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Committee on Herbal Medicinal Products (HMPC)

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

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Centella asiatica* (L.) Urb., herba
Herbal preparation(s)	a) Comminuted herbal substance b) Powdered herbal substance
Pharmaceutical form(s)	Comminuted herbal substance as an infusion for cutaneous use. Powdered herbal substance for cutaneous use.
Rapporteur(s)	A. Assisi
Peer-reviewer	I. Chinou

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000

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1. Introduction

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

- Herbal substance(s)

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

The leaves are very variable in size; the petiole is usually 5-10, even 15 times longer than the lamina, which is 10-40 mm long and 20-40 mm, sometimes up to 70 mm, wide. Dried, fragmented aerial parts contain minimum 6.0% of total triterpenoid derivatives, expressed as asiaticoside (Ph. Eur. 10th ed., ref.: 1498).

Centella asiatica is synonym with *Hydrocotyle asiatica* L. In German, the medicinal plant is also known by the colloquial name of Indischer Wassernabel. Further names used are: Indian Pennywort (English), Hydrocotyle Asiatique (French), Idrocotile (Italian), Brahma-manduki and Brahmi-Buti (Hindi), Tsubokusa (Japanese), 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).

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 ($C_{48}H_{78}O_{19}$; Mr 959.15) (IUPAC name: *O*-6-Deoxy-α-L-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl 2α,3β,23-trihydroxy-4α-urs-12-en-28-oate) (Ph. Eur. 10th ed., ref.: 1498).

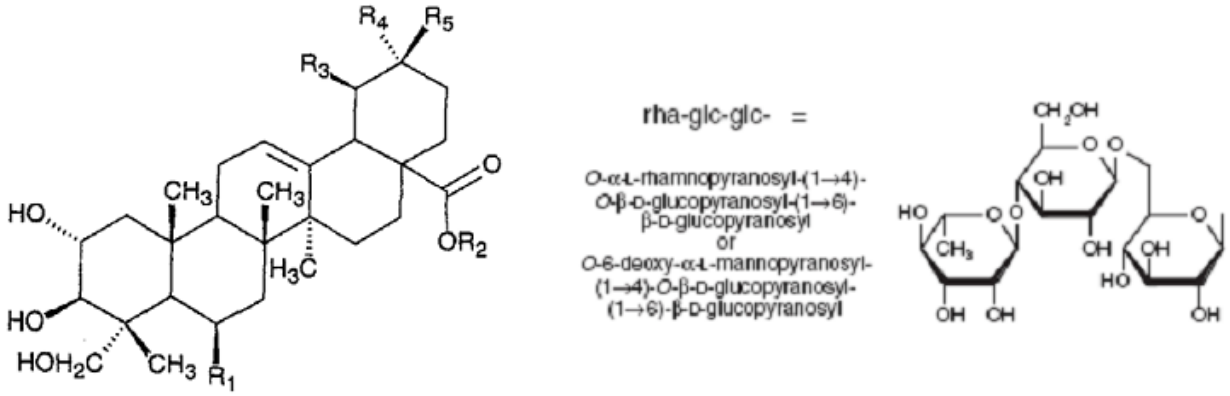
In addition to about 0.1% essential oils and other volatile constituents, *Centella asiatica* contains a wide range of metabolites. These derive mostly from the metabolism of phenylpropane and acetate and belong to the flavonoid and terpenes (Brinkhaus *et al*., 2000). The species is most known for its high content of pentacyclic triterpenoids (C30), collectively referred also as 'centelloids'. The saponins, asiaticoside and madecassoside, and their aglycones, asiatic acid and madecassic acids, are the most abundant pentacyclic triterpenoids in *Centella*. Saponins account for up to 8% of the dry mass of the herb. The levels of saponins and sapogenins vary widely depending on the geographical origin, genetic, environmental and growth conditions. *Centella asiatica* contains many phenolic constituents, including flavonoids, such as catechin, epicatechin, kaempferol, quercetin and related glycosides (Gray *et al*., 2018a). The plant is also rich in chlorogenic acids, a diverse group of compounds formed by quinic acid esterified to cinnamic acid derivatives.

The plant contains about 36% of volatiles and fatty oils. The fatty oil consists of glycerides of palmitic, stearic, lignoceric, oleic, linoleic, and linolenic acids. The major constituent present in *Centella asiatica* oil comprises of terpenic acetate, while other prominent constituents were β-caryophyllene, farnesene, trans-β-farnesene, germacrene-D, α-humulene, bicyclogermacrene, sesquiterpene, and p-cymol (Chandrika and Kumarab 2015). A small percentage (0.21%) of pulegone was found in the essential oil (Joshi and Chaturvedi 2013).

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

tetrahydroxyurs-12-ene-28-oic acid and of 2β,3β,6β,23β-tetrahydroxyolean- 12-ene-28-oic acid (Sahu *et al.,* 1989).

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



	R_1	R_2	R_3	R_4	R_5
asiatic acid	-H	-H	-CH3	-CH3	-H
madecassic acid	-OH	-H	-CH3	-CH3	-H
asiaticoside	-H	1)-β-D-glc-(6-1)- β-D-glc-(4-1)- α-L-rha	-CH3	-CH3	-H
asiaticoside A[1]	-OH	1)-β -D-glc-(6-1)- β-D-glc-(4-1)- α-L-rha	-CH3	-CH3	-H
terminolic acid	-OH	-H	-CH3	-CH3	-CH3
asiaticoside B	-OH	1)-β -D-glc-(6-1)- β-D-glc-(4-1)-α-L-rha	-H	-CH3	-CH3

- Herbal preparation(s)

Aqueous extracts of *Centella asiatica*, as well as refined and purified extracts have been used.

Literature reports studies on the following extracts: TECA (titrated extract of *Centella asiatica*), TTFCA (total triterpenoid fraction of *Centella asiatica)* and TTF (total triterpenoid fraction), 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 and TECA. 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 occasionally 'ETCA' and 'CATTF' are different acronyms to designate the same extract, commercially known with different brand names containing 40% of asiaticoside and 60% of asiatic acid and madecassic acid.

Other herbal substances/preparations historically in medicinal use include powdered fresh and dried leaves, powdered dried whole plant (without roots), alcoholic and aqueous extracts.

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

Not applicable.

1.2. Search and assessment methodology

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

The keywords "*Centella asiatica*", "*Hydrocotyle asiatica",* "Gotu kola", "Indian pennywort", "asiaticoside" in all text fields were used.

The following databases were searched in November 2019:

Medical databases: Pubmed, Embase

Toxicological databases: Toxnet

Pharmacovigilance resources: EudraVigilance

Data from EU and non-EU regulatory authorities: market overview up to January 2019

1.3. Specific assessment history

The initial assessment to prepare a Community herbal monograph on *Centella asiatica* (L.) Urb., herba in line with the MLWP 2009 work programme ended in November 2010 with a public statement (EMA/HMPC/579663/2009) explaining why a monograph could not be established.

Based on the information on manufacturing process, the HMPC was of the opinion that specific purified preparations (such as 'TECA' extract) cannot be classified as a 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 was considered not possible to propose any monograph for *Centella asiatica* preparations, because the data do not refer to a herbal preparation.

Although some data were available on other preparations considered herbal preparations, these data were not found sufficient and consistent according to requirements of Article 16a(1) of Directive 2001/83/EC.

In 2018, the HMPC initiated a systematic review of available new data and decided in September 2019 based on the Rapportuer's review report that sufficient data appear meanwhile available to establish a monograph for a herbal preparation.

Since the publication of the HMPC public statement in 2010, several articles were published on the pharmacological properties of the herb and its main constituents (asiaticoside acid and madecassoside). In addition, the results of new controlled clinical trials with different *Centella asiatica* extracts and several new review articles were published on phytochemistry, pre-clinical and clinical pharmacology.

The Ph. Eur. monograph for *Centella asiatica*, herba was revised by EDQM (publication of the revised monograph in Pharmeuropa 24.1, year 2012). Main changes involved the inclusion of a test for adulteration with *Bacopa monnieri* L., the increase of maximum limit for loss on drying (from 10.0% to 12.0%), the inclusion of a test for ash insoluble in hydrochloridic acid, and changes to the LC method for the assay. However, the definition section of the monograph has not changed.

An EudraVigilance search revealed 23 reports; out of these, 10 reports were found in subjects taking only Centella asiatica. Three cases were serious, and patients experienced urticaria, eritema/hypersensitivity/skin burning/skin swelling and application site pain; however, these adverse

reactions were not life-threatening, disabling and did not cause hospitalization. All other adverse reactions were not serious and concerned mainly the skin.

The review also revealed that since the publication of the public statement in 2010, no new product has been registered for traditional use or authorised as original or well-established use herbal medicinal product containing *Centella asiatica* in the EU. However, the use of dried leaves of *Centella asiatica* has been reported in the British Herbal Pharmacopoeia (BHP) in rheumatic conditions and cutaneous affections and the topical application for indolent wounds, leprous ulcers and cicatrisation after surgery, at a dosage of 0.6 g thrice daily.

It was concluded that this reference can provide the basis for preparing an EU monograph as the herbal substance/preparation reported in the BHP fulfils the requirement laid down in Article 16a(1)(d) of Directive 2001/83/EC.

2. Data on medicinal use

2.1. Information about products on the market

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

Information on medicinal products marketed in the EU/EEA

An inquiry to get information on herbal medicinal products containing *Centella asiatica* was sent to HMPC members in December 2018. There are no authorised medicinal products containing herbal preparations of *Centella asiatica* as a monocomponent. However, medicinal products containing standardised and highly purified *Centella asiatica* preparations are authorised and marketed in Europe with full application in Belgium, France, Greece, Italy, Portugal and Spain with the following indications:

- Treatment of moderate or benign problems in wound formation such as atonic wounds (BE)
- Treatment of hypertrophic scars, keloids in active phase (BE)
- Local treatment for granulation phase of wounds, cutaneous ulcers and cutaneous gangrene (FR)
- Improvement of symptoms of venous stasis (FR)
- Potent wound healing agent (induce collagen biosynthesis) (EL)
- Symptoms of venous insufficiency and capillary fragility (IT)
- 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 (PT)
- Adjuvant on the cicatrisation of skin injuries (PT)
- Epithelizant agent, to heal injuries, scars, keloid scars, burns (ES)

These medicinal products are marketed with different trade names as tablets (10 mg or 30 mg per tablet, respectively), creams (1%), ointments (1%), cutaneous powders (2%) or sterilised impregnated dressings (1 g of extract per 100 g of mass; 2 g of mass per dm^2).

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.

TECA is obtained by extreme purification steps, which 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 (see EMA/HMPC/579663/2009 Public statement on *Centella asiatica* (L.) Urban, herba).

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. The posology is 1 hard capsule (30 mg) 2 times per day.

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable.

Information on other products marketed in the EU/EEA (where relevant)

Food supplements containing different preparations of *Centella asiatica* are on the market in the European Union, as well as cosmetics.

Food supplements are 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.

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

Not applicable.

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

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

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 1852, Boileau, a French doctor in Mauritius, pointed out the use of the plant (called Bavilacqua in Mauritius) in the treatment of leprosy; in his opinion the use of the whole plant was preferable to that of the leaves alone. A few years later, a number of lepers were treated with the drug in the Madras hospitals and included in the Indian pharmacopoeia. The application of *Centella asiatica* causes a feeling of warmth and tingling of the skin, especially that of the hands and feet. After a few days there is a feeling of heat, which is often hard to bear. The capillary circulation is accelerated, and after a week the appetite begins to improve, the skin gradually becomes softer, its thickenings are repelled, and it regains the ability to perspire (Madaus 1938). In Madagascar, the plant was also used to treat leprosy (Sahu *et al.,* 1989).

The use of *Centella asiatica*, mainly leaves and whole plant, in dermatological conditions such as eczema, psoriasis, lupus was reported by Madaus (1938).

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 (Abdul Hamid *et al.,* 2002).

Fresh extracts of the 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).

In folk medicine different uses of *Centella asiatica* herb, not supported by experimental or clinical data, were reported like: 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. *Centella asiatica* 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).

In Asia, the drug is used to enhance urination, for physical and mental exhaustion, diarrhea, eye diseases, inflammations, asthma and high blood pressure. The drug is used in Indian medicine for skin diseases, syphilis, rheumatism and leprosy. *Centella asiatica* is also used for the treatment of mental illness, epilepsy, hysteria and for dehydration. In Chinese medicine, the herb is used for dysentery and summer diarrhea, vomiting, jaundice, urinary calculi, epistaxis and scabies (PDR 2000).

Centella asiatica extracts have been also used in the treatment of keloids (Hausen 1993).

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).

Centella is used in folk medicine also for the treatment of ulcer, tubercolosis (Sardar *et al.,* 2015), carbuncle, spermatorrhea, stomach ache, dyspepsia (Bhuyan and Baishya 2013), cough, heartburn, aphthous stomatitis, sore throat (Sujarwo *et al.,* 2015), sunstroke, gallstones (Li *et al.,* 2017), diabetes mellitus (Semenya *et al.,* 2012), malaria (Paul *et al*., 2013; Shah *et al.,* 2014), intestinal worms (Panmei *et al*., 2019), abdominal pain, anorexia (Kadir *et al*., 2014), headache, depression (Dey *et al.,* 2017; De Rus Jacquet *et al.,* 2014), gonorrhoea (Das *et al.,* 2013).

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 the use in cutaneous affections with topical application for indolent wounds, leprous ulcers and cicatrisation after surgery. According to Medicaments a base de plantes, the whole plant been used also for sunburns, local and superficial burns, nappy rash, antipruritic local treatment of dermatological disorders (British Herbal Pharmacopoeia 2003).

Table 1: Overview of historical data

Herbal preparation	**Documented Use / Traditional Use**	**Pharmaceutical form Strength (where relevant) Posology Duration of use**	**Reference**
Powdered leaves Fluid extract (further information missing) Roots Dried whole plant (without roots)	Leprosy, eczema, lupus, psoriasis, pruritus	0.6-1.2 g daily 10 drops 3 times daily 0.1-0.4 g 1 tablet of the plant trituration "Teep" 3 times daily (The "Teep" preparation is adjusted to 10% plant substance, i.e. 1 tablet corresponds to 0.025 g Hb. Hydrocotyles as.)	Madaus 1938
Aerial parts	Rheumatic conditions Cutaneous affections Topically: indolent wounds, leprous ulcers and cicatrisation after surgery	0.6 g or as an infusion 3 times daily Taken orally for rheumatic conditions Topically applied for indolent wounds, leprous ulcers and cicatrisation after surgery	BHP 1983; Barnes *et al*., 2007 (both are based on British Herbal Pharmacopoeia, 1983)
Powdered leaves	Vulnerary, dermatic, anti-leprotic, anti-inflammatory Main use for the treatment of skin conditions, particularly ulcers, wounds an for keloid and hypertrophic scars, and as an immunomodulator	0.5-1 g daily or equivalent extract, taken orally (often by infusion) and applied topically	Williamson 2003

2.3. Overall conclusions on medicinal use

Table 2: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indications	Strength Posology	Period of medicinal use
Aerial parts	Traditional herbal medicinal product to aid in healing of minor wounds	0.6 g as a powder or as an infusion 3 times daily Single dose: 0.6 g Daily dose: 1.8 g	BHP 1983; Barnes *et al*., 2007 (both the references are based on British Herbal Pharmacopoeia, 1983)

The traditional use of *Centella asiatica* for the symptomatic treatment of minor inflammations of the skin and wound healing is based on the British Herbal Pharmacopoeia, which reports the use of the aerial parts for cutaneous affections (orally) and wound healing (topically). The use in leprosy, psoriasis and lupus is not adequately documentated by clinical data; in addition, these indications have been excluded by the monograph as they do not fulfil the legal requirements for traditional use as defined by the Art. 16a of the Directive 2001/83/EC, i.e. indications intended and designed for use without the supervision of a medical practitioner for diagnostic purposes or for prescription or monitoring of treatment.

There is evidence of traditional use of aerial parts also as anti-rheumatic. However, this indication has been excluded from the monograph, due to the toxic effects of powdered leaves observed in rats on hepatic and renal function; in addition, there is enough evidence that *Centella asiatica* might impair fertility at doses traditionally used in humans, when taken orally (see section 3). Due to these safety concerns, also the traditional use of aerial parts of *Centella asiatica* administered orally for cutaneous affections, as cited in the British harmacopoeia, cannot be included in the monograph.

In conclusion, only the use of aerial parts of *Centella asiatica* topically applied directly as a powder or as an infusion to aid in healing of minor wounds according to the posology reported in the British Pharmacopoeia fulfils the requirements of the Art. 16a of the Directive 2001/83/CE to be included in the monograph.

The application on the skin of the herbal infusion has been reported in the monograph as impregnated dressing. This is based on a medicinal product (Madecassol®) authorised in France since 1976 to aid in the local treatment for granulation phase of wounds, cutaneous ulcers and cutaneous gangrene which is presented as a sterile dressing impregnated with TECA.

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* 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, including wound healing, ulcer-protective, psychoneuro-pharmacological (cognitive effects), antinociceptive, anti-inflammatory, antimicrobial, immunomodulatory, antiproliferative, antimutagenic, angiogenetic, antioxidant.

Triterpenoid compounds occurring in *Centella asiatica*, mainly including 2 glycosides (asiaticoside and madecassoside) and corresponding aglycones (asiatic acid and madecassic acid), are considered to be major ingredients with pharmacological activities.

3.1.1. Primary pharmacodynamics

Wound healing and collagen-poliferative effects – studies with herbal preparations

In vitro studies

An extract of *Centella asiatica* was prepared cutting, dryng and grounding into powder. Powdered samples were extracted with 36% ethanol (ratio 1:6), the extract was filtered mixed with propylene glycol (80% in water, 40 ml). The extract showed stimulatory effects on collagen synthesis in a dose dependent manner. Collagen was assayed by Sirius red staining method. The vitamin C (25 mg/ml) was used as a positive control and showed 2-fold collagen enhancing response compared to untreated solvent, which was used as negative control. At 50 mg/ml, the *Centella asiatica* extract enhanced 3-fold collagen production compared to the control (untreated), whereas at 30 mg/ml and 10 mg/ml the collagen enhancements were of 2- and 1.4-fold, respectively (Hashim *et al.,* 2011).

Kim *et al.* (2011) showed that an ethanolic extract of *Centella asiatica* represses the process of senescence induced by H_2O_2 through the regulation of specific genes involved in DNA replication and mitosis. Human dermal fibroblasts (HDFs) were cultured in the presence of 10% fetal bovine serum (FBS) for 24 h after pre-treatment with *Centella asiatica* extracts for 4 h and exposure to H_2O_2 (200 μm) for 2 h. H_2O_2-induced premature senescence decreased by 2.05% after treatment with *Centella asiatica* extracts at 2 μg/ml and by 20.83% at 20 μg/ml. However, post-treatment with *Centella asiatica* extracts did not change the percentage of senescent cells. Western blotting analysis showed that H_2O_2 induced a robust increase in the expression of p53, p21 and pRb and that pre-treatment with *Centella asiatica* extracts inhibited the accumulation of these proteins. Microarray analyses showed that the profile of mRNA expression was significantly different between *Centella asiatica* extract-treated cells and nontreated cells. Specifically, the expression of 39 probes changed more than 2-fold in *Centella asiatica* extract-treated cells. *Centella asiatica* extracts downregulated the expression of genes involved in apoptosis, gene silencing, cell growth, transcription and senescence, and upregulated the expression of genes involved in DNA replication and spindle checkpoint. Finally, *Centella asiatica* extracts rescued the H_2O_2-induced repression of replication in HDFs.

