15 January 2020
EMA/HMPC/638244/2018
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Aesculus hippocastanum* L., semen

Final – Revision 1

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)	*Aesculus hippocastanum* L., semen
Herbal preparation(s)	*Well-established use:* a) Dry extracts (extraction solvent ethanol 40-80% V/V) standardised to contain 6.5-10% triterpene glycosides, calculated as protoaescigenin *Traditional use:* a) Dry extract corresponding to a specified amount of triterpene glycosides, calculated as protoaescigenin, extraction solvent ethanol 25-50% V/V b) Liquid extract (DER 1:3.5-5), extraction solvent 50% ethanol V/V c) Dry extract (DER 5-10:1), extraction solvent methanol 80% V/V d) Dry extract (DER 5-8:1), extraction solvent methanol 80% V/V e) Dry extract (DER 4.5-5.5:1), extraction solvent ethanol 50% V/V f) Dry extract (DER 5-7:1), extraction solvent ethanol 60% V/V g) Liquid extract (DER 1:1.5-2.5), extraction

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		solvent ethanol 55% V/V h) Liquid extract (DER 1:2), extraction solvent ethanol 19% m/m i) Dry extract (DER 3-6:1), extraction solvent water
Pharmaceutical form(s)		Herbal preparations in oral dosage forms for modified or immediate release (well-established use) Herbal preparations in semi-solid dosage forms for cutaneous use (traditional use) Herbal preparations in liquid or solid dosage forms for oral use (traditional use)
First assessment	Rapporteur	P Claeson
	Peer-reviewer	M Delbò
Revision	Rapporteur	E Svedlund
	Peer-reviewer	A Assisi

Table of contents

Abbreviations

ADP	Adenosine diphosphate
ALP	Alkaline phosphatase
AUC	Area under the curve
ATC	Anatomical Therapeutic Chemical classification system
BUN	Blood urea nitrogen
CI	Confidence interval
CL	Clearance
Cmax	Maximum plasma concentration
CosIng	European Commission database for information on cosmetic substances and ingredients
CT	Computed tomography
CYP	Cytochrome P450 enzymes
CVI	Chronic venous insufficience
DER	Drug Extract Ratio
EDQM	European Directorate for the Quality of Medicines and Healthcare
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ERC	Endoscopic retrograde cholangiography
ESCOP	European Scientific Cooperative on Phytotherapy
EU	European Union
Eur. Com.	European Commission
GTP	Guanosine triphosphate
HCSE	Horse chestnut seed extract
HMP	Herbal Medicinal Products
HMPC	Committee on Herbal Medicinal Products
i.p.	intraperitoneal
i.v.	intravascular
IVC	Inferior vena cava
LC	Liquid chromatography
LD	Lethal dose
LE	List entry (HMPC)
LoR	List of references
MA	Marketing Authorisation
MLWP	Monographs and List entries Working Party (HMPC)
MS	Mass spectrometry
MS(s)	Member State(s)
NSAIDs	Nonsteroidal anti-inflammatory drugs
PGF2α	Prostaglandin F2 alpha
PhV	Pharmacovigilance
Ph. Eur.	European Pharmacopoeia
RCT(s)	Randomised Controlled Trial(s)
RIA	Radio immunoassay
SD	Sprague Dawley
$t_{1/2}$	Elimination half-time
THMP	Traditional Herbal Medicinal Product
TU	Traditional Use
TUR	Traditional Use Registration

WEU	Well-established Use
WMD	Weighed mean difference

Introduction

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

- Herbal substance(s)

The herbal substance is the whole or fragmented, dried, ripe seeds of *Aesculus hippocastanum* L. According to the definition in the Ph. Eur., the herbal substance should contain a minimum 1.5% of triterpene glycosides, expressed as protoaescigenin ($C_{30}H_{50}O_6$; Mr 506.7) (dried drug) (Ph. Eur. monograph ref.: 1830).

In the first version of the assessment report, the herbal substance was described as the dried seeds of *Aesculus hippocastanum* L., containing not less than 3.0% of triterpene glycosides, expressed as anhydrous aescin ($C_{55}H_{86}O_{24}$; M_r 1131) and calculated with reference to the dried drug (Ph. Eur. draft monograph 1995).

- Herbal preparation(s)

The following herbal preparations have been reported as constituents of medicinal products on the market in the EU/EEA Member States (for further information see section 2 "Data on medicinal use"):

a) Dry extract corresponding to a specified amount of triterpene glycosides*, extraction solvent ethanol 25-50% V/V

b) Liquid extract (DER 1:3.5-5), extraction solvent ethanol 50% V/V

c) Dry extract (DER 5-10:1), extraction solvent methanol 80% V/V

d) Dry extract (DER 5-8:1), extraction solvent methanol 80% V/V

e) Dry extract (DER 4.5-5.5:1), extraction solvent ethanol 50% V/V

f) Dry extract (DER 5-7:1), extraction solvent ethanol 60% V/V

g) Liquid extract (DER 1:1.5-2.5), extraction solvent ethanol 55% V/V

h) Liquid extract (DER 1:2), extraction solvent ethanol 19% m/m

i) Dry extract (DER 3-6:1), extraction solvent water

j) Dry extract (extraction solvent ethanol 40-80% V/V) standardised to contain 6.5-10% triterpene glycosides*

* In the assessment report of the first version of the monograph, the hydroalcoholic extract (40-60% ethanol) was defined to contain not less than 16.0% and not more than 20.0% of glycosides of triterpenes, expressed as anhydrous aescin ($C_{55}H_{86}O_{24}$; M_r 1131) and calculated with reference to the dried extract (Ph. Eur. draft monograph 1996). In 2017 the Ph. Eur. monographs on Horse-chestnut and Horse-chestnut dry extract, standardised were updated. The determination of the content of triterpene glycosides was changed from a non-specific colorimetric method (method A), which, in addition, prescribes the use of chloroform and ether, to the more specific LC method (method B). In order to relate the content of triterpene glycosides determined by the two different methods, they were published in Pharmeuropa and users were requested to compare the content obtained with both assay methods A and B. To guarantee comparability between the results obtained by the different laboratories, aescin qualified sample for absorbance assay and aescin qualified sample for LC assay were made available by the EDQM and had to be used to obtain data. The results were evaluated by the Group of Experts concerned and a new lower acceptance criteria for the content of triterpene

glycosides using the more specific method B (LC Assay) was determined. Method A (absorption assay) was suppressed after enquiry in Pharmeuropa and only method B (LC assay) was published in the Ph. Eur.

Assessor's comment:

The new definitions of the Horse-chestnut and Horse-chestnut dry extract in the Ph. Eur. has been carefully analysed by the EDQM to reflect the extracts in authorised medicinal products on the EU market. The new analytical method in the Ph.Eur. monograph does not change the actual quality of the extract (see figure 1). Thus, the herbal substance and herbal preparation in the EU herbal monograph on A. hippocastanum L.*, semen, have been updated accordingly. The correlation factor (ratio method A:B) is approximately 2.4. Hence, the well-established use dry extract should be standardised to approximately 2.4 times lower content of triterpene glycosides, i.e. 21 mg expressed as protoaescigenin instead of the previous content of 50 mg expressed as aescin (50 mg/2.4=20.8 mg≈21 mg). The same approach applies to the dry extract corresponding to a specified amount of triterpene glycosides*, extraction solvent ethanol 25-50% (V/V). This issue is further discussed in the Overview of comments received on European Union herbal monograph on Aesculus hippocastanum L., semen (EMA/HMPC/611976/2019).*

	Draft Ph. Eur. monographs reported in the first version of the assessment report	**Revised Ph. Eur. monographs (01/2017:1830 and 01/2017:1829)**
Herbal substance	The dried seeds of *Aesculus hippocastanum* L., containing not less than 3.0% of triterpene glycosides, expressed as anhydrous aescin ($C_{55}H_{86}O_{24}$; Mr 1131) and calculated with reference to the dried drug (Ph. Eur. draft monograph 1995).	Whole or fragmented, dried, ripe seeds of *Aesculus hippocastanum* L. Content: minimum 1.5% of triterpene glycosides, expressed as protoaescigenin ($C_{30}H_{50}O_6$; Mr 506.7) (dried drug).
Herbal preparation	Hydroalcoholic extract (40-60% ethanol) containing not less than 16.0% and not more than 20.0% of glycosides of triterpenes, expressed as anhydrous aescin ($C_{55}H_{86}O_{24}$; Mr 1131) and calculated with reference to the dried extract (Ph. Eur. draft monograph 1996).	Standardised dry extract produced from Horse-chestnut. Content: 6.5% to 10.0% of total triterpene glycosides, expressed as protoaescigenin ($C_{30}H_{50}O_6$; Mr 506.7) (dried extract). The extract is produced from the herbal drug by a suitable procedure using a hydroalcoholic solvent equivalent in strength to ethanol (40-80% V/V).

Figure 1: Overview of the draft Ph. Eur. monographs on Horse-chestnut and Horse-chestnut dry extract and the revised monographs.

- Constituents

The seeds of *A. hippocastanum* L. contain 3-10% of a mixture of acylated triterpene glycosides (saponins). These are based on two aglycones–protoaescigenin and barringtogenol C, which differ only at C-24, which is hydoxylated in protoaescigenin (Fig. 2). In the glycosides both aglycones are esterified at C-22 with acetic acid and at C-21 with either angelic acid or tiglic acid.

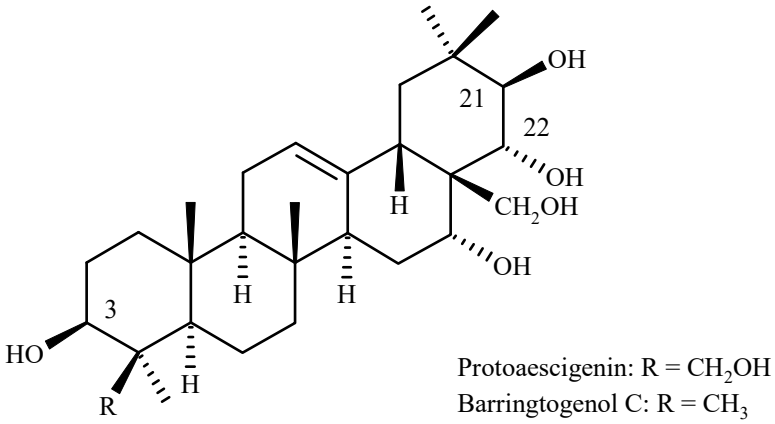

Figure 2: Structure of aescin-aglycones

All saponins have a trisaccharide group at C-3 consisting of glucuronic acid, combined with glucose, galactose or xylose. More than 30 different saponins have been identified in aescin (Fig. 3). The main component constitutes about 60% of the mixture of saponins and is composed of protoaescigenin, esterified with angelic acid at C-21 and with acetic acid at C-22. The sugar part is a trisaccharide consisting of glucuronic acid and 2 molecules of glucose, constituting a 2(β-D-glucopyranosido)-4-β-D-glucopyranosido)β-D-glucuronopyranoside, forming a glycoside bond with C-3 β-OH of the protoaescigenin aglycone (Wulff and Tschesche, 1969).

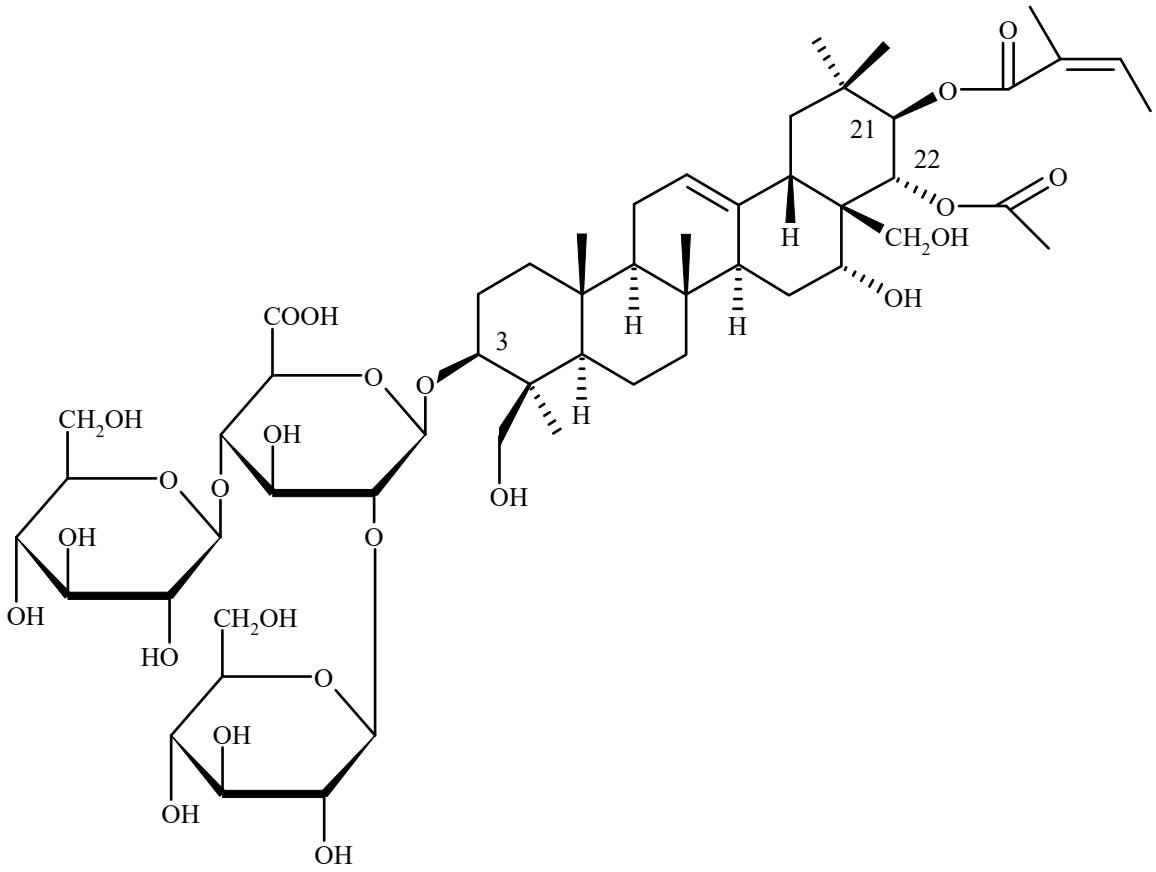

Figure 3: Structure of the main saponin in aescin

Three fractions of aescin, denoted as crypto-, α-, and β-aescin have been described in the literature. Cryptoaescin contains C-28-O-acetyl saponins, and β-aescin contains C-22-O-acetyl saponins, whereas α-aescin is a mixture of crypto- and β -aescin (ESCOP, 2003). β-aescin has haemolytic activity, whereas cryptoaescin has not.

