EUROPEAN MEDICINES AGENCY
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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Calendula officinalis* L., flos

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Calendula officinalis* L., flos
Herbal preparation(s)	a) Comminuted herbal substance b) Liquid extract (1:1), extraction solvent ethanol 40-50% (V/V) c) Liquid extract (1:1.8-2.2), extraction solvent ethanol 40-50% (V/V) d) Tincture (1:5), extraction solvent ethanol 70-90% (V/V) e) Liquid extract (1:10), extraction solvent fatty vegetable oil e.g. olive oil f) Extract (1:5 – 1:25), extraction solvent hardened vegetable fat, petroleum jelly
Pharmaceutical form(s)	Herbal substance or comminuted herbal substance for infusion for oromucosal or cutaneous use Herbal preparations in liquid or semi solid dosage forms for cutaneous use.
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1. Introduction

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

- Herbal substance(s)

Calendulae flos (European Pharmacopoeia 9.0).

Whole or cut, dried, fully opened flowers, which have been detached from the receptacle, of the cultivated, double-flowered varieties of *Calendula officinalis* L. It contains not less than 0.4% of flavonoids, calculated as hyperoside ($C_{21}H_{20}O_{12}$, M_r 464.4) with reference to the dried herbal substance.

Constituents (Willuhn, 2004; Hänsel *et al*., 1992; Hänsel *et al*., 2007; Muley *et al.,* 2009; Ghédira and Goetz, 2016):

Triterpene saponins: 2-10% derivatives of the oleanolic acid with glucuronic acid on C3

Triterpene alcohols: free and esterified (with fatty acids) mono-, di- and triols of the ψ–taraxene-, taraxene-, lupine- and ursine-type. Approximately 0.8% Monols (α- and β-amyrin, lupeol, taraxasterol, ψ-taraxasterol), approximately 4% Diols, mostly in form of the mono esters (faradiols and arnidiol esters)

Ionon- and sesquiterpene glycosides: isolated from Calendula grown in Egypt (officinosids, Marukami *et al*., 2001)

Carotenoids: up to 4.7%; predominately lutein and zeaxanthine (together up to 92% of total carotenoids). The sesquiterpene lactone calendine is not a genuine constituent, the structure is identical with the xanthophyll degradation product loliolide

Flavonoids: 0.3-0.8%; glycosides of isorhamnetin, quercetin

Coumarins: scopoletin , umbelliferone, aesculetin

Volatile oil: 0.2-0.3%, mostly sesquiterpenes (e.g., α– cadinol)

Water soluble polysaccharides: up to 15%

- Herbal preparation(s)

a) Comminuted herbal substance

b) Liquid extract (1:1), extraction solvent ethanol 40-50% (V/V)

c) Liquid extract (1:1.8-2.2), extraction solvent ethanol 40-50% (V/V)

d) Tincture (1:5), extraction solvent ethanol 70-90% (V/V)

e) Liquid extract (1:10), extraction solvent fatty vegetable oil e.g. olive oil

f) Extract (1:5 – 1:25), extraction solvent hardened vegetable fat, petroleum jelly

Some of the preparations mentioned above have specific monographs in DAC:

Calendulae extractum fluidum (Ringelblumenfluidextrakt, DAC 2000): a fluid extract, containing not less than 0.4% of flavonoids, calculated as hyperoside ($C_{21}H_{20}O_{12}$, M_r 464.4). DER corresponds to 1:1 and the extraction solvent is ethanol 50% V/V.

Calendulae tinctura (Ringelblumentinktur, DAC 2002): contains not less than 0.1% of flavonoids, calculated as hyperoside; ratio corresponds to 1:5 and the extraction solvent is ethanol 70% V/V.

Composition of the tinctures

Bilia *et al.,* 2002 investigated, using an HPLC, the compositions of two tinctures (1:5), both prepared using different concentrations of ethanol (40%-60% V/V). The total content of flavonoids was very similar, although the qualitative composition was slightly different.

- 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.

This assessment report includes all data regarding mono-preparations containing herbal substance and herbal preparations from Calendulae flos, literature regarding combination products is not part of the assessment.

1.2. Search and assessment methodology

Databases and other sources used to research available pharmaceutical, non-clinical and clinical data on marigold or its relevant constituents:

- Relevant articles and references retrieved from databases: PubMed, Embase and International Pharmaceutical Abstracts were searched with the search terms ‘Calendula flower’ combined with ‘human’, ‘clinical trial’, ‘randomised controlled trial’ and ‘review’. For updating this Assessment Report with actual information in order to revise the HMPC monograph of 2008, the database Embase has recently been searched with search term: ‘Calendulae flos 2007-‘.
- Textbooks, pharmacopoeias and monographs.

Data was also provided by interested parties after a call for data published on EMA website.

The abstracts of the references found were screened manually and all articles identified that could have a possible impact on the assessment report and monograph were included. This assessment report is based on the summary of the most relevant scientific literature.

2. Data on medicinal use

2.1. Information about products on the market

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

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form**	**Regulatory Status**
1) Herbal substance (Calendulae flos)	Indication a) Internal, local application: inflammation in the mouth and the throat Indication b) External application: for the treatment of wounds, also with poor healing tendency and leg ulcers	Herbal tea Infusion for a) and b) From 0.8-1.6 g herbal substance/150 ml boiling water Indication a) the still warm infusion is used to rinse and gargle 2-3 times daily Indication b) impregnated dressing prepared from the infusion or applied to the wound 2-3 times daily If the symptoms persist in minor inflammation in the mouth - and throat mucosa more than 1 week, or if recurring or unclear symptoms occur, a doctor or a qualified health care practitioner should be consulted. In case of severe redness of the wound edges, wetting or infected wounds, the consultation with a doctor is necessary.	1986, DE, Standard Marketing Authorisation
2) Calendulae flos cum calyce*	Indication a) Oral use: for treatment of minor gastric irritation and gastric spasms Indication b) External use: for treatment of inflammations of oral and pharyngeal mucosa, wounds associated with poor healing, ulcus cruris	Herbal tea Indication a: 1 teaspoon in 0.25 l of boiling water 3-4 times daily; Indication b: 2 tablespoons/1 l of water in a form of compresses	1998-2010, Czech Republic
3) Liquid extract (1:1.8-2.2), extraction solvent ethanol 40 (V/V)	As an aid in healing of superficial wounds	Cream (10 g cream contains 0.4 g liquid extract) Cutaneous use Adults and children > 1 year: apply a small amount of cream in the size of a pea on a skin area corresponding to the size of the palm of the hand 2-3 times daily Duration of use: no longer than 2 weeks	1976-2010, DE, Standard Marketing Authorisation
4) Tincture from Calendulae flos (1:10, extraction solvent ethanol 70% V/V)	Symptomatic treatment of minor skin abrasions, minor wounds, minor inflammation of the skin	Ointment (1 g ointment contains 100mg tincture). Cutaneous use For adolescents from 12 years, adults and elderly: apply 2-4 times daily	WEU, 1999, Latvia

Active substance	Indication	Pharmaceutical form	Regulatory Status
	Symptomatic treatment of minor skin abrasions, minor wounds, minor inflammation of the skin, minor inflammation in the mouth.	Solution (1mL solution contains 1 ml tincture) Cutaneous and oromucosal use For adolescents from 12 years, adults and elderly. For skin disinfection in case of minor skin abrasions: use undiluted tincture. For wounds: use only on wound edges. For treatment of minor skin inflammations: in impregnated dressing use diluted 1:3 with water, 30-60 minutes 2-4 x per day. For minor inflammation in the mouth: use the diluted tincture for gargle 3-5 times daily.	WEU, 1999, Latvia
5) Calendula extract (extraction solvent: CO_2; no further detail)	As an adjuvant for the healing of wounds and skin lesions such as ulcers, bedsores, abrasions, broken skin	Ointment (100 g of the ointment containing 0.4 g of the extract corresponding to 10.0 g of herbal drug) Cutaneous use To be applied on the affected area 3 times daily	1992-2010, Czech Republic

* does not fulfill the Ph. Eur. definition

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)

The Austrian tradition, for more than 30 years includes petroleum jelly as extraction solvent for cosmetic products. The herbal substance may be moistened with ethanol prior to extraction. The ointment base is allowed to melt and subsequently the herbal substance is added. The time for extraction is up to 16 hours. After extraction, the still liquid mixture is filtered; the filtrate congeals as the temperature falls (Kubelka *et al.,* 2007). Semi-solid preparations contain usually 4-20% of the herbal substance (Kubelka *et al.,* 2007). As Article 16c (c) of Directive 2001/83/EC requires documented medicinal use throughout a period of at least 30 years, including at least 15 years within the EU, the data relating to these cosmetic products can be used as traditional evidence for this preparation.

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

No data available.

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

The therapeutic use of Calendula flowers and ointments goes back at least to Hildegard von Bingen (cited in Mayer *et al*., 2000). In fact Calendula flower has been in medical use for many decades. Therefore for Calendula flower a period of at least 30 years in medical use as required by Directive 2004/24/EC for qualification as a traditional herbal medicinal product is fulfilled.

Calendulae flos sine calyce has been the subject of the Czech Pharmacopoeia since 1997, with the following recommended dosages: for oral use: single dose 1 – 2 g, daily dose 4 - 8 g; for local use: 2 – 3 g.

Calendulae flos cum calyce is subject of the Czech Pharmaceutical Codex (Codex Pharmaceuticus Bohemicus, 1993); the recommended dosages are: for oral use single dose 3 g; for local use: 4 – 10 g in 100 g of ointment.

