Assessment report on *Tanacetum parthenium* (L.) Schultz Bip., herba

Draft – Revision 1

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)		*Tanacetum parthenium* (L.) Schultz Bip., herba
Herbal preparation		Powdered herbal substance
Pharmaceutical form(s)		Herbal preparation in solid dosage forms for oral use
First assessment	Rapporteur	G Calapai
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Note: This draft assessment report is published to support the public consultation of the draft European Union herbal monograph on *Tanacetum parthenium* (L.) Schultz Bip., herba. It is a working document, not yet edited, and shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on comments that will be received on this assessment report. The publication of this <u>draft</u> assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

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1. Introduction

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

- Herbal substance(s)

Tanacetum parthenium herba consists of the dried, whole or fragmented aerial parts of *Tanacetum parthenium* (L.) Schultz Bip. It contains no less than 0.20% of parthenolide ($C_{15}H_{20}O_3$; Mr 248.3), calculated with reference to the dried drug. It has a camphoraceous odour (Ph. Eur. 9th edition 2016).

The genus *Tanacetum* includes about 50 species, of those only *T. santolinoides* (D.C.) grows in Egypt (El- Shazly *et al.* 2002).

T. parthenium (L.) Schultz Bip., also known as feverfew, is a member of the *Compositae* family (*Asteraceae*). It is an aromatic, hardy annual herb with chrysanthemum-like leaves and daisy-like flowers that grows prolifically in gardens and other open spaces. It is indigenous to South-East Europe, as far East as the Caucasus, but commonly found throughout Europe and the United States of America (WHO monograph 2004).

The leafy, more or less branched stem has a diameter of up to 5 mm. It is almost quadrangular, longitudinally channelled, and slightly pubescent. The leaves are ovate, 2 to 5 cm long, sometimes up to 10 cm, yellowish-green, petiolate and alternate. They are pinnate or bipinnate, deeply divided into five to nine segments, each with a coarsely crenate margin and an obtuse apex. When present, the flowering heads are 12 to 22 mm in diameter with long pedicels (Ph. Eur. 9[th]edition 2016). Beneficial properties have been associated with consumption of the leaves or aerial parts.

Overview on main active compounds and common qualitative/quantitative characterization

Sesquiterpenes

The main components present in feverfew leaves are a complex series of sesquiterpene α-methylene butyrolactones which are stored in the glandular trichomes on leaves, flowers and seeds. Some of the detected sesquiterpene lactones are known to have biological actions such as cytotoxicity, growth regulation and antimicrobial effects and they cause allergic contact dermatitis. An exocyclic α-methylene function of the sesquiterpene lactones, which may react with sulphydryl groups of proteins, seems to be responsible for these activities (Milbrodt *et al.* 1997). The predominant sesquiterpene lactone present in feverfew is a germacranolide, **parthenolide (PN)** (Figure 1). PN comprises up to 85% of the total sesquiterpene content (Pareek *et al.*2011). This compound was first isolated from *T. parthenium*. Later, the same compound, initially named champakin, was also isolated from the roots of *Michelia champaca*. PN is located in glands on the underside of the leaves in growing plants, in which position it can express its antimicrobial properties. PN contains a highly electrophilic α- methylene-γ-lactone ring and an epoxide residue capable of interacting rapidly with nucleophilic sites of biological molecules. More recent studies have revealed that it also has anti-microbial, antiinflammatory and anticancer activities, which may depend on a wide range of PN-stimulated intracellular signals (Won *et al.* 2004; Pajak *et al.* 2008).

Levels of PN in the dried leaves can be as high as 1%. The remaining sesquiterpene lactones are generally present in much smaller quantities, typically <1 mg/kg. During the growth of *T. parthenium*

the percentage of PN is the highest at an early stage (just before the formation of stems). The yield of PN in the plant gradually increases until the plant is in full bloom. However, PN is present in the leaves and flower heads, but not in the stems.

Drying at ambient temperature and lyophilisation seems to have no negative influence on the yield of PN.

parthenolide   R = H
3β-hydroxyparthenolide R = OH
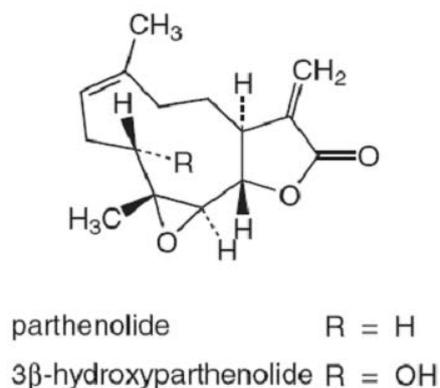
Figure 1. Chemical structure of PN (from WHO monograph).

Since the PN content greatly varies depending on the part used and the season, it has been proposed to distinguish two qualities of feverfew: A) *Tanaceti parthenii* folium (feverfew leaf), harvested at an early stage before the formation of the stems and B) *Tanaceti parthenii* herba (feverfew herb), harvested at full bloom, with a minimum PN content of 0.50% and 0.20% respectively, calculated on a dry weight basis (Hendriks *et al.* 1997).

The aerial parts of feverfew contain a rich mixture of mono- and sesquiterpenes compounds. Sesquiterpenes contained in the aerial parts are germacrene D, β-farnesene and camphor (the most abundant monoterpene in feverfew). In the roots, β-farnesene and bicyclogermacrene are present. The aerial parts contain also smaller amounts (<10 mg kg-1) of chrysanthenyl acetate, the epimeric cischrysanthenol (plus the derived acetic, angelic and isovaleric esters), the isomeric cis-verbenol (an oxidized relative), 4,β-acetoxy-chrysanthenone, bornyl acetate and the corresponding angelate ester. Non-terpenoid spiroketal enol ethers are other substances that are present in small quantities in the aerial parts (Knight 1995).
The presence of different sesquiterpene lactones depends on the habitat of the plant (Milbrodt *et al.* 1997).

Parthenolide metabolites
Other reported germacranolides are PN metabolites: 3β-hydroxyparthenolide, costunolide (common component of the Compositae), 3β-hydroxycostunolide, artemorin and 3β-hydroxyanhydroverlotorin. They are probably formed by epoxidation and/or allylic oxidation of PN. Related epoxides are represented by epoxyartemorin and anhydroverlolorin-4α,5β-epoxide. Costic acid methyl ester and reynosin have also been found in small quantities. Some sesquiterpenes belong to the biosynthetically closely related guaianolide family: 8α-hydroxyestafiatin, with the corresponding isobutyl and angeloyl esters.

Other compounds
The roots of *T. parthenium* contain the coumarinic compound isofraxidin (Kisiel & Stojakowska1997)

and anisofraxidin drimenyl ether named 9-epipectachol B (Pareek*et al.* 2011). (2-glyceryl)-*O*-coniferaldehyde also has been isolated (Pareek *et al.*2011).

Melatonin was identified in four samples from feverfew leaves and in a commercial preparation tested by Murch*et al.* (1997).

Lipophilic flavonoids in the leaf and flower of *T. parthenium* were identified as methyl ethers of the flavonols 6-hydroxykaempferol and quercetagetin. A number of other flavones such as apigenin, luteolin and chrysoeriol and their glucuronides, and glycosides such as apigenin 7-glucuronide, luteolin 7-glucuronide, luteolin 7-glucoside and chrysoeriol 7-glucuronide in feverfew extracts have been found. Apigenin and two flavone glucuronides are present in glandular trichomes on the lower epidermis of the flowers. The vacuolar flavonoids are dominated by the presence of apigenin and luteolin 7-glucuronides. Other substances found are tanetin (previously thought to be a new structure and now formulated as the known 6-hydroxykaempferol 3,6,4 '-trimethyl ether (Williams 1995), and the other three flavonol methyl esters as respective 6-O-methyl ethers instead of 7-O-methyl ethers (Williams 1999) and two closely related flavonols, jaceidin and centaureidin (Long et al. 2003).

Moreover, antioxidant polyphenolic acids were isolated and characterised as 3.5-, 4.5- and 3.4-di-O-caffeoylquinic acids (Wu *et al.* 2007).

Feverfew oil

The analysis of feverfew oil showed the presence of many monoterpenes such as α-pinene, camphene, β- pinene, sabinene, myrcene, α-fellandrene, α-terpinene, p-cymene, γ-terpinene, terpinolene, terpinen-4-ol and α-terpineol. Among them, the oxidized monoterpenes are very well represented especially camphor, trans-chrysanthenyl acetate, linalool, linalyl acetate and bornyl acetate. The essential oil is mostly composed of camphor and trans-chrysanthenyl acetate amounting up to 70% of the oil content. Other minor monoterpenic components are p-cymene (4.77%), linalool (2.28%) and camphene (1.96%). Among the sesquiterpenic compounds there are: β-caryophyllene (1.96%), trans-β-farnesene, germacrene (1.49%) and δ-cadinene. The phenyl propanic compound eugenol is also present (1.09%) (Kalodera *et al.* 1997).

Dutch researchers of feverfew leaf extract, evidently focusing on its essential oil, suggested that the content of trans-chrysanthenyl acetate might be of importance, as this compound inhibits the enzyme prostaglandin synthetase, and it has been described to possess analgesic properties. (Hendricks *et al.* 1996). This constituent declines markedly during the extraction process from 0.25% to just 0.017%; the relevance of this difference has not been determined. Trans-chrysanthenyl acetate and camphor, in contrast, are monoterpenes that are regarded as characteristic constituents of *T. parthenium*, whose content in the essential oil has not shown to vary significantly.

- Herbal preparation(s)

The powdered herbal substance is yellowish-green (Ph. Eur. 9th edition 2016).

PN was found to be the main constituent of the sesquiterpene lactones in ethanol and aqueous extracts of feverfew. The sesquiterpene lactone content of ethanol extracts (ca. 0.5%) were higher than those of the aqueous ones (ca. 0.3%) (Gromek *et al.* 1991).

Commercial preparations of feverfew leaves are known to vary widely in the PN content, as shown by various authors. Feverfew products from the European markets have been simultaneously analysed by HPLC, NMR and biological methods and all were consistent in showing a high variability of the PN

content (Heptinstall *et al.* 1992). Mean PN levels of commercial preparations of feverfew leaves exhibited a range from non-detectable to 1.68% ± 0.97 (per dry weight) based on HPLC-UV-MS (Cutlan *et al.* 2000).

Nelson *et al.* (2002) studied the PN content in capsules containing 25 to 500 mg of feverfew leaf, available to consumers, by means of HPLC. The quantity of feverfew leaf in each capsule was similar to that declared on the label. However, the PN content per dosage form varied 150-fold (from 0.02 to 3.0 mg), while percentage of PN varied 5.3-fold (from 0.14% to 0.74%). Therefore, if a person consumes the daily dose recommended on the label, the intake of dried feverfew leaf would range from 225 to 2246 mg/day, a 10-fold variation, while intake of PN would range from 0.06 to 9.7 mg/day, a 160-fold variation.

Taking into consideration the large variations observed in the PN contents and daily intake as recommended by labelling in commercial feverfew products, it is suggested that manufacturers of feverfew products should use measurements of PN for standardization and quality control (Heptinstall *et al.* 1992). Among the proposed references for establishing quality control of feverfew preparations, a minimum level of 0.2% PN in dried leaves was adopted by the Ph. Eur. and the French Ministry of Health.

Possible differences in physico-chemical properties of extracts from different sources were investigated in the USA on selected formulations of several commercial feverfew extracts. Flowability, hygroscopicity and compressibility were studied in order to develop and validate a suitable extraction method. HPLC was used to determine the PN content of several commercial feverfew extracts. The results of the investigation showed that the extracts exhibited poor to very poor flowability. Hygroscopicity and compactibility varied greatly with the source. Moreover, no extracts contained the PN content labelled. Even different batches from the same manufacturer showed a significantly different PN content (Jin *et al.* 2007).

Another investigation was conducted by the same group of researchers with the aim to evaluate the stability of PN in feverfew solutions versus powdered feverfew (solid state). They further explored the compatibility between commonly used excipients and PN in feverfew. Feverfew extract solution was diluted with different pH buffers to study the solution stability of PN.
Powdered feverfew extract was stored at 40°C/0% to 75% relative humidities (RH) or 31% RH/5 to 50°C to study the influence of temperature and relative humidity on the stability of PN in feverfew solid state. In addition, binary mixtures of feverfew powered extract and different excipients were stored at 50°C/75% RH for excipient compatibility evaluation.

The degradation of PN in feverfew solution appeared to fit a typical first-order reaction. PN is comparatively stable when the environmental pH is in the range of 5 to 7, but becomes unstable when pH is less than 3 or more than 7.

PN degradation in feverfew in the solid state does not fit any obvious reaction model. Both moisture content and temperature play important roles affecting the degradation rate. After 6 months of storage, PN in feverfew remains constant at 5°C/31% RH. However, ~40% PN in feverfew can be degraded if stored at 50°C/31% RH. When the moisture changed from 0% to 75% RH, the degradation of PN in feverfew increased from 18% to 32% after 6-month storage at 40°C. The authors concluded that PN in feverfew exhibits good compatibility with commonly used excipients under stressed conditions in a 3-week screening study (Jin *et al.* 2007). Since the PN stability can vary with storage conditions, feverfew should be stored in a cool, dry environment and in a well-closed container, protected from light and humidity (Heptinstall *et al.* 1992).

The disappointing results of a clinical study carried out by De Weerdt et al. with an ethanolic extract standardized to contain 0,5 mg of PN have raised the doubt that the pharmacological activity of feverfew in the treatment and prophylaxis of migraines could not be attributed to PN content as was previously thought, but to other unidentified constituents (Awang, 1998; de Weerdt *et al.*, 1996). Since the active constituents are unknown, it is recommended that preparations containing the whole leaf (dried or fresh) should be used (ABC Clinical Guide, 2003).

- 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.

Combination products have been designed and their effects on migraine prophylaxis studied. A study with a daily dose of riboflavin 400 mg, magnesium 300 mg and feverfew 100 mg was conducted. Authors concluded that no significant positive effects were observed (Maizels *et al.* 2004).

A prospective, open-label study with a combination product (feverfew extract standardised on PN (>0.2%) 300 mg plus *S. alba* extract standardised on salicilin (>1.5%) 300 mg) investigated the effects of therapy on migraine attack frequency. The combination product reduced the frequency of migraine attacks but also pain intensity and duration. Further investigation of this therapy in a double-blind, randomised, placebo-controlled investigation involving a larger patient population is necessary (Shrivastava *et al.* 2006).

The combined effect of acupuncture and powdered leaves of *Tanacetum parthenium* (150 mg/day) on quality of life in women suffering from migraine attacks was investigated in a randomized clinical study. Acupuncture plus *Tanacetum* was statistically significantly more effective than acupuncture or *Tanacetum* alone in overall health-related quality of life; strong limitations of this study were the lack of a placebo control, the short follow-up period and the use of self-reported outcome measures (Ferro *et al.* 2015).

A prospective observational study was carried out to ascertain the effect of a combination of coenzyme Q10 10 mg, feverfew 100 mg and magnesium 112.5 mg for migraine prophylaxis. Supplementation significantly decreased the number of days with migraine headache during third month of supplementation compared to baseline phase; the reduction was progressive over the period of supplementation and significant from the first month. The proportion of patients with a reduction of at least 50% in the number of days without migraine headache was 75% after 3 months. These results should be confirmed in a randomized placebo-controlled trial (Guilbot *et al.* 2017). Combination products containing *Tanacetum parthenium* herba, are not part of the assessment

1.2. Search and assessment methodology

This assessment report reviews the scientific literature data available for *T. parthenium* and from the WHO monograph, European Pharmacopoeia monograph, PubMed, EMA library, internet as well as available information on products marketed in the European Community, including pharmaceutical forms, indications, posology and methods of administration.

The keywords "*Tanacetum parthenium*", "Tanaceti herba", "*Chrysanthemum parthenium*", "feverfew", "parthenolide" in all text fields were used.

Clinical studies conducted on the effects of PN or other single active principles were excluded.

For revision of the monograph the same databases were searched in October 2017.

Medical databases: Pubmed

Toxicological databases: Toxnet

Pharmacovigilance resources: EudraVigilance

Data from EU and non-EU regulatory authorities: market overview up to August 2018.

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

Feverfew products are currently available in Europe as traditional herbal medicinal products for the prevention of migraine headaches based on traditional use only. They can be bought without prescription from pharmacies and other outlets. The hard capsules contain as active ingredient powdered feverfew.
Between the early 1970's and 1988, feverfew products held product licenses in the UK. Information on indication and posology was not available as these details for "product licenses of right" are not held on the MHRA electronic database (Table 2).

Table 2. Licensed feverfew HMPs since the early 1970s in the UK (data from MHRA).

