebo group and received capsules with liquid paraffin for the adults and olive oil for the children. The patients were administered 6 capsules twice a day. Children who could not swallow the capsule were allowed to open them. The patients could use topical steroids and emollients during the treatment. Patients taking antihistamines could continue their treatment. The study demonstrated no improvement in the active treatment and no effect of EFA supplementation in atopic dermatitis patients.

Assessor's comment: The assessment scale can be discussed. As a consequence, the study is of moderate quality.

Humphreys *et al.* (1994) carried out a double-blind, parallel, placebo-controlled study with 58 adults with moderately severe atopic eczema. The patients were divided in 3 groups: 26 women with premenstrual exacerbation of eczema, 17 women without the premenstrual exacerbation of eczema and a group of 15 men. They were separated based on sex, age and duration of eczema. The whole study comprised 30 weeks, with 4 weeks of run-in, 16 weeks of treatment and 8 weeks of evaluations. The patients continued their usual treatment with topical corticosteroids, emollients or/and systemic treatment. Fifty two patients completed the trial. Four patients of the placebo and 2 of the active group were withdrawn. During the active treatment the patients received 12 evening primrose oil capsules a day, with 500 mg *Oenothera* and 10 mg vitamin E. The placebo group received capsules containing 500 mg liquid paraffin and 10 mg vitamin E. Results of the study demonstrated that there was no conclusive evidence for the two female groups regarding the different reactions in the different stages of the menstrual cycle. A highly significant difference is marked in erythema and surface damage between *Oenothera* and placebo after 4 months and post treatment. There was no significant effect in lichenification. A more sustained fall of serum soluble IL-2 receptor levels was noted in treated patients with *Oenothera*. No significant difference was seen in the use of topical corticosteroid between *Oenothera* and placebo. Women with a premenstrual flare had the greatest improvement of the eczema with GLA. In patients with chronic atopic dermatitis, adjunctive therapy with *Oenothera* should be considered.

Assessor's comment: The study is of low quality because of the limited number of patients and variability between patients. Moreover, the symptoms were scored on a visual analogue scale of 100, whereas values exceeding 100 were reported. This puts the limits of the scale under discussion.

Senapati *et al.* (2008) performed a randomised placebo-controlled trial with Indian people, suffering from mild, moderate or severe **atopic dermatitis**. Sixty eight persons entered the trial. The subjects received *Oenothera* or placebo capsules (Table 5). An *Oenothera* capsule contained 500 mg *Oenothera*, of which 8-10% GLA and 10 IU vitamin E. The placebo capsule consisted of 300 mg sunflower oil and 10 IU vitamin E. Twenty-six out of 29 patients of the *Oenothera* group and 27 of 36 patients of the placebo group ended the study. The study took 5 months and included young and old persons with atopic eczema. At the end of the first month, the intensity and itching were significantly reduced. At the end of the study, 96% of the *Oenothera* group marked an improvement compared to 32% in the placebo group.

Table 5: Posology dependent upon the age.

The amount of capsules given twice a day dependent on the age. Age (Year)	Amount capsules a day
1	1-4
2-5	5-6
6-10	7-8
11-16	9-10
> 16	12

Assessor's comment: This study is of low quality due to the limited number of patients and patients' diversity (age).

Bamford et al (2013) made a Cochrane review on the oral treatment of eczema with evening primrose oil (EPO) and borage oil. They describe eczema as a chronic inflammatory skin condition, which usually develops in early childhood. The authors state that many children outgrow this disorder as they reach secondary school age, and although it may improve with age, there is no cure. Constant itch makes life uncomfortable for those with this condition, no matter what age they are, so it may have a significant effect on a person's quality of life. Its prevalence seems to be increasing as populations move from rural locations to cities. Some people, who do not see an adequate improvement or fear side-effects of conventional medical products, try complementary alternatives to conventional treatment. This article is a review of evening primrose oil (EPO) and borage oil (BO) taken orally (by mouth), as these have been thought to be beneficial because of their gamma-linolenic acid content. To assess the effects of oral evening primrose oil or borage oil for treating the symptoms of atopic eczema. The authors searched the following databases up to August 2012: Cochrane Skin Group Specialised Register, CENTRAL in The Cochrane Library, MEDLINE (from 1946), EMBASE (from 1974), AMED (from 1985), and LILACS (from 1982). They also searched online trials registers and checked the bibliographies of included studies for further references to relevant trials. They corresponded with trial investigators and pharmaceutical companies to try to identify unpublished and ongoing trials. A separate search for adverse effects of evening primrose oil and borage oil in November 2011 was performed. The studies were randomised controlled, parallel, or cross-over trials investigating oral intake of evening primrose oil or borage oil for eczema. Two review authors independently applied eligibility criteria, assessed risk of bias, and extracted data. Dichotomous outcomes using risk ratios (RR), and continuous outcomes using the mean difference (MD) were pooled. Where possible, the authors pooled study results using random-effects meta-analysis and tested statistical heterogeneity using both the Chi(2) test and the I(2) statistic test. They presented

results using forest plots with 95% confidence intervals (CI). A total of 27 studies (1596 participants) met the inclusion criteria: 19 studies assessed evening primrose oil, and 8 studies assessed borage oil. For EPO, a meta-analysis of results from 7 studies showed that EPO failed to significantly increase improvement in global eczema symptoms as reported by participants on a visual analogue scale of 0 to 100 (MD -2.22, 95% CI -10.48 to 6.04, 176 participants, 7 trials) and a visual analogue scale of 0 to 100 for medical doctors (MD -3.26, 95% CI -6.96 to 0.45, 289 participants, 8 trials) compared to the placebo group. Treatment with BO also failed to significantly improve global eczema symptoms compared to placebo treatment as reported by both participants and medical doctors, although a meta-analysis could not be conducted as studies reported results in different ways. With regard to the risk of bias, the majority of studies were of low risk of bias; 67% of the included studies were judged as having low risk of bias for random sequence generation; 44%, for allocation concealment; 59%, for blinding; and 37%, for other biases. **Oral borage oil and evening primrose oil lack effect on eczema; improvement was similar to respective placebos used in trials. Oral BO and EPO are not effective treatments for eczema**. In these studies, along with the placebos, EPO and BO have the same, fairly common, mild, transient adverse effects, which are mainly gastrointestinal. The short-term studies included here do not examine possible adverse effects of long-term use of EPO or BO. A case report warned that if EPO is taken for a prolonged period of time (more than one year), there is a potential risk of inflammation, thrombosis, and immunosuppression; another study found that EPO may increase bleeding for people on warfarin. Noting that the confidence intervals between active and placebo treatment are narrow, to exclude the possibility of any clinically useful difference, the authors we concluded that further studies on EPO or BO for eczema would be hard to justify. This review does not provide information about long-term use of these products.

Kmietowicz (2013) reinforced the investigations made by Bamford *et al.*, 2013 by stating that:" *further studies on the use of these complementary therapies to treat eczema would be hard to justify".*

Chung *et al.* (2013) evaluated dose-dependent effects of evening primrose oil in children and adolescents with atopic dermatitis (AD). According to their opinion the optimal dose and duration of treatment with evening primrose oil were not yet been determined. The aim of their study was to investigate the dose-response treatment effects of evening primrose oil on clinical symptoms of AD and serum concentrations of polyunsaturated fatty acids. Forty AD patients were enrolled for the study and randomly divided into 2 groups: those who received evening primrose oil 160 mg daily for 8 weeks and those who received 320 mg of evening primrose oil twice daily for 8 weeks. The authors evaluated the Eczema Area Severity Index (EASI) scores of all AD patients at weeks 0, 2, 4 and 8. In addition, measured the levels of serum fatty acids, including palmitic, linoleic, linolenic and arachidonic acid using gas chromatography. The serum fatty acid levels (linolenic and arachidonic acid) were higher in the 320 mg group than in the 160 mg group, with statistical significance. After evening primrose oil treatment, EASI scores were reduced in the 2 groups. The improvement in EASI scores was greater in the 320 mg group than in the 160 mg group. There were no side effects seen in either group during the study in the 2 groups. According to the authors, the 320 mg and 160 mg groups might be equally effective in treating AD patients and showed dose-dependent effects on serum fatty acid levels and EASI scores.

Assessor's comment: The study is of low value because of the limited number of patients and the absence of a placebo group.

Simon *et al.* (2014) demonstrated that gamma-linolenic acid levels correlated with clinical efficacy of evening primrose oil in patients with atopic dermatitis. They state that in patients with atopic dermatitis (AD) there is an imbalance in fatty acid metabolism related to a deficiency in the delta-6-desaturase, an enzyme responsible for the conversion of linoleic acid (LA) to gamma-linolenic acid (GLA). Evening primrose oil (EPO, *Oenothera biennis*) is extracted from seeds of this plant which contains high amounts of GLA (approximately 80 mg per 1 g of EPO). The aim of the study was to

investigate whether EPO supplementation results in an increase in plasma GLA and its metabolite dihomo-gamma-linolenic acid (DGLA) correlating with clinical improvement of AD, assessed by the SCORing Atopic Dermatitis (SCORAD) index. The authors conducted an open study including 21 patients with AD (intention to treat population; ITT). Thereof seven patients had to be excluded from the ITT population because of poor treatment compliance (< 75% of study medication intake) and so the per-protocol population comprised 14 patients. EPO (4 - 6 g) was administered daily for 12 weeks. Before treatment, and 4 and 12 weeks after initiation of EPO supplementation, objective SCORAD was assessed and plasma concentrations of GLA and DGLA were determined by gas chromatography. A significant increase in plasma GLA and DGLA levels and a decrease in the objective SCORAD were observed 4 and 12 weeks after initiation of EPO treatment. In the per-protocol population (n = 14), a significant inverse correlation between the changes in plasma GLA levels and SCORAD was found (p = 0.008). The authors' conclusion: the clinical disease activity under EPO treatment correlates with the individual increase in plasma GLA levels. Thus, the results of this pilot study indicate that an increase in plasma GLA might be used as predictive parameter for responsiveness of AD to EPO therapy.

