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EMA/HMPC/291177/2009
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Centella asiatica* (L.) Urban, herba

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

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

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	<i>Centella asiatica</i> (L.) Urban, herba
Herbal preparation(s)	The following designations are synonyms to define a highly refined and purified ethanolic (70% V/V) extract containing asiaticoside (40%) and 60% of triterpenic genins: asiatic acid (29-30%), madecassic (acid 29-30%) and madasiatic acid (about 1%). TECA (titrated extract of <i>Centella asiatica</i>) TTFCA (total triterpenoid fraction of <i>Centella asiatica</i>) TTF (total triterpenic fraction) CATTF (<i>Centella asiatica</i> total triterpenic fraction) ETCA (Estratto Titolato di <i>Centella asiatica</i>) It is not considered to be a herbal preparation
Pharmaceutical forms	Herbal substance for tea preparation; Herbal preparations in solid dosage form for oral use (tablets, containing 10 mg or 30 mg of TECA extract per tablet). Herbal preparations in solid or semisolid dosage forms for cutaneous use (cream or ointment containing 1% dry extract, cutaneous powder containing 2% dry extract, sterilised impregnated dressing with 2 g of mass 1%/ dm ²)
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ABBREVIATIONS

ASR	Acoustic Startle Response
CATTF	Synomim of TTFCA and TECA
CVI	Chronic Venous Insufficiency
EMA	European Medicines Agency
ESCOPE	European Scientific Cooperative on Phytotherapy
ETCA	Synomim of TTFCA and TECA
HPLC	High Pressure Liquid Chromatography
HUVEC	Human Umbilical Vein Endothelial Cell
LD50	Lethal Dose 50% (dose that kills 50% of experimental animals)
SMD	Standardised Mean Differences
TECA	Titrated Extract of <i>Centella asiatica</i>
TTF	Total Triterpenic Fraction comprises asiatic acid and madecassic acid (60%) in a ratio that is not clearly defined, in combination with asiaticosides (40%) (Synonym of TTFCA and TECA)
TTFCA	Total Triterpenic Fraction of <i>Centella asiatica</i> (Synonym of TECA)
VAS	Visual Analog Scale

1. Introduction

The objective of this report is to assess the preclinical and clinical available data on *Centella asiatica* (L.) Urban, as well as available information on products marketed in the European Community, including pharmaceutical forms, indications, posology and methods of administration, with the aim to prepare the Community monograph on the plant and its preparations. This report is based on the documentation provided by the European Medicines Agency (EMA) completed by further searches and information taken from international literature. Bibliographic searches have been performed in PubMed and MEDLINE until 2008; EMBASE (1974-2008). The search terms were: *Centella asiatica*, asiaticoside, Gotu Kola, Indian Pennywort, *Hydrocotyle asiatica*

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

- Herbal substance(s)

Centella asiatica (L.) Urban is a stoloniferous perennial herb belonging to the plant family *Apiaceae* (*Umbelliferae*) (see Table 1), which contains 20 different species. It is a slender, creeping plant, rooting at the nodes, growing in the damp areas in different tropical countries.

Apart from this name, which is usually employed in scientific work, the synonym *Hydrocotyle asiatica* L. is the designation most commonly found. In German, the medicinal plant is also known by the colloquial name of "Indischer Wassernabel". Further names are: Indian Pennywort (English), Hydrocotyle Asiatique (French), Idrocotile (Italian), Brahma-manduki and Brahmi-Buti (Hindi), Tsubokusa (Japanese), and Tungchian and Luei Gong Gen (Chinese), Blasteostimulina (asiaticoside) (Spanish). In India it is commonly known as 'Indian Pennywort' 'Jal Brahmi' or 'Mandookaparni' or Gotu Kola (Brinkhaus *et al.* 2000).

Table 1.

Systematic classification (Taxonomy)	
Classification	Name
Kingdom	<i>Eukaryota</i>
Subkingdom	<i>Embryophyta</i>
Division	<i>Spermatophyta</i>
Subdivision	<i>Angiospermae</i>
Class	<i>Dicotyledoneae</i>
Subclass	<i>Rosidae</i>
Superorder	<i>Aralianae</i>
Order	<i>Araliales (Umbelliflorae)</i>
Family	<i>Apiaceae or Umbelliferae</i>
Subfamily	<i>Hydrocotyle</i>
Genus	<i>Centella</i>
Species	<i>Centella asiatica</i>

Synonyms:

Hydrocotyle asiatica L., herba

Hydrocotyle lunata Lam., herba

Trisanthus cochinchinensis Lour, herba

Centella coriacea Nannfd., herba

Centella cordifolia, (Hooker fil) Nannfd., herba

Centella dusenii Nannfd., herba
Centella floridana (C.et R.) Nannfd., herba
Centella repanda (Pers.) Small, herba
Centella triflora (R.et P.) Nannfd., herba
Centella uniflora (Col) Nannfd., herba

According to the European Pharmacopoeia the herbal substance consists of the dried, fragmented aerial parts, containing minimum 6% of total triterpenoid derivatives, expressed as asiaticoside (C₄₈H₇₈O₁₉; M_r 959.15) (IUPAC name: 6-[[[3,4-dihydroxy-6-(hydroxymethyl)-5-(3,4,5-trihydroxy-6-methyl-oxan-2-yl)oxy-oxan-2-yl]oxymethyl]-3,4,5-trihydroxy-oxan-2-yl]10,11-dihydroxy-9-(hydroxymethyl)-1,2,6a,6b,9,12a-hexamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylate) (European Pharmacopoeia).

In addition to about 0.1% essential oils and other volatile constituents, *Centella asiatica* contains a wide range of other substances. In summary the constituents of *Centella asiatica* are the following (modified from Brinkhaus *et al.* 2000):

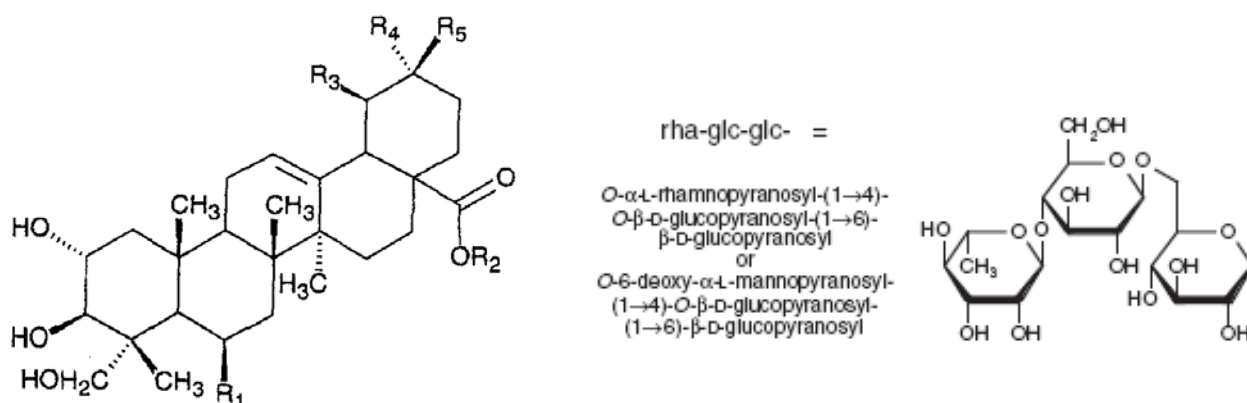
Table 2. Chemical constituents

Main Groups	Constituents
Essential oil (0.1% of the plant)	Terpene acetate Germacrene Caryophyllene p-Cymol Pinene
Flavone derivatives	Quercetin glycoside Kaempferol, glycoside and in free form Astragalin
Sesquiterpenes	Caryophyllene Elemene and bicycloelemene Trans-farnesene Ermacrene D
Triterpenic steroids	Stigmasterol Sitosterol
Triterpenic acids	Asiatic acid 6-hydroxy asiatic acid Madecassic acid Madasiatic acid Betulinic acid Thankunic acid Isothankunic acid
Triterpenic acid sugar esters (= saponins or pseudosaponins) (1-8% depending on country or origin)	Asiaticoside (major component) Asiaticoside A Asiaticoside B Asiaticoside A (Madecassoside) and B Braminoside Brahmoside Brahminoside Thankuniside Isothankuniside

The substances of therapeutic interest are the saponin-containing triterpene acids and their sugar esters, the most important being: asiatic acid, madecassic acid and the three asiaticosides, asiaticoside, asiaticoside A and asiaticoside B (Fig. 1 - Brinkhaus *et al.* 2000). The structures of the three triterpenoid trisaccharides asiaticoside, asiaticoside-A and asiaticoside-B, have been elucidated by spectroscopic analysis as the [O-β-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl(1→6)]-O-β-D-glucopyranose esters of 2β,3β,23β-trihydroxy-urs-12-ene-28-oic acid, of 2β,3β,6β,23v-tetrahydroxy-urs-12-ene-28-oic acid and of 2β,3β,6β,23β-tetrahydroxyolean-12-ene-28-oic acid (Sahu *et al.* 1989).

The main active principles of *Centella asiatica*, are the triterpenoids glycosides asiaticoside and madecassoside (asiaticoside A), used for the quantification of this species as described in the European Pharmacopoeia, and their respective aglycons (asiatic acid and madecassic acid). Significant differences in active constituent contents have been observed between samples of *Centella asiatica* originating from different countries (Randriamampionona *et al.* 2007). Depending on the source of the material, the amount of the triterpenoids may vary widely from 1% to 8% (ESCOP 2009).

Fig. 1 (from Brinkhaus *et al.* 2000)



	R ₁	R ₂	R ₃	R ₄	R ₅
asiatic acid	-H	-H	-CH ₃	-CH ₃	-H
madecassic acid	-OH	-H	-CH ₃	-CH ₃	-H
asiaticoside	-H	1)-β-D-glc-(6-1)- β-D-glc-(4-1)- α-L-rha	-CH ₃	-CH ₃	-H
asiaticoside A ¹	-OH	1)-β -D-glc-(6-1)- β-D-glc-(4-1)- α-L-rha	-CH ₃	-CH ₃	-H
terminolic acid	-OH	-H	-CH ₃	-CH ₃	-CH ₃
asiaticoside B	-OH	1)-β -D-glc-(6-1)- β-D-glc-(4-1)-α-L-rha	-H	-CH ₃	-CH ₃

Centella asiatica plants are reported to contain also the following glycosides: indocentelloside, brahmoside, brahminoside, theankunside and isothankunside (Tiwari 2000).

The major component of *Centella asiatica* essential oil is an unidentified terpenic acetate [peak 14; M^r 274 (base 2592 157)]. Other compounds: β-caryophyllene, trans-p-farnesene and germacrene D have been detected in *Centella asiatica* in respectable amount (Asakawa *et al.* 1982).

Other chemical constituents found in *Centella asiatica* are: vallarine, hydrocotylin, pectic acids, steroids, hersaponin, bacogenin, monnierin, tannins (Sathya & Uthaya Ganga 2007).

Centella asiatica leaves are rich in carotenoids, vitamins B and C (Tiwari *et al.* 2000). Recently, two new flavonoids named castilliferol 1 and castillicetin 2 (both exhibiting antioxidant activity), as well as

¹ Madecassoside

a known compound, isochlorogenic acid 3, were isolated from the whole plant of *Centella asiatica* (Subban *et al.* 2008). The flavonoids apigenin, rutin and quercetin have been detected in methanolic extracts of *Centella asiatica* (Bhandari *et al.* 2007).

A polysaccharide, isolated from ethanolic extracts of *Centella asiatica*, is a complicated arabinogalactan (AG), which contain a little α -(1→4)-linked GalpA and α -(1→2)-linked Rhap residues (Wang *et al.* 2004).

- Herbal preparation(s)

Alcoholic or aqueous extracts of *Centella*, as well as refined and purified extracts have been used.

Literature reports studies on the following extracts: TECA, TTFCA and TTF, all containing 40% of asiaticoside and 60% of the aglycons (asiatic acid and madecassic acid). In particular clinical studies were published describing the use of the following preparations: TTFCA, TECA and, where the name of the commercial extract is mentioned, Madecassol® (titrated extract of *Centella asiatica*) or Centellase® (total triterpenoid fraction of *Centella asiatica*). The extracts TECA and TTFCA in literature are reported to contain 30% of asiatic acid and 30% of madecassic acid, while the extract TTF is reported to comprise 60% of asiatic acid and madecassic acid in a ratio that is not clearly defined. In all extracts the remaining 40% is purified asiaticoside (Brinkhaus *et al.* 2000).

Information coming from literature and licensed medicinal products confirms that all the above mentioned TECA, TTFCA, TTF as well as ETCA and CATTFF are different acronyms to designate the same extract, commercially known as Madecassol® or Centellase® or Blastostimulina®, containing 40% of asiaticoside and 60% of asiatic acid and madecassic acid.

TECA is a highly purified extract, fractionated and enriched in triterpenic acid and triterpenic sugar ester fractions to reach about the 40% of asiaticoside and about the 60% of the triterpenic genins: asiatic acid and madecassic acid. The purification steps are extreme and involve chemical treatments that remove the herbal matrix. The final extract is a recombination of a highly refined extract with an isolated constituent and the natural proportion of the components is not maintained. Therefore, based on the information on manufacturing process, the HMPC is of the opinion that TECA extract cannot be classified as an herbal preparation due to the manufacturing steps and composition.

Insufficient information about the quality of other herbal preparation historically in medicinal use is available.

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

Not applicable

1.2. Information about products on the market in the Member States

Medicinal products containing *Centella asiatica* preparations are authorised and marketed in Europe with full application in the following Members states:

Member State	Trade name	Extract	Pharmaceutical Form	Year of authorisation
Belgium	Madecassol®		Cream 1% for cutaneous use Tablets for oral use	since 1969
France	Madecassol®	TECA	Tablets for oral use Cream 1% for cutaneous use Cutaneous powder 2% Sterilised impregnated dressing (1 g of extract/100 g of mass ; 2 g of mass/ dm ²)	since 1974 since 1975 since 1975 since 1976
Greece	Bilim	(like French Madecassol®)	Ointment 1% cutaneous powder 2%	since 1997
Italy	Centellase®	TECA (like French Madecassol®)	Tablets for oral use	since 1982
Portugal		TECA (like French Madecassol®)	Tablets for oral use Ointment 1% Cutaneous powder 2%	since 1973 since 1968
Spain	Blastoestimulina	TECA	Cutaneous powder 2%	

The active substance of Centellase® and of the product Blastoestimulina® marketed in Spain is TECA extract and information obtained by the Italian Marketing Authorisation Holder confirms that Centellase® is the Italian name of the French Madecassol®. Portugal and Greece have confirmed that the products marketed in their Country contain the same extract of Madecassol®.

