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Evaluation of Medicines for Human Use

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COMMITTEE ON HERBAL MEDICINAL PRODUCTS (HMPC)

ASSESSMENT REPORT ON *AESCULUS HIPPOCASTANUM* L., SEMEN

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 75 23 70 51
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

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I. REGULATORY STATUS OVERVIEW[1]

MA: Marketing Authorisation;
TRAD: Traditional Use Registration;
Other TRAD: Other national Traditional systems of registration;
Other: If known, it should be specified or otherwise add 'Not Known'

Member State	Regulatory Status				Comments[2]
Austria	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Denmark	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Estonia	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Finland	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☒ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Latvia	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Norway	☐ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	
Poland	☒ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Slovenia	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Sweden	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

[1] This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.
[2] Not mandatory field.

Member State	Regulatory Status				Comments[2]
United Kingdom	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	

II. ASSESSMENT REPORT

BASED ON ARTICLE 10A OF DIRECTIVE 2001/83/EC AS AMENDED

(WELL-ESTABLISHED USE)

BASED ON ARTICLE 16D(1), ARTICLE 16F AND ARTICLE 16H OF DIRECTIVE 2001/83/EC AS AMENDED

(TRADITIONAL USE)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Aesculus hippocastanum* L., semen
Herbal preparation(s)	Dry extract (40-80% ethanol), standardised to contain 16-28% triterpene glycosides calculated as aescin (well-established use) Dry extract (ethanol 25-50% v/v) (traditional use) Tincture (1:5; extraction solvent: 25-50% ethanol v/v) (traditional use; topical use)
Pharmaceutical forms	Herbal preparations in oral dosage forms for prolonged or immediate release (well-established use) Herbal preparations in dosage forms for cutaneous use (traditional use)
Rapporteur	Per Claeson
Assessor	Per Claeson

II.1 INTRODUCTION

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

- Herbal substance(s) [3] : *Aesculus hippocastanum* L., semen. 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). A monograph is also available in DAB 10.
- Herbal preparation(s): 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 ($C_{55}H_{86}O_{24}$; M_r 1131) and calculated with reference to the dried extract (Ph. Eur. draft monograph 1996).

Information on period of medicinal use in the Community regarding the specified indication
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 *et al.* 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 1996).
Topically/externally, horse-chestnut extract (aescin 1%) has been used for contusions, non-penetrating wounds and sportinjuries involving oedema (Rote Liste 1969,1980; Mills *et al.* 2000; Bradley 2006).
Aescin has been used orally and topically in the prevention and preatment of various peripheral vascular disorders including traumatic swellings and post-operative oedema (Reynolds, 1982).
Based on the survey of products avaliable in the Member states, *A. hippocastanum* containing products have been in use for at least 30 years:
Germany: Ointment (since 1976) containing 20% tincture (1:5; extraction solvent 50% ethanol v/v); traditionally used for the improvement of conditions in tired legs; apply 1-3 times daily, thinly and evenly on the intact skin above the affected regions of the body and massage gently
Austria: Ointment (since 1968) containing dry extract (extraction solvent: ethanol 50% v/v) corresponding to 760 mg aescin per 100 g ointment; pain in the legs, heavy legs, pruritus, varicosis; apply several times daily.

Conclusions on traditional use
Based on information obtained from Member states and data retreived from handbooks it can be concluded that the following extracts and uses of horse chestnut seed fulfil the criteria for traditional use:

- Dry aqueous ethanol (25-50% v/v) extract in a strength corresponding to ca 1% aescin in the ointment base, for cutaneous use.
- Tincture (1:5; extraction solvent: 50% ethanol v/v), 20% in an ointment base, for cutaneous use.

Indications:

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

B) Traditional herbal medicinal product for relief of symptoms of bruises, such as oedema and haematoma.

3 According to the 'Procedure for the preparation of Community monographs for traditional herbal medicinal products' (EMEA/HMPC/182320/2005 Rev.2) and the 'Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use (EMEA/HMPC/182352/2005 Rev.2)

Posology:

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

B) 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.