Assessor's comment: The study must be considered as a hybride between a pharmacokinetic and a therapeutic evaluation. Its value is limited because of its open design and the limited number of patients and the relatively short duration.

Table 6: Clinical studies on humans with atopic dermatitis

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Lovell *et al.*, 1981 Therapeutic effectiveness of EPO in Atopic eczema	R, DB, PL, CO 6 weeks 3 weeks per treatment period	EPO 500 mg capsules Oral Adults: 4 capsules/day Children: 2 capsules/day	n=32 (total) n=17 children (18 months to 13 years)	Atopic eczema for at least 6 monhts	Linear scale (10 points) Scoring by patients and doctors Improvement: Patients ($p<0.05$) Doctors ($p<0.01$)	ITT No quality score	Low quality: n=low (no power calculation); assessment tool not validated
Wright & Burton 1982 Therapeutic effectiveness of EPO in Atopic dermatitis	R, DB, CO, PL 12 weeks	EPO 500 mg capsules Oral Adults: 3 posologies 2 capsules 2x daily 4 capsules 4x daily 6 capsules 2x daily Children: 2 posologies 1 capsule 2x daily 2 capsules 2x daily	n=99 (total) n=60 adults (15-58 years) n=39 children (8 months to 14 years) Drop out: Adults: 16 Children: 3	Moderate to severe atopic dermatitis	Lowest posologies: itching improved: $p<0.05$ Higher posologies: Improvement of score on linear scale (10 points): $p<0.01$ to 0.002) Doctor's assessment: improvement: $p<0.002$ Overall severity: improvement of 30%	No quality score	Moderate quality, but no validated assessment tool. Use of topical steroids and emollients allowed
Bamford *et al.*, 1985	R, DB, PL, CO 6 months 3 months per study period	EPO 500 mg + 10 IU vit E acetate capsules Oral Children: 2 posologies 2 capsules 2x daily 4 capsules 2x daily Adults: 6 capsules 2x daily 8 capsules 2x daily	n=154 Drop out: n=31: 14 EPO 17 PL Remaining: n=74 adults (16-66 years) n=49 children	Atopic eczema patients Topical steroids, emollients and oral antihistamines allowed	No more therapeutic benefit than with placebo (patients' as well as physicians' scoring) Drop ouot: 3 patients allergic reactions; 1 increased dermatitis: 1 child hyperactivity.	Not specified. No quality score given	Low quality, due to the limited compliance

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
			(2-15 years)		Compliance: 80 patients = 50% 56 patients = 75%		
Schallin-Karrila *et al.*, 1987	R, DB, PL 12 weeks	EPO 500 mg capsules oral 4 capsules 2x daily	n=25 young adults (19-31 years)	Patients with a family history of atopy and atopic respiratory symptoms Topical steroids allowed	EPO significantly improved all clinical parameters. 30% less topical steroid use	Not specified. No quality score given	Moderate quality, but low number of patients
Berth-Jones & Graham-Brown (1993)	C, DB, PL, 16 weeks Wash-out period 8 weeks	EPO 500 mg capsules or EPO 430 mg + 107 marine fish oil capsules oral 6 capsules 2x daily 16 weeks	n=123 (>12 years old)	Atopic eczema Topical steroids and oral antihistamines allowed	No difference with PL. No improvement	Not specified. No quality score given	Moderate quality cf. assessment
Humphreys *et al.*, 1994	DB, PL 30 weeks: 4 weeks run in; 16 weeks treatment; 8 weeks evaluation	EPO 500 mg + 10 mg Vit E Oral 12 capsules per day	n=58 adults drop out = 6	26 patients with premenstrual eczema; 17 patients without 15 men Topical corticosteroids, emollients, systemic treatment allowed	Significant improvement for EPO in patients with erythema ($p<0.017$) and surface damage ($p<0.007$). Sustained fall of IL-2 receptor levels. No difference in use of topical corticoids	Not specified. No quality score given	Low quality: variability in patient population and limited number of patients

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Senapati *et al.*, 2008	R, PL 5 months	EPO 500 mg + 10 mg VitE oral Posology varied from 1-4 capsules per day to 12 capsules per day	n=68 (age from 1 to > 16 years) Drop out: 12	Mild, moderate or severe atopic dermatitis	Improvement: 96% of patients EPO 32% of patients PL	Not specified. No quality score given	Low quality: limited number of patients; diversity in age
Chung *et al.*, 2013	Dose finding study 8 weeks	2 groups: EPO 160 mg EPO 320 mg oral 2x per day	n=40 adults	Children and adolescents with Atopic dermatitis	Circulating fatty acids rise = dose dependent EASI scores dose dependently improved; no difference between groups	Not specified. No quality score given	Low quality: limited number of patients and absence of PL
Simon *et al.*, 2014	Open pilot study 12 weeks	EPO orally: 4-6 g per day	n=21 adults Drop outs: n=7 because of low compliance (< 75%)	Atopic dermatitis	SCORAD index improvement $p<0.008$; correlates with the rise of GLA in plasma	PP evaluation	Limited value: Low number of patients; Open design; short duration

Other dermatological studies

Chalmers & Shuster (1983) first performed an uncontrolled pilot study. Six children with atopic dermatitis participated in the trial and there was a benefit response seen in 2 patients with **ichthyosis vulgaris**, but not with atopic eczema. The researchers expanded this study to a randomised, double-blind trial with 30 patients with ichthyosis vulgaris. Twenty patients (3 to 35 years) were atopic: 11 had active eczema, 6 had a history of atopic eczema and 3 had rhinitis or asthma. Ten other patients (6 to 69 years) were not atopic. The trial took 9 weeks. The placebo was liquid paraffin. The adults were administered 3 g daily, while the children received only 2 g a day. There were no patients with severe eczema. Patients topically treated could continue their therapy. A slight improvement in the mean scores of ichthyosis vulgaris was seen in all groups. No progress was seen in the eczema patients, both *Oenothera* (6 subjects) and placebo (5 subjects) treated. In both atopic and non atopic patients, there was no improvement in ichthyosis vulgaris noted. In atopic eczema treated with *Oenothera*, no benefit was remarked.

Assessor's comment: This study is of low quality, because of the limited number of patients and the inconsistency of the results for which different assessment scales were used.

Whitaker *et al.* (1996) studied 39 patients with stable chronic hand dermatitis during 24 weeks. The patients were between 19 and 75 years. During 16 weeks, 20 of them took 12 capsules of 500 mg *Oenothera*, which is equal to 50 mg GLA. The other 19 subjects received capsules of 500 mg sunflower. After the treatment, the study was continued by a wash out period of 8 weeks. The patients could take unlimited amounts of standard emollients and limited amounts of a semi potent group III topical steroid cream. Five patients dropped out, of which 1 from the *Oenothera* group and 4 from the placebo group. After 16 weeks, no statistically significant difference was noted in both groups. At the end of week 24, the *Oenothera* group gave statistically significant clinical improvement in all parameters, while the placebo group did not. The study demonstrated an improvement of clinical parameters in *Oenothera* and placebo, but no significant difference between the two groups. During the treatments, there was no change in lipid composition of plasma RBC or the epidermis. This double-blind placebo-controlled trial with parallel design demonstrated that GLA has no superior therapeutic value to the placebo.

Assessor's comment: This study is of low quality, because of the co-medication allowed, the limited number of patients and the way of therapeutic assessment.

Muggli (2005) studied the biophysical skin parameters in 22 healthy volunteers. Twenty-two non-pregnant women and 18 men between 32 and 56 years participated in a 12 weeks continuous randomised, double-blind, placebo-controlled study. The active treatment group received soft gel capsules which contained 500 mg *Oenothera* and 8 mg dl-alpha tocopherol acetate. The placebo and the *Oenothera* group received 3 capsules twice a day, each time during the meal, which means that the active groups received 345 mg GLA a day. The subjects did not suffer from any skin disease. They could not use topical preparations on the treated skin area 1 week before the study until the end. Only water or mild synthetic detergent could be used for washing the skin. There was no significant difference between baseline and 4 weeks in both groups. After 12 weeks, skin moisture, transepidermal water loss, firmless, elasticity, fatigue resistance and roughness improved in the *Oenothera* group compared to the placebo group. There was no significant improvement for redness.

Assessor's comment: This study is not a clinical trial for medicinal use, as there was no real therapeutic indication.