From the comparison of the information on production and testing of the active substance provided by Member States where these products are authorised it clearly appears that all the above mentioned medicinal products contain the same type of extract, which is purified, fractioned and enriched in triterpenic acid and triterpenic sugar ester fractions to reach about the 40% of asiaticosides and about the 60% of the triterpenic acids: asiatic acid and madecassic acid.

Food supplements containing different preparations are also on the market in the European Community, as well as cosmetics.

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
Belgium	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Full MA (cream)
Bulgaria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	A few food supplements and cosmetics
Cyprus	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Czech Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP

Member State	Regulatory Status				Comments
Denmark	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No products
Estonia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
Finland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
France	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	40% of asiaticoside and 60% of madecassic acid + asiatic acid
Germany	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	NO authorised/reg. HMP
Greece	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	TECA (40% of asiaticoside and 60% of madecassic acid + asiatic acid)
Hungary	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Iceland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Ireland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No licensed products
Italy	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Triterpenic fraction (40% of asiaticoside and 60% of madecassic acid + asiatic acid) tablets Food supplements
Latvia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Several food supplements
Liechtenstein	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Lithuania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No products
Luxemburg	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Malta	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
The Netherlands	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Norway	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
Poland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
Portugal	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Triterpenic Extract 20 mg/gr Triterpenic Extract 10 mg/gr Triterpenic Extract 10 mg/gr equivalent to 7 mg of asiatic acid Asiatic acid 30 mg
Romania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
Slovak Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
Slovenia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
Spain	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Sweden	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/reg. HMP
United Kingdom	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No licensed products

MA: Marketing Authorisation

TRAD: Traditional Use Registration

Other TRAD: Other national Traditional systems of registration

Other: If known, it should be specified or otherwise add 'Not Known'

This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

This assessment report reviews the scientific literature data available for *Centella asiatica* and from the WHO monograph, European Pharmacopoeia monograph, from PubMed, from the European Medicines Agency (EMA) library, the Internet, information taken from international literature as well as available information on products marketed in the European Community, including pharmaceutical forms, indications, posology and methods of administration. Bibliographic searches have been performed in PubMed and MEDLINE until 2008; EMBASE (1974-2008). The search terms were: *Centella asiatica*, asiaticoside, Gotu Kola, Indian Pennywort, *Hydrocotyle asiatica*.

2. Historical data on medicinal use

Centella asiatica (L.) is an ethnomedical plant used in different continents by diverse ancient cultures and tribal groups. The plant is native in countries like Sri Lanka, Madagascar, South Africa and Malaysia.

Centella asiatica has been referred to in the ancient traditional Chinese Shennong Herbal about 2,000 years ago and in Indian Ayurvedic medicine about 3,000 years ago.

The Chinese prescribed the leaves in curing leucorrhoea and toxic fever while in India, this plant is used in the Ayurvedic system of medicine, usually described under the name of Mandukaparni, to treat various diseases: asthma, bronchitis, dropsy, elephantiasis, gastric catarrh, kidney troubles, leprosy, leucorrhoea, skin diseases and urethritis (Husain *et al.* 2007).

In Madagascar, the plant was also used to treat leprosy (Sahu *et al.* 1989).

In Malaysia, although this herb is commonly eaten fresh as a vegetable (salad), especially among the Malaysia communities, it is also said to have beneficial effects in improving memory and in treating mental fatigue, anxiety, and eczema (Goh *et al.* 1995; Abdul Hamid *et al.* 2002).

Fresh extracts of this plant have been used by the people of Java and the Malay Peninsula for many years, as both topical and internal agents, for healing of wounds and constituent of brain tonics for the cognitive retard (Kartnig, 1988). These properties have been ascribed to the pentacyclic triterpenes (asiatic acid, asiaticoside, madecassic acid and madecassoside), found to display CVI, varicose vein and wound healing properties (Inamdar *et al.* 1996). Further experimental studies showed several biological properties of *Centella asiatica* extracts among which antioxidant activity (Jayashree *et al.* 2003, Gnanapragasam *et al.* 2004) and antiproliferative effect (Yoshida *et al.* 2005; Randriamampionona *et al.* 2007).

In folk medicine different uses, not supported by experimental or clinical data, were reported. They are: therapy of albinism, anaemia, asthma, bronchitis, cellulite, cholera, measles, constipation, dermatitis, diarrhoea, dizziness, dysentery, dysmenorrhoea, dysuria, epistaxis, epilepsy, haematemesis, haemorrhoids, hepatitis, hypertension, jaundice, leucorrhoea, nephritis, nervous disorders, neuralgia, rheumatism, smallpox, syphilis, toothache, urethritis, and varices. It was used as an antipyretic, analgesic, anti-inflammatory, and "brain tonic" agent (WHO monographs, 1999). Poultices have been used to treat contusions, closed fractures, sprains, and furunculosis (WHO monographs, 1999).

Centella asiatica extracts have been also used in the treatment of keloids (Hausen *et al.* 1993) but there are not sufficient clinical data confirming their efficacy in this indication.

2.1. Information on period of medicinal use in the Community

Centella asiatica has been referred in the French Pharmacopoeia ed. 1884. Despite its long history of traditional use, *Centella* appeared in the Codex in 1884 and the first dry extract was not created until 1941. Three years after its triterpenoid molecules were isolated by the French scientist, P. Boiteau.

The British Herbal Pharmacopoeia reports the systemic use of the aerial part of *Centella asiatica* in rheumatic condition and cutaneous affections and the topical application for indolent wounds, leprosy ulcers and cicatrisation after surgery (British Herbal Pharmacopoeia 1983).

External use

TECA has a long history of use in Europe as wound healing drug.

Medicinal products containing triterpenic extract from *Centella asiatica* are authorised in Europe for cutaneous use (powder, cream and ointment) since 1968.

Active constituents of *Centella asiatica* are components of many cosmetic preparations worldwide in the area of skin care. The leaves of *Centella asiatica* found application in clinical practice for dermatological disorders and in particular for improving the healing process of wound, burns, skin and vein ulcers (Randriamampionona *et al.* 2007).

Internal use

Medicinal products containing Madecassol® in tablets for oral use are authorised in Europe since 1969 (BE). Tablets for oral use are authorised in Belgium, France, Greece, Italy and Portugal. During 1980's several clinical studies showed that the Total Triterpenic Fraction of *Centella asiatica* (TTFCA= TECA) (at the oral dose of 60-120 mg daily, for 30-90 days) was able to improve subjective and objective symptoms associated with primary or secondary CVI of the lower limbs (Husain *et al.* 2007).

Food supplements are also available in several countries with healing claims related to the microcirculation and the tissue draining. No information about the type of extracts used in food supplements is available.

2.2. Information on traditional/current indications and specified substances/preparations

***Centella asiatica* preparations**

Medicinal products containing TECA extract (Madecassol®, Blastostimulina®, Centellase®) are authorised in Europe with full application.

External use

The **cream 1%** for cutaneous use is authorised in Belgium since 1969 for the treatment of moderate or benign problems in wound formation such as atonic wounds, hypertrophic scars, keloids in active phase, in France since 1975 to aid in the local treatment for cutaneous ulcerations and since 2000 to aid in the local treatment for granulation phase of wounds, cutaneous ulcers and cutaneous gangrene. The ointment 1% is used in Greece as a potent wound healing agent (induce collagen biosynthesis).

The **cutaneous powder 2%** is authorised in France since 1975 to aid in the local treatment for cutaneous ulcerations and in Greece since 1997 as a potent wound healing agent (induce collagen biosynthesis).

The **sterilised impregnated dressing** (1 g of extract/100 g of mass ; 2 g of mass/ dm²) is also authorised in France since 1976 to aid in the local treatment for granulation phase of wounds, cutaneous ulcers and cutaneous gangrene.

In Portugal medicinal products containing Triterpenic extract of *Centella asiatica* are authorised since 1968 in form of:

- **Ointment 1%** for leg ulcers, decubitus scabs, gangrene, defective scars, fistula, traumatic and surgical wounds, burns, skin grafts, radio-dermites, ORL, ophthalmology and gynaecology cutaneous-mucosal injury;
- **Cutaneous powder 2%** as an adjuvant on the cicatrisation of skin injuries;

In Spain is authorised in form of cutaneous powder 2% as epithelizant agent, to heal injuries, scars, keloid scars, burns.

Internal use

The tablets for oral use containing TECA extract are authorised in Belgium since 1969 for atonic wounds -hypertrophic scars, keloids in active phase, in Greece as a potent wound healing agent (induce collagen biosynthesis); in France since 1974 for improvement of symptoms of venous stasis, in Italy since 1982 for the treatment of pre-varicose syndromes: leg heaviness, orthostatic oedema, vein pains, night cramps and lower limbs itching as well as for the adjuvant therapy of idiopathic or secondary CVI and of complications of varicose veins: phlebitis, varicose ulcers, cutaneous dystrophy.

Tablets containing 10 mg of triterpenic extract equivalent to 7 mg of asiatic acid are authorised in Portugal since 1973 for the treatment of leg ulcers, decubitus scabs, gangrene, defective scars, fistula, traumatic and surgical wounds, burns, skin grafts, radio-dermites, ORL, ophthalmology and gynaecology cutaneous-mucosal injury.

Asiatic acid

Hard capsules containing 30 mg of asiatic acid are authorised in Portugal since 1989 for prevention and recovery of venous insufficiency: heavy legs, fatigability, oedema, itching, pain and leg ulcer.

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

***Centella asiatica* preparations**

Alternative Medicine Review (2007):

The recommended daily dosages of crude herb and 1:5 tincture are 0.5-6 g and 10-20 ml, respectively.

ESCOP monograph (2009):

Internal use:

Adult dose: 0.6 g dried drug as an infusion, tincture or extract, up to four times daily.

No information on the extract or tincture is available.

Children: proportion of adult dose according to body weight and/or age.

External use:

Semi-solid preparations containing 1% of extract or tincture.

No information on the extract or tincture is available.

Potter's Herbal Cyclopaedia (2009):

The posology of powdered leaf is 0.5-1 g daily (Williamson 2003).

TTFCA (TECA) extracts (as authorised):

Internal use:

TTFCA (TECA) has been the most used preparations in clinical practice in the therapy of CVI. In adults, the dosages more frequently used are in the range of 60-180 mg/day, in divided doses 3 times daily, of TTFCA (TECA). The duration of use is ranging between 2-8 weeks but the median period of use is about 6-7 weeks.

For atonic wounds -hypertrophic scars, keloids in active phase, for improvement of symptoms of venous stasis and as a potent wound healing agent (induce collagen biosynthesis):

- adults : 1-2 tablets (10 mg/tbl) 3 times per day, before meals;
mean duration of treatment is 4 weeks
- children > 3 years: according to doctor's advice (in general half the dose for adults).

For improvement of symptoms of venous stasis: 6 tablets (10 mg/tbl) for oral use daily (loading dose); 3 tablets containing 10 mg daily (maintenance dose);

For the treatment of pre-varicose syndromes: leg heaviness, orthostatic oedema, vein pains, night cramps and lower limbs itching. For the adjuvant therapy of idiopathic or secondary CVI and of complications of varicose veins: phlebitis, varicose ulcers, cutaneous dystrophy:
1-2 tablets (30 mg/tbl) per day preferably at the meals

For the treatment of leg ulcers, decubitus scabs, gangrene, defective scars, fistula, traumatic and surgical wounds, burns, skin grafts, radio-dermites, ORL, ophthalmology and gynaecology cutaneous-mucosal injury:

- adults: 6 tablets (10 mg/tbl) divided in 3 times/day as an acute treatment;
afterwards 3 tablets/day
- children (over 30 months): half dose

External use:

Cream 1% for cutaneous use:

- to aid in the local treatment for granulation phase of wounds, cutaneous ulcers and cutaneous gangrene 1 to 2 times daily
- For the treatment of moderate or benign problems in wound formation such as atonic wounds - to be applied after disinfection, upon changing of the wound dressing,
- For hypertrophic scars/keloids in active phase: apply directly on the wounds.

The use is based on clinical experience.

Ointment 1% for cutaneous use as a potent wound healing agent (induce collagen biosynthesis):
2 to 3 times daily covering wounds or dry skin or on skin after radiotherapy. For leg ulcers, decubitus scabs, gangrene, defective scars, fistula, traumatic and surgical wounds, burns, skin grafts, radio-

dermites, ORL, ophthalmology and gynaecology cutaneous-mucosal injury: local application depending on the dressing.

Cutaneous powder 2%:

- To aid in the local treatment for cutaneous ulcerations and as a potent wound healing agent (induce collagen biosynthesis): 1 to 3 times daily covering wounds or dry skin or on skin after radiotherapy. Not to be used on deep infective wounds.
- As an adjuvant on the cicatrisation of skin injuries: local application 1-2 times daily, depending on the need. Do not use in deep wounds (risk of granuloma due to the powder). It is particularly indicated in wet wounds.
- As epithelizing agent, to heal injuries, scars, keloid scars, burns: no information on posology.

Sterilised impregnated dressing (2 g of mass 1%/dm²) to aid in the local treatment for granulation phase of wounds, cutaneous ulcers and cutaneous gangrene: once daily.

Asiatic acid

For prevention and recovery of venous insufficiency: heavy legs, fatigability, oedema, itching, pain, leg ulcer: 1 hard capsule (30 mg) 2 times/day.

3. Non-Clinical Data

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

In literature there are numerous articles describing the pharmacological properties of alcoholic or aqueous extracts of *Centella asiatica* L. and its constituents.

The pharmacological activity of *Centella asiatica* extracts has been investigated basically in the laboratory animals and it is thought to be due to saponin constituents, including asiaticoside, asiatic acid, and madecassic acid. The pharmacodynamic effects of *Centella asiatica* have been investigated in numerous animal experiments and *in vitro* studies. Demonstrated activities include, wound healing, ulcer-protective, psychoneuro-pharmacological (cognitive effects), antinociceptive, anti-inflammatory, antimicrobial, immunomodulatory, antiproliferative, antimutagenic, angiogenetic, antioxidant of the *Centella* extracts or asiaticoside (Brinkhaus *et al.* 2000).

3.1.1. Primary pharmacodynamic studies:

Wound healing and collagen-polymerization effects

Alcoholic *Centella* extracts when topically applied accelerate wound healing stimulating epithelisation and increasing the rate of wound contraction. Reduction in granuloma weight and increase in the force needed to cause rupture of the wound (rupture strength) were also observed. These effects are associated to the increased granulation tissue levels of DNA, protein and total collagen (Suguna *et al.* 1996).