In Germany, Commission E published in 13.3.1986 the monograph Calendulae flos (defined as cut dried flowers, so included the comminuted herbal substance) with the following uses: internal and topical use-inflammatory changes of the oral and pharyngeal mucosa; external use-wounds, including those with tendency to heal poorly; ulcus cruris. Dosage (unless otherwise prescribed): 1-2 g dried Calendula flowers as an infusion in one cup of water (150 ml) or 1-2 teaspoons (2-4 ml) tincture per 1/4 -1/2 liter water or as a preparation in ointments corresponding to 2-5 g dried Calendula flowers in 100 g ointment. The same indications and posology are described by ~~Wichtl~~ Willuhn (2004).

British Herbal Pharmacopoeia (BHP 1976) mentioned the following specific indications: enlarged or inflamed lymphatic nodes; sebaceous cysts; duodenal ulcer, inflammatory skin lesions, acute or chronic.

Dosage (thrice daily):

Dried florets: dose 1-4 g or by infusion

Liquid extract 1:1 in 40% ethanol: dose 0.5-1 ml

Tincture 1:5 in 90% ethanol: dose 0.3-1.2 ml

A similar tincture is included in DAC (2002) Calendulae tinctura monograph ("Ringelblumentinktur"): Composition corresponds to 1:5 and the extraction solvent is ethanol 70% V/V. The indication proposed in DAC refers to the HMPC monograph, while the dosage proposed is:

- as a gargle or mouth wash: in a 2% solution

- in semi-solid preparations: at concentrations equivalent to 2 to 10% herbal substance;

- in impregnated dressings: tincture diluted at least 1:3 with freshly boiled water.

A liquid extract (DER 1:1.8-2.2, extraction solvent ethanol 50% V/V) is also included in DAC (2000): "Calendulae extractum fluidum" The indication proposed is: traditionally used for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds. Dosage: in semi-solid preparation at concentration equivalent to 4 -10% herbal substance.

Ointments containing this liquid extract in a concentration of 10% have been on the Austrian market for more than 30 years, and in a concentration of 4% on the German market.

"Calendulae flos" ESCOP monograph (2003) mentions as therapeutic indications: symptomatic treatment of minor inflammations of the skin and mucosa and as an aid to the healing of minor wounds.

The posology indicated for external use:

Infusion for cutaneous application: 1-2 g dried flower/150 ml water.

Fluid extract 1:1 in 40% ethanol or tincture 1:5 in 90% ethanol; for the treatment of wounds the tincture is applied undiluted; for compresses the tincture is usually diluted at least 1:3 with freshly boiled water (ESCOP monograph 2003 with reference to Van Hellemont, 1988)

Semi-solid preparations containing 2-10% fluid extract 1:1

Duration of use: no restriction

Hänsel *et al.*, 1992 refers not only to cutaneous use but also to internal use of Calendula flowers: for the treatment of cholecystitis, cholangitis, gastritis, as diaphoretic. Externally can be used for the treatment of: atheromas, perianal eczema, proctitis, acne, dry dermatosis

According to Hänsel *et al.,* 1992, which is referring to older references as Lindemann (1982) and Auster and Schafer (1958) Calendula flowers can be used topically in ointment (100 g ointment contain 5 g flowers or 15 g herba). A product which was on the market at that time contained 10 g flowers in 100 g base of ointment (Adeps suillus and Maydis oleum). Other ointments can contain 2-5 g herbal substance/100 g ointment.

Hänsel *et al.*, 1992 is referring also to some preparations, such as calendula oil, fluid extract 1:1 in 40-50% ethanol or tincture 1:5 in 90% ethanol.

"Calendula oil" is prepared by extraction with olive oil or peanut oil (1 part Calendula: 10 parts oil). Ointments contain 20-80% Calendula oil and are used externally.

Assessor comment: Peanut oil, which is mentioned in literature, is not recommended because of the higher probability of adverse reactions.

The tincture 1:5 in ethanol 90% is used:

a) Externally: 1-2 teaspoons in 1/2 L water on dressing wounds or for gargle. Can be also used 1:10 in water on wounds. Ointment contains 10-20% herbal drug

b) Internally: acute cases: 15-25 drops of tincture/hour, or 15-25 drops of tincture /warm water, 3-4 times daily; daily dose: 2-4 g tincture

It is also mentioned that semi-solid preparations can contain 2-10% fluid extract 1:1; the amount corresponds to 2-10% herbal substance (data cited from Spaich, 1977)

In the literature other extracts, prepared with supercritical CO_2 and liquid solvents – in addition to water or ethanol (e.g., isopropylmyristate, propyleneglycol, glycerol, diethylenglycol, polyethylenglycol) are described, but do not fulfil the requirements for traditional use. The same is true for the so called LACE-extract (laser activated Calendula extract), described by Jimenez-Medina *et al*., 2006.

Bradley (1992-2006) states the following external use indications: minor wounds, particularly those with a tendency to poor healing; minor burns and scalds (up to second degree), including sunburn, inflammatory skin lesions and a wide range of skin problems including abrasions, sores and rashes.

Other uses are also mentioned, based on experience or tradition: external use (conjunctivitis and other ocular irritations, sebaceous cysts, enlarged of inflamed lymphatic nodes, sprains and bruises, proctitis); local internal use (inflammation of the mouth and throat mucosa, including stomatitis, apthous ulcer, gingivitis and periodontitis) and internal use (inflammatory complaints or digestive system such as gastric and duodenal ulcer, gastritis, colitis, amenorrhoea and dysmenorrhoea).

Dosage:

External use: ointment containing the equivalent to 2-5 g, or in more concentrated form, 10-12 g of dried flower/100 g ointment. Infused oil and ointments, creams or gels containing about 10% tincture or fluid extract are also used.

Semi-solid preparations or liquids (infusion or diluted tincture) are often applied to wounds and skin conditions with a compress, changed several times daily.

Oromucosal use: as a gargle or mouth rinse, a warm infusion or a 2% solution of tincture, every 2 hours

Oromucosal use: 2-3 g dried flower as an infusion in 150 ml water; liquid extract 1:1 in ethanol 40% 0.5-1 ml; tincture 1:5 in 90% ethanol, 0.3-1.2 ml. Up to 10 g of tincture daily, divided into 3 doses has also been recommended.

In Table 2 information is given regarding the documented medicinal use, strength and posology for the main preparations of Calendula flowers as found in the phytotherapeutic handbooks.

Table 2: Overview of historical data

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
A) Dry herbal substance for infusion	Inflammatory skin lesions, acute or chronic	Infusion Single dose: 1-4 g herbal substance by infusion; 3 times daily	BHP (1976)
	Symptomatic treatment of minor inflammations of the skin and mucosa and as an aid to the healing of minor wounds	Infusion 1-2 g dried flower/150 ml water.	ESCOP (2003)
	1) Internal used in inflammatory complaints or digestive system 2) Inflammations in the mouth or the throat mucosa	1) Internal use Infusion: 2-3 g dried flower/150 ml water 2) External use- warm infusion as a gargle or mouth rinse, every 2 hours	Bradley (1992- 2006)
B) Comminuted herbal substance for infusion	Internal and topical use-inflammatory changes of the oral and pharyngeal mucosa; External use-wounds, including those with tendency to heal poorly; ulcus cruris	Infusion: 1-2 g dried herbal substance/150 ml	Commission E (1986)
C) Liquid extract 1:1 in 40% ethanol	Inflammatory skin lesions, acute or chronic.	Single dose: 0.5-1 ml 3 times daily	BHP (1976)
	Internal used in inflammatory complaints or digestive system	Single dose: 0.5-1 ml	Bradley (1992-2006)
	Minor wounds, particularly those with a tendency to poor healing	Semi-solid preparations containing 10% extract; several times daily	Bradley (1992- 2006)
	Healing of minor wounds	Semi-solid preparations; amount equivalent to 2-10% herbal substance	Spaich, 1977 (cited by Hänsel *et al.*, 1992)
D) Liquid extract 1:1 in 50% ethanol	Healing of minor wounds.	Semi-solid preparations; amount equivalent to 2-10% herbal substance	Spaich, 1977 (cited by Hänsel *et al.*, 1992)
E) Liquid extract (DER 1:1.8-2.2, extraction solvent ethanol 50%	Traditionally used for the symptomatic treatment of minor inflammations of the skin (such as	Semi-solid preparations; amount equivalent to 4-10% herbal substance	DAC (2000); Bradley (1992- 2006)

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
V/V)	sunburn) and as an aid in healing of minor wounds		
F) Tincture (1:5) extraction solvent ethanol 90% V/V	Symptomatic treatment of minor inflammations of the skin and mucosa and as an aid to the healing of minor wounds	For the treatment of wounds, the tincture is applied undiluted; for compresses the tincture is usually diluted at least 1:3 with freshly boiled water	Van Hellemont, 1988 cited by ESCOP (2003)
	Inflammatory skin lesions, acute or chronic	Single dose: 0.3-1.2 ml 3 times daily	BHP (1976)
	Externally: Wounds or for gargle Internal use: Cholecystitis, cholangitis, gastritis, as diaphoretic	External use: 1-2 teaspoons in 1/2 L water on dressing wounds or for gargle. Can be also used 1:10 in water on wounds. Ointment contains 10-20% preparation Internal use: acute cases: 15-25 drops of tincture/hour, or 15-25 drops/warm water, 3-4 times daily 2-4 g tincture	Spaich, 1977, cited Hänsel *et al.*, 1992
	1) Internal used in inflammatory complaints or digestive system 2) Inflammations in the mouth or the throat mucosa 3) Minor wounds, particularly those with a tendency to poor healin	1) Internal use Single dose:0.3-1.2 ml; daily dose: up to 10 g tincture 2) Local use: as a gargle or mouth rinse- 2% solution of tincture, every 2 hours 3) Semi-solid preparations containing 10% tincture; several times daily	Bradley (1992-2006)
G) Tincture (1:5) extraction solvent ethanol 70% V/V	1) Treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds. 2) Treatment of minor inflammations in the mouth or the throat	- as a gargle or mouth wash: in a 2% solution - in semi-solid preparations: at concentration equivalent to 2 to 10% herbal substance; - in impregnated dressings: tincture diluted at least 1:3 with freshly boiled water	DAC (2002)

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
H) Liquid extract (1:10), extraction solvent fatty vegetable oil (e.g. olive oil)	As an aid to the healing of minor wounds	Ointments contain 20-80% preparation (amount equivalent to 2-8% herbal substance)	Hänsel *et al.*, 1992- referring to Lindemann (1982)
I) Calendula extract (1:5 – 1:25), extraction solvent hardened vegetable fat, petroleum jelly[1]	As an aid to the healing of minor wounds	Semi-solid preparations[2]	Lindemann (1982) and Auster and Schafer (1958) cited by Hänsel *et al.*, 1992

2.3. Overall conclusions on medicinal use

Based on the documentation found in handbooks, as listed above and the actual market data received from the Competent Authorities sufficient information was found for the herbal substance, comminuted herbal substance, liquid extracts and tincture to justify at least 30 years of medicinal use including at least 15 years of the EU for the herbal substance Calendula flower (Table 3).