Licence no.	Product name	Date granted	Date cancelled
PLR 00250/5674	Liquid Extract Feverfew Herb	Assumed to be the early 1970's	13/02/1977
PLR 00250/5843	Tincture Feverfew	Assumed to be the early 1970's	27/05/1975
PLR 0076/5631	Feverfew Liquid Extract	Assumed to be the early 1970's	28/10/1976
PLR 01252/5448	Chrysanth. Parthen. Liquid Extract	Assumed to be the early 1970's	11/05/1979
PLR 02167/5183	Feverfew Extract	Assumed to be the early 1970's	17/07/1979

In Spain and France, the powdered herbal substance has been registered as a traditional herbal medicinal product for headache from 1991, in form of capsules as a single preparation containing 200 mg of powdered feverfew; in 1997 the single dose was changed to 260 mg/capsule, but the posology remained the same. Starting from 2018 this product is marketed as a food supplement in Spain. In addition, two fixed combinations containing 200 mg of *T. parthenium* and 100 mg of *Anthemis nobilis* or 150 mg of *T. parthenium* and 150 mg of *Artemisia* have been registered as a traditional herbal medicinal product from 1991 to 2009 with the same indication.

In France, feverfew dry extract (solvent: ethanol 30% V/V, DER 4.5-5.5:1) has been authorised since 1994 and the comminuted herbal substance was authorised from 1996 to 2000. These preparations have been authorised as medicinal products traditionally used in the prevention of headaches.

In Denmark, medicinal products containing Tanaceti extracts quantified to PN (0.1-0.2 mg) in form of tablets were on the market from 1990 to 2004 as herbal medicinal products for the prevention of milder forms of migraine, when a doctor has excluded other reasons for the condition (ATC code N02CX).
An extract corresponding to 0.1 mg of PN had been authorised as a medicinal product from 1993 to 2002 and an extract containing 0.2 mg of PN had been authorised from 1997 to 2004. As a fixed combination with *Achillea millefolium* herba and *Populus tremula,* the extract corresponding to 1 mg of PN had been authorised from 1993 to 2000. No further information on the type of the above-mentioned extracts is available. All these products were withdrawn by the Companies and no information about their possible presence on the EU market as food supplements is available.

In Hungary capsules containing 50 mg of a CO_2 extract of feverfewherb (DER not known) quantified to 0.4% PN content were on the market from 1993 to 2005to reduce the frequency of migraine headaches.

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, MemberState)
CO_2 extract (DER not known)	To reduce the frequency of migraine headaches	Capsules 1 capsule containing 50 mg of CO_2 extract two times daily	HU, Herbal medicinal product authorised from 1993 to 2005
Dry extract (DER 5.0-7.2:1), extraction solvent ethanol 30% V/V	Prevention of headaches	Hard capsules 1 capsule containing 200 mg of dry extract 0ne-two times daily	FR, since 1994

Powdered feverfew herb	prophylaxis of migraine headaches	Capsules 1 capsule containing 200 mg of feverfew herb three times daily	Switzerland[1] Herbal medicinal product authorised from 1989 to 2014
Powdered feverfew herb	Prevention of migraine attacks	Capsules 1 capsule containing 200 mg of feverfew herb three times daily	ES, registered as a traditional medicinal product since 1991 up to 1997.
Powdered feverfew herb	Prevention of migraine attacks	Capsules 1 capsule containing 260mg of feverfew herb three times daily	ES registered as a traditional medicinal product 1997 - 2007
Powdered feverfew herb	Prevention of migraine attacks	Capsules 1 capsule containing 260 mg of feverfew herb three times daily	FR, 1997-2014
Powdered feverfew herb (*Tanacetum parthenium* (L.) Schultz Bip.)	prevention of migraine headaches	Hard capsule Adults & elderly: one capsule daily, corresponding to 100 mg of feverfew herb. If symptoms worsen, or persist after 2 weeks, a doctor or qualified healthcare practitioner should be consulted.	UK, 2007, TUR
Powdered feverfew herb (*Tanacetum parthenium* (L.) Schultz Bip.)	prophylaxis of migraine headaches after serious conditions have been excluded by a medical doctor.	1 capsule contains 100 mg of the powdered herbal substance Adults: 1 capsule daily, corresponding to 100 mg of powdered feverfew herb Duration of use: several months; if symptoms worsen or do not improve within 2 months a doctor should be consulted	AT, 2012 TUR

Powdered feverfew herb (*Tanacetum parthenium* (L.) Schultz Bip.)	prophylaxis of migraine headaches after serious conditions have been excluded by a medical doctor.	1 capsule contains 100 mg of the powdered herbal substance Adults: 1 capsule daily, corresponding to 100 mg of powdered feverfew herb Duration of use: several months; if symptoms worsen or do not improve within 2 months a doctor should be consulted	DE, 2015 TUR
Powdered feverfew herb (*Tanacetum parthenium* (L.) Schultz Bip.)	prophylaxis of migraine headaches after serious conditions have been excluded by a medical doctor.	1 capsule contains 100 mg of the powdered herbal substance Adults: 1 capsule daily, corresponding to 100 mg of powdered feverfew herb	SE, 2018 TUR

[1] The products authorized in Switzerland are also authorized in Lichtenstein. According to the Q&A published on the EMA website, Norway, Iceland and Liechtenstein have, through the EEA agreement, adopted "*the complete Union acquis on medicinal products and are consequently parties to the Union procedures. Where in this chapter reference is made to Member States of the Union this should be read to include Norway, Iceland and Liechtenstein*".

Therefore, for the purpose of Article 16c(1)(c) of Directive 2001/83/EC, the Union shall be understood as including all countries of the EEA (including EEA EFTA States). The medicinal use which has taken place on the territory of the EEA States should be taken into account for the purpose of traditional-use registration, irrespective of when they joined the EEA.

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

Feverfew has been described since ancient times as having beneficial medicinal effects and has been recommended for centuries for its medicinal properties.

The herb has also been known under other names such as *Matricaria parthenium* (L.), *Leucanthenum parthenium* (L.) Gren. and Gordon, *Pyrethrum parthenium* (L.) Bernh, *Chrysanthemum parthenium* (L.) Bernh.

The origin of the term *parthenium* is not certain. According to the ancient Greek author Plutarch, following an incident occurred in the 5th century, feverfew was used to save the life of a person fallen from the Parthenon during its construction. Another explanation is based on the Greek word *parthenios* meaning 'virgin', probably because of the reputation of the herb as an antidote for women's ailments (Groenewegen *et al.* 1992).

Feverfew is derived from the Old English name 'febrifuge' from the Latin 'febrifugia', pointing to one of its benefits in reducing fever. In some other European countries this herb is referred to as 'motherherb" for example, 'Mutterkraut' in Germany, indicating its acclaimed beneficial properties in various women's conditions. Other names for feverfew include featherfoil, flirtwort and bachelor's buttons.

The use of feverfew as a medicinal plant can be traced back to the Greek herbal 'Materia Medica' by Dioscorides, and was successively described by Dodoens in 1619, by Gerard in 1636 and Culpeper in 1650.

T. parthenium has a long history of usage in Europe to prevent headache and migraine, for relief in arthritis and for treatment of psoriasis. In recent years, it has become popular also in the United States of America.

Because of all its folk fields of application, feverfew has been long referred to as a 'medieval aspirin'. In 1772 John Hill claimed that 'in the worst headache this herb exceeds whatever else is known' (Heptinstall 1988).

Feverfew has been used for 'intermittent fevers' and for a variety of other conditions and disorders including toothache, rheumatism, psoriasis, insect bites, asthma, stomach ache, menstrual problems and treatment of miscarriage. During the 17th century, it was also used for aiding to stimulate after births and still births, cleansing the kidneys and bladder, strengthening the womb as well as for the treatment of vertigo, spots, wind, colic, and for the treatment of disturbances due to the excessive use of opium. Other uses recommended in the ancient times were the alleviation of St. Antoine's fire, inflammatory processes and hot swellings (Knight 1995).

Today's use started in late 1970s when the British press reported that a group of migraine sufferers from Wales had found relief from attacks after taking the leaves of the plant for some time. Studies carried out reported that patients were successfully using the herb in the prophylaxis of both migraine and arthritis (Knight 1995). Thus the interest in feverfew grew quickly, bringing it back into the limelight from obscurity since the Middle Ages.

In addition to migraine, feverfew was also tried in many other conditions and it has now been claimed (but not scientifically proven) to be effective in arthritis, psoriasis and stress, among others (Groenewegen *et al.* 1992).

Ancient uses of feverfew may be categorized broadly into three main groups:
1. treatment for fever, headache and migraine;
2. women's conditions such as difficulties in labour, threatened miscarriage, and regulation of menstruation;
3. relief of stomach ache, toothache and insect bites (Groenewegen et al. 1992).

Feverfew is used mainly for migraine, arthritis, rheumatic diseases and allergies. It has also been used in the treatment of tinnitus, vertigo, fever, difficult labour, toothache, insect bites and asthma.
In folk medicine, feverfew has been used for cramps, as a tonic, a stimulant, a digestive substance, blood detoxicant, migraine prophylaxis, intestinal parasites and gynecological disorder. The herb is also used as a wash for inflammation and wounds, as a tranquilizer, an antiseptic and as a mouthwash following tooth extraction. It is used externally as an antiseptic and insecticide.

The herbal infusion has been used for dysmenorrhea. In postpartum care, it has been used to reduce bleeding (lochia) (PDR monographs 2004).

The uterine stimulant effect may explain the folk uses of the plant as abortifacient, emmenagogue and in certain labour difficulties but conflicts with the folk use of the drug in threatened miscarriage (Rateb et al. 2007). This contradictory information supports the common warning of the producers not recommending the use of feverfew during pregnancy.

The use in the prophylaxis of migraine is documented in the British Herbal Pharmacopoeia (1990), but no information is available on the herbal preparation and relevant dose.

The use of feverfew herb with average daily doses of 100 mg of dried leaf for the management of migraine is documented in literature (Pugh 1985).

Table 2: Overview of historical data

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength (where relevant) Posology Duration of use	Reference
Leaf (dried)	Migraine	100 mg daily	Pugh 1985
- Leaf (fresh) - Leaf (dried)	Migraine	2-3 small leaves daily Tablets/capsules containing 50-250 mg of dried leaves. Posology not stated	Heptinstall 1988
- Leaf (fresh)	migraine vertigo	2.5 leaves daily equivalent to about 125	Barnes 2007

	tinnitus	mg of dried herb daily	
	arthritis		
	fever	50 mg daily	
- Leaf (freeze-dried)	menstrual disorders		
	difficulty during labour	50-200 mg daily, equivalent to 0.2 – 0.6 mg PN daily	
- Aerial parts (dried)	stomach ache		
	toothache		
	insect bite		
- Powdered herb	Prophylaxis of migraine	50 – 120 mg daily in adults	ESCOP 2014
- Supercritical CO_2 extract		18.75 mg daily in adults corresponding to 3 g fresh feverfew	

2.3. Overall conclusions on medicinal use

Table 3: Overview of evidence on period of medicinal use

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, MemberState)
Powdered feverfew herb	prophylaxis of migraine headaches	Capsules 1 capsule containing 200 mg of feverfew herb three times daily	Switzerland Herbal medicinal product authorised from 1989 to 2014
Powdered feverfew herb	Prevention of migraine attacks	Capsules 1 capsule containing 200mg of feverfew herb three times daily	ES, registered as a traditional medicinal product since 1991 up to 2007. Note: since 1997, the posology has been changed to 1 capsule containing 260mg of feverfew herb three times daily
Powdered feverfew herb (*Tanacetum parthenium* (L.) Schultz	prevention of migraine headaches	Hard capsule	UK, AT, DE, SE, since 2007, TU

Bip.)		Adults & elderly: one capsule daily, corresponding to 100 mg of feverfew herb	

There is evidence from literature that *Tanacetum Parthenium* has been used for migraine in Europe since late 1970s, whereas first clinical studies investigating the efficacy of feverfew in the prophylaxis of migraine started in mid '80s. Feverfew is currently marketed in several EU countries for the prevention of migraine as hard capsules containing 100 mg of powdered herb to be taken once daily. This posology was already included in the first version of the HMPC monograph published in 2011, and for consistency, it has been retained also in the revised monograph.

Tanacetum Parthenium has been authorized as herbal medicinal product in Switzerland for the prophylaxis of migraine as capsules containing 200 mg of powdered herb to be taken three times daily from 1989 to 2014. The same product has been also registered in France and Spain from 1991 to 2007; however, since 1997 the posology has been changed to one capsule containing 260mg of feverfew herb three times daily. This product is no more marketed as a registered herbal medicinal product in these countries, but it is still marketed in Spain as a food supplement. Therefore, considering the limited number of reports existing for *Tanacetum Parthenium* preparations, as verified in EudraVigilance database, there is no safety issue for the posology of 200 mg trice a day.

Therefore, it can be concluded that there is evidence of traditional use for feverfew in the prevention of migraine when used in capsules containing 100 mg of powdered herb to be taken one time daily or 200 mg of feverfew herb to be taken three times daily (total daily dose 100-600 mg).

The daily dosage of 100 mg may be increased until obtaining an effect, not exceeding the daily dose of 600 mg.

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

Studies on parthenolide (PN)

Although migraine is a complex neurovascular disorder, serotonin based mechanisms are central to its pathophysiology. Antimigraine drugs interact predominantly with receptors of 5-HT1 and 5-HT2 classes. 5-HT2B and 5-HT2A receptor antagonists such as methylsergide, cyproheptadine, and mianserin have been shown to be effective in migraine prophylaxis. PN at a concentration of 1×10^{-5} mol/L was observed to be an inhibitor of neuronal release of 5-HT but without any significant direct effect on 5-HT2B and 5-HT2A receptor sites in both rat fundus, and ileum when the tissues were incubated for 30 min. Increasing the incubation time to 1.5 h resulted in a potent inhibition of both responses to exogenous 5-HT and neuronal release of 5-HT via d-fenfluramine. At a higher concentration (5×10^{-5} mol/L), PN followed a similar trend as with 30-min incubation but its antiserotonergic effect was much more striking when a 1.5-h incubation period was provided. The above results indicate that the antagonism at the 5-HT receptor sites is very slow (Mittra *et al.* 2000).

In another report by Bejar *et al.* (1996), cumulative concentration-response curves to 5-HT and the indirect-acting serotonergics fenfluramine (F) and dextroamphetamine (DA) on fundus were obtained

in the presence and absence of 1 x 10^{-6} to 1 x 10^{-5} mol/L PN. The isolated rat stomach fundus preparation, a sensitive bioassay to evaluate serotonin-(5-HT) like activity, was used as a model to study the effects of PN on 5-HT storage, release and stimulation of the 5-HT2B receptor. PN did not show agonist effects nor antagonism toward 5-HT on rat fundus at all concentrations used. However, PN antagonized non-competitively the effects of F and DA.

The nitric oxide (NO) donor glyceryl trinitrate (GTN) provokes delayed migraine attacks when infused into migraineurs and also causes inducible nitric oxide synthase (iNOS) expression and delayed inflammation within rodent dura mater.
Sodium nitroprusside, a NO donor as well, also increases iNOS expression. As inflammation and iNOS are potential therapeutic targets, Reuter *et al.* (2002) examined transcriptional regulation of iNOS following GTN infusion and the consequences of its inhibition within dura mater. They show that intravenous GTN increases NO production within macrophages, iNOS expression is preceded by significant nuclear factor kappa B (NF-κB) activity, as reflected by a reduction in the inhibitory protein IkBα and activation of NF-κB after GTN infusion. IκBα degradation, NF-κB activation and iNOS expression were attenuated by PN (3 mg/kg).

The anti-inflammatory properties of PN were investigated *in vitro* in cultured lipopolysaccharide (LPS)-stimulated BV-2 mouse microglia. Pre-incubation of the cells (1 h) with PN (200 nM, 1 μM and 5 μM) reduced the secretion of interleukine-6 (IL-6); the effect was comparable to that of indometacine and it was dose-dependent. In the range of concentrations tested, PN only reduced LPS-stimulated tumor necrosis factor alpha (TNF-α) secretion at 5 μM, the highest concentration. Western blotting analysis of cytoplasmic and nuclear extracts showed that PN reduced the translocation in the nucleus of the 52-kDa NF-kB subunit stimulated by LPS. It was suggested that PN covalently interacts with the IκB kinase (IKK) complex, specifically targeting its IKKβ subunit, thus protecting the IκB subunit from phosphorylation and subsequent proteasome-mediated degradation induced by the inflammatory stimulus of LPS. The reduction of microglial activation by inhibition of pro-inflammatory agents may help attenuate the onset and intensity of acute migraine attacks (Magni *et al.* 2012).

The release of the sensory neuropeptide calcitonin gene-related peptide (CGRP) within the trigeminovascular system is also considered involved in the genesis of migraine headaches. It is suggested that CGRP can produce a meningeal vasodilatation via the transient receptor potential ankyrin 1 (TRPA1). To verify if PN targets TRPA1, Materazzi *et al.* 2013 exposed human embryonic kidney (HEK293) cells transfected with the cDNA of human TRPA1 to PN (30–1000 μM), which produced a concentration-dependent increase in intracellular calcium, indicating a stimulatory action. This effect of PN was absent in HEK293 untransfected cells. In addition, the calcium response evoked by PN was abolished by the selective TRPA1 antagonist, HC-030031. Next, the authors examined the effect of PN in cultured rat terminal ganglia (TG) neurons. PN increased intracellular calcium, and the effect was concentration-dependent, and abrogated by HC-030031, but was unaffected by the TRPV1 antagonist, capsazepine. PN, similarly to the selective TRPA1 agonist, allyl isothiocyanate (AITC) 30 μM, produced a measurable calcium response in about 40% of TG neurons isolated from $Trpa1^{+/+}$ mice, an effect that was completely absent in TG neurons isolated from or $Trpa1^{-/-}$ mice. In contrast, calcium response to capsaicin was unchanged in neurons from both $Trpa1^{+/+}$ and $Trpa1^{-/-}$ mice (Fig. 1G). All together, these findings indicate selectivity of PN for the TRPA1 channel. Furthermore, in rat trigeminal neurons PN showed, in addition to a lower potency, a much-reduced efficacy than that of AITC, thus indicating that it behaves as a low potency partial agonist at the TRPA1 channel (Materazzi *et al.* 2013).