Other constituents include flavonoids (0.3%), principally di-and triglycosides of quercetin and kaempferol, sterols, essential oil and a high proportion of starch (30–60%) (ESCOP, 2003). Coumarin derivatives (aesculin and fraxetin) are present in other parts of *A. hippocastanum* L., but not in the seeds or the seed shell (Blaschek *et al.*, 1992).

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

Not applicable

1.2. Search and assessment methodology

Revision 1:

Scientific databases: PubMed (Using the Mesh terms "Aesculus" and "Escin" from 2007 to present, Search date: 19 March 2018), Embase (Using the terms "Aesculus hippocastanum" and "Escin" from 2007 to present, Search date: 19 March 2018)

Medical databases: Cochrane Database of Systematic Reviews (March 2018)

Toxicological databases: ToxNet (March 2018)

Pharmacovigilance resources: EudraVigilance 31 August 2018, active substance (high level): contain *Aesculus hippocastanum*

Data from EU and non-EU regulatory authorities: CosIng (the European Commission database for information on cosmetic substances and ingredients) (March 2018)

Other resources: Literature submitted by Interested Parties

1.3. Major changes introduced in the first revision

The Ph. Eur. monographs on Horse-chestnut and Horse-chestnut dry extract, standardised have been updated. Accordingly, the herbal substance and herbal preparation in the EU herbal monograph on *A. hippocastanum* L., semen, have been updated in section 1.1. 'Description of the herbal substance(s), herbal preparation(s) or combinations thereof'.

According to the market overview, there are several new horse chestnut seed preparations for oral and cutaneous use that fulfils the criteria of medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA. These herbal preparations have been included in the traditional use monograph (see section 2 "Data on medicinal use").

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** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
WEU			
Dry extract (DER 5-6.1:1, extraction solvent ethanol 60%, m/m or V/V not specified) 63-90 mg extract corresponding to 20 mg aescin	Chronic venous insufficiency grade I and II according to Widmer with the symptoms pain in the legs, heavy legs, venous oedemas, cramp in the calf and varicose veins	Film-coated tablet 1 tablet contains: 63-90 mg extract corresponding to 20 mg aescin oral use; 1-2 tablets 3 times daily	Between 2000–2011, WEU, Austria
Dry extract DER 4.5-5.5:1, extraction solvent ethanol 50% m/m 263.2 mg dry extract corresponding to 50 mg anhydrous aescin	Symptoms of chronic venous insufficiency like pain in the legs, heavy legs, cramps in the calf during night, pruritus and venous oedema	Prolonged release capsules 1 capsule contains: 263.2 mg dry extract corresponding 50 mg anhydrous aescin oral use; 1 tablet 2 times daily	Between 2000–2009, WEU, Austria
Dry extract DER 4.5-5.5:1, extraction solvent ethanol 50% V/V (=43% m/m)	Symptoms of chronic venous insufficiency like varicose veins (also during pregnancy), phlebitis, thrombophlebitis, pain in the legs, heavy legs, cramps in the	Prolonged release capsules 1 capsule contains 50 mg aescin oral use; 1 capsule 2 times daily	Since 1989, WEU, Austria

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
	calf during night, pruritus and venous oedema		
Dry extract DER 5:1, extraction solvent ethanol 50% V/V (=43% m/m) 36.5-42 mg dry extract corresponding to 7.60 mg aescin	Varicosis, phlebitis, thrombophlebitis, ulcus cruris, and associated symptoms like pain in the legs, heavy legs, cramps in the calf during night, pruritus and venous oedema	Ointment 1 g contains 36.5-42 mg dry extract corresponding to 7.60 mg aescin cutaneous use: several times daily, during night cover with dressing material	Between 1968–2011, WEU, Austria
Dry extract DER 4.5-5.5:1, extraction solvent ethanol 50% V/V	Symptoms of venous disorders like heavy legs, pruritus, pain in the legs	Oral solution 1 ml contains dry extract corresponding to 20 mg aescin 3 times daily 5-10 drops corresponding to 15-30 mg aescin daily	Between 1961–2007, WEU, Austria
Dry extract DER 4.5-5.5:1, extraction solvent ethanol 50% V/V (=43% m/m)	Symptoms of venous disorders like heavy legs, pruritus, pain in the legs, oedemas, cramps in the calf	Prolonged release capsules 1 capsule contains dry extract corresponding to 75 mg aescin oral use; 2 times daily 1 capsule for 6-8 weeks, then switch to 50 mg	Between 1994–2012, WEU, Austria
Standardised dry extract (DER not specified), extraction solvent ethanol 60% V/V 240–290 mg dry extract corresponding to 50 mg triterpene	Symptoms of chronic venous insufficiency occurring in case of varicose veins such as swollen legs, feeling of heaviness, legs pain, cramps in the calves at night, feeling of itching and tension in legs	1 capsule contains 240–290 mg dry extract (corresponding to 50 mg triterpene glycosides expressed as aescin) 1 capsule twice daily	2001-2005, national legislation, Czech Republic

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
glycosides expressed as aescin			
Dry extract (DER not specified), extraction solvent ethanol 60% m/m 157.5-225 mg dry extract corresponding to 50 mg triterpeneglycosides calculated as aescin	Natural medicinal product for relief of swollen legs, a feeling of heaviness, itching, cramps and restless legs. Treatment of chronic venous insufficiency must only be done after consultation with a doctor.	1 entero-coated tablet contains: 157.5-225 mg extract 1 tablet 2 times daily The patient should contact a doctor, if the symptoms are getting worse during the use of the product, or, if the effect is unsatisfactory after 6 weeks of treatment in order to determine the cause of oedema. Long-term use is possible in consultation with a doctor.	2005, MA, Denmark
Dry extract DER 4.5-5.5:1, extraction solvent: ethanol 50% V/V 240-290 mg corresponding to 50 mg triterpene glycosides, calculated as anhydrous aescin	Symptoms of chronic venous insufficiency (CVI), usually associated with varicose veins, such as oedema of the legs and subjective disturbances, for example pain, heavy or tired legs, nocturnal cramps in the calves, sensation of tension, or itching	1 sustained release capsule contains: 240-290 mg, corresponding to 50 mg triterpene glycosides, calculated as anhydrous aescin *Adults:* 1 capsule twice daily (in the morning and in the evening). The capsules should be swallowed whole with sufficient amounts of liquid before meals. Treatment may be taken long-term	Since 2001, WEU, Norway
Dry extract (DER not specified), extraction solvent ethanol 60% V/V	Herbal medicinal product for treatment of chronic venous insufficiency, which is characterised by swollen legs, varicose	Gastro-resistant tablets 1 tablet contains 157.5 to 225.0 mg of dry extract, corresponding to 50 mg triterpene	Since 2010, WEU, Slovenia

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
157.5 to 225.0 mg dry extract corresponding to 50 mg triterpene glycosides, calculated as anhydrous aescin	veins, a feeling of heaviness, pain, tiredness, itching, tension and cramps in the calves	glycosides, expressed as anhydrous aescin. Posology 1 tablet twice daily Duration of use: In accordance with the HMPC monograph	
Dry extracts, DER 4.5-5.5:1, extraction solvent ethanol 58 % V/V 263.2 mg dry extract corresponding to 50 mg triterpene glycosides, calculated as aescin	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	Prolonged release tablets 1 tablet contains 263.2 mg of dry extract standardised to contain 50 mg triterpene glycosides, calculated as aescin Posology: 1 tablet twice per day (daily dose 100 mg of aescin) Duration of use: 4-8 weeks	Since 1999, MA, Spain
Dry extract DER 4.5-5.5:1 extraction solvent: ethanol 50% V/V 240–290 mg dry extract corresponding to 50 mg of triterpene glycosides, calculated as anhydrous aescin	Herbal medicinal product for the relief of symptoms caused by disturbances in the venous blood flow in the legs, so called chronic venous insufficiency, which is often accompanied by varicose veins, and which is characterised by swelling, a feeling of heaviness, pain, tiredness, itching, tension and cramps in the calf. To be used after chronic venous insufficiency	Hard prolonged-release capsule *Adults and elderly:* 1 capsule 2 times daily 1 capsule contains 240–290 mg of; dry extract, which corresponds to 50 mg of triterpene glycosides, calculated as anhydrous aescin At least 4 weeks of treatment may be required	Since 2002, WEU, Sweden

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
	has been established by a physician	before any beneficial effect is observed. Long-term use is possible in consultation with a doctor.	
Standardised dry extract DER 4.5-5.5:1, extraction solvent: ethanol 50% m/m 240-290 mg dry extract corresponding to 50 mg triterpene glycosides, calculated as anhydrous aescin	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	Prolonged-release capsule, hard 1 capsule contains 240-290 mg dry extract standardised to 50 mg triterpene glycosides, calculated as anhydrous aescin *Adults:* 1 capsule 2 times daily (daily dose 100 mg aescin) After consultation of a physician, long-term use is possible	Since 1983, WEU, Germany
Standardised dry DER 5.3-7.7:1, extraction solvent: methanol 80% m/m 154.4-249.4 mg dry extract corresponding to 40 mg triterpene glycosides, calculated as anhydrous aescin	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 and for swelling of soft tissue following surgery and trauma	Capsule, soft 1 capsule contains 154.4-249.4 mg dry extract standardised to 40 mg triterpene glycosides, calculated as anhydrous aescin *Adults:* 1 capsule 3 times daily	Since 1992, WEU, Germany
Standardised dry extract DER 5-8:1, extraction solvent: methanol 80% m/m 166.7-250 mg dry extract	Herbal medicinal product for treatment of chronic venous insufficiency, which is characterised by swollen legs, varicose veins, a feeling of heaviness, pain,	Capsule, soft 1 capsule contains 166.7-250 mg dry extract standardised to 40 mg triterpene glycosides,	Since 1993, WEU, Germany

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
corresponding to 40 mg triterpene glycosides, calculated as anhydrous aescin	tiredness, itching, tension and cramps in the calves and for swelling of soft tissue following surgery and trauma	calculated as anhydrous aescin *Adults*: 1 capsule 3 times daily (daily dose 120 mg aescin)	
Standardised dry extract (DER not specified), extraction solvent: ethanol 50% m/m 263.2 mg dry extract corresponding to 50 mg triterpene glycosides, calculated as anhydrous aescin	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	Prolonged-release tablet 1 tablet contains 178.5-263.2 mg dry extract standardised to 50 mg triterpene glycosides, calculated as anhydrous aescin *Adults*: 1 tablet 2 times daily (daily dose 100 mg aescin) After consultation of a physician, long-term use is possible.	At least since 1976, WEU, Germany
Standardised dry extract (DER not specified), extraction solvent: ethanol 68% V/V 146.52-202.43 mg dry extract corresponding to 50 mg triterpene glycosides, calculated as anhydrous aescin	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	Capsule, hard 1 capsule contains 146.52-202.43 mg dry extract standardised to 50 mg triterpene glycosides, calculated as anhydrous aescin *Adults*: 1 capsule 2 times daily (daily dose 100 mg aescin) After consultation of a physician, long-term use	At least since 1976, WEU, Germany

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
		is possible.	
Standardised dry extract (DER not specified), extraction solvent: ethanol 60% V/V 130.05 mg dry extract corresponding to 20 mg triterpene glycosides, calculated as anhydrous aescin	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	Film-coated tablet 1 tablet contains 89.6-130.05 mg dry extract standardised to 20 mg triterpene glycosides, calculated as anhydrous aescin *Adults*: Dosage: 2-1-2 (daily dose 100 mg aescin) For use longer than 4 weeks please consult a doctor.	Since 2011, WEU, Germany
Standardised dry extract DER 5-7:1, extraction solvent: ethanol 50% m/m 105.0-141.0 mg dry extract corresponding to 30 mg triterpene glycosides, calculated as anhydrous aescin	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	Coated tablet 1 tablet contains 93.3-162.1 mg dry extract standardised to 30 mg triterpene glycosides, calculated as anhydrous aescin *Adults*: 1 tablet 2 times daily In severe cases: 2 tablets 2 times daily For use longer than 4 weeks a doctor should be consulted.	Since 2012, WEU, Germany
Standardised dry extract DER 5-8:1, extraction solvent: methanol 80% V/V	Herbal medicinal product for treatment of chronic venous insufficiency, which is characterised by swollen legs, varicose veins, a feeling of heaviness, pain,	Coated tablet 1 tablet contains 200-235 mg dry extract standardised to 50 mg triterpene glycosides,	Since 2012, WEU, Germany

