



Review Article

Horse chestnut – efficacy and safety in chronic venous insufficiency: an overview



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ABSTRACT

The extract from horse chestnut seeds (*Aesculus hippocastanum* L., Sapindaceae), standardised for the content of aescin, is used as the treatment for chronic venous insufficiency. It has anti-inflammatory and anti-oedematous properties and indicates a positive effect on the venous tone, rheological properties, and blood coagulability. The mechanism of horse chestnut seed extract/aescin activity was proposed on the basis of *in vitro* and *in vivo* studies, and its effectiveness was documented with numerous randomised clinical trials. The results of the studies have proven that horse chestnut seed extract not only significantly improves subjective symptoms in patients with chronic venous insufficiency like calf spasm, leg pain, pruritus, fatigue, but it also reduced leg volume, the ankle and calf circumference. The preparations containing horse chestnut seed extract are very popular and they have similar effectiveness as compression therapy and a preparation with *O*-(β -hydroxyethyl)-rutosides. Moreover, horse chestnut seed extract has been proven to be safe and very well tolerated. The study was to present the results of the studies that have been conducted so far and that have confirmed the effectiveness of use of horse chestnut seed extract or aescin as the treatment for chronic venous insufficiency.

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Introduction

Chronic venous insufficiency (CVI) is a disorder affecting approximately 25% of the European community, women in particular (Piechal et al., 2005). CVI is caused by inborn or acquired anomalies in the functioning of the venous system, resulting from primary defects in a vein wall and valve structure as well as insufficiency thereof, and by factors influencing the weakened tension and structure thereof, such as hormonal changes, pregnancy, obesity, limited activity, working in a sitting or standing position, and oral contraceptives (Sudoł-Szopińska et al., 2006). As a result of the weakened vascular tension and structure, blood congestion, vascular bed overflow, and hypoxia occur; in consequence, mitochondrial oxidative phosphorylation is inhibited, and the content of adenosine triphosphate (ATP) is lowered. A decrease in ATP content in endothelium cells induces a series of cellular modifications, such as an increase in cytosolic calcium concentration, the release of inflammatory mediators, such as prostaglandins (Michiels et al., 1993), and the platelet-activating factor (PAF) (Arnould et al., 1993) which result in the recruitment, activation and adhesion of

polymorphonuclear neutrophils (Arnould et al., 1996). Leukocytes adhering to the vascular wall release phospholipase A₂ responsible for production of inflammation precursors, toxic oxidative metabolites, and lysosomal enzymes (elastase, collagenase). They also lead to the increased activity of hyaluronidase that degrades hyaluronic acid, the major constituent of capillary endothelium. The increased activity of other enzymes being components of vascular walls, *i.e.* β -*N*-acetylglucosaminidase, β -glucuronidase and arylsulphatase, responsible for degradation of proteoglycans, has also been observed in chronic venous insufficiency. Degradation of hyaluronic acid and proteoglycans results in violation of the integrity of blood vessel walls, increased capillary permeability, and fragility (ESCOPE, 2003). In the course of an inflammatory reaction, histamine and serotonin – the enzymes affecting the increase in capillary permeability – are released, too. The effect of the above-mentioned processes is migration of leukocytes outside the vascular walls, exacerbation of the inflammation, occurrence of oedema, and pathological changes in veins (Fig. 1).

Medicines of plant origin play a significant role in the pharmacological treatment of CVI. The most popular ones include the horse chestnut seed extract or aescin isolated from it and flavonoids: diosmin, hesperidin, rutin, and its derivative – troxerutin.

The aim of the study was to present the results of the studies that have been conducted so far and that have confirmed the

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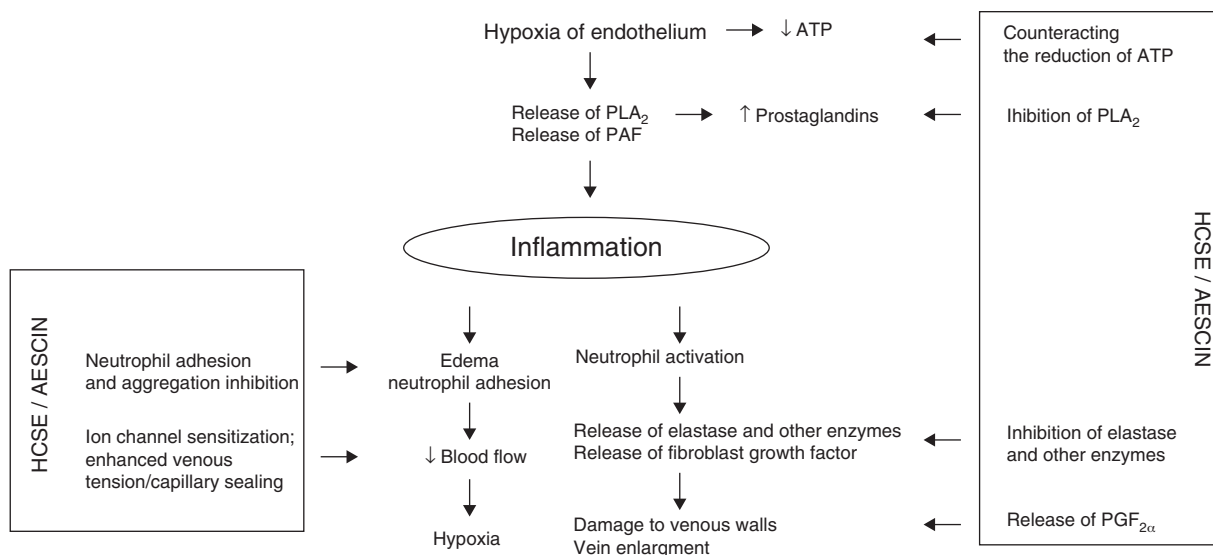