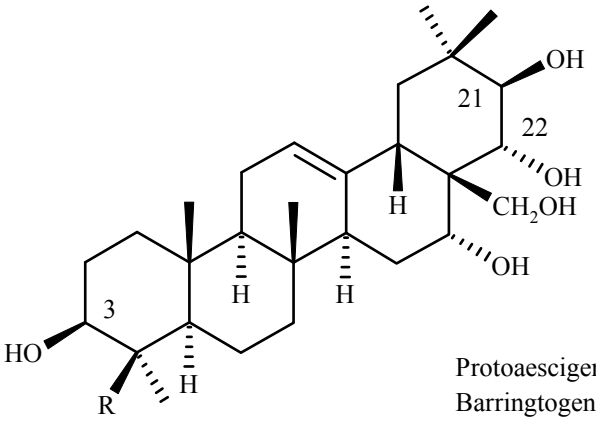
II.2 NON-CLINICAL DATA

II.2.1 Pharmacology

II.2.1.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Constituents

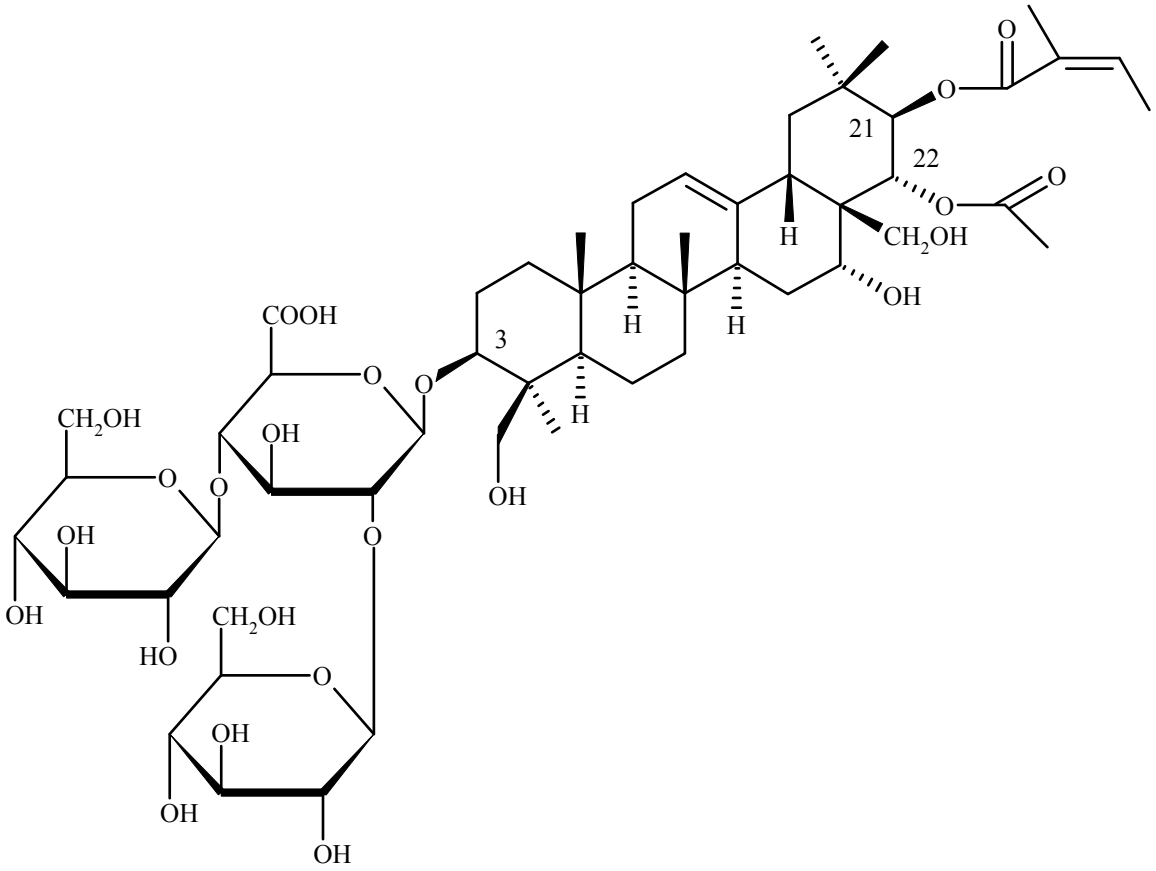
The seeds of *Aesculus hippocastanum* L. contain 3-10% of a mixture of acylated triterpene glycosides (saponins). These are based on two aglycones – protoaescigenin and barringtogenol C, which differ only at C-24, which is hydoxylated in protoaescigenin. In the glycosides both aglycones are esterified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



Protoaescigenin: R = CH_2OH
Barringtogenol C: R = CH_3

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. 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 1969).



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 *Aesculum hippocastanum* L., but not in the seeds or the seed shell (Hagers Handbuch 1992).