An aqueous extract of *Centella asiatica* leaves (obtained extracting dried leaves for 24 h at 100°C) when encapsulated in nanoparticles using gelatin, efficiently reduced the expression of matrix metalloproteinase MMP-1 in UV-irradiated human fibroblasts. UVA (ultraviolet A) irradiation significantly induced MMP-1 expression levels up to 136.1%, when compared with the control group (non-UVA). However, this effect was suppressed down to 106.1% and 77.6%, respectively, by the addition of the unencapsulated crude extracts and nanoparticles at a sample concentration of 0.5 mg/ml. At this concentration, the nanoparticles displayed a stronger hyaluronidase inhibition activity (59.5%) than the crude extract (47.1%). These effects were not mediated by gelatin (Kwon *et al*., 2012).

The effect of an aqueous extract of *Centella asiatica* on the proliferation and migration of rabbit corneal epithelial (RCE) cells was evaluated by Idrus *et al.* (2012). Fresh leaves of *Centella asiatica* were sun-dried and powdered; 250 g of the powder were refluxed with 1.5 l of distilled water at ratio 1:6 for 3 h at temperature approximately 40°C and then cooled, filtered and freeze-dried. RCE cells were cultured with or without supplementation of *Centella asiatica* aqueous extract. Viability and proliferation of the RCE cells was determined by 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. *In vitro* re-epithelisation was studied by scratch assay and migration rate was evaluated quantitatively by image analyser. It was found that supplementation of *Centella asiatica* did not show any significant effect on the RCE cells proliferation at the concentration up to 500 ppm, while at the concentration of 1,000 ppm significantly inhibited RCE cells proliferation ($p<0.05$). However, at the concentration up to 62.5 ppm, RCE cells shows significant enhancement of migration rate compared to the control group ($p<0.05$).

An aqueous solution of *Centella asiatica* obtained dissolving the dry powder in phosphate buffer saline was investigated in human dermal fibroblasts by analysing the *in vitro* proliferation. For this study, 0 µg/ml (control), 7.81 µg/ml, 15.63 µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1,000 µg/ml concentration of *Centella asiatica* in Ham's F12:DMEM (1:1) 10% + 10% FBS were studied. The proliferation assay was performed by using MTT with modifications described by Mosmann, 1983. The result displayed an anti-proliferative effect by the *Centella asiatica* on the growth of dermal fibroblasts when compared to control (0 µg/ml) at high concentrations of *Centella asiatica* after 4 (500 µg/ml and 1000 µg/ml) and 8 (250 µg/ml, 500 µg/ml and 1000 µg/ml) days of treatment. *In vitro* re-epithelisation was assessed by scratch assay and migration rate was evaluated quantitatively by image analyser. *Centella asiatica* aqueous solution inhibits the migration of dermal fibroblasts at the concentration of 194.15 µg/ml, when compared to the control (0 µg/ml). Gene expression of type I collagen, type III collagen, fibronectin and alpha-smooth muscle actin 2 (SMAA2) were studied via real-time RT-PCR. The gene expression decreased with increasing concentration of aqueous extract of *Centella asiatica* up to 194.15 µg/ml, although it was not statistically significant compared to control (0 µg/ml). Finally, supplementation of aqueous solutions of *Centella asiatica* did not alter the cell cycle even at 194.15 µg/ml (Idrus *et al.,* 2018).

In vivo studies

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 (1 ml/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).

The wound healing activities of sequential hexane, ethyl acetate, methanol, and aqueous extracts of *Centella asiatica* in incision and partial-thickness burn wound models have been investigated in rats. Collected aerial parts of *Centella asiatica* were oven-dried at 50°C and then powdered using a milling machine. The powdered plant (1 kg) was macerated with n-hexane (3 times 4 l, 3 days each) at room

temperature. The pooled filtrates were dried under reduced pressure to give the n-hexane extract (HexE) at a constant weight of 17.8 g. The marc was air-dried before it was further macerated with ethyl acetate and methanol, respectively, using the procedure described above to give the ethyl acetate (32.7 g) and methanol (267.5 g) extracts. Subsequently, a portion (300 g) of the dried marc was boiled in distilled water (2 l) for 3 h, then filtered and dried to give 95.1 g of the hot aqueous extract. Tween 20W as a 10% solution in distilled water was used as vehicle for preparation of a 10% w/v of each extract for topical application (0.5 ml of the test substances was topically applied to the burnt area once daily). The general appearance and degree of wound healing of the burn wound were assessed on Days 3, 7, 10 and 14 after burn injury and prior to histopathological evaluation. The degrees of healing in the burn wound with the 4 extracts were significantly higher than that of the control on Days 3, 10 and 14. Histopathological findings on Day 14 after burn injury revealed prominent fibrinoid necrosis and incomplete epithelialisation in the control and untreated groups, whereas fully developed epithelialisation and keratinisation were observed in all extract-treated groups (Somboonwong *et al*., 2012).

The efficacy of a topical spray containing methanolic extract of *Centella asiatica* herb has been evaluated on excision wounds in rats. Hydroxypropyl-*b*-cyclodextrin, Eudragit E100, glycerol, PEG 400, copovidone, ethanol and purified water were included in the formulation. Air-dried aerial parts of *Centella asiatica* were cut into tiny pieces, powdered and sieved. The powder was defatted with petroleum ether and extracted with methanol in a Soxhelet apparatus for 12 h. The extract was concentrated over a water bath (50 °C) and dried at 50 °C (±5 °C) in a hot air oven under atmospheric conditions. Final traces of methanol were removed under reduced pressure (~8 mbar) at 55 °C using a rotary evaporator. The assay content of triterpenes in the extract was 0.12% asiatic acid, 0.54% madecassic acid, 0.25% asiaticoside and 1.02% madecassoside. The animals were assigned into 3 groups (n=6 in each group). Group 1 was untreated and was regarded as the control. Group 2 animals received povidone iodine solution (Betadine®) treatment, while group 3 animals received the *Centella asiatica* extract 5 puffs (~2.5 ml) of topical spray once daily for 14 days starting from the first day of wounding. There was significant wound healing in group 3 treated with *Centella asiatica* extract compared to the control group (group 1). However, group 2 involving conventional wound treatment with povidone iodine solution showed the highest wound healing activity due to its antimicrobial activity (Sawatdee *et al.,* 2016).

In another study, the potential of electrospun gelatin membranes containing methanolic *Centella asiatica* extract (EGC) as topical/transdermal wound dressings was investigated *in vivo* using Sprague Dawley (SD) rats. Thirty-five grams of *Centella asiatica* slices was extracted using 700 ml of 90% methanol under stirring for 5 h. The supernatant from the methanol extraction was directly filtered, evaporated and lyophilised to obtain dry extract (~10 g). The *Centella asiatica* extract was dissolved in 90% methanol, adjusted to a final concentration of 10 mg/ml, and filtered through a 0.45-µm filter membrane. The wound areas of rat skin treated with EGC presented the highest recovery rate compared with those treated with gauze, neat gelatin membranes and commercial wound dressings (Comfeel®, Peterborough, United Kingdom). The results of the histopathological examination support the outcome of the wound models (Yao *et al.,* 2017).

Wound healing and collagen-poliferative effects – studies with pure constituents of *Centella asiatica*

In vitro studies

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 TGFβ receptor 1 (TβR1) 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)-1β levels in burn wound exudates. Asiaticoside (10 pg to 100 ng/ml) enhanced MCP-1 production in human keratinocyte cell line HaCaT cells, but it had no direct effect on VEGF production. Furthermore, asiaticoside (10 pg to 100 ng/ml) increased the IL-1β production in THP-1 macrophages with MCP-1, but it had no effect on IL-1β 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-1β expression in macrophages induced cooperatively by asiaticoside plus MCP-1 (Kimura *et al.,* 2008).

In order to investigate the effects of asiatic acid on cell proliferation, invasion and collagen synthesis, normal and keloid fibroblasts isolated from human skin were exposed to TGF-β1 with or without Asiatic acid. Keloid fibroblasts had a higher baseline level and responded more briskly to TGF-β1 stimulation in collagen type I expression, compared to normal fibroblasts. Asiatic acid (10 and 30 μM) significantly decreased TGF-β1-induced collagen type I expression in keloid fibroblasts, while had little effect on normal fibroblasts as determined by ELISA. This effect seemed to be mediated by peroxisome proliferator-activated receptor-γ (PPAR-γ) activation (Bian *et al.,* 2013).

Higher expression of growth differentiation factor-9 (GDF-9) in keloids compared with hypertrophic scars and normal skin tissues has been reported. Wu *et al*. (2017) showed that GDF-9 could enhance the proliferation, migration, and invasion of keloid fibroblasts (KFs), while it only slightly elevated collagen expression, indicating that the effect of GDF-9 was opposite to that of TGF-β1. The authors also demonstrated that asiaticoside (10 and 30 μM) markedly inhibited cell proliferation induced by GDF-9 stimulation and decreased the invasive capacity of KFs in a concentration-dependent manner.

Lee *et al.* (2012) investigated the effects of asiaticoside at concentrations of 0, 62.5, 125, 250, 500, and 1000 μM on normal human skin cells (adult human dermal fibroblasts and adult normal human epidermal keratinocytes) in a wound closure seeding model. Compared with a control group, asiaticoside-treated cells migrated faster, and the most effective concentration varied with cell type. In fibroblasts, asiaticoside at 250 μM increased migration rate most effectively and improved wound healing by approximately 20% compared with a control group. In keratinocytes, 500 μM asiaticoside improved wound healing by about 20%. By a MTT assay, the authors demonstrated that asiaticoside treatment significantly enhanced the initial skin cell adhesion (the numbers of fibroblasts attached increased by approximately 40% and the numbers of keratinocytes, by more than 10%). Finally, asiaticoside promoted the growth of fibroblasts in a dose-dependent manner. At 62.5 μM asiaticoside, the cell number did not increase significantly compared with a control DMSO until the 5 day assessment; however, at 125 μM and higher concentrations of asiaticoside the numbers of treated cells increased steadily from 1 to 5 days compared with the control group. In contrast, asiaticoside did not influence the growth rate of keratinocytes.

The effects of asiaticoside on proliferation, protein synthesis, and osteogenic differentiation in human periodontal ligament cells (HPDLs) at concentrations of 25, 50, and 100 μg/ml. This compound had no effect on cytotoxicity or cell proliferation as determined by MTT assay. When HPDLs were treated with asiaticoside in serum-free medium, dose-dependent increases in the levels of fibronectin and collagen type I mRNA and protein were observed at 72 h. The increase of fibronectine levels was statistically

significant only with highest concentration of asiaticoside (100 µg/ml), whilst the effect on collagen I protein expression was significant already at 50 µg/ml A significant reduction of MMP-1 mRNA expression was noted at the highest concentration of asiaticoside (100 mg/ml) whereas a significant increase of tissue inhibitor of metalloproteinase-1 (TIMP-1) mRNA expression was observed at lower concentrations (25 and 50 mg/ml) as well. Finally, the addition of asiaticoside to osteogenic medium resulted in an increase in alkaline phosphatase enzymatic activity, up-regulation of osteoblast marker gene mRNA expression, and enhancement of mineralisation by HPDLs (Nowwarote *et al.,* 2013).

The effect of asiaticoside (0, 100, 250, and 500 mg/l) on the proliferation and collagen expression was investigated on fibroblasts isolated from keloid tissue and normal skin tissues. After 3 days of treatment, asiaticoside significantly diminished keloid fibroblast proliferation by 28, 30, and 51%, at 100, 250, and 500 mg/l, respectively ($p<0.05$). On day 5, the reduction of proliferation after asiaticoside treatment was 35, 48, and 63%, at 100, 250, and 500 mg/l, respectively. However, the normal fibroblasts' viability was not significantly decreased by asiaticoside as assayed by MTT. The effects of asiaticoside on the expression of type I and type III collagen in normal and keloid fibroblasts were investigated using RT-PCR analyses and Western blotting. Asiaticoside (100, 250, and 500 mg/l) did not affect the expression of collagen protein and mRNA in normal fibroblasts. Pre-treatment of keloid fibroblasts with asiaticoside (100, 250, and 500 mg/l) significantly reduced the mRNA levels of type I and type III collagen production ($p<0.05$). Moreover, Western blotting showed that type I and type III collagen protein expressions were correlated with their mRNA levels, demonstrating that asiaticoside decreased the expression of collagen. In addition, asiaticoside reduced the expression of both TGF-βRI and TGF-βRII at the transcriptional and translational level. Moreover, it increased the expression of Smad7 protein and mRNA (Tang *et al*., 2011).

Song *et al*. (2011) evaluated of madecassoside on the proliferation and apoptosis of keloid fibroblasts (KFs). Primary KFs, originating from human earlobe keloids, were purified and cultured, and the treated with increasing concentrations of madecassoside (10, 30, and 100 mM) for 48 h-96 h. MTT assay showed that a significant inhibition of madecassoside against the proliferation of KFs occurred at days 3 and 4. At day 4, the inhibitory percentages of madecassoside (10, 30, and 100 mM) were up to 23.4, 36.2, and 45.2%, respectively. The induction of apoptosis was investigated by Hoechst 33258 staining and flow cytometry analysis. In KFs treated with madecassoside (10, 30, and 100 mM) for 48 h, cell nuclei were stained much brighter than control cells. Nuclear shrinkage, condensed chromatin, and fragmented nuclei were also frequently seen in treated cells. Flow ciytometry analysis confirmed that treatment with madecassoside for 48 h resulted in a concentration-dependent increase of apoptosis in KFs. In detail, the apoptosis percentages in madecassoside (10, 30, and 100 mM) treated groups were 9.3, 16.7, and 24.3%, respectively. Furthermore, the authors showed that madecassoside activated caspase-9 and caspase-3 rather than caspase-8, depolarised the mitochondrial membrane potential, and regulated expression of B-cell CLL/lymphoma 2 (Bcl-2) family members in KFs.

The same group of authors treated keloid fibroblasts originating from human earlobe keloids with increasing concentrations of madecassoside (10, 30, and 100 mM) for 24 h to study the potential on the migration by transwell migration assays and scratch-wound-closure assays. Madecassoside strongly prevented wound closure of KFs in scratch assay at 24 h. The inhibitory percentages of madecassoside (30 and 100 mM) as determined by transmembrane assay were up to 52.3% and 71.5% (Song *et al.,* 2012).

In vivo studies

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 per disk concentration (Shukla *et al.,* 1999).

The effects of the 4 major triterpene constituents of *Centella asiatica* were investigated by an *in vivo* burn injury model using male ICR mice (n=120; 18-22 g). Back hair was removed and back skin was burnt by direct contact for 9 seconds with a brass rod (65 g, 1 cm in diameter) heated to 95℃. Equal molar of 4 triterpene compounds were dissolved in distill water, and administered orally (6, 12, 24 mg/kg for asiaticoside and madecassoside; 3, 6, 12 mg/kg for asiatic acid and madecassic acid) for 14 consecutive days. Control group (n=24) was handled in the same way except for administering distilled water. Wound was photographed, and the areas were measured on days 0, 3, 7, 11, 14 by using Image J. At the same time points, the total wounds were biopsied and fixed in 10% formalin for further analysis. Both asiaticoside and madecassoside not only accelerated wound healing, but also resulted in a better wound healing pattern in a view of histological examination. Madecassoside (24 mg/kg) treated group showed significantly better wound healing speed and wound healing results as compared with asiaticoside (24 mg/kg) treated group ($p=0.0057$ and $p=0.0491$, correspondingly). The effects on cell proliferation, collagen synthesis, matrix metalloproteinase-1 (MMP-1) / tissue inhibitor of metalloproteinase-1 (TIMP-1) balance and transforming growth factor-β (TGF-β) / Sma- and Mad-related protein (Smad) signaling pathway were investigated *in vitro* using primary human skin fibroblasts, originating from healthy human foreskin samples. All test compounds at concentrations of 1, 3, 10 μM could not enhance cell proliferation, as compared with control group. Both asiaticoside and madecassoside (3, 10 μM) significantly elevated mRNA levels of collagen type I and type III in fibroblasts as determined by RT-PCR, as well as protein levels of procollagen type I and type III as detected by ELISA. In contrast, neither asiatic acid nor madecassic acid could influence collagen synthesis in fibroblasts as compared with control. Furthermore, madecassoside was more effective than asiaticoside at 10 μM ($p=0.0446$). Although madecassoside (3 μM) could significantly enhanced TIMP-1 mRNA expression, the 4 compounds failed to affect MMP-1/TIMP-1 ratio in human skin fibroblasts as determined by RT-PCR. Both asiaticoside (10 μM) and madecassoside (3, 10 μM) significantly increased TGF-β1 and TGF beta type II receptor (TβRII) mRNA expression, decreasing Smad 7 mRNA expression and elevating phosphorylation levels of Smad 3 in fibroblasts, while they had no influence on TβRI expression. In contrast, neither asiatic acid nor madecassic acid could influence TGF-β/Smad pathway in fibroblasts as compared with control. Madecassoside was more effective than asiaticoside at 10 μM ($p=0.0487$), which was consistent with above results on collagen synthesis (Wu *et al*., 2012).

In another study aimed to investigate the potentiality of ultradeformable vesicles as a possible topical delivery system for asiaticoside, 192 male SD rats weighing 300–350 g were topically treated on the dorsal skin using non-occlusive patches. The rats were divided into 4 groups and treated twice daily: the first group was treated with free asiaticoside, the second was treated with asiaticoside-loaded ultradeformable vesicles at a sodium cholate molar fraction of 0.2, the third group was pre-treated (1 h) with ultradeformable vesicles and then with asiaticoside and the 4th group was left untreated. After 7, 14, and 28 days of treatment the animals were sacrificed, 8 mm skin punches were collected and the degree of collagen biosynthesis was determined. Ultradeformable vesicle-entrapped asiaticoside was able to increase the amount of collagen after just 7 days of treatment (~22%), while the other treatments were not able to significantly increase the biosynthesis of collagen. After 28 days of treatment ultradeformable vesicle-entrapped asiaticoside was able to double the amount of collagen biosynthesis (~105%) compared to untreated animals (Paolino *et al*., 2012).

