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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Aesculus hippocastanum* L., cortex

Draft – Revision 1

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., cortex
Herbal preparations		Powdered herbal substance Dry extract (DER 7.0-8.5:1), extraction solvent water
Pharmaceutical forms		Herbal preparations in solid dosage forms for oral use
First assessment	Rapporteur	A. Sawaya
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Note: This draft assessment report is published to support the public consultation of the draft European Union herbal monograph on *Aesculus hippocastanum* L., cortex. It is a working document, not yet edited, and shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.

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1. Introduction

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

- Herbal substance(s)

Horse chestnut bark. The bark is obtained from the 3-5 year branches (Blaschek, 2016).

The composition of horse chestnut bark is complex. The most characteristic compounds are coumarin derivatives (up to 7%) (Matysik 1994, Stanić 1999, Blaschek 2016, Owczarek & Olszewska 2020):

- Coumarin glucosides:

esculin (6-(β-D-glucopyranosyloxy)-7-hydroxy-2H-1-benzopyran-2-one, or 6,7-dihydroxycoumarin 6-glucoside), soluble in boiling water (1 g of esculin dissolves in 13 ml) and slightly soluble in water in room temperature (1 g in 580 ml, The Merck Index, 1983); a glucoside of esculetin;

fraxin (8-(β-D-glucopyranosyloxy)-7-hydroxy-6-methoxy-2H-1-benzopyran-2-one, or 7,8-dihydroxy-6-methoxycoumarin-8-β-D-glucoside), a glucoside of fraxetin, and a few amount of scopolin (7-(β-D-glucopyranosyloxy)-6-methoxy-2H-1-benzopyran-2-one), a glucoside of scopoletin (Blaschek 2016) (the both hydroxycoumarines slightly soluble in water);

- (-)-epicatechin (Bombardelli et al. 1996; Blaschek 2016, Owczarek & Olszewska 2020), soluble in water (Kandi & Charles, 2018);

- ellagitannins and ellagic acid, soluble in water (Piwowarski, 2011).

Other constituents are: tannins (up to 2 %) (Fournier 1948; Paris & Moyse 1981), flavonoids (quercitrin, quercetin) (Blaschek 2016), poorly or insoluble in water (PubChem 2022), sterols which are soluble alcohols and lipophilic solvents but insoluble in water (Senatore et al. 1989), traces of aescin (Blaschek 2016), procyanidin A2 (Bombardelli 1996, Blaschek 2016, Owczarek & Olszewska 2020), only sparingly soluble in water (FooDB, Showing Compound, 2022).

Subcritical water horse chestnut bark extract, in 150°, contained esculin and fraxin as dominant constituent (41.6 – 44.2 mg/g) and minor constituents chlorogenic acid (0.6 – 0.7 mg/g), neochlorogenic acid (0.3-0.5mg/g) and gallic acid (Gagić T. et al. 2021).

The monograph Horse-chestnut bark (Hippocastani cortex) N° 2945 was accepted (26th of November 2022) for publication by the European Pharmacopoeia. In the monograph the content of esculin in the methanol-water (8:2, V/V) extract of the powdered herbal substance was established at least 3.0% (HPLC/UV).

- Herbal preparation(s)

  - Powdered herbal substance.

  - Dry extract (DER 5-6:1), (genuine DER 7.0-8.5:1), extraction solvent water.

The extract is prepared with hot water and contains 4.4-7.7% of esculin.

- 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

Search engines used: Google, Google Scholar. There were used keywords: "esculin", "aesculin", "hydroxycoumarines + aesculus" and additionally "ellagitiannin" and "procyanidin A2", "procyanidins", "proanthocyanidins", "procyanidin + water + solubility", "epicatechin + solubility", "quercitrin + solubility", "quercetin+ solubility". Period: 1950-2022

Scientific databases: Scopus, Embase, EBSCOhost. There were used keywords: "Aesculus + hippocastanum + bark"; "horse + chestnut + bark + toxic"; "horse+ chestnut + poisoning"; "chestnut + bark + intoxication"; "chestnut + bark + intoxication". Medical databases: Medline complete, PubMed, Polska Bibliografia Lekarska for the period of last 10 years (that is 2012-2022), without language limits.

Toxicological databases: PubMed in the area of human toxicology; developmental toxicology (DART), liver toxicity (LiverTox).

Pharmacovigilance resources: Pharmacovigilance data (e.g. data from EudraVigilance, VigiBase, national databases).

Data from EU and non-EU regulatory authorities: Not reported.