Fig. 1. Mechanisms of HCSE/aescin activity in chronic venous insufficiency. Modified based on Sirtori (2001).

effectiveness of use of horse chestnut seed extract (HCSE) or aescin as the treatment for CVI.

Material and methods

Inclusion and exclusion criteria

Only randomised, controlled trials testing the efficacy of oral preparations containing HCSE as the only active component (a mono-preparation) with the placebo or reference therapy were included. Trials assessing HCSE as one of several active components in a combination preparation or as a part of the combination treatment were excluded. In case of preparations used externally, due to a lack of mono-preparations, the combinations preparations were also included. In all the trials the extract was standardised to aescin.

The studies included if participants were patients with CVI (internal use), and CVI, SVT, and foot ulcers as a result of diabetes complications (external use). The studies using clinical outcome measures were included, whereas the studies focusing exclusively on physiological parameters were excluded.

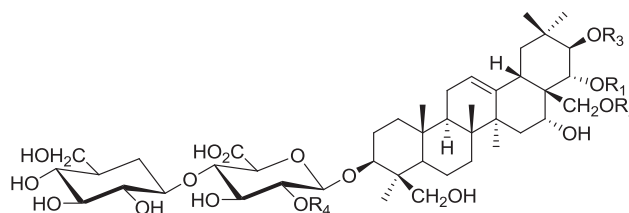
The following electronic English databases were searched: Ovid Medline, Pubmed and The Cochrane Library, from 1976 up to December 2014. They were searched by title and abstract using the following search terms: *Aesculus hippocastanum*, *Hippocastani semen*, horse chestnut seeds, aescin, HCSE, CVI. Hand searches were also conducted for publications not stored in the databases (e.g. webpages).

Reference lists of all the articles were searched for further publications. For the selection of the manuscripts, two independent investigators (MDM, ESS) first assessed all the titles and abstracts and then carried out full text analyses of the publications, against predefined inclusion criteria.

Nineteen trials met the above-mentioned inclusion criteria. Of these, nine were placebo-controlled; two compared HCSE against the reference treatment with compression stockings and the placebo (Diehm et al., 1996; Diehm and Schmidt, 2001); four were controlled against reference medication with *O*- β -hydroxyethyl rutosides (HR) (Erdlen, 1989; Kalbfleisch and Pfalzgraf, 1989; Erler, 1991; Rehn et al., 1996), and one was controlled against medication with pycnogenol (Koch, 2002). Three trials related to preparations used externally were randomised, but their efficacy was not compared with the placebo.

Chemical constituents

Horse chestnut seeds are rich in saponins (3–5%), over thirty of which have been isolated and identified. The main compound is aescin – a mixture of acylated triterpene glycosides. 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. β -Aescin (mainly made up of aescin Ia (1) and aescin Ib (2)) is the major active component of extracts from horse chestnut seeds, whereas α -aescin (made up mainly of iso-aescin Ia (5) and iso-aescin Ib (6)) is less bioactive (ESCOP, 2003). Horse chestnut seeds also contain flavonoids: quercetin and kaempferol derivatives, proanthocyanidins, sterols, and significant amounts of starch (ESCOP, 2003).



- 1 $R_1 = \text{COCH}_3$; $R_2 = \text{H}$; $R_3 = \text{Tig}$; $R_4 = \text{Glc-}p$
- 2 $R_1 = \text{COCH}_3$; $R_2 = \text{H}$; $R_3 = \text{Ang}$; $R_4 = \text{Glc-}p$
- 3 $R_1 = \text{COCH}_3$; $R_2 = \text{H}$; $R_3 = \text{Tig}$; $R_4 = \text{Xyl-}p$
- 4 $R_1 = \text{COCH}_3$; $R_2 = \text{H}$; $R_3 = \text{Ang}$; $R_4 = \text{Xyl-}p$
- 5 $R_1 = \text{H}$; $R_2 = \text{COCH}_3$; $R_3 = \text{Tig}$; $R_4 = \text{Glc-}p$
- 6 $R_1 = \text{H}$; $R_2 = \text{COCH}_3$; $R_3 = \text{Ang}$; $R_4 = \text{Glc-}p$

Glc-*p* = β -D-glucopyranosyl

Xyl-*p* = β -D-xylopyranosyl

Ang: angeloyl

Tig: tiggeloyl

The effectiveness of HCSE and aescin as the treatment for chronic venous insufficiency results from the anti-oedematous and anti-inflammatory activity as well as their influence on the tension of blood vessel walls. The biological activity of both horse chestnut seed extract (HCSE) standardised for the content of aescin and isolated aescin has been confirmed by numerous *in vitro*, *in vivo*, and the clinical studies (Pittler and Ernst, 2012). The results from the studies have been presented below and summarised in chronological order in Tables 1–3.