Another study describes the effects of oral and topical administration of an alcoholic extract of the leaves of *Centella asiatica* on rat dermal wound healing. Leaves of *Centella asiatica* obtained locally were minced and powdered in absolute ethanol (1ml/g) and the homogenate was filtered through a cotton gauze. The crude extract (1 ml) was applied topically or given orally once daily for 24 days. The extract increased cellular proliferation and collagen synthesis at the wound site, as evidenced by an

increase in DNA, protein and collagen content of granulation tissues. Quicker and better maturation and cross linking of collagen was observed in the extract-treated rats. The extract treated wounds were found to reepithelialise faster and the rate of wound contraction was higher, as compared to control wounds (Suguna *et al.* 1996).

A single administration (6, 12, 24 mg/kg) of madecassoside, the major triterpene in *Centella asiatica*, facilitated wound closure in a time-dependent manner and reached its peak effect, nearly completely wound closure, on day 20 in the group receiving the highest dose of 24 mg/kg of madecassoside. Histopathological analysis revealed that madecassoside alleviated infiltration of inflammatory cells as well as enhanced epithelisation resulting from dermal proliferation of fibroblasts. The higher doses (12 and 24 mg/kg) decreased nitric oxide (NO) levels and malondialdehyde (MDA) content in the burn skin tissue. However, reduced glutathione (GSH) and hydroxyproline levels were increased in the same skin tissue. In addition, madecassoside promoted skin angiogenesis *in vivo*. These data suggest that madecassoside has significant wound-healing activity and is one of the major reasons for the use of *Centella asiatica* herb in the successful treatment of burn injury. The effect of madecassoside on wound healing may involve several mechanisms including antioxidative activity, collagen synthesis and angiogenesis (Liu *et al.* 2008).

Centella triterpenes evoke a gene-expression response consistent with their prevailing medical uses in the treatment of connective tissue disorders such as wound healing and microangiopathy. TECA was used on human fibroblasts. TECA effects the expression of genes involved in angiogenesis and the remodelling of extracellular matrix, as well as diverse growth factor genes. The extent of expression change of TNFAIP6, an extracellular hyaluronan binding protein, was found to be largely dose-dependent, to respond most strongly to the free asiatic acid and madecassic acid, and to increase in expression during 48 hours of treatment (Coldren *et al.* 2003).

Angiogenetic activity

The activity of asiaticoside, isolated from *Centella asiatica*, has been studied in normal as well as delayed-type wound healing. Different concentrations of solution in sterile saline were prepared for *in vivo* studies. Methylcellulose disks were loaded with 20–80 µg asiaticoside for *in vitro* tests. A gum acacia suspension in water was used for oral administration. *Centella asiatica* promoted angiogenesis in the chick chorioallantoic membrane model at 40 µg/disk concentration. These results suggested that asiaticoside, the main active constituent of *Centella asiatica*, could have wound healing activity (Shukla *et al.* 1999).

Antioxidant activity

The antioxidant activity of various extracts (ethanol, water and light petroleum) from different parts of *Centella asiatica* (roots, leaves, stolons) was evaluated using a linoleic acid model system and the thiobarbituric acid test. Ten grams of each freeze-dried part of *Centella asiatica* were extracted with three different solvents (ethanol, water and light petroleum). The ethanol extract (best activity at pH 7 and stability up to 50°C) of all parts of *Centella asiatica* exhibited significantly ($P < 0.05$) higher antioxidative activity than the water extract, while the light petroleum ether showed negligible activity. Increasing the concentration of the extract (1000–3000 ppm) resulted in increase in antioxidative activity of both the ethanol and the water extract. From 3000 ppm upward, antioxidative activity of the ethanol extract was not significantly different ($P < 0.05$) from that of α -tocopherol. Roots showed the highest activity of the parts tested. The antioxidative activities of the ethanol extracts were found to be stable up to 50°C and exhibited optimum activity at neutral pH values (Abdul Hamid *et al.* 2002).

The activities of anti-oxidant enzymes like SOD, Catalase and Glutathione peroxidase (GSH Px) and the level of anti-oxidants like Glutathione (GSH) and ascorbic acid were found to be increased significantly

in both the liver and kidney after oral treatment with 50 mg kg⁻¹ day⁻¹ of crude methanolic extract of the *Centella asiatica* whole plant for 14 days on lymphoma-bearing mice. The dried finely powdered plant (20 g) was extracted with MeOH, the solvent was evaporated under reduced pressure to give a solid residue (2 g) (Jayashree *et al.* 2003).

Antioxidative activity and total phenolic compounds of root, leaf and petiole of four accessions of *Centella asiatica* (L.) methanolic extracts, have been investigated by using the ferric thiocyanate (FTC) method and thiobarbituric acid (TBA) test. The antioxidative activities were then compared with that of the natural antioxidant α -tocopherol and the synthetic antioxidant butylated hydroxytoluene (BHT). Results showed that both leaf and root of *Centella asiatica* had high antioxidative activity, which was as good as that of α -tocopherol. The total phenolic content showed strong association ($r^2=0.90$) with antioxidative activity suggesting that phenolic compounds are the major contributors to the antioxidative activities of *Centella asiatica* (Zainol *et al.* 2003).

Increased oxidative stress and antioxidant deficit have been suggested to play a major role in adriamycin induced cardiomyopathy and congestive heart failure due to multiple treatments with adriamycin. The cardio protective effect of *Centella asiatica* on myocardial marker enzymes and antioxidant enzymes in adriamycin induced cardiomyopathy was investigated in rats. The whole plant was shade dried and coarsely ground with grinder. The coarse powder of plant was extracted with 8 parts of distilled water under boiling for 5 hrs and was filtered through a 400-mesh cloth to collect the extract. The extract was concentrated and freeze dried to get a powder of greenish brown in colour. The rats administered with adriamycin (2.5 mg/kg body wt, i.p.) caused myocardial damage that was manifested by the elevation of serum marker (LDH, CPK, GOT and GPT) enzymes and showed significant changes in the antioxidant enzymes (SOD, CAT, GPx, GST). Treatment with *Centella asiatica* (200 mg/kg of body wt/oral) extract significantly prevented these alterations and restored the enzyme activities to near normal levels (Gnanapragasam *et al.* 2004).

A study was designed to determine whether extract of *Centella asiatica*, when administered orally (300 mg/kg body weight/day) for 60 days would prevent age-related changes in antioxidant defense system, lipid peroxidation (LPO) and protein carbonyl (PCO) content in rat brain regions such as cortex, hypothalamus, striatum, cerebellum and hippocampus. Aged rats elicited a significant decline in the antioxidant status and increased the LPO and PCO as compared to control rats in all five regions studied. The increase in LPO and PCO contents were (64%, 34%) in cortex, (86%, 30%) in cerebellum, (51%, 47%) in striatum, (77%, 27%) in hypothalamus and (58%, 45%) in hippocampus, respectively, in aged rats as compared to young rats. The whole plant was cleaned, air-dried and powdered. The powder was soaked in double distilled water, ethanol in shaking incubator at 25G1 8C (1:1) for 2 days. Supplementation of *Centella asiatica* was effective in reducing brain regional LPO and PCO levels and in increasing the antioxidant status. This study shows that *Centella asiatica* acts as a potent antioxidant and consequently could exerted neuroprotective effect in protecting rat brain against age related oxidative damage (Subathra *et al.* 2005).

The effect of *Centella asiatica* extract (not specified) and powder in reducing oxidative stress in SpragueDawley rats was evaluated. Lipid peroxidation was monitored by measuring malonaldehyde (MDA) level in blood. Activities of free radical-scavenging enzymes (superoxide dismutase and catalase) were determined using H₂O₂ decomposition and nitrobluetetrazolium reduction, respectively. Results showed that administration of H₂O₂ (0.1%) in drinking water of the rats, for 25 weeks, increased the malonaldehyde levels in erythrocytes of all the rats. However, rats receiving *Centella asiatica* extract, powder and α -tocopherol had lower MDA levels than did the other rats, which indicates decreased lipid peroxidation in these rats. Increase in catalase activity of the rats appears to be a response to H₂O₂ accumulation. The decrease in the activity of superoxide dismutase in *Centella asiatica*- and α -tocopherol supplemented rats suggested a lower requirement for the enzyme and this indicates the protective effect of the plant in combating oxidative stress undergone by the rats. Results

revealed that *Centella asiatica* extract and powder may ameliorate H₂O₂-induced oxidative stress by decreasing lipid peroxidation via alteration of the antioxidant defence system of the rats (Hussin *et al.* 2007).

3.1.2. Secondary pharmacodynamics studies:

Ulcer-protective and anti-ulcer effects

Antiulcer activity of plant extract has been compared with effects of famotidine (H₂-antagonist) and sodium valproate (anti-epileptic). Fresh whole plants of *Centella asiatica* were collected locally and extracted in a tincture extractor with an ethanol:water (50:50) mixture. All the three treatments: *Centella asiatica*, famotidine and sodium valproate have shown a dose dependent reduction of gastric ulceration. Since *Centella asiatica* increased dose-dependently brain GABA levels, it has been hypothesised a mechanism GABA-dependent. This hypothesis seems to be confirmed by the reversibility of antiulcerogenic activity with pretreatment with bicuculline methiodide (specific GABA_A-antagonist) (Chatterjee *et al.* 1992).

Effects of *Centella asiatica* on the prevention of ethanol induced gastric lesions in rats were investigated. Thirty grams of *Centella asiatica* was boiled in 600 ml of distilled water under reflux for 1 hour. The water extract was filtered and freeze dried to obtain the *Centella asiatica* extract. The gastric transmucosal potential difference (PD) was reduced by the application of 50% ethanol in the gastric *ex vivo* chamber model. *Centella asiatica* extract, orally administered (0.05 g/kg, 0.25 g/kg and 0.50 g/kg) before ethanol administration, significantly inhibited gastric lesions formation (58% to 82% reduction), decreased mucosal myeloperoxidase (MPO) activity, and accelerated its recovery in a dose dependent manner. The data suggest that *Centella asiatica* extract prevents ethanol induced gastric mucosal lesions by strengthening the mucosal barrier and reducing the damaging effects of free radicals (Cheng & Koo 2000).

In another study, the healing effects of *Centella asiatica* water extract and asiaticoside on acetic acid induced gastric ulcers in rats were examined. Water extract was prepared from *Centella asiatica* dry plant and the concentration of asiaticoside in the water extract was quantitatively determined with the use of high performance liquid chromatography analysis. For the preparation of water extract, 30 g of *Centella asiatica* was boiled in 600 ml of distilled water under reflux for one hour. The solution was then filtered and freeze-dried to obtain the powder, which was re-dissolved in distilled water immediately before use. Different concentrations of water extract and asiaticoside were orally administered to rats with kissing ulcers. They were found to reduce the size of the ulcers at day 3 and 7 in a dose-dependent manner, with a concomitant attenuation of myeloperoxidase activity at the ulcer tissues. Epithelial cell proliferation and angiogenesis were on the other hand promoted. The expression of basic fibroblast growth factor, an important angiogenic factor, was also up regulated in the ulcer tissues in rats treated with CE or asiaticoside. Similar effects were observed after 0.25 g/kg *Centella asiatica* water extract or 10 mg/kg asiaticoside administration, suggesting that asiaticoside may be the most active ingredient in *Centella asiatica* water extract that enhanced ulcer healing.

Effects on cognitive function

Different doses of aqueous extract (100, 200 and 300 mg/kg) of the whole plant of *Centella asiatica* have been shown to counter-attack the effect of oxidative stress by decreasing the lipid peroxidation and increasing the endogenous antioxidant enzymes in brain. For the aqueous extract, coarse powder of the plant was extracted with eight parts of water under boiling for 5 hours and filtered to collect the extract. The extract was concentrated and finally dried to powder. For the methanolic and chloroform extract, the coarse powder of the whole plant was extracted with five parts of methanol at 60–65°C for

5 hours and filtered to collect the extract. The extract was concentrated under vacuum and concentration was continued to completely remove the solvent, yielding a viscous liquid. The percentage w/w yields of aqueous, methanolic and chloroform extracts were 41, 12.5 and 1, respectively.

Only the aqueous extract of whole plant (200 mg/kg for 14 days) showed an improvement in learning and memory of male Wistar rats in both shuttle box and step through paradigms. All doses of aqueous extract increased the number of avoidances in shuttle box and prolonged the step through latency in step through apparatus in a dose dependent manner. Only two doses (200 and 300 mg/kg of aqueous extract) showed significant increase in the step down latency in step down apparatus and transfer latency in elevated plus maze. Among doses of aqueous extract tested on oxidative stress parameters, only 200 and 300 mg/kg showed a significant decrease in the brain levels of malondialdehyde (MDA) with simultaneous significant increase in levels of glutathione. There was a significant increase in the levels of catalase at the 300 mg/kg but no significant change in superoxide dismutase (SOD) levels was observed. These findings suggest that the aqueous extract of *Centella asiatica* has cognitive enhancing effect and that an antioxidant mechanism could be involved (Veerendra Kumar & Gupta 2002).

Cognitive impairment in epileptics may be a consequence of the epileptogenic process as well as antiepileptic medication. The effect of aqueous extract of *Centella asiatica* (100 and 300 mg/kg) was evaluated on the course of kindling development, kindling-induced learning deficit and oxidative stress markers in pentylenetetrazole (PTZ) kindled rats. The whole plant was dried and coarsely ground with a grinder. For the preparation of the aqueous extract, the coarse powder of the plant was extracted with 8 parts of water under boiling for 5 hours and was filtered through a 400-mesh cloth to collect the extract. The extract was concentrated and finally spray dried to get a greenish brown powder. The percentage w/w yield of aqueous extract was 41. The loss on drying at 105°C and the ash content of the powder were 5% and 8% w/w, respectively. The concentration of the known active component, asiaticoside, was determined by HPLC and was found to be 3% w/w. Male Wistar rats were injected PTZ (30 mg/kg i.p.) once every alternate day (48 ± 2 h) until the development of the kindling. The administration of *Centella asiatica* aqueous extract (300 mg/kg orally) decreased the PTZ-kindled seizures and showed improvement in the learning deficit induced by PTZ kindling as evidenced by decreased seizure score and increased latencies in passive avoidance behaviour. However, low dose of the *Centella asiatica* aqueous extract (100 mg/kg) showed improvement only in the learning deficit due to the kindling and failed to improve the seizure score. The findings suggest the potential of aqueous extract of *Centella asiatica* aqueous extract as adjuvant to antiepileptic drugs with an added advantage of preventing cognitive impairment (Gupta *et al.* 2003).

Swiss albino mice were injected orally with water (weight to volume ratio of 1:3) whole plant *Centella asiatica* extract 200 mg/kg for 15 days from day 15 to day 30 post partum (p.p.) and the nootropic effect was evaluated on the 31st day and 6 months p.p. Behavioural (open field, dark/bright arena, hole board and radial arm maze tests), biochemical (acetylcholine esterase activity) and histological studies (dendritic arborisation) were carried out. Performance of juvenile and young adult mice was significantly improved in radial arm maze and hole board tests. Treatment resulted in increased acetylcholine esterase activity in the hippocampus. Dendritic arborisation of hippocampal CA3 neurons was also increased in terms of intersections and branching points, both at one month and 6 months. Results of this investigation showed that treatment during postnatal developmental stage with *Centella asiatica* extract can influence the neuronal morphology and promote the higher brain function of juvenile and young adult mice (Rao *et al.* 2005).