Other resources: No data.

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	Regulatory Status
Aesculus hippocastanum L., cortex; powdered herbal substance	Traditional herbal medicinal product for the relief of symptoms associated with mild alterations of the venous circulation such as discomfort and heaviness in the legs and for the symptomatic treatment of haemorrhoids, based exclusively on its traditional use.	Hard capsules containing 275 mg of herbal substance. Posology: 2 capsules 2 to 3 times per day.	National registration in April 1991, since July 2011 TUR, Spain
Aesculus hippocastanum L., cortex; dry extract, DER 5-6:1, extraction	Traditionally used in subjective signs of venous insufficiency, such a heavy legs.	Capsules containing 200 mg of the bark dry extract; (DER 5-6:1, declared in years 1994-	National authorisation 1994, since 2017 TUR, France

Active substance	Indication	Pharmaceutical form	Regulatory Status
solvent water	Traditional herbal medicinal product for symptomatic relief of itching and burning associated with haemorrhoids after serious pathologies have been excluded by a doctor.	2011), in 2011 explained that the genuine DER have been 7.0-8.5:1. Extraction solvent water at 80°C. Posology: one 200 mg capsule 2 times daily.	
Aesculus hippocastanum L., cortex; comminuted herbal substance	Traditionally as an aid in minor swellings and bruises after injuries	Decoction for compresses. Strength: 4 g in 400-500 ml of water, for use as warm compresses	Registration, national, 2001, Poland

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

Changes in products on the market over the review time

Product containing powdered herbal substance (capsules, 275 mg) from France and used in adults and elderly in a dose of 1 capsule (275 mg) 3 to 6 times daily was the basis for the primary monograph when was established in 2012, however, since 2017 the product is no longer present on the French market.

The product from Spain, in the form of capsules, declared to contain 275 mg of powdered herbal substance, which has been registered since 1991 was already reported in the previous assessment report, however, the period on the market was not sufficient at that time to take it up in the monograph. Now it meets the criterion of 30 years of traditional use.

From France is still being reported a product containing 200 mg of the bark dry extract, extraction solvent hot water. Its technological DER that was declared in 1994-2011 to be 5-6:1 but was clarified to be genuine DER 7.0-8.5:1. It was authorised nationally in 1994 in a form of capsules to be used twice daily. In 2017 it was registered as TUR for two indications: *Traditionally used in subjective signs of venous insufficiency, such as heavy legs* and *Traditionally used for the treatment of haemorrhoidal symptoms after serious pathologies had been excluded by a doctor*. The indications (as in Table 1) were not changed since first registration. The product has been on the market for 27 years, since 2017 as TUR.

In Poland a mono-component product in a form of decoction (4 g in 400-500 ml of water) for topical use in the form of compresses (warm impregnated dressings), which was registered in 2001, is still present on the market.

Assessor's comments:

The former French product containing 275 mg of powdered herbal substance in capsules is no longer on a market but similar product from Spain has reached 30 years of tradition during the review period and it may give base for the monograph. The former product was used in posology 3 to 6 capsules daily (single dose 275 mg, daily dose 825 mg – 1650 mg); the product which is currently on the market, is used in posology 2 capsules 2 to 3 times daily (single dose 550 mg, daily dose 1100 to 1650 mg).

On this base p. 4.2 on posology in the monograph should be:

Single dose: 550 mg, 2 to 3 times daily (daily dose 1100 to 1650 mg).

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 important part of the horse chestnut bark preparations is used in a form of combination products, which contain also other non-herbal constituents (other than vitamins and minerals in ancillary quantities) so they are not classified as herbal medicinal products.

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

Not applicable.

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

Although different parts of plant *Aesculus hippocastanum* L. have been used traditionally for medical purposes, the subject of this assessment report is the stem bark. It was mentioned in the French Pharmacopoeia in 1866 to be used as a substitute for cinchona and as astringent for diarrhoea (Fournier, 1948). The author reported the herbal substance was applied: as decoction for topical use (50 g/1000 g); as antiseptic for ulcers and gangrenous wounds; as "tonic", in a form of decoction (30 to 50 g /l, 1 to 2 cups a day) or as powder (1 to 4 g); febrifuge, in a form of powder (doses 15 to 50 g); for hemorrhoids, in a form of medicinal wine (30 to 60 g /l of white wine) or tincture (250 g/l of alcohol) in a doses of 1 tablespoon per day.