Primary pharmacodynamics

The literature on the pharmacodynamics of hydro-alcoholic extracts of *Aesculus hippocastanum* L., semen and of aescin is extensive. Comprehensive reviews are presented by Bombardelli (1996), Hagers Handbuch (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 IV administration of aescin at 0.2 and 2.5 mg per kg body weight. IP administration of 4 mg/kg bw of aescin inhibited dextran-induced oedema in the rat paw model. IV administration to rats of aescin at 2.5 mg/kg bw prevented an increase in vascular permeability, caused by IP injection of egg white.
Similar results are reviewed by Hager (1992)
Lorenz (1960) performed an extensive investigation on several ethanol 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

The effects of extracts (no information on drug/extract ratio or solvent) from horse chestnut (*Aesculus hippocastanum*) 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.
According to ESCOP (2003) similar effects on venous tone were observed with HCSE (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) contained in the commercial preparation Veinotonyl 75®. In the isolated canine saphenous vein, the extract induced concentration-dependent contractions at concentrations above 5×10^{-5} mg/ml; the contraction at 5×10^{-4} mg/ml reached a maximum in 15 minutes and lasted more than 5 hours. In anaestetized dogs, IV 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 ($p<0.001$) (Guillaume 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 antiinflammatory drugs, indicating that the effect was dependent on $PGF_{2\alpha}$ in the venous tissue. This ability to enhance $PGF_{2\alpha}$ generation in the veins, and thus increase tonus, may be of relevance in venous insufficiency (Longiave 1978).
Other investigations showing venotonic effects of various alcoholic horse-chestnut seed extracts are reviewed by Bombardelli (1996). Antioedemic activity of oral doses of horse-chestnut seed extracts was demonstrated in experiments on rats with blocked leg lymphatic circulation (Bombardelli 1996).

Effect on contractility of human veins

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

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* lipopolysaccaride (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 CoCl2 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 2007)

Effect on lysosymal enzymes

Aescin inhibited the enzyme hyaluronidase (IC_{50} = 149.9 μM) (Facino 1995).

Safety pharmacology

No information except toxicity data presented in section II.2.3.

Pharmacodynamic interactions

No information.

II.2.1.2 Assessor's overall conclusions on pharmacology

Aqueous ethanolic extracts of *Aesulus 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 IV and IP administration. No effect was observed after oral administration. The reported inhibitory effect of aescin on lysosymal 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 (ca 15-20% of the HCSE) has *in vivo* antioedematous activity after IV/IP administration in rats, but this experimental model is of very little relevance concerning oral use of HCSE in chronic venous insufficiency.

II.2.2 Pharmacokinetics

II.2.2.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Some pharmacokinetic studies have been performed on aescin, but compelling evidence that the pharmacological activity of HCSE can be ascribed to aescin has not been presented. At present, the pharmacokinetic data on aescin are of limited importance.
There are no indications that toxic metabolites are formed from aescin.

II.2.2.2 Assessor's overall conclusions on pharmacokinetics

No information available on pharmacokinetic interactions. No information available on metabolites.

II.2.3 Toxicology

II.2.3.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Single dose toxicity

LD_{50}-values (mg/kg bw) for the extract of HCSE contained in the preparation Venostasin retard (dry extract, DER 5:1, 50% aqueous ethanol, standardized for a content of 50 mg aescin in 240-290 mg extract) are illustrated in the following table (Liehn 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 the extract contained in the preparation Venostasin retard (dry extract, DER 5:1, 50% aqueous ethanol, standardized to a content of 50 mg aescin in 240-290 mg extract) is available in Hager (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 Hager (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 1977)

Sub-acute intravenous toxicity

Daily IV-doses of the HCSE contained in Venostasin retard (see above) 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. Hemoglobin as well as hematocrit 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 1972).

Chronic oral toxicity

Neither toxic effects, nor organ damage were observed after 34 week oral administration of the HCSE contained in Venostasin retard (see above) to dogs (2 male, 2 female per dose and control group) at 20, 40 or 80 mg/kg bw daily (5 days/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 1972).

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. Hematology indices (white blood cell, red blood cell, platelet and hemoglobin) 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 hemoglobin 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). *Conclusion:* There was significant blood toxicity to SD rats treated with high dose of aescin i.p. (Li 2006).

Genotoxicity

In the Ames mutagenicity test, using *Salmonella typhimurium* strain TA 98, a commercial dry extract (Caesar & Loretz) 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 1994; ESCOP 2003).