Anti-inflammatory and anti-oedematous activity

It has been confirmed that aescin is mainly responsible for the anti-inflammatory and anti-oedematous activity of the horse chestnut seed extract (Table 1). The research results have shown that the complete extract has been around 100 times more effective as the treatment for inflammation and lymphoedema in rats than the extract from which aescin was removed (Guillaume and Padioleau, 1994).

The mechanism of the anti-inflammatory and anti-oedematous activity of HCSE and aescin is multi-directional and has been proposed on the basis of results of *in vitro* and *in vivo* studies (Fig. 1). It was demonstrated that aescin counteracted ATP reduction and an increase in the activity of phospholipase A₂ responsible for the release of precursors of inflammatory mediators (Arnould et al., 1996; Sirtori, 2001). Moreover, aescin reduced neutrophil adhesion and aggregation, which was shown in *ex vivo* studies on an isolated human umbilical vein. Incubation thereof in hypoxic conditions in an aescin solution or without it proved that aescin inhibited adhesion of neutrophils to the vascular endothelium, activated them, and produced leukotriene B₄ and the superoxide anion (Bougelet et al., 1998). In an experimental model of rat pleurisy, HCSE reduced plasma extravasation and leukocyte migration to the pleural cavity as well as the release of inflammatory mediators, which resulted in inhibition of inflammation and oedema (Guillaume and Padioleau, 1994). In *in vitro* studies, the aescin restrained hyaluronidase activity by 93%, which resulted in inhibition of permeability and the loss of plasma from the cells of vascular endothelium and, as a consequence, in a decrease in the occurrence of oedema (Facino et al., 1995). In addition, aescin decreased the activity of lysosomal enzymes, which shifted the proteoglycan synthesis-breakdown balance towards synthesis thereof. *In vivo* studies showed that aescin administered intraperitoneally to rats for three weeks significantly reduced degradation of mucopolysaccharides in the connective tissue (the xiphoid process) (Panigati, 1992). Aescin and HCSE are also characterised by the antihistaminic and antiserotonin activity. It was demonstrated that HCSE administered orally to rats diminished or inhibited excessive permeability of skin capillaries caused by previous administration of histamine and serotonin (Guillaume and Padioleau, 1994). The later research proved that aescin Ib (2), IIa (3), and IIb (4) had the antihistaminic and anti-serotonin activity while aescin Ia (1) mainly inhibited histamine (Matsuda et al., 1997). The anti-exudative activity of aescin is also connected with selective sensitisation of vascular smooth muscles to Ca²⁺ ions, which leads to the increased tension and sealing thereof and, as a result, to a decrease in the inflammation caused by vascular endothelium hypoxia. Results of *in vitro* studies confirmed the above. Aescin caused concentration-dependent contraction rings of inferior vena cava from male rats incubated in normal Krebs. In Ca²⁺-free Krebs there was essentially no contraction to aescin, but in aescin-treated veins incubated in Ca²⁺-free Krebs, stepwise addition of extracellular CaCl₂ caused corresponding increases in contraction (Raffetto and Khalil, 2011).

The effectiveness of aescin and HCSE internal application (*p.o.*, *i.v.*, *i.p.*, *s.c.*) to prevent the formation of oedema was mainly

demonstrated in models of inflammation that reproduce the initial exudative phases, such as oedema of laboratory animal paws induced by a range of irritating agents (albumin from chicken egg white (Girerg et al., 1961) dextran (Damas et al., 1976), cotton pellet, carrageenin (Cebo et al., 1976; Matsuda et al., 1997), bradykinin, compound 48/80, chloroform (Matsuda et al., 1997)), in serous peritonitis caused by injections of formalin in rats or carrageenin in mice (Sirtori, 2001).

Effect on venous tension and blood flow

The effectiveness of HCSE and aescin as the treatment for chronic venous insufficiency is also connected with the influence on the tension of veins (Table 2). As a result of an increase in the tension of vein walls, the blood flow through the vessels is accelerated and venous outflow improves. The consequence is that venous blood congestion decreases, which, in turn, enhances microcirculation as erythrocyte dispersion increases and tissue oxygenation improves, the flow through *vasa vasorum* accelerates, the time of leukocyte migration through the blood vessels and, therefore, the chance for activation thereof initiating the cascade of an inflammatory reaction is lowered.