Since the seventies horse chestnut bark in France have been traditionally used for the "capillary weakness" and the venous system (varicose veins and haemorrhoids) (Bezanger-Beauquesne et al. 1975). Although the tradition was derived from the old observations of the activity of the *Alcoolature de Maron d'Inde* which was made of a fresh seed or immature fruit preparations (with escin) but a view on similar physiological activity of horse chestnut seed and horse chestnut bark preparations was common in manuals and was formally credited by including in the bulletin Médicament à base the plants (1998, 1990).

Table 2: Overview of historical data

Herbal preparation	**Documented use / Traditional use**	**Strength (where relevant) Posology Duration of use**	**Reference**
Powdered herbal substance	Diarrhoea	Per se 1-4 g. Vine decoction of 30-60g. Single doses 60-100g	Garnier et al. 1961
Ethanolic extracts (not specified), stabilised alcoholatures	Capillary fragility. Congestive states in a venous system (varicosis, haemorrhoids)	Dosage specific to the products listed (available on the market).	Bezanger-Beauquesne et al. 1975
Powdered herbal substance. Herbal teas, water extracts. Ethanol	Traditionally in symptomatic treatment of capillary	Powdered herbal substance. Herbal teas, "weak"	Le médicaments à base de plantes, 1988; Médicament à base de

Herbal preparation	Documented use / Traditional use	Strength (where relevant) Posology Duration of use	Reference
extracts and tinctures	fragility such as bruises, petechiae (subcutaneous hemorrhages). Traditionally in subjective symptoms of venous insufficiency like: heavy legs", symptoms of haemorrhoids.	water extracts. Ethanol extracts "strong" and tinctures	plantes, 1990
Powdered herbal substance	Traditionally in symptomatic treatment of capillary fragility such as bruises, petechiae. Traditionally in symptoms of venous insufficiency like: heavy legs" and of haemorrhoids.	Capsules, hard with 275 mg were been used, in adults and elderly. Dose of 1 capsule (275 mg) 3 to 6 times daily.	Product was been in use in France in years 1991-2017. Since 2017 is not present on the market.

2.3. Overall conclusions on medicinal use

One herbal medicinal product on the Spanish market, meets the criterion of 30 years traditional use. The product contains powdered horse chestnut bark and has been used for the following traditional indications: *Traditional herbal medicinal product for the relief of symptoms as discomfort and heaviness in the legs and for the symptomatic treatment of haemorrhoids, based exclusively on traditional use*. The second product is present on the French market since 1994 and is close to reach 30 years of traditional use. It contains per capsule 200 mg of the dried water extract of horse chestnut bark. The product is used in the following indications: *Traditionally used in subjective signs of venous insufficiency, such a heavy legs* and *Traditionally used for the treatment of haemorrhoidal symptoms after serious pathologies have been excluded by a doctor*. In 2017 the product was registered as traditional use registration product, as summarised in Table 2.

The both products, based on the French phytotherapeutic tradition, are proposed to take into account for the monograph (see Table 3).

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
Aesculus hippocastanum L., cortex; powdered herbal substance	Traditional herbal medicinal product for relief of symptoms of discomfort and heaviness of legs and for the symptomatic	Hard capsules containing 275 mg of herbal substance. Posology: 2 capsules 2 to 3 times per day.	31 years National registration in April 1991, since July 2011

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
	treatment of haemorrhoids, based exclusively on traditional use	Single dose: 550 mg Daily dose: 1100 -1650 mg	TUR, Spain
Aesculus hippocastanum L., cortex; dry extract, DER 5.0-8.5:1, extraction solvent water	Traditionally used in subjective signs of venous insufficiency, such a heavy legs. Traditionally used for the treatment of haemorrhoidal symptoms after serious pathologies have been excluded by a doctor.	Capsules containing 200 mg of the bark dry extract, extraction solvent water. Posology: one 200 mg capsule 2 times daily. Single dose: 200 mg Daily dose: 400 mg	28 years National authorisation 1994, since 2017 TUR, France

3. Non-Clinical Data

Non-clinical strategy

Online databases were used to research the available non-clinical data on extracts of horse chestnut bark or its constituents. Only few articles about the non-clinical properties of horse chestnut bark extract were found, however, unfortunately the extracts used in the experiments were not characterised according to the contemporary used criteria.

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

3.1.1. Primary pharmacodynamics

Pharmacodynamics

In the manuals, dictionaries and encyclopaedic publications the main pharmacological activity of horse chestnut bark was claimed to have 'venotonic' activity, increasing vascular resistance and decreasing capillary permeability and also anti-inflammatory activity (Leung & Foster 1996; Fleurentin 2008; Ollier 2000; Girre 1997).

