30 May 2017
EMA/HMPC/464682/2016
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Vitis vinifera* L., folium

Final

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

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Vitis vinifera* L., folium
Herbal preparation(s)	a) Comminuted herbal substance (TU) b) Powdered herbal substance (TU) c) Soft extract (DER 2.5-4:1); extraction solvent water (TU) d) Dry extract (DER 4-6:1); extraction solvent water (WEU)
Pharmaceutical form(s)	Comminuted herbal substance as herbal tea for oral use (TU) Herbal preparation in solid dosage forms for oral use (WEU and TU) Herbal preparation in semi-solid dosage forms for cutaneous use (TU)
Rapporteur(s)	I. Chinou, G Garcia Lorente (first assessment)
Peer-reviewer	B. Kroes

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

Table of contents

1. Introduction

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

- Herbal substance(s)

The herbal substance *Vitis vinifera* L., folium, (Vitaceaea) consists of the dried leaves of the black to pulp-red grapevine and has a faintly perceptible odour. The herbal drug is harvested by hand in the autumn following the grape harvest. Drying takes place under natural conditions in accordance with local climatic conditions.

The crude herbal substance complies with the monograph "Vigne Rouge" French Pharmacopoeia (Pharmacopée Française, 10th ed., 1996).

Constituents

Grapevine leaves contain a wide range of polyphenol flavonoids including flavon(ol)-glycosides and glucuronides, quercetin-3-O-beta-D-glucuronide (most abundant of flavonoids), isoquercitrin, anthocyanins, oligomeric proanthocyanidins (Hmamouchi *et al*. 1997), catechin, epicatechin monomers and dimmers and gallic acid (Fig 1). The phytoalexin trans-resveratrol, another polyphenolic substance belonging to the stilbene group, can also be found in grapevine (Langcake *et al*. 1979). It has been referred in the international literature that $AlCl_3$ is a potent elicitor of resveratrol production in grapevine leaves (Andrian *et al*. 1996). In grapevine leaves, also organic acids appear, mainly malic and oxalic acid but also tartaric acid. Citric, fumaric and succinic acid can be detected in the leaves only in traces. Compared to the grapevine berries, grapevine leaves are richer in the content of carotenoids and vitamin C. According to the French Pharmacopoeia, the dried leaves of grapevine should contain at least 4% of total polyphenols and 0.2% of anthocyanins (Pharmacopée Française, 10th ed., 1996; Jonadet *et al* 1983; Lardos and Kreuter 2000; Laparra and Darné, 1989).

The leaves of the red varieties are very rich in tannins from the catechin group. The composition in tannins of leaves depends on the phase of development and on their position on the plant. In autumn, catechin, gallocatechin and epicatechingallate can be found in the leaves. From catechins and/or leukoanthocyanidines so called oligomeric proanthocyanidins, colourless substances, are formed. The greatest part of anthocyans consists of malvidin glucosides but also delphinidin, cyanidin and petunidin glucosides occur (Laparra *et al*. 1989). The highest content of anthocyans can be detected in the red leaf especially in autumn, in the time between the vintage and the shedding of leaves (Raynaud, 2005).

Overview of main polyphenolic secondary metabolites in grapevine leaf (Bruneton 1999; Bombardelli and Morrazzoni 1995).

Flavonoids	Flavones (Quercetin Kaempferol), Flavanes
Anthocyans	Leucoanthocyanidins, Anthocyanidins • responsible for the blue and red coloring of leaves, flowers and fruits • The concentration of anthocyans in the red colored leaf is high

Catechins	• Grapevine leaf is rich in cathechins • Concentration of cathechins is dependent on: o the leaf's position on the plant o the phase of development of the leaf • In autumn cathechin, gallocathechin, epicathechingallat are present in the leaf
Stilbenes	Resveratrol, *trans*-Resveratrol belong to the stilbenes (phenolic sub group) is a phytoalexin = stress-induced plant metabolite resveratrol can be found only in stressed leaves stress factors, i.e. fungal infection, UV-irritation, injury ->resveratrol is present in different forms depending on the stage of the plant's stress answer

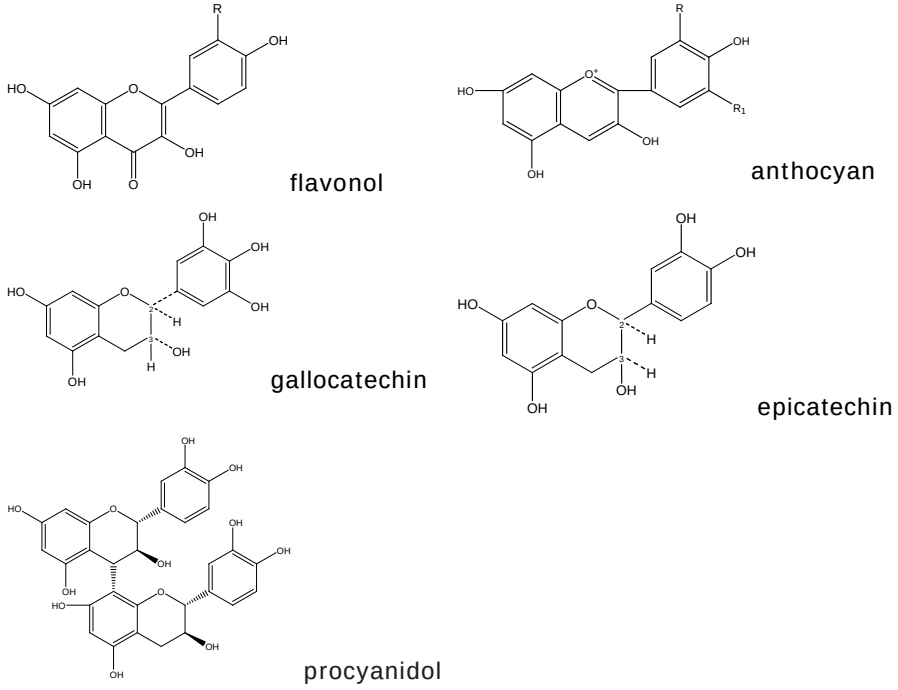

Figure 1. Chemical structures of the main polyphenolic secondary metabolites in grapevine leaf (Bruneton 1999; Bombardelli and Morrazzoni 1995)

- Herbal preparation(s)

a) Comminuted herbal substance

b) Powdered herbal substance

c) Soft extract (DER 2.5-4:1); extraction solvent water

d) Dry extract (DER 4-6:1); extraction solvent water

The powder is reddish-brown. Examined under the microscope, the powdered red grapevine leaf shows the following characteristics: more or less dense, unicellular, long, tapering, covering trichomes, thick-walled, with bulbous or truncated base and a lumen divided into loculi; numerous raphides of calcium oxalate are contained in these cells or scattered about; fragments of parenchyma containing twinned crystals of calcium oxalate; a few fragments of epidermis with polygonal cells and some reticulate

venation. Since the beginning of the last century in many studies the chemical constituents of the different parts of grapevine have been investigated. Fruit acids, tannins and pigments are the substances mainly responsible for taste, odour and colour (PDR for Herbal Medicines, 2004).

For the extract preparation (Extractum *Vitis viniferae* foliae aquosum siccum, 4-6:1), dried leaves of red varieties of *Vitis vinifera* L. (*Vitis viniferae* folium) which comply with the monograph described in Pharmacopée Française (10th edition) for "vigne rouge" are used. Thus, the herbal substance consists of the dried leaves of the black to pulp-red grape which finally undergo a specific production process resulting in defined flavonol content in the dry extract preparation. While the whole dry extract preparation as such is considered as active agent, it is particularly characterised by its content of flavonol glycosides and glucuronides, i.e. quercetin-3-O-β-D-glucuronide, quercetin-3-O-β-glucoside, and kaempferol-3-glucoside. These flavonoids are considered to contribute predominantly to pharmacological effects. One water extract of red vine leaf contains a total of 4-7% of flavonol glycosides, quantified as quercetin-3-O-β-D-glucuronide.

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

This assessment and the monograph refer exclusively to the use of *Vitis vinifera* folium as a single ingredient.

1.2. Search and assessment methodology

The assessment is based on the sources mentioned in the list of references. Publications in other languages than English (at least abstract in English or other language available) were also included in the assessment.

Scientific databases: Scifinder, Scopus; HealLink, Medline, PubMed, search date January–May 2016; Articles were filtered by using the following terms: pharmacological *in vitro* and *in vivo* studies key words: *Vitis vinifera* L., red vine leaf, grapevine leaf, vine leaf.

Only articles found to be relevant for assessment are included in the list of references.

Search engines used: Google, Google Scholar.

Medical databases: Pubmed key words: *Vitis viniferae* L., red vine leaf, grapevine leaf, vine leaf.

For the research, the databases of PubMed, ScienceDirect, Cochrane Database of Systematic Reviews and TOXLINE were used.

Reviews were searched in Cochrane Database of Systematic Reviews, typing in "*Vitis vinifera"*.

Data from EU and non-EU regulatory authorities: European Union Market overview.

Other resources: Library of the National Kapodistrian University of Athens (Pharmacy and Pharmacognosy library.

2. Data on medicinal use

2.1. Information about products on the market

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

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Posology** **Duration of use**	**Regulatory Status**
Dry extract of *Vitis vinifera*, folium (DER 4-6:1), extraction solvent water	Chronic venous insufficiency grade I and II; swelling of feet and lower leg	2 capsules once a day 1 capsule contains: 180 mg dry extract	Austria, since 2000, WEU
	Chronic venous insufficiency; at stage of C2, C3 and C4A according to the international classification system CEAP (Clinical Class, Etiology, Anatomy and Pathophysiology)	2 capsules once a day 1 capsule contains: 180 mg dry extract	Hungary, since 2005-2017 and Since 2009, WEU
Vitis viniferae rubrae folii extract spissum (DER 4-6:1)	For alleviating the discomfort feeling (fatigability, tension of the legs) of the lower extremities occurring in the course of the mild venous circulation disturbance	Oral drops Corresponding to 1.2 mg aesculin/ml 81.4 mg, not further data provided	Hungary (healing product), since 1997-2017
Vitis viniferae folii extractum aquosum siccum (DER 4–6:1)	Prevention and therapy of symptoms of light or starting moderate forms of chronic venous insufficiency related to varicose veins such as swelling of the calves, heavy legs, tingling Prevention and treatment of signs related to chronic venous insufficiency Prevention and treatment of signs related to chronic venous insufficiency, which is usually expressed in connection with varicoses and oedema in the lower parts of legs, fatigue, pins and needles and pain in legs	Capsules of 180 mg standardised to flavonoids content 3-7% 2 capsules (360 mg) in the morning, during first 3 weeks, increase of the dose to 4 capsules is possible to reach faster relief	Czech Republic, since 2001 WEU Latvia since 2004 WEU WEU Slovakia, since 2004 WEU Spain, since 2001

Active substance	**Indication**	**Pharmaceutical form** **Posology** **Duration of use**	**Regulatory Status**
Powdered dried leaf, corresponding to 8% total polyphenols and minimum 0.20% of anthocyanosides	Herbal medicine for the treatment of chronic venous insufficiency characterised by varicose veins, oedema of the calves, heavy or tired legs, cramps in the lower legs, the feeling of tension, tingling and pain	Capsules Oral use For adults: 1 to 2 capsules 3 times daily, during the meals with a large glass of water Maximum daily dose 6 capsules Duration up to 3 months	WEU Belgium, since 2005
Soft aqueous extract (6:1)	Un-infectious conjunctival irritations	Eye-drops: 1 drop 2 to 8 times daily. 1 g of extract/100 ml	France, since 1970
Powder of dried leaf	Traditionally used in the symptomatic treatment of functional disorders of cutaneous capillary fragility, such as ecchymosis, petechias, etc. Traditionally used:, in subjective signs of venous insufficiency, such as heavy legs In haemorrhoidal symptoms	1 hard capsule 3 times daily (5 if necessary) 350 mg of powdered drug/capsule Oral use, in adults	France TU, since 1982
Powdered herbal substance	Traditionally used in the symptomatic treatment of functional disorders of cutaneous capillary fragility, such as ecchymosis, petechias, etc. Traditionally used:, in subjective signs of venous insufficiency, such as heavy legs In haemorrhoidal symptoms	270 mg/capsule 3-5 times daily	Spain TU, since 1982
Dry aqueous extract DER (3:1 and 6:1)	Traditionally used in the symptomatic treatment of functional disorders of cutaneous capillary fragility, such as ecchymosis, petechias, etc. Traditionally used:, in subjective signs of venous insufficiency, such	1 hard capsule 2 times daily, 200 mg of extract per capsules 1 hard capsule 1 to 3 times daily containing 169 mg of extract per caps Oral use, in adults	France TU, since 1990