All above mentioned preparations are for cutaneous use and some for oromucosal use, have a specified strength and posology and have indications suitable to meet the legal requirements in the relevant route of administration.

During the revision process data were evaluated again regarding the criteria and the time frames of WEU and traditional use. No new preparations or indications were added.

[1] petroleum jelly is traditionally used in Austria for more than 30 years
[2] (ratio 1:5-1:25 is equivalent to 4-20% herbal substance in semi-solid preparations)

Table 3: Overview of evidence on period of medicinal use (30 years for traditional use)

Herbal preparation Pharmaceutical form	**Indication**	**Posology, Strength**	**Period of medicinal use**
Herbal substance for infusion	Indication 1: traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds. Indication 2: traditional herbal medicinal product for the symptomatic treatment of minor inflammations in the mouth or the throat.	Indication 1: Single dose: 1-2 g herbal substance in 150 ml water as infusion. The still warm infusion is used to prepare impregnated dressings Daily dose: 2 to 3 times Duration of use: 1 week Indication 2: single dose: 1-2 g in 150 ml water as infusion; the still warm infusion is used for rinsing and gargling Daily dose: 2 to 3 times Duration of use: 1 week	Since 1986, DE;
Comminuted herbal substance for infusion*	Traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds	1-2 g herbal substance in 150 ml water as infusion	Commission E (1986)
Liquid extract (1:1), extraction solvent ethanol 40-50% (V/V)	Traditionally used for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds	In semi-solid dosage forms: amount equivalent to 2-10% herbal substance	Spaich, 1977 (cited by Hänsel *et al.*, 1992)
Liquid extract (1:1.8-2.2), extraction solvent ethanol 40-50% (V/V)	Traditionally used for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds	In semi-solid dosage forms: amount equivalent to 2-5% herbal substance	AT > 30 years DE (1976-2010)
Tincture (1:5), extraction solvent ethanol 90% (V/V)	Indication 1: traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds	Indication 1: In impregnated dressings diluted at least 1:3 with freshly boiled water; In semi-solid dosage forms: amount equivalent to 2-10% herbal substance	Indication 1: Van Hellemont, 1988 cited by ESCOP, 2003 Spaich, 1977, cited

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
	Indication 2: traditional herbal medicinal product for the symptomatic treatment of minor inflammations in the mouth or the throat	Indication 2: As a gargle or mouth wash in a 2% solution	Hänsel *et al.*1992 Indication 2: Bradley (1992-2006)
Tincture (1:5), extraction solvent ethanol 70% (V/V)	Indication 1: treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds Indication 2: treatment of minor inflammations in the mouth or the throat	Indication 1) - in semi-solid preparations: at concentration of 2 to 10%; - in impregnated dressings: tincture diluted at least 1:3 with freshly boiled water Indication 2) As a gargle or mouth wash in a 2% solution	DAC (2002)
Liquid extract (1:10), extraction solvent fatty vegetable oil (e.g. olive oil)	Traditionally used for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds	In semi-solid dosage forms: amount equivalent to 2-8% herbal substance	Lindemann (1982) cited by Hänsel *et al.*, 1992
Calendula extract (1:5 – 1:25), extraction solvent hardened vegetable fat, petroleum jelly**	Traditionally used for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds	In semi-solid preparations equivalent to 4-20% herbal substance	Lindemann (1982) and Auster and Schafer (1958) cited by Hänsel *et al.*, 1992 *AT > 30 years

* Taking into account that the dry herbal substance includes sometimes also comminuted flowers (see Commission E monograph, 1986), the traditional use of herbal substance can be extrapolated to the comminuted substance. The indications and posology are similar.

** in Austria for more than 30 years

Assessor's comment:

All herbal preparations mentioned above except, tincture (1:5), extraction solvent ethanol 70% (V/V) have been in medicinal use for 30 years or more according to the literature and information provided by Member States.

The tincture (1:5), extraction solvent ethanol 70% (V/V) preparation was included in the previous version of the monograph published 2009 and also in the list entry.

It was decided to maintain this herbal preparation in the monograph/list entry because the extraction solvent and the posology is in the range of the other preparations listed in the monograph/list entry.

For the semi-solid dosage forms, the wording proposed in the monograph for the single dose is "apply a thin layer of semi-solid preparation to the affected area''.

Dosage frequency accepted for all preparations in the previous version of the monograph: 2-4 times daily. Taking into account the historical references (Bradley, 1992-2006; Fintelmann *et al.,* 2002) that indicated "several times daily", the frequency was not changed during the revision.

Use in children

In the standard text book on phytotherapy by Weiss (Fintelmann *et al*., 2002) it is stated that Calendula preparations are superior to Chamomile in the topical treatment of nappy rash.

The Council of Europe published in 1989 a document where the use of certain preparations of Calendula flowers is allowed for cosmetic baby toiletries. Cosmetic products are intended to be used on the intact skin. Therefore, the medicinal use of Calendula flowers for minor inflammations of the skin and as an aid in healing of minor wounds cannot be recommended in the same way for babies.

For the preparations included in the monograph, there is no observational data published on the safe use of these in the paediatric population. However, the common use, for example in the treatment of nappy rash, indicates a certain degree of safety. As a compromise, when the monograph was developed the age limits of 6 years (indication a) and 12 years (indication b) were given in the previous version of the monograph.

At time of revision, no new safety data are available after 8 years, therefore so the existing age limits are kept unchanged.

3. Non-Clinical Data

The extracts used in the trials are specified in the comments as far as possible. Unfortunately, in many publications correct specifications of solvent and drug-extract ratio (DER) are missing. In these cases no details can be given, if the extract could not be identified otherwise.

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

3.1.1. Primary pharmacodynamics

Effects on wound healing and anti-inflammatory activity of Calendula flower preparations have been investigated.

Effects on wound healing

In vitro experiments

Herbal preparations

Nicolaus *et al.,* 2017 investigated *in vitro* the effects of three different extracts from Calendula flowers (n-hexanic, ethanolic, aqueous) on the inflammatory phase of wound healing in human immortalized keratinocytes and human dermal fibroblasts. The extracts were obtained using 502 g powdered flower heads of marigold and 5.8-5.9 L solvent, at about 80 °C for 8 h in a Soxhlet apparatus; the solvents were removed under vacuum at 40 °C. The effect of the Calendula extracts on the new tissue formation phase of wound healing was evaluated by studying the migratory properties of these extracts, triterpene mixtures and single compounds in human immortalized keratinocytes using the scratch assay. Finally, the effect of the extracts on the formation of granulation tissue in wound healing was studied using bacterial collagenase isolated from Clostridium histolyticum and the determination of soluble collagen in the supernatant of human dermal fibroblasts. The n-hexanic and the ethanolic extracts (concentrations of 10 and 50 µg/ml) influence the inflammatory phase by activating the transcription factor NF-κB and by increasing the amount of the chemokine IL-8, both at the transcriptional and protein level, in human immortalized keratinocytes. The migration of the keratinocytes during the new tissue formation phase was only marginally influenced in the scratch assay. The ethanolic extract (EE) as well as the aqueous extract (AE) significantly reduced collagenase activity compared to the solvent control in a concentration-dependent manner but the inhibition was only moderate. A concentration of 500 µg/ml of AE diminished the collagenase activity to 45.34 %, whereas a lower concentration of EE, 100 µg/ml, led to a similar reduction (44.48 %). Because of solubility problems the n-hexanic extract could not be tested in the collagenase inhibition assay.

In vivo experiments

Herbal preparations

The wound healing process involves several distinct phases in which the information of the new blood vessels (angiogenesis) plays an important role. In the chick chorioallantoic membrane (CAM) test using incubated hen eggs, a freeze-dried, cold aqueous infusion of Calendula flower proved highly angiogenic, the number of microvessels counted in treated tissue sections being significantly higher than in control CAMs ($p<0,0001$). Hyaluronan, which is known to be involved in the information, alignment and migration of newly formed capillaries, was detected in all Calendula flower treated CAMs, while none was found in untreated CAMs. The high level of neovascularisation observed in treated CAMs was attributed to effects of the Calendula flower extract, in which the predominant constituents were flavonoids (Patrick *et al*., 1996).