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
235 mg dry extract corresponding to 50 mg triterpene glycosides, calculated as anhydrous aescin	tiredness, itching, tension and cramps in the calves	calculated as anhydrous aescin Adults: 1 tablet 2 times daily (daily dose 100 mg aescin) After consultation of a doctor, long-term use is possible.	
Standardised dry extract DER 5-7:1, extraction solvent: ethanol 50% m/m 93.3-162.1 mg dry extract corresponding to 30 mg triterpene glycosides, calculated as anhydrous aescin	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	Coated tablet 1 tablet contains 93.3-162.1 mg dry extract standardised to 30 mg triterpene glycosides, calculated as anhydrous aescin *Adults*: 1 tablet 2 times daily In severe cases: 2 tablets 2 times daily For use longer than 4 weeks a doctor should be consulted.	Since 2015, WEU, Germany
Dry extract DER 4.5-5.5:1, extraction solvent ethanol 50% V/V	Chronic venous insufficiency symptoms like lower extremities oedemas with corresponded symptoms of pain, heavy legs, tension sensation, calf cramps and pruritus	Prolonged-release capsule, 1 capsule contains 240-290 mg dry extract corresponding to 50 mg anhydrous aescin *Adults:* 1 capsule 2 times daily, before meals Long term use possible after doctor's	Since 1999, WEU, Poland

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
		consultation.	
Dry extract (DER not specified), extraction solvent ethanol 60% V/V 100-125 mg dry extract corresponding to 20 mg triterpene glycosides, calculated as anhydrous aescin	In symptoms of chronic venous insufficiency as: swelling lower legs, calf cramps, pruritus and feeling of heavy legs	Capsule, hard 1 capsule contains 100-125 mg dry extract corresponding to 20 mg anhydrous aescin *Adults:* 3 capsules in the morning and 2 capsules in the evening. Advised period of treatment at least 4 weeks. Long term use possible after doctor's consultation. Not intended for children and adolescents.	Since 2001, WEU, Poland
TUR			
Dry extract DER 4.5-5.5:1, extraction solvent ethanol 50% V/V	Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances.	Cream 1 g contains 380 mg dry extract *Adults:* Cutaneous use; 1-3 times daily	Since 2010, TU, Austria
Dry extract (DER not specified), extraction solvent ethanol 60% V/V Standardised to 16-22 % of saponins expressed as aescin	a) for relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances. b) for relief of signs of bruises, such as local oedema and haematoma.	Indication a) for adults only Indication b) for adolescents and adults Cutaneous cream 1 g of the cream contains 50 mg of the extract corresponding to 10 mg of saponins expressed	Since 1992, TU in 2011, Czech Republic

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
		as aescin) Posology: to be spread on the affected area once to three times daily Duration of use: Indication a) 2 weeks Indication b) 5 days	
Dry extract DER 3-6:1, extraction solvent 60% ethanol V/V	- Pains and mild oedema of legs caused by venostasis. - Problems related to venous varices. - Bruises caused by injuries or injection or infusion administration.	Dermal cream 1 g of cream contains 50 mg dry extract in, equivalent to 1% aescin To be applied in a thin layer to the skin around and to the affected area. If necessary, it may be held in place with an elastic bandage.	Since 1992, Slovakia
Dry extract (DER not specified), extraction solvent: 60% V/V ethanol	Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances and for relief of signs of bruises, such as local oedema and haematoma	Cream 1 g of cream contains 50 mg of dry extract standardised to 10 mg aescin. Posology Apply a thin layer of cream 1-3 times daily	Since 1997, Slovenia, from 2011 TU registration
Dry extract DER 5-10:1, extraction solvent:	Traditionally used for the improvement of condition in tired legs	Gel 1 g gel contains 32 mg dry extract	At least since 1976, TU, Germany

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
methanol 80% V/V		>12 years: application of a thin layer on the intact skin of the affected areas 2-3 times daily If symptoms persist longer than 2 weeks, a doctor should be consulted.	
Dry extract DER 5-8:1, extraction solvent: methanol 80% V/V	Traditionally used for the improvement of condition in tired legs	Coated tablet 1 tablet contains 71.43 mg dry extract >12 years: 1 tablet 1 time daily Duration of use: 2-4 weeks	At least since 1976 until 2011, TU, Germany
Dry extract DER 4.5-5.5:1, extraction solvent: ethanol 50% V/V	Traditionally used for the improvement of condition in tired legs	Oral liquid 1 ml (=1.06 g=20 drops) contains 100 mg dry extract >12 years: 5 drops 3 times daily In principle there are no limitations for the duration of use.	At least since 1976, TU, Germany
Liquid extract DER 1:1.5-2.5, extraction solvent: ethanol 55% V/V	Traditionally used for the improvement of condition in tired legs	Oral liquid 20 ml contain 13.6 g liquid extract >12 years: 10 drops (approximately 0.3 g) 2 times daily Traditional duration of use is 2-4 weeks.	At least since 1976 until 2017, TU, Germany

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
Dry extract DER 3-6:1, extraction solvent: water	Traditionally used for the improvement of condition in tired legs	Coated tablet 1 tablet contains 99 mg dry extract adults: 1 tablet 2 times daily If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor should be consulted.	At least since 1976, TU, Germany
Liquid extract DER 1:2, extraction solvent: ethanol 19% m/m	Traditionally used for the improvement of condition in tired legs and for the strengthening of venous system	Oral liquid 100 ml (=103.3 g) contain 770 mg liquid extract *Adults*: 20 ml 3-4 times daily If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor should be consulted.	At least since 1976, TU, Germany
Dry extract DER 4.5-5.5:1, extraction solvent: ethanol 50% V/V	Traditionally used for the improvement of condition in tired legs	Cream 1 g cream contains 38 mg dry extract adults: application of a thin layer on the intact skin of the affected areas 1-3 times daily If the symptoms persist longer than 2 weeks during the use of the medicinal product, a	At least since 1976 until 2013, TU, Germany

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
		doctor should be consulted.	
Dry extract DER 5-8:1, extraction solvent: methanol 80% V/V	Traditionally used for the improvement of condition in tired legs	Cream 1 g cream contains 8.5 mg dry extract adults: application of a thin layer on the intact skin of the affected areas 1-several times daily If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor should be consulted.	At least since 1976, TU, Germany
Liquid extract, DER 1:3.5-5, extraction solvent: ethanol 50% V/V	Traditionally used for the improvement of condition in tired legs	Cream 1 g cream contain 200 mg liquid extract *Adults*: application of a thin layer on the affected areas 3 times daily If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor should be consulted.	At least since 1976 until 2011, TU, Germany
Dry extract DER 5-10:1, extraction solvent: methanol 80% V/V	In line with the monograph (TU) adopted in 2009.	Gel 1 g (=1.03 ml) gel contains 32 mg dry extract adults: application of a thin layer on the intact	At least since 1976 until 2011, TU, Germany

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
		skin of the affected areas 2-3 times daily If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor should be consulted.	
Dry extract DER 5-7:1, extraction solvent: ethanol 60% V/V	Traditionally used for the improvement of condition in tired legs	Cream 1 g cream contains 16 mg dry extract *Adults*: application of a thin layer on the affected areas 1-3 times daily If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor should be consulted.	At least since 1976, TU, Germany
Dry extract DER 4.5-5.5:1, extraction solvent: ethanol 50% V/V	Traditionally used for the improvement of condition in tired legs	Cream 1 g cream contains 38 mg dry extract adults: application of a thin layer on the intact skin of the affected areas 1-3 times daily If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor should be consulted.	Since 2016, TU, Germany

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
Dry extract DER 5-9:1, extraction solvent: ethanol 80% V/V	Traditionally in symptoms of chronic venous insufficiency like swollen legs, calf cramps, pruritus, heavy legs, varicosis	Coated tablets 1 tablet contains 167 mg of the dry extract. Adults and adolescents over 12 years: 1 tablet 3 times daily, after meals.	Since 2004, TU, Poland
Comminuted herbal substance	Traditionally in chronic venous insufficiency symptoms and varicosity	Tablets 1 tablet contains 340 mg of comminuted herbal substance. *Adults*: 2 tablets 3 times daily. If the symptoms perceive during 3-4 weeks of use the product, a doctor should be consulted	Since 2004, TU, Poland
Dry extract DER 4:1, extraction solvent: ethanol 80% V/V	Traditionally used in symptoms of heavy legs with legs oedema and feeling of tired legs, caused by mild venous circulation disturbances	Ointment, 1 g of ointment contains 10 mg of triterpene glycosides counted as aescin. Adults: Apply a thin layer on the skin.	Since 2010, TU, Poland

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)

An ethanolic liquid extract of fresh unripe seeds (DER 1:1), extraction solvent ethanol 96% V/V used traditionally in symptoms of chronic venous insufficiency (lower limbs swellings, calf cramps, pruritus, pain, heavy legs) and supporting in varicosis has been on the market in Poland since 1987. The oral posology (adults only) is 2.5 ml liquid extract (corresponding to 2.34 g of fresh unripe seeds) in a small amount of water 3 times daily.

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

No information available.

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

The medicinal use of horse-chestnut has been documented as a venotonic for treatment of varicose veins (Steinegger and Hänsel, 1972; Newall *et al.,* 1996; Mills and Bone*,* 2000; Ernst *et al.,* 2001), hematoma (Rote Liste, 1980; Ernst *et al.,* 2001), and venous congestion (Wren, 1988). Alcoholic extracts of the seeds of the horse chestnut tree have been used for their venotonic effects since the beginning of the 1900s (Bombardelli *et al*., 1996).

Topically/externally, horse-chestnut extract (aescin 1%) has been used for contusions, non-penetrating wounds and sport injuries involving oedema (Rote Liste, 1980; Mills and Bone*,* 2000; Bradley, 2006).

Aescin has been used orally and topically in the prevention and treatment of various peripheral vascular disorders including traumatic swellings and post-operative oedema (Reynolds and Prasad, 1982).

Table 2: Overview of historical data

Herbal preparation	**Documented use/Traditional use**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Reference**
Horse-chestnut seed extract DER and extraction solvent not specified	Varices, thrombophlebitis, oedema and congestions in the extremities, bruises, hematomas	Cutaneous use Gel 1 g contains 150 mg extract (corresponding to 18 mg aescin) Apply thin layers on affected areas several times daily	Rote Liste 1980

2.3. Overall conclusions on medicinal use

Table 3: Overview of evidence on period of medicinal use

Herbal preparation **Pharmaceutical form**	**Indication**	**Strength** **Posology**	**Period of medicinal use**
WEU			
Dry extract DER 4.5-5.5:1, extraction solvent: ethanol 50-58% V/V	a) 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. b) Symptoms of chronic venous insufficiency like varicose veins (also during pregnancy), phlebitis, thrombophlebitis, pain in the legs, heavy legs, cramps in the calf during night, pruritus and venous oedema.	Prolonged release capsule/tablet One capsule/tablet contains 50 mg of triterpene glycosides, calculated as anhydrous aescin *Adults and elderly*: 1 capsule/tablet 2 times daily	a) At least since 1976, Germany Since 1996, Germany Since 1999, Spain Since 1999, Poland Since 2001, Norway Since 2002, Sweden b) Since 1989, Austria
Dry extract DER 4.5-5.5:1, extraction solvent ethanol 50% V/V	a) Symptoms of venous disorders like heavy legs, pruritus, pain in the legs. b) Traditionally used for the improvement of condition in tired legs.	Oral solution a) 1 ml contains dry extract corresponding to 20 mg aescin 3 times daily 5-10 drops corresponding to 15-30 mg aescin daily b) 1 ml (20 drops) contains 100 mg dry extract >12 years: 5 drops 3 times daily	a) Between 1961–2007, Austria b) At least since 1976, Germany
Dry extract (DER not specified), extraction solvent: ethanol 60% m/m.	Natural medicinal product for relief of swollen legs, a feeling of heaviness, itching, cramps and restless legs	1 entero-coated tablet contain: 157.5-225 mg extract corresponding to 50 mg triterpeneglycosides calculated as	Since 2005, Denmark

Herbal preparation **Pharmaceutical form**	**Indication**	**Strength** **Posology**	**Period of medicinal use**
	Treatment of chronic veneus insufficens must only be done after consultation with a doctor	aescin Adults and elderly: 1 tablet 2 times daily	
Standardised dry extract (DER not specified), extraction solvent: ethanol 68% V/V	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	Capsule, hard 1 capsule contains 50 mg triterpene glycosides, calculated as anhydrous aescin *Adults*: 1 capsule 2 times daily	At least since 1976, Germany
Standardised dry extract DER 5-8:1, extraction solvent: methanol 80% V/V	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 and for swelling of soft tissue following surgery and trauma	Capsule, soft 1 capsule contains 40 mg triterpene glycosides, calculated as anhydrous aescin *Adults*: 1 capsule 3 times daily	Since 1992, Germany
Dry extract DER 5-6.1:1, extraction solvent 60% ethanol 63-90 mg extract corresponding to 20 mg aescin	Chronic venous insufficiency grade I and II according to Widmer with the symptoms pain in the legs, heavy legs, venous oedemas, cramp in the calf and varicose veins	Film-coated tablet 1 tablet contains: 63-90 mg extract corresponding to 20 mg aescin oral use; 1-2 tablets 3 times daily	Between 2000–2011, WEU, Austria
Dry extract (DER not provided), extraction solvent ethanol 60% V/V	In symptoms of chronic venous insufficiency as: swelling lower legs, calf cramps, pruritus and feeling of heavy legs	Capsule, hard, 1 capsule contains: 100-125 mg dry extract corresponding to 20 mg anhydrous aescin	Since 2001, Poland