Psychoneuro-pharmacological profile

Anxiolytic plants may interact with either glutamic acid decarboxylase (GAD) or GABA transaminase (GABA-T) and ultimately influence brain GABA levels and neurotransmission. The purpose of a study was to determine the possible influence on the primary brain enzymes responsible for γ -aminobutyric acid (GABA) metabolism. Two *in vitro* rat brain homogenate assays were developed to determine the inhibitory concentrations (IC₅₀) of aqueous and ethanolic plant extracts. Extracts from *Centella asiatica* (gotu Kola) stimulated GAD activity by over 40% at a dose of 1 mg/ml. The alcoholic *Centella asiatica* extract dose-dependently increased the GABA level in rats. The stimulatory effect of the *Centella asiatica* extract and the anticonvulsant agent valproic acid on this inhibitory transmitter in the central nervous system was inhibited by the specific GABA-antagonist bicuculline methionine. When the stimulating effect of GABA was inhibited by bicuculline methionine, no anti-ulcerative effect of either *Centella asiatica* extract or valproic acid was demonstrable (Awad *et al.* 2007).

Axonal regeneration is important for functional *recovery* following nerve damage. *Centella asiatica* herb has been used in Ayurvedic medicine for centuries as a nerve tonic. It has been shown that *Centella asiatica* ethanolic extract (100 μ g/ml) elicits a marked increase in neurite outgrowth in human SH-SY5Y cells in the presence of nerve growth factor (NGF). A water extract of *Centella* was ineffective at 100 μ g/ml. Sub fractions of *Centella* ethanolic extract, obtained through silica gel chromatography, were tested (100 μ g/ml) for neurite elongation in the presence of NGF. Highest activity was found with a non polar fraction (GKF4). Relatively polar fractions (GKF10 to GKF13) also showed activity, albeit less than GKF4. Asiatic acid, a triterpenoid compound found in *Centella* ethanolic extract and GKF4, showed marked activity at 1 μ M. Asiatic acid was not present in GKF10 to GKF13, further indicating that other active components must be present. Neurite elongation by asiatic acid was completely blocked by the extracellular-signal regulated kinase (ERK) pathway inhibitor PD 098059 (10 μ M). Male Sprague-Dawley rats given *Centella* ethanolic extract in their drinking water (300-330 mg/kg daily) demonstrated more rapid functional recovery and increased axonal regeneration (larger calibre axons and greater numbers of myelinated axons) compared with controls, indicating that the axons grew at a faster rate. The findings above indicate that components in *Centella* ethanolic extract may be useful for accelerating repair of damaged neurons (Soumyanath *et al.* 2005).

The effect during the rat growth spurt period of *Centella asiatica* fresh leaf extract treatment on the dendritic morphology of hippocampal CA3 neurons, one of the regions of the brain concerned with learning and memory, was investigated. Fresh leaf juice was extracted from these leaves after washing, air-drying and homogenising in a glass vessel and finally filtering through a sterile gauge cloth. Neonatal rat pups (7 days old) were fed with 2, 4 or 6 ml/kg body weight of fresh leaf extract of *Centella asiatica* for 2, 4 or 6 weeks. The results showed a significant increase in the dendritic length (intersections) and dendritic branching points along the length of both apical and basal dendrites in rats treated with 4 and 6 ml/kg body weight per day of *Centella asiatica* for longer periods of time (i.e. 4 and 6 weeks). The authors concluded that the constituents/active principles present in *Centella asiatica* fresh leaf extract have a neuronal dendritic growth stimulating property; hence, the extract could be used for enhancing neuronal dendrites in stress and neurodegenerative and memory disorders (Mohandas Rao *et al.* 2006).

The role of *Centella asiatica* fresh leaf extract treatment on the dendritic morphology of hippocampal CA3 neurons, one of the regions concerned with learning and memory, in adult rats, has been investigated. Rats (2.5 months old) were fed with 2, 4 and 6 ml/kg body weight of fresh leaf juice extract of *Centella asiatica* for 2, 4 and 6 weeks, respectively. The fresh leaf juice was fed to the rats as such, through a gastric tube, a capillary tube attached to a 1-ml hypodermic syringe. After the treatment period, the rats were killed, brains were removed and hippocampal neurons were impregnated with silver nitrate (Golgi staining). Hippocampal CA3 neurons were traced using camera

lucida, and dendritic branching points (a measure of dendritic arborisation) and intersections (a measure of dendritic length) were quantified. The results showed a significant increase in the dendritic length (intersections) and dendritic branching points along the length of both apical and basal dendrites in rats treated with 6 ml/kg body weight/day of *Centella asiatica* for 6 weeks. However, the rats treated with 2 and 4 ml/kg body weight/day for 2 and 4 weeks did not show any significant change in hippocampal CA3 neuronal dendritic arborisation. The authors concluded that constituents present in *Centella asiatica* fresh leaf extract has neuronal dendritic growth stimulating properties. (Gadahad *et al.* 2008).

A study reports that phosphorylation of cyclic AMP response element binding protein (CREB) is enhanced in both a neuroblastoma cell line expressing amyloid beta 1–42 (A β) and in rat embryonic cortical primary cell culture. The study suggest also that ERK/RSK signalling pathway might mediate this effect of Gotu Kola water extract (Xu *et al.* 2008).

Antimicrobial effects

Antibacterial effects against *Pseudomonas pyocyaneus*, *Trichoderma mentagrophytes* and *Entamoeba histolytica* have been demonstrated. Both the alcoholic and the aqueous extract of *Centella asiatica* developed also an antiviral action against type II *Herpes simplex virus*.

Centella asiatica, has been used in India in leprosy patients. It is considered that asiaticoside, acts on the waxy covering of *M. leprae*. The *in vitro* effect of an indigenously produced dry powder of *Centella asiatica* on the acid-fastness and viability of *M. tuberculosis* was investigated showing that *Centella asiatica* may not have any direct action on the acid-fastness or viability of *M. tuberculosis* H37Rv *in vitro*. For this study *Centella asiatica* stock solution was prepared by weighing 60 mg of the powder of *Centella asiatica* and dissolving it in 3 ml of M/15 phosphate buffer pH 7.0 (Herbert *et al.* 1994).

The antibacterial activity of an ethanolic *Centella asiatica* extract at concentrations of 100, 200, 300 and 400 mg/ml against a battery of enteric pathogens was studied. The inhibitory effect of the extract was best demonstrated at a concentration of 400 mg/ml. The results of this study indicate the antimicrobial activity of *Centella asiatica* against the enteropathogens and suggest its potential as an anti-diarrhoeal drug (Mamtha *et al.* 2004).

The antimicrobial activity of dried leaves *Centella asiatica* methanolic extract against five bacteria strains was assessed by the presence or absence of inhibition zones and MIC values. *Centella asiatica* methanol extract has potential antibacterial activity to both gram positive *S. aureus* ATCC 25923 and methicillin resistant *S. aureus* No antibacterial activities to gram negative *E. coli* ATCC 25922, *K. pneumoniae* (IMR K25/96) and *P. aeruginosa* ATCC 27853 (Zaidan *et al.* 2005).

Immuno-modulatory effects

Immuno-modulatory activity of methanol extracts of whole plant of *Centella asiatica* (0.18% of asiaticoside) at five dose levels (dose-response relationship) ranging from 100 to 500 mg/kg body weight using carbon clearance, antibody titer and cyclophosphamide immunosuppression parameters. *Centella asiatica*, significantly increased the phagocytic index and total WBC count. The F ratio of the phagocytic index was also significant (Jayathirtha & Mishra 2004).

Immunological activity of a pectin extracted from *Centella asiatica* containing arabinose, rhamnose, galactose, xylose and galacturonic acid was also studied. After deacetylation and carboxyl-reduction, the pectin and its degraded product showed immunostimulating activity to different extent *in vitro*. The results suggested that the carboxyl and acetyl groups play important roles in the expression of immunological activity (Wang *et al.* 2005).

A study focused on the influence of whole fresh *Centella asiatica* extract on cell-mediated and humoral immune responses was carried out. *Centella asiatica* water extract significantly increased proliferation and the production of IL-2 and TNF- α in human peripheral blood mononuclear cells (PBMCs). In contrast, an ethanol extract of *Centella asiatica* inhibited human PBMC mitogenesis and the production of IL-2 and TNF- α . BALB/c mice treated with *Centella asiatica* extracts (100 mg/kg body weight) showed higher responses to both primary and secondary antibodies against BSA when compared with non-treated group. The study shows immuno-modulating activity of *Centella asiatica* extracts with regard to both non-specific cellular and humoral immune responses (Punturee *et al.* 2005).

Antinociceptive and anti-inflammatory effects

The antinociceptive activity of the whole plant water extract of *Centella asiatica* (10, 30, 100 and 300 mg/kg) was studied using acetic acid-induced writhing and hot-plate method in mice. The anti-inflammatory activity of *Centella asiatica* was studied in rats by prostaglandin E2-induced paw oedema. Water extract of *Centella asiatica* revealed significant antinociceptive activity with both the models. The activity was statistically similar to aspirin but less potent than morphine. The *Centella asiatica* extract also revealed significant anti-inflammatory activity. This effect was statistically similar to the non-steroidal anti-inflammatory drug, mefenamic acid (Somchit *et al.* 2004).

Cardiovascular effects

The alcoholic extract of *Centella asiatica* whole plant was evaluated for cardioprotective activity against ischemia-reperfusion induced myocardial infarction in rats. Cardioprotective activity was studied by measuring infarct size and estimating lipid peroxide levels in serum and heart tissue. Freshly collected *Centella asiatica* whole plant was dried under shade and the dried material was milled to obtain a coarse powder. The alcoholic extract of the powder was prepared by a process of continuous extraction such that 1 g of alcoholic extract equivalent to 5.88 g of crude drug was obtained. A dose (100–1000 mg/kg) dependent reduction in percent left ventricle necrosis (PLVN) as well as in lipid peroxide levels was observed in rats treated with alcoholic extract of *Centella asiatica* orally for 7 days compared to control animals (Pragada *et al.* 2004).

The *in vitro* effects of a methanol extract from the aerial parts of *Centella asiatica* on shear-induced platelet activation and coagulation were assessed after oral administration to rats, by subjecting non-anticoagulated blood to haemostatometry. Amongst the isolated constituents, only 3,5-di-*O*-caffeoylquinic acid showed significant inhibition of shear-induced platelet activation and dynamic coagulation. The reactive curve of the inhibitory effect on the platelet reaction and the dynamic coagulation showed a bell-shape (Satake *et al.* 2007).

Anti-tumoral activity (antiproliferative effects)

The anti-tumour effects of the methanolic crude extract of *Centella asiatica* as well as of its partially purified fractions (AF) have been investigated both *in vitro* short and long term chemosensitivity and *in vivo* tumour model test systems. Dried powder of the plant was extracted twice with 50 ml of 80% methanol. Fractions dose dependently inhibited the proliferation of the transformed cell lines significantly more than did the methanolic crude extract and other solvent fractions. No toxic effects were detected in normal human lymphocytes. AF also significantly suppressed the multiplication of mouse lung fibroblast (L-929) cells at a concentration of 8 micrograms/ml in long term culture. Oral administration of the extracts (methanolic crude extract and AF) retarded the development of solid and ascites tumours and increased the life span of these tumour bearing mice. Tritiated thymidine, uridine and leucine incorporation assay suggested that the fraction acts directly on DNA synthesis. In addition, the oral administration of methanolic crude extract and AF to normal as well as transplanted tumour

bearing mice did not produce any toxic symptoms even at high concentrations (500 mg/mouse) (Babu *et al.* 1995).

Effects of the fresh plant water extract of *Centella asiatica* on formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) and intestinal tumorigenesis in male F344 rats were investigated. The yield of water extract from fresh plant was 2.5%. Treatment with the extract significantly decreased the number of larger ACF (with four or more crypts per focus) in the large intestine in the early stage, while the number of methylated DNA adducts was not decreased compared with that in the AOM-treated group. In the post-initiation stage, the extract significantly decreased the total number of ACF and the number of larger ACF, accompanied by a decrease in the 5-bromo-20-deoxyuridinelabeling index and an increase in the induction of apoptotic cells in the colonic mucosa. The incidences of neoplasms, the numbers of adenocarcinomas in the small intestines and entire intestines, and sizes of neoplasms in the entire intestines in rats fed *Centella asiatica* extract at a dose of 10mg/kg were smaller than those in rats given AOM alone ($p < 0.05$). The extract at a dose of 100 mg/kg significantly reduced the multiplicity of neoplasms in the small intestine ($p < 0.05$). These results suggest that inhibition of the formation of AOM-induced ACF by *Centella asiatica* extract is associated with modification of cell proliferation and induction of apoptosis in colonic crypts and that the extract has a chemo-preventive effect on colon tumorigenesis (Bunpo *et al.* 2004).

Yoshida *et al.* investigated on the antiproliferative constituents in the methanolic extract from the aerial parts of *Centella asiatica*. The air-dried aerial parts of *Centella asiatica* (722.7 g) were extracted with MeOH at room temperature. The MeOH solution was concentrated *in vacuo* to give a dark green syrup (109.0 g). Activity-guided fractionation of methanolic extract resulted in the isolation of ursolic acid lactone, ursolic acid, pomolic acid, 2a,3a-dihydroxyurs-12-en-28-oic acid, 3-epimaslinic acid, asiatic acid, corosolic acid, and rosmarinic acid. Antiproliferative activity of the isolated compounds against human gastric adenocarcinoma (MK-1), human uterine carcinoma (HeLa), and murine melanoma (B16F10) cells was estimated. The fraction containing rosmarinic acid had the major concentration-dependent activity (Yoshida *et al.* 2005).

Antimutagenic effects

The antimutagenic effect of aqueous extracts of *Centella asiatica* was studied with the cytogenetic assay in somatic cells for the *in vivo* identification of chromosomal aberration and micronuclei in somatic cells (bone marrow cells) of mice. The modulating effects on the genetic damage induced by cyclophosphamide, a commonly used chemotherapeutic drug which is a known mutagen and chromium (potassium dichromate) an extensively used metal were investigated. The results showed that the *Centella asiatica* has a moderate antimutagenic nature against the cyclophosphamide and chromium (Cr) induced genotoxicity (Edwin *et al.* 2000).