The same authors investigated the ability of PN to affect the release of CGRP from both central and peripheral endings of primary sensory neurons (rat spinal cord, trigeminal ganglia and dura mater, and mouse spinal cord). Tissues were stimulated with PN (10–30–100 μM) or its vehicle (1% DMSO) dissolved in modified Krebs solution. Some tissues were pre-exposed to capsaicin (10 μM, 20 min) or

superfused with a calcium-free buffer containing EDTA (1 mM). PN increased the outflow of CGRP-Like Immunoreactivity (CGRP-LI) assay from rat spinal cord, an effect prevented by pre-exposure to a high concentration of capsaicin and in a calcium-free medium. In addition, pre-exposure to an elevated concentration of PN (300 µM, 30 min) prevented PN-evoked CGRP-LI release from rat spinal cord, indicating self-desensitization. Similar results were obtained in slices of rat TG or dura mater. Exposure to PN increased CGRP-LI outflow from dorsal spinal cord slices obtained from $Trpa1^{+/+}$ mice, an effect that was completely absent in tissues taken from $Trpa1^{-/-}$ mice. Thus, PN acts as a TRPA1 partial agonist, causing selective channel desensitization and a non-selective defunctionalization of CGRP-containing sensory neurons (Materazzi *et al.* 2013).

Studies on other single constituents

Parthenolide, michefuscalide and chrysanthenyl acetate, sequiterpenes isolated from feverfew leaves, inhibited the in vitro conversion of arachidonic acid to prostaglandins by the prostaglandin synthetase enzyme system. The IC_{50} for PGE_2 production for PN, michefuscalide and chrysanthenyl acetate were 11.0 ± 0.44 µM, 12.1 ± 0.51 µM and 14.2 ± 0.58 µM, respectively (Pugh WJ & Sambo K 1988).

Studies on extracts

Feverfew inhibits human blood platelet aggregation and secretion induced by a number of agents *in vitro* and this may be related to the beneficial effects in migraine. The inhibitory activity of PN was compared with that of crude feverfew leaves extract by Groenewegen & Heptinstall (1990). The effects of both on [14C]5-HT secretion from platelets and on platelet aggregation induced by a number of different stimulants were determined. The activating agents studied included the phorbol ester phorbol myristate acetate, ADP, arachidonic acid, collagen, the thromboxane mimetic U46619, the calcium ionophore A23187, the diacylglycerol analogue 1-oleoyl-2-acetylglycerol (OAG) and adrenaline. Powdered leaves were stirred with chloroform (feverfew, 50 mg/ml chloroform) for 30 min. The dried extract was re-suspended in an equal volume of PBS (phosphate-buffered saline) to obtain a final solution concentration 50 mg/ml. The results show that there are similarities between the effects of feverfew extract and of PN on both [14C]5-HT secretion and platelet aggregation, which is consistent with the effects of feverfew extract on platelets caused by PN or similar compounds in the extract (Groenewegen & Heptinstall 1990).

To study the mechanism of antimigraine activity of *T. parthenium*, its extracts and PN were tested for their effects on 5-HT storage and release, and stimulation of 5-HT2B and 5-HT2A receptors. Dichloromethane feverfew extracts (containing $1x10^{-5}$ mol/L PN and a number of other mono- and sesquiterpenes) showed a potent inhibition of neuronally released 5-HT via d-fenfluramine. In rat fundus and ileum incubated with the extract for 30 min, the 5-HT2B and 5-HT2A receptors were blocked in a manner similar to cyproheptadine (a predominantly 5-HT2B receptor blocker) and risperidone (a 5-HT2A/2C receptor blocker) (Mittra *et al.* 2000).ù

Tassorelli *et al.* (2005) studied the biological effects of different *T. parthenium* extracts and purified PN in an animal model of migraine based on the quantification of neuronal activation induced by nitroglycerin in the rat brain. The methanolic extract enriched in PN (HPLC content 26%) significantly reduced nitroglycerin-induced Fos expression in the nucleus trigeminalis caudalis. Purified PN at a dose of 15 mg/ kg i.p. inhibited nitroglycerin induced neuronal activation in additional brain nuclei and significantly, the activity of nuclear factor κB. The authors concluded that PN proved able to interfere with the activation of a key area in migraine pathogenesis, nucleus trigeminalis caudalis.

Chen & Leung (2007) tested three different feverfew extracts to check possible differences in gene response of human monocytes. The standard reference extract (SRE) was obtained by extraction of 2 g

of dried leaf powder with 20 ml of 90% ethanol under mild conditions (sonication for 30 min with no evaporation or exposure to the air). A carbon dioxide supercritical fluid extract was prepared under conditions giving a spectrum of components similar in ratio of concentration to the SRE which contains most of the volatile components of feverfew including camphor, chrysantenyl acetate and PN. A negative control extract was prepared with the same extraction solvent and DER but under stress condition (extraction or 19 days, moderate heat up to 50°C and evaporation). Both, the standard reference ethanolic extract and the carbon dioxide supercritical fluid extract of feverfew exhibited blockade on lipopolysaccharide-mediated TNF-α release. Extracts effectively suppressed also CCL2 (also known as monocyte chemoattractant protein I, MCP-I), suggesting that CCL2 is a potential cellular target for feverfew's antimigraine effects.

Pro-inflammatory mediators such as TNF-α and cyclooxygenase-2 are involved in the NO-mediated cascade of migraine pathogenesis and pathophysiology. TNF-α is a pro-inflammatory cytokine that is induced by LPS through the NF-kB-IkB signaling pathway. The antimigraine mechanism of feverfew supercritical CO_2 extract, composed of sesquiterpene lactones mainly, 15.6% PN and very small amounts of santamarin and reynosin, was investigated *in vitro* using the mouse macrophage cell line (RAW 264.7). Mouse macrophage cells were treated with LPS in the presence and absence of feverfew extracts up to doses of 50 µg/mL. The feverfew extract inhibited both nitric oxide (NO) and TNF-α production in a dose-dependent manner with complete inhibition of NO occurring at 5 µg/mL of feverfew extract (Aviram *et al.* 2012).

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

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Preparations which are not included in the monograph				
Dichloromethane feverfew extracts (containing 1x10^{-5} mol/L PN and a number of other mono- and sesquiterpenes)		*In vitro*: rat fundus, and ileum tissues	Mittra *et al.* 2000	Feverfew extract showed an inhibition of neuronally released 5-HT via d-fenfluramine; the effect of blockage of 5-HT2B and 5-HT2A receptors was similar compared to those of cyproheptadine and risperidone
Methanolic feverfew extract and purified PN	Extract: 100 mg/kg i.p. Purified PN: 15	*In vivo*: rats	Tassorelli *et al.* 2005	The extract significantly reduced nitroglycerin-

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
	mg/kg i.p.			induced Fos expression in the nucleus trigeminalis caudalis. Purified PN inhibited nitroglycerin induced neuronal activation in additional brain nuclei
Ethanolic feverfew and feverfew supercritical CO_2 extracts	0.01% w/v feverfew extracts	*In vitro*: human monocytic cell line THP-1	Chen & Leung 2007	The extracts of feverfew exhibited blockade on LPS-mediated TNF-α release. Extracts effectively suppressed also CCL2.
Feverfew supercritical CO_2 extract, composed of sesquiterpene lactones mainly, 15.6% PN and very small amounts of santamarin and reynosin	Up to concentrations of 50 µg/ml feverfew extract	*In vitro*: mouse macrophage cell line (RAW 264.7)	Aviram *et al.* 2012	Feverfew extract inhibited both nitric oxide (NO) and TNF-α production in a dose-dependent manner with complete inhibition of NO occurring at 5 µg/mL of feverfew extract
Isolated compound				
parthenolide	1×10^{-5} mol/L and 5×10^{-5} mol/L	*In vitro*: rat fundus, and ileum tissues	Mittra *et al.* 2000	PN has an antiserotonergic effect (inhibition on 5-HT receptor)
	From 1×10^{-6} mol/L to 1×10^{-5} mol/L	*In vitro*: rat fundus tissue	Bejar *et al.* 1996	PN did not show agonist effects nor antagonism toward 5-HT; PN antagonized non-competitively the effects of F and DA
	3 mg/kg	*In vitro*: rodent dura	Reuter *et al.* 2002	PN reduces IκBα degradation,

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
		mater		NF-κB activation and iNOS expression promoted by glyceryl trinitrate
	200 nM, 1 µM and 5 µM	*In vitro*: BV-2 mouse microglia stimulated by LPS	Magni *et al.* 2012	PN reduced the secretion of IL-6 and TNF-α secretion
	30-1000 µM	*In vitro*: hTRPA1-human embryonic kidney (HEK293) cells	Materazzi *et al.* 2012	PN provokes a concentration-dependent increase in intracellular calcium acting as a TRPA1 partial agonist; PN causes selective channel desensitization and a non-selective defunctionalization of CGRP-containing sensory neurons.
	10–30–100 µM	*In vitro*: rat spinal cord, trigeminal ganglia and dura mater, and mouse spinal cord	Materazzi *et al.*2013	
	300 µM	*In vitro*: rat spinal cord, trigeminal ganglia and dura mater	Materazzi *et al.*2013	

3.1.2. Secondary pharmacodynamics

Studies on parthenolide

Citotoxic activity

PN has been shown to interfere with the cell cycle or to promote cell differentiation and finally to induce programmed cell death. Recent advances in molecular biology indicate that this sesquiterpene lactone might evoke the above-mentioned effects by indirect action on genes. In particular, it has been shown that PN inhibits NF-κB- and signal transducers and activators of transcription (STATs)-mediated anti-apoptotic gene transcription. The pro-apoptotic activity of PN seems to be associated with stimulation of the intrinsic apoptotic pathway with the higher level of intracellular reactive oxygen substances (ROS) and modifications of B-cell lymphoma 2(Bcl-2) family proteins (conformational

changes of Bak and Bax, Bid cleavage). On the other hand, PN also amplifies the apoptotic signal through the sensitization of cancer cells to extrinsic apoptosis, induced by TNF-α. These properties suggest that PN could be further studied as a metabolic inhibitor to retard tumorigenesis and to suppress tumor growth (Pajak *et al.* 2008).

Glioblastomas are difficult to treat and frequently aggressive and fatal. Treatment of glioblastoma cells with PN resulted in rapid apoptosis through caspase 3/7 without a suppression of NF-κB activity (Anderson & Bejcek 2008).

PN inhibits cell growth irreversibly at concentrations above 5.0 μM and an exposure time of 24 h. At lower concentrations the effect is reversible; PN acted as a cytostatic over multiple cell generations for mouse fibrosarcoma (MN-ll) and human lymphoma (TK6) cell lines (Ross *et al.* 1999).

Transcription factors such as NF-κB provide targets for drugs to use in the treatment of cancer. PN, inhibitor of NF-κB activity, markedly increases the degree of human leukaemia HL-60 cell differentiation into monocytes via the inhibition of NF-κB activity and evidence has been provided that inhibition of NF-κB activation can be a pre-requisite to the efficient entry of promyelocytic leukaemia cells into a differentiation pathway (Kang *et al.*2002).

Won *et al.* examined the cancer chemopreventive property of PN using a combination of *in vivo* and *in vitro* approaches. First, the anticancer effect of PN in an UVB-induced skin cancer model was tested. Mice fed with PN (1 mg/day) showed a delayed onset of papilloma incidence, a significant reduction in papilloma multiplicity (papilloma/mouse) and sizes when compared with the UVB-only group. The molecular mechanism(s) involved in its anticancer effects using cultured JB6 murine epidermal cells were next investigated. Non-cytotoxic concentrations of PN significantly inhibited UVB-induced activator protein-1 DNA binding and transcriptional activity. In addition, PN pre-treatment also inhibited c-Jun-N-terminal kinase (JNK) and p38 kinase activation. Impaired activated protein 1 (AP-1), JNK and p38 signalling led to the sensitization of JB6 cells to UVB-induced apoptosis. These data confirmed the anticancer property of PN in an animal model and provided evidence that the inhibitory effects on AP-1 and mitogen-activated protein kinases could serve as one of the underlying mechanisms for the cancer chemopreventive property of PN (Won 2004).

In another study it was observed that PN is able to induce robust apoptosis in primary human acute myeloid leukemia (AML) cells and blast crisis chronic myeloid leukaemia (CML) cells while sparing normal hematopoietic cells. Furthermore, analysis of progenitor cells using *in vitro* colony assays as well as stem cells using the non-obese diabetic/severe combined immunodeficient xenograft model showed, that PN also preferentially targets AML progenitor and stem cell populations. Notably, in comparison to the standard chemotherapy drug cytosine arabinoside (Ara-C), it was observed that PN is much more specific to leukemia cells. The molecular mechanism of PN mediated apoptosis is associated with inhibition of nuclear factor KB (NF-κB), pro-apoptotic activation of p53, and increased reactive oxygen species (ROS). Based on these findings, the authors proposed that the activity of PN triggers leukemia stem cells (LSC)-specific apoptosis (Guzman *et al.* 2005).

To investigate PN anticancer activity in ultraviolet B (UVB)-induced skin cancer in SKH-1 hairless mice, the role of protein kinase C (PKC; the subtypes novel PKCd and atypical PKCz) in the sensitization

activity of PN on UVB-induced apoptosis has been studied. The results have demonstrated that PN sensitizes UVB-induced apoptosis via PKC-dependent pathways (Won *et al.* 2005).

The effects of PN induced apoptosis in pre-B acute lymphoblastic leukemia (ALL) lines, including cells carrying the t(4; 11) (q21; q23) chromosomal translocation were investigated. PN induced rapid apoptotic cell death distinguished by loss of nuclear DNA, externalization of cell membrane phosphatidylserine and depolarization of mitochondrial membranes at concentrations ranging from 5 to 100 µM. Using reactive oxygen species (ROS)-specific dyes, an increase in nitric oxide and superoxide anion was detected in the cells by 4 h after exposure to PN. Parthenolide-induced elevation of hypochlorite anion was observed only in the two (4; 11) lines (Zunino *et al.* 2007).

Parada-Turska *et al.* (2007) determined the effect of PN on proliferation of three human cancer cell lines: human lung carcinoma (A549), human medulloblastoma (TE671), human colon adenocarcinoma (HT-29) and human umbilical vein endothelial cells (HUVEC) *in vitro*. Cell proliferation was assessed by means of 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) assay. PN inhibited proliferation of all three types of cancer cells (A549, TE671, HT-29) and HUVEC with the following IC50 values (in µM): 4.3, 6.5, 7.0 and 2.8 respectively.

The inhibitory activity of PN and golden feverfew extract against two human breast cancer cell lines (Hs60ST and MCF-7) and one human cervical cancer cell line (SiHa) was determined *in vitro*. Feverfew ethanolic extract inhibited the growth of all three types of cancer cells with a half-effective concentration (EC50) of 1.5 mg/ml against Hs605T, 2.1 mg/ml against Michigan Cancer Foundation-7 (MCF-7) cell line, and 0.6 mg/ml against SiHa. Among the tested constituents of feverfew (i.e., PN, camphor, luteolin and apigenin), PN showed the highest inhibitory effect with an EC50 against Hs605T, MCF-7 and SiHa of 2.6 µg/ml, 2.8 µg/ml and 2.7 µg/ml, respectively. Interactions between PN and flavonoids (apigenin and luteolin) in feverfew extract were also investigated to elucidate possible synergistic or antagonistic effects. The results revealed that apigenin and luteolin might have moderate to weak synergistic effects with PN on the inhibition of cancer cell growth of Hs605T, MCF-7 and SiHa (Wu *et al.* 2006b).

The inhibitory activity of PN on breast cancer cell proliferation (cell lines MCF-7 and triple negative breast cancer cell line MDA-MB-231) was confirmed later by Wyrebska *et al.* 2013 who observed a mean IC_{50} of 9.5 µM and 7.7 in MCF-7 and MDA MB-231, respectively. The IC_{50} was determined using two assays, a cell viability assay (MTT) and the sulphorhodamine cytotoxicity assay, following incubation up to 48 hours after the addition of various concentrations of PN. A concentration-dependent inhibition of cell proliferation following 24 h incubation with PN was also observed with [3H]Thymidine incorporation assay on both cell lines. A pro-apoptotic mechanism was suggested as a basis for cytotoxicity (Wyrebska *et al.* 2013)

The inhibitory activity of PN against a human breast cancer cell line (MCF-7) and a human cervical cancer cell line (SiHa) has been further investigated *in vitro*. The cytotoxic activity of PN (3.5–21 mM) was examined by MTT and lactate dehydrogenase enzyme (LDH) leakage assays at 24 and 48 h time intervals. Apoptotic activity was evaluated by expression analysis of multiple apoptosis-regulatory genes (i.e., p53, Bcl-2, Bax, caspase-3, -6, and -9) by reverse transcriptase-PCR and DNA fragmentation assay. PN inhibited the growth of SiHa and MCF-7 cell lines in a concentration-dependent manner; the IC_{50} values of PN against SiHa and MCF-7 cells were 8.42 ± 0.76 and 9.54 ± 0.82 µM, respectively.PN-treated cells showed up-regulation of p53, Bax, caspase-3, -6, and -3 genes

and down-regulation of Bcl-2 gene; also, the fragmented genomic DNA in parthenolide-treated cells showed the signs of apoptosis (Al-Fatlawi *et al.* 2015).