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
100-125 mg dry extract corresponding to 20 mg triterpene glycosides, calculated as anhydrous aescin		*Adults:* 3 capsules in the morning and 2 capsules in the evening.	
TU			
Dry extract DER 5-10:1, extraction solvent: methanol 80% V/V	Traditionally used for the improvement of condition in tired legs	1 g gel contains 32 mg dry extract Adults and adolescents: application of a thin layer on the intact skin of the affected areas 2-3 times daily	At least since 1976, Germany
Dry extract DER 4.5-5.5:1, extraction solvent: ethanol 50% V/V	Traditionally used for the improvement of condition in tired legs	1 g cream contains 38 mg dry extract *Adults*: application of a thin layer on the intact skin of the affected areas 1-3 times daily	At least since 1976 until 2013, Germany
Dry extract DER 5-8:1, extraction solvent: methanol 80% V/V	Traditionally used for the improvement of condition in tired legs	1 g cream contains 8.5 mg dry extract *Adults*: application of a thin layer on the intact skin of the affected areas 1-several times daily	At least since 1976, Germany
Liquid extract DER 1:3.5-5, extraction solvent: ethanol	Traditionally used for the improvement of	1 g cream contains 200 mg liquid extract	At least since 1976 until

Herbal preparation **Pharmaceutical form**	**Indication**	**Strength** **Posology**	**Period of medicinal use**
50% V/V	condition in tired legs	*Adults*: application of a thin layer on the affected areas 3 times daily	2011, Germany
Dry extract DER 5-7:1, extraction solvent: ethanol 60% V/V	Traditionally used for the improvement of condition in tired legs	1 g cream contains 16 mg dry extract *Adults*: application of a thin layer on the affected areas 1-3 times daily	At least since 1976, Germany
Dry extract DER 5:1, extraction solvent ethanol 50% V/V 36.5-42 mg dry extract corresponding to 7.6 mg aescin	Varicosis, phlebitis, thrombophlebitis, ulcus cruris, and associated symptoms like pain in the legs, heavy legs, cramps in the calf during night, pruritus and venous oedema	Ointment 1 g ointment contains 36.5-42 mg dry extract corresponding to 7.60 mg aescin cutaneous use: several times daily, during night cover with dressing material	Between 1968–2011, Austria
Liquid extract DER 1:1.5-2.5, extraction solvent: ethanol 55% V/V	Traditionally used for the improvement of condition in tired legs	Oral liquid 20 ml contain 13.6 g liquid extract >12 years: 10 drops (approximately 0.3 g) 2 times daily	At least since 1976 until 2017, Germany
Dry extract DER 3-6:1, extraction solvent: water	Traditionally used for the improvement of condition in tired legs	Coated tablet 1 tablet contains 99 mg dry extract *Adults*:	At least since 1976, Germany

Herbal preparation **Pharmaceutical form**	**Indication**	**Strength** **Posology**	**Period of medicinal use**
		1 tablet 2 times daily	
Liquid extract DER 1:2, extraction solvent: ethanol 19% m/m	Traditionally used for the improvement of condition in tired legs and for the strengthening of venous system	Oral liquid 100 ml (=103.3 g) contain 770 mg liquid extract *Adults*: 20 ml 3-4 times daily	At least since 1976, Germany

Well established use monograph (oral use)

Authorised, as well as registered products containing dry extracts with DER in the range 4.5-8:1, extraction solvent ethanol >50% V/V or methanol 80% and/or standardised on the content of triterpene glycosides for the oral treatment of chronic venous insufficiency can be found on the EU market for more than 10 years. The clinical efficacy of the extracts, based on Article 10a of Directive 2001/83/EC (well-established use), is evaluated in section 4. "Clinical data".

Traditional use monograph (cutaneous use and oral use)

Based on information obtained from Member states and literature, *A. hippocastanum* L., semen containing products for cutaneous use and oral use have been in medicinal use for at least 30 years in the EU. The traditional medicinal uses can be grouped into two indications suitable for traditional herbal medicinal products:

1) Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances.

2) Traditional herbal medicinal product for relief of signs of bruises, such as local oedema and haematoma.

The following extracts and uses of horse chestnut seed fulfil the criteria for traditional use:

Indication 1)

Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances.

Cutaneous use:

- Dry extract, extraction solvent ethanol 25-50% V/V in a strength corresponding to ca 0.4% triterpene glycosides, calculated as protoaescigenin, in an ointment base, for cutaneous use.

  Assessor's comment:

  The dry extract, extraction solvent ethanol 25-50% V/V, in a strength corresponding to ca 1% aescin in an ointment base, for cutaneous use, was included in the first version of the monograph, published in 2009. A product on the Austrian market between 1968-2011, literature reference from 1980 (Rote Liste) and products on the EU market since 1992 are in support of the traditional use of extracts corresponding to ca 1% aescin, but consistent information on the extraction solvent is missing. Since there are no known safety concerns, the preparation is retained in the revision of the monograph.

  In addition, a new lower acceptance criteria for the content of triterpene glycosides using a more specific method, i.e. LC assay, have been introduced in the Ph.Eur. monographs 1830 and 1829 and the previous method, i.e. absorption assay was suppressed. A correlation factor of approximately 2.4 (ratio old method:new method) has been used in this revision. For further information see section 1.1. 'Description of the herbal substance(s), herbal preparation(s) or combination thereof'.

- Liquid extract (DER 1:3.5-5; extraction solvent: ethanol 50% V/V, 20% in an ointment base, for cutaneous use.
- Dry extract (DER 5-10:1), extraction solvent: methanol 80% V/V, 100 g gel contain 3.2 g dry extract
- Dry extract (DER 5-8:1), extraction solvent: methanol 80% V/V, 100 g cream contain 0.085 g dry extract

- Dry extract (DER 4.5-5.5:1), extraction solvent: ethanol 50% V/V, 100 g cream contains 3.8 g dry extract
- Dry extract (DER 5-7:1), extraction solvent: ethanol 60% V/V, 100 g cream contains 1.6 g dry extract

Posology: Adults and elderly: Apply a thin layer on the affected area 1-3 times per day. Apply on intact skin only.

Oral use:

- Liquid extract (DER 1:1.5-2.5), extraction solvent ethanol 55% V/V
  Posology: 300 mg 2 times daily
- Liquid extract (DER 1:2), extraction solvent ethanol 19% m/m
  Posology: 154 mg 3-4 times daily
- Dry extract (DER 3-6:1), extraction solvent water
  Posology: 99 mg 2 times daily

Indication 2)

Traditional herbal medicinal product for relief of signs of bruises, such as local oedema and haematoma.

Cutaneous use:

- Dry extract, extraction solvent ethanol 25-50% V/V, in a strength corresponding to ca 0.4% triterpene glycosides, calculated as protoaescigenin, in an ointment base, for cutaneous use.

  Assessor's comment:

  The dry extract, extraction solvent ethanol 25-50% V/V, in a strength corresponding to ca 1% aescin in an ointment base, for cutaneous use, was included in the first version of the monograph, published in 2009. A product on the Austrian market between 1968-2011, literature reference from 1980 (Rote Liste) and products on the EU market since 1992 are in support of the traditional use of extracts corresponding to ca 1% aescin, but consistent information on the extraction solvent is missing. Since there are no known safety concerns, the preparation is retained in the revision of the monograph.

  In addition, a new lower acceptance criteria for the content of triterpene glycosides using a more specific method, i.e. LC assay, have been introduced in the Ph. Eur. monographs 1830 and 1829 and the previous method, i.e. absorption assay was suppressed. A correlation factor of approximately 2.4 (ratio old method: new method) has been used in this revision. For further information see section 1.1. 'Description of the herbal substance(s), herbal preparation(s) or combination thereof'.
- Liquid extract (DER 1:3.5-5; extraction solvent: ethanol 50% V/V), 20% in an ointment base, for cutaneous use.

Posology: Adolescents over 12 years, adults and elderly: Apply a thin layer on the affected area 1-3 times per day. Apply on intact skin only.

Duration of use:

Indication 1)

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

Indication 2)

If the symptoms persist longer than 5 days during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

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

The literature on the pharmacodynamics of hydro-alcoholic extracts of *A. hippocastanum* L., semen and of aescin is extensive. Comprehensive reviews are presented by Bombardelli *et al*., 1996, Blaschek *et al.,* 1992, ESCOP (2003) and Sirtori (2001).

Anti-inflammatory and anti-oedematous effect

The following information was retrieved from ESCOP (2003): Egg albumin-induced oedema in the rat paw was significantly ($p<0.001$) inhibited by i.v. administration of aescin at 0.2 and 2.5 mg per kg body weight. I.p. administration of 4 mg/kg bw of aescin inhibited dextran-induced oedema in the rat paw model. I.v. administration to rats of aescin at 2.5 mg/kg bw prevented an increase in vascular permeability, caused by i.p. injection of egg white.

Similar results are reviewed by Blaschek *et al.* 1992: Lorenz (1960) performed an extensive investigation on several ethanolic extracts of horse-chestnut seeds. On parenteral application, the extracts inhibited ovalbumin-induced oedema of the rat paw. Oral doses had no effect. The effect was long-lasting and reached its peak 16 hours after application. Parenteral administration of the extracts also reduced vascular fragility in rat skin as determined by the petechien test. Also, here the peak effect was obtained 16 hours after application. Aescin was shown to be responsible for these effects.

Effect on venous tone

Lochs *et al*., 1974, reported that the effects of extracts (standardised to 16% aescin, no information on drug/extract ratio or solvent) from horse chestnut (*A. hippocastanum* L.) and of aescin were investigated on isolated veins (bovine *Vena metacarpalis*, human *Vena saphena*) which either were perfused or used as strips with isotonic recording. They produced in high doses (0.2 mg/ml and more) a slow and irreversible contraction in both experimental procedures. The contractions were very similar to those produced by the same doses of saponin, but dissimilar to those produced by much smaller doses of noradrenaline ESCOP (2003). According to ESCOP (2003) similar effects on venous tone were observed with alcoholic horse-chestnut seed extract at 0.2-1 mg/ml on isolated veins of rabbits and with aescin at 1 ng/ml to 1 mg/ml on human vein (*Vena saphena*) preparations. The effects were comparable to those of essential phospholipids, serotonin or dihydroergotamine, and significantly greater than those of acetylcholine or vasopressin.

The following results were obtained with an extract (containing 75% aescin, no other details given): In the isolated canine saphenous vein, the extract induced concentration-dependent contractions at concentrations above 5 times 10^{-5} mg/ml; the contraction at 5 times 10^{-4} mg/ml reached a maximum in 15 minutes and lasted more than 5 hours. In anaesthetised dogs, i.v. administration of 50 mg of the extract significantly increased femoral venous pressure ($p<0.001$). Oral administration to rats of 200 mg/kg of the extract significantly decreased cutaneous capillary hyperpermeability induced by histamine or serotonin (Guillaume and Padioleu, 1994).

Aescin, in concentrations of 5-10 mg/ml, increased the tension of isolated human saphenous veins and rabbit portal veins. The effect was abolished by non-steroidal anti-inflammatory drugs, indicating that the effect was dependent on PGF2α in the venous tissue. This ability to enhance PGF2α generation in the veins, and thus increase tonus, may be of relevance in venous insufficiency (Longiave *et al*., 1978).

Other investigations showing venotonic effects of various alcoholic horse-chestnut seed extracts are reviewed by Bombardelli *et al*., 1996. Anti-oedemic activity of oral doses of horse-chestnut seed extracts was demonstrated in experiments on rats with blocked leg lymphatic circulation (Bombardelli *et al*., 1996).

In vitro, aescin contracted vein segments derived from normal vessels whereas no contraction was observed in segments from varicose vessels (Brunner *et al*., 2001).

In *inferior vena cava* (IVC) segments from male rats incubated in normal Krebs (2.5 mM Ca^{2+}), aescin caused concentration-dependent contraction (max 104.3±19.6 at 10^{-4} M). Aescin-induced contraction was not a rigor state, because after washing with Krebs the veins returned to a relaxed state. In Ca^{2+}-free Krebs, there was essentially no contraction to aescin. In aescin-treated veins incubated in 0 Ca^{2+} Krebs, stepwise addition of extracellular $CaCl_2$ caused corresponding increases in contraction (max 80.0±11.1 at 2.5 mM). In the absence of aescin, the α-adrenergic agonist phenylephrine (PHE, 10^{-5} M), angiotensin II (AngII, 10^{-6} M), and membrane depolarization by KCl (96 mM) caused contraction (122.5±45.1, 114.2±12.2 and 221.7±35.4, respectively). In IVC segments pretreated with aescin (10−4 M), the contractile response to PHE (9.7±2.6), AngII (36.0±9.1) and KCl (82.3±10.2) was reduced (Raffetto and Khalil, 2011).

Endothelium effects

Human vascular endothelial cells (HUVECs) were exposed to $CoCl_2$ as an *in vitro* model of hypoxia. Expression of VCAM-1 (vascular cell adhesion molecule), reduction of PECAM-1 (platelet endothelial cell adhesion molecule) and cytoskeletal changes without alterations in cell viability were observed. HUVECs were also exposed to *Escherichia coli* lipopolysaccharide (LPS) as an *in vitro* model of inflammation: significant IL-6 release was measured. Pre-treatment of HUVECs with aescin prevented, in a concentration-dependent fashion (0.1–1 μM), the action of $CoCl_2$ on VCAM-1 and PECAM-1, also preserving endothelial cell morphology. Furthermore, aescin pre-treatment reduced IL-6 release from LPS-activated vascular endothelium (Montopoli *et al*., 2007).

Effect on lysosomal enzymes

Aescin inhibited the enzyme hyaluronidase (IC50=149.9 μM) (Facino *et al*., 1995).