Dry 70%-ethanolic (E) and aqueous (A) extracts of Calendula flower, applied topically as 5% ointments, accelerated the healing of surgically inflicted skin wounds in rats; the degree of epithelialisation was 73% (E) and 65% (A) by the 5^{th} day, and 90% (E) and 88% (A) by the 10^{th} day compared to 60% and 79% in control animals treated with vehicle only. In similar experiments, addition of allantoin to the ointment enhanced the effect of the extracts; by the 14^{th} day, compared to 70% in controls and 79% with allantoin alone, the degree of healing was 80% with A + E, and 90% with A + E + allantoin in a 2:2:1 ratio ($p<0.01$) (Klouchek-Popova *et al*., 1982 cited in Hänsel *et al*., 1992).

A topically applied Calendula ointment had better influence on epithelisation of artificially infected wounds (Staphylococcus epidermides) in rats than a combination of Comfrey, propolis and honey (Perri de Carvalho *et al*., 1991).

A Calendula ointment (containing 5% dry extract; no further detail) enhanced the healing of experimental wounds in buffalo calves (Ansari *et al*., 1997 cited in ESCOP 2003).

Shafeie *et al.*, 2015 compared *in vivo* the effects of different concentrations of *Calendula officinalis* gels (containing a dry ethanolic extract; extraction solvent: ethanol 70% V/V) on cutaneous wound healing in rats. The male rats were randomly divided into three groups (control, placebo, and treatment group). Under sterile conditions, a 2×2-cm piece of cervical skin for histopathological groups and a rectangular shape with a metal ruler from cervical to lumbar region for biomechanical groups, were excised in each animal. Treatment group received a daily topical application of 5%, 7%, and 10% Calendula gel, the placebo group received a daily topical application of the base gel, and the control group received no treatment during this experimental study. Fourteen and 21 days later, the rats were euthanized and biopsies were taken from the site of the initial incisions and samples were collected for histopathological and biomechanical investigation. Histopathological and biomechanical restorations in the group treated with 7% gel were significantly more than the placebo and control group. Upper and lower doses seem to be less effective, although the reasons for this remain unclear.

Dinda *et al.,* 2016 studied the mechanism of *Calendula officinalis* flowers dry ethanolic extract (CEE; extraction solvent: ethanol 50% V/V) and its active fraction (dry water fraction of ethanolic extract, WCEE) on primary human dermal fibroblasts (HDF). *In vivo*, CEE or WCEE were topically applied on excisional wounds of BALB/c mice and the rate of wound contraction and immunohistological studies were carried out. The authors found that CEE and its WCEE (in the range 50–100µg/mL) significantly stimulated the proliferation as well as the migration of HDF cells. In addition, they up-regulated the expression of connective tissue growth factor (CTGF) and α-smooth muscle actin(α-SMA) *in vitro*. *In vivo*, CEE or WCEE treated mice groups (150mg extract/kg body weight) showed faster wound healing and increased expression of CTGF and α-SMA compared to placebo or control group. The authors considered that the increased expression of both the proteins during granulation phase of wound repair demonstrated the potential role of Calendula flowers in wound healing.

Because of the differences in the structure of the skin between humans and animals these data should be interpreted carefully (Wissinger-Gräfenhahn U, 2000).

Anti-inflammatory effect

Herbal preparations

A 70% alcoholic extract and a CO_2 extract were applied topically in the croton oil ear oedema test in mice. The 70% hydroalcoholic extract had a mild dose-dependent effect, inhibiting oedema by 20% at dose of 1200 µg/ear. The CO_2 extract produced 30% inhibition at 150 µg/ear and 71% inhibition at 1200 µg/ear. The activity at the higher concentration was comparable to that of indometacin at 120 µg/ear (Della Loggia *et al*., 1990 cited by ESCOP 2003).

In the same model, triterpenoids were shown to be the most important anti-inflammatory principles in the CO_2 extract (Della Loggia *et al.,* 1994).

Isolated compounds

The esters of faradiol are about 50% less active as anti-oedematous agents in croton-oil induced oedema of the mouse ear, while the free monols (like taraxasterol, lupeol) are less active than the diols. The most active substance in the croton oil test is faradiol, its molar activity is comparable to indometacin (Zitterl-Eglseer *et al*., 1997).

Isorhamnetin 3-glycosides isolated from Calendula flower inhibited lipoxygenase (a key enzyme in the synthesis of leukotrienes) from rat lung cytosol at a concentration of 1.5×10^{-5} M (Bezakova *et al*., 1996).

Ukiya *et al.*, 2006 tested ten oleanane-type triterpene glycosides along with five flavonol glycosides from the flowers of *Calendula officinalis*. Eight triterpenes exhibited a marked anti-inflammatory activity in the TPA-induced inflammation in the mouse ear with ID_{50} values of 0.05-0.32 mg/ ear.

They were almost comparable and in part even more inhibitory than the anti-inflammatory agent indomethacin (ID_{50} 0.3 mg/ear) used as positive control.

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				
n-hexanic, ethanolic and aqueous extracts	10-500 µg/ml	*In vitro* Human keratinocytes and dermal fibroblasts	Nicolaus *et al.,* 2017	Influenced the inflammatory phase dose-dependently manner
Dry ethanolic extract of *Calendula officinalis* flower (extraction solvent 70% ethanol) and dry aqueous extract (no further detail)	Topically as 5% ointments (±alantoin)	*In vivo* Rats	Klouchek-Popova *et al*., 1982 (cited in Hänsel *et al*., 1992)	Accelerated wounds healing compared with the control group
Dry ethanolic extract of *Calendula officinalis* flower (extraction solvent 70% ethanol; no further detail)	Topically 5%, 7%, and 10% as gel	*In vivo* Rats	Shafeie *et al*., 2015	Histopathological and biomechanical restorations in the group treated with 7% gel were significantly more than the placebo and control group. 5% and 10% seem to be less effective
Dry ethanol extract of *Calendula officinalis* flower (extraction solvent ethanol 50% V/V) Dry water fraction of the ethanolic extract	50–100 µg/mL *(in vitro)* Topically, 150 mg/kg bw *(in vivo)*	*In vitro* Human dermal fibroblasts *In vivo* BALB/c mice	Dinda *et al.,* 2016	*In vitro*: both preparations had anti-inflammatory effect. *In vivo*: both preparations showed faster wound healing compared to placebo group.
A 70% alcoholic extract and a CO_2 extract	Topically 150-1200 µg/ear	Mice *In vivo*	Della Loggia *et al*., 1990 (cited in ESCOP, 2003)	Both preparations exerted anti-inflammatory effect dose-dependent; CO_2 extract was more potent
Isolated compounds				
Isorhamnetin 3-glycosides	1.5×10^{-5} M	*In vitro*	Bezakova *et al*., 1996	Inhibited lipoxygenase
10 oleanane-type triterpene glycosides and 5 flavonol glycosides	Topically; no detail	Mice *In vivo*	Ukiya *et al.*, 2006	Some compounds exhibited anti-inflammatory effect (ID_{50} values of 0.05-0.32 mg/ ear)

3.1.2. Secondary pharmacodynamics

Antimicrobial activity

The essential oil of Calendula flowers inhibited the growth of *Bacillus subtilus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The activity was attributed to the terpene alcohols and terpene lactones (Janssen *et al*., 1986).

The essential oil exhibited also a weak fungicide activity against dermal fungi like *Trichophyton mentagrophytes* var. *interdigitale*, *Trichophyton rubrum*, *Trichophytum concentricum* and *Epidermophyton floccosum* (Hänsel *et al.,* 1992).

A fraction containing flavonoids isolated from the leaves inhibited the growth of *Sarcina lutea*, *S. aureus*, *E. coli*, *Klebsiella pneumoniae* and *Candida monosa*, the saponins were not effective (Tarle *et al.,* 1989). The water soluble components of ethanolic extracts are active against *Staphylococcus aureus* (Dumenil *et al.,* 1980). An antimicrobial activity against several bacteria is also documented for infusions with a DER 1:10 (Gasiorowska, 1983, cited in Hänsel *et al*., 1992).

The methanol extract (no further detail) exhibited a weak activity against periodontopathic bacteria, a decoction showed even less potential (Iauk *et al*., 2003). Compared to a solution of NaF and sodium lauryl sulphate an extract of Calendula flowers had no antimicrobial effect on biofilms and oral microorganisms from children (Modesto, 2000).

Efstratios *et al.,* 2012 investigated the antimicrobial potential of *Calendula officinalis* methanolic and ethanolic extracts (10 g flowers/150 ml solvent) against a panel of microorganisms including *Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cereus, E. coli, Klebsiella aerogenes, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae* and pathogenic fungi (*Candida albicans, Aspergillus flavus Exophiala*) using the disc diffusion assay. The methanolic extract exhibited better antibacterial activity against most of the bacteria tested than the ethanolic extract. Both extracts were also reported to have excellent antifungal activity against some tested strains of fungi, when compared with fluconazole.

Healing ulcerative colitis

Mehrabani et *al.,* 2011 investigated *in vivo* the efficacy of a *Calendula officinalis* extract in treatment of experimentally induced ulcerative colitis in a dog animal model. Ulcerative colitis (UC) was induced with 6% acetic acid as enema and the method of treatment was retrograde (via enema) too. After histological confirmation of induction of UC in all animals, they were randomly divided into two groups. Group A received *Calendula officinalis* extract (no further detail) via enema (40% solution, 3 mL/day until 30 days) and Group B that received a saline enema (3 mL/day). The Calendula preparation could successfully resolve the damages of UC. On days 30th and 45th, the mucosal healing was statistically significant compared to the 7th and 14th day.