The findings obtained by Al-Flatawi *et al.* were confirmed in a recent *in-vitro* study that investigated the pro-apoptotic effect of PN in SW620 cells, representative human colorectal cancer (CRC) cells. The MTT assay showed that PN significantly reduced the viability of CRC cells with a dose-dependent effect (tested concentrations of PN were 5, 10, 20 or 40 µM). The Transwell assay showed that PN inhibited cell migration in a concentration dependent manner; the quantification of migrating cells by crystal violet staining demonstrated the 20 µM PN significantly inhibited the migratory ability of SW620 cells by approximately 80%. Furthermore, 20 µM PN significantly inhibited the invasive ability of SW620 cells by approximately 80% as determined by Matrigel invasion assay. Finally, Western blotting showed that PN markedly suppressed migration/invasion related protein expression; PN also inhibited the expression of antiapoptotic proteins (Bcl-2 and Bcl-xL) and activated apoptosis terminal factor (caspase-3) in a dose-dependent manner (Yu Chuan *et al.* 2017)

A suppressive effect on angiogenesis has been suggested by SE-Lim *et al.* who investigated the effects and potential mechanisms of action of PN on angiogenesis in human colorectal cancer (CRC) *in vitro*. The authors used cultured human umbilical vein endothelial cells (HUVECs) and the human CRC cell lines, HT-29, SW620 and HCT116. PN markedly inhibited vascular cell migration and capillary-like structure formation even at a dose which had not effects on cell viability. PN also suppressed the expression of angiogenic biomarker proteins [vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR)1 and VEGFR2] in both the HUVECs and CRC cells. Additionally, PN at a dose of 4 mg/kg effectively inhibited tumor neovascularization *in vivo* in a HT-29 xenograft model (Se-Lim *et al.*2014).

Recently, the chemotherapeutic properties of PN were attributed to its impact on epigenetic mechanisms, which are frequently altered in cancer. Cancer cells express elevated histone deacetylase 1 (HDAC1) activity and are more sensitive to the actions of HDAC inhibitors than are normal cells. PN is the first example of a small molecule that specifically depletes HDAC1 proteins without affecting other class I/II HDACs in several types of tumor cell. In fact, PN causes proteasomal-mediated degradation of HDAC1 and modulates histone structure specifically at the p21 promoter, leading to increased transcription of this gene and p21-mediated cell death (Ghantous *et al.* 2013).

Another epigenetic role for PN in cancer is its ability to alter DNA methylation. PN induces global DNA hypomethylation *in vitro* and *in vivo* by specifically inhibiting DNA methyltransferase 1 (DNMT1) in myeloid leukemias and skin cancer. Furthermore, PN decreases promoter methylation and, thus, reactivation, of the tumor suppressor high in normal-1 (*HIN-1*) gene (Ghantous *et al.* 2013).

Anti-inflammatory activity

The protein tyrosine kinase (PTK) inhibitors radicicol and herbimycin A inhibit the expression of the mitogen-inducible cyclooxygenase (COX-2) and proinflammatory cytokines. Radicicol and herbimycin A possess polarized double bonds which can conjugate sulphydryl groups of proteins. PN contains α-methylene-gamma-lactone (MGL) and an epoxide in its structure. These moieties can interact with biological nucleophiles such as a sulfhydryl group. Hwang et al. (1996) showed that PN inhibits the expression of COX-2 and pro-inflammatory cytokines (TNFα and IL-1) in lipopolysaccharide (LPS)-stimulated macrophages. The structure-function relationship indicates that the MGL moiety confers the inhibitory effect. PN suppressed LPS-stimulated protein tyrosine phosphorylation in the murine macrophage cell line (RAW 264.7). This suppression was correlated with its inhibitory effect on the expression of COX-2 and the cytokines (Hwang *et al.* 1996).

Excessive nitric oxide production by inducible nitric oxide synthase (iNOS) in stimulated inflammatory cells is thought to be a causative factor of cellular injury in inflammatory disease states. Compounds

inhibiting iNOS transcriptional activity in inflammatory cells are potentially anti-inflammatory. It has been demonstrated that PN exerts potent dose-dependent inhibitory effects on the promoter activity of the iNOS gene in THP-1 cells. PN suppressed iNOS promoter activity at concentrations higher than 2.5 mM, with an IC_{50} of about 10 mM. A tumor-promoting phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), significantly increased the iNOS promoter-dependent reporter gene activity, and the TPA-induced increase in iNOS promoter activity was effectively suppressed by PN, with an IC_{50} of approximately 2 mM. These findings may further explain the anti-inflammatory property of PN (Fukuda *et al.* 2000).

In order to identify the molecular mechanisms of parthenolide's anti-inflammatory activity, a PN affinity reagent was synthesized by Kwok *et al.* (2001) and shown to bind directly to and inhibit IUB kinase L (IKKL), the kinase subunit known to play a critical role in cytokine-mediated signaling. Mutation of cysteine 179 in the activation loop of IKKL abolished sensitivity towards PN. Moreover, the authors showed that parthenolide's *in vitro* and *in vivo* anti-inflammatory activity is mediated through the K-methylene Q-lactone moiety shared by other sesquiterpene lactones. According to the authors, PN targets this kinase complex providing a possible molecular basis for the anti-inflammatory properties (Kwok *et al.*2001).

The decrease of NF-κB activity induced by PN has been also demonstrated *in vitro* by Zhang *et al.* 2014 who investigated the effects of PN on the inflammatory and osteoclastogenic response of human periodontal ligament-derived cells (hPDLCs) and revealed the signaling pathways in this process. hPDLCs are a vital cell type involved in periodontitis as these cells maintain the homeostasis of periodontal tissues and regulate bone formation and resorption. Lipopolysaccharide (LPS), a key pathogenic component of gram-negative bacteria, can stimulate the production of pro-inflammatory cytokines and osteoclastogenic-related cytokines from hPDLCs. PN decreased NF-κB activation, I-κB degradation, and extracellular signal-regulated kinases (ERK) activation in hPDLCs. PN significantly reduced the expression of inflammatory (IL-1β, IL-6, and TNF-αand osteoclastogenic (receptor activator of nuclear factor kappa B ligand, osteoprotegerin, and Macrophage colony stimulating factor) genes in LPS-stimulated hPDLCs (Zhang *et al.* 2014).

Pharmacological control of interleukin-12 (IL-12) production may be a key therapeutic strategy for modulating immunological diseases dominated by type-1 cytokine responses. Kang *et al.* (2001) showed that PN potently inhibited the lipopolysaccharide-induced IL-12 production in a dose-dependent manner. The authors suggested that PN-induced inhibition of IL-12 production in macrophages may explain some of the biological effects of PN including its anti-inflammatory activity.

The massive hyperplasia of synovial fibroblasts is one of the most striking features of rheumatoid arthritis. The effect of PN on the proliferation of rabbit synoviocytes cell line HIG-82, rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) and human skin fibroblasts (HSF) *in vitro* was investigated. Cell proliferation was assessed by means of 3-(4.5- dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide and 5'-bromo-2'-deoxy-uridine methods. PN inhibited proliferation of HIG-82 and human RA-FLS, whereas the proliferation of HSF was inhibited less effectively (Parada-Turska *et al.* 2008).

Antimicrobial activity

PN showed significant activity against the promastigote form of *L. amazonensis* with 50% inhibition of cell growth at a concentration of 0.37 µg/ml. For the intracellular amastigote form, PN reduced by 50% the survival index of parasites in macrophages when it was used at 0.81 µg/ml. The purified compound

showed no cytotoxic effects against J774G8 macrophages in culture and did not cause lysis in sheep blood when it was used at higher concentrations that inhibited promastigote forms. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis with gelatin as the substrate showed that the enzymatic activity of the enzyme cysteine protease increased following treatment of the promastigotes with the isolated compound. This finding was correlated with marked morphological changes induced by PN, such as the appearance of structures similar to large lysosomes and intense exocytic activity in the region of the flagellar pocket, as seen by electron microscopy. These results provided new perspectives on the development of leishmanicidal activities of PN (Tiuman *et al.* 2005).

Antioxidant activity

A study was performed to investigate the protective effect of PN against oxidative stress-induced apoptosis of human lens epithelial (HLE) cells and the possible molecular mechanisms involved. HLE cells (SRA01-04) were incubated with 50 µM H_2O_2 in the absence or presence of different doses of PN (10, 20 and 50 µM). The expression of caspase-3 and caspase-9 induced by H_2O_2 in HLE cells was significantly reduced by PN both at the protein and mRNA levels, and the activation of caspase-3 and caspase-9 was also suppressed by PN in a dose-dependent manner. The authors concluded that PN prevents HLE cells from oxidative stress-induced apoptosis through inhibition of the activation of caspase-3 and caspase-9, suggesting a potential protective effect against cataract formation (Yao *et al.* 2007).

Effects on smooth muscle contractility

Extracts of feverfew (details unkown) and PN inhibit smooth muscle contractility in a time-dependent, non-specific and irreversible manner. The hypothesis that this toxic effect is caused by the presence of the potentially reactive α-methylene function in the sesquiterpene lactone was tested on rabbit isolated aortic ring preparations. The α-methylene functions in PN were chemically inactivated by reaction with cysteine.
The results showed the characteristic smooth muscle inhibitory profile for PN but not for the compound lacking this functional group or by cysteine inactivated compounds. Thus, the α-methylene function is critical for this aspect of the toxic pharmacological profile of the sesquiterpene butyrolactones (*Hay et al.* 1994).

Studies on extracts

Extracts of *T. parthenium* (L.) showed antimicrobial, analgesic, anti-inflammatory, antipyretic, antispasmodic, antithrombotic, antioxidant and uterine-stimulant activities in addition to the *in vitro* cytotoxic effects. The effect of extracts on vessels and smooth muscle cells has also been studied.

Anti-inflammatory, analgesic and antipyretic activities

The alcoholic extracts of flowers and leaves (details unknown) and PN showed significant analgesic, anti-inflammatory and antipyretic activities which confirmed the folk use of feverfew herb for treatment of headache, fever, common cold and arthritis. These effects are attributed to the sesquiterpene lactones and flavonoids present in the leaves and/or flowers (Milbrodt *et al.* 1997). The roots showed no or mild biological activities due to the absence of sesquiterpene lactones and flavonoids; thus confirming the hypothesis of these compounds as active constituents.

Two ethanolic dry extract obtained by feverfew flowers (DER 4.5:1) and leaves (3.6:1) were tested in mice after acute oral administration using the Writhing test (intraperitoneal 0.6% acetic acid to induce visceral pain).The number of abdominal contractions was dose dependently reduced by the flower extract administered at 30, 100 and 300 mg/kg (up to about 50% with the higher dose), whereas the leaf extract was effective (30%) at 1000 mg/kg only. Furthermore, the effects of feverfew extracts on

the acute phase of inflammation was evaluated in the rat model of carrageenan-induced paw edema. The administration of carrageenan induced a significant hypersensitivity to a mechanical stimulus (Paw pressure test) 2 h after the intraplantar injection. The feverfew flower extract at doses of 100 mg/kg and 300 mg/kg (p.o. administered 30 min before the measurement) significantly reduced mechanical hypersensitivity and the effect was comparable to those elicited by diclofenac and ibuprofen (100 mg/kg). The leaf extract was ineffective. In the osteoarthritis model induced in rodents by intra articular injection of monoiodoacetate the flower extract significantly increased the pain threshold peaking 30 min after treatment, whilst the leaf extract was ineffective. Moreover, the flower extract (30, 100 and 300 mg/kg) was effective in the chronic constriction injury model of neuropathic pain showing activity similar to gabapentin in rats at doses of 100 mg/kg and 300mg/kg; the leaf extract was ineffective. Finally, the flower extract activity was confirmed in rat models of chemotherapy-induced neuropathic pain. The mechanical hypersensitivity induced by repeated treatments with oxaliplatin and with dideoxycytidine was significantly reduced after a single injection of feverfew flower extract (Di-Cesare Mannelli *et al.* 2015).

The effect of feverfew as a whole plant on an aqueous extract equivalent to 20 mg dried plant per ml, has been examined on both cyclo-oxygenase and lipoxygenase activity in rat leucocytes *in-vitro*. At 10-25 µg ml^{-1} feverfew had no effect on the formation of arachidonate metabolites; while at the highest concentrations (50-200 µg ml^{-1}) it inhibited both cyclooxygenase and lipoxygenase metabolic products (Capasso 1986).

A feverfew extract produced a dose-dependent inhibition of histamine release from rat peritoneal mast cells stimulated with anti-IgE or the calcium ionophore A23187. The extract was obtained by extraction of 1 g of air-dried leaves using chloroform (20 ml). Greater inhibition of anti-IgE-induced histamine release was achieved with feverfew compared with the inhibition of A23187-induced release. Inhibition of anti-IgE-induced histamine release by feverfew extract was observed when the drug was added simultaneously with anti-IgE and the inhibitory activity increased only slightly when the drug was pre-incubated with the cells for 5 min before anti-IgE stimulation. In this respect feverfew differs from cromoglycate and quercetin. Feverfew extract inhibited anti-IgE-induced histamine release to the same extent in the absence and or the presence of extracellular glucose. The authors concluded that feverfew extract contains a novel type of mast cell inhibitor (Hayes & Foreman 1987).

Crude chloroform extracts of fresh feverfew leaves (rich in sesquiterpene lactones) and of commercially available powdered leaves (lactone-free) produced dose-dependent inhibition of the generation of thromboxane B2 (TXB2) and leukotriene B, (LTB) by ionophore- and chemoattractant-stimulated rat peritoneal leukocytes and human polymorphonuclear leukocytes. Approximate IC$_{50}$ values were in the range 5-50 µg/ml, and inhibition of TXB2 and LTB, occurred in parallel. Isolated lactones (PN, epoxyartemorin) were also inhibitory, with approximate IC$_{50}$ values in the range 1-5 µg/ml, as were crude extracts treated with cysteine (to neutralize reactive ɑ-methylene butyrolactone functions of the sesquiterpenes). Inhibition of eicosanoid generation appeared to be irreversible but not time-dependent. The authors concluded that feverfew contains a complex mixture of sesquiterpene lactone and non-sesquiterpene lactone inhibitors of eicosanoid synthesis of high potency, and that these biochemical actions may be relevant to the claimed therapeutic activity of the herb (Sumner *et al.* 1992).

The bioactivity of feverfew leaf extracts has been analysed by use of a human polymorphonuclear leukocyte (PMNL) bioassay to assess the relative contributions of solvent extraction and PN content to

the biological potency of the extract. Extracts prepared in acetone-ethanol contained significantly more PN (mean ± s.d. 1.3 ± 0.2% dry leaf weight) than extracts in chloroform-PBS (phosphate-buffered saline; 0.1 ± 0.04% dry leaf weight) or PBS alone (0.5 ± 0.1% dry leaf weight). Extract bioactivity was measured as inhibition of phorbol 12-myristate 13-acetate-induced 5-amino-2.3-dihydro-I,4-phthalazinedione (luminol)-enhanced PMNL chemiluminescence. Extracts inhibited phorbol 12-myristate 13-acetate-induced oxidative burst by amounts which, if solely attributable to PN, indicated PN concentrations for the respective solvent systems of 2.2 ± 0.6%, 0.2 ± 0.1% and 0.9 ± 0.1% dry leaf weight. The mean ratio of PN concentration to the PN equivalent/PMNL-bioactivity value for acetone-ethanol and PBS extracts were both 1:1.7. The results indicated that PN, although a key determinant of biological activity for *T. parthenium* leaf extracts based on the PMNL-bioassay, seems not to be the sole pharmacologically-active constituent. The identical and elevated bioactivity-PN ratios for both organic and aqueous-phase leaf extracts suggested that a proportion of the other bioactive compounds have solubility similar to that of PN (Brown *et al.* 1997).

Oral administration of a water feverfew extract (composition unknown) led to significant anti-nociceptive and anti-inflammatory effects against acetic acid-induced writhing in mice and carrageenan-induced paw edema in rats, respectively. These responses were dose-dependent (10, 20, 40 mg/kg, p.o.). PN (1.2 mg/kg i.p.) also produced anti-nociceptive and anti-inflammatory effects. Naloxone (1 mg/kg i.p.), an opiate antagonist, failed to reverse feverfew extract and PN-induced anti-nociception. Feverfew extract in higher doses (40, 60 mg/kg p.o.) neither altered the locomotor activity nor potentiated the pentobarbitone-induced sleep time in mice. It also did not change the rectal temperature in rats. Feverfew extract exerted anti-nociceptive and anti-inflammatory effects without altering the normal behaviour of animals (Jain & Kulkarni 1999).