Asiaticoside - various effects

The activity of asiaticoside, saponin component isolated from *Centella asiatica*, was studied in normal as well as delayed-type wound healing. In guinea pig punch wounds topical applications of 0.2% solution of asiaticoside produced 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content and better epithelisation. In streptozotocin diabetic rats, where healing is delayed, topical application of 0.4% solution of asiaticoside over punch wounds increased hydroxyproline content, tensile strength, collagen content and epithelisation thereby facilitating the healing. Asiaticoside was active by the oral route also at 1 mg/kg dose in the guinea pig punch wound model. It promoted angiogenesis in the chick chorioallantoic membrane model at 40 mg/disk concentration (Shukla *et al.* 1999).

The putative anxiolytic activity of asiaticoside was examined in male mice by using a number of experimental paradigms of anxiety, with diazepam being as a positive anxiolytic control. In the elevated plus-maze test, diazepam (1 and 2 mg/kg) or asiaticoside (5 or 10 mg/kg) increased the percentage of entries into open arms and of time spent on open arms. In the light/dark test, as with 1 mg/kg diazepam, asiaticoside (10 and 20 mg/kg) increased the time spent in the light area and the movement in the light area without altering the total locomotor activity of the animals. In the hole-board test, asiaticoside at 10 mg/kg significantly increased head-dipping counts and duration as well as diazepam (0.3 mg/kg). These findings indicated that asiaticoside exhibited an anxiolytic-like effect (Chen *et al.* 2006).

A study assessed the effects of Gotu Kola plant materials of different genotypic origin; hexane, ethyl acetate and methanolic extracts of Gotu Kola; and asiaticoside (3, 5 and 10 mg/kg body weight). Various paradigms were used to assess the anxiolytic activity, including the elevated plus maze (EPM), open field, social interaction, locomotor activity, punished drinking (Vogel Test) and novel cage tests. The EPM test revealed that Gotu Kola, its methanol and ethyl acetate extracts as well as the pure asiaticoside, imparted anxiolytic activity. Furthermore, the asiaticoside did not affect locomotor activity, suggesting this compound does not have sedative effects in rodents (Wijeweera *et al.* 2006).

Asiaticoside has been shown to induce type I collagen synthesis in human dermal fibroblast cells. However, the mechanism underlying asiaticoside-induced type I collagen synthesis, especially at a molecular level, remains only partially understood. The conclusions of a study conducted on cultured human dermal fibroblast cells suggest that asiaticoside can induce type I collagen synthesis via the activation of the T β R1 kinase-independent Smad pathway (Lee *et al.* 2006).

The application of asiaticoside at low doses of 10⁻⁸ to 10⁻¹²% (w/w) facilitated burn wound repair. To clarify the accelerating mechanisms of asiaticoside on burn wound repair, the effects of asiaticoside on the levels of various cytokines produced at the site of the burn wound was examined. The topical application of a low dose (10 pg, 1 ng, or 100 ng/wound area) of asiaticoside increased monocyte chemo-attractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), and interleukin (IL)-1 β levels in burn wound exudates. Asiaticoside (10 pg to 100 ng/ml) enhanced MCP-1 production in HaCaT cells, but it had no direct effect on VEGF production. Furthermore, asiaticoside (10 pg to 100 ng/ml) increased the IL-1 β production in THP-1 macrophages with MCP-1, but it had no effect on IL-1 β production without MCP-1 or with lipopolysaccharide (LPS). Findings suggest that the enhancement of burn wound healing by asiaticoside might be due to the promotion of angiogenesis during skin wound repair as a result of the stimulation of VEGF production caused by the increase in MCP-1 expression in keratinocytes and the increase in IL-1 β expression in macrophages induced cooperatively by asiaticoside plus MCP-1 (Kimura *et al.* 2008).

In vivo studies in rats have shown that asiaticoside given subcutaneously exhibits a protective action against stress-induced gastric ulcers and orally administered accelerates the healing of chemical-induced duodenal ulcers (Barnes *et al.* 2007).

Other effects

Radiation injury to the skin (radiation dermatitis) is one of the major limiting factors in radiotherapy. Rats were irradiated and treated with the product Madecassol® (an ointment containing TTFA or TECA) at the dose of 16 mg per cm² skin per day or with Tetrandine a bisbenzylisoquinoline alkaloid from the dried root of *Stephania tetrandra* at the dose of 1.6 mg per cm² skin per day or with vaseline. The acute skin reaction in Madecassol® treated rats appeared earlier but less severe than in control group treated with vaseline (1%). At the high dose irradiation the healing effect of tetrandine was better than Madecassol® and vaseline. Histological observations suggested that Madecassol® was able to reduce acute radiation action probably through anti-inflammatory activity (Chen *et al.* 1999).

Psoriasis is a hyperproliferative skin disorder estimated to be present in 1–3% of most populations. A rapid-throughput, *in vitro* bioassay has been utilised to examine plants for inhibitory effects on the growth of SVK-14 keratinocytes. *Centella asiatica* has been compared against the psoralen-containing seeds of *Psoralea corylifolia* and the synthetic anti-psoriatic agent dithranol (anthralin). Dried ground plant material, (2 g), was boiled for 30 minutes in distilled water (50 ml). The extract was freeze dried to 306 mg and 210 mg from *Centella asiatica* and *Psoralea corylifolia* respectively. An appropriate portion of each residue was reconstituted in water (2.5 ml) and passed through wet polyvinylpyrrolidone (PVPP) packed to a height of 1 cm in a syringe, followed by elution with distilled water (10ml). Aqueous extracts of *Psoralea corylifolia* and *Centella asiatica* inhibited keratinocyte replication with IC₅₀ values of 18.4 ± 0.6 µg/ml and 209.9 ± 9.8 mg/ml respectively prior to treatment with polyvinylpyrrolidone (PVPP) and 36.3 ± 3.3 mg/ml and 238.0 ± 2.5 mg/ml respectively after PVPP treatment of the extracts. The effect produced by *Centella asiatica* was unlikely to be due to phenolic compounds. The authors hypothesised that could be due to its two constituents, triterpenoid glycosides madecassoside and asiaticoside, and suggest the potential use of *Centella asiatica* extracts as a topical anti-psoriatic agent (Sampson *et al.* 2001).

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

Pharmacokinetics

No information is available on *Centella asiatica* extracts.

In animal experiments, an *in vivo* transformation of asiaticoside into asiatic acid was established. The maximum plasma concentration of asiatic acid increases significantly with increasing dose administered, while the time point of maximum plasma concentration and the elimination half-life does not significantly change with increasing dose. In comparison with a single oral administration, repeated oral doses have been shown to significantly increase the maximum plasma concentration and the elimination half-life. The possible mechanism behind this accumulation phenomenon is a modification of asiaticoside metabolism induced by the prolonged administration of TTFCA.

The comparison of oral and subcutaneous administration of madecassoside, asiaticoside, asiatic acid and madecassic acid in rats reveals a bio-availability varying between 30% and 50% (Brinkhaus *et al.* 2000).

Madecassic acid crosses the skin rapidly but only to a limited extent. The plasma concentration did not exceed 0.02 to 0.05% of the initial applied concentration. Madecassoside is at the skin application site only 0.04% of the applied dose at 1 hour and about 0.06% after 24 hours. For asiatic acid similar results were observed. In man, active ingredients of *Centella asiatica* are excreted principally by faeces in a 24–76 hour period; smaller quantities are eliminated through the kidneys (EMEA 1998).

Neither a possible pharmacokinetic difference between aqueous and alcoholic extracts, nor a difference between the forms of application has been investigated (Brinkhaus *et al.* 2000).

No pharmacokinetic interactions with other medicinal products are known.

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

Single/repeat dose toxicity

In the mouse, acute toxicity was not shown for oral administration of 1 g/kg body weight of an ethanolic 50% extract of *Centella*. Following intraperitoneal administration, the maximum tolerated

dose (MTD) in mice was found by Dhar *et al.* (1968) to be 250 mg/kg body weight. Alcoholic extracts of Gotu Kola have shown no toxicity at doses of 350 mg/kg when given i.p. to rats (Alternative Medicine Review 2007).

The oral LD50 of a 70% ethanolic dry extract from *Centella* 6:1 in rats was found to be higher than 675 mg/kg body weight (ESCOP 2009).

In mice, a dose of 1 g/kg body weight of an alcoholic (50:50) *Centella asiatica* extract did not lead to any toxic effects (no raw data are available). No mortality were recorded. High concentrations of asiaticoside applied on derma did not give rise to any signs of systemic toxicity.

Chronic oral administration of the extract to rats at 150 mg/kg for 30 days led to no significant differences in body weight or consumption of food and water, nor to changes in plasma glucose, proteins, cholesterol or triglycerid levels, compared to controls. No macroscopic alteration in internal organs was evident (ESCOP 2009).

Repeated topical application of an asiaticoside mixture has been associated with a tumour-growth-promoting effect in mice Laerum and Iversen (1972). Since empirical material is lacking, it is not possible to extrapolate the data to humans.

Cytotoxicity and genotoxicity and antimutagenic effects

Cytotoxic, genotoxic and antimutagenic effects of *Centella asiatica* water extracts have been investigated on *Salmonella typhimurium* TA98 and TA100 and in human lymphocytes. Edible parts of eight plants were washed well with water. The plant (300 g) was homogenised with 300 ml distilled water using a home-mixer, and the mixture was then centrifuged at 4°C, 9000 g for 30 minutes. The supernatants of the extract was freeze-dried and then stored at -20°C. *Centella asiatica* showed antimutagenic activity in the Ames test and any toxic activity (Yen *et al.* 2001).

Pure triterpenoids of *Centella asiatica* have been reported to cause alteration in gene expression in human fibroblast and recently asiaticoside has been shown to induce type I collagen synthesis in human dermal fibroblast. Leaf samples dried in the dark at 50°C for 48 hours were powdered. Powder (1 g) was extracted by sonication (3×10 min) with 3×10 ml of 90% MeOH. The dried crude extract was dissolved in 90% MeOH and filtered (pore size: 0.45 µm) prior to HPLC analysis (Randriamampionona *et al.* 2007).

Asiaticoside has been implicated as a possible skin carcinogen in rodents after repeated topical application. Asiaticoside was tested for carcinogenicity by topical applications to the skin of hairless mice. A short-term tetrazolium test indicated that compound was weak carcinogens. The compound was painted twice weekly on the dorsal skin up to about 20 months; some of the mice had previously been initiated with a small dose of 20-methylcholanthrene (MCA). A control group which received only the solvent benzene after MCA initiation was also studied. These carcinomas did not appear before about 16 months of observation. Before this, the painted, MCA-initiated animals had a significantly lower number of papillomas of the skin than the corresponding control group with only benzene treatment. By systematic autopsy it was found that about 30% of the corresponding MCA-initiated, benzene or asiaticoside-treated animals had such neoplasms. Asiaticoside was used at a concentration of 0.10% in benzene. Asiaticoside dissolved in benzene gave an increased yield of papillomas and also 2.5% skin sarcomas of the animals, indicating an effect on the dermis as well. Asiaticoside did not produce necrosis or acantholysis of the skin and did not appear to be toxic (Laerum & Iversen 1972).

The antigenotoxic effect of *Centella asiatica* extract was studied on human lymphocytes using chromosomal aberrations and sister chromatid exchanges as parameters against the genotoxic effect induced by cyproterone acetate (CPA), a synthetic progestin known to be not only a genotoxic agent

but also a tumour initiating agent. *Centella asiatica* leaves were collected, dried and ground to fine powder. Extraction was performed by soaking samples (30 gm of dry weight) in 300 ml of acetone for 8–10 hours at 40–60°C in Soxhlet's apparatus. A clear dose dependent decrease in the genotoxic damage of CPA was observed, suggesting a protective role of *Centella asiatica* extract during CPA therapy. The results suggest that the leaf extract *per se* does not have genotoxic potential, but can modulate the genotoxicity of CPA on human lymphocytes *in vitro* (Siddique *et al.* 2008).

Local tolerance

A sensitising effect of the triterpene fraction potential cause of allergic contact dermatitis has been confirmed in animal experiments. Skin hyperkeratinisation at the application site has been observed after the application of high concentrations of asiaticoside applied on derma (EMEA 1998).

Reproductive toxicity

Poor information without details is available on reproductive toxicity and teratogenicity. *In vitro* fertility investigations indicate a detrimental effect of asiaticoside on human and rat sperm. Orally administered crude extract of *Centella asiatica* has been reported to significantly reduce fertility of female mice. Teratogenicity studies in rabbit with *Centella asiatica* extracts reported negative results (EMEA 1998).

In vitro anti-infertility activity against human and rat sperm has been described for the total saponin fraction. Asiaticoside and brahminoside are thought to be active components, although no spermicidal or spermostatic action has been demonstrated for the pure saponins. A crude *Centella* extract has been reported to significantly reduce the fertility of female mice when orally administered.

Teratogenicity studies were conducted in rabbits and they reported negative results for *Centella* extract containing asiatic acid, madecassic acid, madasiatic acid and asiaticoside (Barnes *et al.* 2007).

3.4. Overall conclusions on non-clinical data

3.4.1. Pharmacodynamics

***Centella asiatica* extracts**

Several pharmacological activities of *Centella asiatica* extracts and of its chemical constituent asiaticoside have been observed in the laboratory animals.

Studies on wound healing and collagen-proliferative effects, angiogenetic activity and antioxidant activity:

Both topical (alcoholic extract) and systemic (by oral route) treatments with *Centella asiatica* extracts accelerate wound healing by stimulating epithelisation. The mechanisms involved seem to be: enhancement of collagen synthesis, stimulation of angiogenesis and antioxidant activity. These mechanisms are probably involved in the beneficial effects of *Centella asiatica* extracts in the treatment of chronic venous sufficiency.

A concentration-dependent antioxidant activity of various *Centella asiatica* extracts (ethanol, water and light petroleum) was observed. Ethanolic extracts seem to have the higher antioxidant activity followed by water extracts. Also methanolic extracts exert *in vitro* antioxidant activity. Phenolic compounds appear as main contributors to the antioxidative activities of *Centella asiatica*.

Studies on ulcer-protective and anti-ulcer effects, effects on cognitive function, psychoneuro-pharmacological profile, antimicrobial effects, immuno-modulatory effects, antinociceptive and anti-inflammatory effects, antiproliferative effects, antimutagenic effects:

Ethanollic and water extracts exert ulcer-protective and anti-ulcer effects. An antioxidant mechanism has been suggested for this effect and asiaticoside may be the most active antiulcer ingredient.

Aqueous *Centella asiatica* extracts showed improvement in learning and memory apparently associated to antioxidant effects. At the same doses *Centella asiatica* is a potential adjuvant to antiepileptic drugs with the added advantage of preventing cognitive impairment. *Centella asiatica* fresh leaf juice showed to influence the neuronal morphology and to enhance memory retention of neonatal rats.