It was shown that HCSE and aescin affected venous tension by increasing production of prostaglandin F₂ (PGF₂), which participates in regulating the contractile action of veins, and inhibiting the catabolism of venous tissue mucopolysaccharides (Sirtori, 2001). The increased venous tension may also be connected with the effect of HCSE on serotonin 5-HT_{2A} receptors, which was demonstrated in *in vitro* studies on an isolated vein and artery. Their incubation in HCSE caused a contraction, whereas in a simultaneously conducted experiment their pre-incubation in a solution of ketanserin, an antagonist for 5-HT_{2A} receptors, did not show any contraction after HCSE. This proved that the contraction mechanism was connected with stimulation of 5-HT_{2A} receptors (Felixsson et al., 2010). The improvement of blood flow in vessels by the extract from horse chestnut seeds was also associated with its effect on blood rheology, by decreasing viscosity in particular (Klemm, 1982). The influence of aescin and HCSE on the increase in venous tension was confirmed in *in vitro* studies on isolated veins of various animals and human veins as well as in *in vivo* studies (Annoni et al., 1979; Felixsson et al., 2010; Guillaume and Padioleau, 1994; Lochs et al., 1974).

Clinical studies

Evaluation of the effectiveness of HCSE-containing preparations was performed on patients with CVI; only two clinical trials involved healthy volunteers (Table 3). These were randomised, double-blind, placebo-controlled studies, part of which was conducted by the cross-over method where each patient took both HCSE and the placebo in separate treatment cycles while the rest were parallel studies in which patients were divided into two groups – taking either HCSE or the placebo.

In the studies a daily dose of 600 mg of HCSE (which corresponds to 100 mg of aescin/day) was administered most frequently, mainly in two doses; the patients took the medicine for 2–16 weeks.

In the research carried out between 1976 and 1978, a 0–3 scale was used to evaluate the degree of intensity of the complaints typical for CVI. A significant reduction in the symptoms was observed in patients administered HCSE in comparison with those taking the placebo (Friederich et al., 1978; Neiss and Böhm, 1976).

In later years (1986–2002), water displacement plethysmometry (Diehm et al., 1992; Lohr et al., 1986; Rudofsky et al., 1986; Steiner and Hillemanns, 1990; Steiner, 1991) and measurement of the ankle and calf circumference (Diehm et al., 1992; Lohr et al.,

Table 1
Studies of anti-inflammatory and antioedema activity of HCSE and aescin.

Extracts/compounds	Dose/route of administration	Model/effect	Authors
<i>In vitro</i> Aescin	300 µM	Inhibition of activity of hyaluronidase by 93% (IC ₅₀ = 150 mM), respectively less at lower concentrations	Facino et al. (1995)
Aescin (Reparil)	100–750 ng/ml	Human umbilical vein endothelial cells activated by hypoxia, inhibition of decrease in ATP content (EC ₅₀ = 260 ng/ml), inhibition of activation of phospholipase A ₂ (EC ₅₀ = 90 ng/ml), inhibition of adhesion of neutrophils (EC ₅₀ = 90 ng/ml)	Arnould et al. (1996)
Aescin	10 ⁻⁹ –10 ⁻⁴ M	In rings of inferior vena cava from male rats segments incubated in normal Krebs (2.5 mM Ca ²⁺), aescin caused concentration-dependent contraction (max 104.3 mg/mg tissue at 10 ⁻⁴ M; 48.3% of control KCl contraction). In Ca ²⁺ free Krebs, there was essentially no contraction to aescin. In aescin-treated veins incubated in Ca ²⁺ free Krebs, stepwise addition of extracellular CaCl ₂ caused corresponding increases in contraction (max 80.0% of control at 2.5 mM)	Raffetto and Khalil (2011)
<i>In vivo</i> Aescin	<i>i.v.</i> ; 0.2–2.5 mg/kg	Reduction of oedema of rat paw induced by ovalbumin	Girerg et al. (1961)
Aescin	<i>s.c.</i> ; 4 mg/kg	Reduction of dextran induced rat paw oedema	Damas et al. (1976)
Fraction of saponins	<i>i.v.</i> – 3.75 mg/kg <i>p.o.</i> – 7.5 mg/kg	Inhibition of hind rat paws inflammation induced by carrageenan, analgesic activity	Cebo et al. (1976)
Aescin (Reparil <i>i.v.</i> form) (<i>ex vivo</i>)	250 ng/ml (0.22 µM)	Inhibition of neutrophil adhesion to hypoxic endothelium, activation and release of free radicals	Bougelet et al. (1998)
Aescin	<i>i.p.</i> ; 1 mg/kg/day (3 weeks)	Reduction of degradation of mucopolysaccharides in rat connective tissues (xiphoid cartilage); studies of sulphomucopolysaccharides marked S ³⁵	Panigati (1992)
HCSE (70% aescin) (Veinotonyl 75)	<i>p.o.</i> ; 200–400 mg/kg <i>i.v.</i> ; 1–10 mg/kg	Lymphatic oedema in rats; reduction of plasmatic extravasation, leukocytes migration, release of inflammatory mediators, leading to inhibition of inflammation	Guillaume and Padioleau (1994)
	<i>p.o.</i> ; 50–300 mg/kg <i>i.v.</i> ; 2.5–5 mg/kg <i>p.o.</i> ; 150–400 mg/kg (60 min before the administration of histamine and serotonin) <i>p.o.</i> ; 50–400 mg/kg	Reduction of capillary hyperpermeability induced by chloroform in rabbits Reduction of capillary hyperpermeability induced by serotonin or histamine in rats (maximum effect at a dose of 200 mg/kg)	
Aescins Ia (1), Ib (2), IIa and IIb	<i>p.o.</i> ; 50–200 mg/kg	Increase in skin capillary resistance in guinea pigs fed a scorbutogenic diet for 3 weeks Dose-dependent inhibition of increased vascular permeability induced by subcutaneous administration of acetic acid to mice, and histamine (aescin Ia, Ib, IIa, IIb) and serotonin (aescin Ib, IIa, IIb) to rats Inhibition of hind paw oedema induced by carrageenin at first phase in rats (aescin Ia, Ib, IIa and IIb) Dose-dependent inhibition of scratching behaviour induced by subcutaneous injection of compound 48/80 in mice (aescin Ib, IIa and IIb, aescin Ia – the weakest activity, only at a maximum dose)	Matsuda et al. (1997)

Table 2
Studies of venotonic activity of HCSE and aescin.