A horse chestnut bark lyophilised water extract caused inhibition of elastase release *in vitro* by human neutrophils. The protective effect was more effective than of the well-known elastase release inhibitor quercetin (Piwowarski et al., 2011). The effect was attributed to the ellagitannins contained in the water-extract. The extract contained also ellagic acid.

The effect of ellagitannins on vascular health was reviewed by Larossa (2010). *In vitro* studies on ellagitannines in concentrations 10-100 µM indicated anti-thrombotic, anti-atherogenic and anti-inflammatory effects, although a direct evidence from animal models and humans is scarce.

Ellagic acid shown also protective effects on vascular oxidative inflammatory processes in endothelial tissue (Papoutsi et al. 2007, Lee et al. 2010, Ding et al. 2014, Rozentsvit et al. 2017).

Bombardelli et al. (1996) reviewed data on horse chestnut bark and its compounds and referred coumarines such as esculin and its aglycone, alike some flavonoids (rutoside, xanthorutoside, quercetine) as capable of increasing capillary resistance. Esculin was referred by the author to possess an anti-inflammatory activity in the UV-induced erythema in animals and humans. Esculetin was known as a 5-lipoxygenase inhibitor that inhibits the production of leukotrienes and 5-hydroxyeicosatetraeinoic acid through lipoxygenase pathway what may explain its anti-inflammatory effects (Kaneko et al. 2003).

Proanthocyanidin A2 was shown to stimulate the processes of skin healing, which was measured by wound scar resistance in mice. The compound was also referred to show wound healing activity in prednisone-treated rats after topical or oral administration and to have antioxidant and anti-enzymatic activity. *In vitro*, it was able to inhibit all the stages of the peroxidative phenomenon in a dose-dependent manner and to inhibit the activity of some proteolytic enzymes (β-glucuronidase, elastase, collagenase)(Bombardelli et al. 1996). Although, the proanthocyanidin A2 is known to be law soluble in water, even more difficultly than other procyanidines (Bate-Smith 1975). Pure proanthocyanidin A2 or the oligomeric procyanidin fraction in the experimental conditions were very poorly absorbed (Schötz 2015) and the absorption is affected by the intestine microbiota (Engemann 2012, Yang S et al. 2021).

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

Herbal preparation tested	**Posology**	**Experimental model**	**Reference**	**Main non-clinical conclusions**
Preparations comparable/similar to preparations of the monograph				
Water extract. 5g of the herbal substance extracted 3 x with 50ml of water in t. 40°C and lyophilised	Extract concentration 10µg/mL	*In vitro* Extract influence on elastase release of human neutrophils of young healthy donors (20-35y non-smokers) was compared to the known elastase release inhibitor quercetin.	Piwowarski et al. 2011	64.9+/-6.9% inhibition of elastase release by the tested horse chestnut bark extract. More effective than quercitin (46.1±4.8% inhibition)
Other preparations				
Dry petrol extract of horse chestnut branch bark (1.4 g from 500g of the herbal substance)	Oral administration of the petrol extract at dose of 100 mg/kg	*In vivo* rat paw oedema model. Inhibition of carrageenan-induced oedema male Wistar rats, in comparison with calcium phenylbutazone.	Senatore et al. 1989	The administration of horse chestnut bark extract produced a 30% inhibition of paw oedema in comparison to the comparator calcium phenylbutazone. Weak anti-inflammatory activity
Single substances				