Premenstrual syndrome

PMS is a condition characterised by a number of physical and mental symptoms during the luteal phase of the menstrual cycle; this is between 7 and 14 days before the onset of the menstrual period. A lot of symptoms are attributed to PMS, the most common are headache, backache, swollen abdomen, breast discomfort (including mastalgia), irritability, depression, anxiety, changes in sexual drive and lack of energy (Larsson *et al.,* 1989).

Puolakka *et al.* (1985) carried out a placebo controlled, randomised cross-over study over 4 cycles to investigate the value of *Oenothera* in premenstrual tension. Thirty subjects, suffering from severe premenstrual syndrome, took 3 evening primrose capsules (500 mg) twice a day, from the 15th day of the cycle until the next menstrual period. Nineteen symptoms were recorded and scored. The comparison of EPO with placebo suggested no difference in effectiveness as both decreased the PMS score. The authors concluded that more patients obtained relief on EPO than on placebo.

Casper (1987) studied 66 patients with PMS in a double-blind, placebo-controlled cross-over trial. The premenstrual self-rating scores were reduced at 3 months with both placebo and *Oenothera* (posology not specified), but no difference was found between placebo and *Oenothera*.

Larsson *et al.* (1989) studied the effects of *Oenothera* fatty oil in a pilot study with 19 women between 25 and 48 years old. Two women left the study. The subjects received 4 capsules of *Oenothera* (1000 mg oil per capsule) in the morning and 4 in the evening during the last 2 weeks before menstruation in 5 consecutive cycles. There were 8 premenstrual symptoms listed and scored every day: irritability, swollen abdomen, breast discomfort, depression, anxiety, swollen fingers or ankles, tiredness and headache. The symptom scores were significantly lower for 6 symptoms during the treatment cycles 1 and 2 compared to control. The scores were lower for 7 symptoms during the 5th cycle. Also the total PMS score was significantly lower in the 5th cycle compared to the pre-treatment cycle.

Khoo *et al.* (1990) studied the therapeutic effectiveness of *Oenothera* fatty oil. Thirty-eight women, age 20-40 years, with PMS were observed for 7 menstrual cycles of which one pre-treatment cycle. The preparation, containing 72% linoleic acid and 12% oleic acid, was studied in a randomised, double-blind placebo-controlled, cross-over study. Ten symptoms associated with PMS and menstrual symptoms were studied in categories: (a) fluid retention, (b) breast and (c) mood changes. Subjects taking systemic steroids and non-steroidal anti-inflammatory drugs were excluded from the trial because these drugs interfere with the essential fatty acid metabolism. The patients were randomly assigned to two treatments A or B. The placebo treatment consisted of liquid paraffin capsules. The subjects took 8 capsules (500 mg oil per capsule) a day, four in the morning and four in the evening. On the first day of the first cycle, the placebo or *Oenothera* treatment was started and was continued until the end of the third cycle. The other treatment was started on the first day of the fourth cycle and continued for 3 cycles. Before the onset of the trial, every subject filled in an assessment report. A four-point scoring system was used to rate the severity of the 10 symptoms, menstrual pain and blood loss. Body weight was measured in the beginning and at the end in order to count the weight gain. There were no dropouts in this study. There was no significant difference found in the scores between the two treatment groups. There was a possible carry-over effect in the data. This was tested and there was no carry-over noticed. *Oenothera* fatty oil had no advantage over placebo in the scoring of the 10 symptoms of PMS or menstrual symptoms. The scores increased in the fourth cycle after the cross-over independently from active or placebo treatment. This suggested that the improvements were due to a placebo effect.

Collins *et al.* (1993) carried out a randomised, double-blind, cross-over study with 27 women suffering from PMS to evaluate the effect of essential fatty acids. The age of the subjects ranged from 30 to 45 years. Thirty-eight subjects completed the study, but 11 women were excluded due to non-significant cyclicity or due to the absence of ovulation during the assessment cycle. The symptoms

were self-reported by the women throughout the study, which consisted of 10 cycles for the women with PMS and 1 cycle for the controls: 1) happiness and feelings of well-being, 2) depressed feelings and crying spells, 3) irritability and short temper, 4) breast swelling and discomfort, 5) headache, 6) fatigue, 7) sexual need and positive feelings toward sex, 8) energetic feelings, and 9) tension and anxiety were rated. The subjects received 12 capsules of EPO every day which contained 4.32 g linoleic acid and 0.54 g gamma-linoleic acid. The placebo contained 500 mg paraffin oil and was given 3 times a day. The drug-first group started the treatment in cycle 3 of the study, while the placebo-first group started treatment with EPO in cycle 7. (Figure 2) Blood samples were taken in the assessment cycle and in cycles 1,5,6,9 and 10. The samples were drawn 1 time during the follicular phase (cycle days 3-5) and 3 times in the luteal phase (cycle days 22-26) and were set for hormone analysis. The results showed no significant effect for mood ratings, but there was an effect in time noticed for tension and anxiety, irritability and short temper, depression and crying spells. The longer the women stayed in the study, the better they felt, independently of the treatment they were receiving, which indicates a placebo-effect.

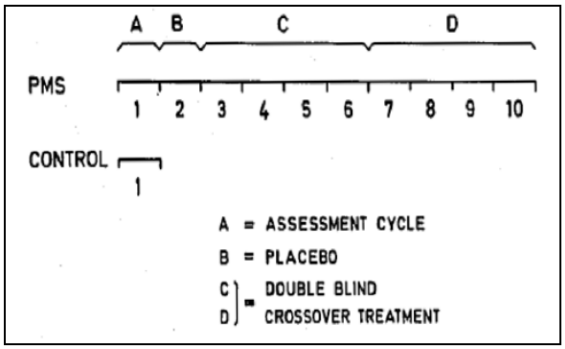

Figure 2: treatment schedule.

Budeiri *et al.* (1996) carried out a systematic literature search of clinical trials of evening primrose oil (EPO) for the treatment of the premenstrual syndrome (PMS). Seven placebo-controlled trials were found but only in five trials was randomization clearly indicated. Inconsistent scoring and response criteria made statistical pooling and hence a rigorous meta-analysis inappropriate. The two most well-controlled studies failed to show any beneficial effects for EPO. The trials were relatively small and modest effects cannot be excluded. According to the authors, EPO was of little value in the management of premenstrual syndrome.

Mastalgia

A lot of women suffer from cyclical premenstrual breast pain, which resolves with menstruation. It is physiological, hormonally driven and normal. Non-cyclical breast pain with nodularity is more severe and can interfere with the daily activities, it is considered to be clinically relevant (Qureshi & Sultan 2005).

Pashby *et al.* (1979: cited by Anonymous 1981) investigated the effect of *Oenothera* fatty oil in mastalgia in 73 patients in a randomised double-blind cross-over study. Over 3 months, the subjects took *Oenothera* (posology not specified). Nineteen patients dropped out of the experiment. Pain and tenderness were significantly reduced in the non-cyclical group. This was less marked in the cyclical group.

Blommers *et al.* (2002) carried out a randomised, double-blind, controlled trial to evaluate the effect of *Oenothera* fatty oil and fish oil on breast pain in premenopausal women with severe chronic mastalgia. One hundred twenty women were randomly assigned to 4 groups: (1) fish oil and control oil (FC), (2) *Oenothera* fatty oil and control oil (EC), (3) fish and *Oenothera* fatty oils (EF), or (4) both control oils (CC). The control oils were corn oil and corn oil with wheat germ oil. The study took 6

months. The subjects were categorised in cyclic (94 patients) or non-cyclic mastalgia (26 patients). Every day the women received 3 g of two oils. The capsules with *Oenothera* fatty oil contained 9.6% γ-linoleic acid, 71.2% linoleic acid and 5 mg of vitamin E. Vitamin E was added to every oil to prevent oxidation. Patients did not change their diet during the trial. The patients had to fill in a questionnaire about the changes in their breast complaints at the time of randomisation and after 3 and 6 months. There was a significant decrease of percentage of days with pain found for the total study population. The severity of pain was not significantly decreased. *Oenothera* showed less decrease in the percentage of pain days than the control oils. However, none of these results were significant.

Srivastava *et al.* (2007) conducted a meta-analysis on randomised trials on mastalgia. The studies of Pashby *et al.* (1979), Blommers *et al.* (2002), Preece and Mansel (1982) and the trial of Goyal and Mansel (2005) were evaluated. The results indicated that *Oenothera* is ineffective.

Jafarnezhad (2016) published results on the comparative effect of flaxseed and evening primrose oil with vitamin E on severity of cyclic mastalgia in women. As he used a combination, the separate effect of evening primrose oil cannot be evaluated separately.

Sharma *et al.* (2012) compared a nonsteroidal, selective anti-estrogen oral contraceptive to evening primrose oil in patients with mastalgia. In a clinical trial, 135 patients were randomized into two study groups: group 1 (67 patients) received anti-estrogenic compound30 mg on alternate days and on left over days a placebo was given to eliminate bias; group 2 (68 patients) received evening primrose oil 3 g / day. On follow-up after 6 months, 44 patients (86%) showed complete response in group 1 as compared to only 12 patients (23%) in group 2. This difference is statistically significant ($p < 0.05$). This study is of limited value, due to the unknown nature of the comparator.