The therapeutic effects of asiaticoside-microspheres on wound healing and skin appendages regeneration were investigated. Asiaticoside was dissolved in small volume of ethanol and then added to the internal phase (20 ml of dichloromethane containing drug and ethyl cellulose); this preparation was dropped into 60 ml distilled water containing polyvinyl alcohol (PVA) in various concentrations as the emulsifying agent. The mixture of internal and external phases was stirred for 8 h at 25°C to evaporate the dichloromethane. The wound healing properties of a free asiaticoside solution and of asiaticoside-based microsphere was evaluated in SD rats. Full thickness skin excision wounds (1.5 cm) were made on the rats. The rats with wounds were then divided into 4 groups randomly, including: (1) blank control group in which no treatment was provided; (2) blank microspheres group, no drug was loaded in microspheres; (3) asiaticoside solution group; and (4) asiaticoside-loaded microspheres group, with 6 in each group. Treatments were topically delivered to the wound site once every 2 days. The dose of asiaticoside in the asiaticoside microsphere and asiaticoside solution groups was 0.5 mg per each time. The gloss appearance images of the wound sites in the various groups were examinated on days 0, 3, 7, 14, and 20. All of the wounds expressed the gradual healing throughout the experimental period. Accurate wound closure rates of the skin excision were determined from the percentage of wound surface covered by regenerating epidermis. Compared with the blank control group, both the asiaticoside solution and asiaticoside-microsphere expressed a trend to accelerate the wound closure within the tested 20 days. However, only the acceleration rates at day 7 and day 14, expressed by the asiaticoside-microsphere treatment were significant. Scar was one of the most common complications of wound healing. In contrast to those of blank control and vehicle control groups, the scars in the asiaticoside solution and asiaticoside loaded microsphere treated groups appeared small or less within the tested period. On the days 14 and 20, the healed skins of the asiaticoside-microsphere treated group, appeared smooth without scar appearance. The healed skins were analysed with hematoxylin and eosin staining for histological analysis. The wounds in the blank control and blank microsphere groups had wide areas of dense dermis with the characteristics of scars. In the asiaticoside solution treated group, small skin appendage follicles could be observed. However, in the asiaticoside-microsphere group, obvious skin appendages and similar structure to those of normal skin were shown in the regenerated skin sections. The Masson's trichrome staining on the newly formed skin tissues at day 20 post-wounding revealed that, the amount of collagenous fibers in drug group and drug loaded microsphere groups slightly was increased compared with those of blank control and blank microsphere treated groups. A pro-angiogenic effect could be detected in both asiaticoside solution treated group and in the asiaticoside-microsphere group based on CD31 immunohistochemical staining (Zhang *et al.,* 2016).

The wound healing activity of an asiaticoside-rich polyvinyl alcohol/polyethylene glycol (PVA/PEG) hydrogel has been evaluated in rabbits using an incision model. This fraction was obtained subjecting an ethanolic extract of aerial parts of *Centella asiatica* to Vacuum Liquid Chromatographic procedure. The wounds were divided into 4 groups in 6 rabbits as follows: Group 1: treated with asiaticoside-rich hydrogel, Group 2: treated with Madecassol cream (Bayer) (positive control), Group 3: treated with blank hydrogel, Group 4: no treatment (negative control). The wounds were treated once daily for 12 days. The hydrogel formulation did not cause any signs of irritation on the rabbits' skin and enhanced wound healing 15% faster than the commercial cream and >40% faster than the untreated wounds. The skin healing process was seen in all wounds marked by formation of a thick epithelial layer, keratin, and moderate formation of granulation tissues, fibroblasts and collagen with no fibrinoid necrosis detected (Ahmed *et al.,* 2019).

Table 3: Overview of the main non-clinical data/conclusions

Herbal preparation tested	**Strength** **Dosage** **Route of administration**	**Experimental model** ***In vivo/*** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
Ethanolic extract (from powdered leaf)	Topical (1 ml/g) Oral (1 mg/g) once daily for 24 days	*In vivo*	Suguna *et al*., 1996	Increased cellular proliferation and collagen synthesis at the wound site; wounds reepithelialise faster, and the rate of wound contraction was higher, as compared to control wounds
Different extracts (from powdered plant)	Topical (0.5 ml of extract at 10% w/v) once daily	*In vivo*	Somboongwong *et al*., 2012	Improvement of healing in the burn wound compared to control after 14 days; complete epithelialisation and keratinisation compared to control
Dry methanol extract (from powdered aerial parts)	Topical spray containing 1% m/m of extract; 2.5 ml of spray once daily for 14 days	*In vivo*	Sawatdee *et al*., 2016	Significant wound healing in group 3 treated with *Centella asiatica (CA)* extract compared to the control group, but less than observed with Betadine®
Dry methanol extract	Topical/transdermal wound dressings based on electrospun gelatin membranes (ECG) containing 31.2 mg/ml of extract placed on the wound surfaces for 14 days	*In vivo*	Yao *et al*., 2017	The wound areas of rat skin treated with EGC presented the highest recovery rate compared with those treated with gauze (control), neat gelatin membranes and commercial wound dressings (Comfeel®, Peterborough, UK)
Aqueous extract (from dried whole plant)	Skin fibroblast cell line, CCD-986sk treated with unencapsulated crude extracts and nanoparticles at a sample	*In vitro*	Kwon *et al*., 2012	The nanoparticles reduced the expression of MMP-1 in UV-irradiated cells from 136.1% to 77.6% (UV-irradiated control) and inhibited hyaluronidase expression (>60%) at a concentration of 0.5 mg/ml,

Herbal preparation tested	**Strength** **Dosage** **Route of administration**	**Experimental model** ***In vivo/*** ***In vitro***	**Reference** **Year of publication**	**Main non-clinical conclusions**
	concentration of 0.5 mg/ml			which was higher than the levels produced by the unencapsulated crude extracts
Aqueous extract (from dried powdered leaves)	Rabbit corneal epithelial (RCE) cells supplemented with 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 ppm of extract for cell viability and proliferation; with 7.8, 15.6, 31.2, 62.5 and 125 ppm for cell migration	*In vitro*	Idrus *et al*., 2012	No significant effect on the RCE cells proliferation up to 500 ppm, but of 1000 ppm significantly iinhibition of proliferation; up to 62.5 ppm, RCE cells shows significant enhancement of migration rate compared to the control group

3.1.2. Secondary pharmacodynamics

Due to the large amount of *in vitro* and *in vivo* studies investigating the pharmacological effects of *Centella asiatica* on a wide spectrum of physiological functions, mainly as water or alcoholic extracts, this section **focuses only on studies carried out with water extracts or powdered herb** which are considerd more relevant taking ino account the herbal preparations included in the EU herbal monograph.

Antinociceptive and anti-inflammatory effects

The potential of *Centella asiatica* as an anti-inflammatory agent was evaluated on fibroblast cells incubated for 24 h with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and methanolic, ethanolic and aqueous extracts prepared from 50 g of coarsed plant powder and with asiaticoside and madecassoside. Treatments with 30 g/ml concentration of each extract on the TPA-induced fibroblast cells produced significantly less PGE2 compared to control (TPA alone). Ethanol and methanol extracts of CA were more potent than aqueous extract. In addition, at 30 µg/ml, the ethanol, methanol and aqueous extracts inhibited COX-1 and COX-2 enzymes by 97.84% and 97.91%, 98.18% and 96.16%, 83.06% and 72.10%, respectively. Aspirin was used as positive control at 5 mg/ml concentration and inhibited COX-1 and COX-2 by 80.98% and 88.86%, respectively (Nurlaily *et al.,* 2012).

The whole plant water extract of *Centella asiatica* (10, 30, 100 and 300 mg/kg) revealed significant antinociceptive activity using acetic acid-induced writhing and hot-plate method in mice. The activity was statistically similar to aspirin but less potent than morphine. The *Centella asiatica* extract also revealed significant anti-inflammatory activity in rats by prostaglandin E*2*-induced paw oedema. This effect was statistically similar to the nonsteroidal anti-inflammatory drug, mefenamic acid (Somchit *et al.,* 2004).

The anti-inflammatory effects of dried, powdered water and ethanolic extracts from whole plant of *Centella asiatica* were investigated in male albino rats by paw oedema. The ethanolic extract at a dose of 200 mg/kg bw/oral possessed anti-inflammatory activity, comparable to the standard Ibuprofen at a dose of 100 mg/kg bw/oral (% inhibition of oedema after 3 h was 71.18% and 66.66%, respectively; mean oedema volume after 3 h was 0.212±0.023 ml, respectively). The aqueous extract at a dose of 200 mg/kg bw/oral showed a lower effect (% inhibition of edema after 3 h was 46.31% and mean oedema volume after 3 h was 0.192±0.024 ml), although superior to the control (1% sodium carboxy methyl cellulose suspension) (George *et al*., 2009).

Effects on vascular function

The cardiovascular effects of a single oral administration of lyophilised powder of *Centella asiatica* (CA) leave juice at doses 16, 24 and 32 g of fresh leaves/kg (equivalent to 0.26, 0.38 and 0.52 g of lyophilised powder/kg) on blood pressure (BP), heart rate and regional cerebral blood flow (rCBF) were investigated in deoxycorticosterone acetate (DOCA)-salt (1 g/kg bw) hypertensive male Wistar rats. CA leave juice had BP lowering and slight negative chronotropic effect in DOCA-salt hypertensive group, but not in normal. Prior administration of CA leave juice, the rCBF level of DOCA-salt hypertensive group was significantly low, compared to that of the normal. After CA leave juice administration at the dose of 32 g/kg bw, rCBF increased significantly at 5-90 min and 5-120 min in normal and DOCA-salt hypertensive groups, respectively. The increased rCBF was accompanied with significant decreased BP at 15-120 min only in DOCA-salt hypertensive group. The maximum decrease of systolic BP and dyastolic BP were 13.86% and 14.10% at 60 min (Thirawarapan *et al.,* 2019).

Anti-pruritic activity

In vivo studies

The antipruritic activity of aqueous and alcoholic extracts of *Centella asiatica* was evaluated by examining the incidence of scratching in albino rats of either sex weighing between 120-150 g. Powdered dried plant (20 g) was macerated with 500 ml of water or ethanol for 4 days; then, the solvent was evaporated from the filtrate to get the dried extract.

Rats were divided into 4 groups:
Group I: Control, received only vehicle (2% gum acacia solution, 2 ml/kg p.o.)
Group II: Treated with Chlorpheniramine maleate (1 mg/kg p.o.)
Group III and Group IV: Treated with aqueous/ethanolic extract of *C. asiatica* (100 and 100mg/kg p.o.)
The incidence of scratching was significantly lower for Groups II, III and IV compared to control group (George *et al.,* 2009).

Immuno-modulatory effects

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 bw) 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).

Antioxidant activity

The antioxidant activity of various extracts 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 3 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 (Abdul Hamid *et al.,* 2002).

An aqueous extract of *Centella asiatica* (50 g/l), obtained by infusion followed by cold maceration for 24 h, showed elevated DPPH scavenging activity, with an IC_{50} value of 31.25 µg/ml. Ascorbic acid and butylated hydroxytoluene (BHT) produced IC_{50} values of 2.50 µg/ml and 7.58 µg/ml, respectively (Pittella *et al.,* 2009).

Anand *et al*. (2010) assessed the antioxidant potential *in vitro* of extracts of *Centella asiatica*. Extracts were prepared from 50 g of crushed leaf by sequential extraction of *Centella asiatica* from nonpolar to polar solvents: hexane, chloroform, ethyl acetate, acetone, methanol and water. Determination of antioxidant activity was based on DPPH radical scanvenging activity and Hydroxyl radical scavenging activity (TBARS). Methanol extract was observed with highest of the DPPH and hydroxyl radical scavenging with IC_{50} values of 0.07 mg/ml and 500 µg/ml respectively, while hexane fraction is least potent (Anand *et al.*, 2010).

An ethanolic extract of *Centella asiatica* was assayed for its antioxidative activity (1 mg/ml) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was also estimated: the antioxidant activity of *Centella*

asiatica (84%) was compared to grape seed extract (83%) and Vitamin C (88%), used as positive controls (Hashim *et al.,* 2011).

The antioxidant potential of a water extract of *Centella asiatica* (4-64 µg/ml) was assessed by its free radical scavenging activity such as DPPH as well as 2, 2'-azino bis (3-ethyl) benzothiazoline -6-sulphonic acid ($ABTS^+$) radical cation decolourisation assay; in addition, hydrogen peroxide and nitric oxide radical scavenging activities were determined. Ascorbic acid and trolox were utilized as a standard. The dried plant powder (100 g) was dissolved in 1000 ml of distilled water for 72 h with occasional shaking. The extract was further filtered through Whatman No. 1 filter paper and the filtrate was concentrated using a bench top lyophiliser under reduced pressure. At a concentration of 64 µg/ml, the IC_{50} values of water extracts of *Centella asiatica*, trolox, and ascorbic acid were 9.62±0.88, 14.32±1.6, and 6.93±0.76 µg/ml, respectively. The results from the $ABTS^+$ radical scavenging ability was found to be high in *Centella asiatica* (IC_{50}=27.21 µg/ml) followed by ascorbic acid (IC_{50}=12.06±1.06 µg/ml) and trolox (12.76±2.1 µg/ml). H_2O_2 radical scavenging ability of extracts of *Centella asiatica,* ascorbic acid and trolox at the concentration of 64 µg/ml were 59.93±1.07, 69.96±1.07, and 66.43±1.4%, respectively. The IC_{50} values of *Centella asiatica,* ascorbic acid and trolox found to be 18.23±2.1, 7.6±2.51, and 13.43±1.4 µg/ml, respectively. Finally, the water extract of *Centella asiatica* showed the highest inhibitory effect with the IC_{50} value of 28.15±2.01 µg/ml at the concentration of 64 µg/ml. In contrast, trolox and ascorbic acid showed the inhibitory effect with the IC_{50} value 18.53±1.7, and 21.93±3.2 µg/ml, respectively (Kumari *et al.,* 2016).

The cardioprotective 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 h and was filtered through a 400-mesh cloth to collect the extract. The extract was concentrated and freeze dried. Pre- and co-treatment with *Centella asiatica* (200 mg/kg bw/oral) extract significantly prevented alterations of serum marker (LDH, CPK, GOT and GPT) enzymes and of the antioxidant enzymes (SOD, CAT, GPx, GST) activities induced by adriamycin (2.5 mg/kg bw, i.p.), restoring them to near normal levels (Gnanapragasam *et al*., 2004).

A study was designed to determine whether extract of *Centella asiatica* would prevent age-related changes in in rat. The whole plant was cleaned, air-dried and powdered. The powder was soaked in double distilled water, ethanol in shaking incubator at 25±1°C (1:1) for 2 days. Oral supplementation of *Centella asiatica* (300 mg/kg bw per day) for 60 days to aged rats, reduced brain regional lipid peroxidation (LPO) and protein carbonyl (PCO) levels and increased the antioxidant status (Subathra *et al*., 2005).

In a further study, H_2O_2-treated male SD rats were randomly divided into 6 groups of 4 rats and treated for 6 weeks as follows: (1) normal diet; (2) normal diet + 0.03% V/V H_2O_2; (3) normal diet + 0.03% V/V H_2O_2 + 0.3% w/w *Centella asiatica* extract; (4) normal diet + 0.03% V/V H_2O_2 + 1.5% w/w *Centella asiatica* powder; (5) normal diet + 0.03% V/V H_2O_2 + 5.0% w/w *Centella asiatica* powder and (6) normal diet + 0.03% V/V H_2O_2 + 0.3% w/w α-tocopherol. Dietary supplementation of *Centella asiatica* (extract and powder) and α-tocopherol significantly ($p<0.05$) reduced lipid peroxidation induced by 0.03% V/V H_2O_2. However, there were no significant differences in dietary intake and histopathology observations of the organs of the rats (Mahanom *et al.,* 2011).

Neuroprotective effects, including effects on cognitive function

The fresh leaf extract of *Centella asiatica* was given to adult mice at 2, 4, and 6 ml/kg doses during 2, 4, and 6 weeks, respectively. After these periods, the removed brains of mice were investigated under microscope, which pointed out to the evidence that the extract given at 6 ml/kg dose during 6 weeks caused a significant augment in dendritic arborisation in neurons (Gadahad *et al*., 2008).

The powdered leaf of Centella asiatica given intragastrically to male Wistar rats at a dosage of 2 mg/0.5 ml of distilled water per day (12 mg/kg bw) for 10 days, only enhanced retention of memory but did not improve the learning process (Jared 2010).

Amyloid beta (Aβ) is the major pathological and etiological factor implicated in Alzheimer's disease (AD). Aβ (42) inherently self assembles to form oligomers and fibrils which may lead to neuronal dysfunction. Dried leaves (40 g) of *Centella asiatica* were extracted with distilled water by steam extractor, then filtered and lyophilised to get dry powder. The anti-amyloidogenic property of this aqueous extract of *Centella asiatica* (100 µg) was examined using both thioflavin-T test and transmission electron microscope; however, it was observed not to cause any inhibition on aggregation of the monomers and oligomers (Ramesh *et al.,* 2010).

In SH-SY5Y neuroblastoma cells treated with exogenous Aβ as well as in MC65 neuroblastoma cells that overexpress amyloid precursor protein that an aqueous extract of *Centella asiatica* at 100 µg/ml can reverse the increase in reactive oxygen species observed in response to Aβ. The extract was prepared by refluxing *Centella asiatica* (60 g) with water (750 ml) for 2 h, filtering the solution and freeze drying to yield a powder (~6–8 g) (Gray *et al.,* 2015).

The same group of authors carried out an *ex vivo* study, using cultures of primary hippocampal neurons isolated from embryonic Tg2576 mice and their WT littermates. Isolated neurons were treated with a *Centella asiatica* water extract (CAW) at a concentration of 50 ug/ml for 7 days. CAW was prepared by refluxing *Centella asiatica* (160 g) with water (2 l) for 2 h, filtering the solution and freeze drying to yield a powder (~16–21 g). The Tg2576 line expresses the human APPswe double mutation (K670N-M671L), resulting in an accumulation of Aβ1-42 in the brain and the development of agedependent Aβ plaques. CAW enhanced arborisation and spine densities in WT neurons and prevented the diminished outgrowth of dendrites and loss of spines caused by Aβ exposure in Tg2576 neurons (Gray *et al.,* 2017).

Different doses of aqueous extract (100, 200 and 300 mg/kg) of the whole plant of *Centella asiatica* have been shown to counterattack the effect of oxidative stress by decreasing the lipid peroxidation and increasing the endogenous antioxidant enzymes in brain. Coarse powder of the plant was extracted with 8 parts of water under boiling for 5 h and filtered to collect the extract. The extract was concentrated and finally dried to powder. 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 2 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. Aqueous extract at 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 (Veerendra Kumar and Gupta 2002).

The effect of the same aqueous extract of *Centella asiatica* (100 and 300 mg/kg) as reported above was evaluated on the course of kindling development, kindling-induced learning deficit and oxidative stress markers in pentylenetetrazole (PTZ) kindled rats. 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 (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 day 31 and 6 months p.p. 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 (Rao *et al.,* 2005).