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

Herbal preparation tested	**Strength Dosage Route of administration**	**Experimental model**	**Reference**	**Main non-clinical conclusions**
Extracts (standardised to 16% aescin) from horse chestnut seed aescin	5 μg/ml – 0.2 mg/ml	*Ex vivo:* isolated veins (bovine *Vena metacarpalis*, human *Vena saphena*) which either were	Lochs *et al*., 1974	In high doses of the extract (0.2 mg/ml and more) and aescin (0.1 mg/ml) a slow and irreversible contraction in both experimental

Herbal preparation tested	Strength Dosage Route of administration	Experimental model	Reference	Main non-clinical conclusions
		perfused or used as strips with isotonic recording		procedures
Extract containing 75% aescin, no other details given	*Various concentrations*	*Ex vivo:* isolated canine saphenous vein	Guillaume and Padioleau, 1994	concentration-dependent contractions at concentrations above 5 times 10^{-5} mg/ml
Extract containing 75% aescin, no other details given	i.v. 50 mg	*In vivo*: anaestetised dogs	Guillaume and Padioleau, 1994	administration of 50 mg of the extract increased femoral venous pressure
extract containing 75% aescin, no other details given	Oral administration, 200 mg/kg	*In vivo*: rats	Guillaume and Padioleau, 1994	200 mg/kg of the extract decreased cutaneous capillary hyperpermeability induced by histamine or serotonin
aescin		*Ex vivo:* isolated human saphenous veins and rabbit portal veins	Longiave *et al.*, 1978	concentrations of 5-10 mg/ml, increased the tension of isolated human saphenous veins and rabbit portal veins
aescin		*Ex vivo:* *inferior Vena cava* (IVC) segments from male rats	Raffetto and Khalil, 2011	in rat IVC, aescin induces extracellular Ca2+-dependent contraction, but disrupts α-adrenergic and AT1R receptor-mediated pathways, and depolarisation-induced contraction.

3.1.2. Secondary pharmacodynamics

Protective effects against gastric ulcer

Wang *et al*., 2014, investigated the possible mechanisms underlying the gastroprotective effect of aescin against indomethacin-induced gastric ulcer in mice. The mice underwent intragastric treatment with aescin at doses of 0.45, 0.9 or 1.8 mg/kg. Gastric lesion was estimated morphometrically and

histopathologically 6 hours after the indomethacin administration. The authors report that aescin reduced the ulcer index and the attenuation of histopathologic changes (Wang *et al.*, 2014).

Effects on platelet aggregation

The effect on platelet aggregation of a dry hydroalcoholic extract, standardised for 16–22% triterpene saponins was investigated. Platelet-rich plasma was prepared from heparinized venous blood from healthy blood donors. The donors had refrained from drugs known to affect platelet aggregation (e.g., NSAIDs) for two weeks prior to blood donation. The platelet-rich plasma (500 µl) was preincubated with horse chestnut extract (5 µl), with or without 10^{-5} M ketanserin, for 5 minutes prior to stimulation with 10^{-6} or 10^{-5} M adenosine diphosphate (ADP). The authors report that no aggregatory effect of the horse chestnut extract or ketanserin alone was seen on platelet aggregation. However, ADP-induced platelet aggregation was reduced by horse chestnut extract. A further reduction was seen by the extract in the presence of the 5-HT_{2A} receptor antagonist ketanserin (Felixsson *et al.*, 2010).

3.1.3. Safety pharmacology

No information available.

3.1.4. Pharmacodynamic interactions

No information available.

3.1.5. Conclusions

Aqueous ethanolic extracts of *A. hippocastanum* L., seed (horse-chestnut seed extract; HCSE), and pure aescin, have contracting effects on veins in (very) high concentrations. This seems to involve a stimulated formation of prostanoids in the venous tissue.

Anti-oedematous effects of HCSE (and aescin) have been observed *in vivo* after i.v. and i.p. administration. No effect was observed after oral administration. The reported inhibitory effect of aescin on lysosomal enzymes (hyaluronidases) has been obtained with very high concentrations that probably exceed the concentrations obtained *in vivo*.

Based on available preclinical data, it can be concluded that the mechanism of action of orally administered HCSE in connection with chronic venous insufficiency is not known. Concerning aescin, it has been shown that pure aescin has *in vivo* anti-oedematous activity after i.v./i.p. administration in rats, but this experimental model is of very little relevance concerning oral use of HCSE in chronic venous insufficiency.

None of the reported pharmacological studies constitute any cause for safety concern.

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

Treatment with a single dose or multiple doses of aescin had inductive effects on rat CYP1A2, while CYP2C9 and CYP3A4 enzyme activities were inhibited. Moreover, aescin has no inductive or inhibitory effect on the activity of CYP2E1. The authors concluded that further clinical studies are required to fully assess the safety of aescin in terms of CYP (Huang *et al*., 2014).

Assessor's comment:

Some non-clinical pharmacokinetic studies have been performed on aescin, but compelling evidence that the pharmacological activity of horse chestnut seed extract can be ascribed to aescin has not been

presented. At present, the pharmacokinetic data on absorption, distribution and elimination of aescin are of limited importance. There are no indications that toxic metabolites are formed from aescin. Clinical confirmation on the effect of aescin on CYP-enzymes is lacking.

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

3.3.1. Single dose toxicity

LD_{50}-values (mg/kg bw) for an extract of horse chestnut seed extract (dry extract, DER 5:1, extraction solvent 50% V/V, standardised for a content of 50 mg aescin in 240-290 mg extract) are illustrated in the following table (Liehn *et al.*, 1972):

Animal	**Oral**	**Intraperitoneal**	**Intravenous**
Mouse	910-990	342	138
Rat	2150		165
Guinea pig	1120		465
Rabbit	1530		180
Dog	>130		

The following data (LD_{50} mg/kg bw) on a horse chestnut seed extract (dry extract, DER 5:1, extraction solvent ethanol 50% V/V, standardised to a content of 50 mg aescin in 240-290 mg extract) is available in Blaschek *et al.*,1992:

Animal	**Oral**	**Intraperitoneal**	**Intravenous**
Mouse	990–1050	98	6.8
Rat	2150–2600	175	12.0
Guinea pig	1120		
Rabbit	1530		180
Dog	>130		

For aescin the following data are available in Blaschek *et al.,*1992: LD_{50} (mg/kg bw) intravenous: Mouse 9.3, rabbit 5.0, rat 16.8, guinea pig 9.1, pig 4, dog 3. By intraperitoneal administration an LD_{50} of 17 mg/kg was determined for rats (von Kreybig and Prechtel, 1977).

3.3.2. Repeat dose toxicity

Daily i.v. doses of a horse chestnut seed extract (dry extract, DER 5:1, extraction solvent 50% V/V, standardised for a content of 50 mg aescin in 240-290 mg extract) at 9, 30 or 90 mg/kg bw were administered to rats for 60 days. With the 90 mg/kg dose, 8 out of 30 animals died during the first few days, but the others developed normal weights. During the period of treatment there was a rise in the consumption of drinking water and a fall in the renal concentration capacity. Haemoglobin as well as haematocrit values were slightly, not always significantly, reduced. 30 mg/kg caused a slight, not always significant rise in the consumption of drinking water. 9 mg/kg was tolerated virtually without symptoms. Histopathological studies of rat organs prepared after treatment for 4 and 8 weeks showed an accumulation of Lepehne-positive material in the epithelium of the proximal renal tubule in 58% of

the animals receiving the highest dose and 10% of the animals receiving the medium dose. The no-effect dose level was considered to be around 30 mg/kg bw (Liehn *et al*., 1972).

Neither toxic effects, nor organ damage were observed after 34 week oral administration of the horse chestnut seed extract (dry extract, DER 5:1, extraction solvent ethanol 50% V/V, standardised for a content of 50 mg aescin in 240-290 mg extract) to dogs (2 male, 2 female per dose and control group) at 20, 40 or 80 mg/kg bw daily (5 days per week) and to rats (20 male, 20 female per dose and control group) at 100, 200 and 400 mg/kg bw daily. The highest dose level used in dogs corresponded to 8 times the usual therapeutic dose in humans. The highest dose studied in rats amounts to 40 times the human therapeutic dose (Liehn *et al*., 1972).

3.3.3. Genotoxicity

In the Ames mutagenicity test, using *Salmonella typhimurium* strain TA 98, a commercial dry extract of seeds (no further information on the extract available in the reference) gave a negative response without activation, but a weekly positive response (factor 2-3) with S9 activation. Fluid extracts of horse-chestnut seed gave a weakly positive response (factor 2-3) without activation and a negative response with activation. The authors suggested that quercetin is possibly the main mutagenic principle in these extracts (Schimmer *et al*., 1994; ESCOP, 2003).

3.3.4. Carcinogenicity

No information available.

3.3.5. Reproductive and developmental toxicity

Following daily oral administration of a horse chestnut seed extract (dry extract, DER 5:1, extraction solvent ethanol 50% V/V, standardised for a content of 50 mg aescin in 240-290 mg extract) to rats and rabbits at 100 and 300 mg/kg bw, no significant effects compared to control animals were observed in teratogenicity studies. At 300 mg/kg bw to rabbits, a significant reduction ($p<0.001$) in the mean weight of the foetuses was observed. 300 mg/kg bw is approximately 30 times the recommended therapeutic dose for humans (Liehn *et al*., 1972).

Juvenile rats were treated with 2 times 5 mg/kg aescin at age 32 days. After they had reached fertility, kidneys, testes and sperm were examined. The high dose of aescin used did not affect fertility and a nephrotoxic activity could not be detected (von Kreybig and Prechtel, 1977).

3.3.6. Local tolerance

No information available.

3.3.7. Other special studies

Blood toxicity

SD rats were treated with different doses of aescin (15, 10 and 5 mg/kg, i.p.) once per day for 7 days. Hematologic indices (white blood cell, red blood cell, platelet and haemoglobin) and blood coagulation indices (prothrombin time, thrombin time, activated part thromboplastin and coagulation time) were selected as observational indices. Comparing rats treated with aescin with the controls, the number of white blood cell was decreased ($p<0.05$). The number of red blood cell and platelet, and the content of haemoglobin were enhanced markedly ($p<0.05$, <0.01). At the same time, all the blood coagulation indices in rats treated with aescin 10 and 15 mg/kg shortened significantly ($p<0.05$, <0.01), and in

rats treated with 5 mg/kg, prothrombin time and thrombin time were reduced ($p<0.05$, <0.01) (Li *et al*., 2006).

Assessor's comment:

There was significant blood toxicity to SD rats treated with high dose of aescin i.p. The study is considered not relevant for oral or cutaneous use.

3.3.8. Conclusions

With an LD_{50} ranging between 1 and 2.6 g/kg bw, the acute oral toxicity of horse chestnut seed extract is low in all animals studied. The subacute studies were performed with i.v. administration and indicated that a daily dose amounting to ¼ of the i.v. LD_{50} has no untoward effects when given during 8 weeks. Also the data on oral chronic toxicity indicate a low toxicity of the extract. Data on teratogenic effects are incomplete. Equivocal results on mutagenic activity of horse chestnut seed extract have been reported.

3.4. Overall conclusions on non-clinical data

Oral use:

Based on available preclinical data, it can be concluded that the mechanism of action of orally administered horse chestnut seed extract in connection with chronic venous insufficiency is not known. Concerning aescin, it has been shown that pure aescin (ca 15-20% of the horse chestnut seed extract) has *in vivo* antioedematous activity after i.v./i.p. administration in rats, but this experimental model is of very little relevance concerning oral use of horse chestnut seed extracts in chronic venous insufficiency.

Cutaneous use:

Results from relevant experimental studies in support of the proposed traditional use indications for cutaneous use are limited. None of the reported pharmacological studies constitute any cause for safety concern.

Oral use and cutaneous use:

With an LD_{50} ranging between 1 and 2.6 g/kg bw, the acute oral toxicity of horse chestnut seed extract is low in all animals studied. The subacute studies were performed with i.v. administration and indicated that a daily dose amounting to ¼ of the i.v. LD_{50} has no untoward effects when given for 8 weeks. Also, the data on oral chronic toxicity indicate a low toxicity of the extract.

Adequate tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed. As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

4. Clinical Data

4.1. Clinical pharmacology

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

The effect of an extract (DER 5:1, extraction solvent ethanol 50% V/V), contained in capsules with 240 to 290 mg of the extract, standardised to 50 mg aescin/capsule on trans-capillary filtration has been assessed by measuring capillary filtration coefficients in two clinical studies.

In the first study (Pauschinger *et al*., 1953), oral administration of a single dose of the extract (300 mg, n=12) or placebo (n=14) to healthy volunteers, produced a significantly lower capillary filtration coefficient in the extract group.

The second study (Bisler *et al*., 1986) had a double-blind, crossover design and involved 22 female patients with proven chronic venous insufficiency. The capillary filtration coefficient and the intravascular volume of the lower leg were determined by venous-occlusion plethysmography. 3 hours after oral administration of a single dose of 2 capsules (=100 mg of aescin) the capillary filtration coefficient had decreased significantly by 22% (p=0.006), compared to a slight increase with placebo. The intravascular volume was reduced 5% or more in comparison with administration of placebo, but this decrease was not significant. It was concluded that the extract had an inhibitory effect on oedema formation via a decrease in trans-capillary filtration and thus improved oedema-related symptoms in venous diseases of the legs.

In a study of venous tone, a single dose of 150 mg of extract was administered orally to 23 healthy young subjects. A further 14 subjects received either 80 mg of extract or identical placebo capsules in a crossover design. Plethysmographic measurements taken before and 2 hours after administration showed that the extract dose-dependently increased venous tone (Nehring, 1966).

Comparable results were obtained from a further study in which 12 healthy volunteers firstly received placebo and then a single oral dose of extract (360 mg, standardised to 90 mg of aescin). In contrast, intravenous administration of 20 mg of aescin had no effect on venous tone (Ehringer, 1968).