Tanideh et *al.,* 2016 also investigated the healing effects of *Calendula officinalis* flower hydroalcoholic extract in experimentally induced ulcerative colitis in male rats. The extract was obtained with ethanol: water (80:20) solution for 72 h using the percolation method. The extract was filtered and evaporated in a rotary evaporator under reduced pressure then dried in temperature of 50°C for 72 h. Ulcerative colitis was induced by 3% acetic acid and oral doses of *Calendula officinalis* extract, 1500 and 3000 mg/kg, and enema (gel 10% and 20%) were given. Two groups as positive controls were given asacol (enema) and oral mesalamine. Negative control groups were given normal saline and base gel. On days 3 and 7, intestinal histopathology and weight changes, plus oxidative stress indices including malondialdehyde (MDA) level and myeloperoxidase (MPO) activity were assayed. A significant increase in the body weight of rats was seen in the group given *Calendula officinalis* extract 3000 mg/kg orally,

oral mesalamine, and 20% intracolonic gel form of marigold extract compared with negative control and base gel groups during the experimental period. Acute inflammation and granular atrophy after UC induction were resolved completely by both 20% intracolonic gel and 3000 mg/kg orally. An increase in MPO activity and a decrease in MDA level in response to oral and intracolonic gel form of *Calendula officinalis* were observed 3 and 7 days after treatment ($P < 0.05$).

Antiviral activity

A tincture of Calendula flower suppressed the replication of herpes simplex, influenza A2 and influenza APR-8 viruses *in vitro* (Bogdanova *et al*., 1970), however, an aqueous extract was not active (May *et al*., 1978). A chloroform extract inhibited the replication of HIV Type I in acutely infected lymphocytic Molt-4 cells *in vitro*. A chloroform extract also inhibited the HIV-I reverse transcriptase activity in a dose dependent manner (Kalvatchev *et al*., 1997).

Immunostimulation

Polysaccharide fractions from Calendula flower (molecular weight in the range of 25000-500000) showed significant immunostimulating activity in the granulocytes – and carbon clearance tests (Wagner *et al*., 1985).

Isolated polysaccharides from Calendula flower were found to stimulate phagocytosis of human granulocytes (Varljen *et al*., 1989).

Amirghofran *et al.*, 2000 found that extracts of Calendula flowers do not show a direct mitogenic effect on human lymphocytes and thymocytes.

Antitumoral activities

The monodesmosides Arvenoside B and D exhibit an *in vitro* cytotoxic effect on HeLa-cells, B 16-melanoma cells, 3T3 fibroblasts and human 2002-cells (Quetin-Leclerque *et al*., 1992). Triterpenes like faradiol and taraxasterol inhibit experimental tumor promotion and are therefore considered as inhibitors of tumor growth (Yasukawa *et al*., 1996).

Dietary lutein from Calendula flowers increased tumor latency and inhibited mammary tumor growth in mice (Chew *et al*., 1996; Park *et al*., 1998).

Two triterpenes of Calendula flowers showed cytotoxic effects against colon cancer, leukaemia, and melanoma cells (Ukiya *et al*., 2006).

Spasmogenic and spasmolytic activities

Activity directed fractionation of an aqueous-ethanol extract of Calendula flowers showed that the spasmolytic activity was concentrated in the organic fraction, while the aqueous fraction exhibited a marked atropine sensitive spasmogenic effect (Bashir *et al*., 2006).

Hepatoprotective activity

Calendula extract (liquid extract DER 1:1, solvent ethanol 70%) was examined in CCl_4-intoxicated rat livers. It was able to reduce the hepatocytolysis by 28% compared to control, to reduce histological modifications as well as enzyme and steatosis modifications (Rusu *et al.,* 2005).

Antioxidant activity

The butanolic fraction of Calendula flowers possesses a significant free radical scavenging and antioxidant activity (Cordova *et al*., 2002, Herold *et al*., 2003).

The antioxidant activity of a cream with 0.9% of dry Calendula extract (5:1, no further detail) was demonstrated *in vitro* on the mitochondria of rat cardiac muscles by Bernatoniene *et al*., 2011.

Another ethanolic extract (1:9, extraction solvent: 50% ethanol V/V) demonstrated *in vitro* its antioxidant efficacy activity against various radicals. The IC_{50} values were 97.1±2.1 µg/mL, 350.0±13.1 µg/mL and 4.4±0.9 µg/mL for the DPPH system, lipid peroxidation assay and xanthine/luminol/XOD assay, respectively. *In vivo* the protective effect against UVB-induced oxidative stress in the skin was evaluated by determining reduced glutathione (GSH) levels and monitoring the secretion/activity of metalloproteinases. Oral treatment of hairless mice with 150 and 300 mg/kg of ME maintained GSH levels close to non-irradiated control mice. In addition, this extract affects the activity/secretion of matrix metalloproteinases 2 and 9 (MMP-2 and -9) stimulated by exposure to UVB irradiation (Fonseca *et al*., 2010)

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

The medicinal use of Calendula flower preparations for the symptomatic treatment of minor inflammations of the skin or the oral mucosa, and as an aid in healing minor wounds is documented in a number of handbooks.

Some *in vitro* and *in vivo* studies were conducted to investigate the anti-inflammatory activities of Calendula preparations or on some isolated compounds, but the relevance of these data is uncertain, as none of the extracts tested are comparable/similar to preparations included in the monograph**.**

Some secondary pharmacodynamic effects were identified but the relevance of these data is limited as the results refer to isolated compounds or are on herbal preparations which are not included in the monograph.

The published non-clinical data give some but limited support to the traditional uses.

The indications included in the monograph are supported by the traditional topical use of preparations from Calendula flowers in the proposed indications for a period of at least 30 years in Europe.

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

No specific data are available on Calendula flowers.

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

3.3.1. Single dose toxicity

a) Herbal substance

No data available.

b) Herbal preparations

For an aqueous extract from Calendula flower administered to mice, the intravenous LD_{50} was determined as 375 mg/kg body weight and the intraperitoneal LD_{100} as 580 mg/kg (Manolov *et al.*, 1964).

Largato *et al.*, 2010 observed that 2000 mg/kg of an aqueous extract (obtained by decoction, solvent: herbal substance ratio 3:1) administered orally by gavage to Wistar rats did not induce any signs of toxicity.

For a hydroalcoholic extract (DER 1:1, 30% ethanol) the subcutaneous LD_{50} was 45 mg in mice and the intravenous LD_{50} was 526 mg/100g in rats (Boyadzhiev *et al.,* 1964).

An ethylene glycol extract (DER 2:1) was non-toxic in albino mice after subcutaneous administration of 10 ml/kg (Russo, 1972).

Calendula oil has a LD_{50} of 20 ml/kg rat p.o. (cited in Blaschek *et al*., 2006).

Hydroethanolic dry extracts showed no signs of toxicity when administered orally to mice and rats up to a dose of 5 g/kg (Silva *et al*., 2007).

3.3.2. Repeat dose toxicity

a) Herbal substance

No data available.

b) Herbal preparations

An aqueous extract was reported to be non-toxic on chronic administration to mice (Manolov *et al.,* 1964).

No symptoms of toxicity were observed after oral administration of a Calendula flower extract (solvent unspecified) at 0.15 g/kg body weight to hamsters over 18 months and to rats over 21 months (Avramova *et al*., 1988).

No death of experimental animals was detected during oral administration of a hydroethanolic dry extract in a dose of 1 g/kg for 30 days. The biochemical profile showed no changes for most of the parameters, however, there was a dose dependent increase of blood urea nitrogen and of the ALAT level in doses up from 0.25 g/kg extract. The authors interpret these findings as due to hepatic overload (Silva *et al*., 2007).

Largato *et al.*, 2011 studied the oral toxicities of *Calendula officinalis* flower aqueous extract (obtained by decoction of plant material for 45 min and the ratio of solvent volume to the weight of the plant material was 3:1) in male and female Wistar rats. Doses of 50, 250 and 1000 mg/kg/day were administered in drinking water. Several of the blood elements were significantly affected in males and females after 90 days. Hemoglobin concentration was significantly decreased in females at dosages of 250 and 1000 mg/kg/day. Hematocrit level was significantly depressed in the male high dose group. Erythrocyte and leukocyte levels, in both males and females, were significantly increased in a dose-dependent manner and blood clotting time was significantly prolonged in males at 250 and 1000 mg/kg/day. Of the biochemical parameters tested, ALT and AST appeared to be affected. ALT levels were significantly decreased in males in a dose-dependent manner and significantly increased in females at of 50 and 250 mg/kg/day. AST levels were significantly increased in females at doses of 50 and 250 mg/kg/day. Histopathological examination of tissues showed slight abnormalities in hepatic parenchyma that were consistent with biochemical variations observed. Authors concluded that toxicity of *Calendula officinalis* extract is low.

c) Isolated compounds

No toxic symptoms appeared in rats after daily oral administration of calenduloside B at 200 mg/kg body weight for 2 months (Yatsuno *et al.,* 1978).

3.3.3. Genotoxicity

a) Herbal preparations
In the Ames test using *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100, a fluid extract (60% ethanol) was non-mutagenic at concentrations of 50-5000 µg/plate. With *Aspergillus nidulans* diploid strains genotoxic effects with mitotic crossing over and chromosome malsegregation were observed at higher concentrations of 0.1-1.0 mg/ml, at which a concentration-dependent increase of cytotoxicity also occurred. These findings were not confirmed *in vivo* in the mouse bone marrow micronucleus test; after oral administration of the extract up to 1 g/kg body weight for two days no increase in the number of micronucleated polychromatic erythrocytes was observed (Ramos *et al*., 1998).

An aqueous extract showed no genotoxic effects in the Drosophila Wing Somatic Mutation and Recombination Test (SMART) (Graf *et al.,* 1994).