Extracts/compounds	Dose/route of administration	Model/effect/route of application	Authors
<i>In vitro</i> HCSE	0.2 mg/ml	Increased tension of cow vein and human saphenous vein incubated in tested solutions, the effect lasted for 3 h	Lochs et al. (1974)
Aescin	0.1 mg/ml		
Aescin	1 ng/ml–1 mg/ml	Increased tension of human saphenous vein incubated in tested solutions, the pharmacological effect comparable to serotonin and dihydroergotamine, significantly stronger than acetylcholine and vasopressin	Annoni et al. (1979)
HCSE (70% aescin)	0.05–0.5 mg/ml (0.5–5 × 10 ⁻⁴)	Increased tension of dog saphenous vein incubated in tested solutions	Guillaume and Padioleau (1994)
HCSE	25–50 mg	Increased tension of perfused dog saphenous vein	
	1 mg/ml	Inhibition of aggregation of ADP-induced human platelets	Felixsson et al. (2010)
	0.1–10 mg/ml	Contraction of isolated vein and artery incubated in HCSE solution, previous incubation in ketanserine solution (10 ⁻⁶ and 10 ⁻⁵ M) resulted in inhibition of contraction after HCSE	
<i>In vivo</i> HCSE (70% aescin)	<i>i.v.</i> ; 50 mg	Increased venous pressure, venous and lymphatic flows in femoral dog vein	Guillaume and Padioleau (1994)

Table 3
Clinical trials of efficacy of HCSE in CVI.

Daily dose Preparation	Duration	Design of study, number of patients	Indications	Improvement of signs and symptoms	Authors
<i>HCSE – internal use</i>					
300 mg HCSE caps. ^a 2×/day (brand not stated)	20 days	R, DB, PC, PG, CO n = 226	CVI, varicosis	Significant alleviation ($p < 0.05$) of leg pain, oedema and pruritus in participants treated with HCSE compared to <i>placebo</i>	Neiss and Böhm (1976)
300 mg HCSE caps. ^a 2×/day (brand not stated)	20 days	R, DB, PC, CO n = 95	CVI, varicosis	Significant reduction ($p < 0.05$) of calf spasm, pain, fatigue, and tenseness compared to <i>placebo</i>	Friederich et al. (1978)
300 mg HCSE caps. ^a 2×/day Venostasin® Retard	8 weeks	R, DB, PC, PG n = 74	CVI	Reduction of leg volume of 16.5 ml compared to 3.8 ml reduction with <i>placebo</i> , reduction of formation of oedema (21.0 ml) with HCSE and increase (0.2 ml) with <i>placebo</i> during oedema-provoking period	Lohr et al. (1986)
2 × 300 mg HCSE caps. ^a 1×/day Venostasin® Retard	4 weeks	R, DB, PC, CO n = 22	CVI	Significant reductions ($p < 0.01$) of capillary filtration coefficient by 22% compared to <i>placebo</i>	Bisler et al. (1986)
300 mg HCSE caps. ^a 2×/day (brand not stated)	4 weeks	R, DB, PC, PG n = 39	CVI grade I or II	Significant reduction ($p < 0.001$) in leg volume (78 ml) compared to increase with <i>placebo</i> (34 ml), significant reduction ($p < 0.01$) in calf and foot circumference, significant reduction in subjective parameters (pain, tiredness, tension, and pruritus in legs) compared to <i>placebo</i>	Rudofsky et al. (1986)
300 mg HCSE caps. ^a 2×/day (brand not stated)	20 days	R, DB, PC, PG n = 28	CVI	Significant reduction ($p < 0.05$) of 0.8 cm in leg circumference and oedema at ankle compared with <i>placebo</i> , alleviation of subjective symptoms ($p < 0.05$)	Pilz (1990)
300 mg HCSE caps. ^a 2×/day Venostasin® Retard	20 days	R, DB, PC, CO n = 52	Pregnant women with oedema due to CVI	Significant reduction ($p < 0.01$) in foot volume before and after oedema provocation and greater resistance to oedema provocation compared to <i>placebo</i> , reduction in foot circumference and subjective symptoms compared to <i>placebo</i>	Steiner and Hillemanns (1990)
300 mg HCSE caps. ^a 2×/day Venostasin® Retard	2 weeks	R, DB, PC, CO n = 20	CVI, varicosis during pregnancy	Significant reduction ($p = 0.009$) in leg volume (114 ml in patients with varicose veins and 126 ml in patients with CVI) and subjective symptoms ($p < 0.05$) compared to <i>placebo</i>	Steiner (1991)
300 mg HCSE caps. ^b 2×/day (brand not stated)	6 weeks	R, DB, PC, PG n = 39	Venous oedema in chronic deep vein incompetence (CVI grade II)	Significant reduction ($p < 0.01$) in leg volume by an average of 84 ml compared with baseline, while reduction of 4 ml with <i>placebo</i> , alleviation of subjective symptoms (feelings of heaviness, tension, fatigue, and paresthesia) in legs, itching not helped	Diehm et al. (1992)
<i>HCSE – external use</i>					
Esseven gel	4 weeks	R n = 30	SVT	Alleviation of subjective symptoms, decrease in body temperature	De Sanctis et al. (2001)
Esseven gel 1×/day	4 weeks	R n = 22	CVI, acute lower leg ulcers	Improved blood flow parameters and microcirculation, alleviation of subjective symptoms	Cesarone et al. (2001a)
Esseven gel	4 weeks	R, DB n = 15	Foot ulcers as a result of diabetes complications	Improvement of microcirculation, reduction of skin ulcers	Cesarone et al. (2001b) and Incandela et al. (2001)
<i>HCSE vs. reference medications</i>					
300 mg HCSE caps. ^a 2×/day vs. reference medication (probably rutosides, brand not stated)	4 weeks	R, DB, PG, Cm n = 30	CVI	Significantly reduced (p value not reported) ankle circumference by 0.4 cm and improved subjective symptoms compared with baseline, ankle circumference reduced by 0.4 cm (rutosides)	Erdlen (1989)