Active substance	Indication	Pharmaceutical form Posology Duration of use	Regulatory Status
	as heavy legs In haemorrhoidal symptoms in combination		
Soft extract (2.5-4:1), extraction solvent: water	Traditionally used to relieve symptoms of tired legs	Cream for cutaneous use in adults 10 g cream contain 282 mg soft extract	Germany TU, since 1976
Soft extract (4-6:1), extraction solvent: water	For symptomatic treatment of chronic venous insufficiency, which is characterised by swollen legs, a feeling of heaviness, pain, itching, cramps in the calves at night	Oral liquid 1 time daily 6 ml liquid If necessary 1 times daily 12 ml liquid 10 ml (= 11.013 g) liquid contain 0.6 g soft extract	Germany WEU, since 1976
Dry extract (4-6:1), extraction solvent: water	For symptomatic treatment of chronic venous insufficiency, which is characterised by swollen legs, a feeling of heaviness, pain, itching, cramps in the calves at night	Film-coated tablet for oral use in adults. 1-2 tablets x 360 mg (max daily dose 720 mg)	Germany WEU, since 2005
Dry extract (4-6:1), extraction solvent: water	For symptomatic treatment of chronic venous insufficiency, which is characterised by swollen legs, a feeling of heaviness, pain, itching, cramps in the calves at night	Hard capsules. 1 capsule contains 180 mg dry extract. For oral use in adults 2 capsules 1 time daily, if necessary 4 capsules Daily dose 360-720mg	Germany WEU, since 1976
(DER 4-6:1), extraction solvent water	Chronic venous insufficiency grade I and II; swelling of feet and lower leg	Film coated tablet 1 tablet contains: 360 mg dry extract 1 tablet daily (up to 2 tablets) maximum 3 months	Austria WEU, since 2005

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

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

Not applicable

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

Not applicable

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

Not applicable

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

The knowledge of the medicinal potential of grapevine leaf can be traced back far in history:

- In Europe, the leaves of *Vitis vinifera* are documented in the literature of traditional medicines for their astringent and homeostatic properties where they are utilised in the treatment of diarrhoea, bleeding, haemorrhoids, varicose veins and other circulatory and venous diseases (Anonymous 2004; 2005; 2006; Bombardelli and Morazzoni 1995).
- In Turkish folk medicine, grapevine leaves are known to have a diuretic effect, while the juice of leaves has been used as an eye bath (Kosar *et al*. 2007).
- Native North American indigenous peoples used the leaf tea of related fox grape (*Vitis labrusca* L.) for treating diarrhoea, as well as for hepatitis, stomach aches and thrush and externally poulticed the wilted leaves for sore breasts, rheumatism, headaches and fevers. Other closely related Vitis species have been used similarly. (Anonymous 2004; 2005; 2006).
- In Indian Medicine: Grape is used for headache, dysuria, scabies, skin disease, gonorrhoea, haemorrhoids and vomiting (PDR for herbal Medicines 2004).

Since ancient times, beneficial effects on health have been ascribed to wine and grapevine leaves, as confirmed by numerous "recipes" reported in Egyptian papyri, the Sumerian tablets, the writings of Hippocrates of Cos (5^{th}-4^{th} century BC), Celsus (1^{st} century AD), Galen (130-201 AD) and Paracelsus (1493-1541). Retracing therapeutic literature from France showed the origin of using red leaves shortly after first incidence of the respective grapevine varieties called "teinturiers" (Schneider, 2007i). The herbal substance and herbal preparations have a long history of use in folk medicine where French winegrowers collected the red grapevine leaves at the time of the grape harvest to make infusions and paste-like poultices from them. The infusions were filled into bottles and regularly ingested in small quantities. The paste from grapevine leaves was used for the treatment of swollen, painful legs. Nowadays, the extracts of grapevine leaves were developed into herbal medicinal products mainly used against venous diseases and for its effects on microcirculation (Rabe *et al*. 2005; Volonté and Petrini, 2004; Volonté *et al*. 2003). The grapevine, fresh and brined or fermented, are used as food (mixtures of rice and spices with or without meat are wrapped with vine leaves) and have been widely consumed as traditional foods around the Mediterranean countries (Kosar *et al*. 2007). They are also used for diarrhoea, vomiting and varicose treatment (Gharib Naseri and Heidari 2006).

There is evidence of the use of grapevine leaf outside France, in Italy in 1957, where the Biosedra preparation was also tested in vascular disorders in gynaecology, producing a positive effect on capillary fragility (Schneider, 2007i).

As early as 1960, a clinical study was published in Germany on the venous efficacy of a product, which contained a fluid extract of grapevine leaf, with a drug/extract ratio of 1:1. The content of anthocyanin was set at 600 µg/ml, which was subsequently changed to 1.2 mg bioflavonoids per ml (German Rote Liste 1974). In 1969, a company registered the preparation as a medicinal product, with the indications of varices, phlebitis, thrombophlebitis, calf cramps and leg oedema. The successor product,

has been marketed since 1971. The capsules and tablets contain 180 mg and 360 mg of dried extract of grapevine leaves.

An overview of grapevine leaf components and its supposed pharmacological action as well as the polyphenol composition of *Vitis vinifera* can be found in the literature (Anonymous 2004; 2005; Petrini *et al*. 2003; Schneider, 2007i; 2007ii; Schneider *et al*. 2008; Schaefer *et al.* 2003).

Grapevine leaves and extracts thereof have been traditionally used for the treatment of symptoms associated with venous insufficiency for more than 70 years, in France. Extracts have been introduced in a number of European countries, e.g. Austria, Belgium, Czech Republic, Spain, Switzerland, Italy, United Kingdom etc. It is indicated for the prevention and treatment of chronic venous insufficiency (CVI), associated with varicose veins including oedema of the lower leg, heavy or tired legs, sensation of tension, tingling and pain.

Red grapevine leaf has been included in the French official list entitled “Avis aux fabricants concernant les demandes d’autorisation de mise sur le marché” by virtue of which, the products containing it were subjected to a simplified registration procedure before the implementation of the Directive 2004/24/EC.

In France: "Vigne Rouge" (= red grapevine leaf) is regarded as one of the herbal medicinal products whose efficacy and safety has been proven by thorough literature studies and long-term traditional use. The Pharmacopée Française 10th edition includes the monographs of "Vigne rouge" and "Extrait de vigne rouge (sec)" and the traditional use of red grapevine leaves is discussed in "Rotes Weinlaub" (Schneider, 2007i; 2007ii).

Herbal medicinal products with red grapevine leaf have a long tradition in France. The official compendium "Médicaments à base de plantes", issued by the French department "Ministère des Affaires sociales et de la solidarité", released and comprised the long tradition of diverse herbals in France in 1990. In this Official French list, there are cited many herbals that have been used in France for a long time (at least for about 15–20 years). Therefore, the use of red grapevine leaf for symptomatic relief of chronic venous disease has been stated in Les Cahiers de l'Agence 1998 state ("No 015+016: "Traditionnellement utilisée dans le traitement symptomatique des troubles fonctionnels de la fragilité capillaire cutanée, tels que ecchymoses, pétéchies, etc."; No. 017+018: "Traditionnellement utilisée: - dans les manifestations subjectives de l'insuffisance veineuse telles que jambes lourdes; - dans la symptomatologie hémorroïdaire").

Different products prepared of water extracts of "Vigne Rouge" (3:1; 4:1; 5:1) have been available in oral forms in France to treat blood vessels fragility in order to reduce the feeling of heavy legs and haemorrhoids related disorders. The posology range from 169 mg to 200 mg, two to three times a day, for a max period of 4 weeks.

An article from 'Deutsches Medizinisches Journal' published in 1960 by K. Güthenke, "Wesen und Behandlung der Bindegewebsschwäche, insbesondere der Veneninsuffizienz mit Weinblattextrakt" (Nature and Treatment of weak connective tissues, in particular venous insufficiency, ref.) describes the long traditional use of grapevine leaves for treatment of diseases of the leg veins, and demonstrates that vine leaf extracts have been already traditionally used before 1960, in Germany.

Table 2: Overview of historical data

Herbal preparation	**Documented use / Traditional use**	**Pharmaceutical form**	**Reference**
A solution for oral use contained 1-2	Venous insufficiency	Oral use has been available. 20 ml contained	German Rote Liste

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
g of extract from leaves of *Vitis vinifera* (1:4-5)		1-2 g of extract from leaves of *Vitis vinifera* (1:4-5)	1974 Kosar *et al*. 2007, Rabe *et al*. 2005; Volonté and Petrini, 2004 Volonté *et al*. 2003, Schneider, 2007i, PDR for herbal Medicines 2004
Grapevine leaf extracts	"Wesen und Behandlung der Bindegewebsschwäche, insbesondere der Veneninsuffizienz mit Weinblattextrakt" (Nature and Treatment of weak connective tissues, in particular venous insufficiency, ref.) traditional use of grapevine leaves for treatment of diseases of the leg veins, and demonstrates that have been already	Different pharmaceutical forms for oral and external uses	'Deutsches Medizinisches Journal' published in 1960 by K. Güthenke
A red grapevine leaf water extract preparation	Vascular disorders in gynaecology, producing a positive effect on capillary fragility	Not confirmed and specified posology from old products	Italy 1957 (Schneider, 2007i)
Vigne rouge, capsules: 1 capsule contains 200 mg of grapevine leaves dry aqueous extract is used, a DER of about (4-6:1)	Traditionally used to reduce fine blood vessels fragility, in order to reduce the feeling of heavy legs and haemorrhoids related disorders symptomatic relief of chronic venous disease	The product is registered since 1990. The manufacturer recommends the use of 2 capsules per day (1 capsule in the morning and 1 in the evening, with a glass of water)	(Les Cahiers de l'Agence 1998) for ("No 015+016: " Traditionnellement utilisée dans le traitement symptomatique des troubles fonctionnels de la fragilité capillaire cutanée, tels que ecchymoses, pétéchies, etc."; No. 017+018"
Dried leaves in herbal teas	Traditional use to reduce fine blood vessels fragility, and the feeling of heavy	40 g/l One cup (10 g/250 ml) 2-4 times per day	Les Cahiers de l'Agence 1998

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
	legs and for relief of mild venous disease		Duke JA. 2002 PDR 2004 ESCOP 2009
Vigne rouge, capsules: 1 capsule contains 169 mg of grapevine leaves dry aqueous extract is used, a DER of about (3:1)	Traditionally used to reduce fine blood vessels fragility, in order to reduce the feeling of heavy legs and haemorrhoids related disorders symptomatic relief of chronic venous disease	Single dose 169 mg Daily dose 3 x 169 daily	Registered product since 1990. Les Cahiers de l'Agence 1998) for ("No 015+016: " Traditionnellement utilisée dans le traitement symptomatique des troubles fonctionnels de la fragilité capillaire cutanée, tels que ecchymoses, pétéchies, etc."; No. 017+018"
Comminuted herbal substance (Vitis dried leaf)	Herbal medicinal product to relieve problems related to varicosis as painful and heavy legs Medicinal product for symptomatic treatment of cutaneous capillary fragility Medicinal product for symptomatic relief of itching and burning associated with haemorrhoids	Comminuted herbal substance as herbal tea 5-10 g/250 ml, 2 times daily	Van Helemont 1986, Valnet 1992 ESCOP 2009 (Les Cahiers de l'Agence 1998) for ("No 015+016: " Traditionnellement utilisée dans le traitement symptomatique des troubles fonctionnels de la fragilité capillaire cutanée, tels que ecchymoses, pétéchies, etc."; No. 017+018

2.3. Overall conclusions on medicinal use

Information obtained from member states and data retrieved from handbooks confirm the medical use of red grapevine leaves in the European Union.

Registrations for the active ingredient *Extractum vitis viniferae foliae aquosum siccum* exist since 1969. Since 1974 a solution for oral use has been available. 20 ml contained 1-2 g of extract from leaves of *Vitis vinifera* (1:4-5) and 0.02 g of aesculin. In 1999 aesculin was removed from the

formulation. The recommended dosage is 2-3 times 30 drops/day. The product is traditionally used to treat diseases of the leg veins (e.g. varicose veins, chronic venous insufficiency), to reduce pains and feeling of heavy legs, night time cramps in the calf, itching and swollen legs. The use of this product can be proven for over 30 years.

In 1991 the galenic form of hard gelatine capsules was registered and marketed for the first time. One capsule contained a combination of 180 mg of *Extractum vitis viniferae foliae aquosum siccum* and 3 mg aesculin (a coumarine glucoside that naturally occurs in the horse chestnut *Aesculus hippocastanum*). This herbal medicinal product was indicated for the treatment of venous insufficiency, varices, haemorrhoids, heavy tired legs and feet, essential oedema and calf cramps. The daily posology was 2-3 capsules a day. In 1999, aesculin was removed from the product and the only active substance declared was the dry extract of grapevine leaves. The capsules contain 180 mg of *Extractum vitis viniferae* (extraction solvent: water, DER: 4–6:1), with a recommended use of 2-4 capsules/day.

A cream (ointment), containing soft extract (4-6:1; water) in a cream base (10 g cream contain 282 mg soft extract), has been traditionally used since 1976 to relieve symptoms of tired legs applying it several times daily.

Based on information obtained from Member states and data retrieved from handbooks it can be concluded that the following extracts of grapevine leaf extracts have been on the European market for a period of at least 30 years fulfil the criteria for traditional use in accordance with Directive 2004/24/EC and are proposed for the monograph on traditional use (see table 3):

- Comminuted herbal substance
- Powdered herbal substance
- Soft extract (DER 2.5-4:1); extraction solvent water in a cream base

Concerning the preparation: dry extract (3:1; water), the product has been on the market since 1990 (France) and does not comply with the requirements of Directive 2004/24/EC for THMP. There is no complete information on the manufacturing process and DER of this preparation, and it is not possible to evaluate the similarity with other dry extracts under WEU.