Perez-Carreon *et al.*, 2002 investigated whether dry extracts prepared from Calendula flowers by several solvents are able to induce unscheduled DNA synthesis in rat liver cell cultures and whether they can reverse diethylnitrosamine induced unscheduled DNA synthesis. Polar extracts (solvents water and water/ethanol) completely reversed the effect of diethylnitrosamine in very low concentrations (50 ng/ml and 0.4-16 ng/ml, respectively). In the absence of diethylnitrosamine these two extracts induced at higher concentrations (three orders of magnitude above total protection) unscheduled DNA synthesis. Lipophilic extracts showed no or only slight effects in this model. The authors concluded that flavonoids may act in lower concentrations as radical scavengers, while in higher concentrations their oxidizing potential is dominating.

In a model using diethylnitrosamine treated rats the protecting effect could be demonstrated up to a dose of 10 mg/kg; at higher concentrations increased altered hepatocyte foci could be detected (Barajas-Farias *et al*., 2006).

Bakkali F *et al.*, 2005 found that the essential oil of *Helichrysum italicum* (the authors declare this name as a synonym of *Calendula officinalis*) exhibits only a weak cytotoxicity; in contrast to other essential oils it did not induce cytoplasmatic petite mutations indicating damage to mitochondrial DNA.

Assessor's comment: the value of this paper is limited, because in the botanical literature Helichrysum italicum is not mentioned as a synonym of Calendula officinalis, therefore the plant source for the tested essential oil remains unclear.

b) Isolated compounds

Six saponins from Calendula flower (at 400 µg/plate) were non-mutagenic in the Ames test using *Salmonella typhimurium* TA98 with and without S9 activation (Elias *et al*., 1990).

3.3.4. Carcinogenicity

Carcinogenicity studies with Calendula flower extract (solvent not mentioned) have been performed in rats over a period of 22 months and in hamsters over a period of 18 months with a daily oral dose of 0.15 g/kg body weight. The extract was not carcinogenic in either species (Avramova *et al*., 1988).

Because the preparation used is not well characterized (undefined extract), the test is not considered adequate, therefore section 5.3 of the monograph states "Adequate tests on carcinogenicity have not been performed".

3.3.5. Reproductive and developmental toxicity

Water infusion of Calendula flower (no further details) was shown to have an uterotonic effect when applied *ex vivo*, to isolated rabbit and guinea pig uterine horn (Shipochliev, 1981 cited in Mills, 2006).

Silva *et al.,* 2009 evaluated the effects of the administration of an ethanolic extract of Calendula flowers (extraction solvent: ethanol 70% V/V; 0.4% total flavonoids; no further detail) on the reproductive function of Wistar rats. Four groups of adult male rats were treated orally at doses of 0, 0.25, 0.5 and 1.0 g/kg for 60 consecutive days. From day 53 to 60 of treatment, rats were mated with untreated and fertile female rats. Reproductive parameters including testicular morphology, reproductive organ weights, fertility index and offspring viability were evaluated. In another protocol, groups of pregnant rats were treated orally with the same doses of preparation from days 1 to 6 (preimplantation period), 7 to 14 (organogenic period) or 15 to 19 (fetal period) of pregnancy. On day 20 of pregnancy, rats were killed for evaluation of maternal and fetal parameters. The results showed that the treatment with ethanolic extract did not affect male reproductive parameters. Besides, it was non-toxic in the preimplantation and organogenic periods of pregnancy. However, the preparation induced a decrease of the maternal weight gain when administered during the fetal period. Authors concluded that the preparation did not affect male fertility nor had toxic effects in early and middle periods of pregnancy, but caused maternal toxicity when administered during the fetal period of pregnancy.

Because the preparation used by Silva *et al*., 2009 is not well characterized, the test is not considered adequate, therefore section 5.3 of the monograph states "Adequate tests on reproductive toxicity have not been performed".

3.3.6. Local tolerance

According to Andersen *et al*., 2010 the dermal irritation potential of a 10% aqueous *Calendula officinalis* flower extract (no further details) was tested on rabbits in a single-insult occlusive patch test (SIOPT). The material produced no irritation, had a primary irritation index of 0.0, and was not considered an irritant.

The same authors reviewed the information regarding other preparations not included in the monograph (e.g. water/ propylene glycol extracts) and concluded that were non-irritant. These results have no impact on the monograph.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

Single dose toxicity tests and repeated-dose studies with Calendula flower preparations show low toxicity in animals.

Genotoxicity data are only available for hydroethanolic liquid extracts. *In vitro* data (Ames test with *S. typhimurium* strains TA1535, TA1537, TA98 and TA100) with a fluid ethanolic extract (60% ethanol)

showed a negative outcome. *In vivo*, the mouse bone marrow micronucleus test was also negative. Therefore a European Union list entry is proposed for this type of extract only.

Ex vivo an aqueous preparation exhibited an uterotonic effect, while *in vivo* in pregnant rats another preparation caused maternal toxicity when administered during the fetal period of pregnancy. Because the preparation used *in vivo* is not well characterized, the test is not considered adequate.

Negative carcinogenicity data are available in two species (rats and hamsters) for a preparation not well characterized, therefore the results are not considered adequate to be included in the monograph.

3.4. Overall conclusions on non-clinical data

Results from *in vitro* and *in vivo* studies with extracts and isolated constituents, support the indications.

Specific data on pharmacokinetics and interactions are not available.

Toxicological data indicate low toxicity in animals.

Genotoxicity data are only available for hydroethanolic liquid extracts, therefore, a European Union list entry is proposed for this type of extract.

Developmental toxicity data revealed that an ethanolic extract caused maternal toxicity when administered during the fetal period of pregnancy. Based on this information, and taking into account the uterotonic effect exhibited by another preparation, the use during pregnancy and lactation cannot be recommended.

Adequate tests on reproductive toxicity and carcinogenicity have not been performed.

During the longstanding use as a medicinal product in the European Union no serious side effects have been reported. Therefore, the cutaneous and oromucosal use of Calendula preparations can be regarded as safe under the conditions of use described in the monograph.

4. Clinical Data

The extracts used in the trials are specified in the comments as far as possible. Unfortunately, in the majority of publications correct specifications of solvent and drug-extract ratio (DER) are missing. In these cases no details can be given, if the extract could not be identified otherwise.

4.1. Clinical pharmacology

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

No specific data are available on Calendula flower.

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

No specific data are available on Calendula flower.

4.2. Clinical efficacy

4.2.1. Dose response studies

There are no dose response studies available.

4.2.2. Clinical studies (case studies and clinical trials)

Several clinical studies involving human patients have been performed to test the efficacy of herbal preparations in patients with skin conditions, such as venous leg ulcers, skin burns or acute dermatitis.

Phase III clinical studies

Controlled study of three ointments for the local management of 2nd and 3rd degree burns (Lievre *et al.,* 1992):

Randomized, controlled, open study with parallel groups (only adults > 18 years of age; 53 patients treated with Pommade au Calendula par digestion (prepared by extraction in vaseline), 53 patients treated with Elase (proteolytic ointment), 50 patients treated with vaseline (control treatment)). A marginally significant difference in favour of Calendula over vaseline was observed (p=0.05) after 17 days of treatment. Calendula was significantly better tolerated than the other treatments.

Assessor's comment:

The study medication was prepared not only from the flowers but also stems and leaves of Calendula officinalis. Therefore, the results of this study are of limited relevance for the assessment of preparations containing the ligulate florets only. However, the results support the safe use of preparations containing Calendula.

Phase III randomized single blinded trial of *Calendula officinalis* compared with Trolamine for the prevention of acute dermatitis during irradiation for breast cancer (Pommier *et al.,* 2004):

254 patients who had surgery for breast cancer and who were to receive postoperative radiation therapy were randomly allocated to application of either trolamine (128 patients) or Calendula (126 patients), twice daily or more on the irradiated fields after each session. The Calendula ointment (Pommade au Calendula par digestion) contained 20% of fresh Calendula aerial parts in petroleum jelly. The primary end point was the occurrence of acute dermatitis of grade 2 or higher. Secondary end points were the occurrence of pain, the quantity of the topical agent used and patient satisfaction. The occurrence of acute dermatitis of grade 2 or higher was significantly lower (41% v 63%; P<0.001) with the use of Calendula than with trolamine. Moreover, patients receiving Calendula had less frequent interruption of radiotherapy and significantly reduced radiation-induced pain.

No allergic reactions occurred in the group using the Calendula preparation, whereas four patients using trolamine developed allergic-type reactions (pruritis and urticaria).

Assessor's comment:

The study medication was prepared not only from the flowers but also stems and leaves of Calendula officinalis. Therefore, the outcome of the study does not justify the well-established use of Calendula flower in the prevention of acute dermatitis under postoperative irradiation.

*This may be the reason why in an overview and practice guideline for prevention and management of acute skin reactions related to radiation therapy the authors conclude that there is insufficient evidence to support or refute topical agents (*Bolderston *et al.,* 2006*).*

Observational studies

Protective effects of different cream preparations containing Calendula extract against sodium-lauryl-sulfate induced irritant contact dermatitis (Fuchs *et al*., 2005):

The extract (prepared with supercritical CO_2) in a base cream according to DAC was tested in 20 healthy volunteers with experimentally induced irritant contact dermatitis in a 4-day repetitive irritation test. A statistically significant protective effect was observed; the sequential treatment (post-irritation) was without any effect (Fuchs *et al*., 2005).

Assessor's comment:

The extract which has been used in this study does not fulfil the criteria for traditional use.