Table 3 (Continued)

Daily dose Preparation	Duration	Design of study, number of patients	Indications	Improvement of signs and symptoms	Authors
300 mg HCSE caps. ^a 1×/day vs. 500 mg HR ^c 1×/day (brand not stated)	8 weeks	R, DB, PG, Cm n = 30	CVI	Ankle and calf circumference reduced by 0.2 and 0.18 cm, respectively, compared to baseline, values not significantly different compared with HR	Kalbfleisch and Pfalzgraf (1989)
300 mg HCSE caps. ^b 2×/day vs. 2000 mg HR ^c 1×/day	8 weeks	R, DB, PG, Cm n = 40	CVI and peripheral venous oedema	HCSE significantly protected calf and ankle from oedema provocation compared with baseline, both HCSE and HR comparable in reducing oedema, more pronounced protective effect of HCSE	Erler (1991)
300 mg HCSE caps. ^a 2×/day vs. 1000 mg HR ³ /day (4 weeks) 500 mg HR ³ /day (4 weeks) (brand not stated)	12 weeks	R, DB, PG, MC, Cm n = 137	Postmenopausal CVI grade II	Significant reduction (<i>p</i> value not reported) in leg volume of 28 ml (HCSE), 58 ml (HR 1000 mg/day) and 40 ml (HR 1000 mg/day for 4 weeks then 500 mg/day for 8 weeks) compared to baseline, alleviated subjective symptoms, after 6-week follow-up period both treatments exhibited substantial carry-over effect	Rehn et al. (1996)
300 mg HCSE caps. ^a 2×/day (Venostasin [®] Retard) vs. mechanical compression with bandages and class II elastic stocking	12 weeks	R, SB, PC, PG, Cm n = 240	CVI	Significant reduction in leg volume of 43.8 ml (HCSE), 46.7 ml (compression therapy) and increase -9.8 ml (<i>placebo</i>) (HCSE and compression therapy vs. <i>placebo</i> <i>p</i> = 0.005), HCSE compared with compression therapy <i>p</i> = 0.001), HCSE was well-tolerated (98% compliance), whereas compression treatment was reported as uncomfortable, inconvenient and subject to poor (90%) compliance	Diehm et al. (1996)
300 mg HCSE caps. ^a 2×/day (Venostasin [®] Retard) vs. mechanical compression with bandages and class II elastic stocking	16 weeks	R, DB, PC, PG, Cm n = 286	CVI grade II and IIIa	Reduction of lower leg volume during treatment in both therapy groups, whereas in <i>placebo</i> group, there were no effects	Diehm and Schmidt (2001)
300 mg HCSE caps. 2×/day (Venostasin [®] Retard) vs. 360 mg Pycnogenol/day	4 weeks	R, PG, O, Cm n = 40	CVI	Pycnogenol: significant reduction of circumference of lower limbs, significantly improved subjective symptoms of pain, cramps, night-time swelling, feeling of 'heaviness', and reddening of skin, decrease in cholesterol and LDL values in blood, whereas HDL remained unaffected, Venostasin: insignificant reduction of circumference of lower limbs, marginally improved symptoms, no influence on the determined lipid values, both medications were equally well tolerated	Koch (2002)

Essaven gel: *Hippocastani seminis extractum spissum*, 83.5 mg/g aescin; *Hyperianum natricum*, 0.42 mg/g.

CVI, chronic venous insufficiency; Cm, comparison; CO, cross-over; DB, double-blind; MC, multicentre; O, open; PC, placebo-controlled; PG, parallel group; R, randomised; SB, single blind.