The psychoneuro-pharmacological activity includes anxiolytic-like effects (stimulating effect of GABA shown *in vitro* with ethanolic extract), improvement of recovery and increased axonal regeneration after nerve damage (orally administered ethanolic extract), and neuronal dendritic growth stimulating property (orally administered fresh juice leaf extract) in brain areas involved in memory and learning processes. It has been suggested that the ERK/RSK signalling pathway mediate central effects.

Both ethanolic and water extracts of *Centella asiatica* showed antibacterial effects against *Pseudomonas pyocyaneus*, *Trichoderma mentagrophytes*, *Entamoeba histolytica* and antiviral action against type II *Herpes simplex* virus. Due to its activity against the enteropathogens, the Ethanolic *Centella asiatica* extract has been suggested as an antidiarrhoeal drug.

The *Centella asiatica* methanolic extract has potential antibacterial activity to both gram positive *S. aureus* ATCC 25923 and methicillin resistant *S. aureus*.

Immuno-modulating activity with regard to both non-specific cellular and humoral immune responses has been shown with both ethanolic and water *Centella asiatica* extracts. Positive immuno-modulatory effects were demonstrated also with methanolic extracts. Effects on immune system could be due to a pectin contained in *Centella asiatica*.

Antinociceptive and anti-inflammatory effects of water extract of *Centella asiatica* were shown in a single study in rats.

Cardiovascular effects are described including studies showing cardioprotection with an ethanolic extract and anti-aggregation of platelets activity of a methanolic extract.

Concentration-dependent anti-tumoral activity was shown *in vitro* with methanolic crude extract of *Centella asiatica*. Fractions of extracts containing rosmarinic acid have the highest anti-tumoral activity.

A single experiment shows that a water extract of *Centella asiatica* is moderately antimutagenic against cyclophosphamide and chromium (Cr) induced genotoxicity.

Water extracts of *Centella asiatica* exert *in vitro* anti-hyperproliferative effects on keratinocytes suggesting the potential use of *Centella asiatica* extracts as a topical anti-psoriatic agent.

Asiaticoside

Asiaticoside exerts wound healing, anxiolytic and antiulcer effects. Wound healing might be due to the stimulation of angiogenesis as a result of the stimulation of VEGF production and the induction of type I collagen synthesis probably via the activation of the TβR1 kinase-independent Smad pathway.

In conclusion, pharmacological studies show that *Centella asiatica* extracts and its main compound possesses different pharmacobiological properties. Among these, the most important are **wound healing, antiulcer properties, and nootropic-like effects**. Pre-clinical pharmacological studies

designed to clarify the mechanism of action of *Centella asiatica* in treatment of CVI have not been performed, however, induction of type I collagen synthesis induced by asiaticoside could explain some positive effects.

3.4.2. Pharmacokinetics

In humans, after single oral administration of TTFCA 30-60 mg, asiatic acid reaches maximum plasma levels at about 4.2-4.5 hours. Plasma half-life is about 2.2-3.4 hours. No detectable saponin is present 24 hours after single dosing. After repeated doses higher peak plasma concentrations, longer half-lives, and greater area-under-the curve values are observed. In animal experiments, an *in vivo*, transformation of asiaticoside into asiatic acid occurs. After oral or subcutaneous administration of madecassoside, asiaticoside, asiatic acid and madecassic acid in rats, the bio-availability is varying between 30% and 50%, respectively.

Minimal cutaneous absorption of active principles has been demonstrated. Active ingredients of *Centella asiatica* have minimal dermal absorption and they are excreted principally by faeces except for smaller quantities through the kidneys. No pharmacokinetic interactions are reported.

In animal experiments, an *in vivo* transformation of asiaticoside into asiatic acid was established. The maximum plasma concentration of asiatic acid increases significantly with increasing dose administered, while the time point of maximum plasma concentration and the elimination half-life does not significantly change with increasing dose. In comparison with a single oral administration, repeated oral doses have been shown to significantly increase the maximum plasma concentration and the elimination half-life. The possible mechanism behind this accumulation phenomenon is a modification of asiaticoside metabolism induced by the prolonged administration of TTFCA.

The comparison of oral and subcutaneous administration of madecassoside, asiaticoside, asiatic acid and madecassic acid in rats reveals a bio-availability varying between 30% and 50% (Vogel *et al.* 1990).

3.4.3. Toxicology

Centella asiatica water extract showed anti-mutagenic activity. Pure triterpenoids of *Centella asiatica* have been reported to cause alteration in gene expression in human fibroblasts. Repeated topical application of an asiaticoside mixture has been associated with a tumour-growth-promoting effect in mice. A recent study suggests that the leaf extract *per se* does not have a genotoxic potential.

The sensitising effect of the triterpene fraction could be the potential cause of allergic contact dermatitis.

No toxic effects after acute oral administration of 1 g/kg body weight of an ethanolic 50% extract of *Centella*. Alcoholic extracts of Gotu Kola have shown no toxicity at doses of 350 mg/kg when given i.p. to rats.

The total saponin fraction and asiaticoside cause *in vitro* detrimental effects on human and rat sperm. Orally crude extract of *Centella asiatica* significantly reduces fertility of female mice. Teratogenicity studies in rabbit with *Centella asiatica* extracts reported negative results. Teratogenicity studies were conducted in rabbits and they reported negative results for *Centella* extract containing asiatic acid, madecassic acid, madasiatic acid and asiaticoside.

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

TTFCA in chronic venous insufficiency (CVI)

TTFCA 30 mg twice daily for three months, reduced in humans serum enzymes involved in mucopolysaccharide metabolism in patients with varicose veins causing a protecting effect.

Six studies, 4 of which being RCTs, assessing the efficacy of TTFCA in CVI patients at any stage of the disease have been considered. Overall 357 patients were enrolled in all six studies. Five of the six studies were randomised and results compared with placebo. The endpoints investigated were: filtration rate, PO₂, PCO₂, resting flux, permeability, oedema, ankle swelling, and subjective symptoms. The results show that treatment with TTFCA improves the endpoints investigated. The doses used were ranging between 60 and 180 mg/day generally divided in two or three times during the day. The period of treatment was between 2 and 8 weeks; the median period of treatment in the six studies was about 6 weeks; the median period of treatment calculated per patient was 7.14 weeks (Table 3).

In another study the combination of alpha tocopherol, rutin, melilotus, and *Centella asiatica* (TTFCA) in the treatment of thirty patients with CVI randomised in two groups of fifteen subjects (control and treatment group) was investigated. Therapeutic efficacy and tolerability have been valued after 15 and 30 days of treatment. At the end of the observation period, a significant improvement of clinical symptoms was obtained, characterised by a diminution of the sovralfascial oedema (Cataldi *et al.* 2001) (Table 3).

Incandela *et al.* published in 2001 a review on the effects of TTFCA in improving venous wall alterations in chronic venous hypertension and in protecting the venous endothelium. The authors concluded that TTFCA is active on the microcirculation in venous and diabetic microangiopathy and that signs and symptoms of venous hypertension and oedema are also improved by treatment. The remodelling on collagen synthesis is cited as one of the possible mechanisms of actions of TTFCA in the remodelling of echolucent plaques at the carotid and femoral bifurcation. TTFCA was considered safe and well tolerated (Incandela *et al.* 2001a).

A study was designed to evaluate whether TTFCA, was effective, by modulating collagen production, in a period of 12 months, increasing the echogenicity of echolucent plaques at the femoral bifurcation. TTFCA was used at the dose of 60 mg three times daily (oral tablets). 60 subjects were recruited: 26 completed the study in the treatment group and 24 in the placebo group. No significant changes were observed in controls while a qualitative increase in homogeneity was observed in the TTFCA group. Plaque size measured at the beginning and at the end of the study showed a median increase in size, in controls (23%; range 0%-44%); it was unchanged in the TTFCA group (variation 7%; 4%-26%). In conclusion in the treatment group plaques increased in echogenicity and in homogeneity; size and stenosis remained unchanged. For the authors these observations indicate a positive action of TTFCA on the stabilisation of hypoechoic, low-density femoral plaques (Incandela *et al.* 2001b).

Fifty patients with diabetic microangiopathy were studied by laser Doppler flowmetry (measuring skin blood flow at rest) (RF) and the venoarteriolar response (VAR), by transcutaneous PO₂ and PCO₂ measurements, and by capillary permeability evaluation (rate of ankle swelling [RAS]). Thirty of these patients were treated for 6 months with TTFCA (60 mg twice daily). A control group of ten patients was treated with placebo and another group of ten patients was left without treatment thus acting as a

second control group. After six months there were no significant changes in the two control groups. There was a significant improvement of microcirculatory parameter in patients treated with TTFCA. RF (abnormally increased at the beginning of the treatment) decreased, and the VAR (impaired at the beginning of the study) improved. PO₂ increased and PCO₂ decreased the abnormally increased capillary permeability was also improved (decreased). The data showed that TTFCA was useful in diabetic microangiopathy by improving microcirculation and decreasing capillary permeability and protects against the deterioration of microcirculation due to diabetic microangiopathy (Cesarone *et al.* 2001).

4.1.2. Assessor's overall conclusions on pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Chronic treatment with TTFCA 30 mg, reduced in humans serum enzymes involved in mucopolysaccharide metabolism in patients with varicose veins. TTFCA at oral doses ranging between 60 and 180 mg/day for a period of treatment between 2 and 8 weeks improves filtration rate, PO₂, PCO₂, resting flux, permeability, oedema, ankle swelling in CVI patients.

TTFCA improves venous wall alterations in chronic venous hypertension protecting the venous endothelium. TTFCA is active on the microcirculation in venous and diabetic microangiopathy and signs and symptoms of venous hypertension and oedema are also improved by treatment. The remodelling on collagen synthesis is cited as one of the possible mechanisms of actions of TTFCA in the remodelling of echolucent plaques at the carotid and femoral bifurcation.

Fifty patients with diabetic microangiopathy were studied by laser Doppler flowmetry (measuring skin blood flow at rest) (RF) and the venoarteriolar response (VAR), by transcutaneous PO₂ and PCO₂ measurements, and by capillary permeability evaluation (rate of ankle swelling [RAS]). TTFCA (60 mg twice daily) induced a significant improvement of microcirculatory parameters: TTFCA decreases resting flux (abnormally increased at the beginning of the treatment), and improves VAR (impaired at the beginning of the study); increases PO₂ and decreases PCO₂.

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

No information is available on *Centella asiatica* extracts.

In a randomised cross-over study involving 12 subjects, Grimaldi investigated the absorption behaviour of TTFCA following both single and repeated oral doses (30 and 60 mg). The results suggest that active ingredients in TTFCA are well absorbed in human volunteers (Grimaldi *et al.* 1990). After single oral administration of the extract, maximum plasma levels of asiatic acid were reached at 4.5 and 4.2 hours, with 30 and 60 mg, respectively. Plasma half-lives were 2.2 hours in the 30-mg dose and 3.4 hours in the 60-mg dose. No detectable saponin was present 24 hours after single dosing. After repeated doses (7-day treatment) higher peak plasma concentrations, longer half-lives, and greater area-under-the curve values were observed.

4.2. Clinical Efficacy

4.2.1. Dose response studies

Three dose-response studies were published. These studies show that the *Centella asiatica* extract TTFCA ameliorated the endpoints investigated. The doses used were ranging between 60 and 180 mg/day generally divided in two or three (see Table 3).

4.2.2. Clinical studies (case studies and clinical trials)

Centella is stated to be valuable in the treatment of wide range of ailments, such as: chronic venous insufficiency, periodontitis, ulcer cicatrisation and burns recovery, anxiety, atherosclerosis.

This paragraph is describes the following studies:

- A) Reviews of the clinical studies on CVI
- B) Clinical studies on the effects on CVI
- C) Clinical studies on other indications (Ulcer cicatrisation and burns recovery; anxiety; atherosclerosis)
- D) A clinical study on psoriasis
- E) Clinical studies with combined products containing *Centella asiatica* (Periodontitis; Mild to moderate atopic dermatitis)
- F) Cosmetic studies on skin ageing

Clinical studies were published describing the use of the following preparations containing *Centella asiatica* extracts:

- TTFCA (total triterpenoid fraction of *Centella asiatica*)
- TECA (titrated extract of *Centella asiatica*);
- MADECASSOL® (gauze (10 x 10cm) impregnated with a mixture of titrated extract of *Centella asiatica*)
- The above mentioned extracts are different declaration used to designate the same type of extract containing asiaticosides (40%), asiatic acid (29-30%), madecassic acid (29-30%) and madasiatic acid (1%);
- Gotu kola extract
- Combined product with Alpha tocopherol, Rutin, Melilotus, and *Centella asiatica* (TTFCA)
- Combined alcoholic extract from *Centella asiatica* and *Punica granatum*
- Ointment containing *Mahonia aquifolium*, *Viola tricolor* and *Centella asiatica*

Varicose veins

Varicose veins are the most common manifestation of CVI, affecting 25% of women and 15% of men. Varicose veins are swollen, twisted, painful veins that have filled with an abnormal collection of blood. In normal veins, valves in the vein keep blood moving forward toward the heart. With varicose veins, the valves do not function properly, allowing blood to remain in the vein. Pooling of blood in a vein causes it to enlarge.

Current treatments include measures primarily aimed at reducing venous hypertension and preventing progression to chronic inflammation and ulcerations. Surgical procedures including saphenous vein stripping, ligation of the saphenofemoral junction, and ambulatory phlebectomy are effective in the treatment of varicose veins but are associated with a high complication rate and recovery time.

Centella asiatica (TTFCA; 30 mg twice daily for three months) reduced serum enzymes involved in mucopolysaccharide metabolism (betaglucuronidase, beta-N-acetylglucosaminidase and arylsulfatase) in patients with varicose veins causing a protecting effect on connective tissue of varicosities and showing regulatory effects of the extract of *Centella asiatica* on metabolism in the connective tissue of the vascular wall (Arpaia *et al.* 1990).

Chronic venous insufficiency (CVI)

CVI is a common disorder that affects the veins of the legs. It is a long-term condition in which the veins do not send adequately the blood from the legs back to the heart. In people with CVI, the veins do not work properly and blood pools in the legs, leading to increased pressure in the veins and alteration of blood flow to the legs and feet. It occurs because of partial vein blockage or blood leakage around one or more valves of the veins, which are damaged, or blocked from a clot (deep vein thrombosis).

Risk factors for venous insufficiency include: genetic factors, age, sex (women), obesity, pregnancy, works implying prolonged sitting or standing.

Symptoms can be mild or severe and include leg heaviness and/or cramping in legs, itching, tingling, pain (worsening when standing), dilated or unsightly veins, oedema (swelling), skin colour changes and rashes on the legs and ankles, superficial varicose veins, recurrent skin infections and chronic ulcers.