Three hydrolases, β-N-acetylglucosaminidase, β -glucuronidase and arylsulphatase, catalyze the breakdown of proteoglycans, which constitute part of capillary walls. In the serum of varicose in patients the activity of these enzymes has been found to be markedly increased (by 60-120%) compared to healthy subjects; this may render the capillaries more permeable and fragile. In two studies, one with 10 patients and the other with 15 patients, oral administration of an extract of horse-chestnut seeds (dry extract, 5:1, extraction solvent ethanol 50% V/V, 900 mg, standardised to 150 mg of aescin) daily for 12 consecutive days led to significant reductions in the activity of these enzymes ($p<0.01$ and $p<0.05$ respectively), of the same order of magnitude (about 30%) for each enzyme. It was hypothesized that horse chestnut seed extract does not inhibit the individual enzymes but has a protective action towards the site of enzyme release, the fragile lysosomal membrane (Enghofer *et al*., 1984).

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

After i.v. administration, the pharmacokinetics of aescin was found to correspond to an open three compartment model. With an i.v. dose of 5 mg of aescin (infusion rate: 718 µg per minute) the elimination half-life $t_{0.5\alpha}$ was 6.6 minutes; $t_{0.5\beta}$ was 1.74 hours and $t_{0.5\gamma}$ was 14.36 hours. The

distribution volume under steady state conditions was 100.9 l, total plasma clearance 21.8 ml per minute and renal clearance 1.7 ml per minute. Urinary excretion from 0 to 120 hours after injection comprised 8.2% of the dose. After oral administration of an aescin solution the absolute bioavailability was determined as only 1.5%. This low bioavailability is due to a pronounced first pass effect (metabolism and biliary excretion). The relative bioavailability of aescin from a horse-chestnut seed extract was 100% compared to an aescin solution (Hitzenberger, 1989).

Several clinical studies have been published comparing the bioavailability of aescin in a prolonged release formulation containing 240-290 mg of horse chestnut seed extract (providing 50 mg of aescin) and other pharmaceutical formulations. Some pharmacokinetic parameters for aescin have been reported from these studies, see below.

In single dose experiments (Schrader *et al*., 1995; Dittgen *et al*., 1996 and Oschmann *et al*., 1996), horse chestnut seed extract containing 50 mg aescin resulted in C_{max} values ranging from 3.2-9.8 ng/ml and AUCs between 92.2 and 276. A considerable inter- and intra-individual variability in serum concentrations was observed (Loew *et al*., 2000).

In repeated dose experiments (Oschmann *et al*., 1996; Schrödter *et al*., 1998; Kunz *et al*., 1998), horse chestnut seed extract containing 50 mg aescin resulted in C_{max} values ranging from 6.5-16.7 ng/ml (Loew *et al*., 2000). During these steady state conditions a considerable variability in serum concentrations between the different clinical studies was also observed (Loew *et al*., 2000).

In all the published clinical studies the same radio immunoassay (RIA), under identical conditions, in the same laboratory, was employed. The immunoassay had been validated for a specific batch of aescin, and for this particular batch of aescin it was reported to work satisfactorily. However, HPLC-analyses of various batches of horse chestnut seed extract showed a significant quantitative variability of the more than 30 individual triterpene saponins in aescin between the different batches. Given the potential sensitivity of an immunoassay for certain subtypes of structures, this analytical problem offers an explanation to the unexpectedly high variability in pharmacokinetic parameters that can be derived from the quoted studies. The variability of the C_{max} values in the different clinical studies may simply reflect the natural variation of individual aescin components between different batches of horse chestnut seed extract. The absolute values of the pharmacokinetic parameters obtained with this RIA are thus not reliable (Loew *et al*., 2000; Bässler *et al*., 2003).

Liu *et al*., developed a LC-MS/MS method for simultaneous quantification of aescin Ia and aescin Ib in human plasma and performed a pharmacokinetic study after a single i.v. infusion of sodium aescinate (containing 10 mg aescin) in 10 healthy male volunteers. The 10 mg aescin used in the study contained 3.0 mg aescin Ia and 2.0 mg aescin Ib. Blood samples (3 ml) were collected at 0 hours (pre-dose), 0.5, 1.0, 2.0, 2.08, 2.17, 2.25, 2.33, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10.0, 14.0 and 24.0 hours post-dose (drip infusion was started at time 0 hours and completed at time 2.0 hours). In this study, aescin Ia and aescin Ib were rapidly cleared from human plasma. There were some differences between distribution and elimination of the two aescins. Aescin Ib was eliminated more quickly than aescin Ia from human plasma. The values of Cmax for aescin Ia and aescin Ib were 138 and 74.5 ng/ml, respectively, and $t_{1/2}$ were 2.32 and 1.87 hours, respectively (Liu *et al*., 2010).

Wu *et al*., developed a LC–MS/MS method for the simultaneous determination of four isomeric aescin saponins (aescin Ia, aescin Ib, isoaescin Ia and isoaescin Ib) in human plasma. The method was used in a pharmacokinetic study of aescins in 10 healthy male volunteers (20-30 years with a body mass index of 20-24) after oral administration of sodium aescinate tablets containing 60 mg aescin. After 12 hours fast, the participants were given a single oral dose of two sodium aescinate tablets containing 60 mg aescin saponins with 250ml water. Blood samples (3.0 ml) were collected by venepuncture into heparinised tubes prior to and at 0.5, 0.67, 0.83, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 hours after dosing. In some subjects, plasma concentration–time profiles exhibited multiple peaks suggesting

some form of redistribution or enterohepatic recycling occurs in certain individuals. B-aescins were cleared more rapidly from human plasma than α-aescins. The values of C_{max} for aescin Ia, aescin Ib, isoaescin Ia and isoaescin Ib were 0.77, 0.38, 2.4 and 2.1 ng/ml, respectively, and $t_{1/2}$ were 8.5, 4.7, 13.7 and 8.4 hours, respectively (Wu *et al*., 2010).

4.2. Clinical efficacy

4.2.1. Dose response studies

No information available.

4.2.2. Clinical studies (case studies and clinical trials)

Only clinical studies on horse chestnut seed extracts in indications that have been in medicinal use within the EU for at least ten years are included in this section. Clinical studies on horse chestnut seed extracts in indications that has not been in medicinal use within the EU for at least ten years do not introduce the possibility for new indications in the well-establish use monograph.

A Cochrane review of the efficacy and safety of oral horse chestnut seed extract (HCSE) versus placebo, or reference therapy, for the treatment of chronic venous insufficiency (CVI) was first published in 2002. The review has been updated in 2004, 2006, 2008, 2010 and 2012 (Pittler and Ernst, 2006, 2012). No new included studies have been identified since 2005. The authors have marked the review as stable and will only be updated when new studies are identified (Pittler and Ernst, 2012).

Criteria for the review:

- Randomised, controlled trials (RCTs), i.e. trials with a randomized generation of allocation sequences.
- Studies assessing acute effects only were excluded.
- No restrictions regarding the language of publication
- Studies were included if participants were patients with CVI.
- Trials were included if they compared oral preparations containing horse chestnut seed extract as the only active component (mono-preparation) with placebo or reference therapy.
- Trials using clinical outcome measures were included. Studies focusing exclusively on physiological parameters were excluded.

Primary outcomes: The primary outcome measures were CVI-related symptoms (e.g. leg pain, pruritus (itching), oedema (swelling)).

Secondary outcomes: Secondary outcomes were, leg volume and leg circumference at ankle and calf. Adverse events were assessed as reported in the included trials.

The following 17 trials met the inclusion criteria: Cloarec, 1992 (unpublished), Diehm *et al*., 1992, 1996, Diehm and Schmidt, 2001), Erdlen, 1989, Erler, 1991, Friederich *et al*., 1978, Kalbfleisch and Pfalzgraf, 1989, Koch, 2002, Lohr *et al*., 1986, Morales and Barros, 1993, Neiss and Bohm, 1976, Pilz, 1990, Rehn *et al*., 1996, Rudofsky *et al*., 1986, Steiner and Hillemanns, 1986 and, Steiner and Hillemanns, 1990. Of these, ten were placebo-controlled, two compared horse chestnut seed extract against reference treatment with compressing stockings and placebo, four were controlled against reference medication with O-β-hydroxyethyl rutosides and one was controlled against medication with pycnogenol. In all of these studies horse chestnut seed extract was administered in capsules,

permitting the preparation of adequate placebos. In all trials, the extract was standardised on aescin, but information on the preparation of the extracts such as drug/extract ratio and solvent is not reported in the review. The original papers, however, show that 14 of the studies were performed with a preparation consisting of capsules containing 240-290 mg of a dry extract (DER 5:1, extraction solvent ethanol 50% V/V) corresponding to 50 mg of aescin. The daily dose was 2 capsules=100 mg of aescin. Two studies (Diehm *et al*., 1992 and Erler, 1991) were performed with capsules containing 360-412 mg of the same extract, corresponding to 75 mg of aescin. Here the daily dose was 2 capsules=150 mg of aescin. One study (Cloarec, 1992) was unpublished and therefore no information on the extract is available. The duration of the trials ranged from 2 to 16 weeks. All the included trials except one were double blinded. They scored at least one out of five points on the Jadad scale (Jadad *et al*., 1996). Three trials scored A and the remaining fourteen scored B for the method of allocation concealment. The symptoms related to chronic venous insufficiency studied were: Leg pain, oedema, pruritus, leg volume and circumference.

Results:

A total number of 1443 patients participated in the trials. The number of patients varied between the studies from 20 to 286. Eleven studies comprised less than 50 participants. The majority of the included studies diagnosed the patients according to the classification by Widmer (Widmer and Stähelin, 1978). Fourteen trials reported inclusion criteria for CVI patients relating to this classification. Eighty-two percent of the participants in these trials were categorised into CVI stages II or I-II. Three trials, comprising 22% of the total number of participants did not refer to this classification. Overall, the included placebo-controlled trials suggested an improvement in the CVI related symptoms of leg pain, oedema and pruritus.

Leg pain: Leg pain was assessed in seven placebo-controlled trials (Cloarec, 1992; Friederich *et al*., 1978; Lohr *et al*., 1986; Morales and Barros, 1993; Neiss and Bohm, 1976; Rudofsky *et al*., 1986; Steiner and Hillemanns, 1990). Six studies (n=543) reported a statistically significant reduction ($p<0.05$) of leg pain on various measurement scales in participants treated with horse chestnut seed extract compared with placebo, while another reported an improvement compared with baseline (Steiner and Hillemanns, 1990). One study (Cloarec, 1992), reported adequate data (i.e. data that are included within RevMan Analyses 1.0.4 and can be used for meta-analysis) assessed on a 100 mm VAS, suggesting a weighted mean difference (WMD) of 42.40 mm (95% confidence interval (CI) 34.90 to 49.90). Other studies which compared horse chestnut seed extract with hydroxyethyl rutoside (Kalbfleisch and Pfalzgraf, 1989), pycnogenol (Koch, 2002) or compression (Diehm and Schmidt, 2001) reported no significant intergroup differences for leg pain or a symptom score including leg pain.

Oedema: Oedema was assessed in six placebo-controlled trials (Cloarec, 1992; Friederich *et al*., 1978; Lohr *et al*., 1986; Morales and Barros, 1993; Neiss and Bohm, 1976; Steiner and Hillemanns, 1990). Four trials (n=461) reported a statistically significant reduction of oedema in participants treated with horse chestnut seed extract compared with placebo, whilst one (Steiner and Hillemanns, 1990) reported an improvement compared with baseline. One study (Cloarec, 1992) reported adequate data suggesting a WMD of 40.10 mm (95% CI 31.60 to 48.60) in favour of horse chestnut seed extract assessed on a 100 mm VAS. Another study (Koch, 2002) reported that horse chestnut seed extract was inferior to pycnogenol, whereas a further trial (Diehm and Schmidt, 2001) reported no significant differences for a score including the symptom oedema compared with compression. Oedema provocation before and after treatment with horse chestnut seed extract revealed oedema protective effects (Erler, 1991).

Pruritus: Pruritus was assessed in eight placebo-controlled trials (Diehm *et al*., 1992; Friederich *et al*., 1978; Lohr *et al*., 1986; Morales and Barros, 1993; Neiss and Bohm, 1976; Rudofsky *et al*., 1986; Steiner and Hillemanns, 1986; Steiner and Hillemanns, 1990). Four trials (n=407) suggested a

statistically significant reduction of pruritus in participants treated with horse chestnut seed extract compared with placebo ($p<0.05$). Two trials (Steiner and Hillemanns, 1986; Steiner and Hillemanns, 1990) suggested a statistically significant difference in favour of horse chestnut seed extract compared with baseline ($p<0.05$). Another trial (Kalbfleisch and Pfalzgraph, 1989), which compared horse chestnut seed extract with hydroxyethylrutosides, but failed to include a placebo group, seemed to corroborate these findings. A further trial (Diehm and Schmidt, 2001) reported no significant differences for a score including the symptom pruritus compared with compression.

Leg volume: Leg volume was assessed in seven placebo-controlled trials (Diehm *et al*., 1992; Diehm *et al*., 1996; Diehm and Schmidt, 2001; Lohr *et al*., 1986; Rudofsky *et al*., 1986; Steiner and Hillemanns, 1986; Steiner and Hillemanns, 1990). All of these studies used water displacement plethysmometry to measure this outcome. Meta-analysis of six trials (Diehm *et al*., 1992; Diehm *et al*., 1996; Diehm and Schmidt, 2001; Rudofsky *et al*., 1986; Steiner and Hillemanns, 1986; Steiner and Hillemanns, 1990; n=502) suggested a WMD of 32.1 ml (95% CI 13.49 to 50.72) in favour of horse chestnut seed extract compared with placebo (pooled standardised mean difference 0.34; 95% CI 0.15 to 0.52). One trial (Rehn *et al*., 1996) reported findings suggesting that horse chestnut seed extract was equivalent to HR, and another (Diehm *et al*., 1996, n=194) suggested that it may be as efficacious as treatment with compression stockings (WMD-2.90 ml; 95% CI-30.42 to 24.62).