Menopause

Chenoy *et al.* (1994) evaluated the efficacy of oral GLA provided by *Oenothera* fatty oil on menopausal flushing in a double-blind, placebo-controlled study. The study consisted of 56 women who had hot flushes at least 3 times a day, and increased gonadotrophin concentrations and/or women with amenorrhoea for at least 6 months. In the first month the women did not receive any treatment in order to establish a baseline level. The placebo group received 500 mg of liquid paraffin and the treatment group received 500 mg *Oenothera* fatty oil with 10 mg of natural vitamin E. The subjects took 4 capsules twice a day for 24 weeks. The severity and the amount of sweating episodes during the day and night were written on diary cards. Eighteen women receiving *Oenothera* and 17 receiving placebo completed the study. The frequency of daytime hot flashes decreased in the placebo group, but not in the treatment group. The night time hot flashes were decreased in both groups. The trial shows that *Oenothera* offers no benefit over placebo for the vasomotor symptoms of the menopause.

Farzaneh (2013) conducted a randomized placebo clinical trial in order to investigate the effect of evening primrose oil (EPO) on menopausal symptoms. In a 6-week randomized clinical trial, a total of 56 menopausal women aged 45-59 years selected. The patients asked for their hot flash characteristics and responded to HFRDIS (hot flash related daily interference scale) questionnaire before and after the intervention. The participants were randomly assigned to take two capsules per day of placebo or EPO (500 mg soft capsules) for continuous 6 weeks. The improvement in hot flash was compared between two groups. The improvement in hot flash frequency, severity, duration was 39%, 42%, 19%, in EPO group VS 32%, 32%, 18% in placebo group, respectively. Although, only hot flash severity was significantly better in EPO group VS placebo group ($p<0.05$), all 3 characters of hot flash were ameliorated in first arm (not significant). All HFRDIS score were significantly improved in two groups, but in social activities, relations with others, and sexuality were significantly superior to placebo group ($p<0.05$). The author concluded that the severity of hot flash might be decreased using EPO as non-hormonal treatment and that larger studies are needed.

Cervical dilatation

Tanchoco and Aguilar (2015 conference abstract) conducted a randomized controlled study comparing the efficacy of laminaria Versus EPO for cervical priming prior to operative hysteroscopy. The study objective was to compare the ease of cervical dilatation to allow insertion of a 10-11 mm Hegar's dilator prior to operative hysteroscopy with intravaginal EPO versus intracervical Laminaria. The two-arm randomized controlled trial was done in a tertiary training hospital. Patients included were women admitted for operative hysteroscopy. The laminaria group received intracervical laminaria 12 hours prior to hysteroscopy. The EPO group received six soft gel capsules (strength not communicated in the abstract) 6 hours prior and another 4 soft gel capsules 1 hour prior to hysteroscopy. Initial cervical dilatation and time required to reach Hegars 10 mm were recorded. The ease of dilatation and patient acceptability were measured using 5-point Likert scale. A total of 84 patients will be included. An initial analysis on 18 randomized patients (n1 and n2 = 9) was done using the percentage frequency distribution, arithmetic mean, and standard deviation. For both groups, mean age was 40 $\pm$ 9, in majority premenopausal, underwent vaginal deliveries and presented with abnormal uterine bleeding. Initial cervical dilatation for the luminaria group was 5 mm while for the EPO was 7 mm. Median time to Hegar's 10 mm was 5 minutes and 45 seconds for the laminaria group and 1 minute and 57 seconds for the EPO group. Cervical dilatation of patients on the laminaria group was good while that in the EPO was easy. There was significant difference on pain on application and discomfort during priming between the two groups. The EPO group experienced no pain (M=4.78, Var=0.19: extent of the scale not communicated), while laminaria group experienced tolerable pain (M=3.67, Var=0.50). From this preliminary analysis it can be concluded that both agents were effective in dilating the cervix. Cervical dilatation was easier and required less time in the EPO group compared to those in the laminaria group. Nonetheless, EPO is more acceptable and convenient due to ease of administration.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the synovium. This causes pain, stiffness, swelling, deformity and eventually loss of function in the joints (Soeken *et al.,* 2003).

Jäntti *et al.* (1989) studied the effect of 10 ml of *Oenothera* fatty oil and olive oil twice daily in 18 patients with RA. Twenty patients were randomly assigned to two groups of 10 patients, each group with 9 women and 1 man. One group (mean age of 50 years) received *Oenothera* containing 9% of gamma-linolenic acid twice a day. The other group took olive oil (mean age of 38 years). The duration of RA was 13 years in the *Oenothera* group and 10 years in the olive oil group. In each group, one patient left the study. Blood was collected from the antecubital vein and the plasma PGE2, 6-keto-PGF1α and TxB2 were measured. Urine was collected over a 24-hour period. No significant changes in Erythrocyte sedimentation rate (ESR), blood hemoglobin or platelet count, serum C reactive protein (CRP) or immunoglobulin concentrations were seen in either of the treatment group; neither in the number of swollen or tender joints, pain, duration of morning stiffness or grip strength of hands. PGE2 decreased and TxB2 increased more in the *Oenothera* group, but the changes between the groups in plasma prostanoids, PGE2, TxB2 and 6-keto PGF1α concentrations were not statistically significant. Neither the urinary excretion of these products changed significantly.

Brzeski *et al.* (1991) carried out a 6-month double-blind placebo-controlled study on 40 patients with rheumatoid arthritis and upper gastrointestinal lesions due to non-steroidal anti-inflammatory drugs. The subjects had classical or definite RA, and were between 16 and 75 years old. The NSAIDs, H2 blockers and analgesia medication was not stopped during the trial. The patients were divided into 2 groups, 19 of them received *Oenothera* fatty oil 6 g a day, 21 others received placebo, olive oil 6 g a day, in identical capsules. The *Oenothera* fatty oil contained 540 mg GLA and 10 mg of alpha-tocopherol as antioxidant a day. Six subjects from the *Oenothera* group and 4 from the placebo group

withdrew from the study. At 0, 3 and 6 months, assessments were performed of the daily use of NSAID and analgesia, morning stiffness, pain and well-being, Ritchie articular index (AI) and health assessment questionnaire, haemoglobine (Hb), platelets, ESR, CRP, globulins and plasma fatty acid analysis. After 3 months the patients tried to reduce the NSAID and analgesic medication. In each group, 3 subjects reduced their NSAID dose with one tablet. In the *Oenothera* treated patients the morning stiffness was significantly reduced at 6 months, in the placebo treated patients the AI was significantly reduced at 6 months. The trial found that only 23% of the subjects receiving *Oenothera* treatment could reduce their NSAID dose and none could stop, which was the same for the placebo group.

Belch *et al.* (1988) treated patients (age between 28 and 74) with 12 capsules *Oenothera* or *Oenothera*/fish oil daily for 12 months to determine whether *Oenothera* or *Oenothera*/fish oil could replace NSAID treatment in RA. Liquid paraffin was used as placebo (18 patients). Sixteen and 15 patients received 540 mg GLA or 240 mg EPA, respectively, plus 450 mg GLA a day. There was a wash out phase from 12 to 15 months. All patients received placebo capsules without vitamin E to assess whether any improvement was due to the antioxidant and radical scavenging effect of the vitamin E. From three months the patients were instructed to decrease or stop their NSAID and from 12 to 15 months they were told to maintain the dose, if possible. One patient in the *Oenothera* group and two in the *Oenothera*/fish oil group were withdrawn. Assessment of morning stiffness, grip strength and the Ritchie articular index was performed at 0, 3, 6, 12, and 15 months, also blood samples were taken at these times, ESR, CRP, Hb and rheumatoid factor estimation were measured. Eleven *Oenothera* treated patients and 12 *Oenothera*/fish oil treated patients reduced or stopped their NSAIDs by 12 months compared to 5 out of 15 patients from the placebo group. No significant changes were noted in the laboratory and clinical measurements. After the 3 months placebo phase, almost all patients from the *Oenothera* and *Oenothera*/fish oil groups returned to baseline or became worse compared to 14% of the placebo group. The results showed that it is possible to reduce or stop the NSAID medication in some RA patients by using *Oenothera* or *Oenothera*/fish oil treatment. The improvement, however, is purely subjective as there are no measurements found to support it. Therefore, the authors claim that it is unlikely that long-term therapy with these essential fatty acids would alter the course or prognosis of the disease (Belch & Hill 2000).

Sjögren syndrome

Sjögren syndrome (SS) is a common systemic autoimmune disease. It occurs most in menopausal women, frequent symptoms are fatigue, oral and ocular dryness. The secondary Sjögren syndrome is often associated with Rheumatoid Arthritis (RA). In these patients, the GLA concentrations are reduced (Belch & Hill 2000, Theander *et al.,* 2002).

Manthorpe *et al.* (1984) evaluated 36 patients in a randomised, double-blind, cross-over study. A week wash out period was used which is not long enough, when EFA therapy is evaluated. Nevertheless, 3 capsules (500 mg with 9% GLA and 73% LA) or 3 tablets of a combination (125 mg vitamin C, 25 mg pyridoxine, 25 mg niacin, 5 mg zinc sulfate) twice a day improved results during the treatment period compared to placebo.