As CVI results from venous hypertension secondary to superficial or deep venous valvular reflux, treatment modalities are aimed at reducing venous valvular reflux. Compression therapy using pumps, bandaging, and/or graded compression stockings is the mainstay of treatment for CVI. Long periods of sitting or standing should be avoided and care has to be taken of wounds and infections, if occur. Pharmacologic agents such as diuretics and topical steroid creams reduce swelling and pain short term but offer no long-term treatment advantage. Herbal substances may ameliorate the symptoms but vary in their efficacy, quality, and safety. Several randomised controlled trials using horse chestnut seed extract containing aescin have shown short-term improvement in signs and symptoms of CVI. Endovascular and surgical techniques aimed at treatment of primary and secondary venous valvular reflux have been shown to improve venous haemodynamics promoting healing of venous ulcers and improving quality of life. The newer endovascular treatments of varicose veins using laser, radiofrequency ablation, and chemical foam sclerotherapy show some promise.

A) Reviews of *Centella asiatica* clinical studies on CVI

Three reviews with comments on effects of *Centella asiatica* on CVI have been published. One of them is centred on *Centella asiatica* and the two others report general comments on the protecting effects of different substances on CVI.

A Cochrane review to assess the efficacy of oral or topical phlebotonics was published in 2005; studies published from January 1966 to April 2005 were analysed. Randomised, double blind, placebo-controlled trials (RCTs) assessing the efficacy of *Centella asiatica* and other phlebotonics such as rutosides, hidrosmine, diosmine, calcium dobesilate, chromocarbe, disodium flavodate, French maritime pine bark extract, grape seed extract and aminaftone in CVI patients at any stage of the disease were collected. The effects of treatment were estimated by relative risk or by standardised mean differences (SMD) by applying a random effects statistical model. Sensitivity analyses were also performed. Fifty-nine RCTs of oral phlebotonics were included, but only 44 trials involving 4413 participants contained quantifiable data for the efficacy analysis: 23 of rutosides, ten of hidrosmine and diosmine, six of calcium dobesilate, two of *Centella asiatica*, one of French maritime pine bark extract, one of aminaftone and one of grape seed extract. Outcomes included oedema, venous ulcers, trophic disorders, subjective symptoms (pain, cramps, restless legs, itching, heaviness, swelling and paraesthesias), global assessment measures and side effects. The authors concluded that there was not enough evidence to globally support the efficacy of phlebotonics for CVI. There is a suggestion of some efficacy of phlebotonics on oedema but this is of uncertain clinical relevance. Due to the limitations of current evidence, there is a need for further randomised, controlled clinical trials with greater attention paid to methodological quality. *Centella asiatica* effects were assessed in two studies. One study showed non-significant results compared with placebo in the dichotomous variable heaviness. The other study showed favourable results for *Centella asiatica* in the dichotomous variable global assessment by the patient (Martinez *et al.* 2005).

A more recent review has been focused on CVI and in particular on drugs that have been evaluated by randomised prospective controlled trials. Horse chestnut seed extracts, flavonoids, red vine leaves extracts, TTFCA, procianidins, calcium dobesilate, and pentoxifylline are discussed together with the microcirculatory effects of compression therapy using bandages or stockings. The review comments that the major microcirculatory effects that have been shown are the reduction of capillary filtration rate and improvements in levels of transcutaneous partial pressures of oxygen and carbon dioxide (TcPO₂ and TcPCO₂). Data suggest that a combination of pharmacologic and compression therapy may have some additive effects. The review analyses two controlled studies carried out on the efficacy of TTFCA. The author underlines that data support TTFCA having a favourable effect on cutaneous microcirculation improving capillary filtration rate, ankle circumference, and ankle oedema decreased, with the higher dosage (120 mg per day) being more effective (Wollina *et al.* 2006).

B) Clinical studies on the effects on CVI

TTFCA

Six studies assessing the efficacy of TTFCA in CVI patients at any stage of the disease have been considered. Five studies were randomised and results compared with. The endpoints investigated were: filtration rate, PO₂, PCO₂, resting flux, permeability, oedema, ankle swelling, and subjective symptoms. These studies show that TTFCA ameliorated the endpoints investigated and where different doses were used dose-relation improving effects were showed. The doses used were ranging between 60 and 180 mg/day generally divided in two or three times. The total number of patients treated in the six studies was 357. The period of treatment was between 2 and 8 weeks; the median period of treatment in the six studies was about 6 weeks; the median period of treatment calculated for patient was 7.14 weeks (Table 3).

TECA

A multicenter, randomised, double-blind versus placebo study was conducted to investigate on the effects of the TECA in the treatment of venous insufficiency. Ninety-four patients suffering from venous insufficiency of the lower limbs were recruited and divided in three groups treated with TECA 120 mg/day, TECA 60 mg/day, or placebo, respectively. The treatment period was two months. A significant difference (p less than 0.05) in favour of TECA was shown for the symptoms of heaviness in the lower limbs and oedema, as well as for the overall evaluation by the patient. The venous distensibility was improved for the TECA groups but aggravated for the placebo group (Pointel *et al.* 1987).

Table 3. Clinical studies on TTFCA in the therapy of CVI.

Indication	N° subjects	Randomisation and control	Endpoints	Posology and Duration of treatment	Statistical analysis	Efficacy	Authors
Venous hypertension	10 normal controls 22 pts with moderate venous hypertension 12 pts with severe venous hypertension	Healthy subjects	Permeability, microcirculatory parameters and subjective symptoms	60 mg twice/day 2 weeks	Yes	Improvement of capillary permeability and microcirculation and signs and symptoms	Belcaro <i>et al.</i> i G.1990
Venous hypertensive microangiopathy	90 patients	Placebo Double blind Randomisation	Resting perimalleolar skin flux, PO ₂ , PCO ₂	30 mg and 60 mg twice daily 2 months	Yes	Dose-dependent improvement of resting perimalleolar skin flux, increase of PO ₂ , decrease of PCO ₂	Cesarone <i>et al.</i> 1994
Venous Hypertension	10 normal controls 52 patients	Placebo and health subjects Blindness not specified Randomisation	Capillary filtration rate, ankle oedema, subjective symptoms	30 mg and 60 mg thrice daily 4 weeks	Yes	Dose related improvement of signs and symptoms was well correlated with that of CFR and ankle oedema	De Sanctis <i>et al.</i> 2001
Venous microangiopathy	40 patients	Placebo Blindness not specified Randomisation	Resting flux, PO ₂ , PCO ₂ , leg volume	60 mg twice/day 6 weeks	Yes	Improvement of microcirculation and leg volume associated with decrease in local filtration and reduction of oedema	Cesarone MR, <i>et al.</i> 2001

Venous hypertension and microangiopathy	40 patients	Placebo Blindness not specified Randomisation	Resting flux, ankle swelling, skin flux, symptoms	60 mg twice/day 8 weeks	Yes	Improvement of microcirculation, ankle swelling and oedema	Cesarone <i>et al.</i> 2001
Venous hypertension	99 patients	Placebo Single blind Randomisation	Transcutaneous PO ₂ , PCO ₂ , venoarteriolar reflex, flow variation, symptom evaluation	60 mg and 120 mg twice daily Dose was divided in two daily administrations 2 months	Yes	Dose related improvement of symptoms and microcirculatory parameters after 1 and 2 months	Incandela <i>et al.</i> 2001

Incandela *et al.* published in 2001 a review on the effects TTFCA in improving venous wall alterations in **chronic venous hypertension and in protecting the venous endothelium**. The authors concluded that TTFCA is active on the microcirculation in venous and diabetic microangiopathy and that signs and symptoms of venous hypertension and oedema are also improved by treatment. The remodelling on collagen synthesis is mentioned as one of the possible mechanisms of actions of TTFCA in the remodelling of echolucent (soft; therefore, with risk of thrombosis and embolisation) plaques at the carotid and femoral bifurcation. TTFCA was considered safe and well tolerated (Incandela *et al.* 2001a; Incandela 2001b).

C) Clinical studies on atherosclerosis other indications

TTFCA

Atherosclerosis is a condition in which fatty material collects along the walls of arteries. This fatty material thickens, hardens, and may eventually block the arteries. Atherosclerosis is a type of arteriosclerosis. The two terms are often used to mean the same thing.

A study was designed to evaluate whether TTFCA was effective, by modulating collagen production, in a period of 12 months, increasing the echogenicity of echolucent plaques at the femoral bifurcation in patients with **atherosclerosis**. In hypoechoic atherosclerotic plaques stromal composition is limited as the collagen component is generally very low; the plaque composition is mainly due to lipid accumulation or thrombosis. Echogenicity of hyperechoic plaques and how it could be modified by drug acting on the modulation of collagen synthesis was evaluated. Antiplatelet agents were used in all patients; cholesterol-lowering agents were used in 34% of patients in the treatment group and in 36% in the placebo group. TTFCA was used at the dose of 60 mg three times daily (oral tablets). 60 subjects were recruited: 26 completed the study in the treatment group and 24 in the placebo group. No significant changes were observed in controls while a qualitative increase in homogeneity was observed in the TTFCA group. Plaque size measured at the beginning and at the end of the study showed a median increase in size, in controls (23%; range 0-44%); it was unchanged in the TTFCA group (variation 7%; 4-26%). In conclusion in the treatment group plaques increased in echogenicity and in homogeneity; size and stenosis remained unchanged. For the authors these observations indicate a positive action of TTFCA on the stabilisation of hypoechoic, low-density femoral plaques (Incandela *et al.* 2001c).

Fifty patients with **diabetic microangiopathy** were studied by laser Doppler flowmetry (measuring skin blood flow at rest) (RF) and the venoarteriolar response (VAR), by transcutaneous PO₂ and PCO₂ measurements, and by capillary permeability evaluation (rate of ankle swelling [RAS]). Thirty of these patients were treated for 6 months with TTFCA (60 mg twice daily). A control group of ten patients was treated with placebo and another group of ten patients was left without treatment thus acting as a second control group. After six months there were no significant changes in the two control groups. There was a significant improvement of microcirculatory parameter in patients treated with TTFCA. RF (abnormally increased at the beginning of the treatment) decreased, and the VAR (impaired at the beginning of the study) improved. PO₂ increased and PCO₂ decreased the abnormally increased capillary permeability was also improved (decreased). Data showed that TTFCA was useful in diabetic microangiopathy by improving microcirculation and decreasing capillary permeability and protects against the deterioration of microcirculation due to diabetic microangiopathy (Cesarone *et al.* 2001).

MADECASSOL® (corresponding to TECA or TTFCA)

The effects of impregnated dressing of Madecassol® on ulcer cicatrisation and burns recovery were investigated. Madécassol® individual gauze (10x10cm) impregnated with a mixture of TECA was used. The study was conducted on 76 patients (43 males, 33 females) who had experienced previous allergic

dermatitis or were affected by dermatosis of different kinds. The results are favourable after a treatment period of 8-10 days (Basset 1978).

Gotu kola extract

Centella is used in Ayurvedic medicine for the treatment of anxiety. A double-blind, placebo-controlled study was undertaken to evaluate the anxiolytic activity of Gotu Kola (*Centella asiatica*). The authors evaluated the effects of Gotu Kola on the acoustic startle response (ASR) in humans. The ASR model is based on the universally occurring startle reaction to unexpected stimuli (e.g., loud acoustic signals) characterised in humans by a sequence of physical responses, including an eye blink. The ASR is augmented by fear and anxiety and potentiated by anxiogenic substances. The ASR paradigm has been extensively used to investigate stress-related disorders and anxiety in humans. Subjects were randomly assigned to receive either a single 12 g orally administered dose of Gotu Kola (N = 20) or placebo (N = 20). Gotu Kola was administered as crude powder herb blended with grape juice and celery salt in order to make *verum* and placebo solutions identical in colour, smell and taste. The results revealed that compared with placebo, Gotu Kola significantly attenuated the peak ASR amplitude 30 and 60 minutes after treatment. The authors concluded that these preliminary findings suggest that Gotu Kola has anxiolytic activity in humans but it remains to be seen whether this herb has therapeutic efficacy in the treatment of anxiety syndromes (Bradwejn *et al.* 2000).

A randomised, placebo-controlled, double-blind study investigated the effect of *Centella asiatica* on cognitive function of twenty-eight healthy elderly volunteers. Participants received the plant extract at various doses ranging 250, 500 and 750 mg once daily for 2 months. A-day capsule contained a specialised aerial part extract containing total phenolic content equivalent to tannic acid = 29.9 mg/g. In addition, the extract also contained asiaticoside and asiatic acid were presented at concentration of 1.09 and 48.89 mg/g of crude extract, respectively. Cognitive performance was assessed using the computerised test battery and event-related potential whereas mood was assessed using Bond–Lader visual analogue scales prior to the trial and after single, 1 and 2 months after treatment. The results showed that the high dose of the plant extract enhanced working memory and increased N100 component amplitude of event-related potential. Improvements of self-rated mood were also found following the *Centella asiatica* treatment. The present findings suggest the potential of *Centella asiatica* to attenuate the age-related decline in cognitive function and mood disorder in the healthy elderly (Wattanathorn *et al.* 2008).

D) Clinical study on psoriasis

The effects of a topical application of a cream containing the **water and oil extract from *Centella asiatica*** were investigated in seven patients affected by psoriasis in a not controlled study. Lesions completely cleared in five of them, majority of lesions disappeared in one patient, partial but definitive improvement was observed in one case. Preparation: the leaves of the plant were dried and powdered. Dry powder (100 g) was put in a flask containing 500 ml of water. Duration of treatment was from 3 to 8 weeks. Patients received no other systemic therapies. There was no evidence of systemic or local toxicity. None of the patient experienced any side effect (Natarajan & Paily 1973).

Assessor's comment: It is a not controlled study carried out with a very small number of patients.

E) Clinical studies with combined products containing *Centella asiatica*

Combined product with Alpha tocopherol, Rutin, Melilotus and *Centella asiatica* (TTFCA)

The combination of alpha tocopherol, rutin, *Melilotus* and *Centella asiatica* (TTFCA) in the treatment of patients with CVI was investigated. Thirty patients with CVI have been randomised in two groups of

fifteen subjects (control and treatment group). The therapeutic efficacy and the clinical tolerability have been valued with clinical-instrumental evaluations and by a control after 15 and 30 days. Functional complaints, cramps and the oedema have been evaluated in function, presence and severity with a clinical-score between 0 and 4. At the end of the observation period, a significant improvement of the clinical symptoms was obtained, characterised by a diminution of the suprafascial oedema (Cataldi *et al.* 2001).