The following herbal preparation is on the European market for a period of at least 10 years and was proposed for the monograph on well establish use (see table 3 and section 4 ‘Clinical data’):

WEU herbal medicinal product:

- Dry extract (4-6:1; water)

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	**Indication**	**Posology, Strength**	**Period of medicinal use**
Herbal tea from the comminuted herbal substance	Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances Traditional herbal medicinal product for symptomatic relief of itching and burning associated with haemorrhoids	Herbal tea: 5-10 g of dried leaves in 250 ml of boiling water as herbal infusion, 2 times daily	France since 1970 Van Helemont 1986 Les Cahiers de l'Agence 1998

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
	after serious conditions have been excluded by a medical doctor Traditional herbal medicinal product for symptomatic treatment of cutaneous capillary fragility		Duke JA. 2002 PDR 2004 ESCOP 2009
Powdered herbal substance	Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances Herbal medicinal product for symptomatic treatment of cutaneous capillary fragility Traditional herbal medicinal product for symptomatic relief of itching and burning associated with haemorrhoids after serious conditions have been excluded by a medical doctor	270-350 mg, 3-5 times per day	Since 1982, France, Spain
Soft extract (DER 2.5-4:1); extraction solvent water	Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances	In a cream base, (10g cream contain 282 mg soft extract), a thin layer should be spread on the affected area 1-3 times per day	Since at least 1976 Germany
Dry extract (DER 4-6:1); extraction solvent water	Herbal medicinal product for treatment of chronic venous insufficiency, which is characterised by swollen legs, varicose veins, a feeling of heaviness, pain, tiredness, itching, tension and cramps in the calves.	Single dose: 360-720 mg Daily dose: 360-720 mg	Since 2003 WEU Austria Since 2005 WEU Germany

Indications 2)

For safety reasons, as preliminary haemorrhoids symptoms could be associated with other serious medical conditions and in accordance with recent monographs sharing the same indication, the phrase “after serious conditions have been excluded by a medical doctor” has been added to the indication “Traditional herbal medicinal product for symptomatic relief of itching and burning associated with haemorrhoids”.

Duration of use TU

Indication 1)

The recommended duration of use is 4 weeks.

If the symptoms persist for more than 2 weeks during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

Indications 2) and 3)

If the symptoms persist for more than 1 week during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

Duration of use WEU

The recommended duration of use is 12 weeks.

2 to 3 weeks of treatment may be required before beneficial effects are observed.

Long term use is possible in consultation with a doctor.

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

Non-clinical *in vitro* and *in vivo* studies suggested protective effects of components from extracts of grapevine leaves on the venous system; e.g. procyanidines (Maffei *et al*. 1994; Constantini *et al*. 1999), and flavonoids (Nees *et al*. 2003i).

The phenolic acids present in grapevine leaves are mainly derivatives of cinnamic acid, vanillic acid and caffeic acid (Boucheny and Brum-Bousquet 1990). These compounds are ubiquitous in vegetables and preparations of herbal origin and therefore not expected to contribute to the specific pharmacological effects of grapevine leaf extract.

Anti-inflammatory and anti-oedematous effect

In an *in vitro* study, venular endothelial cells were isolated from Wistar rats and cultivated on porous filters to confluent monolayers. These preparations respond to certain release products from simultaneously activated blood platelets and polymorphonuclear granulocytes (PMN) with a rise in hydraulic conductivity that, *in-situ*, would lead rapidly to local oedema, arteriolar constriction and venular thrombosis. In this model, selectively activated PMN alone induced only a modest increase in endothelial hydraulic conductivity that could be prevented by uric acid, an antioxidant. ASA prevented the activation of the blood cells. A standardised water extract from grapevine leaves (4-6:1), containing in particular the flavonoids quercetin-3-O-ß-D-glucuronide and isoquercitrin (quercetin-3O-ß-D-glucoside), not only prevented the deleterious effect of the release products on the venular endothelial monolayers but, applied promptly to an endothehium damaged by prior exposure to these release products, resulted in the repair of the endothehium (Nees *et al*. 2003i).

In another study, the scavenge by procyanidines (polyphenol oligomers from *Vitis vinifera* seeds CAS 85594-37-2) of reactive oxygen species (ROS) involved in the onset and the maintenance af microvascular injury has been studied in phosphatidylcholine liposomes (PCL) using two different models of free radical generation. In an iron-promoted (Fenton-driven) model, procyanidines had a remarkable, dose-dependent antilipoperoxidant activity (IC_{50} = 2.5 µmol/L), more than one order of

magnitude greater than that of the monomeric unit catechin (IC_{50} = 50 µmol/L). In the second model, procyanidines were effective in preventing conjugated diene formation in both the induction (IC_{50} = 0.1 µmol/L) and propagation (IC_{50} = 0.05 µmol/L) phases. The scavenging effect of tocopherol was weaker with IC_{50} of 1.5 and 1.25 µmol/L (Maffei Facino *et al*. 1994; Wollina *et al*. 2006).

The grapevine leaf extract is characterised by its content in flavonol glycosides and glucuronides, in particular quercetin-3-O-β-D-glucuronide, isoquercitrin (quercetin-3-O-β-glucoside), and kaempferol-3-O-glucoside. The most informative investigations were performed using confluent venular endothelial cells from animal and from human origin (Nees, 2003i). An attack by release products from simultaneously activated blood platelets (P) and polymorphonuclear granulocytes (PMN) leads to a breakdown of the venular endothelial barrier. Clinically this would result in formation of oedema and constriction of microvessels. Red vine leaf extract (RVLE) was able to support the repair of the venular barrier after an attack by said release products. These effects could be demonstrated on cells of animal origin as well as on cells isolated from the human heart (Nees *et al*. 2003i, 2003ii). Quercetin-3-O-ß-D-glucuronide that had been isolated from the extract and was shown to be the major metabolite being present in human plasma after ingestion of RVLE acted in the same way. The extent to which cellular gaps opened and allowed the supernatant to flow through could be measured as “hydraulic conductivity”. 0.7 mg dry Red vine leaf extract /ml incubation medium prevented the opening of the barrier in the presence of activated PMN/P. Preincubation of the venular cell layer with 50 µM quercetin-3-O-ß-D-glucuronide for 7 days markedly reduced the hydraulic conductivity compared to untreated cells.

In a publication by Jonadet *et al*. (1983), the authors describe studies conducted with anthocyanosides extracted from *Vitis vinifera* (a), *Vaccinium myrtillus* (b) and *Pinus maritimus* (c). The results obtained *in vitro* indicated that these compounds inhibit elastase, a proteolytic enzyme which plays a role in the deterioration of conjunctive tissue and elastic fibers and is involved in certain pathological vascular conditions. The IC_{50} values are 0.13 mg/ml for (a), 0.20 mg/ml for (b) and 0.31 for (c). Lineweaver-Burk curves revealed that the inhibition was not competitive. Results obtained *in vivo* show that the angioprotective activities of these compounds can be classified in decreasing order as follows: (a), (b) and (c).

Nees developed a measurable and reproducible *in vitro* experimental model to investigate the effect of substances capable of modifying the hydraulic conductivity of the endothelial barrier of the venules. In *in vitro* experiments, this extract has been shown to have a "sealing" effect on the endothelium of the venules and a protective action against fluid extravasation induced by incubation with chemical mediators of inflammation (Nees *et al*. 2003i).

Red vine leaf extract (RVLE) prevented the deleterious effect of the release products of P and PMN on venular endothelial cells. In addition, the extract was able to support the repair of the venular barrier after an attack by said release products (Smith, 1999).

The anti-inflammatory activity of the oligomeric stilbene a-viniferin from redvine leaf has been also tested as well as its mode of action though inhibition of cyclooxygenase-2 and inducible nitric oxide synthase (Chung *et al*. 2003).

Vasorelaxant effect on isolated rat aorta

The relaxant effect of *Vitis vinifera* leaf hydroalcoholic extract (VLHE) on isolated rat thoracic aorta contractions induced by phenylephrine and KCl, and the role of aorta endothelium on this action has been investigated. Rat aorta was removed and placed in an organ bath containing Krebs-Henseleit solution and aorta contractions were recorded isometrically. VLHE at 0.125, 0.25, 0.5, 1 and 2 mg/ml reduced the endothelial intact aorta contracted by phenylephrine (1 µM) significantly and dose-dependently. Endothelial denuded aorta showed the same relaxation but in much less extent. The IC_{50}

of these two groups were 0.454±0.08 and 1.73±0.23 mg/ml respectively. VLHE also reduced the aorta contractions induced by KCl (80 mM). The relaxatory effects of VHLE on KCl-induced contractions were less than those evoked by phenylephrine. Soluble guanylate cyclase inhibitor (methylene blue, 10 µM) and nitric oxide synthase inhibitor (L-NAME, 100 µM) reduced the VHLE-induced relaxation in the intact aorta significantly, but atropine (1 µM) was unable to decrease this vasorelaxant effect. These results suggest that the most vasorelaxant effect of VHLE on rat aorta is endothelium-dependent and also nitric oxide (NO) and cGMP are involved in this action (Gharib Naseri *et al.* 2004).

Spasmolytic effect

The effect of grape leaf hydroalcoholic extract (GLHE) on rat colon contractions induced by some spasmogens has been investigated. A piece of distal colon from male adult Wistar rats was dissected and mounted in an organ bath containing Tyrode solution and colon contractions recorded by an isotonic transducer under 1 g resting tension. The GLHE (0.5- 4 mg/ml) reduced the contractions induced by KCl (60 mM), $BaCl_2$ (4 mM), acetylcholine (1 µM) dose-dependently ($P<0.001$). The spasmolytic effect of GLHE on ACh-induced contraction was unaffected by propranolol (1 µM), phentolamine (1 µM), L-NAME (300 µM), and naloxone (1 µM). In Ca^{2+}-free but rich in KCl (120 mM) Tyrode solution, cumulative concentrations of $CaCl_2$ induced colon contractions, which were inhibited by the extract. Glibenclamide (3 µM) had no effect on the extract spasmolytic activity, but tetraethylammonium (5 mM) contracted the pre-relaxed colon induced by the extract. Results suggest that the grape leaf hydroalcoholic extract spasmolytic effect is due to the blockade of the voltage dependent calcium channels and activation of Ca^{2+}-operated potassium channels (Gharib Naseri and Heidari 2006i).

The effect of *Vitis vinifera* leaf hydroalcoholic extract (VLHE) on isolated rat tracheal contractions induced by KCl and acetylcholine has been also studied. The trachea was removed from male adult Sprague Dalwey rat and placed in an organ bath containing Krebs-Henseleit solution. The tracheal contractions were recorded isometrically under 1.5 g initial tension. The results demonstrate that the VLHE at 0.5, 1, 2, 4 and 8 mg/ml reduces the tracheal contractions induced by KCl (60 mM) significantly and dose-dependently ($P<0.0001$). Acetylcholine (55 µM) - induced tracheal contractions were also attenuated by the same extract doses significantly ($P<0.0001$). The VHLE-induced relaxation in the KCl-induced contraction in trachea was not affected neither by nitric oxide synthase inhibitor (L-NAME, 100 µM) or beta-adrenoceptor antagonist (propranolol 1 µM) and by muscarinic receptors antagonist (atropine 30 µM). These results suggested that the relaxant effect of VHLE on rat trachea is evoked via voltage dependent calcium channel blockage and beta-adrenoceptors, NO and cholinergic receptors are not involved in this relaxant effect of VHLE (Gharib Naseri and Heidari, 2006ii).