Teratogenicity

Following daily oral administration of the HCSE contained in Venostasin retard (see above) 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 1972).
Juvenile rats were treated with 2 x 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 1977).

II.2.3.2 Assessor's overall conclusions on toxicology

With an LD_{50} ranging between 1 and 2.6 g/kg bw, the acute oral toxicity of HCSEs is low in all animals studied. The subacute studies were performed with IV administration and indicated that a daily dose amounting to ¼ of the IV 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 HCSE have been reported.

II.3 CLINICAL DATA

II.3.1 Clinical Pharmacology

II.3.1.1 Pharmacodynamics

II.3.1.1.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

The effect of an extract (drug/extract ratio 5:1, 50% ethanol), contained in capsules with 240 to 290 mg of the extract, standardized 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 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 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, standardized 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, 50% aqueous ethanol, 900 mg, standardized to 150 mg of aescin = Venostasin®) 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 HCSE does not inhibit the individual enzymes but has a protective action towards the site of enzyme release, the fragile lysosomal membrane (Enghofer 1984).

II.3.1.1.2 Assessor's overall conclusions on pharmacodynamics

An increase in venous tone has been reported for oral HCSE 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 HCSE 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 HCSE would prevent the action of enzymes which catalyze 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 HCSE 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.

II.3.1.2 Pharmacokinetics

II.3.1.2.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

After intravenous administration, the pharmacokinetics of aescin was found to correspond to an open three compartment model. With an intravenous dose of 5 mg of aescin (infusion rate: 718 µg/min) 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 litres, total plasma clearance 21.8 ml/min and renal clearance 1.7 ml/ min. Urinary excretion from 0 to 120 hours after injection comprised 8.2% of the dose. After oral administration of an aescin solution the absolute bioavailabiIity was determined as only 1.5 %. This low bioavailability is due to a pronounced first pass effect (metabolism and biliary excretion). The relative bioavailabiIity of aescin from a horse-chestnut seed extract (Venostasin retard ®) 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 (Venostasin retard®, 240-290 mg of HCSE, providing 50 mg of aescin) and other pharmaceutical formulations. Some pharmacokinetic parameters for aescin have been reported from these studies.
In single dose experiments (Schrader 1995; Dittgen 1996 and Oschmann1996), HCSE (Venostasin retard®) contataining 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 intraindividual variability in serum concentrations was observed (Loew 2000).

In repeated dose experiments (Oschmann 1996; Schrödter 1998; Kunz 1998), HCSE (Venostasin retard®) contataining 50 mg aescin resulted in C_{max} values ranging from 6.5-16.7 ng/ml (Loew 2000). Also during these steady state conditions a considerable variability in serum concentrations between the different clinical studies were observed (Loew 2000).
In all the published clinical studies the same RIA, under identical conditions, in the same laboratory, was employed. The immuno assay 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 HCSE 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 HCSE. The absolute values of the pharmacokinetic parameters obtained with this RIA are thus not reliable (Loew 2000; Bässler 2003).

II.3.1.2.2 Assessor's overall conclusions on pharmacokinetics

There is no compelling evidence that aescin is the therapeutically active substance in HCSE, 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 2000; Bässler 2003). They are thus not suitable for adjusting a dosing regimen of HCSE in clinical practice.

II.3.2 Clinical Efficacy[4]

II.3.2.1 Dose response studies

II.3.2.2 Clinical studies (case studies and clinical trials)

A systematic review of 29 randomised controlled clinical trials assessing oral mono-preparations containing extracts of *Aesculus hippocastanum* L. semen (HCSE) has been published (Pittler 2006). The following 17 trials met the inclusion criteria: Cloarec 1992 (unpublished), Diehm 1992, 1996, 2001), Erdlen 1989, Erler 1991, Friederich 1978, Kalbfleisch 1989, Koch 2002, Lohr 1986, Morales 1993, Neiss 1976, Pilz 1990, Rehn 1996, Rudofsky 1986, Steiner 1986 and, Steiner 1990. Of these, ten were placebo-controlled, two compared HCSE 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 HCSE 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 the preparation called Venostasin retard, consisting of capsules containing 240-290 mg of a standardized dry extract (5:1.50 % ethanol) corresponding to 50 mg of aescin. The daily dose was 2 capsules = 100 mg of aescin. Two studies (Diehm 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 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.

[4] In case of traditional use the long-standing use and experience should be assessed.