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Esculetin Esculin		*In vitro*. Inhibition of platelet cyclooxygenase and lipoxygenase in rat blood Model: Preincubation of sonicated platelets from rat blood with esculin and then with [1-14C]arachidonic acid. Analysis of radioactive metabolites. Metabolites identification by GC-MS.	Sekiya et al. 1982	Esculetin IC50 Lipoxygenase: 0.647 µM Cyclooxygenase: 447 µM Esculin IC50 Lipoxygenase: 287 µM Cyclooxygenase: >104 µM. Anti-inflammatory activity and inhibition of platelet aggregation may be due to inhibition of lipoxygenase. Inhibition of lipoxygenase and stimulation of cyclooxygenase by esculetin.
Esculetin	Esculetin was added (in concentrations: 1.17, 1.68, 20.2µmol/ear) to the Croton oil acetone solution and was applied on the inner ear cover in mice.	*In vivo* inflammatory ear test on CD male mice (28-32g).The left ears were treated with the solution of Croton oil (15 µl) in acetone. The tested esculetin was added to the tested inflammatory-induced solution and similarly applied. After the test (6 or 24 h after) the mice were killed, ears cut off and difference between inflammatory response was monitored as a difference in weight. The contribution of granulocyte infiltration was assessed by measuring the peroxidase activity	Tubaro et al., 1988	Esculetin, in a dose 0.84 µmol/ear reduced the weight of the ear by 18.2% ($p<0,05$) in doses 1.17, 1.68 µmol/ear by 20.2 and 24.7% ($p<0.005$). Significant concentration-dependant reduction in oedema after 6 and 24 hours. Peroxidase activity reduction was observed in highest dose, by 38.5% ($p<0.005$)
Esculin Esculetin	Cotreatment of the TIG-7 cells with esculin and esculetin (50µM)	*In vitro* model of DNA oxidation on human diploid fibroblasts (TIG-7 cells), cultured with Earle's solution containing linoleic acid hydroperoxide (LOOH) and/or FeCl3. Esculetin was concurrently added or cells were pretreated with esculin or esculetin (50µM). Quantitation of 8-oxo-7,8-dihydro-29-deoxyguanosine (8-oxodG) by electrochemical detection.	Kaneko et al. 2003	Pre-treatment of the TIG-7 cells with esculetin exhibited a suppressive effect on the formation of DNA base oxidation product, 8-oxodG in cells treated subsequently with LOOH and iron(III) ion. Esculin also had a suppressive effect on the increase in 8-oxodG content but the effect was not significant.
Esculin, isolated of Cortex Fraxini, with structure confirmed by 1H, 13C NMR	Oral administration of esculin in doses 5, 10, 20 mg/kg (or dexamethasone as a positive control, 5mg/kg) before induction of ear edema Oral administration of	*In vivo*. Xylene-induced ear edema in mice. Male and female Kunming mice (18-22g) *In vivo.* Carrageenan – induced rat paw edema. Male	Niu X et al. 2015	Oral pre-treatment with the tested esculin concentrations reduced the extent of edema compared with the control group. The inhibition rates in esculin (5, 10, 20 mg/kg) were 26.8%, 47.75, 54.8%, (dexamethasone, positive control 64.2%). Carrageenan–induced edema was significantly

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
	esculin in doses 5, 10, 20 mg/kg (or dexamethasone as a positive control, 5 mg/kg) before injection of carrageenan	Sprague-Dawley rats, 8-9 weeks (220-250g)		attenuated by pre-treatment with esculin (5, 10, 20 mg/kg) (and dexamethasone as a positive control)
	Oral administration of esculin in doses 5, 10, 20 mg/kg (or dexamethasone as a positive control, 5 mg/kg) 0.5 h before injection of carrageenan	*In vivo*. Effect of esculin on TNF-α and IL-6 production in carrageenan-induced pleurisy in mice. Carrageenan injection into the pleural cavity in mice induced the increase of TNF-α and IL-6 in the supernatant of centrifuged pleural exudates. *In vivo*. Effect of esculin on myeloperoxidase activity as indicator of polymorphonuclear accumulation in pleural exudates		TNF-α and IL-6 in the supernatant of centrifuged pleural exudates were markedly reduced in esculin treated mice in a dose – dependent manner.(Dexamethasone effectively inhibited both TNF-α and IL-6) Carrageenen-induced myeloperoxidase activity was markedly reduced by the pre-treatment with esculin in the tested concentrations
	In vitro. Peritoneal macrophages were pretreated with esculin in concentrations 10^{-3} – 10^{-1} mg/ml, 24 h before stimulation with LPS (10μg/ml, 12 h)	*In vitro.* Effect of esculin on the levels of TNF-α and IL-6 in LPS stimulated peritoneal macrophages (ELISSA assay)		Pre-treatment with esculin reduced LPS-induced production of TNF-α and IL-6. Results of Western blotting indicate that protein expression of TNF-α and IL-6 increased in LPS group and this effect was decreased significantly by treatment with esculin and positive control.
	Peritoneal macrophages were pretreated with esculin in concentrations 10^{-3} – 10^{-1} mg/ml, 24 h before stimulation	Effect of esculin on phosphorylation of Mitogen-activated protein kinases (p38MAPK, JNK, ERK/1/2). The peritoneal macrophages were pretreated with esculin. The positive control was pre-treatment with specific inhibitors for the MAPKs		In the experiment LPS significantly elevated the levels of phospho-p38MAPK, phosphor-JNK, phosphoERK1/2 in peritoneal macrophages lysate supernatants while pre-treatment with esculin markedly inhibited protein expression of the phosphor-MAPKs. Authors suggested that esculin effectively inhibits phosphotylation of MAPKs in LPS induced peritoneal macrophages which may responsible for the inhibitory effect of esculin on TNF-α, IL-6 production
Esculin	Oral administration of esculin in doses 20 and 40 mg/kg (or dexamethasone as a positive control, 5 mg/kg) 1 h before	An experimental model of protection against LPS-induced acute lung injury. BALB/c mice were randomly divided into five groups with 10 mice: control, treated with saline, LPS group, LPS+ dexamethasone (Dex, 2 mg/kg), LPS + esculin 20 mg/kg, LPS + esculin 40	Tianzhu, Shumin 2015	Pre-treatment with esculin significantly decreased the number of total cells and neutrophils in bronchoalveolar fluid compared to those in LPS group. Esculin and dexamethasone significantly decreased the levels of superoxidismutase,