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

Herbal preparation tested	**Posology**	**Experimental model**	**Reference**	**Main non-clinical conclusions**
A standardised water extract from grapevine leaves (4-6:1) and isolated flavonol glycosides and glucuronides (quercetin-3-O-β-D-glucuronide, isoquercitrin (quercetin-3-O-β-	Different doses of extracts and isolated flavonoids Active dose of 50 µM quercetin-3-O-ß-D-glucuronide for 7 days	*In vitro* study in venular endothelial cells were isolated from Wistar rats	(Nees *et al*. 2003i; Smith, 1999)	Anti-inflammatory and anti-oedematous effect

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
glucoside), and kaempferol-3-O-glucoside)				
The stilbene a-viniferin from red vine leaf has been tested (*Vitis vinifera*)	Many Different doses have been assayed	*In vitro* study	(Chung *et al*. 2003)	The anti-inflammatory activity of the oligomeric stilbene a-viniferin and its mode of action though inhibition of cyclooxygenase-2 and inducible nitric oxide synthase was shown
A standardized water extract from grapevine leaves (4-6:1)	IC_{50} = 2.5 µmol/l in comparison with unit catechin (IC_{50} = 50 µmol/l) and tocopherol was weaker with IC_{50} of 1.5 and 1.25 µmol/l	An iron-promoted (Fenton-driven) model*, in vitro* study	(Maffei Facino *et al*. 1994; Wollina *et al*. 2006)	Procyanidines showed a dose-dependent antilipoperoxidant activity (IC_{50} = 2.5 µmol/l), higher than catechins and tocopherol
Quercetin-3-O-ß-D-glucuronide isolated from Vitis extract and was shown to be the major metabolite being present in human plasma after ingestion of RVLE	0.7 mg dry RVLE/ml incubation medium with 50 µM quercetin-3-O-ß-D-glucuronide for 7 days markedly reduced the hydraulic conductivity compared to untreated cells nearly for 7 days	*In vitro* study	(Nees *et al*. 2003i, 2003ii)	Anti-oedematous effect
Anthocyanosides extracted from *Vitis vinifera* (a), compared with *Vaccinium myrtillus* (b) and *Pinus maritimus* (c)	The IC_{50} values 0.13 mg/ml for (a), 0.20 mg/ml for (b) and 0.31 for (c)	*In vitro* study *In vivo* study	(Jonadet *et al*. 1983),	*In vitro* inhibition of elastase and elastic fibres involved in certain pathological vascular conditions The *in vivo* results showed angioprotective activities of the tested compounds can be classified in decreasing order as follows: (a), (b) and

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
				(c)
Vitis vinifera leaf hydroalcoholic extract (VLHE)	The IC_{50} of these two groups were 0.454±0.08 and 1.73±0.23 mg/ml respectively. VLHE also reduced the aorta contractions induced by KCl (80 mM)	*In vitro* study on isolated rat thoracic aorta contractions induced by phenylephrine and KCl, and the role of aorta endothelium on this action has been investigated	(Gharib Naseri *et al*. 2004)	Spasmolytic and vasorelaxant effect. The results demonstrate that the VLHE at 0.125, 0.25, 0.5, 1 and 2 mg/ml reduces the endothelial intact aorta contracted by phenylephrine (1 µM) significantly and dose-dependently
Grapevine leaf hydroalcoholic extract (GLHE)	The GLHE (0.5- 4 mg/ml) reduced the contractions induced by KCl (60 mM), BaCl2 (4 mM), acetylcholine (1 µM) dose-dependently (P<0.001)	*In vitro* study on rat colon (from male and tracheal adult Wistar rats)	(Gharib Naseri *et al*. 2006ii)	The spasmolytic effect of GLHE which, proved to be due to the blockade of the voltage dependent calcium channels and activation of Ca2± operated potassium channels

3.1.2. Secondary pharmacodynamics

Flavonoid-containing herbal preparations and isolated flavonoids have been reported to exhibit a wide range of biological effects, including antioxidant and enzyme-modulating actions and anti-allergic, anti-atherosclerotic, antithrombotic, antiviral, antibacterial, anti-inflammatory, antiproliferative, anticarcinogenic, antispasmodic and diuretic effects (Middleton *et al*. 1992; Middleton, 1996; Hertog and Hollmann 1996; Hollmann and Katan 1999; Pietta, 2000).

Platelet aggregation inhibition

In the context with chronic venous insufficiency (CVI) it would be of interest to evaluate whether grapevine leaf extract exhibits any effects on platelet aggregation. The effect of flavonoids on platelet aggregation was studied *in vitro* using platelet-rich plasma from four healthy male volunteers aged 24, 29, 35 and 47 years. The flavonol glycoside quercetin 3-O-β-D-glucoside and various flavonoid aglycones including quercetin, apigenin and (+)-catechin were added to both platelet-rich plasma and washed platelets at concentrations of 0, 0.25, 2.5, 25, 250 and 2500 µmol/l. Indomethacin was used as a positive control. The flavonoid aglycones inhibited platelet aggregation but that quercetin-3-O-β-D-glucoside did not significantly affect aggregation at any of the concentrations tested (Janssen *et al*. 1998).

The catechins found in grapevine leaves are mainly catechol and epicatechol (Boucheny and Brum-Bousquet 1990). Of the condensed catechins (proanthocyanidins) procyanidins B1 and B2 are the prevailing compounds, followed by procyanidins B3 and B4. Procyanidins have been reported to possess antioxidant and anti-oedematous activity (Bombardelli and Morrazoni 1995). The study did not investigate the form in which the flavonoids were present prior to administration. Onions are known to be rich in quercetin glucosides, especially quercetin-4´-glucoside and quercetin-3, 4´-O-diglucoside. After administration of fried onions quercetin glucuronides can be detected in plasma (Aziz *et al*. 1998; Graefe *et al*. 2001). It can be supposed that quercetin glucuronides do not influence platelet aggregation *in vivo*.

Hepatoprotective activity

The hepatoprotective effect of ethanolic extract and its four different fractions ($CHCl_3$, EtOAc, n-BuOH, and remaining water fraction) of *Vitis vinifera* L. leaves was investigated against carbon tetrachloride (CCl_4)-induced acute hepatotoxicity in rats. The ethanolic extract was found active at 125 mg/kg dose (per os). The ethanolic extract was fractionated through successive solvent-solvent extractions and the n-BuOH fraction in 83 mg/kg dose possessed remarkable antioxidant and hepatoprotective activities. Liver damage was assessed by using biochemical parameters (plasma and liver tissue MDA (malondialdehyde), transaminase enzyme levels in plasma (AST-aspartate transaminase, ALT-alanine transferase) and liver GSH (glutathione) levels). Additionally, the pathological changes in liver were evaluated by histopathological studies. A *silybum* registered extract was used as standard natural originated drug (Orhan *et al*. 2007).

Antimicrobial activity

Ethanol extracts of *Vitis vinifera* (leaves, raw fruits, young branches; 2:1:1, V/V/V), were investigated for their antimicrobial activity against 14 pathogenic bacterial species and a yeast, *Candida albicans*, using the agar well diffusion method, and 19 Turkish traditionally used medicinal plants. *Vitis* leaves showed broad-spectrum antimicrobial activity (Oskay and Sari 2007).

Antioxidative activity

The effect of grapevine leaf extract (GLEt) (extraction solvent not declared) on antioxidant and lipid peroxidation states in liver and kidney alcohol induced toxicity were evaluated. *In vitro* studies with DPPH and ABTS (cation radical) showed that GLEt possesses antioxidant activity. *In vivo* administration of ethanol (7.9 g/kg bw per day) for 45 days resulted an activity of liver marker enzymes (AST, ALT, ALP and GGT), lipid peroxidation markers (TBARS, lipid hydroperoxides) in liver and kidney with significantly lower activity of SOD, CAT, GPx, GST and non-enzymatic antioxidants (vitamin E, vitamin C and GSH) in liver and kidney as compared with control rats. Administration of ethanol along with GLEt significantly decreased the activities of liver markers enzyme in serum towards near normal level. GLEt at a dose of 100 mg/kg was more effective than 25 and 50 mg/kg body weight. In addition GLEt also significantly reduced the levels of lipid peroxidation and in addition, significantly restored the enzymic and non-enzymatic antioxidants level in liver and kidney of alcohol administration rats. This observation was supplemented by histopathological examination in liver and kidney. These data suggest that GLEt exerts its protective effect by decreasing the lipid peroxidation and improving antioxidants status, thus proving itself as an effective antioxidant in alcohol induced oxidative damage in rats (Pari and Suresh 2008).

The antioxidative activity of an ethanolic extract of *Vitis vinifera* L. leaves was also investigated. The ethanolic extract of *Vitis vinifera* leaves at 250 mg/kg dose was found to have the highest antioxidant activity (Sendogdu *et al*. 2006). Comparable results have been published by Kosar *et al*. (2007), from extracts from fresh, dried and brined leaves of Vitis (Monagas *et al*. 2006).

Anthocyanins have been reported to possess antioxidant and vasoprotective properties. There are some doubts whether these effects measured in *in vitro* experiments or in animal studies using high doses are relevant for humans (Prior, 2004).

Bronchodilatory activity

The effect of grape leaf hydroalcoholic extract was investigated on isolated rat tracheal contractions induced by KCl and acetylcholine. The trachea was removed from male adult Sprague-Dawley rat and placed in an organ bath containing Krebs-Henseleit solution and contractions were recorded isometrically. The results demonstrate that the grapevine leaf extract at 0.5, 1, 2, 4 and 8 mg/ml significantly reduces the tracheal contractions induced by KCl (60 mM) dose-dependently ($P<0.0001$). Acetylcholine (55 µM)-induced tracheal contractions were also attenuated at the same concentration of the extract ($P<0.0001$). The grape leaf extract induced relaxation in the KCl-induced contraction in trachea was unaffected neither by nitric oxide (NO) synthase inhibitor (L-NAME, 100 µM) nor by beta-adrenoceptor antagonist (propranolol 1 µM). According to the authors, the results suggest that the bronchodilatory effect of grape leaf extract is mediated via the voltage dependent calcium channels on the smooth muscle cells membrane. Furthermore, the beta-adrenergic receptors and NO are not involved. The extract was prepared from dried grapevine leaves 50 g which were mixed with 230 ml of ethanol 70% for 72 hours at room temperature and stirred four times a day. The mixture was filtered and the solvent evaporated yielding 9.5 g of extract (Gharib Naseri and Heidari, 2006i).

Diuretic activity

Aqueous and alcoholic extracts of *Vitis vinifera* leaves were tested for diuretic activity in rats. The parameters studied on individual rat were body weight before and after test period, total urine volume, urine concentration of Na^+, K^+ and Cl^-. In the present study alcoholic and aqueous extracts of *Vitis vinifera* leaves (100 mg/kg of body weight) showed increase in urine volume, cation and anion excretion. Furosemide was used as a reference diuretic (Shastry *et al*. 2002).

Hypocholesterolemic effect

Oral administration of procyanidins (OPCs) from grape seed produced a hypocholesterolemic effect in a high-cholesterol animal feed model (rats). Specifically it prevented an increase in total and LDL plasma cholesterol and a decrease in HDL (Tebib *et al*. 1994).

In normal and hypercholesterolemic rabbit aortas, OPCs significantly lowered the amounts of cholesterol bound to aortic elastin compared to controls (Wegrowski *et al*. 1984).

Antiproliferative activity

Procyanidin-rich fractions from grape as well as leaf *Vitis vinifera* extract showing different mean degrees of polymerisation, percentage of galloylation (percentage of gallate esters) and reactive oxygen species-scavenging capacity were tested on HT29 human colon cancer cells. It has been observed that the most efficient fractions in inhibiting cell proliferation, arresting the cell cycle in G_2 phase and inducing apoptosis were the grape fractions with the highest percentage of galloylation and mean degree of polymerisation. Additionally, the antiproliferative effects of grape fractions were consistent with their oxygen radical-scavenging capacity and their ability to trigger DNA condensation-fragmentation (Lizarraga *et al*. 2007).

Antidiabetic activity

The acute and subacute antidiabetic activities of the ethanolic extract of *Vitis vinifera* L. leaves were investigated. The acute effect was studied on the normoglycaemic, glucose hyperglycaemic and streptozotocin-induced diabetic rats; and the subacute effect was studied on same diabetic rats for 15 days. The blood glucose levels were measured by using blood glucose measuring strips based on

glucose-oxidase method. The ethanolic extract of *Vitis vinifera* leaves at 250 mg/kg dose was found to possess a high antidiabetic activity. Mainly condensed tannins and flavonoids were suggested to contribute in the activity (Sendogdu *et al*. 2006).

The acute and subacute (15 days) hypoglycaemic and antihyperglycaemic effect of the two different doses (250, 500 mg/kg) of the aqueous extract from the leaves of *Vitis vinifera* L. were evaluated in this study. The aqueous extract was further fractionated through successive solvent extractions and the acute effect of different doses of its subfractions, 25 mg/kg for ethylacetate fraction, 80 mg/kg for *n*-butanol fraction and 375 mg/kg for remaining aqueous fraction were investigated using normal, glucose-hyperglycaemic and streptozotocin-induced diabetic rats. Blood glucose levels were measured according to the glucose oxidase method. Tolbutamide was used as a reference drug at a dose of 100 mg/kg. The antioxidant activity of the test samples was studied in the liver, kidney, heart tissues of diabetic rats by measuring malondialdehyde (MDA) and glutathion (GSH) levels. All results were compared to the diabetic control groups. The results showed that the EtOAc fraction, which is rich in polyphenolics, possess antihyperglycaemic and antioxidant activities equipotent with the reference hypoglycaemic agent (tolbutamide), when evaluated in diabetic rats (Orhan *et al*. 2006).

Other activities

The anti-herpes as well as the anti-mutagenic activities from the leaves and procyanidins from *Vitis vinifera* have been also assayed and published in the international literature (Girre *et al*. 1990; Liviero *et al*. 1993).

A reversible inhibition of intestinal enzyme activity (alkaline phosphatase, sucrose and dipeptidyl peptidase) was demonstrated in animal models (Tebib *et al*. 1994; PDR for Herbal Medicines, Thomson, 2004).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

Pharmacodynamic drug interactions of the herbal substance, herbal preparations or isolated constituents have not been reported.

3.1.5. Conclusions

The data obtained with herbal preparations and pure secondary metabolites differ, but show in most cases positive spasmolytic, vasorelaxant and anti-oedematous effects.

The data available allow only very limited conclusions on the plausibility of the therapeutic effects of the traditional use preparations of the monograph.

Based on available preclinical data, it can be concluded that the mechanism of action of orally administered extract in CVI is not known (i.e. well-establish use).

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

Data on the pharmacokinetics of the herbal substance, herbal preparations or isolated constituents are not available.