Combined alcoholic extract from *Centella asiatica* and *Punica granatum*

Two clinical studies on the effects of combined extracts from *Centella asiatica* and *Punica granatum* on periodontitis were published. The total number of recruited adult patients was 35. Patients with initial pocket depth 5-8mm were enrolled into the studies. A herbal medication in the form of biodegradable chips for subgingival application was used. *Centella asiatica* L. dried whole plants were ground and refluxed three times for three hours, each with 95% ethanol. The combined alcoholic extracts were subsequently dried under vacuum. *Centella asiatica* dry extract has been analysed for the molecular structure of asiatic acid and madecassic acid by NMR and gas-spectrophotometry. *Punica granatum* L. dried fruit peels were ground and extracted using the same method as for *Centella*. Unmedicated chips were used in the placebo group. Probing pocket depth, attachment level, bleeding on probing, gingival index, and plaque index were recorded at baseline, 3 and 6 months. The results showed significant improvements of pocket depth and attachment level in the test sites when compared with the placebo sites at 3 months and with the placebo and control sites at 6 months. All treatment sites exhibited a similar trend of decreasing plaque score. However, the test sites seemed to show slightly better percentage of bleeding on probing. The results indicate that local application of *Centella asiatica* and *Punica granatum* extracts plus scaling and root planing significantly reduced the clinical signs of chronic periodontitis. In one of the two studies it was also shown that the combined extracts reduced IL-1 beta and IL-6 levels (Sastravaha *et al.* 2003; 2005).

Ointment containing *Mahonia aquifolium*, *Viola tricolor* and *Centella asiatica*

A randomised, double-blind, vehicle-controlled, half-side comparison with a herbal ointment containing *Mahonia aquifolium*, *Viola tricolor* and *Centella asiatica* for the treatment of mild-to-moderate atopic dermatitis was performed. A total of 88 patients with mild-to-moderate atopic dermatitis were enrolled in the study. Patients between 18 and 65 years of age were treated for 4 weeks with the ointment. The primary endpoint was a summary score for erythema, edema/papulation, oozing/crust, excoriation and lichenification according to a 4-point scale. Secondary efficacy variables were assessment of pruritus severity (10 cm VAS) and a global assessment of effectiveness as well as tolerability. The study ointment reduced the primary and secondary endpoints slightly more than the base cream which was used as vehicle; the differences were not statistically significant. Since the climatic conditions during the study duration varied from very mild and sunny to very cold and dry, a post-hoc subanalysis was performed with a subset of 64 patients whose treatment was at a mean outside temperature of 10° C or less. Under these conditions the primary endpoint showed high statistical significance. Author's conclusion: the ointment containing *Mahonia aquifolium*, *Viola tricolor* and *Centella asiatica* could not be proven to be superior to a base cream for patients with mild-to-moderate atopic dermatitis. However, a subanalysis indicated that the cream might be effective under conditions of cold and dry weather (Klövekorn *et al.* 2007).

F) Cosmetic studies on skin ageing

A randomised double-blind study was carried out on photoaged skin of 20 female volunteers with actinically aged facial, neck and forearm skin to investigate the effects of topically applied 5% vitamin C and 0.1% madecassoside on the clinical, biophysical and structural skin properties. A

fingertip unit of the tested cream was applied twice daily for 6 months to the face, as well as the assigned half of the neck and upper chest, and one of the arms of each volunteer whereas the other half of the neck and the other arm received the control cream. After the treatment, a significant improvement of the clinical score for deep and superficial wrinkles, suppleness, firmness, roughness and skin hydration has been observed. These results were corroborated by measurements of skin elasticity and semi-quantitative histological assessment of the elastic fibre network in the papillary dermis. Two-thirds of the subjects showed an improvement and re-appearance of a normally structured elastic fibre network was observed. The results indicated a functional and structural remodelling of chronically sun-damaged skin (Haftek *et al.* 2008).

A study was designed with the aim to evaluate the efficacy of wrinkle improving lipstick containing asiaticoside (0.2% concentration). The digital photograph image of lips before and after lipstick application was assessed from 20 female volunteers. Colour tone and time-related pigment spread was calculated. The efficacy of wrinkle improving lipstick containing asiaticoside was evaluated by using subjective and objective methods including image analysis in a double-blind placebo-controlled fashion. Results: The colour tone and spread phenomenon after lipstick make-up were remarkably affected by lip wrinkles. By using the lipstick containing asiaticoside for 8 weeks, the change of visual grading scores and replica analysis indicated the wrinkle-improving effect. As the depth and number of wrinkles were reduced, the lipstick make-up appearance by image analysis also improved significantly (Ryu *et al.* 2005).

A total of 30 healthy women with signs of skin ageing were studied. Fifteen of the women were treated with a food supplement based on polysaccharides derived from the fish cartilage and a natural mix of antioxidants (*Gingko biloba*, flavonoids, *Centella asiatica*) for 2 months and the other 15 with a placebo. Clinical evaluation and biophysical parameters related to skin function and wrinkle severity, such as silicone replica, skin thickness, mechanical properties, skin colour and capacitance, were measured. The results showed statistically significant changes in the active-treated group in comparison to the placebo. In particular, dermal thickness (treatment: from 1.13 to 1.23 mm; $P < 0.001$), skin wrinkling (treatment: from 9.5 to 3.5 Ra; $P < 0.002$), skin colour (treatment: brighter and less pigmented; $P < 0.02$) and viscoelasticity (treatment: from 0.70 to 0.97%; $P < 0.02$) showed considerable improvement (Distante *et al.* 2002).

Prophylaxis with an antistriae cream (*Centella asiatica* extract, α -tocopherol, and collagen-elastin hydrolisates) was assessed by a double blind trial in 80 pregnant women. In the placebo group 22 women (56%) presented striae, whereas in the treated group only 14 women (34%) developed striae in this pregnancy; this difference was significant ($p < 0.05$; χ^2 test). An arbitrary score was designed to assess the intensity of striae (from 0 to 3); this score was 1.42 (s.d. 0.5) in the treated group and 2.13 (s.d. 1.32) in the placebo group and this difference was also significant ($p = 0.014$; Mann-Whitney test). In women with a history of striae during puberty, the active cream induced a significant absolute prevention in 59% of the cases whereas in the placebo group all the women developed striae ($p = 0.00014$; χ^2 test) (Mallol *et al.* 1991).

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

According to Siddha literature, *Centella asiatica* leaves are used in the treatment of syphilis, all types of fever, children's abdominal disorder (Sathya & Uthaya Ganga 2007), but no information on clinical studies in special populations is available.

4.3. Overall conclusions on clinical pharmacology and efficacy

Clinical efficacy of TTFCA and TECA (corresponding to the same type of *Centella asiatica* extract) in the treatment of CVI was shown in different clinical studies. Improvement of microcirculation and leg

volume associated with reduction of oedema and symptoms were observed. Three studies showed dose-dependent ameliorating effects. The safety profile of *Centella asiatica* extracts appears as acceptable and well tolerated as it is emerging from clinical studies in patients affected by CVI and from its use from products on the market. No drug-related serious adverse events were reported during the clinical trials.

Pharmacokinetics

No information is available on *Centella asiatica* extracts.

In a randomised cross-over study involving 12 test subjects, Grimaldi investigated the absorption behaviour of TTFCA following both single and repeated oral doses (30 and 60 mg). The results suggest that active ingredients in TTFCA are well absorbed in human volunteers (Grimaldi *et al.* 1990). After single oral administration of the extract, maximum plasma levels of asiatic acid were reached at 4.5 and 4.2 hours, with 30 and 60 mg, respectively. Plasma half-lives were 2.2 hours in the 30 mg dose and 3.4 hours in the 60 mg dose. No detectable saponin was present 24 hours after single dosing. After repeated doses (7-day treatment) higher peak plasma concentrations, longer half-lives, and greater area-under-the curve values were observed.

Madecassic acid crosses the skin rapidly but only to a limited extent. The plasma concentration did not exceed 0.02 to 0.05% of the initial applied concentration. Madecassoside is at the skin application site only 0.04% of the applied dose at 1 hour and about 0.06% after 24 hours. For asiatic acid similar results were observed. In man, active ingredients of *Centella asiatica* are excreted principally by faeces in a 24-76 hour period; smaller quantities are eliminated through the kidneys (EMA 1998).

Neither a possible pharmacokinetic difference between aqueous and alcoholic extracts, nor differences between the forms of application have been investigated (Brinkhaus *et al.* 2000).

No pharmacokinetic interactions with other medicinal products are known.

5. Clinical Safety/Pharmacovigilance

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

Although a putative causal effect of adjunctive substances has also been discussed, a sensitising effect of the triterpene fraction has been confirmed in animal experiments (Hausen 1993). In a large-scale case study observation, occasional burning pain following i.m. injections and the local application of a powder has been reported (Wolfram 1965). Following oral administration of *Centella* preparations, gastric complaints and nausea have occasionally been reported, but were not significant versus placebo (Brinkhaus *et al.* 2000).

The tolerability of oral *Centella asiatica* preparations has generally been reported to be good in all the studies cited.

5.2. Patient exposure

See section 4.2.2.

5.3. Adverse events and serious adverse events and deaths

Allergic contact dermatitis has been reported after the topical application of various creams and ointments containing *Centella* extracts (Rotblatt & Ziment 2001). However, further testing revealed

that reactions may be due to other ingredients in the preparations (Hausen BM. *Centella asiatica* Indian pennywort), an effective therapeutic but a weak sensitiser (Rotblatt & Ziment 2001).

Following oral administration of *Centella* preparations, gastric complaints and nausea have occasionally been reported, but they were not significant versus placebo (Kartnig and Hoffmann-Bohm, 1992). Pain and burning sensation, following intramuscular injection or the topical application of preparations available as powders, and allergic contact dermatitis have been reported (Allegra *et al.* 1981; Marastoni *et al.* 1982; Pointel *et al.* 1987).

Adverse reaction from Greece: During the oral use rarely mild gastrointestinal pain (nausea, vomit etc.) Gotu Kola has been reported to cause hyperglycaemia and hypercholesterolemia in a single trial from 1969 (Rotblatt & Ziment 2001).

Three women (61, 52 and 49 years old) developed jaundice after taking *Centella asiatica* for 30, 20 and 60 days. Respective laboratory tests: ALT: 1193, 1694 and 324 U/L; ALP: 503, 472 and 484 U/L; bilirubin: 4.23, 19.89 and 3.9 mg/dl. The first patient also had ASMA (Anti-Smooth Muscle Antibody) 1/160 and AMA (Antimitochondrial Antibody) 1/320. The respective pathological diagnoses were: granulomatous hepatitis with marked necrosis and apoptosis; chronic hepatitis with cirrhotic transformation and intense necroinflammatory activity, and granulomatous hepatitis. All the three patients improved with *Centella asiatica* discontinuation, and ursodeoxycholic acid 10 mg/kg/day. The first patient took *Centella asiatica* again, with recurrence of the damage. The second one had taken this herb a year before.

It was hypothesised that terpenic active principles of *Centella* can produce hepatic injury by promoting apoptosis and altering cell membranes. The presence of autoantibodies and granulomas also favours an immune-mediated mechanism (Jorge & Jorge 2005).

No drug interactions have been reported.

Caution should be advised in patients who are already taking substances having sedative properties with the aim to avoid additive effect.

Contraindications: allergy to plants of the *Apiaceae* family (WHO monographs, 1999).

5.4. Laboratory findings

Chronic treatment with TTFCA extract 30 mg twice daily for three months, reduced in humans serum enzymes involved in mucopolysaccharide metabolism in patients with varicose veins. TTFCA at oral doses ranging between 60 and 180 mg/day for a period of treatment between 2 and 8 weeks improves filtration rate, PO₂, PCO₂, resting flux, permeability in CVI patients.

The effects of Gotu Kola on the ASR were studied in humans. The ASR model is based on the universally occurring startle reaction to unexpected stimuli (e.g. loud acoustic signals) characterised in humans by a sequence of physical responses, including an eye blink. The ASR is augmented by fear and anxiety and potentiated by anxiogenic substances. The ASR paradigm has been extensively used to investigate stress-related disorders and anxiety in humans. Subjects were randomly assigned to receive either a single 12 g orally administered dose of Gotu Kola (N = 20) or placebo (N = 20). Gotu Kola was administered as crude powder herb diluted in grape juice and added with celery salt. Gotu Kola significantly attenuated the peak ASR amplitude 30 and 60 minutes after treatment. The authors concluded that these preliminary findings suggest that Gotu Kola has anxiolytic activity in humans (Bradwejn *et al.* 2000).

5.5. Safety in special populations and situations

Drug interactions

None reported.

Use in pregnancy and lactation

Centella asiatica is reputed to be abortifacient and to modify the menstrual cycle (Barnes *et al.* 2007). Gotu Kola should be avoided during pregnancy, due to its emmenagogue action (Alternative Medicine Review 2007).

Not be used during pregnancy without medical advice (ESCOP 2009).

Drug abuse, withdrawal and rebound

None reported.

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

No data.

Contraindications

Hypersensitivity to plants of *Apiaceae* family (ESCOP 2009).

5.6. Overall conclusions on clinical safety

The tolerability of oral *Centella asiatica* preparations has generally been reported to be good in all the studies cited.

Occasional burning pain following i.m. injections and the local application of a powder has been reported. Following oral administration of *Centella* preparations, gastric complaints and nausea have occasionally been reported.

6. Overall conclusions

Medicinal products containing *Centella asiatica* (L.) Urban refined extracts, corresponding to TECA, are authorised and have been marketed in Europe in several Members States: Belgium, France, Greece, Italy, Portugal and Spain and the time elapsed since the first marketing authorisation is longer than 30 years.

The medicinal use of TECA for a period of 30 years has been established on the basis of published literature and decisions taken by National Competent Authorities to grant marketing authorisations.

There are several clinical studies on the efficacy of TECA extract in CVI. They involve small numbers of patients, but they are all positive and no negative results are reported. Moreover the period of long standing use since more than 30 years of products containing TECA extract in the EU Community, the pharmacological data, the plausibility of the efficacy in wound healing support the traditional use in this indication.

No reasons of concerns relating the safety are reported and the tolerability of oral *Centella asiatica* preparations has generally been shown to be good in all studies. No adverse events from pharmacovigilance data are known.

Based on the information on manufacturing process, the HMPC is of the opinion that TECA extract cannot be classified as an herbal preparation due to the manufacturing steps and composition.

Therefore, despite the existing data on the safety and efficacy and the historical use within the Community of products containing TECA extract, it is not possible to propose any monograph for this preparation, because all the data do not refer to a herbal preparation.

Other products containing preparations from *Centella asiatica* are also available in several EU Countries with medicinal claims related to the microcirculation and the tissue draining. The traditional use of some of these *Centella asiatica* preparations may date back to more than the 30 years required by Directive 2004/24/EC.

Although some data are available on other herbal preparations, these data were not found sufficient and consistent according to requirements of Article 16a(1) of Directive 2001/83/EC. Should such information be provided, a monograph could be prepared.

Further investigation on products on the market and their use is needed, because all available information are insufficient to draft a monograph for the traditional used based on:

- the description of the herbal preparation
- the period of use in the proposed indication in and outside Europe
- the posology and route of administration

Annex

List of references