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
		mg/kg. 30 min after treatment (esculin, Dex, saline) LPS was administered intratracheally. 6 h after the animals were sacrificed and bronchoalveolar lavage fluid (BALF) was collected, total leucocytes were counted, samples were centrifuged. Supernatant was used for cytokines determination, Activities of myeloperoxidase, superoxide dismutase and methyldismutase were determined. Cytokines: TNF-α, IL-1β, IL-6in BALF were determined by ELISA kits. At the end right lungs were removed and the wet-dry ratio was determined. Histopathological evaluation was performed.		methyldismutase and myeloperoxidase compared to the LPS group. Pre-treatment with esculin efficiently decreased cytokines TNF-α, IL-1β, IL-6in BALF. Esculin and dexamethasone decreased lung wet-dry ratio. Histopathological evaluation indicate reduction degree of pathological inflammatory changes in acute lung injury.
Esculin isolated from horse chestnut bark	Oral doses 5, 10, 20 mg/kg, before evoking gastric lesions by ethanol	Male Kunming mice (18-22g). Protection before acute gastric ethanol lesions by pre-treatment with gastroprotective cimetidine and tested esculin solutions. Inflammatory state assessment of NO, TNFα, IL-6 determination and by histopathology index after gastric tissue fixation and staining.	Li W et al 2016	Pre-treatment of mice with esculin reduced TNFα and IL-6 (P>0.05) versus cimetidine (p>0.01). NO levels in gastric tissues of mice were clearly lower than in the control group. Esculin effect at a dose 20 mg/kg was comparable with those of cimetidine (p>0.01). Esculin in tested doses inhibited increase of myeloperoxidase (p>0.05), cimetidine (p>0.01). Histopathological indexes shown positive effect of pre-treatment with esculin.

3.1.2. Secondary pharmacodynamics

Water extract

Dry water extract of 30 g grinded dry horse chestnut bark, extracted with 270 ml of distilled water and evaporated to dryness in 40°C with yield 13,4% [corresponding to DER 7.5:1]. The extract tested on 10 Gram-positive and Gram-negative strains of bacteria, isolated from patients with urinary infections , had overall weak antibacterial activity (MIC≥0.625 mg/mL) and bacteriostatic potential (MBC/MIC≥16). (Khar'kov et al. 2022)

Coumarines

Kong et al. (2002) tested anty uricemic potential of esculin. Potassium oxonate, uricase inhibitor, was used to increase urate level in serum of male mice (ICR) and male Spraque-Dawley rats. Serum uric acid was determined by the phosphotungistic acid method. 1 h after the injection of the uricase inhibitor the esculin in concentration 20mg/ml (dispended in 0.8% sodium carboxymethylcellulose was administered intraperitoneally and orally. Homogenized mouse or rats livers were used for *in vitro* enzymes assays, xanthine oxidase or dehydrogenase activities were assayed by using spectrophotometric methods, in 80mM sodium phosphate buffer (pH=7.4) centrifuged. Oral

administration the same doses 100mg/ml in both rodents did not produced hypouricemic effects. In tests *in vitro* on rats and mice liver homogenates aesculin did not elicit any measurable inhibitory action on xanthine oxidase or xanthine dehydrogenase activity. Esculin administered i.p. to the oxonate-induced hyperuricemic rodents elicited dose-dependently hypouricemic effects after 1.5 h. At a dose 100mg/ml the serum urate levels in rats were not different from normal rats.