Flavonoids are the substances which are regarded to be responsible for the pharmacological properties of grapevine leaf extract. In the plant, flavonoids are generally present in genuine form as glycosides. Grapevine leaf extract as it has been referred previously contains as major flavonoid components quercetin-3-O-glucuronide, isoquercitrin (quercetin-3-O-glucoside) and kaempferol-3-O-glucoside. The substances present in blood after ingestion of plant extracts containing polyphenols usually differ from the native compound, as they are metabolites resulting from digestive and hepatic activity. The examination of pharmacokinetics and metabolism of the major and active constituents of such plant extracts in humans is therefore strongly recommended (Manach *et al*. 2005).

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

3.3.1. Single dose toxicity

The toxicity of grapevine leaf aqueous extract (4-6:1) following a single oral dose was also determined in SPF Wistar rats (3 males and 3 females per group). The doses used were 2000, 4000 and 10000 mg/kg. There were no signs of toxicity and no mortalities over the 14-day observation period, and no pathological or histological changes were seen in autopsy (Rabe *et al*. 2005; ESCOP, 2009).

3.3.2. Repeat dose toxicity

Repeated dose - chronic toxicity

The toxicity of grapevine leaf soft extract when given over a 90-day period was determined in SPF Mol-Wistar rats (12 males and 12 females per group) in a study which was subjected to inspection and carried out in accordance with GLP standards. The animals were given oral doses of 25, 125 or 250 mg/kg per day, or a placebo. A satellite group given 250 mg/kg per day was subjected to follow-up observation. No mortalities occurred during the study period. Observations made during the study period on bodyweight, food intake etc. revealed no evidence of any systemic toxic effects due to the extract. The values found during haematological and clinical chemistry testing were within biological limits. The autopsy findings and the results of histological tests did not reveal any evidence of changes due to the extract. The doses of extract used were therefore judged to be non-toxic in the rat.

The maximum daily dose of extract is 720 mg, which represents a dose of 10.3 mg/kg for a person weighing 70 kg. This is about one-thousandth of the maximum dose of extract used in the single-dose toxicity studies. As the maximum dose used in these studies did not give rise to any signs of toxicity, it is extremely unlikely that any toxic effects will occur in man. Preparations containing grapevine leaf extract have been used in European Union countries for many years and there have been no reports of any harmful effects in man to date. On this basis, it is reasonable to assume that the products reviewed in this report will not pose any safety risk on therapeutic administration (ESCOP, 2009).

Crowell *et al*. 2004: Resveratrol-Associated Renal Toxicity. Resveratrol, (3, 5, 4'-trihydoxystilbene) is a compound found in grapes, mulberries, and peanuts. To evaluate the potential toxicity of resveratrol, rats were administered by gavage 0, 300, 1000, and 3000 mg trans-resveratrol per kilogram body weight per day for 4 weeks. Most of the adverse events occurred in the rats administered 3000 mg per kilogram body weight per day. These included increased clinical signs of toxicity; reduced final body weights and food consumption; elevated BUN (blood urea nitrogen), creatinine, alkaline phosphatase, alanine aminotransferase, total bilirubin, and albumin; reduced hemoglobin, hematocrit, and red cell counts; and increased white cell counts. Increases in kidney weights and clinically significant renal lesions, including an increased incidence and severity of nephropathy, were observed. Diffuse epithelial hyperplasia in the bladder was considered, equivocal and of limited biological significance. No

histological effects on the liver were observed, despite the clinical chemistry changes and increased liver weights in the females. Effects seen in the group administered 1000 mg resveratrol per kilogram body weight per day included reduced body weight gain (females only) and elevated white blood cell count (males only). Plasma resveratrol concentrations in blood collected 1 h after dose administration during week 4 were dose related but were relatively low given the high dosage levels; conjugates were not measured. Under the conditions of this study, the no observed adverse effect level was 300 mg resveratrol per kilogram body weight per day in rats (corresponding to 21 g in a 70 kg human).

3.3.3. Genotoxicity

The mutagenic potential of grapevine leaf water extract (4-6:1), was assessed in a range of established *in vitro* and *in vivo* test models (Ames test, point mutation assay and chromosomal aberration assay). It was assessed in an Ames *Salmonella*/microsome plate assay using *Salmonella typhimurium* TA-100, TA-1535, TA-98, TA-1537 and TA-1538 with and without metabolic activation; S9 mix prepared from rat liver was used as the activation system. The assay was carried out in accordance with EU recommendations and OECD Guideline 471. Five concentrations of the grapevine leaf extract ranging from 8 to 5000 µg per plate were used, and positive and negative controls were also included. The results obtained did not provide evidence to suggest that vine leaf extract has mutagenic potential (ESCOP, 2009).

Mutagenic potential of grapevine leaf extract was also assessed in two independent point mutation assays carried out on the HGPRT locus of Chinese hamster V79 cells with and without metabolic activation; S9 mix prepared from rat liver was used as the activation system in each case. The assays were designed and conducted in accordance with the guidelines and recommendations on genotoxicity testing that were current at the time. On the basis of preliminary toxicity tests, a concentration range of 3.0 - 5000 µg/ml was selected for use in the assays. Positive controls as well as a vehicle control were included. Neither of the assays provided evidence to suggest that the extract used had mutagenic potential in the concentration range tested (ESCOP, 2009).

A micronucleus assay was carried out on NMRI mice (5 males and 5 females per group) to establish the potential of grapevine leaf soft extract for causing chromosomal aberrations. The assay, which complied with the relevant EU and OECD guidelines, was carried out using oral doses of the extract of 1.0, 3.0 and 10.0 ml/kg. Positive controls (cyclophosphamide 40 mg/kg) and vehicle controls (sodium chloride 0.9%) were included. Under the conditions employed, there was no significant increase in micronuclei frequencies in polychromatic erythrocytes. The methanolic and aqueous extracts from Greek varieties of *Vitis vinifera* demonstrated *in vitro* antimutagenic effects against mutagenicity induced by bleomycin and hydrogen peroxide in *Salmonella typhimurium* strain TA102 (Stagos *et al*. 2006).

Oligomeric proanthocyanidins from grape seed have demonstrated *in vitro* antimutagenic activity (Liviero *et al*. 1993).

In addition, among the pharmacologically active flavonoids (quercetin, quercetin 3-O-β-D-glucuronide and isoquercitrin) in the grapevine leaf extract used, it is known that quercetin has given positive results in the Ames test. However, the mutagenic potential of quercetin has not been confirmed in *in vivo* tests carried out in rats and mice (Hertog and Hollman 1996).

3.3.4. Carcinogenicity

No data available.

So far, there is no evidence in the literature to indicate that flavonoids have any carcinogenic properties. On the contrary, a large number of *in vitro* studies have been carried out which suggest that flavonoids have anti-carcinogenic effects (see section 3.1.2).

In toxicity and carcinogenicity studies carried out on quercetin, promoted by the FDA, four groups of 50 male and female F344 rats were given 40, 400 or 1900 mg/kg of quercetin, or a placebo, in feed over a period of two years. No carcinogenic activity was observed in the female rats and only slight carcinogenic activity in male rats. The carcinogenic effects, which manifested as renal tubule hyperplasia and nephropathy, were observed only in the high-dose group. No evidence of chronic toxicity was found (NTP: Toxicology and carcinogenesis studies of quercetin (CAS No. 117-39-5) in F344 rats (feed studies) 1992).

3.3.5. Reproductive and developmental toxicity

The maternal and embryofoetal toxicity of an orally administered aqueous preparation of grapevine leaf soft extract was investigated in pregnant Himalayan rabbits in a study which was carried out in accordance with GLP standards and subjected to QAU inspection. Four groups of animals (12 per group) were used. One group received a placebo whilst the other three groups were given doses of 300, 1000 or 3000 mg/kg per day on days 6-18 of gestation. All doses were administered in a volume of 10 ml/kg. No signs of maternal toxicity were seen during the observation period and there was no visible evidence of foetal retardation. Slight, non-significant skeletal retardations and variations were seen in the foetuses of animals receiving the highest dose (3000 mg/kg of the extract per day). These variations were within biological limits in all cases and did not occur in any of the other groups. There was no evidence of changes due to the extract in any of the other findings, and no teratogenic effects were seen. On the basis of the results obtained, the NOEL for the extract was defined as > 3 g. In the reproduction study conducted with rabbits no evidence of teratogenic effects was found. The recommended maximum dose is equivalent to a daily intake of 10.3 mg/kg of grapevine leaf extract. In the Leuschner study up to 3000 mg/kg per day of the extract, were applied without resulting in any teratogenic effect. The recommended maximum human dose of grapevine leaf extract is about 300 times lower than the highest dose used in the Leuschner study (Leuschner and Mitterer 1993; ESCOP, 2009).

Daily doses of 3000 mg/kg extract from grapevine leaves administered to female rabbits during organogenesis (6th to 18th day of pregnancy) did not exhibit teratogenic effects by a commercial water extract from grapevine leaves (4-6:1) (Anonymous, 2005).

3.3.6. Local tolerance

No data available.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

Data on oral single-dose and repeat-dose toxicity, as well as on genotoxicity and reproduction toxicity of grapevine leaf have been reported. The results give no reasons for safety concerns.

In vitro and *in vivo* studies on the mutagenic potential of grapevine leaf extracts did not show mutagenic activity.

3.4. Overall conclusions on non-clinical data

The reported pharmacological effects are not considered contradictory to the traditional uses.

Non-clinical data of grapevine leaf extracts in chronic venous insufficiency supports the traditional use.

Specific data on pharmacokinetics and interactions are not available.

Data on oral single-dose and repeat-dose toxicity, as well as on genotoxicity and reproduction toxicity of grapevine leaf have been reported. The results do not give any reason for safety concerns.

In vitro and *in vivo* studies on the mutagenic potential of grapevine leaf extracts did not show mutagenic activity.

Tests on carcinogenicity have not been performed.

As there is not sufficient information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

Oral and cutaneous administration of grape leaf can be regarded as safe at traditionally used doses with the exception of patients with severe renal or cardiac disease e.g. renal and heart failure.

The data on safety are considered sufficient to support a European Union list entry for the following herbal preparation: Soft extract (2.5-4:1; extraction solvent water).

4. Clinical Data

4.1. Clinical pharmacology

Pathophysiology of chronic venous insufficiency (CVI) describes a clinical picture in which chronic venous diseases of diverse aetiology are manifested by similar or related symptoms and complaints. CVI is mostly caused by venous and capillary hypertension, which causes a persistent, chronic, venous stasis in the skin of the lower leg, manifested most characteristically upon postural challenge (Alguire and Mathes 1997). The impairment of venous return that is responsible for the venous capillary hypertension can be caused by degenerative, dilating venous conditions of the superficial (primary varices), the transfascial (insufficiency of the perforating veins), or the deep system (“deep” varicosis, insufficiency of the main veins) (Jünger *et al.* 1994). Chronically disturbed haemodynamics of deep or superficial veins due to obstructed venous segments or valvular incompetence lead usually to trophic changes in the inner ankle area of the lower limbs and disturbances in the microcirculation of the skin have been considered to be major contributors for skin changes associated with chronic venous hypervolaemia and venous hypertension. Cutaneous microangiopathy of clinical relevance such as enlarged, tortuous capillaries surrounded by micro-oedema contributes to the skin alterations in the lower limbs and determines the course of CVI (Fagrell 1995; Jünger *et al.* 1996). CVI can be classified according to its haemodynamic, morphological, and clinical aspects. The clinical grading system, based on Widmer’s classification, as well as other international classification systems, e.g. CEAP (Clinical Class, Etiology, Anatomy and Pathophysiology), (Widmer *et al.* 1981; Partsch 1994; Porter and Moneta 1995). The most important therapeutic approaches include compression therapy, physiotherapy, surgical treatment and sclerotherapy.

Drug therapy is a treatment option in incipient and mild to moderate chronic venous insufficiency (CVI) (Widmer grades I and II, CEAP Clinical classes 2, 3, or 4a).

Topical drug treatment - Antioedema drugs are mostly plant derived substances for which an antiexudative and antioedematous action and efficacy have been confirmed in experimental studies

and clinical trials. Experimentally, most of them have a membrane stabilising action that leads to a decrease in capillary permeability. Some of these substances also have venotonic properties.

Long-term treatment with antioedema drugs can also be considered in primary and secondary CVI or if invasive measures are not applicable (Gallenkemper *et al*. 1998). The majority of these products contain plant extracts or semisynthetic derivatives of plant-derived substances. The most commonly used are coumarins (α-benzopyrone derivatives); saponins/horse chestnut seeds extracts; flavonoids (γ-benzopyrone derivatives); rutin, diosmin, and hesperidin; anthocyanins and procyanidins.

Procyanidins, like the anthocyanins, are flavonoids that are not available as monosubstances. Procyanidins are present in relevant amounts in standardised extracts such as grapevine leaf extracts (*Vitis vinifera*) and are formed through condensation reactions of flavonols (Hostettmann *et al*. 1994).

The complex syndrome of symptoms involved in CVI led to investigate the activity of grapevine leaf preparations on two distinct parameters affecting CVI. On the one hand the effects of the product on microcirculation, on the other on the typical objective and subjective symptoms of CVI such as the presence of oedema and the typical CVI complaints “tired, heavy, and swollen legs”, or tension and pain in the legs.