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 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 1978; Lohr 1986; Morales 1993; Neiss 1976; Rudofsky 1986; Steiner 1990a). Six studies (n = 543) reported a statistically significant reduction ($P < 0.05$) of leg pain on various measurement scales in participants treated with HCSE compared with placebo, while another reported an improvement compared with baseline (Steiner 1990a). 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 HCSE with hydroxyethyl rutoside (Kalbfleisch 1989), pycnogenol (Koch 2002) or compression (Diehm 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 1978; Lohr 1986; Morales 1993; Neiss 1976; Steiner 1990a). Four trials (n = 461) reported a statistically significant reduction of oedema in participants treated with HCSE compared with placebo, whilst one (Steiner 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 HCSE assessed on a 100 mm VAS. Another study (Koch 2002) reported that HCSE was inferior to pycnogenol, whereas a further trial (Diehm 2001) reported no significant differences for a score including the symptom oedema compared with compression. Oedema provocation before and after treatment with HCSE revealed oedema protective effects (Erler 1991).

Pruritus: Pruritus was assessed in eight placebo-controlled trials (Diehm 1992; Friederich 1978; Lohr 1986; Morales 1993; Neiss 1976; Rudofsky 1986; Steiner 1986; Steiner 1990). Four trials (n = 407) suggested a statistically significant reduction of pruritus in participants treated with HCSE compared with placebo ($P < 0.05$). Two trials (Steiner 1986; Steiner 1990a) suggested a statistically significant difference in favour of HCSE compared with baseline ($P < 0.05$). Another trial (Kalbfleisch 1989), which compared HCSE with HR, but failed to include a placebo group, seemed to corroborate these findings. A further trial (Diehm 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 1992; Diehm 1996; Diehm 2001; Lohr 1986; Rudofsky 1986; Steiner 1986; Steiner 1990). All of these studies used water displacement plethysmometry to measure this outcome. Meta-analysis of six trials (Diehm 1992; Diehm 1996; Diehm 2001; Rudofsky 1986; Steiner 1986; Steiner 1990; n = 502) suggested a WMD of 32.1m1 (95% CI 13.49 to 50.72) in favour of HCSE compared with placebo (pooled standardised mean difference 0.34; 95% CI 0.15 to 0.52). One trial (Rehn 1996) reported findings suggesting that HCSE was equivalent to HR, and another (Diehm 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 HCSE standardised to 100-150 mg aescin daily. Three studies, using 100 mg escin daily, reported a statistically significant reduction of mean leg volume after two weeks of treatment compared with placebo ($P < 0.01$) (Rudofsky 1986; Steiner 1986; Steiner 1990). Persistence of treatment effects was suggested by one study (Rehn 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 1992; Lohr 1986; Pilz 1990; Rudofsky 1986; Steiner 1986; Steiner 1990). Five studies (n = 172) suggested a statistically significant reduction at the ankle, and three (n = 112) at the calf in favour of HCSE compared with placebo. At the ankle, meta-analysis of three trials (Cloarec 1992; Pilz 1990; Steiner 1986),

The authors of the systematic review conclude that HCSE 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 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 (2006, see above). The controlled studies comprised 1051 patients and the number in the observational studies was 10725. Of the controlled studies, 11 were also evaluated by Pittler (2006) while 2 were not included in Pittler's analysis. Data from the meta-analysis of the controlled trials supported a clinically relevant and statistically significant effect of HCSE in the treatment of chronic venous insufficiency. Leg volume, ankle and calf circumference and edema 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 HCSE in routine settings and confirmed the findings from the controlled trials.

II.3.2.3 Clinical studies in special populations (e.g. elderly and children)

No information available.

II.3.2.4 Assessor's overall conclusions on clinical efficacy

Two meta-analyses, comprising 19 controlled clinical trials and 3 observational studies of a preparation (Venostasin retard) containing a dry extract (5:1, 50% aqueous ethanol, standardized to a content of aescin of 50 mg/ 240-290 mg of extract) of *Aesculus 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 (1996) where HCSE 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 HCSE 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 1996). The oedema reduction obtained in this study was ca 55 ml (for both HCSE 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 HCSE compared to placebo, although the data are less convincing.

II.3.3 Clinical Safety/Pharmacovigilance

II.3.3.1 Patient exposure

The controlled studies evaluated in the meta-analysis published by Pittler (2006) comprised 1443 patients. The open studies, evaluated by Siebert (2002) comprised 10725 patients.