Oxholm *et al.* (1986) carried out a randomised, double-blind, placebo-controlled cross-over trial in order to determine whether long-term evening primrose oil (EPO) treatment of patients with primary SS would improve their clinical status. Furthermore, the increase of the levels of EFA in plasma and erythrocytes during the treatment with EPO was investigated. Twenty-four women and 4 men with a mean age of 51 years entered the study. All the subjects had keratoconjunctivitis sicca (KCS) and xerostomia. The subjects were treated with 3 g EPO (73% cis-LA, 9% GLA) a day, 6 capsules, for a period of 8 weeks and, for another 8 weeks, they received placebo capsules. The patients' ocular and oral status, fatty acid levels in plasma and erythrocytes were evaluated after 0, 4, 8, 12 and 16 weeks.

The values after 8 weeks of EPO treatment were compared with scores after placebo treatment and the values after 8 weeks of EPO treatment were compared with EPO start values. Significant improvements were found in the ocular status when start values were compared with values after EPO treatment. Results of the fatty acid analyses showed a pronounced increase of DGLA values after *Oenothera* treatment, although compared to the placebo treatment it is thought not to be significant. No significant difference was observed between DGLA erythrocyte values at the end of placebo and EPO treatments. The authors concluded that, in the absence of further data, this treatment cannot be recommended to patients with Sjögren syndrome.

Raynaud's phenomenon

Raynaud's phenomenon is characterised by local vasospasm, cyanosis and rubor. It is evoked by cold or emotions and blood flow, mainly in the limbs, slows down due to vasospasms, most often followed by hyperemia. Enhanced platelet aggregation, decreased RBC deformability, increased leukocyte aggregation and release are also associated with Raynaud's phenomenon (Belch & Hill 2000).

Belch *et al.* (1986) assessed the effect of *Oenothera* on the manifestations of Raynaud's phenomenon (RP) in 21 patients in a double-blind placebo-controlled trial. All subjects endured a 2 weeks run-in period, taking 12 placebo capsules a day. Eleven patients received 12 *Oenothera* capsules daily for 8 weeks (540 mg GLA), 6 of them suffered from Raynaud's syndrome (RS) associated with SS and 5 from Raynaud's disease (RD). The other 10 subjects, 5 RS with SS and 5 RD, took placebo capsules 12 times daily. One subject was withdrawn. The *Oenothera* group experienced less and shorter vasospastic attacks then the control group when the weather became colder. There was no significant improvement in hand temperature and cold challenge, both measured to indicate the blood flow. The drug appears to be able to stimulate production of PGE_2 and decrease production of TXB_2, possibly leading to some antiplatelet effect. The trial demonstrated that some patients with RP experience benefit from *Oenothera* treatment, but larger controlled studies are needed.

Diabetic neuropathy

Peripheral neuropathy is a complication of both insulin dependent (type 1) and non-insulin-dependent (type 2) diabetes mellitus. It is characterised by a progressive loss of nerve fibres which leads to painful or insensitive extremities, neuropathic ulceration and finally amputation. It was caused by a long-term elevated plasma glucose levels. Elevated plasma and nerve glucose levels contribute to nerve degradation by a number of proposed mechanism (Halat & Dennehy 2003).

Three randomised, double-blind, placebo-controlled trials were completed with type 1 and 2 diabetes mellitus patients (Halat & Dennehy 2003).

Jamal & Carmichael (1990: cited by Halat & Dennehy 2003) included 22 diabetic patients with also a mild distal diabetic neuropathy for a mean of 3 years. Twelve subjects received 360 mg *Oenothera* fatty oil daily, 10 subjects got a placebo during a period of 6 months. The *Oenothera* treated patients had statistical significant improvements in nerve function measurements, wrist and ankle heat threshold values and overall symptoms scores. Glycohemoglobin (HbA1C) was not significantly different between the 2 groups and indicated that GLA had no effect on glucose control.

NOTE: In view of the fact that one of the investigators in this study was found guilty of research fraud in the clinical trials on Evening Primrose oil for diabetic neuropathy. The HMPC considers that no further use should be made of this publication and it is included here solely for completeness. BMJ 2003; 326:730.2 http://www.bmj.com/content/326/7392/730.2

Keen *et al.* (1993: cited by Halat & Dennehy 2003) included 111 diabetic patients with a mild or moderate neuropathy. They received 480 mg *Oenothera* a day or a placebo (liquid paraffin) during 12 months. Eighty-four patients left the study. It was noted that 13 neural function parameters improved

more in the intervention group. The improvements were significantly greater in patients with HbA1C values of less than 10%.

Purewal *et al.* (1997: cited by Halat & Dennehy 2003) studied 51 diabetic patients with autonomic peripheral neuropathy for 12 months. They administered a dose of 480 mg daily of *Oenothera* or placebo. No improvements were shown in the vibratory perception threshold compared to placebo.

Safaahussain (2016) investigated the anti-inflammatory, anti-oxidant, and vasodilating effect of evening primrose oil in type 2 diabetic patients. Evening primrose oil (EPO) is a substantial source of omega-6 essential fatty acids, mostly gamma-linolenic acid (GLA). Linolenic acid (LA) forms GLA by delta-6-desaturase enzyme. The activity of delta-6-desaturase enzyme is compromised in patients with type 2 diabetes. The study aims to evaluate the effect of evening primrose oil in reducing the complications of type 2 diabetes mellitus. Twenty six Iraqi patients newly diagnosed with type 2 diabetes who are either overweight or obese were enrolled. Thirteen patients received metformin 500 mg tablets twice daily alone, and 13 patients received metformin 500 mg plus evening primrose oil 2 gm twice daily for a three-month therapy. Serum CRP, Tumor necrosis factor alpha and malondialdehyde (MDA) were measured. There was statistically significant elevation in baseline levels of serum MDA, CRP, and TNF-alpha, and in both systolic and diastolic blood pressure in both patient groups compared with control subjects, ($p<0.001$). High reduction after three months of treatment was found in these parameters compared with a pre-treatment level, significantly with serum MDA, TNF-alpha, and in both systolic and diastolic BP in patients group receiving evening primrose oil ($p<0.001$). It can be concluded that early intervention with natural oil rich in gamma linolenic acid, which possess anti-angiogenic, anti-inflammatory, and anti-oxidant activities, with traditional hypoglycemic drugs can improve therapeutic benefits and represent a promising strategy to restrain the progression of diabetes complications. A weakness of the study is the fact that the added value of EPO is not clearly demonstrated.

Lipid lowering effect

Gupta *et al.* (2012) studied the effect of a mixture policosanol, tomato extract, orally bioavailable grape procyanidins and *Oenothera biennis* oil against placebo in the management of patients with primary hypercholesterolemia and mixed dyslipidemia. Despite positive results, this, study cannot be taken into consideration, because of the fact that the effect of *Oenothera biennis* oil is not evaluated separately.

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

Patients treated with bortezumib

Auberger *et al.* (2013) reported on topical use of evening primrose oil (EPO) for reduction of bortezomib-induced skin reactions. Bortezomib is an effective and established drug in the management of patients with multiple myeloma (MM). Skin reactions at the injection site are frequent adverse events when the drug is injected subcutaneously. They normally resolve spontaneously in a median of 6 days; however, in 3 % of patients, skin reactions are classified as severe. In analogy to the reported alleviation of skin reactions with 5-azacitidine, topical EPO was applied in 10 myeloma patients who already experienced mild to moderate skin reactions during previous bortezomib SC cycles, immediately after each injection. In 10 out of 10 patients, the administration of EPO clearly reduced skin reactions at injection site compared to previous cycles. The median time to alleviation of symptoms objectively and subjectively was 3 days (range 2–5 days). From the patients' view, the injection site itself was less painful and the intensity of inflammation (degree of redness) was reduced.

Additionally, the time until complete resolution of the skin reactions was shortened, too (median 4 days, range 3–5 days). No anaphylactic skin reactions were observed with the topical use of EPO.

Pregnant patients

Diansuy. and Aguilar (2017) investigated the effectiveness of evening primrose oil gel capsule as a cervical ripening agent during labor induction as measured by Bishop score on term singleton pregnant patients. A quasi-experimental cross-sectional study was conducted from May to September 2016. All patients age 18 years and above with accurate dating of gestation, singleton, cephalic, term pregnancy, unfavorable cervix (Bishop score of 4), intact amniotic membranes, and biophysical profile of 10/10 or 8/8 admitted for labor induction and with any of the following were included: (1) 37 weeks age of gestation with preeclampsia, (2) 38 weeks age of gestation with gestational or overt diabetes mellitus, and (3) 41 weeks age of gestation. Six 1000 mg capsules were inserted vaginally. The change in Bishop score was measured after four hours. The difference in the Bishop scores was analyzed using paired t-test. Thirteen patients with age of 27 ± 6 years and gestational age of 40 ± 1 weeks were included. Seven (54%) were nulliparous, two (15%) were primiparous, and four (31%) were multiparous. Seven (54%) patients had preeclampsia, one (8%) had diabetes mellitus, and five (38%) had post-term pregnancies. No adverse events were reported. Eleven (85%) had significant change in Bishop scores (p=0.001). Specifically, improvements were noted in terms of dilatation (p=0.027), effacement (p=0.006) and consistency (p=0.002). Four (31%) patients delivered vaginally but nine (69%) underwent primary low segment cesarean section, six (46%) of which for non-reassuring fetal status, two (15%) for arrest in cervical dilatation, and one (8%) for intra-amnionic infection.

Assessor's comment: This study adds to the knowledge of evening primrose oil gel capsule as a potential benefit in cervical ripening during labor induction. Larger scale, randomized, blinded, and controlled studies are warranted to establish its direct effect as a cervical ripening agent.