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

No data available.

Assessor’s overall conclusions on pharmacodynamics:

At present, the mechanism of action of grapevine leaf water extract in chronic venous insufficiency is not known.

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

The grapevine leaf extract is a complex extract containing more than 200 different identified substances. The clinical effects of the grapevine leaf extract cannot be attributed to a specific individual constituent but should be ascribed to the extract as a whole. It is impossible to perform classical pharmacokinetic studies with the complete product and pharmacokinetic studies of individual constituents such as flavonoids will only provide limited information with regard to clinical efficacy.

Flavonoids (quercetin glucuronide, kaempferol glucosides) are quantitatively important constituents of the extract. The systemic bioavailability of flavonoids is probably relatively low and variable. Orally administered flavonoids are susceptible to pre-systemic metabolism by the intestinal flora. It is likely that the absorbed metabolites formed by the microflora in the gut contribute to the biological action of orally administered flavonoids. Systemic flavonoids are metabolised in the liver primarily by conjugation (methylation or sulfuronidation) and/or glucuronidation. The resulting metabolites are excreted either with the urine or with the bile into the intestine, where they undergo further metabolism. The pharmacokinetic input function of systemic flavonoids may well be further extended by this enterohepatic recycling process (Manach and Donovan 2004).

Various sources in the literature have suggested that the desired therapeutic effect of many flavonoids is usually obtained only after repeated oral intake of the respective preparations. This implies that the flavonoids and their metabolites accumulate in the body, and do not exert the pharmacodynamic effects seen in *in vitro* studies until a pharmacological threshold dose and relevant plasma levels have been reached. This hypothesis was confirmed by the results of a clinical study by Kiesewetter *et al*.

(2002). A significant improvement in key CVI symptoms in the active treatment groups versus placebo could not be observed after 6 weeks, but became evident after 12 weeks of treatment. This could also explain why earlier studies reported a "small" or "undetectable" effect following single oral administration.

The grapevine leaf extract represents a complex mixture of different classes of compounds, each having their own pharmacokinetic characteristics. An important portion of the components of grapevine leaf extract belongs to the group of polyphenols, such as anthocyanins, proanthocyanidins, catechins and phenolic acids (Bombardelli and Morazzoni 1995; Boucheny and Brum-Bousquet 1990). Manach *et al*. (2005) have presented a review of various bioavailability studies in humans concerning polyphenols, through various sources in the literature have suggested that the desired therapeutic effect of *Vitis* leaf could be based to its polyphenols content.

Assessor's overall conclusions on pharmacokinetics:

Data on pharmacokinetics of vine leaf extract or relevant components are not available in humans. No reliable methods exist to determine simultaneously the plasma levels of all active ingredients present in the whole extract.

4.2. Clinical efficacy

Several publications referring to human clinical studies with medicinal products containing *Vitis vinifera* were found in the literature.

The clinical information available for aqueous extracts of grapevine leaf includes the activity on mainly two distinct parameters affecting chronic venous insufficiency (CVI). On the one hand the effects of the product on microcirculation, on the other on the typical objective and subjective symptoms of CVI such as the presence of oedema and the typical CVI complaints “tired, heavy, and swollen legs”, or tension and pain in the legs.

In all clinical studies, changes in leg volume and/or calf and ankle circumference were included in the endpoints studied. All confirmatory studies were designed to show efficacy on subjective symptoms of CVI used the lower leg volume determined by water displacement plethysmography as the primary efficacy endpoint. Other endpoints included the lower leg diameter (at calf height and at the ankle), visual analogue scales (VAS: 0-10 cm) for the main symptoms (“tired, heavy legs”, “sensation of tension”, “prickling sensation”, and “pain in the legs”), and the global assessment of efficacy and tolerability by the patient and by the doctor according to a 4-point verbal rating scale (VRS: “good”, “satisfactory”, “not satisfactory”, and “poor”). These parameters are consistent with the guidelines of the German Society for Phlebology.

In the double-blind, placebo-controlled cross-over study with two treatment sequences of 6 weeks each separated by a 4-weeks placebo washout period, microcirculation in the most affected CVI leg was measured by laser doppler fluxmetry (685 nm, penetration depth approximately 1 mm) and transcutaneous oxygen monitoring(Kiesewetter *et al*. 2000; Kalus *et al*. 2004; Schaefer and Petrini 2004; Limoni, 1996).

Patients were questioned about their subjective well-being. They were also instructed to spontaneously report any adverse events experienced. Laboratory tests (haematology, clinical chemistry, and urinalysis) and blood pressure and pulse measurements were carried out in most studies to record safety.

4.2.1. Dose response studies

In all published clinical trials (Limoni C, 1996; Monsieur and Van Snick 2006; Schaefer *et al*. 2003; 2004; Kalus *et al*. 2004; Vix *et al*. 2007) film coated red grapevine leaf tablets, extract *Vitis viniferae* (4-6:1 water) were used in male and female patients, aged ≥ 18 years with a single dose of 180 mg and in daily dose of 360 mg from a period of 6-12 weeks.

4.2.2. Clinical studies (case studies and clinical trials)

Several publications referring to human clinical studies investigating the safety and efficacy of a dry extract of red *Vitis vinifera* leaves (4-6:1) extraction solvent water (RVLE) were found in the literature. Some unpublished studies that originate from confidential in house files of Boehringer Ingelheim are also assessed in this AR. The company allowed using those data for assessment and monograph/list entry establishment.

Double-blind, placebo-controlled studies

Kiesewetter *et al*. 2000: In a 12-week, randomised, double-blind, placebo-controlled, parallel-group, multicentre study conducted in accordance with the principles of Good Clinical Practice (GCP), the efficacy and safety of once daily doses of 360 and 720 mg RVLE were compared to placebo in male and female outpatients aged between 21 and 80 years old (with 52% ≥ 60 years) with body weights ranging from 46 to 120 kg (Broca index: median 7.3, range: -30 to +82%; this correspond to a BMI of approximately 25 kg/m^2) and stage I and incipient stage II chronic venous insufficiency (CVI) with no major dystrophy of the skin. The majority (72%) of the patients were women. Compression therapy and the administration of diuretics were not permitted during the study period.

Patients were randomly assigned to a double-blind treatment with placebo, 360 mg RVLE or 720 mg RVLE once daily for 12 weeks, preceded and followed by a single-blind 2-week placebo treatment for baseline run-in and end-of-trial washout, respectively. Study criteria were evaluated at baseline, after 6 and 12 weeks of treatment and 2 weeks after discontinuation of treatment. Of the 260 patients enrolled and randomised, 219 completed the study in accordance with the protocol, 86 test subjects took a single daily dose of 360 mg RVLE, 84 took 720 mg RVLE per day and the remaining 87 test subjects received a placebo. During weeks 13 and 14 none of the participants received any further medication.

Subjectively, there was an improvement in CVI symptoms at week 6 with all treatments, but a further improvement at week 12 was seen only in the active treatment groups: at week 12, the changes compared to baseline were significantly greater ($p < 0.001$) in both active treatment groups than in the placebo group. The study demonstrated that once daily doses of 360 and 720 mg RVLE were effective in the treatment of mild CVI, reducing significantly lower leg oedema and improved CVI-related symptoms to a clinically relevant extent.

The treatments were well tolerated. All 260 patients who were enrolled and randomised were included in the safety analysis. This included three patients who withdrew from the study before visit 2 (i.e. before entering the treatment phase). 257 patients received at least one dose of the randomised study medication. Most patients completed the study up to 84 days of treatment. The mean duration of treatment for the 360 mg dose of RVLE was 81.9 days (n = 86) whilst the mean duration of treatment for the 720 mg dose of RVLE was 79.7 days (n = 84). Placebo was taken by 87 patients during the randomised treatment period (mean duration of treatment: 79.3 days) and by all patients during the 14-day run-in period (n = 260) and the 14-day follow-up period (n = 246). A total of 34 adverse events (AEs) were reported in 31/260 patients: 3 adverse events in 3/260 subjects treated with placebo during the run-in phase; 3 adverse events in 3/246 patients treated with placebo during the

follow-up period; 10 adverse events in 10/87 patients treated with 360 mg RVLE; 2 adverse events in 2/85 patients treated with 720 mg RVLE; and 16 adverse events in 13/88 patients treated with placebo. Most adverse events were considered to be mild (n = 21) or moderate (n = 9). Four adverse events were classified as severe, three in patients taking the placebo (phlebitis of the large saphenous vein; knee surgery; abdominal discomfort) and one in a patient taking 360 mg RVLE (leg hematoma following minor trauma). Six adverse events were rated as likely to be causally related to the study medication by the investigator; of these, two (mild constipation and mild hair thinning) were reported in patients treated with 360 mg RVLE, whilst the other four occurred in patients treated with placebo. Gastrointestinal disorders (abdominal discomfort, diarrhoea, dyspepsia, dry mouth or retching) were the most frequently cited adverse events (n = 11), followed by infections (n = 6), headache (n = 4) and disorders of the musculoskeletal system (n = 4). Two adverse events which occurred during treatment with placebo required hospitalisation and were labelled as “serious”; there was one incident of vaginal haemorrhage during the run-in phase and one incident of severe acute osteoarthritic complaints in the knee of the affected leg during the randomised treatment period. Three other patients were withdrawn following an AE: 1 patient was withdrawn during the placebo run-in period due to moderate abdominal discomfort; 2 patients were withdrawn from randomised treatment with the placebo, one because of phlebitis of the greater saphenous vein and one because of severe abdominal discomfort. The clinical laboratory data, blood pressure and pulse rate data and 12 lead ECG recordings did not indicate any significant mean or individual changes which were likely to have been due to the study medications. Overall tolerability was rated as follows for the 3 treatments: 360 mg AS 195: good 81%, satisfactory 19%; 720 mg RVLE: good 94%, satisfactory 6%; placebo: good 68%, satisfactory 30%, not satisfactory 2%. The study was conducted in multicentric settings, but most measurements were carried out by only two investigators, thus this trial can be considered basically a monocentric one.

Kalus *et al*. 2004: The effect of RVLE on cutaneous microvascular blood flow, transcutaneous oxygen pressure (tcpO2), and leg oedema was investigated in a randomised, double-blind, placebo-controlled, crossover study in male and female patients, aged ≥ 18 years with an average age of 66 years, with confirmed CVI stage I or II. In this efficacy study, 71 patients (70 during the second phase) were treated with either placebo or 360 mg RVLE film coated tablets. The first group (n=36) received special RVLE extract (AS 195) 360mg once daily during a first 6-week treatment period, followed by a 4-week placebo washout period and then placebo during the second 6-week treatment period. The second group (n = 35) started with placebo and received RVLE 360 mg after the placebo washout. After 6 weeks, patients in the RVLE group had increased microvascular blood flow values and trans-cutaneous oxygen pressure (tcpO2). The active substance group showed a statistically significant increase of 241.8±18.7 Arbitrary Units (AU), while a reduction of 41.0±18.7 AU was recorded in the placebo group ($p<0.0001$) (fig. 2).

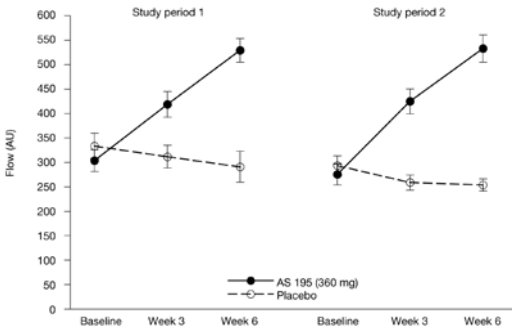

Figure 2. Microcirculation, measured in arbitrary units (AU) (laser Doppler flow measurement at Doppler frequencies of 10.1–37.2 kHz, mean ± SEM, after 10 minutes of standing) (from Kalus *et al.* 2004).

The TcpO2 oxygen reading also rose significantly from baseline levels in the RVLE (AS 195) group, by 1.35±0.97 mmHg, contrasting with a reduction in the placebo group of 7.27±0.97 mmHg (fig. 3).

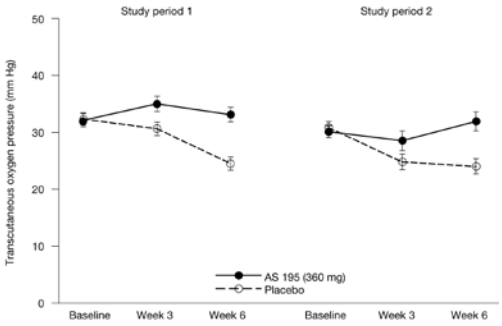

Figure 3. Transcutaneous oxygen pressure (mean ± SEM, after 10 minutes of standing) (from Kalus *et al*. 2004).