Both crude ethanol feverfew extracts and purified PN were examined for their ability to modulate adhesion molecule expression in human synovial fibroblasts. Pre-treatment of synovial fibroblasts with either feverfew extracts or purified PN could inhibit the expression of intercellular adhesion molecule-1 (ICAM-1) induced by the cytokines IL-1 (up to 95% suppression), TNF-α (up to 93% suppression) and, less strongly, interferon-γ (up to 39% suppression). Inhibition of ICAM-1 was dose and time dependent; as little as a 30-min pretreatment with feverfew resulted in inhibition of ICAM-1. The decrease in ICAM-1 expression was accompanied by a decrease in T-cell adhesion to the treated fibroblasts. The modulation of adhesion molecule expression may be an additional mechanism by which feverfew mediates anti-inflammatory effects (Piela-Smith & Liu 2001).

Antimicrobial activity

The activity of crude extracts, fractions and PN (pure compound) obtained from *T. parthenium* against two forms of the parasite *Trypanosoma cruzi* was investigated. One thousand grams of dried aerial parts were sequentially extracted by exhaustive maceration in ethanol/water 9:1. The powder resulting from lyophilization, soluble in water, was termed the aqueous crude extract (WCE). The residue was dissolved in ethanol or ethylacetate. Activity against epimastigote forms was observed for aqueous (WCE), ethanolic (ACE) and ethyl-acetate (ECE) crude extracts, fractions and PN, and a progressive increase in the antitrypanosomal effect was observed in the course of the purification process. These extracts were assayed for their activity against epimastigote forms of *T. cruzi*. At 1000 μg/ml, all the crude extracts showed similar effects inhibiting more than 90% of the parasites' growth. At low concentrations, ACE was the most effective extract. The pure compound showed IC_{50}/96h and IC90/96h of 0.5 μg/ml and 1.25 μg/ml, respectively. The cytotoxic effect of PN in LLMCK2 cells was 3.2 μg/ml (CC_{50}/96h) and the selectivity index was 6.4. No haemolysis was detected for the pure

compound. The internalization index of *T. cruzi* in LLMCK2 cells was reduced to almost 51% at the concentration of 2 μg/ml of PN and 96.6% at 4 μg/ml. Scanning and transmission electron microscopy permitted observation of morphological modifications and ultrastructural alterations (Izumi *et al.* 2008).

Other investigations showed that the ethanolic extracts possess high activity against all *Gram* positive bacteria, on some *Gram* negative bacteria and on some fungi (Kalodera *et al.* 1997).

Antioxidant activity

A PN-depleted extract of feverfew (PD-feverfew), which was free of sensitization potential, was found to possess free radical scavenging activity against a wide range of reactive oxygen species and with greater activity than Vitamin C. *In vitro*, PD-feverfew restored cigarette smoke-mediated depletion of cellular thiols, attenuated the formation of UV-induced hydrogen peroxide and reduced pro-inflammatory cytokine release. *In vivo*, topical PD-feverfew reduced UV-induced epidermal hyperplasia, DNA damage and apoptosis. In a clinical study, PD-feverfew treatment significantly reduced erythema versus placebo 24 h post-UV exposure. The authors suggested that through the ability to scavenge free radicals, preserve endogenous antioxidant levels, reduce DNA damage and induce DNA repair enzymes, which can help repair damaged DNA, PN-depleted extract of feverfew may protect skin from the numerous external aggressions encountered daily by the skin and reduce the damage to oxidatively challenged skin (Martin *et al.* 2008).

In another study, the antioxidant activities of an ethanolic feverfew extract and its bioactive components were determined in terms of their free radical-scavenging activities against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and their $Fe2^+$-chelating capacities. In addition, the bioactive constituents in feverfew were determined by GC–MS and HPLC–UV. Feverfew powder extracted by 80% alcohol contained camphor, PN, luteolin and apigenin in 0.30 ± 0.08%, 0.22% ± 0.03%, 0.84% ± 0.10% and 0.68% ± 0.07%, respectively. Total phenolic content of the feverfew extract was measured in 21.21 ± 2.11 μg gallic acid equivalent per mg dry material. The feverfew alcoholic extract possessed a strong DPPH free radical-scavenging activity of 84.4% and moderate Fe^{2+}-chelating capacity of 53.1%. Luteolin also showed strong DPPH scavenging activity of approximately 80% at 0.52 mg/ml. PN exhibited weak DPPH scavenging activity of 15% and moderate Fe^{2+}-chelating capacity of nearly 60%. Similar moderate Fe2+-chelating activity (approximately 60%) was observed for luteolin and apigenin at 2 mg/ml (Wu *et al.* 2006a).

The antioxidant activity of a methanolic dry extract of feverfew (aerial parts, extraction solvent methanol 70%) has been recently evaluated *in vivo* on 54 male Wistar rats whose liver was damaged after treatment with carbon tetrachloride (CCl_4). These rats were divided into 9 groups each consisting of 6 rats. Two of the groups were control groups (normal and damage control groups); 4 groups were exposure groups which were respectively administered with 40, 80, and 120 mg/kg feverfew extract and silymarin for 14 days before being damaged with CCl_4; the other 3 groups were post-treatment groups which received 80 and 120 mg/kg feverfew extract and silymarin 2, 6, 24 and 48 h after being injected with CCl_4.

Pre-treatment with all doses of feverfew extract for 14 day as well as post-treatment with all doses of feverfew extract for 2, 6, 24, and 48 h after the injection of CCl_4 led to the decrease of ALT, AST, ALP, TC, TG, LDL, and glucose levels and increase of albumin and HDL levels (however a statistically significant effect on some biochemical factors was not seen at doses of 40 mg/kg in pre-treatment and 80 mg/kg in post-treatment group). Furthermore, pre-treatment with the doses of 40, 80, and 120 mg/kg feverfew extract and post-treatment with the dose of 120 mg/kg feverfew extract could prevent

the decrease in the activities of catalase, superoxide dismutase and tissue glutathione peroxidase compared to the group without treatment (Mahmoodzadeh *et al.* 2017).

Effects on vessels and smooth muscle cells

Barsby *et al.* (1992) showed that samples prepared from chloroform extracts of fresh leaves of feverfew strongly inhibited responses of rabbit aortic rings to phenylephrine, 5-hydroxytryptamine, thromboxane mimetic U46619 (9,11-dideoxy-11α,9α-epoxy-methano-PGF2α), and angiotensin II. In contrast, the inhibition of potassium-induced depolarization was much less pronounced. The inhibition was concentration- and time-dependent, non-competitive, irreversible and also occurred in endothelium-denuded preparations. The feverfew extracts also caused a progressive loss of tone of pre-contracted aortic rings and appeared to impair the ability of acetylcholine to induce endothelium dependent relaxations of the tissue. These effects were mimicked by PN, obtained from the extract. The results suggest a non-specific and potentially toxic response to feverfew on the vessels. (Barsby *et al.*1992)

Thakkar *et al.* (1983) demonstrated that phospholipase A activity, measured in homogenates and acid extracts of smooth muscle cells from rat aorta and mesenteric artery, was inhibited by an extract from the leaves of feverfew plant.

The effects of a chloroform feverfew extract of fresh leaves on potassium currents in smooth muscle were studied by Barsby *et al.* (1993). The currents were recorded from single cells dissociated from the rat anococcygeus and the rabbit ear artery using the whole-cell patch-clamp technique. When applied to cells isolated from the rat anococcygeus, the extract reduced the inactivating voltage-dependent potassium current in a concentration-related manner, with an IC_{50} value of 56 μg/ml. A complete block of the current occurred at 1 mg/ml. In addition to reducing the peak current, feverfew decreased the time to peak of the current and increased the rate of delay of the current. These effects could be explained by the feverfew extract blocking open potassium channels. In single cells isolated from rabbit ear artery, the feverfew extract again reduced the voltage-dependent potassium current, whilst at the same time having no effect on the spontaneous ion of calcium-dependent potassium channels. These results suggest that chloroform extracts of feverfew leaf contain a yet unidentified substance capable of producing a selective, open-channel block of voltage-dependent potassium channels (Barsby *et al.*1993).

Antithrombotic activity

Chloroform and water extracts of feverfew inhibited secretory activity in blood platelets and polymorphonuclear leucocytes (PMNs). Release of serotonin from platelets induced by various aggregating agents (adenosine diphosphate, adrenaline, sodium arachidonate, collagen, aM U46619) was inhibited. Platelet aggregation was consistently inhibited but thromboxane synthesis was not. Feverfew also inhibited release of vitamin B12-binding-protein from PMNs induced by the secretagogues formyl-methionyl leucy1-phenylalanine, sodium arachidonate and zymosan-activated serum. Feverfew did not inhibit the secretion induced in platelets or PMNs by the calcium ionophore A23187. The pattern of the effects of the feverfew extracts on platelets was different from that obtained with other inhibitors of platelet aggregation. The effect on PMNs was more pronounced than that has been obtained with very high concentrations of non-steroidal anti-inflammatory agents (Heptinstall *et al.* 1985).

Loesche *et al.* (1988) demonstrated that chloroform feverfew leaves extract inhibits aggregatory and secretory responses in human platelets and granulocytes, and such inhibition may be relevant to the

beneficial effects. It has been suggested that feverfew extracts inhibit platelet behaviour via effects on platelet sulphydryl groups. In another study, researchers found evidence that feverfew inhibits uptake as well as liberation of arachidonic acid into/from platelet membrane phospholipids.

The same group studied the effect of feverfew on the interaction of platelets with different types of collagen-immobilized plastic as well as on the integrity of endothelial cells monolayer in perfused rabbit aorta. Feverfew leaves were dried in air, powdered and extracted with chloroform (20 ml/g leaves).

The extract was dried under nitrogen and the residue dissolved in phosphate-buffered saline. Feverfew extract inhibited in a dose-dependent way deposition of platelets and inhibited the formation of surface-bound aggregates. The results of the study indicate that feverfew extract may have antithrombotic potential (Loesche *at al.* 1988).

Chloroform/methanolic and water extracts (details not known) of the herb were found to inhibit mitogen-induced tritiated thymidine ([3H]-TdR) uptake by human peripheral blood mononuclear cells (PBMC), interleukin 2 (IL-2)-induced [3H]-TdR uptake by lymphoblasts and prostaglandin E2 (PGE2) release by interleukin 1 (IL-1)-stimulated synovial cells. Both crude organic and aqueous extracts and PN proved cytotoxic to mitogen-induced PBMC and IL-1 stimulated synovial cells, the cytotoxic effect being functionally indistinguishable from the inhibitory effects. The authors suggest that pharmacological properties of feverfew may thus be due to cytotoxicity (O'Neill *et al.* 1987).

Studies on the essential oil

The essential oil showed bactericidal and fungicidal activity. *Gram* positive species demonstrated a significantly lower sensitivity than the *Gram* negative ones, moulds, the dermatophytes and some fungi. Regarding the *Gram* positive species, the essential oil has a strong bactericidal effect only on the Bacillus species, while this effect is negligible on other species (*Sarcina flava, Staphylococcus aureus, Enterobacter sp.*). The oil has a strong bactericidal effect on many of the *Gram* negative species: *Escherichia coli*, *Klebsiella oxitoca*, *Salmonella, sp.*, *Shigella sonnei*, *Serratia marcescens* and *Citrobacter freundii*. On *Candida tropicalis*, *C. pseudotropicalis* and *C. apicola*, the essential oil has a strong fungicidal effect. The microbicidal effect on moulds (*Aspergillus flavus*, *A. ochraceus*, *A. niger*) and dermatophytes (*Microsporum gypseum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*) has also been determined (Kalodera *et al.* 1997).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

Many pre-clinical studies investigated the role of feverfew in the pathogenesis of migraine.

The role of PN in the antimigraine effects of feverfew was investigated mostly *in vitro* and antagonism of serotonin receptors and inhibition of neuronal released 5-HT have been suggested for the mechanism of action. In addition, PN seems to play a role in the blockade of NO cascade and of the

release of pro-inflammatory citokines (TNF-α and IL-6) which might be involved in the pathogenesis of migraine. Finally, PN acts as a TRPA1 partial agonist, causing selective channel desensitization and a non-selective defunctionalization of CGRP-containing sensory neurons. The release of the sensory neuropeptide calcitonin gene-related peptide (CGRP) within the trigeminovascular system has also been considered involved in the genesis of migraine headaches.

Similar findings were observed *in vitro* with alcoholic, dichloromethane and supercritical CO_2 feverfew leaf extracts.

In summary, the pathogenesis and pathophysiology of migraine is still far to be fully understood; literature data suggest the PN and feverfew extracts could be involved at different levels in the pathways supposed to be involved in the migraine pathogenesis and pathophysiology, although there is no clear demonstration that the pharmacological activity of feverfew in the treatment and prophylaxis of migraines can be attributed to PN content.

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

Methanolic extract of feverfew leaf at aliquot of 25 mg/ml inhibited CYP2C9 (51.1% ± 10.81% of inhibition), 2C19 (46.2% ± 5.27% of inhibition), 2D6 (54.1% ± 1.10% of inhibition), and most markedly 3A4 (64.7% ± 1.69% of inhibition) *in vitro* (Foster *et al.* 2003). A further *in vitro* study showed that methanolic extract of feverfew inhibited CYP1A2/2C9/2C19 and CYP2C8/3A4, with IC_{50} values below 100 mg/mL and 100–200 mg/mL, respectively (Unger & Frank 2004).

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

No data available.

3.3.3. Genotoxicity

No data available.

3.3.4. Carcinogenicity

No data available.

3.3.5. Reproductive and developmental toxicity

Yao *et al.* (2006) carried out a preliminary screen of a commonly used formulation of feverfew in order to determine its potential for reproductive toxicity. The extract of feverfew used consisted of a commercial preparation of the dried leaf of the feverfew plant extracted in 60% ethanol in a 1:2 dilution, giving a final concentration of 200 mg/ml feverfew standardized to 0.7 mg/ml of PN. The reported teratogenic threshold of ethanol is 2 g/kg in the Long-Evans rat, used in the study. The upper dose of the ethanol content of the feverfew concentrate was therefore limited to 1.98 g/kg. This meant that the maximum dose of feverfew that could be delivered to the rat was 839 mg/kg/day. Treated

rats (weight 200-300 g) were dosed with 12.8 ml/kg/day of a 65.2 mg/ml feverfew solution. Control rats received either 12.8 mg/kg of distilled water or 20% (v/v) ethanol. Five female rats were orally dosed with 839 mg/kg feverfew daily on either gestation days (GD) 1-8 or 8-15. Two pregnant rats became very sick (exhibited piloerection, reduced mobility, reduced response to stimuli) before the 8 days of feverfew treatment were completed. They were immediately euthanized and replaced. The appearance and behaviour of the remaining rats were normal during the treatment period. On GD20, rats were sacrificed and fetuses, placentae and ovaries were collected. The fetuses were weighed and examined for malformations. While maternal weight gain appeared to be reduced, ANCOVA analysis suggested that the difference was due to the litter size, rather than treatment. Pre-implantation loss appeared increased, but this was not statistically significant in the feverfew GD 1-8 group. Fetuses exposed to feverfew from GD 8-15 were smaller than ethanol controls perhaps as a result of the increased frequency of runts in treated litters. Feverfew induced toxicity when GD 10.5 embryos were cultured for 26 h in rat serum to which extract was added. The results of this study suggest that a comprehensive reproductive study of feverfew is warranted.

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

Very limited data on feverfew are available.

Single/repeat dose toxicity, genotoxicity, carcinogenicity and local tolerance studies have not been performed.

3.4. Overall conclusions on non-clinical data

Data on primary pharmacodynamics with powdered *T. Parthenium* herba are not available; however, several *in vitro* studies with alcoholic, dichloromethane and supercritical CO_2 extracts of *T. Parthenium* support the plausibility of efficacy of feverfew in the prophylaxis of migraine.

Specific data on pharmacokinetics are not available. Feverfew resulted to inhibit cytochrome P450 (CYP3A4, 1A2, 2C8, 2C9, 2C19, 2D6) *in vitro*, but *in vivo* information are missing.

Non-clinical information on the safety of *T. Parthenium* is poor.

Tests on genotoxicity and carcinogenicity have not been performed.

A preliminary study, to determine the potential of *T. Parthenium* for reproductive toxicity, was conducted in the rat. Treatment induced both maternal and embryo-toxicity at doses of feverfew slightly higher than the maximum human dose reported in the monograph; however, the small number of animals used in this study limited clear interpretation of results. In addition, a clear concentration to effect relation cannot be established. Therefore, the results of the preclinical reproductive study were not conclusive and should be deeper investigated in a comprehensive reproductive study of feverfew.

4. Clinical Data

4.1. Clinical pharmacology

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

No data available.