The effects of a water extract of *Centella asiatica* in the Tg2576 mouse, a murine model of AD with high *β*-amyloid burden were investigated. The dried water extract was prepared by refluxing *Centella asiatica* (120 g) with water (1.5 l) for 2 h, filtering to remove plant debris and freeze-drying to yield a residue (11.5 g). A dose of 200 mg/kg of extract was administered for 2 weeks. Open-field behavior and Morris water maze testing were performed at the end of this period. Orally administered *Centella asiatica* water extract attenuated *β*-amyloid-associated behavioral abnormalities in these mice (Soumyanath *et al.,* 2012).

A standardised (no further detail provided) aqueous extract of *Centella asiatica* orally administered to male Wistar rats at doses of 150 and 300 mg/kg for a period of 6 weeks improved memory performance compared to aluminum chloride treated rats (100 mg/kg). Chronic *Centella asiatica* (150 and 300 mg/kg) administration significantly attenuated oxidative damage as compared to aluminum chloride treated rats; higher dose of *Centella asiatica* (300 mg/kg) significantly reversed the decreased activity of mitochondrial enzymes as compared to aluminum-treated rat. Finally, chronic *Centella asiatica* (150 and 300 mg/kg) treatment significantly attenuated AChE and caspase-3 activity in hippocampus and cortex compared to control rats (aluminum chloride treated group) (Prakash and Kumar 2013).

Giribabu *et al*. (2014) investigated the protective role of *Centella asiatica* on the hippocampus in diabetes. An aqueous extract was prepared by soaking 1 kg of powdered dried leaves in 3 l of distilled water for 48 h. The extracted material was then fitered 3 or 4 times until the extract was rendered colorless. The extract was distilled and concentrated under reduced pressure in rotary evaporator at 50±5°C and lyophilised using freeze-dryer. Streptozocin (STZ)-induced diabetic rats received 100 and 200 mg/kg per day bw *Centella asiatica* leaf aqueous extract for 4 consecutive weeks. *Centella asiatica* extracts 100 or 200 mg/kg per day bw or glibenclamide 600 µg/kg bw resulted in a significantly ($p<0.01$) lower MDA, TNF-α, IL-1, and IL-6 level in the hippocampal homogenates as compared to non-treated diabetic rats and resulted in significantly higher SOD ($p<0.01$), CAT ($p<0.05$) and GPx levels ($p<0.01$) in the hippocampus as compared to nontreated diabetic rats. In addition, administration of *Centella asiatica* leaf aqueous extract to diabetic rats maintained near normal ATPases activity levels. Lesser signs of histopathological changes were observed in the hippocampus of *Centella asiatica* leaf aqueous extract treated diabetic rats (Giribabu *et al.,* 2014).

Mitha *et al*. (2016) studied the effect of *Centella asiatica* (CA) on cognitive impairment in offspring of alcoholic rats. Pregnant rats (n=6) in alcoholic group were orally fed with 30% alcohol at a dose of 5 g/kg body weight during their gestation period. Pregnant rats in control group (n=6) were given water. Offspring from alcoholic group were divided into treated group and untreated group (n=8 each group). Offspring in treated group were orally given whole plant aqueous extract of CA at a dose of 20 ml/kg bw. Treatment with CA increases the learning capacity, spatial memory, memory retention and decreases the anxiety like behavior as determined by Morris Water Maze, Passive avoidance test, Elevated Plus Maze.

The cognitive enhancing effects of a water extract of *Centella asiatica* (CAW) were determined using Morris Water Maze (MWM), on old and young C57BL/6 mice treated with CAW 2 mg/ml in their drinking water. CAW exposure continued for 2 weeks prior to the beginning of behavioral testing and

throughout the test period. CAW improved performance in the MWM in aged animals and had a modest effect on the performance of young animals. CAW also increased the expression of mitochondrial and antioxidant response genes in the brain and liver of both young and old animals. Expression of synaptic markers was also increased in the hippocampus and frontal cortex, but not in the cerebellum of CAW-treated animals (Gray *et al*., 2016).

Chintapanti *et al*. (2018) evaluated the protective effects of *Centella asiatica* (CA) leaf extract on behavioral deficits and neurotoxicity in adult rat exposed to lead during perinatal period. Powdered dried leaves (100 g) were extracted in cold percolation with water for 24 h. The extract was recovered and this process was repeated 4 times. The extracts were pooled together, combined, and filtered. The filtrate was concentrated to dryness under reduced pressure and then air-dried, giving 11.09±1.29 g of extract. Adult Wistar rats were exposed to 0.15% lead acetate (Pb) from gestation day 6 through drinking water and the pups were exposed lactationally to Pb till weaning. Oral supplementation of CA (200 mg of the crude extract/kg bw per day) during post-weaning period provided significant protection against Pb-induced behavioral impairments (mainly significant improvements in open-field behaviour and in water maze behaviour were obtained). In addition, CA induced a partial recovery of AChE activity in different brain regions of Pb-exposed rats. Finally, dministration of CA to Pb-exposed rats, showed significant increases in SOD, catalase, GPx and glutathione reductase activities, and GSH levels, and a decrease in MDA level in different regions of the brain, indicating recovery from oxidative stress. CA did not chelate Pb in brain regions and blood, suggesting that the possible neuroprotective effects may be due to its antioxidant potential but not by lowering effects of brain Pb content (Chintapanti *et al.,* 2018).

The effects of a water extract of *Centella asiatica* (CAW) was evaluated in the 5xFAD mouse model of Aβ-accumulation. A total of 1200 g of dried aerial parts were refluxed with 15 l of water for 1.5 h, in several small batches. After filtering the extract to remove plant debris, the liquid was freeze dried to produce 245 g of CAW powder. Seven-month-old female 5xFAD mice and their female WT littermates were either exposed to CAW in their drinking water at 2 g/l or to untreated water for 2 weeks prior to the beginning of behavioral testing and throughout the tests. Learning, memory and executive function were assessed using the object location memory task (OLM), conditioned fear response (CFR) and odor discrimination reversal learning (ODRL) test. CAW improved performance in all behavioral tests in the 5xFAD but had no effect on WT animals (Gray *et al.,* 2018b).

Anticonvulsivant effects

Anxiolytic plants may interact with either glutamic acid decarboxylase (GAD) or GABA transaminase (GABA-T) and ultimately influence brain GABA levels and neurotransmission. An aqueous extract from *Centella asiatica* 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 (Awad *et al.,* 2007).

The anticonvulsant effect of *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water leaf extracts of *Centella asiatica* were investigated with respect to cholinergic activity on pentylenetetrazol (PTZ)-induced seizures. Convulsions were induced in adult male Wistar rats with an intraperitoneal injection of PTZ 60 mg/kg bw dissolved in saline. Treatment with all the extracts except aqueous extract at 200 mg/kg bw 1 week prior to dose of PTZ showed significant protection against PTZ-induced convulsions. *N*-hexane, ethyl acetate and *n*-butanol extracts resulted in a more pronounced effect than chloroform extract (Visweswari *et al*., 2010a).

The same group of authors found that the activities of Na^+, K^+-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase in brain (cerebral cortex, cerebellum, pons medulla and hippocampus) were significantly increased after treatment with different *Centella asiatica* extracts which in general is decreased after PTZ induced epilepsy in all the above brain regions (Visweswari *et al*., 2010b).

Manasa and Sachin (2016) evaluated the anticonvulsant action of aqueous extract of *Centella asiatica* and compared it with sodium valproate in PTZ-induced seizures in albino mice. The aqueous extract was prepared by cold maceration method. The plants were air dried and powdered. Thirty grams of the dry powder was soaked in 200 ml of cold water at room temperature. The extract was filtered, and the filtrate was dried at room temperature in a steady air current. Animals, which received 100 mg/kg of aqueous extract of *Centella asiatica*, exhibited significant delay in the onset of seizures ($p<0.001$) and suppression of clonic seizure ($p<0.01$) when compared with control non-treated group, and it was statistically significant. The aqueous extract of *Centella asiatica* at a dose of 300 mg/kg exhibited complete suppression of seizures, and its anticonvulsant activity is comparable to sodium valproate (Manasa and Sachin 2016).

The anticonvulsivant activity of aqueous leaf extract of *Centella asiatica* was evaluated in Maximal Electroshock induced (MES) Seizures and PTZ-induced seizures in Wistar albino rats. About 500 g of powdered dried leaves were boiled in hot water for 30 min, allowed to cool and filtered using a piece of white cotton gauze. The filtrate was evaporated to dry at room temperature. The aqueous leaf extract of *Centella asiatica* significantly increased the time of onset of Tonic Hind Limb Extension (THLE) in all the doses (200 mg/kg and 400 mg/kg) used and decreased the duration of THLE significantly with 400 mg/kg when compared to control group. The percentage of protection of MES seizures with 400 mg/kg is 67%. In PTZ-induced seizures, the aqueous extract of *Centella asiatica* significantly increased the mean latency period in all the doses used. The percentage of protection of PTZ-induced seizures with 200 mg/kg of extract was 33% and 400 mg/kg was 67%. Standard drugs like phenytoin in MES-induced seizures and diazepam in PTZ-induced seizures possesses 100% seizure protection (Umamageswari *et al*., 2015).

Cytotoxic and anti-proliferative effects

An aqueous extract of *Centella asiatica* (50 g/l), obtained by infusion followed by cold maceration for 24 h, was active against human breast cancer (MDA-MB 231) and mouse melanoma (B16F1) (648.0 and 698.0 µg/ml, respectivelly), while that for rat glioma (C6) the IC_{50} was 1,000.0 µg/ml. On the other hand, the extract was not cytotoxic at the tested concentrations (up to 1,000.0 µg/ml) towards the human lung carcinoma (A549) and normal hamster kidney (BHK-21) cell lines (Pittella *et al.,* 2009).

An aqueous extract of *Centella asiatica* enhanced the cytotoxic effect of bleomycin in the adenocarcinoma human alveolar basal epithelial A549 cell line. Dried and milled leaves and tender stems of *Centella asiatica* were extracted with distilled water to obtain a 10% w/v concentration. The mixture was incubated at 4°C for 1 h, refluxed, vacuum filtered, and then sterilised using 0.4 micron filters followed by a 0.2 micron filter. Ten grams of the plant powder was extracted using 100 ml of distilled water to obtain a final concentration of 10% V/V. A549 cells exposed to 25% and 30% V/V *Centella asiatica* extract showed cell granulation and debris in the culture medium. IC_{50} value of bleomycin on A549 cells was 100 µg/ml and was reduced to 80 µg/ml when cells were co-incubated with 20% V/V of *Centella asiatica* extract (Wu *et al.,* 2016).

The cytotoxic effects of the plant different extracts (methanol, ethyl acetate, dichloromethane and distilled water) of *Centella asiatica* were determined using MTT cell proliferation assay against MCF-7, human colorectal carcinoma cells (Caco-2), A549 and HeLa cancerous cell lines. *Centella asiatica* acetone extract showed the most significant activity, with IC_{50} value of 53.65±0.06 µg/ml against MCF-7 and 46.49±0.04 µg/ml for A549. The water extracts were the least active and showed IC_{50} value of >100 µg/ml for MCF-7, Caco-2 and A549 and IC_{50} value of 76.3±0.06 µg/ml for HeLa. Doxorubicine, used as a reference drug, showed the following IC_{50}: (1.26±0.03 µg/ml for MCF-7, 2.76±0.06 µg/ml for Caco-2, 1.43±0.24 µg/ml for HeLa, and 2.10±0.11 µg/ml for A549) (Soyingbe *et al.,* 2018).

Rai *et al*. (2011) examined the chemopreventive potential of *Centella asiatica* extract on 7,12-dimethylbenz(a)anthracene (DMBA)-induced skin tumorigenesis in male Swiss albino mice (15-20 g). DMBA was used to initiate skin carcinogenesis and croton oil to promote it to a visible tumour. Twenty-five grams of powdered dried whole plant was taken for aqueous extraction through soxhlet apparatus and refluxed for 2-3 days at 60ºC. After the complete extraction, the extract was kept it in water bath 45ºC for removing the solvent and the dry powder was obtained. *Centella asiatica* extract, topically applied at the dose of 500 and 1000 mg/kg bw at the pre promotion phase, showed a significant reduction in tumor incidence, and cumulative number of papillomas compared to carcinogen control group (DMBA + cotron oil). The effect was more pronounced with the dose of 1000 mg/kg bw (Rai *et al*., 2011).

The effects of *Centella asiatica* leaves water extract on decreasing the number of benzo(a)pyrene-induced lung tumor nodules was investigated on 30 of 1-day-old baby mice. The average number of tumor nodules obtained in the positive control group of benzo(a) pyrene is 5.60±2.07. The mean number is significantly different ($p<0.05$) compared with the group which treated with water extract of *Centella asiatica* leaves doses of 500 and 750mg/kg bw (2.20±0.83 and 2.60±1.14, respectively). Whereas, when compared to extract with dose of 250 mg/kg bw shows no significant difference ($p>0.05$). The smallest number of tumor was observed in the groups of mice given tamoxifen (2.00±1.58 on average). The results show no significant differences ($p<0.05$) in the group of mice which given water extract of *Centella asiatica* doses 500 and 750 mg/kg bw (average 2.20±0.83 and 2.60±1.14, respectively). Microscopic examination of lung histopathological features of *Centella*-treated mice showed a decrease on tumor foci in bronchus, alveolar septum and an inhibition of bronchial epithelial cell hyperplasia (Hamid *et al.,* 2016).

Hypolipidemic effects

The anti-hyperlipidemic properties of *Centella asiatica* were studied in in hyperlipidemic rats fed with high-colesterol diet. The dried plant powder (100 g) was dissolved in 1000 ml of distilled water for 72 h with occasional shaking. The extract was further filtered and the filtrate was concentrated under reduced pressure. *Centella asiatica* extracts at doses of 0.25 g/kg, 0.5 g/kg or 1 g/kg bw per day up to 28 days and fenofibrate remarkably lowered the level of TC, TG, LDL-C, and showed elevated levels of HDL-C, SOD. The histopathological observations further demonstrated clear differentiation and structural changes in liver of high colesterol fed and *Centella asiatica* treated groups (Kumari *et al*., 2016).

Antimicrobial effects

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 antimicrobial effects of methanol and water extracts of *Centella asiatica* were investigated against *E. coli*, *Entetobacter aerogens*, *Kleibsella pneumonea*, *Mycobacterium glutamicus*, *Proteus vulgaris* micro-organisms. The methanolic extracts showed highest inhibition against *M. glutamicus* (25 mm), followed by *K. pneumonea* and *E. coli* and lowest against *E. aerogens* and *P. vulgaris* (15 mm) with 50 mg/ml DMSO concentration. The water extracts showed highest inhibition against *M. glutamicus* (40 mm) and least against *K. pneumonea* (18 mm) with 250 mg/ml DMSO concentration (Kunta and Vadlapudi 2009).

Methanol, acetone, chloroform and water extracts of leaf and callus of *Centella asiatica* in varying concentrations ranging 10 to 100 µg/l were evaluated for *in vitro* antibacterial activity against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by agar plate well diffusion method. All the leaf and callus extracts showed dose dependent anti-bacterial activity. The maximum growth of inhibition (30 mm) was observed in methanol extract of leaves at 100 µg/ml against *E. coli*, which was followed by *B. cereus* (29 mm), *P. aeruginosa* and *S. aureus* (28 mm). Similarly, methanol extract of *in vitro* grown callus at the concentration of 100 µg/ml showed maximum growth of inhibition (29 mm) against *P. aeruginosa*, *E. coli* and *S. aureus* which was followed by *B. cereus* (28 mm). Methanol extract of leaf and callus at the concentration of 10, 25 and 50 µg/ml also showed significant activity (24-27 mm of zone of inhibition) against all the tested organisms. The aqueous and acetone extracts of leaf and callus were found to be less effective and chloroform extract of leaf and callus showed moderate zone of inhibition against all the tested organisms in the present study (Arumugam *et al*., 2011).

Soyingbe *et al*. (2018) evaluated the anti-microbial activity of different extracts of *Centella asiatica* on 14 microorganisms, *Enterococcus avium*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus*, *Enterococcus hirae*, *Enterococcus faecalis*, *Enterococcus gallinarium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Acinetobacter calcaoceuticus anitratus* and *Salmonella typhi*. The dried leaves were extracted (1:10 w/v) in methanol, ethyl acetate, dichloromethane and distilled water. The dried extracts were used. In the agar diffusion assay, the methanolic extract had the highest antibacterial activity towards *S. agalactiae* with zone of inhibition of 29±0.4 mm; the inhibition zones for methanolic extracts on other microorganisms ranged 9±1.0 mm (*P. vulgaris*) to 19±2.0 mm (*E. avium*). The MIC for all the extracts of *Centella asiatica* ranged 0.63 mg/ml (acetone extract towards *E. Coli*) to >10 mg/ml. The water extract has the lowest antimicrobial activity (Soyingbe *et al.,* 2018).

Gastro-protective and anti-ulcer effects

Centella asiatica extract, prepared boiling 30 g of the plant in 600 ml of distilled water under reflux for 1 h, 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 and 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. Thirty grams of *Centella asiatica* was boiled in 600 ml of distilled water under reflux for 1 h. The solution was then filtered and freeze-dried to obtain the powder, which was re-dissolved in distilled water immediately before use. The water extract and asiaticoside 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 *Centella asiatica* or asiaticoside. Similar effects were observed after 0.25 g/kg *Centella asiatica* water extract or 10 mg/kg asiaticoside administration (Cheng *et al*., 2004).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

The wound healing effects of *Centella asiatica* have been extensively investigated both *in vivo* and *in vitro* mainly using alcoholic extracts of the whole plant, herb and leaves. Incision, excision and burn wound models have been used. *Centella asiatica* extracts, mainly applied topically, accelerate wound healing stimulating epithelisation and increasing the rate of wound contraction by promoting cellular proliferation and collagensynthsis at wound site.
Asiaticoside and madecassoside are the main single constituents studied in wound healing. The mechanisms involved seem to be enhancement of collagen synthesis and antioxidant activity. Although animal studies have been carried out with herbal preparations different from those reported in the monograph, taken together, the above summarised non-clinical *in vivo*/*in vitro* evidence supports the traditional use of *Centella asiatica* to aid in healing of minor wounds.

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*, except for standardised and highly purified extracts.