Pilot study with a Calendula jelly containing 10% of a homoeopathic mother tincture in 30 patients with first- and second-degree burns (Baranov, 1999):

In an open, uncontrolled pilot study, 30 patients with burns or scalds (degree 1 and 2a) were treated 3 times per day for up to 14 days with a hydrogel containing 10% of hydroethanolic extract. The symptoms reddening, swelling, blistering, pain, soreness and heat sensitivity were scored before, during and at the end of treatment. The total score and individual scores for each symptom improved.

Assessor's comment:

The lack of a control group makes the evaluation of the efficacy impossible. However, the study medication was well tolerated and no adverse effects have been observed. This study supports the traditional use of Calendula for the treatment of minor inflammations of the skin because of the chemical similarity of homoeopathic mother tinctures and phytotherapeutic tinctures.

Observational study of an ointment containing Calendula in the treatment of venous leg ulcers (Duran *et al*., 2005):

34 patients were divided into an experimental group (21 patients with 33 venous ulcers) receiving a Calendula preparation (65 g flowers were extracted with absolute ethanol then dried and dispersed in a neutral base at concentration of 7.5%) and a placebo group (13 patients with 22 venous ulcers) receiving saline solution dressing. The therapy was applied twice daily for 3 weeks. In the experimental group, a significant acceleration of wound healing (expressed as the surface of the ulcers) could be observed. No allergic reactions occurred in the group given the Calendula preparation.

Assessor's comment:

This observational study does not fulfil the criteria for classification of this type of treatment in the category 'well established use'. The indication 'topical treatment of venous leg ulcer' is not considered as suitable for traditional use without medical supervision.

During revision 1: Two new trials were identified (Buzzi *et al.,* 2016(a and b)), that investigated the efficacy of a specific preparation, *Plenusdermax* (a hydroglycolic extract of *Calendula officinalis*, with a total flavonoid content of 120 mg/ml), in patients with venous leg ulcers.

(Buzzi *et al*., 2016a):

In an observational cohort study on 41 patients with a diagnosis of pressure ulcer that was stable in size for more than three months, the therapeutic benefits of the *Calendula officinalis* special preparation for pressure ulcer healing have been evaluated. Patients were evaluated every two weeks, for 30 weeks, for: reduction of the wound area, infection control, types of tissue and exudate, and ulcer microbiology. The proportions of patients who were completely healed after 15 and 30 weeks of

treatment were 63% and 88%, respectively, and the mean healing time was 12.5±7.8 weeks. No adverse events were observed during treatment.

(Buzzi *et al.,* 2016b):

In a prospective non-randomised controlled study patients treated with *Calendula officinalis* extract (n=38) and control patients (n=19) were evaluated every two weeks for 30 weeks or until their ulcers healed. The patients in the control group were treated using the hospital standard procedure, which included the use of collagenase (0.6 U/g), chloramphenicol (10 mg/g), and 1% silver sulfadiazine. Twice a day, the ulcers were cleaned with 25 ml sterile physiological saline solution immediately before application of the *Calendula officinalis* extract or the standard of care product. The extract was sprayed on the wound bed and allowed to dry for a few minutes. Conventional dressings with sterile non-adherent gauze and non-elastic supportive bandages were used in all patients. Assessments included determination of the wound area by planimetry, infection control, and evaluation of the clinical aspects of the wounds. The percentage of healing velocity per week (% HVw), taking the initial area at baseline into account, was also determined. The proportion of the treatment patients achieving complete epithelialisation was 72% and 32% in the treatment and control groups, respectively. The average healing time was approximately 12 weeks in the treatment group and 25 weeks in control patients. Patients with ulcers treated with *Calendula officinalis* extract had a significant 4-fold increase in percentage healing velocity per week, 7.4%, compared with 1.7% in the control group.

No adverse events were observed during the *Calendula officinalis* extract treatment.

Assessor's comment:

Because the herbal substance used for the extract is not well defined (it is not known if it contained only the flowers or also the stems) both studies cannot be used to support a well-established use indication.

Another blinded, randomized clinical trial was conducted on 411 patients in order to compare the effects of Calendula cream (contains extract of Calendula plant 10%; no further detail) and another product (Essex - a standard skin care treatment) in reducing the risk of severe acute radiation skin reactions (ARSR) in relation to adjuvant radiotherapy (RT) for breast cancer (Sharp *et al*., 2013). The primary endpoint was the difference in proportion of patients with ARSR, assessed with the Radiation Therapy Oncology Group/The Organization for Research and Treatment of Cancer Acute Radiation Morbidity Scoring Criteria (RTOG/EORTC scale) at follow-up. The incidence of severe ARSR (RTOG/EORTC grade 2) at the follow-up visit was 23% (n=45) in the Calendula group and 19% (n=38) in the Essex group. No difference in severe ARSR between the groups at any point of assessment was found.

Table 5: Clinical studies on humans

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Lievre *et al.,* 1992	Randomised controlled, open study	Control: vaseline Verum: Calendula preparation (flowers, leaves and stems) Group Elase: proteolytic ointment Topically Duration: 17 days	156 patients (>18 years) Control (n=50) Verum (n=53) Group Elase (n=53)	Patients with 2nd and 3rd degree burns	Principal outcome: efficacy	Student's t-test	The results of this study are of limited relevance as the Calendula preparation includes leaves and stems
Pommier *et al.,* 2004	Randomised, placebo-single-blind study	Control: trolamine Verum: Calendula ointment (20% of fresh Calendula aerial parts in petroleum jelly) Topically, twice daily or more Duration: 7 days	254 women (18-75 years old) Control (n=128) Verum (n=126)	Acute dermatitis during irradiation for breast cancer	The primary end point: the occurrence of acute dermatitis of grade 2 or higher. Secondary end points: the occurrence of pain, the quantity of the topical agent used and patient satisfaction.	Fischer test	The results of this study are of limited relevance as the Calendula preparation includes leaves and stems
Baranov, 1999	Open-uncontrolled study	Verum: hydrogel containing 10% of a homoeopathic mother tincture Topically, 3 times daily Duration: 14 days	30 patients Verum (n=30)	Patients with 1st and 2nd degree burns	Outcome measures: reddening, swelling, blistering, pain, soreness and heat sensitivity	None	Limited value
Duran *et al.*, 2005	Observational study	Verum: ethanolic extract Placebo: saline solution dressing Topically, twice daily 1 g ointment/ cm^2 of ulcer Duration: 3 weeks	34 patients Verum (n=21) Placebo (n=13)	Patients with venous ulcers	The clinical outcome: wound healing (expressed as the surface of the ulcers)	Student's t-test	Limited value

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Buzzi *et al*., 2016a	Observational study	Verum: hydroglycolic extract of *Calendula officinalis*, with a total flavonoid content of 120 mg/ml Topically Duration: 30 weeks	41 patients Group J (n= 51) Group K (n=51)	Patients with pressure ulcer	Primary outcomes: reduction of the wound area, infection control, types of tissue and exudate, and ulcer microbiology	None	Limited value
Buzzi *et al*., 2016b	Non-randomised controlled study	Verum: hydroglycolic extract of *Calendula officinalis*, with a total flavonoid content of 120 mg/ml + collagenase (0.6 U/g), chloramphenicol (10 mg/g), and 1 % silver sulfadiazine; Control: saline solution + collagenase (0.6 U/g), chloramphenicol (10 mg/g), and 1 % silver sulfadiazine. Topically, twice daily Duration:30 weeks	57 patients Verum (n=38) Control (n=19)	Patients with pressure ulcer	The clinical outcomes: the wound area, infection control and the percentage of healing velocity per week	None	Limited value
Sharp *et al.,* 2013	Randomised controlled, blinded study	Verum: Calendula cream (contains extract of Calendula 10%; no further details) Control: standard skin care treatment at the RT Topically, twice daily Duration: 10 days	411 patients Verum (n=203) Control (n=208)	Prevention of acute radiation skin reactions	The primary endpoint: patients with severe ARSR	Fisher's test	No difference between the groups

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

Panahi *et al.,* 2012 compared in a randomized double-blind trial the efficacy of Aloe vera cream and *Calendula officinalis* ointment on the frequency and severity of diaper dermatitis in children.

Sixty-six infants with diaper dermatitis (aged < 3 years) were randomized to receive either Aloe cream (n = 32) or Calendula flower ointment (n = 34). Calendula ointment contained 1.5% of total extract obtained from *Calendula officinalis* flowers; no further details are provided. Infants were treated with these preparations 3 times a day for 10 days. The severity of dermatitis was graded at baseline as well as at the end of trial using a 5-point scale. The adverse effects of the study medications were assessed during the trial. Although improvement in the severity of diaper dermatitis was observed in both treatment groups (P<0.001), patients receiving Calendula ointment had significantly fewer rash sites compared to the Aloe group (P = 0.001). No adverse effect was reported from either of the medications.

Assessor's comment:

The extract which has been used in this study is not well defined, therefore the results have a limited value.

4.4. Overall conclusions on clinical pharmacology and efficacy

There are some trials that have evaluated the use of Calendula flower preparations for the treatment of skin conditions, such as venous leg ulcers, skin burns or acute dermatitis. However, the majority of the studies are small with methodological weakness, therefore the evidence of efficacy in not sufficient for a well-established use indication.