Jamilian *et al.* (2016) studied the effect of Vitamin D combined with EPO on glycemia and lipid profiles in women with **gestational diabetes**. The mixture improved insulin resistance and lipid profiles, but it is not possible to evaluate the effect of EPO separately. No adverse effects were reported.

Patients with polycystic ovary syndrome

Nasri *et al.* (2017) investigated the effects of vitamin D and EPO co-supplementation on lipid profiles and biomarkers of oxidative stress in vitamin D-deficient women with polycystic ovary syndrome in a randomized, double-blind, placebo-controlled trial. Vitamin D and EPO co-supplementation for 12 weeks among vitamin D-deficient women with PCOS significantly improved triglycerides, VLDL cholesterol, GSH, and MDA levels. However, it is not possible to evaluate the effect of EPO separately, as a combination was used.

Nulliparous women

Pineda (2017) investigated the effectiveness of intravaginal evening primrose oil as a cervical ripening agent in nulliparous women in a double blinded randomized controlled clinical trial. There were 68 patients included in the study, who were randomly assigned to two groups, 32 (49%) of which were categorized under the experimental drug EPO while the remaining 33 (51%) received placebo. Both were given 4 capsules intravaginal every 4 hours for 3 doses. Among sixty-five subjects who completed the study, there was a significant improvement in Bishop score in the EPO group with a mean difference of 3.59 ± 2.17 compared to 1.97 ± 1.28 for placebo (p = 0.0005). In comparison in terms of Bishop score, there was a significant difference in terms of proportion of success between the

two groups. Specifically, 68% treated with intravaginal evening primrose oil has a significantly higher success rate in achieving Bishop score >/=5 as compared to only 33% for the placebo group. Intravaginal Evening Primrose Oil given 4 capsules every 4 hours for 3 doses has s significant effect in Bishop score compared to placebo. Significant improvement in the amount of change in Bishop score during treatment period was also seen. No adverse effects on maternal and fetal safety profile were noted.

Atopic eczema in children

Cutaneous application

Ferreira *et al.* (1998: cited by Hoare *et al.* 2000) examined 23 patients, aged 3 to 15 years, for 4 months in a randomised controlled trial with a parallel design. The patients had an **atopic dermatitis which was in remission**. Two patients were withdrawn from the study. The emollients contain 10% GLA (Gamma Linolenic Acid) versus borage oil (24% GLA) versus rose hip oil (35%-40% GLA). The placebo was an emollient without EFA (Essential Fatty Acids). Clinical assessment of xerosis and pruritis revealed improvement in all 4 groups, slightly more pronounced in the 3 GLA groups. The changes were not statistically significant.

Also see 4.2.2 for studies on children Anstey *et al.* (1990).

Oral intake

Bordoni *et al.* (1987) studied 24 children with **atopic dermatitis**, of which 14 boys and 10 girls aged 2 to 4 years. The children were divided in 2 equal groups. One group received 6 capsules of evening primrose oil a day. The other group swallowed the same amount capsules which contained 0.5 g of olive oil. An *Oenothera* capsule consisted of 0.5 g *Oenothera* fatty oil, of which 74.7% LA; 8.9% GLA; 6.8% palmitic acid and 1.9% stearic acid. During the parallel, double-blind, randomised, placebo-controlled trial, the patients continued to take emollients and weak topical steroids.Only in the *Oenothera* treated children, there was an increase of DGLA and AA and a decrease of 18:2/20:4 ratio measured.

After 4 weeks, the trial showed an improvement of 2/3 of clinical symptoms in EFA-treated children in comparison with that of placebo-treated children. No side effects were noticed.

Assessor's comment: The study is of low quality due to the limited number of patients and the imprecision of outcome measures.

Biagi *et al.* (1988) studied 12 children, 8 boys and 4 girls, with **atopic dermatitis** between 2 and 4 year during 20 weeks in a double-blind, parallel, placebo-controlled trial. They took 6 capsules every day with 0.5 g *Oenothera*, which contains 74.7% LA and 8.9% GLA. The patients could continuously make use of emollients and also topical steroids, if necessary. Blood samples were taken at the beginning of the trial and after 4 and 20 weeks. AA levels, plasma LA/AA ratio and other fatty acids were measured. No important side effects were noticed. The results proved a significant improvement of the clinical status of atopic eczema after 4 weeks of treatment. There was no improvement between week 4 and 20 of the treatment.

Assessor's comment: The study is of low quality due to the limited number of patients and the fact that after 4 weeks no further improvement was seen.

Hederos & Berg (1996) studied children, between 1 and 16 years, with, atopic dermatitis who met the criteria of Hanifin and Rajka and who needed the regular treatment with topical skin corticosteroids. Sixty children started the study and were divided in 2 equal groups. Two subjects group dropped out of the study. Twenty-two of the patients also had asthma symptoms. The double-blind, randomised, placebo-controlled trial with a parallel design took 16 weeks. The placebo capsules

contained 50 mg sunflower oil and 10mg vitamin E. An *Oenothera* capsule contained 500 mg *Oenothera* fatty oil (= 40 mg GLA) and 10 mg vitamin E. Children between 1 and 12 years took 4 capsules twice a day. Children above 12 years took 6 capsules twice daily. The use of topical steroids, antihistamines and asthma medication was allowed. Children, who could not swallow the capsule were allowed to open it. Two patients dropped out of the study: 1 because of the taste and 1 refused to undergo further assessments. Some side effects (5 of *Oenothera* group and 6 of the placebo group) were mentioned, but these effects were not considered as serious. Over the 16 weeks, a compliance of minimum 87% was obtained in each patient. In the *Oenothera* group, there was a highly significant raise of DGLA and AA concentration in the blood analyses. Both groups improved with respect to the baseline, but no significant differences were found between both groups. Furthermore, the use of asthma medication and topical steroids showed no differences between the groups.

Assessor's comment: The study is of low quality, because of the limited number of patients and indirect measures as outcome.

Yoon *et al.* (2002) studied 14 children with atopic eczema, which had an itchy dry scale skin. The persons, who had an apparent erythema or oozing were excluded. Five boys and 9 girls, with an average age of 5.5 years, participated in the trial. Seven of them had a mild atopic dermatitis; the other 7 had a severe atopic dermatitis. They received 2 capsules twice a day. Each capsule contained 40 mg GLA. The control group consisted of 4 boys and 2 girls, with an average age of 7.2 years. Before the start of the study the serum interferon gamma was lower and the serum IgE concentration was higher than these of the normal group. After 2 weeks treatment with *Oenothera,* there was a significant raise of serum interferon gamma and decrease of serum IgE found. The study marked a reduced level of skin lesions and pruritus. No serious side effects were noticed. This study demonstrated that both supplementation of GLA and modulation of immunological abnormalities improved atopic dermatitis.

Assessor's comment: This study has an observational character and is of limited value due to the low number of patients.

Also see 4.2.2 for studies on children: Lovell *et al.* (1981), Wright & Burton (1982), Bamford *et al.* (1985), Berth-Jones & Graham-Brown (1993), Senapati *et al.* (2008).

Other skin studies

See 4.2.2 for studies on children: Chalmers & Shuster (1983).

Attention deficit hyperactivity disorder (ADHD)

ADHD is a development and behaviour disorder. The three principal symptoms are hyperactivity, concentration problems and impulsiveness. To date, the mechanism is unprecedented.

Aman *et al.* (1987) conducted a study with 31 hyperactive children (4 girls and 27 boys; age not specified) in Australia. Twenty-six had attention problems and 5 had an attention deficit disorder with or without hyperactivity. Six children had a history of febrile convulsions and one of epilepsy. The children did not swallow medication and they had no neurological disorders. The double-blind, placebo-controlled, cross-over study took 4 weeks. Three capsules twice a day were given to each patient. An *Oenothera* capsule contained 360 mg LA and 45 mg GLA. The placebo consisted of 500 mg liquid paraffin. A wash out period of 1 week took place. Blood samples were taken and analysed. The results showed a significant decrease of palmitoleic acid and a 14% increase of DGLA during *Oenothera* treatment. Other EFA did not change, but there was a non-significant trend of decreased alpha-LA. Some measurements showed treatment-related changes, but the majority of measurements failed to show an effect in psychomotor improvements.

Xerotic cheilitis

Park *et al.* (2014) conducted a pilot study on the effect of evening primrose oil for the prevention of xerotic cheilitis in acne patients being treated with isotretinoin. Forty Korean volunteers of Fitzpatrick skin types III and IV, having moderate acne, were enrolled and randomized to receive either isotretinoin with or without EPO (450 mg 3x daily) for 8 weeks. The efficacy of treatment was evaluated on the basis of global acne grading system scores, number of inflammatory and noninflammatory lesions, TEWL (Trans Epidermal Water Loss), corneometry, physician's global assessment, and patient satisfaction. The results after 8 weeks of treatment showed that the TEWL of the lip increased significantly during isotretinoin treatment, whereas the TEWL of the hand dorsum showed no significant change. The increase of the TEWL of the lip was more definite in the control group than in the experimental group. The number of acne lesions decreased significantly in both groups, and there were no differences between them.