The potential modulatory effects of asiaticoside, asiatic acid and madecassic acid and 4 extracts (hexane, dichloromethane, ethanol and water) prepared sequentially from whole plant of *Centella asiatica* were examined on 3 major cDNA-expressed human cytochrome P450 (CYP) isoforms. *Centella asiatica* aqueous, hexane extracts and asiaticoside weakly inhibited CYP2C9 activities in general with IC_{50} values of more than 100 µg/ml (IC_{50} of 599.0, 117.9 and 1070.2 µg/ml, respectively). On the other hand, *Centella asiatica* ethanol extract, dichloromethane extract, asiatic acid and madecassic acid showed significant inhibition on CYP2C9 activities with IC_{50} values less than 100 µg/ml (IC_{50} of 28.3, 17.2, 33.1 µg/ml and 40.8 µg/ml, respectively). The 4 *Centella asiatica* extracts and asiaticoside did not exhibit significant inhibition on catalytic activity of CYP2D6 in this study (IC_{50} values above 100 µg/ml). Only asiatic acid and madecassic acid significantly inhibited CYP2D6 activities (IC_{50} 67.9 µg/ml and 30.7 µg/ml, respectively). No *Centella asiatica* extracts showed significant inhibition on CYP3A4 activity, while asiaticoside and madecassic acid did not significantly inhibit CYP3A4 activities as well (all having IC_{50} above 100 µg/ml). Asiatic acid was the only component exhibiting significant inhibition on CYP3A4 with an IC_{50} value of 53.6 g/ml (Pan *et al.,* 2010).

An ethanolic extract of *Centella asiatica* leaves showed significant inhibition of both CYP1A2 (IC_{50}=42.23±3.65 µg/ml) and 2C9 enzyme (IC_{50}=48.41±4.64 µg/ml) in a competitive manner with respect to methanolic, hydromethanolic (methanol and water in equal ratio) and water extracts of *Centella asiatica* leaves (IC_{50}>100 µg/ml) in human liver microsomes. In addition, the flavonoids, quercetin and kaempferol showed potent (IC_{50} values less than 10 µM) inhibition of CYP1A2 activity with no significant inhibition of CYP2C9 enzyme (Savai *et al.,* 2015).

The IC_{50} determined for a methanolic extract of whole plant of *Centella asiatica* on human CYP3A4, CYP2D6, CYP2C9 and CYP1A2 were 225.71±2.26, 139.99±1.73, 184.68±3.79, and 288.83±1.61 µg/ml, respectively; the IC_{50} for asiaticoside were 457.62±5.14, 235.35±2.63, 292.43±1.67, and 504.02±4.45, respectively (Kar *et al*., 2017).

Centella asiatica was demonstrated to potentiate the anti-epileptic activity of gabapentin, phenytoin and valproate in PTZ-induced seizure in ICR mice but decrease the anti-epileptic effect of phenobarbital in maximal electroshock-induced seizure model in Wistar rat (Fong *et al*., 2014).

The effects of *Centella asiatica* on the pharmacokinetic of amitriptyline in rats was studied by Khurshid *et al*. (2018). Male Wistar albino rats were randomly divided into 2 groups (n=6 each) which were served as a control (amitriptyline alone) and treatment group (amitriptyline with *Centella asiatica*), respectively. Rats were administered vehicle saline or *Centella asiatica* (62.5 mg/kg, p.o. daily for 7 days), then administered a single amitriptyline dose (25 mg/kg, p.o.) on day 8. The coadministration of *Centella asiatica* with amitriptyline resulted in increased C_{max}, AUC_{0-24} and $t_{1/2}$ by 14.34, 22.84, and 13.81%, respectively (Khurshid *et al*., 2018).

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

3.3.1. Single dose toxicity

In the mouse, acute toxicity was not shown for oral administration of 1 g/kg bw of an ethanolic 50% extract of *Centella asiatica*. Following intraperitoneal administration, the maximum tolerated dose (MTD) in mice was found by Dhar *et al.* (1968) to be 250 mg/kg bw. Alcoholic extracts of *Centella asiatica* have shown no toxicity at doses of 350 mg/kg when given i.p. to rats (Alternative Medicine Review 2007).

The oral LD_{50} of a 70% ethanolic dry extract from *Centella asiatica* 6:1 in rats was found to be higher than 675 mg/kg bw (ESCOP 2009).

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

The standardised extract ECa 233 was administrated at 10.0 g/kg into 10 male and 10 female ICR mice (20–22 g). The extract at the given dose did not cause any toxic signs and death within the observation period of 14 days (Chivapat *et al*., 2011).

Oral single dose acute toxicity study of aqueous mixture of *Centella asiatica* whole plant powder on female SD rats showed no toxic effects on behaviour, body weight, food and water intake. All rats were observed for 14 days prior to necropsy. No death was found throughout the study period. Necropsy revealed no significant abnormality. LD_{50} value was determined as >2000 mg/kg. (Global Information Hub On Integrated Medicine, *Centella asiatica* (L.) Urban. Malaysian Herbal Monograph)

The acute toxicity of an ethanolic extract (obtained by Soxhlet extraction of the whole plant at 72-82°C for 72 h) was evaluated in Swiss albino mice (25–30 g). The animals were divided into experimental groups of 6 animals each (3 male and 3 female). Group 1 received 10 µl/g of distilled water and served as control; the other groups were treated with ethanolic extract of *Centella asiatica* (ECA) at doses of 300, 600, 1200 and 2000 mg/kg respectively. All treatments were administered once by oral gavage. Animals were closely observed for 4 h following administration and once a day for 14 days. No deaths were recorded within 72 h in LD_{50} assay after administration of the extracts and no death was recorded after 14 days. No signs of toxicity were observed in animal groups after the treatment with ECA 300, ECA 600, ECA 1200 and ECA 2000 mg/kg. It was not observed any significant alterations in serum levels of SGOT, SGPT, and alkaline phosphatase and histopathological analysis did not show liver damage (Yadav *et al*., 2019).

3.3.2. Repeat dose toxicity

Chronic oral administration of a hydoalcoholic extract from *Centella asiatica* 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.

Oral administration of dried, powdered, muslinised, aerial parts of *Centella asiatica* in Wistar albino rats (n=24; weight not reported) in different doses (250, 500 and 1,000 mg/kg bw) over a period of 30 days did not produce any clinical signs of toxicity, morbidity, mortality with no effect on gross behavioural effects. All the treated rats exhibited normal activities as that of the control group (administered with distilled water). All the 4 groups (control and treatment) of rats showed a steady gain in their body weight during the entire study period. The changes in hematological parameters between the control and treatment groups were statistically significant but all are in normal physiological range. After 30 days, oral administration of *Centella asiatica* resulted in a significant increase in the levels of ALT, AST in a dose-dependent manner. The concentration of tissue total protein in treated groups were also significantly higher than the control group. The concentration of BUN and creatinine in treated groups were also higher than the control group. There was statistically significant decrease in liver viability count in treatment groups in comparison to the control group. The viable count in kidney was also reduced mainly in groups treated with 500 and 1,000 mg/kg compared to the control group. No significant differences were seen for the total viable count in heart between treated and control groups. Histopathology also revealed a significant hepatic damage and a moderate degree of changes in the renal tissue. The authors concluded that the administration of *Centella asiatica* at 1,000 mg/kg bw for a period of 30 days may cause a significant damage to liver tissue in rats (Oruganti *et al*., 2010).

Sub-chronic toxicity study of ECa 233 was investigated in 4 groups of Wistar rats (180–220 g), each of 24 rats (12 of each sex). Control group was orally given distilled water and 3 experimental groups were orally administered with ECa 233 in distilled water at the doses of 10, 100, and 1,000 mg/kg per day for 90 days. All ECa 233-treated rats showed no difference with regards to body weight, food consumption and organs weight in comparison to the control group except that female rats receiving 1,000 mg/kg per day of ECa 233 had lower food consumption, relative right adrenal weight, and higher white blood cell counts than the control group ($p<0.05$). Eosinophils in the female groups receiving ECa 233 at 10 and 1,000 mg/kg per day were significantly lower than those of their control group ($p<0.05$). Clinical chemistry values of all ECa 233-treated, male and female, groups were not significantly different from those of their sex-corresponding control groups, except male rats receiving 1,000 mg/kg per day of ECa 233 which had significantly higher sodium level, but still within normal range, than those of control group ($p<0.05$). Male rats receiving ECa 233 at 10 mg/kg per day had a significantly higher incidence of lymphoid proliferation in the lung and centrilobular fatty degeneration in the liver than their control group. However, there was clearly no exacerbation of changes or increase of effect at higher dose of 100 and 1,000 mg/kg per day were noted. The incidences of gut associated lymphoid tissue (GALT) proliferation in the small intestine of female rats receiving ECa 233 at the doses of 10 and 1,000 mg/kg per day were significantly lower than those observed in their respective control groups. In summary, histopathological results of internal organs did not demonstrate any incidence or degree of lesions in a dose-dependent manner with the increasing dose of ECa 233 (Chivapat *et al*., 2011).

The sub-chronic toxicity of an ethanolic extract (obtained by Soxhlet extraction of the whole plant at 72-82°C for 72 h) was evaluated in Swiss albino mice (25–30 g). The animals were divided into experimental groups of 6 animals each (3 male and 3 female). Group 1 received 10 µl/g of distilled water and served as control; the other groups were treated with ethanolic extract of *Centella asiatica* (ECA) at doses of 300, 600, 1200 and 2000 mg/kg respectively. All treatments were administered once by oral gavage daily 7 days each week for 28 days. No death was recorded for 28 days extract administration. Animal treated with ECA showed weight gain throughout the entire experiment duration. The increase was the same in treated and control group animals, and the treatment did not affect relative organs weights, food, and water intake. Treatment with ECA at all doses did not produce any changes on animal haematological parameters and did not produce any statistically significant changes on urea, creatinine, SGOT, SGPT and alkaline phosphatase. At the 4 evaluated doses, it was not observed any tissue damage on the kidney, heart, liver and brain of mice (Yadav *et al*., 2019).

3.3.3. Genotoxicity

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 8 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 min. The supernatants of the extract was freeze-dried and then stored at -20°C. *Centella asiatica* at doses of 2 mg/plate and 5 mg/plate 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 h were powdered. Powder (1 g) was extracted by sonication (3 times 10 min) with 3 times 10 ml of 90% methanol. The dried crude extract was dissolved in 90% methanol and filtered (pore size: 0.45 µm) prior to HPLC analysis (Randriamampionona *et al.,* 2007).

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 h 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).

3.3.4. Carcinogenicity

Asiaticoside has been implicated as a possible skin carcinogen in rodents after repeated topical application while was tested for carcinogenicity by topical applications to the skin of hairless mice. A short-term tetrazolium test indicated that compound was weak carcinogen. 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 and Iversen 1972).

3.3.5. Reproductive and developmental toxicity

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. Orally administered crude extract of *Centella asiatica* has been reported to significantly reduce fertility of female mice. Teratogenicity studies were conducted in rabbits and they reported negative results for *Centella* extract containing asiatic acid, madecassic acid, madasiatic acid and asiaticoside (EMEA 1998, Barnes *et al*., 2007).

The effects of *Centella asiatica* ethanolic leaf extract on the induction of spermatogenic cells were studied in adult Wistar albino male rats (250±10 g). Dried leaves were powdered and then subjected to exhaustive extraction in ethanol. Forty adult male rats were equally divided in 5 groups: one control and 4 experimental groups. During the experiment, the crude extract was dissolved in distilled water and administered orally to rats at a concentration of 10, 50, 80, and 100 mg/kg body weight in 1 ml volume as single daily doses for 8 weeks using animal feeding gavage. Similarly, the controls received gavage of 1 ml of distilled water in the same schedule as the experimental rats. When compared to the control group, statistically significant ($p<0.01$ or $p<0.001$) reductions in sperm viability and motility were noted in each group dosed with *Centella asiatica* leaf extract. In each experimental group, histopathological examination of the testis revealed a significant (p value not stated) decrease in the number of spermatogenic cells (spermatogonia, spermatocyte, spermatid, and sperm) in the seminiferous tubules. Also, when compared to the control group, intertubular spaces and venous congestion were increased in experimental groups. The authors noted that the reported loss in testicular weight likely corresponded to a dose-dependent decrease in mean spermatogenic cells in seminiferous tubules. At the 100 mg/kg per day dose, the mean number of sperms from the cauda epididymis (times 10^6) was 36.7±4.8, compared to a mean value (control) of 61.60±2.34; this difference was statistically significant ($p<0.001$). Additionally, degeneration of seminiferous tubules was reported. It was concluded that *Centella asiatica* leaf extract was toxic to the reproductive system of male rats (Heidari *et al.,* 2012).

A study was performed to evaluate the effects of *Centella asiatica* (ethanol extract) on the rat testis. The following group of 8 male SD rats (dosed orally) were used in the study: low-dose group (100 mg/kg), mid-dose group (200 mg/kg), high-dose group (300 mg/kg), and control group (distilled water). The groups were force fed for 42 consecutive days, after which the animals were killed and the testis removed for histological examination. Animals of all dose groups had some degeneration of spermatogenic cells and reduction of spermatozoa in the lumen of the seminiferous tubules. When compared to the control group, the serum testosterone level decreased in a dose-dependent manner and there was a significant decrease in cauda epididymal sperm count. A statistically significant reduction ($p<0.05$) in sperm count was observed in the 200 mg/kg and 300 mg/kg dose groups, but not in the 100 mg/kg dose group. Differences in sperm motility were also observed. Slow or sluggish progressive sperm motility was reported for the control and 100 mg/kg dose group. Non-progressive motility (<5 µm per second) was reported for both the 200 mg/kg and 300 mg/kg dose groups. In control animals, the testis had normal features, with successive stages of transformation of the seminiferous epithelium into spermatozoa. However, abnormalities in seminiferous tubules were observed in all dose groups. Complete arrest of the seminiferous tubules was observed only in the 300 mg/kg dose group. It was concluded that *Centella asiatica* extract (ethanol extract) was a reproductive toxicant in male rats (Cosmetic Ingredient Review Expert Panel, 2015).

The antifertility properties of an ethanolic extract of *Centella asiatica* were studied in 32 male SD rats (250-300 g). The ethanol extract was prepared by extracting *Centella asiatica* powder macerated with ethanol (90%) at 55°C to 60°C for 36 h. Then, the ethanol was removed from the extract and the extract was concentrated to dryness using a rotary evaporator. Rats were divided into 2 groups: control group (n=16) received distilled water and treatment group (n=16) which received 300 mg/kg of *Centella asiatica* extract orally for 42 days. After the treatment period, the number of implantation sites was recorded, and the sperm proteomic changes were analysed by 2D gel electrophoresis. In addition, the expression of protein spots was quantified by MALDI-TOF analysis. Female SD rats were placed individually in a cage with a treated male rat for 5 days. The day that the sperm appeared in the vaginal smear was considered as day 1 of pregnancy. On the 17th day, the animals were laparotomised under light ether anesthesia and the number of implants in the uterine horns was recorded. *Centella asiatica* extract resulted in low number of implantation sites in the treatment group (100.00±2.82) compared to the control group (183.00±2.14). The percentage of infertile male rats in the treatment group was higher (43.75%) compared to the control group (18.75%). Proteomic analysis showed the expression of protein spots identified in the treatment group decreased with 234 spots compared to the control group with 282 spots (Yunianto *et al.,* 2017).

Akbar *et al*. (2018) examined the influence of the ethanol extract of *Centella asiatica* towards the birth rates or fertility in the early post-implantation. Dried powdered leaves were macerated with 70% polar ethanol for 24 h and repeatedly 3 times to obtain soluble and soft; the chopped leaf extract was used for the experiment. The research design used was complete randomised design with 4 treatments and 6 replications respectively. A total of 24 adult SD female rats were distributed into 12 trays/cages grouped into 4 groups. After mating, pregnant rats were grouped into 4 treatment groups: Group I (control group), Group II (dose 175 mg/kg bw), Group III (a dose of 200 mg/kg bw) and Group IV (dose 225 mg/kg bw). Rats were treated on the 6th day until the 9th day of pregnancy. After 15 days, the value of post-implantation death percentage is calculated by counting the number of implantation, either containing a live fetus, or dead fetus and embryo resorb. In Group III and Group IV treatments, it was clear that there were no living fetuses and there was an acceptably higher embryo rate than Group I and Group II treatments. The percentage of post-implantation death was significantly higher in Groups III and IV compared to control group (57,23% and 56.23%, respectively vs 0%; $p<0.05$).

The deterioration of male reproductive health is one of the major manifestations of occupational and/or environmental exposure to Pb toxicity. Sainath *et al.* (2011) investigated the effects of an aqueous extract of *Centella asiatica* (powdered whole plant) on lead-induced oxidative stress and suppressed reproductive performance in male rats. The animals were equally randomised to 4 groups, 6 per group. The rats in group I served as control and was allowed *ad libitum* access to tap water. The animals in groups II were allowed *ad libitum* access to tap water containing 819 mg/l lead (0.15% lead acetate) for 70 days. Rats in group III were given orally aqueous extracts of *Centella asiatica* (200 mg/kg bw per day) over a period of 70 days and the animals in group IV received same experimental regimen as that of group II and in addition, the aqueous extracts of *Centella asiatica* (CA) was also given orally for a period of 70 days. The indices of testis and epididymis were significantly decreased in lead treated rats over control animals. No significant changes in the testis and epididymis indices were observed in CA alone treated rats when compared with control rats, whilst in rats coadministrated with CA the weights of testis and epididymis were comparable to control group and significantly higher than those observed in rats treated with Pb. No significant changes in sperm parameters were observed in rats after CA administration. On the other hand, significant decrease was observed in epididymal sperm count, sperm viability, sperm motility and also percentage of number of hyposmotic swelling (HOS)-tail coiled sperms in rats exposed to lead for 70 days. Co-administration of CA treatment significantly increased the selected sperm variables in lead acetate exposed rats when compared with Pb treated rats. Significant decrease in the testicular 3β- and 17β-hydroxysteroid dehydrogenase activities were observed in Pb exposed rats as compared to control rats. No effect of CA on these enzymes was noted

when administered to normal animals. On the other hand, co-administration of CA with lead had significantly increased both these enzymes when compared with Pb treated rats but still lower than the control levels (Sainath *et al.,* 2011).

3.3.6. 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).

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

No signs of acute oral toxicity were seen in mice and rats with *Centella asiatica* up to doses of 2000 mg/kg.

In the study by Oruganti *et al.* (2010) signs of liver and kidney toxicity were observed for the dried, powdered, muslinised, aerial parts of *Centella asiatica* when orally administered for 30 days at doses of 500 mg/kg and 1000 mg/kg in rats. Specifically, a significant rise of ALT, AST, BUN and creatinine was seen, compared to the control group; in addition, with these doses the liver and kidney cells viability was significantly decreased. The dose of 500 mg/kg corresponds to a human equivalent dose (HED) of 4 g for an adult human weighting 50 kg. On the other hand, in this study the 250 mg/kg dose of powdered aerial parts of *Centella asiatica* provoked a significant increase of ALT, AST, BUN and creatinine without alteration of viable cell count in liver and kidney compared to control group; no changes of liver and kidneys with the lower dose appeared by histopathological examination. The dose of 250 mg/kg corresponds to a human equivalent dose (HED) of 2 g for an adult human weighting 50 kg.