II.3.3.2 Adverse events

Fourteen studies in the meta-analysis, referred to above, reported on adverse events. Four studies (Cloarec 1992; Diehm 1996; Pilz 1990; Rudofsky 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 six studies (Diehm 2001; Friederich 1978; Morales 1993; Neiss 1976; Rehn 1996; Steiner

The meta-analysis published by Siebert (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%)

According to Hitzenberger (1989) the preparation Venostasin retard was used in 895 362 500 single doses (= 447 681 250 daily doses) between 1968 and 1988. Only 15 cases of mild adverse effects were reported.

II.3.3.3 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 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 i.v. daily for 3 days, eight were given 20 mg 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. bid for 6 days-the highest recommended therapeutic dose-except in the two children, who received 0.2 mg kg^{-1} daily) for the treatment of postoperative 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, urin 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 HCSE (Sirtori 2001).

Assessors comment

As the bioavailabillity of orally administered aescin is only about 1.5% of the given dose (see II.3.1.2.1), there are no safety concerns in this aspect when HCSE is given orally in the normal dose.

II.3.3.4 Laboratory findings

None reported.

II.3.3.5 Safety in special populations and situations

No reports.

II.3.3.5.1 Intrinsic (including elderly and children) /extrinsic factors

No reports

II.3.3.5.2 Drug interactions

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

II.3.3.5.3 Use in pregnancy and lactation

Pregnant women participated in one clinical trial (Steiner 1990). No adverse events were reported.

II.3.3.5.4 Overdose

No information.

II.3.3.5.5 Drug abuse

No information.

II.3.3.5.6 Withdrawal and rebound

No information.

II.3.3.5.7 Effects on ability to drive or operate machinery or impairment of mental ability

No information.

II.3.3.6 Assessor's overall conclusions on clinical safety

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. During 20 years only 15 mild adverse events were reported from the consumption of 447 million daily doses of the HCSE contained in the product Venostasin. This extract is therefore acceptable with respect to clinical safety.
Only oral administration is foreseen for treatment of chronic venous insufficiency.

II.4 ASSESSOR'S OVERALL CONCLUSIONS

The clinical efficacy and safety of an extract (1:5, 50% aqueous ethanol, standardized to contain 50 mg of aescin in 240-290 mg extract) of *Aesculus hippocastanum,* semen in a daily oral dose corresponding to 100 mg aescin, is well documented. At present, the mechanism of action of HCSE in chronic venous insufficiency cannot be considered clarified, but it seems to involve an influence on venous tone and capillary filtration rate. The therapeutically active constituent in HCSE is not known with certainty. The data in support of aescin as the active substance in chronic venous insufficiency is very weak. In the present terminology of Ph. Eur., this extract should then be classified as a quantified extract. This extracts has been used in Europe for treatment of chronic venous insufficiency since 1968. This extract can therefore be regarded as an active substance with a well-established medicinal use. The clinical studies were performed with a preparation contained in slow-release capsules. Several pharmacokinetic studies indicate that the availability of aescin is the same in retarded and non-retarded preparations. However, as no evidence is available that aescin is the (only) active ingredient in HCSE, the validity of these bioequivalence studies is of questionable relevance. The fact that all clinical efficacy studies of HCSE have been performed with a prolonged release formulation remains. In the absence of comparative clinical efficacy and safety studies, the monograph should only cover prolonged release formulations of horse chestnut seed extracts, quantified on aescin.

II.5 FINAL CONCLUSIONS

During the meetings of the MLWP and at the public consultation several items were discussed. The following points led to amendments in the monograph:

The data in support of aescin as responsible for the therapeutic effect of HCSE 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 HCSE should be classified as a standardised or a quantified extract in the sense of Ph. Eur. In the final discussion of the MLWP, the notation "standardised extract" was considered most appropriate. A consequence of classifying HCSE 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 50 mg aescin 2 times daily.
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 extract is standardised on aescin, led to an agreement to include HCSE in pharmaceutical forms both for immediate and modified release in the monograph.

III. ANNEXES

III.1 COMMUNITY HERBAL MONOGRAPH ON *AESCULUS HIPPOCASTANUM* L., SEMEN[5,6]

III.2 LITERATURE REFERENCES

[5] Prepared according to the 'Procedure for the preparation of Community monographs for traditional herbal medicinal products' (EMEA/HMPC/182320/2005 Rev.2)

[6] Prepared according to the 'Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use' (EMEA/HMPC/182352/2005 Rev.2)