^a HCSE capsules standardised to 50 mg aescin each.

^b HCSE capsules standardised to 75 mg aescin each.

^c *O*-(β-Hydroxyethyl)-rutosides.

1986; Pilz, 1990) were additionally applied to measure the effectiveness of HCSE-containing preparations.

The research involved people with diagnosed CVI, varicose veins, including those developed during pregnancy. It was shown that the leg volume considerably decreased in the groups taking HCSE, whereas in the patients administered the *placebo* it lowered only slightly or not at all (Diehm et al., 1992; Lohr et al., 1986; Rudofsky et al., 1986; Steiner and Hillemanns, 1990). Alleviation of the subjective complaints and an improvement in the general physical activity was observed in the patients taking HCSE, in contrast to those taking the *placebo*.

The effect of HCSE on capillary filtration and the intravascular volume of the lower limb vein were also assessed in patients with CVI. Three hours after administration of the preparation, the capillary filtration increased insignificantly in the patients taking the *placebo* while in those taking HCSE it decreased. A decrease in the intravascular volume (-5%) was not considerable, which proved that the effectiveness of HCSE resulted from the impact on the ability to reduce capillary permeability to a larger extent than from the influence on the vein tension (Bisler et al., 1986).

Moreover, the studies comparing the effectiveness and safety of use of HCSE therapy and compression therapy were conducted.

Using both class II elastic stockings and HCSE caused a significant decrease in leg volume while in the case of the *placebo* leg volume slightly increased. HCSE and compression therapy were both well tolerated and no serious complications were reported. The results suggested that use of elastic stockings as well as HCSE ensured similar effectiveness in treating patients with oedema caused by CVI (Diehm et al., 1996; Diehm and Schmidt, 2001).

The research that aimed at a comparison of the effectiveness of HCSE with that of *O*-(β -hydroxyethyl)-rutosides (HR) was done, too. HR showed similar effectiveness to HCSE (reduction in the ankle and calf circumference, a leg volume reduction) (Erdlen, 1989; Erler, 1991; Kalbfleisch and Pfalzgraf, 1989; Rehn et al., 1996). In the other studies, the efficacy of HCSE and pycnogenol was compared. Pycnogenol significantly reduced the circumference of the lower limbs, improved subjective symptoms and decreased cholesterol, and LDL values in the blood, whereas HDL remained unaffected. HCSE only moderately but not significantly reduced the circumference of the lower limbs, marginally improved symptoms and had no influence on the determined lipid values. Pycnogenol was found to be more efficacious than HCSE for the treatment of CVI (Koch, 2002).

The effectiveness of local application of a preparation containing HCSE with an addition of heparin (Essaven gel) was also evaluated. Alleviation of the symptoms and a fall of skin temperature were observed in patients with superficial vein thrombosis (SVT) (De Sanctis et al., 2001) while in those with CVI and acute lower leg ulcers a significant improvement in the blood flow and microcirculation parameters was found (Cesarone et al., 2001a). Improved microcirculation and a considerable decrease in skin ulcers within the foot were also observed after application of this preparation in diabetics (Cesarone et al., 2001b; Incandela et al., 2001).

The effect of HCSE on pedal oedema in people travelling on long-haul flights was assessed ($n = 19$; double-blind trials). The results demonstrated lesser foot oedema in people prophylactically taking 600 mg of HCSE before the flight (Marshall and Dormandy, 1987). Other studies on healthy volunteers ($n = 12$; double-blind, *placebo*-controlled trial) showed that standardised HCSE administered once at a dose of 600 mg decreased vascular volume and the capillary filtration rate (Pauschinger, 1987).

Pharmacokinetics

A few of the reports on the pharmacokinetic and pharmacovailability of aescin were published. The conducted studies allowed to compare the bioavailability of aescin (50 mg contained in 240–290 mg of HCSE) in a prolonged release formulation and other pharmaceutical formulations after oral administration. In a single dose experiment C_{max} was 3.2–9.8 ng/ml while in a repeated dose experiment C_{max} was 6.5–16.7 ng/ml. In both of the discussed clinical studies the differences in the concentrations of aescin in the blood/plasma were observed (Loew et al., 2000).

Furthermore, after oral administration of an aescin solution, the absolute bioavailability was low and determined as only 1.5%. This fact was probably due to the first pass effect (metabolism and biliary excretion). The relative bioavailability of aescin from a HCSE (Venostasin retard®) was 100% compared to an aescin solution (EMEA, 2012).

The pharmacokinetics of aescin, after intravenous administration, corresponded to an open three compartment model. The eliminations half-times of 5 mg aescin (infusion rate: 718 μ g/min) were: $t_{0.5\alpha} - 6.6$ min; $t_{0.5\beta} - 1.74$ h and $t_{0.5\gamma} - 14.36$ h. Moreover, the distribution volume was 100.91, binding to plasma proteins was 84%, total plasma and renal clearances were 21.8 ml/min and 1.7 ml/min respectively. Following 120 h after the dose administration, aescin excreted in the urine (EMEA, 2012).