A subsequent study carried out on rats showed that a standardised extract of *Centella asiatica*, ECa233, did not cause significant chronic toxicity when given orally in doses of 10-1000 mg/kg for 90 days. ALT, AST, BUN and creatinine in treated rats did not differ significantly compared to control group. The histopathological examination did not show any alteration in kidneys of treated rats up to 1000 mg/kg. A centrilobular fatty degeneration was seen in liver of 5/12 rats treated with 10 mg/kg of ECa233 ($p<0.05$ compared to control group), but this alteration was less frequent in rats receiving 1000 mg/kg (1/12, no statistical difference with the control group) (Chivapat *et al*., 2011).

The study by Yadav *et al*. (2019) did not show any significant effect of an ethanolic extract of *Centella asiatica* given orally to mice for 30 days up to doses of 2000 mg/kg (HED ~8 g for an adult human weighting 50 kg). No alteration of urea, creatinine, SGOT, SGPT, alkaline phosphatase and no damage to liver and kidney tissues were observed.

There were no relevant abnormalities/no adverse effects of *Centella asiatica* on hematology. Histopathological examination showed no effect of *Centella asiatica* on heart and brain of rats and mice at very high doses. A bronchiolar associated lymphoid tissue proliferation was detected in lung of male rats in the study by Chipavat *et al*. (2011), although this was statistically significant only at doses of 10 mg/kg and not at higher doses. The dried, powdered, aerial parts of *Centella asiatica* caused an increase of weight of the spleen without pathologal lesions; the microscopic section of spleen showed hypercellularity and hyperplasia with doses of 500 mg/kg or higher which was associated by the

authors to immunosuppressive effect of *Centella asiatica*. The effects on the spleen were not observed in the study by Chipavat *et al*. (2011).

Centella asiatica water extract was not mutagenic in an Ames test (pre-incubation method) up to 5 mg/plate; only strains TA98 and TA100 were assayed and no information on positive control was provided, therefore further studies are needed to confirm the absence of mutagenicity. *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 further study suggests that the leaf extract *per se* does not have a genotoxic potential. Adequate tests on carcinogenicity of *Centella asiatica* have not been performed.

Asiaticoside caused *in vitro* detrimental effects on human and rat sperm (EMEA 1998). A recent study demonstrated that *Centella asiatica* leaf ethanolic extract was toxic to the reproductive system of male rats when administered orally at a concentration of 10, 50, 80, and 100 mg/kg. The sperm motility and viability were significantly reduced compared to the control group with the lower dose of 10 mg/kg of extract, whereas a significant decrease of sperm count and testis weight was seen at doses of 50 mg/kg or higher. In addition, doses of 50 mg/kg or higher caused a significant reduction of testosterone, LH and FSH levels compared to control. A descending trend of spermatogenic cell survival with increase in extract dose was also observed, starting with doses of 50 mg/kg. Histopathological studies of testis tissue demonstrated a significant decrease in the number of spermatogenic cells (spermatogonia, spermatocyte, spermatid and sperm) in the seminiferous tubules in all experimental groups relative to the control group. Intertubular spaces and venous congestion were increased in the treatment groups compared with those seen in the control group (Heidari *et al*., 2012).

The oral dose of 50 mg/kg, which caused the antifertility effects observed in male rats in the above-mentioned study, corresponds to a HED of ~480 mg for an adult human weighting 50 kg. Although referred to a different herbal preparation (ethanolic extract), this dose is below the dose range of 0.6 – 1.8 g reported in literature for the traditional use of dried powdered leaves of *Centella asiatica*. On the other side, no effect was seen on the weight of testis, ovaries and uterus of male and female rats up to doses of 1000 mg/kg of ECa233 in the study of Chivapat *et al*. (2011).

The antifertility effects of an ethanolic extract of *Centella asiatica* were confirmed by a more recent study. An oral dose of 300 mg/kg given to male rats, corresponding to a HED of ~2.8 g for an adult human weighting 50 kg, reduced significantly the number of implants in the uterine horns of female rats compared with the control group (Yunianto *et al.,* 2017).

Orally administered crude extract of *Centella asiatica* has been reported to significantly reduce fertility of female mice.

An ethanol extract of *Centella asiatica* at doses of 200 and 225 mg/kg bw significantly reduced birth rate compared to control group when given orally to pregnant rats. Also the percentages of post-implantation death were significantly higher in these dose groups copmpared to control (Akbar *et al*., 2018).

In contrast with the antifertility effects observed with ethanolic extracts reported above, an aqueous extract of *Centella asiatica* (200 mg/kg per day) showed beneficial effects on lead-induced suppressed reproductive performance in male rats. This extact when co-administered with Pb, counteracted the weight reduction of testis and epididymis and the decrease of epididymal sperm count, viable sperms, motile sperms and HOS-tail coiled sperms induced by lead (Sainath *et al*., 2011).

3.4. Overall conclusions on non-clinical data

Several non-clinical studies, mainly *in vivo* in rats and mice, carried out with alcoholic and water extracts of dried, powdered leaves, aerial parts or whole plant of *Centella asiatica*, ECa 233, TECA and main single constituents such as asiatic acid, asiaticoside and madecassoside support the traditional use of *Centella asiatica* to aid in healing of minor wounds.

Pharmacokinetic data for *Centella asiatica* extracts are not available.

Alcoholic, dichloromethane, hexane and aqueous extracts did not show any significant inhibition of CYP enzymes *in vitro* (IC_{50}>100 µg/ml for CYP 2D6, 3A4, 2C9, 1A2, 2C19, 2E1 and 2B6); ethanolic extracts strongly inhibited only CYP 2C9 and CYP 1A2, and dichloromethane extract inhibited only CYP 2C9 *in vitro*, with IC_{50}<100 µg/ml. *Centella asiatica* increased bioavailability of amitriptyline in rats.

Centella asiatica was well tolerated by mice and rats when administered orally up to single doses of 2000 mg/kg. Repeated-dose toxicity studies revealed signs of liver and kidney toxicity of dried, powdered, muslinised, aerial parts of *Centella asiatica* when orally administered for 30 days at doses of 500 mg/kg (HED ~4 g) and 1000 mg/kg in 24 rats; however, ECa233 did not cause significant chronic toxicity, including liver and kidney toxicity, when given to rats orally in doses of 10-1000 mg/kg for 90 days. An ethanolic extract of *Centella asiatica* given orally to mice for 30 days up to doses of 2000 mg/kg (HED ~8 g) did not show any toxic effect.

A couple of reproductive toxicity studies showed that ethanolic extracts of *Centella asiatica* were toxic to the reproductive system of male rats when administered orally at a concentration range of 10 - 300 mg/kg. A significant reduction in sperm viability and motility, in the number of spermatogenic cells in the seminiferous tubules with concomitant decrease of weight testis and a degeneration of seminiferous tubules were observed. These effects were dose-dependent and started with doses of 50 mg/kg corresponds to a HED of ~480 mg for an adult human weighting 50 kg.

Furthermore, an ethanol extract of *Centella asiatica* at oral doses of 200 and 225 mg/kg bw significantly reduced birth rate and increased the percentages of post-implantation death compared to control group when given orally to pregnant rats.

Some shortcomings in these studies that limit the extrapolability of the results observed to humans included the limited number of rats for treatment/control groups and the lack of investigation on potential for reversibility of the effect. In addition, these studies have been carried out using an ethanolic extract, which does not correspond to the herbal preparation reported in the monograph. On the other side, the above-mentioned studies provide evidence of a detrimental effect of ethanolic extracts of *Centella asiatica* on fertility of rats at HED doses even lower than 0.6 – 1.8 g for which there is enough evidence of traditional use. Therefore, the potential for anti-fertility effects of *Centella asiatica* in humans when taken orally cannot be completely ruled out.

Adequate tests on genotoxicity and carcinogenicity have not been performed.

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

Clinical pharmacology on *Centella asiatica* is not well documented in humans.

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

Clinical pharmacokinetic data on herbal substance/preparation of *Centella asiatica* are not available. Data on single constituents and highly purified and standardised extracts (ECa 233, TECA and synonims) have not been included in this assessment report as not deemed relevant for the monograph.

4.2. Clinical efficacy

Several clinical studies reported from literature refer to the following preparations containing *Centella asiatica* extracts:

- TTFCA (total triterpenoid fraction of *Centella asiatica*)
- TECA (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%).

Information coming from literature and licensed medicinal products confirms that TECA and TTFCA are different acronyms to designate the same extract, containing 40% of asiaticoside and 60% of asiatic acid and madecassic acid.

TECA is a highly purified extract, fractioned and enriched in triterpenic acid and triterpenic sugar ester fractions to reach about 40% of asiaticoside and about 60% of the triterpenic genins: asiatic acid and madecassic acid. The purification steps are extreme and involve chemical treatments that remove the herbal matrix so that 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.

Clinical efficacy of TTFCA and TECA in the treatment of chronic venous insufficiency (CVI) was investigated in several clinical studies. Improvement of microcirculation and leg volume associated with reduction of oedema and symptoms were reported with dose-dependent ameliorating effects. Small clinical studies to evaluate the efficacy of these refined extracts in atherosclerosis, diabetic microangiopathy, ulcer cicatrisation and burns recovery were also carried out.

TECA extract cannot be classified as an herbal preparation due to the manufacturing steps and composition (see EMA Public statement on *Centella asiatica* (L.) Urban, herba); therefore, only clinical data coming from other herbal preparations of *Centella asiatica* have been reported in this section.

4.2.1. Dose response studies

No dose-finding studies have been conducted with *Centella asiatica* based on data obtained from literature search.

4.2.2. Clinical studies (case studies and clinical trials)

Clinical studies on wound healing

The clinical efficacy and side effects of the oral *Centella asiatica* extract capsule in the diabetic wound healing was studied in a prospective randomised control study. Two hundred of diabetic foot ulcer Thai patients were enrolled into the study. The exclusion criterion were low immune patients, oral steroid intake, age more than 80 year and less than 18 years, serum albumin less than 3.0 gm/dl, uncorrected peripheral arterial diseased patients, and uncontrolled infective wound. The termination criterion were

patient refusal, wound infection, delayed primary sutured wound, secondary healing wound. The patients were divided into 2 groups randomly, group A was *Centella asiatica* extract capsule group (type of extract not specified) and group B was placebo group. The administration was 2 capsules after meal 3 times a day (50 mg of extracted asiaticoside/capsule in group A). The general symptoms, wound characteristic, wound size and depth were examined at day 7, day 14 and day 21 by the same investigator. Wound contraction was detected by the decrease of the volume of the wound; wound granulation tissue forming was detected by the decrease of wound depth. There were 20 cases dropped out from the study. One hundred and seventy cases were analysed. The author reports significance changes of wound contraction between placebo and study group. The study group trend to be good contraction earlier than placebo group and there were more wound granulation tissue forming in the placebo group than the study group (Paocharoen 2010).

The efficacy of an ointment containing *Centella asiatica* on partial-thickness burn wounds was compared to silver sulfadiazine in a randomised controlled clinical trial. Fresh leaves of the plant were air-dried at 40°C and powdered. Then, they were subjected to exhaustive extraction using ethanol (96%) and distilled water with the relation of 60:40. The dark green liquid extract was dried and then partitioned between butanol and petroleum ether. It made 2 separate portions in which butanol fraction was concentrated under rotary evaporator and vacuum. Dried butanol fraction was mixed with vaseline and glycerin to make the ointment which contained about 3% of the final extract. Patients with second-degree burn wounds on their limbs treated in Velayat Burning Hospital at Rasht, Iran entered the study. The difference in duration of treatment for complete wound healing was considered as the primary outcome measure. It was calculated that 30 subjects would be required per group in order to detect a difference of 5 days in this parameter with 80% power and 5% probability of type I error. Seventy-five subjects aged 30.67±9.91 years were randomised of whom data were analysed for 30 patients treated with 1% sulfadiazine (SSD) cream and 30 patients treated with the ointment containing *Centella asiatica*. Burn wounds were treated once daily at home. All of the wounds were evaluated till complete healing occurred and at the admission, days 3, 7, 14 objective signs; visual acuity score (VAS) and subjective signs were recorded. Re-epithelialisation time and complete healing days were recorded. The mean of complete healing in burning wounds was 14.67±1.78 days in *Centella asiatica* versus 21.53±1.65 days in SSD group. Starting time of complete healing in *Centella asiatica* group patients was on the 10^{th} day, which was the 18^{th} day in SSD group. The average time of re-epithelialisation was 6.8 days sooner in *Centella asiatica* group versus SSD group patients. Multivariate analysis showed a significant difference in complete healing between *Centella asiatica* and SSD groups ($p=0.001$). All of the objective indexes, that is, pliability, vascularity, pigmentation, height, and VAS, significantly led to better healing of the burning wound in *Centella* group rather than SSD group ($p<0.05$). All of the subjective indexes, that is, dryness, itching, and irritation, significantly led to better healing of the burning wound both in time and efficacy in *Centella asiatica* group rather than SSD group ($p<0.05$). Mean of re-epithelialisation in burning wounds was 13.7±1.48 days in *Centella asiatica* group versus 20.67±2.02 days in SSD group (Saeidinia *et al*., 2017).

A prospective randomised, double-blind control study was performed to evaluate the efficacy of *Centella asiatica* cream in 30 patients who underwent a split-thickness skin graft (STSG) operation. The *Centella asiatica* was prepared by being extracted with 70% alcohol in cream preparation. The *Centella asiatica* extract comprised asiaticoside 5.12% and madecassoside 5.1%. Thai subjects (20 years or older) were divided randomly into 2 groups, with various cream applied to each part of the donor's scar site. One gram of Cream A (7% w/w *Centella asiatica* extract in cream preparation) or Cream B (placebo) was randomly applied on the subjects at least 2 weeks after epithelialisation was completed. The gels were applied for a total treatment period of 12 weeks. A scar assessment using the Vancouver Scar Scale (VSS) to determine pigmentation, vascularity, pliability, and height was taken at 4, 8, and 12 weeks. Only 23 of 30 patients completed the study protocol. Two patients were excluded due to rash at the scar, which may have been caused by an allergic response to the products. Five patients

were lost to follow-up because they lived far away. For the *Centella asiatica* cream group, there were differences from baseline including the pigmentation score at 8 and 12 weeks and between 4 and 12 weeks (-0.443, *p* value 0.019; -0.707, *p* value 0.001; -0.557, *p* value 0.001) and the overall VSS scores between 4 and 12 weeks (-1.279, *p* value 0.041). However, for height, it was worse at 4 weeks (0.300, *p* value 0.043). For the placebo group, there were 2 differences from the baseline including the pigmentation score between 4 and 12 weeks (-0.399, *p* value 0.020) and after 12 weeks (-0.549, *p* value 0.002), while the pliability and the height scores of both groups were compared before and after treatment and still were not different (Jenwitheesuk *et al.,* 2018).

Clinical studies on psoriasis

The effects of a topical application of a cream containing the water and oil extract from *Centella asiatica* were investigated in 7 patients affected by psoriasis in a not controlled study. The authors report lesions completely cleared in 5 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 (Natarajan and Paily 1973).

Clinical studies on anxiety and general anxiety disorder (GAD)

The effects of Gotu Kola on the acoustic startle response (ASR) was evaluated in subjects who were randomly assigned to receive either a single 12 g orally administered dose of Gotu Kola (n=20) or placebo (n=20). The ASR paradigm has been extensively used to investigate stress-related disorders and anxiety in humans. 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 authors report that compared with placebo, Gotu Kola significantly attenuated the peak ASR amplitude 30 and 60 min after treatment (Bradwejn *et al.,* 2000).

The role of 70% hydro-ethanolic extract of dried powdered *Centella asiatica* (CA) on generalised anxiety disorder (GAD) was investigated in man. Thirty-three Indian participants (18 male and 15 female; average age 33 years) were medicated with gelatin capsules containing the CA in a fixed dose regime (500 mg/capsule, twice daily, after meal for 60 days). Hamilton's Brief Psychiatric Rating Scale (BPRS) was used to screen the subjects. The authors report that the results indicate that CA ingestion significantly attenuated stress-anxiety-depression related disorders. The baseline score of anxiety index declined to 13.1% in 30 days and 26.0% in 60 days after the treatment of CA. An improvement (12.5%) in self-perceived stress within 30 days and 23.2% within 60 days were noted. Depression index also reduced from 10.2% (30 days) to 21.8% (60 days) in case of CA trial. After treatment, adjustment score finally improved by 35.2% and attention level improved by 27.8%. Each of these results was statistically significant with *P-values* all <0.01 (Jana *et al.,* 2010).

Clinical studies on cognitive function

A randomised, placebo-controlled, double-blind study investigated the effect of *Centella asiatica* on cognitive function of 28 healthy elderly volunteers. Participants received the plant extract (information on extraction method is missing) at various doses ranging 250, 500 and 750 mg once daily for 2 months. According to the authors, 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 (Wattanathorn *et al.,* 2008).

Farhana *et al.* (2016) determined the effectiveness of *Centella asiatica* extract (DER 10:1, extraction solvent ethanol 70%) in improving cognitive function in patients with vascular cognitive impairment (VCI). This study used a quasi-experimental design. Subjects in this study were hospitalised Indonesian patients with post-stroke cognitive impairment. Seventeen subjects were treated with 1000

mg per day of *Centella asiatica* extract, 17 subjects treated with 750 mg per day of *Centella asiatica* extract, and 14 subjects treated with 3 mg per day of folic acid for 6 weeks. According to the authors, all treatment groups showed significant improvement in Montreal Cognitive Assessment-Indonesian version (MoCA-Ina) score after 6 weeks. The mean difference in score of MoCA-Ina at the 6th week minus the baseline for the *Centella asiatica* 1000 mg group was 5.6±4.61 ($p<0.001$; 95% CI), *Centella asiatica* 750 mg was 4.94 ± 2.16 ($p<0.001$; 95% CI), and 3 mg of folic acid was 4.06±3.11 ($p<0.001$; 95% CI). Not one therapy was statistically more effective than the others in increasing MoCA-Ina score ($p=0.39$) (Farhana *et al.,* 2016).

Puttarak *et al*. (2017) carried out a systematic review and meta-analysis to determine the effects of *Centella asiatica* on cognitive function and mood related outcomes. Of the 11 included studies, 5 studies compared *Centella asiatica* alone to placebo, and 6 studies compared a combination of *Centella asiatica* versus other herbs. Nine studies were conducted using double-blind parallel designs, one used an open-labeled parallel design, and one used a cross-over design. Most studies were conducted in healthy volunteers, while one study was conducted in children with attention deficit hyperactive disorder. In 4 studies powdered plant was used (part of the plant not specified) whilst in 5 studies an extract was used, but only in 2 studies the extraction solvent was reported (standardised water extract of aerial parts). In addition, the doses of *Centella asiatica* in each study were different, ranging from 40–12,000 mg per day. Three of the studies had a high risk of bias, 7 studies were unclear, and only one study had a low risk of bias. Although, all studies stated that they were randomised controlled trials, 4 of the trials were found to have unclear risk of bias for "sequence generation" because there was no description of the sequence generation methods. Most studies did not describe the "allocation concealment" method. In the bias domain of "blinding", one study was an open-label study which was categorised as having a high risk of bias. Of the included studies, 60 cognitive function tests were described, but only 27 of the tests had sufficient data for a meta-analysis. The 27 cognitive function tests were each categorised into specific cognitive domains for the purpose of evaluating the cognitive improvement effect of *Centella asiatica*. The domains included: 1) overall cognitive status, 2) attention and concentration, 3) executive function, 4) working memory, 5) information processing speed, 6) language, 7) verbal memory, 8) visuospatial skill, and 9) visual memory. The meta-analysis indicated no significant difference between *Centella asiatica* and comparators (placebo) on any cognitive function domain on a total of 153 subjects (Puttarak *et al*., 2017).