4.4. Overall conclusions on clinical pharmacology and efficacy

Oenothera oil has been studied in patients with various pathologies: atopic dermatitis, ichtyosis vulgaris, premenstrual syndrome, mastalgia, menopauzal symptoms, patients undergoing cervical dilatation, rheumatoid arthritis, Sjögren syndrome, Raynaud's phenomenon, diabetic neuropathy and hyperlipidemia. Some special populations include patients treated with bortezumib and patients treated with isotretinoin with the purpose to treat side effects of the primary therapy. Furthermore pregnant patients during delivery and children suffering from ADHD were studied. Some additional studies were done with mixtures and will not be further discussed as the effect of evening primrose oil is difficult to evaluate.

The studies concerned with patients suffering from moderate or severe atopic dermatitis are in closest relation with the therapeutic indication of the monograph. Personal characteristics of the patients included were representative for a usual population. The age started from 8 months to adult age (until 66 years when specified). A total of 620 patients were included in 9 studies dealing with oral administered *Oenothera* oil. The number of patients per study varied from 21 to 154. In most cases, a double-blind, placebo controlled, parallel or cross-over design was chosen. The patients received an equivalent to 360 mg LA and 45 mg GLA, mostly as soft capsules containing 500 ml of *Oenothera* oil. Treatment periods were up to 6 months. In 7 out of 9 trials, a positive clinical outcome was seen. When measured, plasma levels of DGLA, GLA and AA were increased. There seemed to be a relation between the increase in these plasma levels and the clinical outcome. However, the level of evidence is low to moderate.

Four small scale studies included only children between 1 and 16 years old (n=110), suffering from atopic dermatitis or eczema. Two of these studies – of which one with an open design - resulted in a positive outcome. However, the number of subjects in these studies is lower, compared to studies with adults. It should be noted that children were also included in the other clinical trials mentioned before. The level of evidence is low to very low.

Other indications were non-specified dermatitis, PMS, mastalgia, menopausal complaints, RA, SS, Raynaud's phenomenon, cervical dilatation and diabetic neuropathy. It is notable that the results in the case of PMS failed to show efficacy. In 3 double-blind, placebo-controlled studies, there was no significant difference between *Oenothera* and placebo with regard to clinical outcomes. In one study, only partial results were obtained. In most cases, the condition of all patients improved, therefore the therapeutic efficacy was considered to be due to a placebo effect. The same can be concluded when patients with mastalgia or menopausal symptoms were studied, and when *Oenothera* was used in case of RA. Clinical trials in the other conditions mentioned did not create perspective for a well-established use, mostly by lack of placebo group. There were no serious adverse events reported during the clinical trials taken into consideration.

Outcomes described in patients with Raynaud's phenomenon may point to possible antiaggregating effects on blood platelets.

Overall conclusions on clinical efficacy of the evening primrose oil of:

- Positive outcomes were only seen for eczema/atopic dermatitis. The evidence generated by the studies is very low to moderate even by using a less conservative approach with respect to some of the parameters applied. One study with moderate evidence had a negative outcome.

- Negative results were reported for studies on other therapeutic uses including non-specified dermatitis, PMS, mastalgia, menopausal complaints, RA, SS, Raynaud's phenomenon and diabetic neuropathy.

- Although several hundreds of patients participated in the clinical studies, the number is low in the individual studies. Furthermore, in most cases children and adults are included in the same study which makes the study population diverse and the drawing of conclusions difficult.

- Most studies have involved preparations of 500 mg *Oenothera* oil which facilitates comparison between studies.

- The instruments used for therapeutic evaluation are diverse and subjective.

In conclusion, the evidence for a well-established use in the case of eczema/atopic dermatitis is not sufficient. A traditional use can be considered for oral use as *Oenothera* oil was on the market before 1981 and plausibility is supported by some clinical studies. With regard to the use of cutaneous preparations, the studies fail to support a well-established medicinal use. In addition, there is no evidence to demonstrate 30 years of cutaneous use, therefore traditional use is not substantiated for this route of administration.

5. Clinical Safety/Pharmacovigilance

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

No serious side effects were reported from clinical studies.

5.2. Patient exposure

More than 600 patients (of whom more than 100 children) were exposed in clinical studies dealing with atopic dermatitis. For other conditions (e.g. PMS, menopausal complaints, SS, Raynaud, diabetes, inflammatory conditions) another 600 patients were involved.

5.3. Adverse events, serious adverse events and deaths

The World Health Organization's Uppsala Monitoring Centre (WHO-UMC) received 291 reports from national pharmacovigilance centres of 12 different countries. These reports describe 489 suspected adverse drug reactions for products reported to contain *Oenothera* fatty oil as the active ingredient. Only mild adverse effects are reported when *Oenothera* fatty oil is taken in the recommended dosage. The most common are gastro-intestinal effects, indigestion, nausea, softening of stool and headache (Barnes *et al.* 2007).

The most serious side effects reported in the above-mentioned WHO-UMC related to the use *Oenothera* oil were convulsions, hepatitis, bronchospasms and interference with platelet function.

In the European Union herbal monograph side effects are limited to the most common symptoms: gastrointestinal effects, indigestion, nausea, softening of stool, rise in temperature, hypersensitive reactions like exanthema and headache. The frequency is not known.

5.4. Laboratory findings

Laboratory findings are limited to serum lipids (see clinical trials).

5.5. Safety in special populations and situations

Intrinsic (including elderly and children) / extrinsic factors

Puri (2007) criticises the fact that two trials concluded that *Oenothera* leads to a higher risk of epileptic seizures in schizophrenic patients. The first trial, which was performed by **Vaddadi (1981: cited by Puri 2007)**, was an open study. Three long-term hospitalised schizophrenic patients, for whom conventional therapy failed, participated in the trial. The 3 subjects took phenothiazine-type antipsychotics while being treated with *Oenothera*. They had a history of abnormal electroencephalographs and loss of consciousness. All developed characteristics of temporal lobe epilepsy. The second trial was carried out by **Holman & Bell (1983: cited by Puri 2007)**. It was a double-blind placebo-controlled study with 23 schizophrenic patients. Three patients generated epileptic seizures, 1 from the placebo and 2 from the *Oenothera* group. One *Oenothera* and one placebo treated patient had tonic-clonic seizures. These patients were administered with phenothiazines, which are known to decrease the attack threshold. Therefore, Puri concluded that *Oenothera* is not a risk for having epileptic seizures. In rats, researchers demonstrated that a long-term oral intake of LA and GLA protects rats of seizures and that *Oenothera*-derived omega-6 fatty acid arachidonic acid inhibits sodium ion currents and synaptic transmission. *Oenothera*-derived eicosanoid PGE1 seems to have an anticonvulsive activity. Therefore, according to the author, *Oenothera* can be seen as a safe product which does not cause epileptic attacks as side effects.

5.5.1. Use in children and adolescents

See clinical studies and studies on special populations.

5.5.2. Contraindications

The only contraindication is related to hypersensitivity to *Oenothera* oil.

5.5.3. Special Warnings and precautions for use

Despite the fact that children were included in clinical trials, the oral use in children under 12 years of age has not been established due to lack of adequate data.

5.5.4. Drug interactions and other forms of interaction

Lopinavir

Williamson *et al.* (2013) describe a case with raised lopinavir levels and persistent diarrhoea, which developed in an HIV-positive man after evening primrose oil and a product containing aloes, rhubarb and liquorice were started.

Assessor's comments: as several herbals are mentioned in this case, it is not scientifically justified to incriminate Oenothera oil.

Li-concentrations

Osman and Badawi (2016) report on a case wherein EPO reduced serum lithium concentration. A 38-year-old woman with an established diagnosis of treatment-resistant depression has achieved remission for 10 years on a combination of thyroxin 25 µg and nortriptyline 150 mg daily augmented with lithium carbonate at a daily dose of 800 mg. She has comorbid chronic acne vulgaris with acne scarring that was stabilized on a combination of topical preparations, namely adapalene gel 0.3% and clindamycin/benzoyl peroxide gel and Tetralysal 300 mg daily. Serum levels of lithium were within the therapeutic range (0.69 mmol/l). However, she noted deterioration in her acne and was commenced on EPO 500 mg daily capsules. Yet, on regular 3-monthly checkup of lithium levels, after taking EPO for 2 months, a reduction in serum lithium level was noted (0.37 mmol/l). As she reported no affective symptoms and was functioning optimally, no change in the dose of lithium was warranted and she remained on the daily 800 mg dose. After 3 months, serum lithium level fell to 0.23 mmol/l. The EPO capsules were discontinued. After a further 6 weeks, the lithium level was observed to have risen to the therapeutic window level at 0.73 mmol/l. No other medication was altered during the 6-month period and no difficulties in terms of physical well-being or compliance noted. All the biochemical investigations were unremarkable. The total duration of EPO therapy was 5 months. According to the authors a potential mechanism, by which EPO could have led to a reduction in the serum level of lithium, is *via* increasing the production of renal prostaglandin that may have resulted in increased renal blood flow and decreased renal reabsorption of lithium. The ingredients of the EPO capsule are omega-6 fatty acids, including linoleic acid, natural vitamin E, beef gelatin, humectant (vegetable glycerol), and D-alpha tocopherol. None of these ingredients was reported to have an effect on lithium carbonate metabolism.

Assessor's comment: as this appearant interaction did not lead to therapeutic complications and the mechanism of action is not clear, this finding is not translated to the monograph.