Kaneko et al. (2004) studied protective effect of oral esculin administration, in 0.05% aquous solution, to Syrian hamsters on a model of pancreas carcinogenesis induced by the administration of N-nitroso-bis-(2-oxopropyl)-amine (BOP). The esculin was administered for 7 days before application of the carcinogen. 4 hours after induction of carcinogenesis the animals were killed and their pancreas were assessed. As indicator of oxidative DNA damage was a content of 8-oxo-2'-deoxyguanosine (8-oxodG). The incidence of invasive tumors in animals given esculin was significantly lower than in control group. The results suggest that intake of esculin has an inhibitory effect on the BOP-induced oxidative DNA damage and carcinogenesis in hamster pancreas.

Kaneko et al. (2007) studied effects of esculin and esculetin on 8-oxo-2'-deoxyguanosine (8-oxodG) formation and on carcinogenesis induction by 1,2-dimethylhydrazine (DMH)(chemical carcinogen) in the colons of rats. To 344 male Fisher rats were administered a water solution of esculin (0.02, 0.05%) and esculetin (0.01%) for 7 days and then DMH was subcutanaeously injected (20 mg/kg). 24 h after the animals were killed and levels of thiobarbituric acid reactive substances (TBARS) and 8oxodG were determined. Authors further investigated effects of esculin administration on the development of colonic aberrant crypt foci (ACF). To the animals was administered DMH for one week, for induction ACF and then they received esculin water solution for 5 weeks (during initiation phase, group 1), or during next 11 weeks after initiation (post initiation phase, group 2). Animals in the positive control group received DMH + water; animals of negative control normal diet + water and last group on diet + esculin. At the end of the experiment (after 16 weeks), was found that the oral administration of esculin during the initiation phase significantly reduced the incidence of gross tumors, the number of ACF per rat and the average number of aberrant crypt per focus. Esculin treatment during the post-initiation phase significantly decreased only the number of ACF per rat. Authors suggested that esculin intake has an inhibitory effect on DMH-induced oxidative DNA damage and carcinogenesis in rat colons.

Cals-Grierson (2007) tested an extract of horse chestnut bark (named Affilene, not closer characterised) on *in vitro* modulation of the activity of the aquaglyceroporin channel in human and mouse adipocytes. The horse chestnut bark extract was tested in concentrations 4, 20 and 100 µg/ml for its influence on release of glycerol after addition of adrenaline. Evaluation of glycerol elimination due to plant extracts indicated no stimulatory effect (no possible influence on skin aging symptoms). The only slight inhibitory effect was noted in co-stimulation with adrenaline (inhibition of 22% 4% and 10% with 4, 20 and 100 µg/ml respectively).

Rios ERV et al. (2010) studied gastroprotective mechanisms of esculin activity on a model of acute gastric lesions induced by intragastric ethanol administration in male Swiss mice. The animals obtained 12.5, 25, 50 mg/kg of tested esculin (p.o.); control mice obtained the solvent; the reference group obtained cyproheptadine, a non-selective antagonist of 5-HT and histamine receptors. 30 min after ethanol administration the animals were killed, their stomachs were preserved and examined histopatologically. Further experiments were conducted for estimation of possible mechanism of esculin activity in stomach: evaluation of role of nitric acid, ATP-dependent K+ channels (KATP), prostaglandins and TRPV1 in the gastroprotective effect of esculin. In the ethanol-induced ulcer model esculin reduced ulcer lesions (3.9-5.2% ulcerated area) at level similar to the cyproheptadine (10 ml/kg) (4.1%) in compare to the positive control group (18.1% ulcerated area). In the indomethacin induced gastric lesion model esculin in concentrations 25 and 50 mg/kg reduced lesions level (3.6-

4.8%) to the level of ranitidine at concentration 20 mg/kg (5.1%) in compare to the indomethacin (9.5%). Animals pretreated with esculin (25 mg/kg, p.o.) showed less macro- and microscopic mucosal damage than those treated with ethanol. Esculin decreased the ethanol-induced lesions significantly to a similar extent as N-acetyl-L-cysteine (NAC). Additionally for screen a possible esculin mechanism of activity in the stomach, the effect of esculin on nitric acid, ATP-dependent K+ channels (KATP), prostaglandins and TRPV1 in was tested also. The authors concluded that the pre-treatment with esculin possess significant gastroprotective activity.