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

The pharmacokinetic of PN was investigated in a phase I trial in adult patients (≥18 years), with a histological or cytological diagnosis of cancer, with measurable or evaluable evidence of residual, recurrent, or metastatic disease. This single-institution phase I trial was designed as an open-label, non-randomized dose escalation study in which groups of three to six patients were to receive increasing dosages of oral PN from 1 to 5 mg per day in 1 mg increments. PN was administered daily for 28 days. Subsequent 28-day cycles were administered with an intervening break. PN was supplied as feverfew capsules containing 500 micrograms of PN. Plasma PN concentrations were assessed for all patients using a high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS). This assay was validated using purified PN. In cycle 1, blood samples were drawn on day 1 and 29 in the following fashion: pre-treatment, and then 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 12, and 24 hours after the ingestion of the capsules. PN was not detected in any of the patient samples. The authors concluded that low doses of PN are unable to deliver detectable doses of PN with the feverfew formulation, probably due to poor bioavailability (Curry III E.A. *et al.* 2004).

4.2. Clinical efficacy

Migraine is a recurrent moderate-to-severe headache that can be unilateral and often characterized by photophobia, phonophobia, nausea, vomiting, or aggravated by movement. Migraine is further classified into migraine without aura, migraine with aura, basilar-type migraine, familiar or sporadic hemiplegic migraine. These subtypes may be further classified as episodic or chronic.

Migraine is heterogeneous (among sufferers and between attacks) in frequency, duration and disability. Some migraineurs have less than one attack a month, while others have one or more attacks a week. Some are quite disabled by their headaches, while others are not. Therefore, it is appropriate to stratify the care of the migraine population by headache frequency, severity and level of disability. Furthermore, prevention needs to be considered for those patients whose migraine has a substantial impact on their lives.

Migraine is considered a genetic disorder of neuronal hyper-excitability. The genesis of the attack is linked to neuronal activation. Migraine headache has an incidence of 6% in men and 15% to 17% in women (Bamford *et al.* 2009).

There are two ways to treat migraine headache: the acute therapy to terminate the headache when needed and the preventive therapy, in which treatment is given chronically to reduce attack frequency, severity and duration, to improve responsiveness to acute therapy, and to improve function and reduce disability (Bamford *et al.* 2009).
Medications with the best evidence for efficacy in the prevention of migraine are amitriptyline, propranolol, timolol, valproate, and topiramate. Options available include alternative and

complementary therapies, optimized lifestyles with changes as necessary, dietary and substance changes and drug prevention (Taylor 2009).

Although all migraine preventive medications are non-specific and have multiple potential mechanisms for their effects, they often share a tendency to reduce central neuronal hyper-excitability by inhibiting excitatory neurotransmitters, such as glutamate and norepinephrine, increasing inhibitory tone via GABA, reducing the likelihood of cortical spreading depression or favorably altering channelopathies or mitochondriopathies thought to be intrinsic to migraine pathophysiology (Bamford *et al.* 2009).

4.2.1. Dose response studies

The efficacy and safety of T. parthenium (feverfew) in migraine prophylaxis-a double-blind, multicentre, randomized placebo-controlled dose-response study

A study was designed with the primary objective to show a dose–response with a supercritical CO_2 extract (MIG-99) (DER unknown) from feverfew. Furthermore, the study provided data on the safety and tolerability of MIG-99. In a randomized, double-blind, multicentre, controlled trial, the clinical efficacy and safety of three dosages of MIG-99 (2.08 mg, corresponding to 0.17 mg PN; 6.25 mg, corresponding to 0.5 mg PN; 18.75 mg, corresponding to 1.5 mg PN twice in a day) were compared with placebo. The patients (n=147) suffered from migraine with and without aura according to International Headache Society (IHS) criteria and were treated with one of the study medications for 12 weeks after a 4-week baseline period. During the active treatment period patients received either 2.08 mg MIG-99 (corresponding to 0.17 mg PN), 6.25 mg MIG-99 (0.5 mg PN), 18.75 mg MIG-99 (1.5 mg PN) or placebo. The primary efficacy parameter was the number of migraine attacks during the last 28 days of the treatment period compared with baseline. Secondary endpoints were total and average duration and intensity of migraine attacks, mean duration of the single attack, number of days with accompanying migraine symptoms, number of days with inability to work due to migraine as well as type and amount of additionally taken medications for the treatment of migraine attacks. The design of the study included a pre-planned adaptive interim analysis for patients with at least four migraine attacks within the baseline period. With respect to the primary and secondary efficacy parameter, a statistically significant difference was not found between the overall and the confirmatory intention-to-treat (ITT) sample in the exploratory analysed four treatment groups. The frequency of migraine attacks for the predefined confirmatory subgroup of patients (n=49) with at least four migraine attacks during the baseline period decreased in a dose-dependent manner (P=0.001).

The highest absolute change of migraine attacks was observed under treatment with 6.25 mg t.i.d. (mean ± SD = - 1.8 ± 1.5 per 28 days) compared with placebo (- 0.3 ± 1.9; P=0.02). Overall, 52 of 147 (35%) patients reported at least one adverse event.

The incidence of adverse events in the active treatment groups was similar to that in the placebo group, and no dose-related effect was observed in any safety parameter. MIG-99 failed to show a significant migraine prophylactic effect in general. Accordingly, in the ITT analysis a dose–response relationship could not be observed. MIG-99 was shown to be effective only in a small predefined subgroup of patients with at least four attacks during the 28-day baseline period, where the most favourable benefit–risk ratio was observed with a dosage of three capsules of 6.25 mg MIG-99 extract per day. Due to a low number of patients, these findings need to be verified in a larger sample. The incidence of adverse events was similar for all treatment groups. In conclusion, there were no statistically significant effects for either primary or secondary outcome measures. Accordingly, a dose-response relationship could not be observed. Subgroup analysis including patients with at least four migraine

attacks during baseline evaluations (n=49) showed a significant effect when the 6.25-mg dose was compared with placebo (p=0.02) (Pfaffenrath *et al.* 2002) (Table 5).

4.2.2. Clinical studies (case studies and clinical trials)

Clinical studies with feverfew for prevention of migraine
• Efficacy of feverfew as prophylactic treatment of migraine.

A double blind placebo controlled trial including seventeen patients who already used fresh leaves of feverfew daily as prophylaxis against migraine was carried out. Patients who had suffered from classical or common migraine for at least two years, with eight or fewer attacks per month, were allocated randomly to receive either feverfew (freeze-dried leaves) or identical placebo capsules in numbered packs. Diary cards were used to assess the frequency of migraine and the incidence of nausea and vomiting. Patients were instructed how to record the various visual symptoms, nausea, vomiting and headache (including times of onset and relief and any additional treatment) and to grade them according to severity on diary cards provided for each period. The severity of nausea or vomiting was recorded as: 0 = neither nausea nor vomiting; 1=nausea only; 2=vomiting, single episode; 3=vomiting, repeated episodes. Headache was scored: 0=no pain; 1=mild, unpleasant but not affecting work or recreational activities; 2=severe, reducing ability to work or carry out recreational activities; 3=incapacitating, unable to work or carry out recreational activities; 1=duration up to six hours; 2=duration between six and 24 hours; 3=duration greater than 24 hours. Presence of usual visual disturbance scored 1. Use of drugs was scored: 1=use of repeated doses of minor analgesics; 1=use of single dose of ergotamine; 2=use of repeated doses of ergotamine. The cards were reviewed at intervals of one to two months throughout the study. Patients were instructed to take two capsules every morning with food for six periods of four weeks. The mean daily dose of feverfew used by patients before entry to the study was 2.44 leaves (roughly 60 mg). Hence, it was decided that the dose of each capsule should be fixed at 25 mg and that each patient should receive two capsules daily. Eight patients received capsules containing freeze-dried feverfew powder and nine patients received placebo. The results showed a significant (p<0.02) increase in the number of attacks per month in the in the final three months in the placebo group (mean, 3.43; standard error [SE], 1.02) compared with baseline, while attack frequency remained constant in patients receiving feverfew (mean, 1.50; SE, 0.62). 42% and 79% of attacks were associated with nausea and vomiting in the feverfew and placebo groups, respectively (p<0.05). The incidence of bouts of nausea/vomiting was significantly (p<0.05) lower in the feverfew group than in the placebo group (39 and 116, respectively). The global assessment of efficacy by patients indicated a significant (p<0.01) difference in favour of feverfew: 6/8 patients in the feverfew group rated the overall treatment effect as moderately good to excellent, while this result was reported by only 3/9 patients in the placebo group. (Johnson *et al.* 1985) (Table 5).

• Randomised double-blind placebo-controlled trial of feverfew in migraine prevention.

The use of feverfew for migraine prophylaxis was assessed in a randomised, double blind, placebo-controlled crossover study. After a one-month single-blind placebo run-in, 72 patients with common or classical migraine were randomly allocated to receive either one capsule of a chloroform extract (DER unknown) of dried feverfew leaves a day or matching placebo for four months and then transferred to the other treatment arm for a further four months. There was no wash-out period between the treatment periods. Capsule feverfew extract content ranged from 70 to 114 mg (mean 82 mg) and contained the equivalent of 2.19 (SD 0.63) µmol of PN per capsule. Frequency and severity of attacks

were determined from diary cards which were issued every two months; efficacy of each treatment was also assessed by visual analogue scores. Sixty patients completed the study and full information was available for 59. The results suggested a significant (p<0.005) difference in the number of attacks per 2-month period during feverfew treatment (mean, 3.6; SE, 0.2) compared with placebo (mean, 4.7; SE, 0.3). Among patients with classical migraine (n=17), the number of attacks per 2-month period was significantly (p < 0.05) lower with feverfew (mean, 2.9; SE, 0.4) than with placebo (mean, 4.3; SE, 0.5); among patients with common migraine (n=42), headache frequency was similar during the feverfew (mean, 3.9; SE, 0.3) and placebo (mean, 4.9; SE, 0.4) periods (p = 0.06). In the study population as a whole, the total number of attacks rated as severe or very severe was 178/424 (42%) with feverfew and 258/559 (46%) with placebo. Nausea and vomiting accompanied the attacks in 207/424 (49%) and 313/559 (56%) cases treated with feverfew and placebo, respectively (p<0.02). The global assessment of efficacy, measured on a 100-mm visual analogue scale with 'worst ever' and 'best ever' as the two extremes, indicated a significant (p<0.0001) difference in favour of feverfew compared with placebo (mean, 74; SE, 2 versus mean, 60; SE, 3, respectively). Among patients with classical migraine, global assessment scores were significantly (p<0.01) higher during treatment with feverfew (mean, 78; SE, 4) than during treatment with placebo (mean, 57; SE, 5); among patients with common migraine, scores for the two treatment periods were similar (mean, 72; SE, 2 for feverfew and mean, 61; SE, 3 for placebo). In conclusion, treatment with feverfew was associated with a reduction in the mean number and severity of attacks in both two-month periods, and in the degree of vomiting, while the duration of individual attacks was unaltered. Visual analogue scores also indicated a significant improvement with feverfew. There were no serious side-effects (Murphy *et al.* 1988) (Table 5).

- Herbal medicines in migraine prevention: Randomized double-blind placebo-controlled crossover trial of feverfew preparation.

De Weerdt *et al.* (1996) assessed 50 patients diagnosed according to the criteria of the International Headache Society (IHS 1988). Patients suffering from migraine with or without aura received daily either one capsule filled with an alcoholic feverfew extract (143 mg containing 0.5 mg of PN) or placebo in a randomised crossover trial. The extract was obtained subjecting feverfew powder to 19 days of prolonged exposure to 90% ethanol, moderate heat (up to 90°C) and evaporation. A 1-month placebo run-in phase was followed by 4-month treatment periods. There was no wash-out period between the treatment periods. The investigators reported that no significant effects on the number or severity of headaches were observed (Table 5).

- Feverfew (*T. parthenium*) as a prophylactic treatment for migraine: A placebo-controlled double-blind study.

The crossover trial conducted by Palevitch *et al.* (1997) included 57 patients with migraine diagnosed by medical examination (diagnostic criteria not specified). During the preliminary, open phase of the trial, each patient received 100 mg feverfew daily for 2 months. The PN content of the dried leaves was 0.2% as determined by HPLC. 50 mg of fine powdered leaves was packed in small gelatin capsules. Powdered dry leaves of parsley (*Petroselinum crispum*), were prepared in the same way and served as the placebo control. Thereafter, in the double-blind crossover phase, one group received placebo for 30 days, while the other continued taking feverfew. Patients in the active treatment group were then transferred to the placebo arm and vice versa. There was no wash-out period between the treatment periods. A relevant portion of patients (43%) suffered from chronic migraine (more than 10 attacks per

month). The severity of migraine attacks was measured by patients on a numerical scale of 0 ('no pain') to 10 ('most severe pain') and the severity of nausea and vomiting was assessed using a numerical analogue scale and a questionnaire. The results of the preliminary, open phase showed a significant decrease in migraine severity after treatment with feverfew compared with baseline (p<0.001). In the first crossover phase there was a further reduction of migraine severity in the feverfew group (mean, 1.5; SE, 0.7) and an increase in severity in the placebo group (mean, 1.6; SE, 0.9) (p<0.01). In the second phase of the crossover, these trends continued: migraine severity decreased among patients taking feverfew (mean, 4.0; SE, 1.1) and increased among patients taking placebo (mean, 1.4; SE, 1.1). In addition, there was a significant (p<0.001) difference in the severity of nausea and vomiting in favour of feverfew (Table 5).

• Efficacy and safety of 6.25 mg t.i.d. feverfew CO_2-extract (MIG-99) in migraine prevention, a randomized, double-blind, multicentre, placebo-controlled study.

The efficacy and tolerability of a CO_2-extract of feverfew (MIG-99, 6.25 mg t.i.d.) for migraine prevention were investigated in a randomized, double-blind, placebo-controlled, multicentre, parallel-group study. Patients (N=170 intention-to-treat; MIG-99, N=89; placebo, N=81) suffering from migraine, according to the International Headache Society criteria, were treated for 16 weeks after a 4-week baseline period. The primary endpoint was the average number of migraine attacks per 28 days during the treatment months 2 and 3 compared with baseline. Predefined secondary efficacy parameters were the number of migraine attacks during each 28-day period of therapy, the clinical global impression of efficacy (very good, good, moderate, none), change of migraine intensity, the total duration of migraine attacks, mean duration of the single attack, number of migraine days, number of attacks with confinement to bed or inability to work or accompanying migraine symptoms per 28 days, number of migraine attacks with aura per 28 days, type and amount of analgesics and migraine preparations taken and number of drop-outs due to insufficient efficacy. Safety parameters included adverse events, laboratory parameters, vital signs and physical examination. The migraine frequency decreased from 4.76 by 1.9 attacks per month in the MIG-99 group and by 1.3 attacks in the placebo group (P=0.0456). Logistic regression of responder rates showed an odds ratio of 3.4 in favour of MIG-99 (P=0.0049). Adverse events possibly related to study medication were 9/107 (8.4%) with MIG-99 and 11/108 (10.2%) with placebo (P=0.654). The authors concluded that MIG-99 is effective and shows a favourable benefit–risk ratio (Diener *et al.* 2005) (Table 5).

Table 5: Clinical studies on humans, in prophylaxis of migraine

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
Johnson et al. 1985	Randomised, double-blind, placebo-controlled, parallel group study.	Powdered freeze-dried feverfew leaves 25 mg in capsules twice daily (n=8) or placebo (n=9) taken orally for six periods of four weeks.	17 patients. Mean age of patients taking feverfew was 44.9; mean age of patients taking placebo 51.2. Two patients in the placebo group withdrew from the study due to re-appearance of migraine attacks.	Patients who had suffered from classical or common migraine for at least two years, with eight or fewer attacks per month; patients had been treating themselves with raw feverfew leaves every day for at least three months before starting the study. Diagnostic criteria for migraine have not been specified.	Fewer headaches were reported in each month in the last three months by patients taking feverfew capsules (1.50 ± 0.62) than by those taking placebo (3.34 ± 1.02) (p<0.02); Less attacks associated with nausea and vomiting in the feverfew compared to placebo group (p<0.05). Incidence of bouts of nausea/vomiting was significantly (p<0.05) lower in the feverfew group.	Non-parametric statistical methods such as the Wilcoxon rank sum test used for comparisons between the treatments.	Explorative due to a very small sample size.

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
Murphy et al. 1988	Randomised, double-blind, placebo-controlled, crossover study.	Chloroform extract (DER not declared) of dried feverfew leaves in one capsule containing 2.19 ± 0.63 µmol of PN daily or placebo for 4 months and then transferred to the other treatment arm for a further four months. Oral administration.	72 patients; 60 patients completed the study and full information was available in 59	Patients with common (n=42) or classical (n=17) migraine diagnosed as defined by Blau 1984.	Overall, the number of attacks per 2-month period during feverfew treatment (mean, 3.6; SE, 0.2) was reduced compared with placebo (mean, 4.7; SE, 0.3) (p<0.005). The effect was still statistically significant in patients with classical migraine (n=17; p < 0.05) but not in patients with common migraine.(n=42; p = 0.06). Severe or very severe attacks were 178/424 (42%) with feverfew and 258/559 (46%) with	Analysis was restricted to 59 patients who completed the study. ANOVA used to compare the number and duration of attacks, the visual analogue scores.	The study has a limited value due to the small sample size. Other potential bias in the study design were: - lack of a wash-out period; - random sequence generation not described; - details of allocation concealment

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
					placebo. Nausea and vomiting accompanied the attacks in 207/424 (49%) and 313/559 (56%) cases treated with feverfew and placebo, respectively (p<0.02). VAS score was significantly higher in feverfew group than in the placebo group both in the whole population(p<0.0001) and patients with classical migraine but not in patients with common migraine.		not provided.