Table 4: Clinical studies on humans, in wound healing

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
Clinical efficacy of oral *Centella asiatica* (CA) extract in the diabetic wound healing Paocharoen 2010	Prospective RCT; placebo used as a control; duration 21 days	CA freeze-dried extract (no further information available, suspected to be water extract, being freeze dried); 2 capsules each containing 50 mg of asiaticoside taken orally 3 times a day or placebo	200 diabetic wound patients enrolled; n=84 received CA extract, n=86 took placebo; mean age 58.59 years in both groups; 20 dropped out	Diabetic foot ulcer Thai patients	Wound contracted earlier and less granulation tissue forming in CA than in the placebo group	Pearson Chi-square tests	No clinical relevance due to shortcomings in reporting and/or design (e.g. no key information on kind of extract; no information on blinding and wound size of baseline; high rate of drop-out)
Efficacy of CA on partial-thickness burn wounds Saeidinia *et al*., 2017	Prospective, parallel group, RCT; cream containing 1% sulfadiasine used as a control; duration 14 days	Ointment containing 3% dry ethanol-water (60:40) extract from dried, powdered leaves of CA; the ointment was applied once daily	75 Iranian patients: n=35 allocated to SSD and n=40 to CA treatment; mean age 30.67±9.91 years; 19 males (31.7%) and females (68.3%); 10 participants in the CA group	Subjects with partial thickness, burning wound < 10% of total body surface area (TBSA) and in the limbs, burning event < 48 h, no other concurrent injury except burning,	Primary outcome: mean of complete healing in burning wounds CA: 14.67± 1.78 days SSD: 21.53±1.65 days (p=0.001) Secondary outcomes: all objective indexes, pliability (vascularity, pigmentation, height, and VAS), significantly	Per protocol (General linear model test, Student and indep. T tests)	No clinical relevance due to the lack of a placebo group, missing information on blinding and by the limited sample size DER of the hydro-alcoholic extract has not been reported

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
			and 5 participants in the control group dropped out	general physical and mental health, 14 - 60 years old	better in CA group than SSD group (p<0.05); all subjective indexes (dryness, itching, and irritation), significantly better in CA group than SSD group (p<0.05) Mean of re-epithelialisation in burning wounds: CA: 13.7±1.48 days CA SSD: 20.67±2.02 days (p=0.001)		
Efficacy of CA in patients who underwent a split-thickness skin graft (STSG) operation Jenwitheesuk	Prospective, double-blind, RCT; placebo cream used as a control; duration 12 weeks	Cream containing 7% w/w CA ethanolic extract (containing 5.12% asiaticoside and 5.1% madecassoside) 1 g of the cream was applied on the skin twice daily	30 Patients (13 males and 10 females; mean age 54 years); 5 patients were lost to follow-up and 2 patients discontinued intervention	Patients who underwent split-thickness skin graf harvesting. Inclusion criteria: 1) donor site of patients who underwent STSG	CA; signifcantly improved the overall Vancouver scores between 4-12 weeks (p=0.04) and pigmentation from the baseline since 8th week (p<0.05) Placebo: significantly improved the	Not clear	No clinical relevance due to the small number of patients included

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
et al., 2018			due to allergy to the product	operation completed more than 14 days of epithelialisatio n; 2) aged 20 years or older	pigmentation score between 4-12 weeks (p=0.020) and afer 12 weeks (p=0.002)		

Table 5: Clinical studies on humans, in psoriasis

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
Natarajan and Paily 1973	Preliminary clinical study of the effect of CA on psoriasis; study not controlled; duration of tretatment was variable based on the individual response (range 3-8 weeks)	Cream containing the water and oil extract from dried, powdered leaves of CA administered to 5 patients or concentrated expressed juice from fresh leaves of CA administered to 2 patients	7 patients (6 males and 1 woman; mean age 32 years); all patients completed the study	Clinical diagnosis of psoriasis (from 1 month to 8 years)	5 patients showed complete clearance of the lesions; one patient showed clearance of most of the lesions; one patient experienced flattering reduction of scales, decrease in size of the lesions but not complete clearing	No statistical analysis performed	Not clinically relevant

Table 6: Clinical studies on humans, in anxiety and general anxiety disorder

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
Effect of CA on the acoustic startle response (ASR) Bradwejn *et al.,* 2000	Double-blind, parallel group, RCT; placebo used as a control	Single dose of 12 g of CA orally administered as capsules containing crude powder herb blended with grape juice and celery salt	n=40 (21 men and 19 women, age from 18 to 45 years)	Healthy subjects with no lifetime history of rnental disorders no family history of psychiatric disorders, no hearing problems, and a normal physical examination, blood, and urine laboratory test results	Compared with placebo, CA significantly attenuated the peak ASR amplitude 30 (p<0.05) and 60 min (p<0.001) after treatment, but not 90 and 120 min after treatment	ANOVA post hoc Duncan's test, Cohen's index d values The signiffcance level was set at p<0.05 using two-tailed tests	No clinical relevance (healthy subjects; low number of patients with unclear grouping, single dose)
Therapeutic role of CA in generalised anxiety disorders (GAD)	Clinical trial not including a control group; no information on blinding and randomisation; duration 60	Capsule containing 500 mg of a lyophilised ethanolic extract (70% ethanol) from dried, powdered aerial parts of CA given	35 Indian participants, 33 completed the trial (18 males and 15 females, mean age 33 years);	Patients with diagnosis of GAD	The baseline score of anxiety index declined to 13.1% in 30 days and 26.0% in 60 days after the treatment	*Chi-square test* and percentile change compared to baseline	No clinical relevance due to too many shortcomings in the design (lack of a control group, no information on blinding,

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
Jana *et al*., 2010	days	orally twice daily, after meal	2 dropped-out		Improvement (12.5%) in self-perceived stress within 30 days and 23.2% within 60 days; Depression index reduced from 10.2% (30 days) to 21.8% (60 days) After treatment, adjustment score finally improved by 35.2% and attention level by 27.8%. All results were statistically significant (p<0.01)	results	randomisation, validation of the assessment scales, small sample size etc.)

Table 7: Clinical studies on cognitive function in humans

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
Effect of CA on cognitive function.	Randomised, placebo-controlled,	Specialised dry extract from aerial parts of CA	28 participants (mean age 65.05±3.56	Healthy elderly Thai subjects	CA 750 mg per day significantly increased the subject's N100	Analysis of variance (ANOVA)	No clinical relevance mainly due to the small number of

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
Wattanathorn *et al*., 2008	double-blind study; Duration 2 months	standardised to contain 29.9 mg/g of tannic acid, 1.09 mg/g of asiaticoside and 48.89 mg/g of asiatic acid, Patients were given capsules containing placebo or CA extract at various doses ranging 250, 500 and 750 mg once daily	years; 4 males and 24 females)		amplitude compared to placebo; no significant change in N100 latency. No significant changes in either the amplitude or latency of P300. CA decreased the reaction time (significantly only in choice, spatial, numeric and picture reaction times), while increased the % accuracy of working memory (significantly only in spatial, numeric, word and picture recognition)	Statistical significance was set at $p<0.05$	subjects included; no adequate information on the standardised dry extract; the study was performed in healthy subjects
Improvement of cognitive function in patients with vascular cognitive impairment after the use of CA Farhana *et*	Comparative clinical study of CA extract 750 mg per day and 1000 mg per day vs folic acid 3 mg per day for 6 weeks	2 capsules containing 375 mg, 500 mg of a standardised dry extract (ethanol 70%; DER 10:1) from the whole plant or 1.5 mg of folic acid each, taken orally once daily	51 patients, whose 48 completed the study (n=17 in CA 1000 mg per day group; n=17 in CA 500 mg per day group and n=14 in folic	Ischemic stroke patients, with MoCA-Ina values ≤ 26, age ≥ 18 years, and good liver function	All treated groups showed significant improvement in MoCA-Ina score. Mean difference in score of MoCA-Ina at the 6th week minus the baseline: CA 1000 mg: 5.6±4.61 ($p<0.001$; 95% CI); CA 750 mg:	One-way ANOVA or paired *t*-test; Wilcoxon signed-rank test or Kruskal-Wallis test	No clinical relevance (small sample size, lack of information on randomisation and statistical power, lack of a placebo control group, lack of further information on the extract)

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
al., 2016			acid 3 mg per day group) Mean age 60.27±11.83 years, 60.41% males and 39.58% females		4.94±2.16 (p<0.001; 95% CI); Folic acid 3 mg: 4.06±3.11 (p<0.001; 95% CI) No statistical difference among treatment goups in increasing MoCA-Ina score (p=0.39), and for all domains tested, except for memory domain (delayed recall memory) which showed statistically significant improvement in CA groups compared to folic acid		

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

There are no clinical studies carried out with *Centella asiatica* alone in special population groups.

4.4. Overall conclusions on clinical pharmacology and efficacy

Clinical pharmacology on *Centella asiatica* is not well documented in humans. Pharmacokinetic data on *Centella asiatica* herbal substance/preparations are missing.

A few clinical studies investigating the effects of *Centella asiatica* extracts in wound healing have been carried out, but deficiencies in the clinical designs and the lack of sufficient information on the herbal preparations used render the outcomes of these studies not clinically relevant. As a consequence, available data are inadequate to substantiate a well-established use of *Centella asiatica* in wound healing.

Several clinical studies with herbal preparations of *Centella asiatica* have been also carried out in different therapeutic areas (i.e., in the treatment of psoriasis, general anxiety and in the management of skin ageing), but also in these cases the number and the quality of the trials do not provide sufficient clinical relevance to acknowledge a therapeutic indication based on well-established use.

A few clinical studies on the the nootropic effect of *Centella asiatica* have been carried out (Wattanathorn *et al*., 2008; Farhana *et al*., 2016). A meta-analysis including 11 clinical studies to determine the effects of Centella asiatica on cognitive function indicated no significant difference between *Centella asiatica* and comparators (placebo) on any cognitive function domain on a total of 153 subjects, although findings in some trials indicated that *Centella asiatica* alone may improve working memory. Most of the studies presented many methodological deficiencies. Finally, full details on the different herbal preparations used in these studies were often missing and the doses of *Centella asiatica* were too wide (40–12,000 mg per day). Half of the studied were carried out using *Centella asiatica* in combination with other treatments. Therefore, there are no grounds to support a well-established use of *Centella asiatica* in improving cognitive function in humans.

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 asiatica* preparations, gastric complaints and nausea have occasionally been reported, but were not significant versus placebo (Brinkhaus *et al.,* 2000).

The efficacy of TTFCA in the treatment of sign and symptoms of chronic venous insufficiency was examined in a systematic review by Chong and Aziz (2012). Eight clinical studies including a total of 522 patients were included. Two trials reported on the adverse effects. In one trial, 2 patients given *Centella asiatica* extract experienced minor stomach pain while one patient had to stop treatment due to severe nausea. In the other trial, 4 patients given TTFCA withdrew from the trial: 3 due to nausea and gastric pain and one because of "neurological absence".

The clinical efficacy and side effects of the oral *Centella asiatica* extract capsule in the diabetic wound healing was studied in a prospective randomised control study on 200 Thai patients. Patients were treated with 2 capsules of *Centella asiatica* extract after meal, 3 times a day (50 mg of extracted

asiaticoside /capsule in group A; n=84) or placebo (group B; n=86). There were no systemic side effect or complications reported in the clinical study and there was no significant difference of wound infection between 2 groups; however, the reason why 20 patients dropped out was not provided (Paocharoen 2010).

There were no adverse reactions such as severe itching, hypersensitivity, systemic symptoms in the *Centella asiatica* group (n=40) during the trial carried out by Saeidinia *et al*. (2017) in 75 patients with partial thickness burning.

A cream containing 7% w/w of an ethanolic extract of *Centella asiatica* was administered for 12 weeks in 30 patients who underwent a split-thickness skin graft (STSG) operation. Only 23 of 30 patients completed the study protocol. Two patients were excluded due to rash at the scar, which may have been caused by an allergic response to the products. The allergic reaction occurred with both Centella cream and placebo. Five patients were lost to follow-up because they lived far away. No information on the adverse events experienced during the treatment is available (Jenwitheesuk *et al*., 2018).

The role of 70% hydro-ethanolic extract of *Centella asiatica* (500 mg capsule, twice daily for 60 days) on generalised anxiety disorder (GAD) was investigated in 33 patients, but no information was provided on adverse events (Jana *et al*., 2010).

Forty-eight subjects with vascular cognitive impairment were included in a clinical study aimed to compare the effectiveness of a *Centella asiatica* extract and folic acid in improving cognitive function. Seventeen subjects were treated with 1000 mg per day of Gotu kola extract, 17 subjects treated with 750 mg per day of Gotu kola extract, and 14 subjects treated with 3 mg per day of folic acid for 6 weeks. Side effects that arose as a result of a *Centella asiatica* extract therapy of 1000 mg per day were constipation and itching (11.11%). The subject who experienced constipation continued with the therapy while the subject who experienced itching discontinued treatment on the 7th day. A side effct that occurred in the *Centella asiatica* extract group of 750 mg per day was a bloated feeling (5.5%); that subject continued therapy. The adverse effcts of folic acid therapy at 3 mg per day were nausea and heartburn (14.2%). Two subjects who suffered the condition continued the therapy after getting information and symptomatic treatment for their conditions (Farhana *et al*., 2016).

In the systematic review carried out by Puttarak *et al*. (2017) 5 randomised controlled trials (RCTs) conducted to determine the effect of *Centella asiatica* alone and 6 RCTs conducted to determine the effect of *Centella asiatica*-containing products on cognitive function and mood related outcomes. 466 patients were included. No adverse effcts were reported in any studies looking at *Centella asiatica* alone. However, for studies of combination products, 4 studies reported mild adverse events of *Centella asiatica*-containing products. Two studies reported adverse event rates comparable to the placebo rate, while another 2 studies reported lower rates of adverse event for *Centella asiatica*-containing products. Common adverse events were gastrointestinal discomfort, fltulence, nausea, headache, decreased appetite, sedation, and rash. Hepatotoxicity was not observed in any of the included RCTs.

None of 7 patients with psorias treated with 100 g of dried powdered leaf of *Centella asiatica* experienced side effects (Natarajan and Paily 1973).

Lou *et al*. (2018) examined the effects of CAST, a highly purified, standardised *Centella asiatica* extract containing triterpenes, on neuropathy symptoms in Type II diabetic subjects in a 52-week, randomised, double-blind, placebo-controlled trial. The triterpene content reported in the certificate of analysis was 37.03% AS, 34.26% MA, and 23.01% AA by weight, which were within the specifications for CAST of 36–44% AS and 56–64% of combined MA and AA. Forty-three subjects (age 42–80 years) with a history of Type II diabetes enrolled in the study. Thirty-three subjects completed the study. Ten dropped out of the study for the following reasons: bladder cancer (1), abnormal liver function (1),

flare-up of a pre-existing skin condition (1), breast tenderness of unknown etiology (1), other unstable medical condition (1), non-compliance (5), and moved out of state (1). Patients were treated either CAST or Placebo for 52 weeks. CAST was well tolerated up to 240 mg per day for 1 year. Twenty-nine of 43 randomised subjects (67%) experienced at least one adverse event (AE). The proportion of patients who experienced at least one AE in the Placebo (56%) and in the CAST (80%) groups was not significantly different (p=0.1). However, the odds ratio of having an AE was 3.5 times higher, and the relative risk of an AE was 1.4 times higher for subjects on CAST compared to placebo. AEs included transient abnormal liver and kidney function or gastrointestinal symptoms, which resolved on their own. Abnormal electrocardiograms (ECGs) were noted in some subjects and were linked to either pre-existing conditions or returned to normal on subsequent tests. All AEs were minor.