Significant beneficial effects for CVI patients were reported in trials which administered horse chestnut seed extract standardised to 100-150 mg aescin daily. Three studies, using 100 mg aescin daily, reported a statistically significant reduction of mean leg volume after two weeks of treatment compared with placebo ($p<0.01$) (Rudofsky *et al*., 1986; Steiner and Hillemanns, 1986; Steiner and Hillemanns, 1990). Persistence of treatment effects was suggested by one study (Rehn *et al*., 1996). At the end of a six-week follow-up period mean leg volume was similar to post-treatment values.

Circumference: Circumference at calf and ancle was assessed in seven placebo-controlled trials (Cloarec, 1992; Diehm *et al*., 1992; Lohr *et al*., 1986; Pilz, 1990; Rudofsky *et al*., 1986; Steiner and Hillemanns, 1986; Steiner and Hillemanns, 1990). Five studies (n=172) suggested a statistically significant reduction at the ankle, and three (n=112) at the calf in favour of horse chestnut seed extract compared with placebo. At the ankle, meta-analysis of three trials (Cloarec, 1992; Pilz, 1990; Steiner and Hillemanns, 1986), which reported adequate data, suggested a statistically significant reduction in favour of horse chestnut seed extract compared with placebo (WMD 4.71 mm; 95% CI 1.13 to 8.28; pooled standardised mean difference 0.60; 95% CI 0.15 to 1.05). At the calf, the pooled analysis of three trials (Cloarec, 1992; Pilz, 1990; Steiner and Hillemanns, 1986), suggested a statistically significant reduction in favour of horse chestnut seed extract compared with placebo (WMD 3.51 mm; 95% CI 0.58 to 6.45; pooled standardised mean difference 0.42; 95% CI-0.04 to 0.88).

The authors of the systematic review conclude that horse chestnut seed extract appears to be effective and safe as a symptomatic, short-term treatment for chronic venous insufficiency. However, caveats exist and more rigorous, large studies are required to assess the efficacy of this treatment option.

In another meta-analysis (Siebert *et al*., 2002) 75 studies were identified. Sixteen of these (13 controlled and 3 observational) studies were included in the meta-analysis. Eleven of the controlled studies were also analysed by Pittler and Ernst (2006, see above) while 2 were not included in Pittler's analysis. The controlled studies comprised 1051 patients and the number in the observational studies was 10725. Data from the meta-analysis of the controlled trials supported a clinically relevant and statistically significant effect of horse chestnut seed extract in the treatment of chronic venous insufficiency. Leg volume, ankle and calf circumference and oedema improved significantly, while improvements in pain and itching were of borderline statistical significance. For leg fatigue/heaviness and calf cramps, no consistent significant improvement was demonstrated across studies. Data from

the observational studies also suggested a benefit of horse chestnut seed extract in routine settings and confirmed the findings from the controlled trials.

Table 5: Clinical studies on humans, in CVI

Type of study	**Study Design**	**Herbal preparation, pharmaceutical form, dosage**	**Number of subjects**	**Type of subjects**	**Outcomes**	**Statistical analysis**	**Comments**
Cochrane review of the efficacy and safety of oral horse chestnut seed extract (HCSE) versus placebo, or reference therapy, for the treatment of chronic venous insufficiency (CVI) Pittler and Ernst (2012)	Meta-analysis 17 studies included 10 placebo-controlled, 2 compared HCSE against reference treatment with compressing stockings and placebo, 4 controlled against reference medication with O-β-hydroxyethyl rutosides and 1 controlled against pycnogenol. The duration ranged from 2 to 16 weeks.	In all of the studies HCSE was administered orally and the extract was standardised on aescin 14 studies with 240-290 mg of a standardised dry extract (5:1 ethanol 50% V/V) corresponding to 50 mg of aescin 2 times daily. 2 studies with 360-412 mg standardised dry extract (5:1 ethanol 50% V/V), corresponding to 75 mg of aescin 2 times daily	1443 patients	Patients with CVI. The majority of the included studies diagnosed the patients according to the classification by Widmer (Widmer 1978)	Primary outcomes: The primary outcome measures were CVI-related symptoms (e.g. leg pain, pruritus (itching), oedema (swelling)). Secondary outcomes: Secondary outcomes were, leg volume and leg circumference at ankle and calf. Conclusion: The included placebo-controlled trials suggested an improvement in the CVI related symptoms of leg pain, oedema and pruritus. HCSE appears to be effective and safe as a symptomatic, short-term treatment for CVI.	Data-pooling of continuous data was performed using the weighted mean difference; for dichotomous data the odds ratio was used. Summary estimates of the treatment effect were calculated using a random effects model. The chi-square test was used for the assessment of heterogenicity. Methodological quality was assessed using the Jadad score and the Cochrane risk of bias tool.	Significant beneficial effects of HCSE daily oral doses corresponding to 100 mg of aescin for symptomatic treatment of CVI.

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

No information available.

4.4. Overall conclusions on clinical pharmacology and efficacy

An increase in venous tone has been reported for oral horse chestnut seed extract in clinical pharmacology studies, but pure aescin (i.v.) was reported not to have this effect. Furthermore, a decrease in capillary filtration coefficient has been reported for horse chestnut seed extract in human pharmacological studies. Both these effects (which probably are related) appear relevant in connection with chronic venous insufficiency.

A mechanism proposed for this effect, is that horse chestnut seed extract would prevent the action of enzymes which catalyse the breakdown of proteoglycans, constituting part of the capillary walls. It has been proposed that this effect would not be via direct inhibition of the enzymes, but by a protective action on the lysosomal membrane that is the site of enzyme release. However, it appears unlikely that a saponin containing extract would stabilize membranes.

At present, the mechanism of action of horse chestnut seed extract in chronic venous insufficiency cannot be considered clarified, but it seems to involve an influence on the venous tone and capillary filtration rate. The effect does not seem to be due to aescin.

There is no compelling evidence that aescin is the therapeutically active substance in horse chestnut seed extract, so the pharmacokinetic data available are of very limited value.

Some data on the pharmacokinetic parameters of the marker substance aescin have been reported in the literature. The absolute figures of the parameters are not reliable for analytical methodological reasons (Loew *et al*., 2000; Bässler *et al*., 2003). They are thus not suitable for adjusting a dosing regimen of horse chestnut seed extract in clinical practice.

Two meta-analyses, comprising 19 controlled clinical trials and 3 observational studies of a preparation containing a dry extract (5:1, ethanol 50% V/V, standardised to a content of aescin of 50 mg/240-290 mg of extract) of *A. hippocastanum* L., semen, in daily oral doses corresponding to 100 mg of aescin indicate that the extract is effective for symptomatic treatment of chronic venous insufficiency.

Of particular importance is the study by Diehm *et al*., 1996, where horse chestnut seed extract was compared both with placebo and with standard treatment with compression stockings, which showed that the reduction of leg volume after 12 weeks of treatment was equal between horse chestnut seed extract and compression stockings and significantly ($p<0.005$ and 0.002, respectively) different from placebo. The oedema volume in the leg of a patient with chronic venous insufficiency has been estimated to approximately 220 ml (Diehm *et al*., 1996). The oedema reduction obtained in this study was ca 55 ml (for both horse chestnut seed extract and compression stockings), i.e. an effect size of approximately 25%, which is considered clinically relevant.

The effects on subjective symptoms, such as pain and pruritus have also been found to be significantly reduced by horse chestnut seed extract compared to placebo, although the data are less convincing.

5. Clinical Safety/Pharmacovigilance

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

No new safety concerns have been identified in the updated Cochrane review published in 2012 (Pittler and Ernst, 2012). Fourteen studies in the meta-analysis by Pittler and Ernst, reported on adverse

events. Four studies (Cloarec, 1992; Diehm *et al*., 1996; Pilz, 1990; Rudofsky *et al*., 1986) reported that there were no treatment-related adverse events in the horse chestnut seed extract group. Gastrointestinal complaints, dizziness, nausea, headache and pruritus were reported as adverse events in six studies (Diehm and Schmidt*,* 2001; Friederich *et al*., 1978; Morales and Barros, 1993; Neiss and Bohm, 1976; Rehn *et al*., 1996; Steiner and Hillemanns, 1990). The frequency ranged from 1 to 36% of treated patients. Four other studies (Diehm *et al*., 1992; Koch, 2002; Lohr *et al*., 1986; Steiner and Hillemanns, 1986) reported good tolerability with horse chestnut seed extract.

The meta-analysis published by Siebert *et al*., 2002, comprised three observational studies with a total of 10725 patients. No severe adverse events were reported. Mild adverse event rates reported in these studies were 2.89% (95% CI, 2.41-3.46%), 0.61% (95% CI, 0.43-0.86%) and 0.85% (95% CI, 0.43-1.60%) respectively, yielding a sample-size weighted average of 1.51% (95% CI, 1.29-1.76%).

Table 6: Clinical safety data from clinical trials

Type of study	Study Design	Herbal preparation, pharmaceutical form, dosage	Number of subjects	Type of subjects	Adverse reactions	Comments
Cochrane review of the efficacy and safety of oral horse chestnut seed extract (HCSE) versus placebo, or reference therapy, for the treatment of chronic venous insufficiency (CVI) Pittler and Ernst (2012)	Meta-analysis 17 studies included 10 placebo-controlled, 2 compared HCSE against reference treatment with compressing stockings and placebo, 4 controlled against reference medication with O-β-hydroxyethyl rutosides and 1 controlled against pycnogenol. The duration ranged from 2 to 16 weeks.	In all of the studies HCSE was administered orally and the extract was standardised on aescin 14 studies with 240-290 mg of a standardised dry extract (5:1. ethanol 50 % V/V) corresponding to 50 mg of aescin 2 times daily. 2 studies with 360-412 mg standardised dry extract (5:1. ethanol 50% V/V), corresponding to 75 mg of aescin 2 times daily	1443 patients	Patients with CVI. The majority of the included studies diagnosed the patients according to the classification by Widmer (Widmer 1978)	Out of 17 studies, 14 reported on adverse events. 4 studies (Cloarec 1992; Diehm *et al.*1996; Pilz 1990; Rudofsky *et al.* 1986) reported that there were no treatment-related adverse events in the HCSE group. Gastrointestinal complaints, dizziness, nausea, headache and pruritus were reported as adverse events in 6 studies (Diehm and Schmidt 2001; Friederich *et al.* 1978; Morales and Barros 1993; Neiss and Bohm 1976; Rehn *et al.* 1996; Steiner and Hillemanns1990a). The frequency ranged from 1 to 36% of treated patients. 4 other	Gastrointestinal complaints, vertigo, itching, headache and allergic reactions are listed in section 4.8 of the WEU monograph (oral use). The reported adverse events in this meta-analysis are considered as listed in the monograph.

Type of study	Study Design	Herbal preparation, pharmaceutical form, dosage	Number of subjects	Type of subjects	Adverse reactions	Comments
					studies (Diehm *et al.* 1992; Koch 2002; Lohr *et al.* 1986; Steiner and Hillemanns 1986) reported good tolerability with HCSE.	
Meta-analysis on effect of HCSE in the treatment of chronic venous insufficiency (CVI) Siebert *et al*., 2002	Meta-analysis 16 studies included 13 controlled and 3 observational studies The duration ranged from 3-12 weeks for the RCTs and 4 weeks–6 months for the observational studies	Horse chestnut seed extracts, no further details included in the meta-analysis	10725	Patients with CVI	No severe adverse events were reported. Mild and transient adverse events reported were gastrointestinal disorders such as constipation, diarrhoea, vomiting, and nausea. Headache, dizziness, flushing, fatigue, and itching were also reported.	Gastrointestinal complaints, vertigo, itching, headache and allergic reactions are listed in section 4.8 of the WEU monograph (oral use). There are no new safety issues identified to add any adverse event in section 4.8 of the monograph.

5.2. Patient exposure

Oral use:

The controlled studies evaluated in the meta-analysis published by Pittler and Ernst (2012) comprised 1443 patients. The open studies, evaluated by Siebert *et al*., (2002) comprised 10725 patients.

According to Hitzenberger (1989) a specific commercial preparation (a preparation consisting of a dry extract DER 5:1 extraction solvent ethanol 50% V/V corresponding to 50 mg of aescin) was used in 895 362 500 single doses (=447 681 250 daily doses) between 1968 and 1988.

Cutaneous use:

Aside from market presence and data from studies, there are no concrete data concerning patient exposure. There is no restriction of use in cosmetics for *A. hippocastaum* L., seed extract according to CosIng (the European Commission database for information on cosmetic substances and ingredients).

5.3. Adverse events, serious adverse events and deaths

A serious safety issue was raised more than 25 years ago, i.e. the risk of acute renal failure, when patients, who had undergone cardiac surgery, were given high doses of horse chestnut seed extract i.v. for post-operative oedema. This led to three clinical trials to assess the effects of aescin on renal function. The total number of subjects studied was 83, comprising 18 healthy volunteers. Ten were administered 10 mg aescin i.v. daily for 3 days, eight were given 20 mg aescin i.v. for 6 days; 40 in-patients (38 adults and two children aged 4 and 8 years) with intact renal function, given aescin (10 mg i.v. twice a day for 6 days-the highest recommended therapeutic dose-except in the two children, who received 0.2 mg/kg daily) for the treatment of post-operative oedema after reconstructive surgery of the hand and extremities following trauma; 12 patients with cerebral oedema and normal renal function, who were given a massive i.v. dose on the day of surgery (49.2±19.3 mg) and 15.4+9.4 mg daily for the following 10 days; 13 patients with impaired renal function due to glomerulonephritis or pyelonephritis, who were given 20-25 mg i.v. daily for 6 days. In all studies renal function was monitored daily resorting to the usual tests of renal function: BUN, serum creatinine, creatinine clearance, urine analysis. In a selected number of cases para-aminohippurate and labelled EDTA clearance were also measured. No signs of development of renal impairment in the patients with normal renal function or of worsening of renal function in the patients with renal impairment were recorded. All of these studies were carried out with whole horse chestnut seed extract (no further information on the herbal preparation available in the reference) (Sirtori, 2001).