Side-effects, toxicity

Preclinical data

Weak genotoxic activity of a commercial dry extract as well as fluid extracts of horse chestnut seeds was demonstrated in the Ames test on strains of *Salmonella typhimurium* TA 98 (EMEA, 2012; ESCOP, 2003).

HCSE studies on rats and rabbits (100 and 300 mg/kg bw) showed no teratogenic activity. A fall in the average body weight of the foetus was only observed after application of large doses of HCSE. HCSE single dose toxicity studies (Venostatin retard® preparation, standardised for the content of 50 mg of aescin in 240–290 mg extract) conducted on animals (mice, rats, guinea pigs, and rabbits) demonstrated greater toxicity of the extract when administered intravenously and intraperitoneally (LD_{50} 6.8–465 mg/kg bw) than after oral administration (LD_{50} 910–2600 mg/kg bw). The LD_{50} values ranged from 3 to 17 mg/kg bw after intravenous and intraperitoneal administration of aescin to laboratory animals (EMEA, 2012).

The studies of chronic oral toxicity of HCSE were carried out on dogs (20, 40, 80 mg/kg bw; 5 days/week/3 month) and rats (100, 200, 400 mg/kg bw; 5 days/week/3 month). The obtained results proved a lack of toxicity of the administered extract.

In the study of sub-acute intravenous toxicity, HCSE was administered to rats at doses of 9, 30, 90 mg/kg/day for 60 days. The dose of 90 mg/kg caused death of 8 out of 30 animals as early as in the first days of the study; the doses of 30 and 9 mg/kg did not lead to any significant disorders and were safe for the animals (Liehn et al., 1972).

Aescin, at a dose of 2×50 mg/kg bw, given to young, 32-day old rats, did not affect fertility, and nephrotoxicity was not detected either (EMEA, 2012).

A lack of the nephrotoxic properties of aescin was also confirmed in a study on the influence of free and albumin-bound aescin on renal tubular transport performed on an isolated, artificially perfused frog kidney. It was proven that the low harmfulness resulted from the ability of aescin to bind with plasma proteins (50%), and the concentration of free aescin filtered through the kidney was considered too low to be able to have a toxic effect (Barnes et al., 2007).

Clinical data

The results of three clinical studies proved a lack of toxic effects of HCSE and aescin on the renal function after *i.v.* administration. The studies involved healthy volunteers or patients with impaired renal function, both adults and children. The kidney function was monitored every day; BUN, serum creatinine and creatinine clearance were determined, and the urine analysis was performed. It was shown that aescin did not cause decreased kidney efficiency in the individuals from the examined groups (EMEA, 2012).

The research showed that use of HCSE was safe. Ailments connected with HCSE application were transient and of little intensity; gastrointestinal disorders, dizziness, headaches, and itch were observed (Barnes et al., 2007; Diehm et al., 1996; EMEA, 2012; Neiss and Böhm, 1976; Rehn et al., 1996; Steiner and Hillemanns, 1990).

Contra-indications, warnings

The safety of use of the horse chestnut seed extract during pregnancy and lactation has not been deeply investigated. The results of the only study conducted so far with the participation of pregnant women showed that use of HCSE did not cause any undesirable activity (Steiner, 1991; EMEA, 2012). However, use of the

pharmacologically active constituents of *A. hippocastanum* should be avoided during pregnancy and lactation (Barnes et al., 2007).

Despite the literature data describing interactions between preparations containing HCSE and other medicinal agents, application of the extract simultaneously with other medicines, especially those of similar or opposite activity, requires caution. It was also shown that aescin bound with plasma proteins; however, the influence on binding of other drugs or a lack thereof was not confirmed (Barnes et al., 2007; EMEA, 2012).

There is no information about overdose, drug abuse, withdrawal, and rebound effect or the impact on the ability to drive or operate machinery or impairment of mental ability.

Conclusions

CVI is a common disorder of venous vessels affecting mainly the elderly. Preparations containing HCSE/aescin have long been used to alleviate both the subjective symptoms, such as the feeling of tiredness, itching, cramps, paresthesia, and the objective ones, *i.e.* decreased leg volume. HCSE standardised for the content of aescin or aescin isolated from the extract are used. α -Aescin is industrially obtained in the amorphous form, which is characterised by better bioavailability due to better solubility. Most frequently, 600 mg of HCSE, which corresponds to 100 mg of aescin, is applied daily. Randomised clinical studies showed beneficial effects of such a treatment after 2–16 weeks, and its effectiveness was similar to that of compression therapy or administration of hydroethylrutoside or pycnogenol.

The activity of HCSE is mainly connected with the presence of aescin, which is the major active compound of the extract. Numerous *in vitro* and *in vivo* studies enabled to investigate the mechanism of HCSE/aescin activity, which is mainly connected with anti-oedema, anti-inflammatory, vessel protective, and the venotonic activity.

Moreover, preparations containing HCSE/aescin have been proven to be safe. Over 40 years of research into the undesirable effects of the horse-chestnut seed extract applied internally have proved very good tolerance thereof, and the sporadic ailments, mainly gastrointestinal disorders, have been transient.

Authors' contribution

MD-M participated in data collection and writing of the manuscript. All the authors contributed to the critical reading and final editing of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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