Seizures

There may be an increased risk of seizure in schizophrenic patients, temporal lobe epileptic patients and others who take epileptogenic drugs such as phenothiazines. Patients with a history of these illnesses should use *Oenothera* products with caution (Barnes *et al.* 2007).

Also see paragraph above Puri (2007) for studies on phenothiazines.

Assessor's comment: There is no consensus whether a drug-drug interaction with phenothiazine-type antipsychotics exists in schizophrenic patients, lowering the convulsive threshold. As a consequence, no such interaction is mentioned in the monograph. This is in line with the conclusions by Williamson et al. (2013).

Bleeding

Patients taking antiplatelet or anticoagulant medications should use *Oenothera* with caution or should not use it at all. *Oenothera* consumption decreases thromboxane formation and increases PGE1 formation, which leads to an inhibition of the platelet aggregation with increased risk of bleeding (Halat & Dennehy 2003).

Assessor's comment: Use of Oenothera oil concomitantly with antiplatelet drugs could enhance the effect of the latter. This combination should be discouraged. However, as there are no clinical cases reported, no interaction warning is included in the monograph. This is also the conclusion by Williamson et al., 2013).

Anti-inflammatory drugs

Theoretically, there is a risk of interaction with anti-inflammatory drugs, corticosteroids, beta-blockers and antipsychotics (Huntley 2004).

Morse & Clough (2006) made a meta-analysis of 26 clinical RCTs of *Oenothera* oil. The open, not placebo-controlled or ongoing trials were not included in the meta-analysis. A number of 1207 patients participated in these studies: in the parallel trial 616 patients took *Oenothera* and 591 placebo; in the cross-over studies there were respectively 212 and 217 patients in both groups. The number of patients per study was between 15 and 154. The patients of the studies differ from age, sex, baseline severity of eczema and nationality. The placebos used in the trials were different: liquid paraffin, olive oil, safflower oil or sunflower oil. The duration of the trials was between 3-16 weeks. The dose (in mg GLA a day) taken was between 2 to 16 capsules of 500 mg *Oenothera* a day (160-480 mg GLA). The most important potential factor of heterogeneity between the trials was the use of steroids. The patients in the trial were classified as 'low' users or as 'high' users. The low users were the ones taking mild to moderate potent steroids. High users took potent, very potent or oral steroids. If a study consisted of less than 25% of the high users, it was considered as a 'low' usage. If more than 25% were high users in a trial, it was regarded as a 'high' usage trial. Twelve studies used low steroids, 13 were high use trials. The meta-analysis noted that, in the presence of low steroid usage, there is a beneficial effect of *Oenothera* for itching and this is apparent between 4 to 8 weeks. Other parameters like sleep loss, crusting, excoriation and oedema showed a trend in favour of *Oenothera*. The magnitude of the effects is reduced in association with an increased frequency of potent steroid use.

Assessor's comment: as this study does not indicate an interaction with steroids, it is not translated into the monograph. This is in line with the conclusionof Williamson et al. (2013).

5.5.5. Fertility, pregnancy and lactation

Use in pregnancy and lactation

Oral treatment given to atopic pregnant and nursing mothers and *Oenothera* fatty oil given to newborns with an increased risk of atopic eczema may prevent atopic dermatitis. During the growth of the thymus, it can compensate the D6D deficit and the lack of lymphocytic PGE receptors (Kerscher & Korting, 1992). However, not enough data are available on the safety of *Oenothera* during pregnancy and therefore it is not recommended to use *Oenothera* products during pregnancy. *Oenothera* treatment is, however, possible during pregnancy when the potential benefits outweigh the potential harms, but attention should be paid to high doses, above 4 g daily (Barnes *et al.,* 2007; Bédard, 2003).

Dove & Johnsons (1999) studied the effect of oral *Oenothera* fatty oil on the length of pregnancy and selected *intrapartum* outcomes in low-risk nulliparous women. Fifty-four women received a standard dose of *Oenothera*. At week 37, they received 500 mg 3 times a day for 1 week, then once a day 500 mg until labour started. The control group consisted of 54 women who did not take *Oenothera* during their pregnancy. One subject was withdrawn of the study. There were no significant differences found in age, Apgar score and days of gestation between the control group and the *Oenothera* group. A borderline significant difference was observed in birth weight: the *Oenothera* group infants were on average 156 g heavier. A significant variation was observed on the 5-minute Apgar score and length of labour between the *Oenothera* and control groups. Women administered with *Oenothera* had a longer labour as compared to the control group. The *Oenothera* group had a tendency to a greater risk of more protracted active phase, prolonged rupture of membranes, oxytocin augmentation, and arrest of descent.

Oral use

No malformations were observed. However, when using primrose fatty oil during the first three months of the pregnancy, a benefit-risk evaluation has to be done. Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

5.5.6. Overdose

Symptoms of overdosage are loose stool and abdominal pain, treatment is not necessary (Barnes *et al.,* 2007). These symptoms are translated into the European Union herbal monograph in section 4.9: "the symptoms of overdosing are mild diarrhoea and abdominal pain. No special treatment is required."

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

Not applicable

5.6. Overall conclusions on clinical safety

There are no major concerns related to serious adverse reactions. Possible implication of *Oenothera* fatty oil lowering the convulsive threshold is debated. The HMPC did not consider that the data justify including in the monograph a statement on drug interactions during the use of *Oenothera* in patients taking anticonvulsive drugs or antipsychotics. *Oenothera* has been used during pregnancy without major problems. Use during in the perinatal period is not recommended, due to interference with delivering. The HMPC concluded that safety during pregnancy and lactation has not been established; in the absence of sufficient data, the use during pregnancy and lactation is not recommended.

6. Overall conclusions (benefit-risk assessment)

Quality

The herbal preparation is a fatty oil, described in the European Pharmacopoeia. It contains unsaturated fatty acids:at least 65-85% linoleic acid, 7-14% gamma-linolenic acid and a maximum of 0.5% alpha-linolenic acid. Besides oxidation after exposure to air and light, the oil is sensitive to heat and humidity. Consequently, the storage conditions (cool dark place) are important. There are no cases of contamination or adulteration mentioned in literature, but the quality parameters/specifications for *Oenothera* oil are referred to in the European Pharmacopoeia, especially as far as the percentage non oxidized linoleic acid is concerned.

Safety

The use of *Oenothera* oil can be considered as well known, as its use has been reported in the 17th century in Europe, coming from North America (Indians). In the Vigisearch database of the World Health Organization's Uppsala Monitoring Centre reported side effects are listed. As most serious side effects, convulsions, hepatitis, bronchospasms and interference with platelet function were related to the use *Oenothera* oil. A variety of minor side effects was also collected in the database. The type of preparation and the dose are not always specified. Ingestion of overdoses can lead to fatty diarrhoea. This effect may be considered as self-limiting when ingesting large doses. There is no consensus

whether a drug-drug interaction with phenothiazine-type antipsychotics exists in schizophrenic patients, lowering the convulsive threshold. Use of *Oenothera* oil concomitantly with antiplatelet drugs could enhance the effect of the latter. This combination should be considered carefully. The HMPC decided that the data do not justify including a statement in the European Union herbal monograph. There is limited information from clinical observations in pregnant women. *Oenothera* oil did not cause any harm, but more systematically gathered reports are needed, also in case of breast-feeding. There is a suggestion from animal experiments that *Oenothera* oil can protect animals (rats) against induced breast cancer. In chronic feeding experiments with rats, no carcinogenic potential could be seen. No specific genotoxicity or mutagenicity testing was performed.

Taking into consideration available information on side effects and drug-drug interactions, the use of *Oenothera* oil in the specified conditions of use is safe.

Efficacy

Oenothera oil has been studied for atopic dermatitis in adults and children (from 8 months of age). The quality of evidence varied from very low to moderate. This indication was taken to the monograph. The outcome was not consistent but was mainly negative for other indications such as premenstrual syndrom, rheumatoid arthritis, mastalgia, menopausal symptoms, Sjögren syndrome, Raynaud's phenomenon, ADHD and diabetic neuropathy. Concerns have been raised about the quality of the evidence to support the therapeutic claims for *Oenothera* oil. In 2002, the UK Medicines Agency withdrew all marketing authorisations for oral evening primrose oil capsules. This followed a review by the UK Medicines Agency of all the relevant information, including new studies and statistical analyses. The UK Medicines Agency concluded that the data did not support the current standards of efficacy required for authorisation of these products as medicines for the treatment of eczema and mastalgia.

Some studies on the use of *Oenothera* oil for diabetic neuropathy have been marred by the fact that one of the investigators in the studies was found guilty of research fraud in the clinical trials.

In view of the uncertainties concerning the available bibliographic data together with the poor quality of the evidence of efficacy in the published studies, the HMPC considers that there is insufficient evidence on which to base a 'well-established use' indication for *Oenothera* oil. Clinical studies with commercial *Oenothera* oil preparations were reported from 1981 onwards, and a traditional use of the oil in atopic dermatological conditions (for the symptomatic relief of itching in acute and chronic dry skin conditions) can be accepted. Because there are only limited data on the specific use in children the use of the oil was finally restricted to adolescents and adults.

A European Union list entry is not supported due to lack of data on genotoxicity.

Annex

List of references