These changes in the measured parameters were associated with corresponding volume changes in the lower leg. After just 3 weeks of treatment, statistically significant differences were also observed in ankle and calf circumference. After 6 weeks, these circumferences had fallen by 0.39 and 0.54 cm respectively, in the active group, compared to increases in the placebo group of 0.29 and 0.14 cm respectively. The authors of the study concluded that the administration of RVLE could have a positive effect on the course of CVI. The minimum number of days of exposure was 38 in the RVLE and 24 in the placebo group, the maximum number 46 in the RVLE and 45 in the placebo group. Thirteen (18.3%) subjects out of 71 experienced 16 adverse events (AEs) during the 16-week trial. Ten adverse events (14.1%) were of mild, 4 (5.6%) of moderate and 1 (1.4%) of severe intensity. None of the adverse events were assessed as related to the trial medication. One patient died from a cardiac arrest. He had been treated with placebo and never received RVLE in this trial. All patients assessed the overall tolerability as good or satisfactory. Laboratory parameters did not change during the study (Kalus *et al*. 2004).

Limoni, 1996: A 4-week, multicentre, randomised, double-blind versus placebo, parallel-group safety trial to evaluate the tolerability profile of certain capsules (water extract *Vitis vinifera* siccum 4-6:1) in male and female patients suffering from chronic venous insufficiency. The study population consisted of 105 patients, male and female, aged between 20 and 70 years with chronic venous insufficiency (CVI) of grade I, II, and III according to Widmer. The patients received 3 capsules of that extract or placebo per day during 28 days. 72 patients were randomised into the active treatment group and 33 into the placebo treatment group. In the absence of a strictly diagnosed CVI, patients with a subjective feeling of heavy and tired legs were admitted as well. Primary endpoints were the incidence of adverse events and the rating of the overall tolerability. Secondary endpoints were laboratory tests and physical examination. There were no differences in tolerability between the two treatment groups. The proportion of volunteers reporting adverse events was similar in both treatment groups: 40.2% in the active treatment group and 36.4% in the placebo group. The most frequent adverse events were mild gastrointestinal disorders, sleep disturbances, and tiredness. Laboratory values did not change in either treatment group during the trial period. The capsules were well tolerated by 84.9% of the volunteers. No severe adverse events were reported.

Vix *et al*. 2007: This 12-week, double-blind, randomised, placebo-controlled, multicentre trial was carried out to evaluate the efficacy and tolerability of film-coated tablets, extract *Vitis vinifera* (*4-6:1 water*) 360 mg per day orally, in male and female patients suffering from chronic venous insufficiency. The design and methodology used closely reflected those used in Boehringer Ingelheim trial by Schaefer and Petrini 2004.

The time course of the change from baseline in limb volume was similar for both treatment groups with a more marked improvement of leg oedema over time in patients treated with these tablets. After 84 days of treatment, limb volume was reduced by -21.38 (±11.30) g of displaced water in the verum group. The adjusted mean reduction in limb volume in the placebo group was less pronounced with -10.71 (±11.56) g. However, the difference between treatment groups (-10.67 ±14.35 g) was not statistically significant.

For the secondary endpoint "change from baseline in calf circumference", the adjusted mean difference to placebo after 42 days of treatment with these tablets was -0.42±0.22 cm in the full-analysis data set (p=0.0596) vs. -0.31±0.24 cm on study day 84 (p=0.1851). In the Per-Protocol Set (PPS), the difference to placebo was -0.47 (±0.23) cm on study day 42, with a p-value just reaching significance at 0.0445. The evaluation of the four subjective symptoms of CVI, as assessed by visual analogue scale, yielded similar results: after 42 days of treatment, the adjusted mean differences between active treatment and placebo were -0.44 cm for “tired, heavy legs”, -0.73 cm for “sensation of tension in the legs”, and -0.48 cm for “pain in the legs” (vs. -0.38 cm, -0.04 cm, and 0.18 cm on study day 84). There was no difference between treatment groups regarding the symptom “tingling sensation in the legs”. Global efficacy was rated as good or satisfactory by 70.9% of all patients treated with these tablets and 67.7% of all patients allocated to placebo whereas the investigators' ratings were 69.0% and 62.6%, respectively. The assessment of quality of life by the Tuebingen QoL questionnaire resulted in a better mean score for verum group compared with placebo regarding the subscale "leg complaints", the subscale most specifically related to CVI. The differences, however, were not statistically significant (Rabe *et al*. 2011).

Schaefer and Petrini 2004: This placebo controlled, randomised, parallel group study was carried out in 2004. 247 CVI patients (CEAP-Scores 3 or 4a) were entered in the study: 121 were treated with the special grapevine leaf extract (AS 195) 360 mg as a single tablet and 126 with placebo for 12 weeks. For the primary endpoint, the difference related to placebo, adjusted for centre effect and baseline values, in changes of displaced water from baseline until Day 84 was -10.42 cm (SE: 11.84; 95% CI: -33.76, 12.92). The difference in changes between the two treatment groups was not statistically significant. Subjective CVI symptoms diminished in both treatment arms similarly during the first 3 weeks. A statistically significant difference between the two treatment groups in favour of verum, was found for the symptoms “sensation of tension in the legs” at Day 42 (p=0.0211, ANCOVA) and “pain in the legs“ at Day 84 (p=0.0222, ANCOVA). At all other time points no statistically significant difference was detected between the two treatment regimens for the symptoms “tired heavy legs” as well as “tingling sensation” in the legs. After 84 days of treatment, the limb volume as measured by the water displacement method was statistically not significantly more reduced in subjects allocated to verum than in those randomised to placebo. Inspection of the results showed that the unexpected high intra- as well as inter-individual variability of limb volume determinations contributed significantly to the outcome of the study. The variability between 2 or 3 replicates of the limb volume determination, for example, exceeded 20 g of displaced water in more than 20% of patients (range 0% to 44.1%) in 8 out of 23 study sites (35%). This study was negative in terms of a primary endpoint, although there was evidence of symptomatic benefit from the active treatment.

Petrini and Schaefer 2003: A 17-week, randomised, double-blind, placebo controlled cross-over trial to evaluate the efficacy of water extract *Vitis vinifera* 4-6:1 film coated tablets (RVLE) given as 2 capsules of 180 mg of RVLE daily 360 mg per day p.o., in 179 male and female Japanese patients with swellings of calf and ankle due to disorders of venous reflux belonging to Class 1 according to Porter’s classification, in improving microcirculation of the skin in the leg of male and female patients suffering from chronic venous insufficiency. The microcirculation study has demonstrated that objective signs associated with CVI (microvascular blood flow, $tcpO_2$, ankle circumference and calf circumference) can be improved significantly after just 3 weeks of oral treatment with a single daily dose of 360 mg of the

grapevine leaf extract RVLE. These results build on those from Harrison trial (1998) showed that RVLE reduces lower leg oedema and calf circumference in patients suffering from CVI treated once daily for 12 weeks. Out of the 179 evaluable subjects 38.5% experienced a marked and 42.5% a moderate global improvement of CVI symptoms. Improvement was rated as “moderate” by 59.8% of the patients with regards to heaviness/tiredness, 69.9% with regards to tension, 79.2% to tingling, 74.0% to pain, and 74.4% to itching. Circumference of calf (mean±SD) was 33.5±2.99 cm prior to treatment and 33.0±2.94 cm at 12 weeks, of the ankle (mean±SD) 22.3±1.84 cm prior to treatment and 21.9±1.82 cm at 12 weeks after treatment ($p<0.05$). These differences were not statistically significantly different from baseline.

Open-label, Observational studies

Schaefer *et al*. 2003: The 6-week, open, uncontrolled multicentre safety trial was conducted to specifically evaluate the tolerability and safety of RVLE film-coated tablets, 360 mg per day *per os*, in 65 male and female patients, aged 25 - 82 years, suffering from chronic venous insufficiency (CVI) grade I or II (Widmer classification). The verum group received two film-coated tablets once daily for 42 days (360 mg per day). The primary objective of the study was to assess the tolerability and safety of RVLE extr. *Vitis vinifera* siccum *4-6:1*) film-coated tablets. The observational study was conducted in accordance with the requirements of the Swiss licensing authority (Swissmedic: application document) in a total of 11 study centres (general practitioners) between June and October 2002. Three visits (study enrolment, baseline examination at the start of the study, final examination at the end of the study, with a follow-up examination if necessary) were planned. The study was carried out in accordance with GCP. At the end of the study, all subjective symptoms of CVI (tired, heavy legs, sensation of tension in the legs, tingling sensations in the legs, pain in the legs) were statistically significantly improved. The global assessment of efficacy by the patients and by the investigators was rated as good or satisfactory in most of the patients. The primary variable was the number and intensity of adverse events (AEs), with particular emphasis placed on adverse events regarded by the investigator to be treatment-related. Secondary safety variables included the global assessment of tolerability by both the patients and investigators as well as vital sign assessments. RVLE film coated tablets were well tolerated during the whole study. Overall, six patients (9.2%) experienced a total of 7 adverse events of mild or moderate intensity judged by the investigator as potentially causally related to study medication. They included gastrointestinal problems (4 patients), headache (1 patient), anorexia (1 patient), and erythematous rash (1 patient). Four patients of whom experienced a significant AE necessitated study discontinuation in three cases and dose reduction in one case. Most patients rated the global tolerability of RVLE film-coated tablets as good (49 patients, 75.4%) or satisfactory (12 patients, 18.5%). Three patients (4.6%) rated tolerability as bad. One patient (1.5%) gave no rating. The global assessment of tolerability by the investigators was in good agreement with those of the patients. The investigators rated the tolerability as good in 50 patients (76.9%), as satisfactory in 10 patients (15.4%), as not satisfactory in one patient (1.5%), and as bad in two patients (3.1%). No relevant changes of vital signs (systolic and diastolic blood pressure, pulse rate) were measured. Patient´s compliance was rated as good (Schaefer *et al*. 2003).

Monsieur and Van Snick 2006: A small, open observational clinical study was conducted in 39 patients suffering from chronic venous insufficiency (CVI), grade I or II according to the Widmer classification, (grade 2 to 4 of the international CEAP classification). Patients were treated with 180 mg of RVLE (AS 195) twice daily (360 mg in total) for 6 weeks. The parameters investigated were objective measurements of lower leg volume and the circumference of the leg as well as subjective criteria such as heaviness and pain in the leg. A clear and significant improvement of all parameters was observed after 2 weeks of treatment. This effect was still present and increased slightly after four and 6 weeks in all objective and subjective parameters tested in this study.

Table 5: Clinical studies on humans, in venous insufficiency

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Kiesewetter, 2000	randomized, double-blind, parallel, dose response Placebo Efficacy, tolerability, safety	caps 180mg, *per os* 2-0-0 AS 195 capsules 360 mg, *per os* 2-0-0 April Sept-1998 Placebo 12 weeks	260 persons 24/62 56.2±12.4 years (mean±SD) 27/57 55.7±13.8 years (mean±SD) 20/67 59.2±11.5 years (mean±SD): 86/84, 720 mg:84/79 87/80	CVI Grade I, II according to Widmer	Baseline adjusted changes of lower limb volume assessed by water displacement plethysmography	The statistical analyses were based on the arithmetic mean of the measurements	Positive clinical relevance
Schaefer and Petrini 2004	randomized, double-blind, parallel Placebo Efficacy, tolerability	RVLE tablets 360 mg, *per os* 1-0-0 Placebo 1-0-0 2004 12 weeks, 2 weeks single blind placebo	247 persons 20/99 53.0 years (20–83 years) 15/111 53.0 years (23–79 years)	CVI CEAP-Scores 3 or 4a 360 mg: 121/117 126/123	Baseline adjusted changes of lower limb volume assessed by water displacement plethysmography	Not further data given	Negative clinical relevance
Schaefer and	randomized, double-	RVLE tablets 360 mg,	65 males and females	CVI Grade I, II	Baseline adjusted changes of lower limb	Symptoms of CVIs were	Negative clinical

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Petrini 2003	blind, controlled cross over trial Placebo Efficacy	*per os* 1-0-0 Placebo 1-0-0		according to Porter	volume Changes in CVI symptoms	evaluated Not further data given	relevance
Vix *et al*. 2006	randomized, double-blind, parallel Placebo Efficacy, tolerability 211 individuals	RVLE tablets 360 mg, *per os* 1-0-0 Placebo 1-0-0 360 mg: 103/98 99/95 12 weeks	32/71 61.0 years (29–86 years) 26/73 61.0 years (30–87 years)	CVI CEAP Scores 3 or 4a	Baseline adjusted changes of lower limb volume assessed by water displacement plethysmo-graphy	Symptoms of CVIs were evaluated by comparing the end-of-treatment scores with the base line scores	Negative clinical relevance
Kalus 2004	randomized, double-blind, cross-over Placebo Efficacy, tolerability	RVLE tablets 360 mg, *per os* 1-0-0 360 mg: 70/70 6 wks	71 subjects 16/55 65.2±7.7 years (mean±SD) (32–76)	CVI Grade I, II according to Widmer	resting flux, change from baseline measured by Laser Doppler fluxmetry	Statistical analyses were based on the arithmetic mean of the measurements	Increase of microvascular blood flow
Monsieur & Van Snick	Open label observation	RVLE tablets 180 mg, *per os*	39 patients	CVI Grade I, II according to	Baseline adjusted changes of lower limb	Continuous secondary endpoints	Positive clinical

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
2006	al study Tolerability, safety, Efficacy 2006 39	2-0-0 360 mg 6 wks		Widmer	volume Changes in CVI symptoms	(change from baseline in limb volumes and in subjective CVI symptoms were measured	relevance
Limoni 1996	randomized, double-blind, parallel, dose response Placebo Multicentre Tolerability, Safety	RVLE caps. 180 mg, *per os* 1-0-2 Placebo 1-0-2 540 mg: 72/64 33/27 4 weeks	105 6/66 45.2 years (18.4–67.9 years) 4/29 36.7 years (25.6–70.6 years)	CVI Grade I, II. III according to Widmer	Safety Global improvement	Symptoms of CVIs were evaluated by comparing the end-of-treatment scores with the base line scores	Safety is proved and general positive clinical relevance

[1)] RVLE = red *Vitis viniferae* leaves (4-6:1) water extract

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

No information available.