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
De Weerdt et al. 1996	Randomized double-blind placebo-controlled, crossover trial.	Granulate containing dried extract of feverfew, extraction solvent ethanol 90% (DER not declared) mixed with microcrystalline cellulose in one capsule (143 mg of granulate containing 0.5 PN) daily or placebofor 4-months and then transferred to the other treatment arm for a further four months. Oral administration.	50 patients; 48 W and 8 M; age 18-64. 44 patients completed the study. Withdrawals were due to side-effects (1), lack of efficacy (3, 2 out of 3 in the feverfew group) or other causes not related to the study (2).	Patients with migraine with or without aura diagnosed according to the criteria of the International Headache Society with a frequency of at least one attack per month.	The difference in the average of numbers of headache attacks between feverfew and placebo was statistically not significant (0.52, 95% CI: - 4.39 to 5.43). The average response to the two treatments was the same regardless of the order in which they were received (0.16; 95% CI:- 2.96 to 3.28).	PP analysis on 44 patients. The possibility of a period effect and a treatment-period effect was tested by two sample t-tests. The treatment effect was tested by performing a one-sample t-test within subject differences between the	The study has a limited value due to the small sample size. No significant effect on the number or severity of headaches was observed.

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
						two treatments.	
Palevitch et al. 1997	Placebo-controlled double-blind, crossover study.	100 mg powdered feverfew leaves containing 0.2% PN in one capsule daily or placebo for one month (open-label phase) and then transferred to the other treatment arm for a further one month (groups A and B). Oral administration.	57 patients; 47 W and 10 M; mean age 38 (9 – 65) years.	Patients with migraine diagnosed by medical examination (diagnostic criteria not specified); patients received 100 mg feverfew every day for two months before starting the study.	During preliminary, open phase there was a significant decrease in migraine severity after treatment with feverfew compared with baseline (mean decline in the score 4.27 ± 0.38 points; p<0.001). In the first crossover phase there was a further reduction of migraine severity in the feverfew group (mean, 1.5; SE, 0.7) and an increase in severity in the placebo	For each patient the pain intensity was compared by paired t - test individually at the beginning of the study and then at the end of the open-labelled phase. A	The contribution of this study to the clinical evidence is scarce due to the following reasons: - small sample size; - random sequence generation not described; - details of

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
					group (mean, 1.6; SE, 0.9) (p<0.01). In the second phase of the crossover, migraine severity decreased among patients taking feverfew (mean, 4.0; SE, 1.1; p<0.01) and increased among patients taking placebo (mean, 1.4; SE, 1.1). There was a significant (p<0.001) difference in the severity of nausea and vomiting in favour of feverfew	t-test was performed between group A and group B during phases 2 and 3.	allocation concealment not provided; - lack of a wash-out period; - no information provided on drop-outs; - limited duration of the study (two months); - frequency and duration of migraine attacks were not

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
							determined.
Pfaffenrath et al. 2002	Double-blind, multicentre, randomized placebo-controlled dose-response study.	Supercritical CO_2 feverfew extract (DER not declared) 2.08 mg (corresponding to 0.17 mg PN), 6.25 mg (corresponding to 0.5 mg PN), 18.75 mg (corresponding to 1.5 mg PN) t.i.d. or placebo for 12 weeks after a 4-week baseline period. Oral administration.	147 patients.	Patients with migraine with and without aura according to HIS criteria.	There were no statistically significant effects in the number of migraine attacks during the last 28 days of the treatment period compared with baseline Primary outcome). The same was seen secondary outcomes measures. Subgroup analysis including patients with at least four migraine attacks during baseline evaluations (n = 49) showed a significant effect when the 6.25 mg	ITT analysis for both the overall population (n=147) and the confirmatory group (n=49).	The study failed to show superiority of feverfew versus placebo in primary and secondary outcome. Accordingly, a dose-response relationship could not be observed.

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
					dose was compared with placebo (P value = 0.02).		
Diener et al. 2005	Randomized, double-blind, placebo-controlled, multicentre, parallel-group study.	Supercritical CO_2 feverfew (MIG-99) extract 6.25 mg t.i.d. or placebo for 16 weeks after a 4-week baseline period. Oral administration.	170 patients; MIG-99, N=89; placebo, N=81	Patients with migraine, according to the IHS criteria.	The migraine frequency decreased from 4.76 by 1.9 attacks per month in the MIG-99 group and by 1.3 attacks in the placebo group (P=0.0456). Logistic regression of responder rates showed an odds ratio of 3.4 in favour of MIG-99 (P=0.0049). In addition, significant differences versus placebo were reported	ITT.	The clinical relevance of this study is questionable : - the number of nausea and vomiting which are indicative of the intensity of migraine attacks was not determined; - the use of analgesics to

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
					for number of migraine days per 28 days (P value = 0.0353), and global assessment of efficacy by patients (P value = 0.024) and investigators (P value = 0.035). No statistically significant differences were seen for any of the other outcome measures.		treat acute attacks was allowed during this study and results on the amount and distribution of the use of these medications in the study population have not been reported.

Reviews on clinical studies with feverfew for prevention of migraine

The first systematic review on feverfew as a preventive treatment for migraine was published by Vogler *et al.* in 1998. Only randomized, placebo-controlled, double-blind trials were included. The methodological quality of all trials was evaluated using the Jadad score.

Main results:

Five trials met the inclusion/exclusion criteria. The majority favoured feverfew over placebo. Three studies reported positive results in favour of feverfew. In total, 216 patients were included, both men and women suffering from classical and/or common migraine. The author concluded that the effectiveness of feverfew in the prevention of migraine has still not been established beyond reasonable doubt (Vogler *et al.* 1998).

Ernst published in the year 2000 a systematic review on the efficacy and safety of feverfew. Only randomized, placebo-controlled, double-blind trials of feverfew mono-preparations for the prevention of migraine in human subjects were included. Six trials met the inclusion/exclusion criteria. The majority favoured feverfew over placebo. The data also suggested that feverfew is associated with only mild and transient adverse effects and few other safety concerns. The author concluded that feverfew is likely to be effective in the prevention of migraine and that there are no major safety problems (Ernst & Pittler 2000).

A Cochrane review on feverfew for preventing migraine was released in 2004. The aim of the review was to systematically evaluate the evidence from double-blind randomised controlled trials (RCTs) assessing the clinical efficacy and safety of feverfew versus placebo for preventing migraine.

Publications describing (or which might describe) double-blind RCTs of feverfew extract for migraine were considered. Randomised, placebo-controlled, double-blind trials assessing the efficacy of feverfew for preventing migraine were included (Johnson et al. 1985; Murphy et al. 1988; De Weerdt et al. 1996; Palevitch et al. 1997; Pfaffenrath et al. 2002). Trials using clinical outcome measures were included. Trials focusing exclusively on physiological parameters were excluded. There were no restrictions regarding the language of publication.

Five trials (343 patients) met the inclusion criteria. Results from these trials were mixed and did not convincingly establish that feverfew is efficacious for preventing migraine. Only mild and transient adverse events were reported in the included trials.

The authors concluded that there is insufficient evidence from randomised, double-blind trials to suggest an effect of feverfew over and above placebo for preventing migraine. It appeared from the data reviewed that feverfew presents no major safety problems. It has been suggested that the lack of efficacy may be due to the absence of the therapeutic constituents in the granulated feverfew leaves, which were either not sufficiently extracted, or perhaps degraded during the preparation (Pittler & Ernst 2004).

In 2015, an update of the Cochrane review carried out in 2004 by Pittler & Ernst was published. This new review included one larger rigorous randomised, placebo-controlled, double-blind trial (n=218) carried out by Diener *et a*l. (2005), for a total of 561 patients (Wider *et al.* 2015). The methodological quality of the included studies was evaluated using the Oxford Quality Scale developed by Jadad et al. (Jadad 1996). The primary outcome of this systematic review was the frequency of migraine attacks;

secondary outcomes were intensity and duration of migraine attacks, incidence and severity of nausea/vomiting, global assessment of efficacy and adverse events. The authors planned to assess heterogeneity of efficacy outcomes, but the included studies were too heterogeneous in terms of participants, interventions, methods and outcome measures to be statistically combined; for the same reason a meta-analyses of data on primary and secondary outcomes was not feasible.

Overall the results from the six included trials are mixed and provide low quality evidence. With the addition of the most recent and largest study, Diener 2005, there is evidence from one medium-sized, rigorous randomized controlled trial that feverfew may reduce migraine attacks by 0.6 headaches per month compared to placebo. Results for the secondary outcome measures remain unconvincing. The high variability in terms of designs, participants, interventions and outcome measures make difficult to extend the results of this review to the general population.

The authors concluded that the cautiously positive results obtained adding the study by Diener 2005 to the previous Cochrane review need to be confirmed in larger trials, which need to be rigorously executed and reported (Wider *et al.* 2015).

Feverfew in rheumatoid arthritis

• Feverfew in rheumatoid arthritis: a double blind, placebo-controlled study.

Feverfew is reputed by folklore to be effective in arthritis. Forty-one female patients with symptomatic rheumatoid arthritis received either dried powdered feverfew (70-86 mg) leaf (equivalent to 2-3 μmol PN) or placebo capsules once daily for six weeks. One capsule of feverfew corresponded to two medium sized leaves. Allocation was random and not known by patient or observer. Variables assessed included stiffness, pain (visual analogue scale), grip strength, articular index, full blood count, erythrocyte sedimentation rate, urea, creatinine, C reactive protein, complement breakdown products (C3dg), rheumatoid factor titre, immunoglobulins (IgG, IgA, IgM), functional capacity, and patient and observer global opinions. One patient (placebo) withdrew after three days and was not included in the analysis. Treatment and placebo groups (20 patients each) were matched at entry. No differences between the clinical or laboratory variables of the groups were observed during the six week period. This study therefore shows no apparent benefit from oral feverfew in rheumatoid arthritis (Pattrick et al. 1989).

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

No studies performed.

4.4. Overall conclusions on clinical pharmacology and efficacy

Based on the totality of evidence, including clinical trials and systematic reviews, it is concluded that there is insufficient evidence to the use of feverfew as a treatment for migraine. Even though data coming from the clinical study by Diener *et al.* (2005) provide some evidence for efficacy of feverfew in the prophylaxis of migraine, these cautiously positive results obtained in a medium-size clinical trial should be confirmed in a larger trial. Furthermore, taking into account all clinical studies on feverfew, there is there is low quality evidence that feverfew is effective in migraine prevention, because of the small number of patients, effects that were only lightly superior than placebo and the different dosages used. In summary, overall data are inadequate to substantiate a well-established use of *Tanacetum Parthenium* for the prophylaxis of migraine.

The study by Pattrick *et al.* (1989) is the only clinical trial that investigated the effect of feverfew in symptomatic rheumatoid arthritis. This study failed to show any apparent benefit of the use of *Tanacetum Parthenium*.

5. Clinical Safety/Pharmacovigilance

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

Feverfew was well tolerated in the included trials, and adverse events were generally mild and reversible. Controlled studies have demonstrated no effect on blood pressure or body weight after 6 months of regular consumption of encapsulated dried feverfew leaf (Johnson et al. 1985). Three studies (Johnson 1985; Murphy 1988; Diener 2005) reported a higher incidence of adverse events during treatment with placebo than with feverfew. Feverfew did not appear to affect blood pressure, heart rate, body weight or haematological and biochemical safety parameters (Wider *et al.* 2015).

During the study conducted in 1985 by Johnson at the City of London Migraine Clinic, roughly 10% of patients who were switched to placebo after taking feverfew for several years appeared to experience a genuine "post-feverfew syndrome": a cluster of nervous system reactions including rebound of migraine symptoms, anxiety, poor sleep patterns along with muscle and joint stiffness (Johnson 1985). This study included only seventeen patients that were already using the plant by eating fresh leaves of feverfew daily as prophylaxis against migraine in the period before the beginning of the clinical investigation.

In another study conducted by Pfaffenrath *et al.* (2002), the incidence of adverse events was similar for all treatment groups.

The toxicity of feverfew was investigated in a phase I trial in adult patients (≥18 years), with a histological or cytological diagnosis of cancer, with measurable or evaluable evidence of residual, recurrent, or metastatic disease. This single-institution phase I trial was designed as an open-label, non-randomized dose escalation study in which groups of three to six patients were to receive increasing dosages of oral feverfew capsules containing from 1 to 5 mg per day of PN in 1 mg increments. Feverfew was administered daily for 28 days. Subsequent 28-day cycles were administered with an intervening break. Each feverfew capsule contained 500 micrograms of PN. Twelve patients with advanced solid tumours were treated with feverfew. The median age was 73 years (range 48 to 80). There were 11 men and 1 women enrolled.

The median number of four-week cycles was two cycles with a minimum exposure of one and a maximum of eight. There were no dose limiting toxicities (DLTs). Although no drug-related DLTs were seen, the trial was stopped at the dose level of 4 mg daily once it was determined that no PN was identified in the plasma of any of the patients. Toxicities include fever, gastrointestinal side effects, chills, fatigue, and blurred vision. All toxicities resolved after stopping feverfew administration. The fever was more likely related to an intercurrent viral illness and the blurred vision did resolve after withdrawal from the protocol, but it should be noted that the patient was a known diabetic and that causes other than the feverfew were considered more likely (Curry III E.A. *et al.* 2004).

Chewing feverfew leaves produces sometimes a more general inflammation of the oral mucosa and tongue, accompanied by swelling of the lips and occasionally loss of taste. In Johnson's 1983 survey of 300 feverfew users, mouth ulceration (aphthous ulcers) from chewing fresh leaves was reported by 11.3% of users, prompting discontinuance of treatment by some. Mouth ulceration is a systemic

reaction to *Tanaceti Parthenii* observed only in subjects chewing fresh leaves; it requires discontinuation of the product. Inflammation of the mouth and tongue with swelling of the lips appears to be a local reaction that may be overcome by using encapsulated herb products (WHO monograph 2004).

Abdominal pains and indigestion have been reported for feverfew users who chewed the leaves over a period of years. Although long-term toxicity data are presently unavailable, no serious side effects have been noted in patients taking the plant for several years. Digestive disturbances were experienced by 6.5% of respondents (Dennis 1998, Johnson 1983).

The frequency of chromosomal aberrations and sister chromatid exchanges (SCE) were determined from lymphocyte cultures established from blood samples. Samples had been taken over a period of several months in 30 migraine patients who had daily taken leaves, tablets or capsules of feverfew for more than 11 consecutive months. They were compared to 30 feverfew non-user migraine patients who had been individually age and sex matched. Matched pairs were sampled on the same date for two-thirds of the cases, and the greatest difference in sampling time of the remainder was 20 days. Also, the mutagenicity of urine samples from 10 feverfew user migraine patients was compared to that from 10 matched non-user migraine patients using the Ames *Salmonella* mutagenicity test system. Paired samples were given on the same date. The mean frequency of chromosomal aberrations in the feverfew user group was lower than that in the non-user group, both in terms of cells with breaks (2.13% vs 2.76%) and in terms of cells with all aberrations (4.34% vs 5.11%). However, this difference was small and not significant. The mean frequency of SCE in the feverfew exposed group was lower than that in the control group (8.78 vs 8.80 SCE/cell*)*, but this difference was not significant as determined by factorial analysis of variance *(P =0.897)*. There was a highly significant variance between the frequencies of SCE in the matched pairs of migraine patients, but this was not related to age, sex or feverfew exposure. The mean number of revertants in the Ames mutagenicity assay was greater for the urine of the feverfew user migraine patients than that of the non-user migraine patients, in both strains of bacteria, with or without the inclusion of an S-9 metabolizing system. However, the increases were small and insignificant. The data indicate that the prophylactic use of feverfew for the alleviation of migraine symptoms affects neither the frequency of chromosomal aberrations nor the frequency of SCE in the circulating peripheral lymphocytes. Also, the mutagenicity of urine from feverfew user migraine patients is unaffected compared to urine from non-user migraine patients detectable by the methods used in this study (Anderson *et al.* 1988).

Table 6: Clinical safety data from clinical trials

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation ,pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Adverse reactions	Comments on clinical relevance of results
Johnson *et al.* 1985	Randomised, double-blind, placebo-controlled, parallel group study.	Powdered freeze-dried feverfew leaves 25 mg in capsules twice daily (n=8) or placebo (n=9) taken orally for six periods of four weeks.	17 patients. Mean age of patients taking feverfew was 44.9; mean age of patients taking placebo 51.2. Two patients in the placebo group withdrew from the study due to re-appearance of migraine attacks.	Patients who had suffered from classical or common migraine for at least two years, with eight or fewer attacks per month; patients had been treating themselves with raw feverfew leaves every day for at least three months before starting the study. Diagnostic criteria for migraine have not been specified.	No significant differences between feverfew group and placebo group in mean blood pressure and body weight after six months of treatment. The heart rates in the group given feverfew were significantly higher (p<0.05) at the end of treatment. Patients in the feverfew group experienced stiffness or pain in the joints (n=2; they already suffered before starting the study),	Higher incidence of adverse events during treatment with placebo than with feverfew; feverfew was well-tolerated.