Table 8: Clinical safety data from clinical trials

Study, Reference	**Study design, controls, duration**	**Test Product**s (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of** S**ubjects**	**Adverse reactions**	**Clinical relevance**
Effect of *Centella asiatica* (CA) on psoriasis Natarajan and Paily 1973	Preliminary clinical study; not controlled; duration of tretatment was variable based on the individual response (range 3-8 weeks)	Cream containing the water and oil extract from dried, powdered leaves of CA administered to 5 patients or concentrated expressed juice from fresh leaves of CA administered to 2 patients	7 patients (6 males and one female; mean age 32 years); All patients completed the study	Clinical diagnosis of psoriasis (from 1 month to 8 years)	There was no evidence of systemic and local toxicity, neither anything wrong could be assessed by blood and urine tests None of the patients experienced any side effects	No safety issue reported However, only 7 patients included in this small study
Effect of CA on the acoustic startle response (ASR) Bradwejn *et al.,* 2000	Double-blind, RCT; placebo controlled	Single dose of 12 g of CA orally administered as capsules containing crude powder herb blended with grape juice and celery salt	n=40 (21 males and 19 females, age from 18 to 45 years)	Healthy subjects with no lifetime history of rnental disorders no family history of psychiatric disorders, no hearing problems, and a normal physical examination, blood, and urine laboratory test results	CA was well tolerated with no adverse event	An oral high single dose (12 g) of powdered CA herb was well tolerated

Study, Reference	**Study design, controls, duration**	**Test Product**s (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of** S**ubjects**	**Adverse reactions**	**Clinical relevance**
Effect of CA on cognitive function Wattanathorn *et al*., 2008	Randomised, placebo-controlled, double-blind study; Duration 2 months	Specialised dry extract from aerial parts of CA standardised to 29.9 mg/g of tannic acid, 1.09 mg/g of asiaticoside and 48.89 mg/g of asiatic acid, Patients were given capsules containing placebo or CA extract at various doses ranging 250, 500 and 750 mg once daily	28 participants (mean age 65.05±3.56 years; 4 males and 24 females)	Healthy elderly Thai subjects	Adverse events were monitored during the study and laboratory tests were drawn at baseline and follow-up visits and compared to see whether any changes suggested adverse events; However, no information or data were provided on the AEs in the published article	No information or data were provided on the safety outcomes in the article
Therapeutic role of CA in generalised anxiety disorders (GAD) Jana *et al*., 2010	Clinical trial not including a control group; no information on blinding and randomisation; Duration 60 days	Capsule containing 500 mg of a lyophilised ethanolic extract (70% ethanol) from dried, powdered aerial parts of CA given orally twice daily, after meal	35 Indian participants, 33 completed the trial (18 males and 15 females, mean age 33 years); 2 dropped-out	Patients with diagnosis of GAD	None of the patients reported self-perceived adverse events	No safety issue reported in this small study
Clinical efficacy of oral CA extract in the diabetic	Prospective RCT; placebo used as a control; Duration 21 days	CA freeze-dried extract (no further information available); 2 capsules each containing 50 mg of asiaticoside taken	200 diabetic wound patients enrolled; n=84 received CA extract, n=86	Diabetic foot ulcer Thai patients	No systemic side effects or complications reported in this study; No significant difference of wound infection between the	No information available on the collection and the assessment of adverse

Study, Reference	**Study design, controls, duration**	**Test Product**s (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of** S**ubjects**	**Adverse reactions**	**Clinical relevance**
wound healing Paocharoen 2010		orally 3 times a day or placebo	took placebo; mean age 58.59 years in both groups; 20 dropped out		two groups	events; laboratory testings were not performed
Improvement of cognitive function in patients with vascular cognitive impairment after the use of CA Farhana *et al*., 2016	Comparative clinical study of CA extract 750 mg per day and 1000 mg per day vs folic acid 3 mg per day for 6 weeks	2 capsules containing 375 mg, 500 mg of a standardised dry extract (ethanol 70%; DER 10:1) from the whole plant or 1.5 mg of folic acid each, taken orally once daily	51 patients, whose 48 completed the study (n=17 in CA 1000 mg per day group; n=17 in CA 500 mg per day group and n=14 in folic acid 3 mg per day group) Mean age 60.27±11.83 years, 60.41% males and 39.58% females	Ischemic stroke patients, with MoCA-Ina values ≤ 26, age ≥ 18 years, and good liver function	In CA 1000 mg group: constipation and itching (11.11%). Subject with constipation continued; subject with itching discontinued treatment on the 7th day. CA 750 mg group: bloated feeling (5.5%); subject continued therapy. Folic acid group: heartburn and nausea (14.2%). An increase in the liver enzymes AST and ALT was observed. No significant difference between AST and ALT levels before and after treatment with value of all groups (p>0.05), based on a paired t-test analysis One-way ANOVA showed no significant difference between	A trend towards an increase of ALT and AST was observed in CA and folic acid groups after 6 weeks of treatment compared to baseline, but differences were not statistically significant in this small study

Study, Reference	**Study design, controls, duration**	**Test Product**s (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of** S**ubjects**	**Adverse reactions**	**Clinical relevance**
					the treatment groups on the increase in AST and ALT	
Efficacy of CA on partial-thickness burn wounds Saeidinia *et al.,* 2017	Prospective, parallel group, RCT; cream containing 1% sulfadiasine used as a control; Duration 14 days	Ointment containing 3% dry ethanol-water (60:40) extract from dried, powdered leaves of CA; the ointment was applied once daily	75 Iranian patients: n=35 allocated to SSD and n=40 to CA treatment; mean age 30.67±9.91 years; 19 males (31.7%) and females (68.3%); 10 participants in the CA group and 5 participants in the control group dropped out	Subjects with partial thickness, burning wound <10% of total body surface area (TBSA) and in the limbs, burning event <48 h, no other concurrent injury except burning, general physical and mental health, 14 - 60 years old	No adverse reaction such as severe itching, hypersensitivity, systemic symptoms in the CA group, while in the SSD group, 4 patients were infected and received antibiotic therapy and conservative treatment.	An ethanolic extract of CA was well tolerated when topically applied on the burn wounds
Efficacy of CA in patients who underwent a split-thickness	Prospective, double-blind, RCT; placebo cream used as a control;	Cream containing 7% w/w CA ethanolic extract (containing 5.12% asiaticoside and 5.1% madecassoside)	30 Tatients (13 males and 10 females; mean age 54 years); 5 patients were	Patients who underwent split-thickness skin graf harvesting Inclusion criteria:	No information on monitoring and assessment of adverse events	

Study, Reference	**Study design, controls, duration**	**Test Product**s (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of** S**ubjects**	**Adverse reactions**	**Clinical relevance**
skin graft (STSG) operation Jenwitheesuk *et al.,* 2018	Duration 12 weeks	One gram of the cream was applied on the skin twice daily	lost to follow-up and 2 patients discontinued intervention due to allergy to the product	1) donor site of patients who underwent STSG operation completed more than 14 days of epithelialisation; 2) participants aged 20 years or older		
Systematic review of all available evidence to determine the efficacy and safety of CA on cognitive function Puttarak *et al*., 2017	Meta-analysis of 11 clinical studies, 5 comparing a combination of CA versus other herbs and 6 comparing a combination of CA versus other herbs 9 studies were conducted using double-blind parallel designs, one used an open-labeled	4 studies were carried out using a powder and 5 using an extract of CA (2 with water extracts, 3 without indication of the type of extract); In the remaining 2 studies no information on the herbal preparation was given. Standardisation methods were reported in 3 studies but only 2 studies quantitatively described	Total subjects: 642 Subjects trated with CA (alone plus other herbs): 359 Subjects treated with CA alone: 137	9 studies were conducted in healthy adult volunteers, while one study was conducted in children with attention defiit hyperactive disorder and one study was carried out in mentally retarded children	No adverse effects reported in any studies looking at CA alone. However, for studies of combination products, 4 studies reported mild adverse events of CA-containing products. 2 studies reported adverse event rates comparable to the placebo rate, while another 2 studies reported lower rates for CA-containing products Common adverse events: gastrointestinal discomfort, flatulence, nausea, headache, decreased appetite,	Overall, different preparations of CA appeared to be tolerated

Study, Reference	**Study design, controls, duration**	**Test Product**s (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of** S**ubjects**	**Adverse reactions**	**Clinical relevance**
	parallel design, and one used a cross-over design	the standardisation CA was orally administered mainly as a capsule or tablet with daily doses ranging from 40 mg to 12 g			sedation, and rash Hepatotoxicity was not observed in any of the included RCTs	

5.2. Patient exposure

See sections 4.2.2 and 5.1.

5.3. Adverse events, serious adverse events and deaths

An analysis of reports in the Thai Health Product Vigilance Center Database from 2000 to 2008 was performed to describe the characteristics of reported adverse events in patients receiving herbal products in Thailand. Causality assessment was carried out using Naranjo's algorithm by health professionals at the time of report submission. Only reports rated as 'possible' or higher were included in the analysis. Over the 9-year study period, Thai VigiBase had a total of 593 case reports with 1868 adverse events involving 24 different herbal products. *Centella asiatica* registered 42 reports (7.1%) for 200 adverse events (10.8%). Most of adverse events were gastrointestinal system disorders (47.5%; n=95); abdominal pian, flatulence, constipation, dry mouth, nausea and vomiting were the most frequent gastrointestinal adverse events (n≥10). Anorexia (n=21), dizziness (n=13) and palpitations (n=12) were other frequent observed adverse events. One case of serious exfoliative dermatitis was experienced (Saokaew *et al.,* 2011).

Allergic contact dermatitis has been reported after the topical application of various creams and ointments containing *Centella* extracts (Izu *et al*., 1992, Danese *et al*., 1994). However, further testing revealed that reactions may be due to other ingredients in the preparations (Hausen BM. *Centella asiatica* Indian pennywort).

A case-report of contact dermatitis with ointment containing *Centella asiatica* purified branded material (Madecassol®) has been reported by Gomes *et al*. (2010). A 42-year-old non-atopic woman with no relevant past history presented with localised, severe eczema on her neck and upper chest, after treating a hypertrophic thyroidectomy scar with the mentioned ointment. She required systemic corticosteroid therapy while, one month later, patch tests were performed with the ointment (tested 'as is') and its ingredients. Positive reactions to the ointment itself as well as to *Centella asiatica* extract (1% and 5% pet.) and weak reactions (probably of irritant nature) to lavender oil (20% pet.) and propylene glycol (5% pet.) were obtained. Patch tests with *Centella asiatica* extract 1% and 5% in 20 controls were negative.

In a multicentric Italian study to assess use and skin reactions to topical botanically derived products and usefulness of patch tests, 122 patients who reported cutaneous adverse reactions after botanical products application, underwent patch testing with a series of botanical preparations including *Centella asiatica* extract 2% in ethanol. Three patients (2.5%) had positive reactions to this extract (Corazza *et al*., 2013).

Following oral administration of *Centella asiatica* 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 since 1969 (Rotblatt and Ziment 2001).

Three women (61, 52 and 49 years old) developed jaundice after taking orally *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 3 patients improved with *Centella asiatica* discontinuation, and ursodeoxycholic acid 10 mg/kg per 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 and Jorge 2005).

A case report of hepatotoxicity involved 15-year-old girl who presented with a short history of abdominal pain and vomiting. She had been on lymecycline, for acne, over the preceding 8 weeks. Investigations revealed deranged liver function as follows: Serum bilirubin 31 µmol/L, ALT 319 IU/L, GGT 39 IU/L and albumin 35 g/l. ALT rapidly rose to 3222 IU/L within 24 h. Prothrombin time increased to 31 s and an INR was 2.7. She was treated with N-acetyl cysteine at a dose of 100 mg/kg per 24 h as a continuous intravenous infusion that was stopped after 54 h. Intravenous Vitamin K at a dose of 10 mg once daily was given for 4 consecutive days. Her liver function test results and coagulation profile improved rapidly. Investigations showed normal immunoglobulin, caeruloplasmin and alpha-1-antitrypsin levels, and negative autoantibody and urine metabolic screens. Serology for Hepatitis A, B, C viruses, Toxoplasma, Cytomegalovirus, Herpes simplex virus, Mycoplasma and Epstein-Barr virus was negative. Ultrasound revealed a mildly echogenic liver. Further enquiry revealed that she had been on herbal medication (20 mg per day, active ingredient Gotu Kola) for acne that was bought over the Internet, over the preceding 6 weeks. Both lymecycline and the herbal medication were stopped. Tetracyclines are known to cause acute hepatitis and/or cholestasis, but usually within 4–6 days of starting the medication. A temporal association of herbal medication induced acute hepatitis with immediate improvement after cessation of medication was seen in this child. It was considered unlikely that lymecycline could have contributed to the liver injury due to the time frames involved and absence of cholestasis (Dantuluri 2011).

Itching and photosensitivity reactions with the appearance of redness and rashes after oral administration of the TECA (Centellase®, Summary of Product Characteristics, section 4.8).

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 (Umbeliferae) family (WHO monographs, 1999).

EudraVigilane database

A EudraVigilance search on *Centella*, *Centella asiatica*, *Centella asiatica* extract, *Centella asiatica* triterpenoid extract and *Hydrocotyle* revealed 23 report; out of these, 10 reports were found in subjects taking only *Centella asiatica*. Three cases were serious and patients experienced urticaria, eritema/hypersensitivity/skin burning/skin swelling and application site pain; however, these adverse reactions were not life-threatening, disabling and did not cause hospitalisation. All other adverse reactions were not serious and concerned mainly the skin.

5.4. Laboratory findings

Chronic treatment with TTFCA extract 30 mg twice daily for 3 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 per day for a period of treatment between 2 and 8 weeks improves filtration rate, PO_2, PCO_2, resting flux, permeability in CVI patients.

Thirty patients with diabetic microangiopathy were treated for 6 months with total triterpenic fraction of *Centella asiatica* (TTFCA) (60 mg twice daily), whilst a control group of 10 patients was treated with placebo and another group of 10 patients was left without treatment thus acting as a second control group. Fasting blood sugar levels were similar in the 3 groups at the beginning of the study. No significant variation of fasting blood sugar levels or glycosylated hemoglobin levels were observed during or after the study in the group treated with TTFCA or the control group. Blood tests indicating renal and hepatic functions and routine blood tests did not show any significant change after 3 and 6 months of treatment (Cesarone *et al*., 2001).

Seventeen subjects were treated with 1000 mg per day of *Centella asiatica* extract, 17 subjects treated with 750 mg per day of Gotu kola extract, and 14 subjects treated with 3 mg per day of folic acid for 6 weeks to determine the effectiveness of *Centella asiatica* in improving cognitive function in patients with vascular cognitive impairment (VCI). In all groups no significant differences were found in the levels of AST and ALT after the 6-week treatment period when compared with baseline levels of AST and ALT. The between-group analysis also did not show difference in AST and ALT changes among the 3 treatment groups (Farhana *et al.,* 2016).

5.5. Safety in special populations and situations

Acute hepatitis possibly related to the assumption of an herbal medication (active ingredient Gotu Kola) for acne was observed in a case-report on a 15-year-old girl (Dantuluri 2011).

5.5.1. Use in children and adolescents

No data available.

5.5.2. Contraindications

Hypersensitivity to plants of *Apiaceae* family (ESCOP 2009, WHO monograph 1999).

5.5.3. Special Warnings and precautions for use

None reported.

5.5.4. Drug interactions and other forms of interaction

None reported.

5.5.5. Fertility, pregnancy and lactation

Centella asiatica has been referred to be reputed abortifacient and to modify the menstrual cycle (Barnes *et al.,* 2007). *Centella asiatica* should be avoided during pregnancy, due to its emmenagogue action (Alternative Medicine Review 2007). Not be used during pregnancy without medical advice (ESCOP 2009).

There is no clinical evidence from literature on the antifertility effects of *Centella asiatica* in humans.

For cutaneous use, safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

No fertility data available on topical application of *Centella asiatica*.

5.5.6. Overdose

None reported.

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

No data available.

5.5.8. Safety in other special situations

No data available.

5.6. Overall conclusions on clinical safety

Overall, different herbal preparations of *Centella asiatica* alone or in combination with other herbs have been well tolerated in clinical studies, although no specific clinical safety studies have been carried out.

Gastrointestinal system disorders including abdominal pain, flatulence, nausea, and vomiting were the most frequent adverse events mainly associated to oral use experienced during clinical trials and recorded from post-marketing surveillance.

Topical application of *Centella asiatica* can result in allergic reactions although frequency cannot be established. Allergic contact dermatitis has been associated with topical application of *Centella asiatica*. However, these reactions may be due to other ingredients in the products.

Hypersensitivity to plants of Apiaceae (Umbeliferae) family is reported in published monographs of *Centella asiatica* (ESCOP 2009, WHO monograph 1999).

There is no clinical evidence from literature on the antifertility effects of *Centella asiatica* in humans. However, it has been used in folk medicine for dysmenorrhoea (WHO monograph 1999) and is reputed to be abortifacient and to modify the menstrual cycle (Barnes *et al.,* 2007). In addition, there is non-clinical evidence that ethanolic extracts of *Centella asiatica* could significantly impair fertility in rats also at HED lower than those traditionally used in humans topically for wound healing and orally for rheumatoid arthritis and cutaneous affections, based on the British Herbal Pharmacopoeia.

Signs of hepatotoxicity were seen in a non-clinical study on rats treated with oral HED of 4 g of dried, powdered aerial parts of *Centella asiatica*, whilst no evidence of hepatotoxicity could be drawn from clinical studies in humans. However, there are several case reports of hepatotoxicity cited in literature with the assumption of oral *Centella asiatica* preparations, but without a conclusive causal relationship.

Moderate renal toxicity was also observed in non-clinical studies on rats treated with oral HED of 4 g of dried, powdered aerial parts of *Centella asiatica*, but this evidence was not confirmed in humans.

In conclusion, oral use of *Centella asiatica* has not been reported in the HMPC monograph due to concerns on potential anti-fertility and hepatotoxic effects in humans, taking also into account that there are other effective and safe therapeutic options available. Both anti-fertility and hepatotoxic effects are not expected following topical application of *Centella asiatica* as an aid to healing of minor wounds based on clinical data available.

6. Overall conclusions (benefit-risk assessment)

Centella asiatica, also known as "Gotu kola" or "*Hydrocotile asiatica*", is a member of the Apiaceae family and it has been described since ancient times as having beneficial medicinal effects. The plant parts used for medicinal use are the dried leaves, aerial parts or the whole plant.

There are no marketed herbal medicinal products containing herbal preparations of *Centella asiatica* alone in EU, but the British Herbal Pharmacopoeia (1983) reports the use of the herb for the treatment of rheumatic conditions, cutaneous affections, and topically, for the healing of indolent wounds. The posology indicated was 0.6 g of dried herb or as infusion thrice daily.

Reproductive toxicity studies showed that ethanolic extracts of *Centella asiatica* had a detrimental effect on fertility of male and female rats treated with oral doses at HED doses even lower than 0.6–1.8 g reported in the monograph.

Signs of severe hepatotoxicity and moderate nephrotoxicity were also observed in rats treated with 500 mg/kg and 1000 mg/kg (corresponding to a HED of 4 g and 8 g, respectively) due to a decrease of viable cell counts and histopthological evidence of liver damage and change of renal tissue. Clinical case reports of hepatotoxicity following oral assumption of *Centella asiatica* have been reported in literature.

Therefore, the outcomes of above mentioned studies raise concerns on the safe use of *Centella asiatica* when taken orallyfor the therapeutic uses reported in the BHP. As a consequence, this traditional indication has not been included into the monograph considering that other effective and safe therapeutic options (including other herbal preparations) are available.

Differently for oral route, topical application of *Centella asiatica* herb can be regarded reasonably safe. Indeed, there are some shortcomings in the reproductive toxicity studies that limit the extrapolability of the results observed to humans, such as the limited number of rats for treatment/control groups and the lack of investigation of the potential for reversibility of the effect. In addition, these studies have been carried out using an ethanolic extract which is an herbal preparation not included in the monograph. Furthermore, hepatotocity and nephrotoxicity are expected to be associated with the oral use of *Centella asiatica* rather than with cutaneous use.

In summary, based on British Herbal Pharmacopoeia, there is evidence of traditional use according to the requirements set out by art. 16a of the Directive 2001/83/EC for comminuted or powdered *Centella asiatica* herb to aid in healing of minor wounds when topically applied on the affected area of the skin at doses of 0.6 g 3 times a day as a cutaneous powder or as an infusion preparation for cutaneous use (impregnated dressing).

Pre-clinical studies with *Centella asiatica* in wound healing support the plausibility of efficacy based on tradition. Clinical studies showed a trend effect of cutaneous applications of *Centella asiatica* in wound healing but the small sample size, some methodological deficiencies and the lack of full details on the herbal preparations used do not provide sufficient proof of well-established use.

Clinical studies with *Centella asiatica* have been carried out to treat different conditions, such as psoriasis, skin ageing, and anxiety disorders and to improve cognitive function but there are too many methodological issues to support a well-established use.

A duration of use of 1 week is established to be aligned with the HMPC monographs of other plants used topically in wound healing.

Centella asiatica should not be topically applied in case of known hypersensitivity to the active substance and to other plants of the Apiaceae (Umbeliferae) family.

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

Due to the absence of sufficient data, (the antiabortifacient properties and the emmenagogue effect reported in literature), the use of *Centella asiatica* during pregnancy and lactation is not recommended.

Centella asiatica water extract was not mutagenic in an Ames test (pre-incubation method) up to 5 mg/plate; only strains TA98 and TA100 were assayed and no information on positive control was provided, therefore further studies are needed to confirm the absence of mutagenicity.

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

Annex 1

List of references