4.4. Overall conclusions on clinical pharmacology and efficacy

The clinical efficacy of an extract from the red grapevine leaves (RVLE) was investigated in double blind, placebo-controlled studies (Kiesewetter *et al*. 2000; Kalus *et al*. 2004) and in open observational studies (Schaefer *et al*. 2003; Monsieur and Van Snick, 2006) during treatment of chronic venous insufficiency. In these studies, the RVLE showed an acceptable clinical efficacy and tolerability, while there were some others with negative results (Schaefer and Petrini 2003; 2004; Vix *et al*. 2007).

Treatment with RVLE has been shown to lead to a reduction of leg volume, measured using both water plethysmography and calf and ankle circumference, and of the subjective symptoms investigated in the studies reviewed.

5. Clinical Safety/Pharmacovigilance

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

The safety profile of grapevine leaf extracts can be described as acceptable from all clinical studies in chronic venous insufficiency (CVI) patients and from its use from products on the market. The safety results obtained from the clinical studies conducted so far show that the oral use of vine leaf extracts are well tolerated by most patients. No drug-related serious adverse events were reported during the clinical trials. Seven non-serious adverse events (constipation, dermatitis, headache, hair thinning, menometrorrhagia, urticaria) or moderate (erythematous rash) of mild intensity leading to patient withdrawals, were suspected to be related to the active trial medication (Kiesewetter *et al.* 2000; Kalus *et al.* 2004; Schaefer *et al.* 2003; Monsieur and Van Snick, 2006; Schaefer and Petrini 2003; 2004; Vix *et al.* 2007).

5.2. Patient exposure

The controlled studies included 1073 patients in total (Kiesewetter *et al.* 2000; Kalus *et al.* 2004; Schaefer and Petrini 2004; Vix 2006, Limoni 1996). The open studies, evaluated by Schaefer *et al.* 2003, and by Monsieur and Van Snick, 2006, comprised 104 patients.

5.3. Adverse events, serious adverse events and deaths

Brito *et al*. (2008); Mur *et al*. (2006): Vine pollen allergies have been reported with *Vitis vinifera* pollen and an extract thereof in Castilla-La Mancha, Spain, the area with the highest density of vineyards in the world. Two cases of allergic reactions to *Vitis vinifera* pollen and grape have been previously reported. The aim of a prospective study by Brito *et al*. (2008) was to determine the clinical relevance and biochemical characteristics of vine pollen in the Spanish province of Ciudad Real. The authors designed a prospective study of patients treated in the allergy units from Puertollano and Ciudad Real for respiratory symptoms of 4 months' duration in the year 2000. Skin prick tests with a standard aeroallergen battery and *V. vinifera* pollen extract were performed on all patients. The authors also performed conjunctival and bronchial provocation tests and serum specific IgE and sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoblotting on the patients who tested positive for *Vitis vinifera* pollen.

The results of this prospective study show that *Vitis vinifera* pollen has a moderate clinical relevance from an allergic point of view, particularly affecting those subjects who are exposed to it during the working hours and those who perform leisure activities close to vine fields. During a period of four months, the authors performed skin prick tests to vine pollen in patients who attended the outpatient clinic with suspected respiratory allergy, finding sensitisation to *Vitis vinifera* pollen in 9 of the 200 patients seen (98 patients had allergy to other pollen). In conclusion, in areas with a high density of vineyards, vine pollen can reach midrange air concentrations and could be the cause of hay fever in those individuals with the highest level of exposure (Brito *et al*. 2008; Mur *et al*. 2006).

Reports on immunoglobulin E (IgE)-mediated allergic reactions to grapes and wine are limited in the literature. Nevertheless, grapes are widely grown and consumed in Mediterranean countries. The object of a prospective study by Kalogeromitros *et al*. (2005) was to present clinical features, *in vivo* and *in vitro* allergy testing, and human leukocyte antigen (HLA) serotyping in patients with recurring reactions to grapes and grape products. Eleven unrelated Greek patients, six men and five women (aged 16-44 years; mean, 26.9 years) were enrolled based on a documented history of IgE-mediated reactions to grapes, wine, or other grape products. Their evaluation included full history, reaction severity, clinical examination, skin-prick tests with food allergens and moulds, serum IgE, specific IgEs to the same allergen battery, and HLA typing. Patients reported 35 grape-induced episodes of anaphylaxis ranging from moderate to severe. The authors described several cases of repeated IgE-mediated reactions to grapes, vine and other grape products. Similar clinical manifestations in different courses of grape allergen, almost identical prevalent co-sensitisations to other already known allergenic fruits, and the first determination of HLA antigens in grape-allergic patients are the core of this study.

The authors suggest that allergy to grapes may not be as uncommon as generally believed in the literature and should be considered as a possible offending cause in certain episodes of food-induced anaphylaxis probably in genetically at-risk individuals. However, allergic reactions to *Vitis vinifera* are known and reported in rare cases (Kalogeromitros *et al*. 2005).

In summary, there is no safety specific information that is relevant for medical products. The reported adverse events / side effects were mild to moderate. The majority of the above mentioned events are considered to be related to underlying diseases, incidental concomitant disorders or other coincidences but not causally related to *Vitis vinifera*. From these published adverse events, no change of the safety profile of *Vitis vinifera* can be concluded. According to current knowledge, gastrointestinal disorders and hypersensitivity reactions should be labelled in the PIL and SPC.

The Periodic Safety Update Report for *Vitis vinifera* has been compiled within the joint German Medicines Manufacturers' Association (BAH) PSUR project. It summarises safety data from 01/Nov/2003 to 01/Sep/2008.In the report period no new safety relevant issues were found in the literature. There were no reports, clinical or experimental studies identified that give reason for a change in the assessment of the safety and benefit/risk ratio of *Vitis vinifera*.

Serious adverse events and deaths

The case of a 51 years old female patient is reported. She was hospitalised due to an acute icterus, which had developed over 15 days and was associated with asthenia, nausea and anorexia. Beyond the mucocutaneous icterus, the clinical examination did not show any abnormality, especially no chronic hepatopathy. The laboratory parameters suggested a cytolysis due to an ALAT value that was twelve times higher than normal, γ-GT and alcalic phosphatase plasma concentrations two times higher than normal and total bilirubine of 87 µmol/L. Normal findings in the abdominal ultrasonic examination lead to the diagnosis of an acute hepatitis. The patient has a history of euthyroidism. The patient did not consume alcohol and was in a good nutritional condition. The etiologic examination of this hepatitis

was negative for infections, immunological and metabolic causes. A hepatic punction-biopsy, revealed a discrete cholangitis with focal ductal hyperplasia, a major cholestasis with numerous biliary thromboses and intrahepatocytic biliary pigments, a focal hepatocellular necrosis, a lymphohistiocytic infiltrate of weak intensity without significant fibrosis. Altogether this suggested an acute cholestatic hepatitis with either viral or toxic origin. The review of the medical questionnaire revealed sporadic intake of 1 g of acetaminophen and two repeated intakes in an interval of 1 week, when the first symptoms had appeared. Additionally the patient took *Vitis vinifera tinctoria* (extract of red wine leaves) and Fumitory (extract of *Fumaria officinalis*) daily for two months, as "summer cure" for the treatment of heavy legs and as a stimulant. She stopped the intake of these two products 3 weeks prior to hospitalisation, when the first general symptoms appeared. The patient had already taken *Vitis vinifera tinctoria* at the same time one year before. Fumitory was used for the first time. According to the official method used at the pharmacovigilance centres in France, this case is classified as plausible with a chronological C2 and a semiotic S3 score of two products concomitantly used with paracetamol (Bonnet *et al*. 2007).

Assessor's comment:

This case is classified as serious because of a significant medical reaction including hospitalisation.

A causal relationship with *Vitis vinifera* cannot be excluded (plausible temporal relationship). However, the herbal products used are insufficiently described; additionally relevant information on the dosage are lacking. Moreover, the concomitant use of acetaminophen must be considered. Acetaminophen is well known for its hepatotoxicity.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

The product is not suitable for patients with known hypersensitivity against the herbal substance, the plant family, the herbal preparation or to the excipients of the final product.

5.5.1. Use in children and adolescents

In the absence of sufficient safety data, the use of grapevine leaf in children and adolescents below 18 years of age is not recommended.

5.5.2. Contraindications

Hypersensitivity to the active substance.

5.5.3. Special warnings and precautions for use

To ensure a safe use the following statement should be labelled:

All indications:

In the absence of sufficient safety data, the use in children and adolescents below 18 years of age is not recommended.

If the symptoms worsen during the use of the medicinal product, a doctor or a pharmacist should be consulted.

WEU indication and TU indication 1:

If there is inflammation of the skin, thrombophlebitis or subcutaneous induration, severe pain, ulcers, sudden swelling of one or both legs, cardiac or renal insufficiency, a doctor should be consulted.

In the event of inadequate or unsatisfactory symptomatic response within 2 weeks, a doctor should be consulted as oedema may have alternative causes.

Cutaneous use: The product should not be used on broken skin, around the eyes or on mucous membranes.

TU indication 2:

If rectal bleeding occurs during the treatment of haemorrhoids a doctor or a qualified health care practitioner should be consulted.

In the event of inadequate or unsatisfactory symptomatic response within 1 week, a doctor should be consulted.

TU indication 3:

In the event of inadequate or unsatisfactory symptomatic response within 1 week, a doctor should be consulted as oedema may have alternative causes.

5.5.4. Drug interactions and other forms of interaction

Not known

Drug interactions from clinical trials or case studies have not been reported so far.

5.5.5. Fertility, pregnancy and lactation

Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use of grapevine leaf during pregnancy and lactation is not recommended.

No fertility data available.

5.5.6. Overdose

No data available.

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

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

5.5.8. Safety in other special situations

Not applicable

5.6. Overall conclusions on clinical safety

The safety profile of grapevine leaf extracts can be judged as good from clinical studies and from its long term use and market availability. The available literature, on pharmacological and toxicological studies, does not give rise to safety concerns. The proof of safety is further supported by PSURs data and the safety data obtained during the clinical trials.

Overall, *Vitis vinifera,* leaves can be considered as safe in herbal medicinal products.

6. Overall conclusions (benefit-risk assessment)

Well-established use

The clinical efficacy and safety of the dry aqueous extract of grapevine leaves (4-6:1) has been demonstrated in appropriately designed clinical trials and is supported by extensive use of the product in the market since 1999.

The above referred extract showed an influence to the microcirculation and transcutaneous oxygen pressure at the predominantly affected perimalleolar area of the leg in chronic venous insufficiency (CVI) patients who were treated for 6 weeks. Moreover, the assayed grapevine leaves extract has shown to lead to a reduction of the volume of oedema and to improvement of the typical subjective symptoms of CVI such as tired, heavy and swollen legs or pain and tension in the legs.

In several of the clinical trials objective and subjective symptoms of CVI appeared to be effectively reduced. The trials were performed according to ICH-GCP.

An additional placebo-controlled and open safety and tolerability trial provided supportive evidence for the efficacy.

This extract can therefore be regarded as an active substance with a well-established medicinal use.

The use of herbal medicinal products prepared with this extract is not recommended during pregnancy and lactation and should not be taken in children and adolescents under 18 years of age, due to the lack of adequate data.

The proposed indication is:

- Herbal medicinal product for treatment of chronic venous insufficiency, which is characterised by swollen legs, varicose veins, a feeling of heaviness, pain, tiredness, itching, tension and cramps in the calves.

Traditional use

Herbal preparations from grapevine leaves have been widely used. The safe use of the herbal preparations can be concluded on the basis of the well-known, long-lasting and traditional use of preparations of grapevine leaves in the folk medicine and as registered medicines. Based on the evaluation and assessment of the available documentation on the traditional medicinal use and safety, the use as herbal tea or in other oral dosage forms, and the aqueous extracts of grapevine leaf are acceptable as traditional herbal medicinal products.

Sufficient data are available to develop a European Union monograph on the traditional use of *Vitis vinifera* L., leaves. The indications are suitable for self-medication. The proposed indications are:

- Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances.
- Traditional herbal medicinal product for symptomatic relief of itching and burning associated with haemorrhoids after serious conditions have been excluded by a medical doctor.
- Traditional herbal medicinal product for symptomatic treatment of cutaneous capillary fragility.

The use of the traditional herbal medicinal products is not recommended during pregnancy and lactation and should not be taken in children and adolescents under 18 years of age.

The minimum required data on mutagenicity (Ames test) are available for the soft water extract (2.5-4:1). Therefore, inclusion of this herbal preparation in the European Union list of traditional herbal substances and preparations is recommended.

Annex

List of references