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation ,pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Adverse reactions	Comments on clinical relevance of results
					palpitations (n=1) and abdominal pain (n=1). No differences within or between the groups in the incidence of abnormal laboratory findings or in changes of values from normal to abnormal and vice versa.	
Murphy *et al.* 1988	Randomised, double-blind, placebo-controlled, crossover study.	Chloroform extract of dried feverfew leaves (DER unknown) in one capsule containing 2.19 ± 0.63 µmol of PN daily or placebo for 4 months and then transferred to the other treatment arm	72 patients; 60 patients completed the study and full information was available in 59	Patients with common (n=42) or classical (n=17) migraine diagnosed as defined by Blau 1984.	The incidence of adverse events was lower in the feverfew group (28/72) compared to the placebo group (36/72).	Higher incidence of adverse events during treatment with placebo than with feverfew; feverfew was well-tolerated.

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation ,pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Adverse reactions	Comments on clinical relevance of results
		for a further four months. Oral administration.				
De Weerdt *et al.* 1996	Randomized double-blind placebo-controlled, crossover trial.	Granulate containing dried extract of feverfew (DER not declared), extraction solvent ethanol 90% mixed with microcrystalline cellulose in one capsule (143 mg of granulate containing 0.5 PN) daily or placebo for 4-months and then transferred to the other treatment arm for a further four months. Oral administration.	50 patients; 48 W and 8 M; age 18-64. 44 patients completed the study. Withdrawals were due to side-effects (1), lack of efficacy (3, 2 out of 3 in the feverfew group) or other causes not related to the study (2).	Patients with migraine with or without aura diagnosed according to the criteria of the International Headache Society with a frequency of at least one attack per month.	One woman withdrew due to diarrhoea, which she ascribed to the use of the feverfew preparation. No patient who finished the study reported side effects. No serious changes in regards to the physical condition were found concerning body-weight, pulse-frequency, blood pressure, liver function or cholesterol content, all of which were monitored monthly	Feverfew was well-tolerated.

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation ,pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Adverse reactions	Comments on clinical relevance of results
					during the study.	
Palevitch *et al.* 1997	Placebo-controlled double-blind, crossover study.	Powdered feverfew leaves containing 0.2% PN in one capsule daily or placebo for one month (open-label phase) and then transferred to the other treatment arm for a further one month (groups A and B). Oral administration.	57 patients; 47 W and 10 M; mean age 38 (9 – 65).	Patients with migraine diagnosed by medical examination (diagnostic criteria not specified); patients received 100 feverfew every day for two months before starting the study.	No data available.	
Pfaffenrath *et al.*2002	Double-blind, multicentre, randomized placebo-controlled dose-response	Supercritical CO_2 feverfew extract (DER unknown) 2.08 mg (corresponding to 0.17 mg PN), 6.25 mg (corresponding to 0.5 mg PN),18.75 mg	147 patients.	Patients with migraine with and without aura according to HIS criteria.	The percentages of patients who experienced adverse events were between 12.8% and 30.6% in the high-dose and low-dose feverfew	The incidence of adverse events in the active treatment groups was similar to that in the placebo group, and no dose-related effect was

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation ,pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Adverse reactions	Comments on clinical relevance of results
	study.	(corresponding to 1.5 mg PN) t.i.d. or placebo for 12 weeks after a 4-week baseline period.			groups respectively and 28.6% in the placebo group.	observed in any safety parameter.
Diener *et al.* 2005	Randomized, double-blind, placebo-controlled, multicentre, parallel-group study.	Supercritical CO_2 feverfew extract (DER unknown) (MIG-99) 6.25 mg t.i.d. or placebo for 16 weeks after a 4-week baseline period. Oral administration.	170 patients; MIG-99, N=89; placebo, N=81	Patients with migraine, according to the IHS criteria.	8.4% of participants in the feverfew group and 10.2% in the placebo group experienced adverse events.	Higher incidence of adverse events during treatment with placebo than with feverfew; feverfew was well-tolerated.
Curry III E.A. *et al.* 2004	Open-label, non-randomized dose escalation, phase I study	Powdered leaves in capsules each containing 500 µg of PN. Groups of 3 to 6 patients were to receive increasing dosages from 1 to 5 mg per day of PN in 1	12 patients; 11 M and 1 W; median age was 73 years (48 - 80).	Patients with a histological or cytological diagnosis of cancer, with measurable or evaluable evidence of residual, recurrent, or metastatic disease	There were no dose limiting toxicities (DLTs) up to 4 mg daily of PN. Toxicities include fever, gastrointestinal side effects, chills, fatigue, and blurred vision. All	Feverfew was without any significant limiting toxicity. The fever was more likely related to an intercurrent viral illness and the blurred vision did resolve

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation ,pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Adverse reactions	Comments on clinical relevance of results
		mg increments. Feverfew was administered daily for 28-day cycles. The median number of four week cycles was two cycles with a minimum exposure of one and a maximum of eight.			toxicities resolved after stopping feverfew administration.	after withdrawal from the protocol, but it should be noted that the patient was a known diabetic and that causes other than the feverfew were considered more likely.

5.2. Patient exposure

In total, 561 patients participated in the clinical studies with feverfew alone included in this assessment report, but safety data were available only on 504 patients, as the study by Palevich *et al.* (1997) did not give any information on adverse events. In these studies, patients were treated with various single-ingredient preparations containing feverfew.

The proportion of participants experiencing adverse events varied considerably between studies but there were no statistically significant differences in the occurrence of adverse events between the feverfew and placebo groups.

Adverse events were generally mild and reversible. Most common were gastrointestinal problems but a wide range of adverse events in the feverfew and placebo groups were reported. No serious adverse events related to the study medication occurred in any of the included trials. In total, 13 withdrawals were necessitated by adverse events associated with feverfew, compared with eight withdrawals due to adverse events associated with placebo.

Again, adverse events leading to withdrawals were mainly of a gastrointestinal nature. Feverfew did not appear to affect blood pressure, heart rate, body weight or haematological and biochemical safety parameters in any of the included studies (Wider *et al.* 2015).

5.3. Adverse events, serious adverse events and deaths

See also chapters 5.1 and 5.2.

Allergy to Tanacetum and other plants from the Compositae family

Sesquisterpene lactones such as the feverfew constituents and PN are also known to cause contact dermatitis (Dennis 1998, Johnson 1983).

Parthenium dermatitis, in its classical form, is known as airborne contact dermatitis and primarily affects the exposed areas and the flexures. Other clinical patterns are a seborrheic pattern, widespread dermatitis and exfoliative dermatitis.

The allergens responsible for contact dermatitis are sesquiterpene lactones and are present in the oleoresin fraction of the leaf, the stem and the flower, and also in the pollen.

Contact dermatitis has been reported after use of moisturizers containing feverfew (Killoran et al. 2007).

EudraVigilance

EudraVigilance database was consulted in September 2019. A number of 18 reports (two duplicates) were found using the search terms "Feverfew" and "Tanacetum". In only 5 cases feverfew was the only medication taken but all the adverse events were not serious. Seven reports were serious and in 5 cases the patients were hospitalised; serious adverse events included mainly cardiovascular events, although also gastrointestinal, renal and cutaneous adverse reactions were seen, however in no cases a clear causal correlation between the use of feverfew and the observed adverse event could be established. Interestingly, there were two serious cases reporting a potential drug-drug interaction between feverfew and warfarin or propranolol. In the case of warfarin (Kim 2005), patient who was assuming warfarin for atrial fibrillation and vitamin E, gingko biloba and feverfew (dose not available) as dietary supplements, experienced international normalized ratio increased (value 27.9), and he died three day after hospitalization. A comprehensive assessment of any causal correlation was precluded

by the lack of essential information (e.g. medical history, lab data, concurrent medical illness, clinical course etc.). In the case of propranolol (Somes & Donatelli 2011), patient experienced beta-blocker toxicity, hypotension, weakness and low heart-rate; a causal correlation between feverfew (dose not available) and propranolol was rated as possible, based on the interaction of feverfew with cytochrome P450 substrates in the liver, resulting in a reduced clearance of propranolol.

5.4. Laboratory findings

Physical and biochemical parameters after oral assumption of powdered freeze-dried feverfew leaves were examined during the clinical trial by Johnson *et al.* (1985). No significant differences between feverfew group and placebo group in mean blood pressure and body weight after six months of treatment.

The heart rates in the group given feverfew were significantly higher (p<0.05) at the end of treatment.

Blood was taken for full haematological and biochemical analysis on enrolment and at the end of the trial. Complete data were obtained for eight patients in each group. There were no differences within or between the groups in the incidence of abnormal laboratory findings or in changes of values from normal to abnormal and vice versa.

This clinical trial involved only 17 patients therefore no conclusion can be drawn on the effect of feverfew on physical and biochemical parameters.

5.5. Safety in special populations and situations

Occupational or direct exposure has caused eczema and allergic dermatitis.
Feverfew cross reacts with Tansy, Yarrow, Marguerite, Aster, Subflower, Laurel and Liverwort (PDR monograph 2007).

5.5.1. Use in children and adolescents

Because of lack of information on the plant's effect, it has to be advised that children and adolescents should not be treated with feverfew

5.5.2. Contraindications

Based on data reported in chapter 5.3 there is evidence that sesquisterpene lactones such as the feverfew constituents and PN can cause contact dermatitis; the use of *Tanacetum Parthenium* should be contraindicated in patients with known hypersensitivity to the active substance(s) and to other plants of the *Asteraceae* (*Compositae*) family..

5.5.3. Special Warnings and precautions for use

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

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

5.5.4. Drug interactions and other forms of interaction

Some theoretical potential risks have been suggested:

- feverfew inhibits platelet aggregation and it has been suggested that caution should be used in patients treated with other inhibitors of platelet aggregation such as aspirin and dipyridamole (PDR monograph 2007);

- moderate risk of bleeding may result by interactions with anticoagulants, low molecular weight heparins, thrombolytic agents and antiplatelet agents (PDR monograph 2007);

- moderate risk of adverse reactions (i.e. gastrointestinal, renal effects) may result by interactions with non steroidal anti-inflammatory agents (PDR monograph 2007).

However, no *in vivo* drug interaction studies have been reported in literature. Two serious cases reporting a potential drug-drug interaction between feverfew and warfarin or propranolol were found in EudraVigilance database. In the case of warfarin, a pharmacodynamic interaction could be conjectured but a comprehensive assessment of causal correlation was not possible due to the lack of sufficient information; in the case of propranolol a pharmacokinetic interaction was suggested and the causality relation was deemed as possible.

5.5.5. Fertility, pregnancy and lactation

A preclinical reproductive study showed that feverfew has potential maternal toxicity and embryotoxicity. The dose of feverfew used in this study was 839 mg/kg/day, corresponding to a human equivalent dose of 135.38 mg/kg; the dose used in this study was 67.7-fold higher than the minimum human daily dose (100 mg), but only 11.3-fold higher than the maximum human daily dose (600 mg) reported in the monograph. Signs of maternal toxicity (lower weight gain) have been observed, but they did not reach statistical significance. The two mentioned sick animals that were taken out of the study group cannot be weighted as treatment related because there was no real check of the reasons. Embryo-toxicity has been observed (e.g. development of some embryos stopped), but clear interpretation of results was limited by the small number of animals; in addition, a clear concentration to effect relation cannot be established. Therefore, the results of the preclinical reproductive study were not conclusive and should be deeper investigated in a comprehensive reproductive study of feverfew.

In view of its traditional reputation for emmenagogic effect and to affect the menstrual cycle, the use feverfew preparations is not recommended in pregnant or lactating women. This is in agreement with the SmPC of herbal medicinal products containing feverfew reported in table 3.

No fertility data are available.

5.5.6. Overdose

No case of overdose has been reported.

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

No data available.

5.5.8. Safety in other special situations

Withdrawal and rebound

Post-feverfew syndrome. About 10% of migraine patients who abruptly stop taking feverfew fresh leaves may experience rebound headache, insomnia, muscle stiffness, joint pain, fatigue and nervousness (PDR monograph 2007). This "post-feverfew syndrome" was observed in a very small clinical study (Johnson *et al.* 1985) in patients who switched to placebo after taking feverfew for several years, but it has not been confirmed in subsequent larger clinical studies.

5.6. Overall conclusions on clinical safety

Data from clinical studies show that feverfew is well tolerated and adverse events were generally mild and reversible. Although feverfew has a traditional reputation for emmenagocic effect, there are no data suggesting that this plant should be contraindicated in pregnant or lactating woman; the use of feverfew in not recommended in these women due to insufficient data. Because of the lack of data, feverfew treatment is not recommended in children and adolescents. Moderate risks may result from interactions with drugs or substances influencing blood coagulation and platelets aggregation; however, these risks are only theoretical because clinical drug-drug interaction studies have not been carried out. Rebound symptoms may appear after stop taking feverfew. Exposure to feverfew may cause eczema and allergic dermatitis.

6. Overall conclusions (benefit-risk assessment)

Tanacetum parthenium, also known as "feverfew", is a member of the *Compositae* family and it has been described since ancient times as having beneficial medicinal effects. The plant parts used for medicinal use are the dried leaves or the dried aerial parts.

Several investigations support feverfew's traditional medicinal use as treatment for migraine headache. The medicinal use of feverfew for migraine and headaches in Europe is also well documented for centuries.

Feverfew appears to be safe and well tolerated for the indication proposed and side effects are generally mild and reversible. Despite the wide use, no serious adverse reaction was reported. The analysis of data from clinical studies shows that side effects associated with its use such as nausea, heartburn, constipation, flatulence, abdominal bloating and diarrhoea are rarely reported and their frequency is similar to that of placebo.

There is evidence that feverfew in herbal medicinal products for migraines has been available for more than 30 years across European Union.

The proposed medicinal use for *Tanaceti parthenii* herba is:

"Traditional herbal medicinal product for the prophylaxis of migraine headaches".

Based on data in textbooks and information provided by interested parties, only powdered herbal substances has been included in the monograph with a daily dose of 100-600 mg. The highest posology is justified by the medicinal use for 30 years of products on the market both as traditional herbal medicinal products and food supplements. Feverfew at dosages of 50mg – 300 mg b.i.d is

should be considered for patients requiring migraine prophylaxis according to the American Headache Society with a level B of efficacy (Loder *et al.* 2012). Considering the limited number of reports existing for *Tanacetum Parthenium* preparations, as verified in Eudravigilance database, there is no safety issue for the posology of 200 mg trice a day. The daily dosage of 100 mg may be increased until obtaining an effect, not exceeding the daily dose of 600 mg.

Due to the lack of sufficient data on long term use, the duration of use of two months seems to be suitable for self-medication. Treatment should be maintained for 2 to 3 months to evaluate effectiveness. It may take two to three months before a decrease in the frequency or severity of attacks is noticed even after reaching "the beneficial dose."

The use of feverfew in children and adolescents under 18 years of age is not recommended due to the lack of adequate data.

The use of feverfew has been contraindicated in patients with known hypersensitivity to the active substance(s) and to other plants of the *Asteraceae* (*Compositae*) family.

Drug interactions between feverfew and other active substance have not been reported in the monograph, due to the lack of *in vivo* clinical data and the absence of reports in EudraVigilance database showing a definitive causal correlation. Notwithstanding, a report on EudraVigilance on a patient taking feverfew and propranolol suggest that further investigation is needed to clarify the clinical relevance of the inhibition of cytochrome P450 by feverfew observed *in vitro*.

In view of its traditional reputation for emmenagogic effect and to affect the menstrual cycle (Herbal Medicines 2007), the use feverfew preparations is not recommended in pregnant or lactating women. This is in agreement with the SmPC of herbal medicinal products containing feverfew reported in table 3, and with the results of a reproductive toxicity study on rats. In this study, signs of maternal toxicity and embryo-toxicity were observed following treatment of rats with high doses of feverfew (about 11-fold higher than the maximum human daily dose of 600 mg), but these effects were rarely statistically significant and not clearly related to feverfew. In addition, a clear interpretation of data is limited by the reduced number of animals and a concentration to effect relation cannot be established, therefore further investigation is necessary.

Several clinical studies investigating the efficacy of feverfew alone in the prophylaxis of migraine have been carried out in the period from 1985 to 2005 on a total of 561 patients. These studies are heterogeneous in terms of designs, participants, interventions and outcome measures. Overall, there is low quality evidence that feverfew is effective in migraine prevention. Moreover, no feverfew are authorized in the EU under article 8(3) or art 10a of the amended Directive 2001/83/EC. Therefore, a well-established use of *Tanacetum Parthenium* for the prophylaxis of and treatment of migraine is not included in the monograph.

The study by Pattrick et al. (1989) is the only clinical trial that investigated the effect of feverfew (as capsules containing powdered leaves) in symptomatic rheumatoid arthritis. This study failed to show any apparent benefit of the use of *Tanacetum Parthenium*.

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

Therapeutic areas for browse /search at the EMA website: pain and inflammation.

Annex

List of references