Assessor's comment:

No undesirable effects on the renal function were observed in this safety study.

Snow *et al*., 2012, report that a 46-years old female patient taking horse chestnut seed extract (DER and extraction solvent not specified) for venous insufficiency developed bleeding from a 5 cm angiomyolipoma (AML) (diagnosed since 15 years). Routine blood investigations showed an INR of 2.5. The authors state that there is a potentially life-threatening association between horse chestnut seed extract-containing products and renal AML, highlighting the risk associated with horse chestnut seed extract induced anticoagulation.

Assessor's comment:

In the report by Snow et al., 2012, the details about the herbal preparation, dosage, duration of intake and de-challenge/re-challenge are poor.

Renaudin *et al*., 2013 analysed 333 cases recorded by the Allergy Vigilance Network from 2002 to 2010. The following information on horse chestnut extract (DER and extraction solvent not specified) was listed in a table for drug implicated in severe drug anaphylaxis: 1 report, skin test negative, *in vitro* LHRT (leucocyte histamine release test) positive.

Assessor's comment:

There are no details in the report about the herbal preparation, dosage, duration of intake and de-challenge/re-challenge. Hypersensitivity is included as a contraindication in section 4.3 and listed as an undesirable effect in section 4.8 of the current version of the monograph.

Hartleb and Gutkowski, 2015 reported an unexpected liver cirrhosis in a 33-year-old female patient with long-term use of horse chestnut seed extract. The authors reported portal hypertension with esophaegal varices (I/II degree), no ascites and histologically proven liver cirrhosis. The patient had no history of liver disease and had taken horse chestnut seed extract for 5 years (2-times daily 167 mg of a specific commercial dry extract) for telangiectasia on the left calf. No other drugs were used and the patient did not drink excessive amounts of alcohol. Differential diagnosis was ruled out and there was a positive dechallenge. The authors refer to one more case report by Takegoshi *et al*., 1986 on hepatic injury, after using a specific commercial product containing an extract of *A. hippocastanum* L. In this case report, a 37-year-old man was admitted for treatment of a pathological fracture of the left brachial bone. 17 days later (60 days after injection), the patient had pruritus and jaundice and a liver function test showed mild abnormality. Laboratory results revealed moderate elevation of total bilirubin, ALP, gamma-GTP and mild eosinophilia. CT studies and ERC showed no signs of extrahepatic obstructive jaundice. The lymphocyte stimulation test was positive. The liver biopsy demonstrated marked cholestasis with zonal necrosis in the centrilobular areas but showed little or no changes in the portal tracts.

Assessor's comment:

Only these two case reports on liver toxicity have been found in the literature. In the old case report from Japan, the product was injected. There is no information from MS(s) that pharmacovigilance actions have been taken against products in EU.

Zaj *et al*., 2014, has published a case report of a 15-year-old woman who was admitted to the hospital because of symptoms including vomiting, dyspnoea, burning in the nose and throat, and syncope, after intranasal snuff of powdered horse chestnut seeds. Laboratory tests showed no abnormalities. After 2 days of hospitalization the female was discharged home with subjective and objective improvement. The preparation and use of snuff is related to the tradition of the kashubian region. The authors conclude that intoxication by powdered seeds of horse chestnut used nasally as snuff may lead to sudden and self-limiting clinical symptoms.

Assessor's comment:

The undesirable effects observed after the nasal use of powdered horse chestnut seeds are not considered relevant for the monograph.

A 32-year-old male patient who had consumed 3 boxes of horse chestnut (*A. hippocastanum* L.) paste over the previous 1.5 months was referred to a cardiology outpatient clinic with a complaint of dyspnoea (Edem *et al*., 2016). The chest x-ray examination revealed an enlarged cardiac shadow and bilateral pleural effusion. On transthoracic echocardiographic examination, his ejection fraction was found to be 55% with circumferentially extended pericardial effusion that reached 3.9 cm at its maximal thickness. During follow-up, the patient developed signs of jugular venous distention and hepatic congestion, and signs of cardiac tamponade were also found in his transthoracic echocardiogram. Direct examination of the pleural fluid revealed a large number of polymorphonuclear

leukocytes but no bacteria. The pericardial and pleural biopsies, the cell counts and the immunohistochemical assays did not demonstrate any findings that suggested malignancy. Results of testing for rheumatologic and autoimmune disease markers were negative in the blood samples. Based on all these findings, consumption of horse chestnut paste was considered to have been the cause in this case of pericarditis (Edem *et al*., 2016).

Assessor's comment:

The authors state that there are many well-known causes of pericardial effusion, such as cancer metastasis, bacterial or viral pericarditis, and uremic pericarditis, but that no reports exist in the literature demonstrating a pericardial effusion that led to cardiac tamponade following consumption of an herbal preparation. Also, the preparation is described as 'un-purified, traditionally acquired horse chestnut paste', no further information is available. Thus, this report does not trigger any safety concerns for the well-established use monograph on ethanolic extracts of horse chestnut seed.

In a study by Iannitti *et al*., 2013, the efficacy and safety of a new betamethasone valerate medicated plaster to manage facial swelling, oedema, inflammation, ecchymosis, and hematoma, when applied immediately after a facial rejuvenation procedure were investigated. The control group (20 patients) were treated with an aescin 10% cream, which was applied immediately after the procedure, in the evening, and the morning after. Among the 20 patients that received aescin 10% cream, seven reported no swelling, oedema, or inflammation, five reported minimal oedema and inflammation, four patients showed slight swelling, three had moderate swelling and inflammation, and a patient reported a severe degree of inflammation.

Assessor's comment:

No new safety issues were identified in the study.

In addition to literature reports, a screening for adverse reactions in the EudraVigilance database was conducted in August 2018, using the eRMR tool for *Aesculus hippocastanum*.

Assessor's comment:

No new safety issues for the oral or cutaneous use of horse chestnut seed preparations in the monograph were identified from screening the EudraVigilance database.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

No information available.

5.5.1. Use in children and adolescents

The well-established use indication "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" and the traditional use indication "to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances", are not intended for children. Although, chronic venous insufficiency is no longer considered a disorder related to age as the first symptoms can develop between the ages of 15 and 25 years (Pitsch, 2012), the use is not recommended in adolescents under 18 years of age due to a lack of data on safety and efficacy. The traditional use indication "to relieve symptoms of discomfort and heaviness of legs related to minor

venous circulatory disturbances" is not recommended in adolescents under 18 years of age because of concerns requiring medical advice.

In the absence of sufficient safety data, the use in children below 12 years of age is not recommended for the traditional use indication "relief of signs of bruises, such as local oedema and haematoma" (see section "special warnings and precautions for use" in the monograph).

5.5.2. Contraindications

Hypersensitivity to the herbal preparations is included as a contraindication in the well-established use and traditional use monograph.

5.5.3. Special Warnings and precautions for use

The following general warning is included for all indications in the well-established use and traditional use monographs:

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

The following warning is included in the well-established use monograph and for the indication "to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances" in the traditional use monograph:

"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."

The following warning is included for the cutaneous use in the traditional use monographs:

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

5.5.4. Drug interactions and other forms of interaction

It has been stated that aescin could increase the effect of anti-coagulants (Blaschek *et al.*, 1992), but no confirmed case reports have been found in the literature or been identified through the spontaneous reporting system.

5.5.5. Fertility, pregnancy and lactation

Pregnant women participated in one clinical trial (Steiner and Hillemanns 1990). No adverse events were reported. However, the data is not sufficient to recommend the use of any horse chestnut seed preparation during pregnancy and lactation.

No fertility data available.

5.5.6. Overdose

No information 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

No information available.

5.6. Overall conclusions on clinical safety

Well-established use monograph:

As the bioavailability of orally administered aescin is only about 1.5% of the given dose (see 4.1.2.), there are no safety concerns in this aspect when horse chestnut seed extract is given orally in the normal dose.

Only mild adverse events were reported in the 17 clinical trials evaluated in a meta-analysis. Also, in open clinical trials with over 10,000 participants only a small number of mild adverse events were reported.

For oral use, gastrointestinal complaints, vertigo, itching, headache and allergic reactions are listed undesirable effects in section 4.8 of the monograph. The frequencies of the undesirable effects are not known.

No concerns that standardised dry extracts of horse chestnut seed used orally 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, are harmful under normal conditions of use have been identified. Long-term use is possible in consultation with a doctor.

Traditional use monograph:

For oral use, gastrointestinal complaints, vertigo, itching, headache and allergic reactions are listed undesirable effects in section 4.8 of the monograph. The frequencies of the undesirable effects are not known.

For cutaneous use, hypersensitivity reactions of the skin (itching and erythema) are listed undesirable effects in section 4.8 of the monograph. The frequencies of the undesirable effects are not known.

Long-standing medicinal use and experience of horse chestnut seed preparations for oral and cutaneous use to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances or for cutaneous use to relief of signs of bruises, such as local oedema and haematoma, have been documented within the EU. During this time, no concerns that horse chestnut seed preparations are harmful under normal conditions of use have been identified.

6. Overall conclusions (benefit-risk assessment)

Well-established use monograph:

In the first version of the monograph, the dry extract of horse chestnut seed (40-60% ethanol) was defined to contain 16-28% of glycosides of triterpenes, expressed as anhydrous aescin. In 2017 the Ph. Eur. monographs on Horse-chestnut dry extract, standardised, were updated and defined as a dry extract (produced by using a hydroalcoholic solvent equivalent in strength to ethanol 40-80% V/V), standardised to contain 6.5-10% triterpene glycosides calculated as protoaescigenin. The new definition of the Horse-chestnut dry extract in the Ph. Eur. has been carefully analysed by the EDQM to reflect the extracts in authorised medicinal products on the EU market. The new analytical method in the Ph. Eur. monograph does not change the actual quality of the extract. Thus, the herbal preparation in the EU herbal monograph on horse chestnut seed has been updated accordingly.

The data in support of aescin as responsible for the therapeutic effect of dry extract of horse chestnut seed is very weak, but the extracts used in most clinical trials appear to be produced as extracts standardised on aescin. It can thus be debated whether dry extract of horse chestnut seed should be classified as a standardised or a quantified extract in the sense of Ph. Eur. In the final discussion for the first version of the monograph, the notation "standardised extract" was considered most appropriate. A consequence of classifying dry extract of horse chestnut seed as a standardised extract, is that it is reasonable to widen the span of ethanol content of the extraction solvent as long as the dose of the HCSE corresponds to 21 mg protoaescigenin 2 times daily.

The clinical efficacy and safety of the dry ethanolic extracts of horse chestnut seed in a daily oral dose corresponding to 100 mg triterpene glycosides calculated as aescin (42 mg triterpene glycosides calculated as protoaescigenin), are well documented. The extracts have been used in Europe for treatment of chronic venous insufficiency since 1968. The exact mechanism of action of dry ethanolic extracts of horse chestnut seed 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 is not known, but preclinical and clinical pharmacological studies indicate that an effect on venous tone and capillary filtration rate is involved.

Based on a systematic review (meta-analysis) of 17 clinical trials, it can be concluded that horse chestnut seed extract significantly reduces symptoms of chronic venous insufficiency, such as oedema, pain and itching compared to placebo.

The requirements for well-established use according to Article 10a of Directive 2001/83/EC are considered fulfilled for the 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.

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

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

Due to the uncertainty of the analytical data available for the pharmacokinetics of aescin, precise figures of the pharmacokinetic parameters should not be given in the monograph. However, the data seem to allow the conclusion that there is virtually no difference in bioavailability of aescin between retarded and non-retarded preparations. This conclusion, in combination with the view that the extracts is standardised on aescin (protoaescigenin), led to an agreement to include horse chestnut seed extracts in pharmaceutical forms both for immediate and modified release in the monograph.

Proposed ATC-code: C05CX03

Traditional use monograph:

According to the market overview and literature, horse chestnut seed preparations for oral and cutaneous use fulfils the criteria of medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA, i.e. traditional medicinal use according to Directive 2004/24/EC for the following herbal preparations and indications:

Indication 1)

Traditional herbal medicinal product to relieve symptoms of discomfort and heaviness of legs related to minor venous circulatory disturbances. The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.

Cutaneous use:

a) Dry extract corresponding to a specified amount of triterpene glycosides, calculated as protoaescigenin, extraction solvent ethanol 25-50% V/V

b) Liquid extract (1:3.5-5), extraction solvent ethanol 50% V/V

c) Dry extract (DER 5-10:1), extraction solvent methanol 80% V/V

d) Dry extract (DER 5-8:1), extraction solvent methanol 80% V/V

e) Dry extract (DER 4.5-5.5:1), extraction solvent ethanol 50% V/V

f) Dry extract (DER 5-7:1), extraction solvent ethanol 60% V/V

Oral use:

g) Liquid extract (DER 1:1.5-2.5), extraction solvent ethanol 55% V/V

h) Liquid extract (DER 1:2), extraction solvent ethanol 19% m/m

i) Dry extract (DER 3-6:1), extraction solvent water

Indication 2)

Traditional herbal medicinal product for relief of signs of bruises, such as local oedema and haematoma. The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.

Cutaneous use:

a) Dry extract corresponding to a specified amount of triterpene glycosides, calculated as protoaescigenin, extraction solvent ethanol 25-50% V/V

b) Liquid extract (1:3.5-5), extraction solvent ethanol 50% V/V

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

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

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

Therapeutic area Indication 1 and 2: Circulatory disorders.

Annex

List of references