5. Clinical Safety/Pharmacovigilance

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

Table 6: Clinical safety data from the clinical trials

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
Lievre *et al.,* 1992	Randomised controlled, open study	Control: vaseline Verum: Calendula preparation (flowers, leaves and stems) Group Elase : proteolytic ointment Cutaneous Duration: 17 days	156 patients (>18 years) Control (n=50) Verum (n=53) Group Elase (n=53)	Patients with 2nd and 3rd degree burns	No adverse events were reported	Good tolerability of study medication
Pommier *et al.,* 2004	Randomised, placebo-single-blind study	Control (trolamine) Verum: Calendula ointment (20% of fresh Calendula aerial parts in petroleum jelly) Topically, twice daily or more Duration: 7 days	254 women (18-75 years old) Control (n=128) Verum (n=126)	Acute dermatitis during irradiation for breast cancer	Control: 4 patients developed allergic-type reactions (pruritis and urticaria). Verum: No	Good tolerability of Calendula preparation

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
					adverse events were reported	
Duran V *et al.*, 2005	Observational study	Verum: ethanolic extract; Placebo: saline solution dressing Topically, twice daily 1 g ointment/ cm^2 of ulcer Duration: 3 weeks	34 patients Verum (n=21) Placebo (n=13)	Patients with venous ulcers	No adverse events were reported	Good tolerability of study medication
Buzzi *et al.*, 2016a	Observational study	Verum: hydroglycolic extract of *Calendula officinalis*, with a total flavonoid content of 120 mg/ml Topically Duration: 30 weeks	41 patients GroupJ (n= 51) Group K (n=51)	Patients with pressure ulcer	No adverse events were reported	Good tolerability of study medication
Buzzi *et al.*, 2016b	Non-randomised controlled study	Verum: hydroglycolic extract of *Calendula officinalis*, with a total flavonoid content of 120 mg/ml+ collagenase (0.6 U/g), chloramphenicol (10 mg/g), and 1 % silver sulfadiazine; Control: saline solution + collagenase (0.6 U/g), chloramphenicol (10 mg/g), and 1 % silver sulfadiazine. Topically, twice daily Duration: 30 weeks	57 patients Verum (n=38) Control (n=19)	Patients with pressure ulcer	No adverse events were reported	Good tolerability of study medication

5.2. Patient exposure

Apart from market presence and some data from clinical studies, there are no substantive data concerning patient exposure.

5.3. Adverse events, serious adverse events and deaths

Adverse events reported from clinical trials

See section 5.1.

Adverse events reported from literature

The data concerning the risk of skin irritation or allergic reactions are controversial.

Weak skin-sensitization has been reported (WHO, 2004; ESCOP, 2003) but there are no clearly recorded cases of contact dermatitis.

In a review Paulsen concludes that, experimentally the plant extract is only weakly sensitizing which is supported by the scarcity of case reports (Paulsen *et al*., 1993; Paulsen, 2002). This may be explained by the lack of sesquiterpene lactones in Calendula flowers.

In contrast to this, Reider *et al.*, 2001 report that 2% of 443 patients reacted positively to Calendula, while only 1% reacted to Arnica.

In the assessor's opinion, the latter publication is of limited value for the assessment of the safety of Calendula preparations defined in the monograph, because the authors tested the whole aerial parts of Calendula and not the ray florets alone.

There are no reports in the literature that the topical application of preparations of Calendula flowers bears a higher risk for skin irritation for atopic persons.

There is no evidence for phototoxic activity.

On the basis of the available data, section 4.8 of the monograph states: "Skin sensitization has been reported. The frequency is not known".

Serious adverse events:

In the literature one case report of anaphylactic shock after gargling with Calendula tincture is cited (Hänsel *et al.,* 1992); no details are reported.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

According to Mills et *al.*, 2006, Calendula used cutaneously is not reported in the scientific literature as being either safe or contraindicated during pregnancy or lactation.

Assessor's recommendation: Safety during pregnancy and lactation has not been established.

In the absence of sufficient data and taking into account developmental toxicity (see section 3.3.5) the use during pregnancy and lactation is not recommended.

5.5.1. Use in children and adolescents

The Council of Europe published in 1989 a document where the use of certain preparations of Calendula flowers is allowed for cosmetic baby toiletries. Cosmetic products are intended to be used on the intact skin. Therefore, the medicinal use of Calendula flowers for minor inflammations of the skin and as an aid in healing of minor wounds cannot be recommended in the same way for babies.

For the preparations included in the monograph the safety in the paediatric population is not demonstrated, therefore the cutaneous use in children under 6 years of age is not recommended.

The oromucosal use of Calendula preparations is not recommended in children under 12 years due to the lack of adequate data.

5.5.2. Contraindications

Calendula preparations are contraindicated in cases of hypersensitivity to the active substance.

A cross sensitivity with other members of the Asteraceae cannot be excluded but has not been reported to date, therefore section 4.3. of the monograph states: "Hypersensitivity to the active substance and to other plants of the Asteraceae (Compositae) family".

5.5.3. Special warnings and precautions for use

Due to the lack of adequate data cutaneous use in children under 6 years of age and oromucosal use in children under 12 years is not recommended.

5.5.4. Drug interactions and other forms of interaction

No data available.

5.5.5. Fertility, pregnancy and lactation

According to the ESCOP monograph (2003) there are no objections to external use during pregnancy and lactation, however, there are no data to confirm the safety during pregnancy and lactation.

In the absence of data and, taking into account nonclinical data (see 3.3.5 Reproductive and developmental toxicity), the use during pregnancy and lactation is not recommended.

5.5.6. Overdose

No cases of overdose has been reported.

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

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

5.5.8. Safety in other special situations

Not applicable.

5.6. Overall conclusions on clinical safety

The cutaneous and oromucosal use of preparations of Calendula flowers can be regarded as safe, especially at therapeutic doses as there are no reported adverse events. Weak skin-sensitization has been reported in the literature and this is listed as an undesirable effect in section 4.8 of the monograph. The frequency is not known.

Calendula flower preparations are contraindicated in cases of hypersensitivity to the active substance and to other plants of the Asteraceae (Compositae) family, taking into account that cross sensitivity with other members of the Asteraceae cannot be excluded.

There are no data regarding interactions with other medicines.

As there is no information on safety during pregnancy and lactation and taking into account nonclinical data on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

Calendula flower preparations are not recommended for cutaneous use in children under 6 years of age due to the lack of adequate safety data. Furthermore, the oromucosal use of Calendula preparations is also not recommended in children under 12 years due to the lack of adequate data.

6. Overall conclusions (benefit-risk assessment)

Products containing *Calendula officinalis* flower preparations have been registered as traditional herbal medicinal products or as well-established use medicines in some Member States. The medicinal use of Calendula flowers has been documented in a number of medicinal handbooks throughout a period of at least 30 years, including at least 15 years within the EU. Therefore, Calendula flower fulfils the requirements of Directive 2004/24 EC as a basis for classification as a traditional herbal medicinal product.

The clinical data cannot be considered sufficient to fulfil the criteria required for “well-established medicinal use”, according to Directive 2001/83/EC.

The positive effects of Calendula flower preparations on healing of minor wounds and for the treatment of minor inflammations of the skin have long been recognised empirically.

The traditional use indications and duration of use included in the monograph are:

Indication 1)

Traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds. Duration of use: 1 week.

Indication 2) Traditional herbal medicinal product for the symptomatic treatment of minor inflammations in the mouth or the throat. Duration of use: 1 week.

For both indications, the duration is in line with approved SPCs for traditional herbal medicinal products containing Calendula flower preparations. If the symptoms persist longer than 1 week during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

Calendula flower preparations are traditionally used in the following pharmaceutical forms and posology:

a) Herbal substance or comminuted herbal substance

Indication 1) single dose: 1-2 g herbal substance or comminuted herbal substance in 150 ml water. The still warm infusion is used to prepare impregnated dressings**;** daily dose: 2 to 4 times

Indication 2) single dose: Herbal substance or comminuted herbal substance for infusion preparation for oromucosal use: 1-2 g in 150 ml water; the still warm infusion is used for rinsing and gargling; daily dose: 2 to 4 times

b) Liquid extract (1:1), extraction solvent ethanol 40-50% (V/V)

Indication 1) in semi-solid dosage forms: amount equivalent to 2-10% herbal substance; single dose: apply on a thin layer on the affected area; daily dose: 2 to 4 times

c) Liquid extract (1:1.8-2.2), extraction solvent ethanol 40-50% (V/V)

Indication 1) semi-solid dosage forms: amount equivalent to 2-5% herbal substance; single dose: apply on a thin layer on the affected area; daily dose: 2 to 4 times

d) Tincture (1:5), extraction solvent ethanol 70-90% (V/V)

Indication 1) in impregnated dressings diluted at least 1:3 with freshly boiled water; in semi-solid dosage forms: amount equivalent to 2-10% herbal substance; single dose: apply on a thin layer on the affected area; daily dose: 2 to 4 times

Indication 2) as a gargle or mouth wash in a 2% solution; daily dose: 2 to 4 times

e) Liquid extract (1:10), extraction solvent fatty vegetable oil e.g. olive oil

Indication 1) in semi-solid dosage forms: amount equivalent to 2-8% herbal substance; single dose: apply on a thin layer on the affected area; daily dose: 2 to 4 times

f) Extract (1:5 – 1:25), extraction solvent hardened vegetable fat, petroleum jelly

Indication 1) in semi-solid dosage forms: amount equivalent to 4-20% herbal substance; single dose: apply on a thin layer on the affected area; daily dose: 2 to 4 times.

Information on reproductive and developmental toxicity is inadequate. Therefore use during pregnancy and lactation cannot be recommended. No data on fertility is available.

Calendula flower preparations cannot be recommended for cutaneous use in children under 6 years of age and for oromucosal use in children under 12 years of age due to lack of adequate safety data.

The traditional use of the herbal substance and herbal preparations of Calendula flower have a positive benefit risk ratio due to minimal risks.

Reliable data from tests on genotoxicity are only available for hydroethanolic liquid extracts. Therefore, a European Union list entry is proposed for this type of extract only.

Annex

List of references