Li W et al. (2016) studied gastroprotective effect of esculin (extracted of horse chestnut bark) on ethanol-induced lesion in mice (male Kunming mice, 18-22g). Mice were divided in 6 (8 animal) groups, obtained esculin in oral doses 5, 10, 20 mg/kg or cimetidine (5 mg/kg) as positive control. Two hours after administration of tested substances the animals obtained 0.1 ml of absolute alcohol. 3 hour after ethanol administration the animals were sacrificed and were evaluated for macroscopic and histopathological alterations, gastric mucosa lesion index and for myeloperoxidase activity as marker of neutrophil infiltration was determined. Pre-treatment with esculin significantly reduced macroscopic and histopathological damage, gastric lesion index, and inhibited increase of myeloperoxidase in gastric tissues ($p<0.05$), cimetidine ($p<0.05$), in a dose dependent manner. Moreover NO, inducible synthase (iNOS) and inflammatory cytokines were tested. The levels of TNF-α and IL-6 production were reduced in esculin pretreated mice gastric tissues and in cimetidine ($p<0.05$ and $p<0.01$). Esculin at dose 20 mg/kg gave protective effect comparable to the cimetidine.

Naaz F et al (2014) studied nephroprotective effect of esculin on model of Swiss albino mice intoxicated with 66.6 µg/kg bw per day of the aflatoxin B1 for 90 days. In tested group, 30 min. after the intoxication, esculin (150mg/kg per day) or ascorbic acid (300 mg/kg per day) were administrated. Protective effects were assessed by measuring lipid peroxidation, non-enzymatic antioxidants as reduced glutathione-S-transferase, glutathione reductase, superoxide dismutase and catalase in kidney at days 30, 60 and 90 of treatment. The authors observed decrease of lipid peroxidation after esculin daily treatment and protective effect of esculin against nephrotoxic aflatoxine effects. The protective effect was confirmed by histopathological findings in mice.

Procyanidins

Ambrogini et al. (1995) studied the potential influence of procyanidin A2 on nerve regeneration in rats. Procyanidin A2 was obtained from horse chestnut bark and was poorly dissolved in water, but very soluble in methanol, ethanol and acetone. In the experiment Sprague Dawley male rats were divided in two groups treated and control in which at the age of 45 days, the extensor digitorum longum (EDL) and soleus muscles were surgically denervated under aesthesia. The experimental group of rats obtained procyanidin A2 in 0.9% NaCl (5 mg/ml), administered i.p. once a day in dose 20 mg/kg, 6 days a week for 20 days. Over the experiment the EDL and soleus muscles were stimulated by a connected stimulation (isomeric tension transductor) with a single stimulus with a frequency of 1 Hz and a train of stimuli of 100Hz for 1 second. Tension was recorded 12, 14, 16, 18, 20 days after denervation. The difference between un-denervated groups of treated and control was calculated by Student t-test and two-way analyse of variance (ANOVA). There was no observed difference between procyanidine A2 treated and control rats in the experiment. Body weights were not affected by procyanidin treatment. Also time course of re-innervation (regeneration) of EDL and soleus muscles was not affected by the procyanidin A2 treatment. Although in the procyanidin A2 groups a trophic effect was observed, increase in muscle mass in denervated and un-denervated treated rats, compared to controls. Authors suggested trophic effect on muscle.

3.1.3. Safety pharmacology

No data about safety pharmacology are available.

3.1.4. Pharmacodynamic interactions

Herbs with coumarins, salicylate or with antiplatelet properties were suspected to potentially interfere with warfarin because of a theoretical risk of potentiation of anticoagulant activity although there is no direct experimental or clinical evidence available to date. Despite no clinical cases for coumarins (as mono-) have been reported, it has been recommended that patients taking horse chestnut extracts concurrently with medications that have anticoagulant effects, such as warfarin, should be closely monitored for signs of symptoms of bleeding (Heck *et al.* 2000).

3.1.5. Conclusions

There are no available data on powdered horse chestnut bark. The only publication relevant to the products presented above is a study of Piwowarski et al. (2011) on the influence of horse chestnut bark water extract on elastase release. The effect observed by the authors correlated with the traditional use of the products with this herbal substance in diseases with inflammatory background and it was compared to the effect known from quercetin.

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

No information about pharmacokinetics of horse chestnut bark extract is available.

Although esculin and esculetin are only present in trace amounts in Aesculus hippocastanum cortex, some information about pharmacokinetics of esculin and esculetin is available and summarized hereunder.

Rehman et al. (2015) determined esculin and esculetin in rat's plasma. After oral administration of esculin and esculetin in a dose 120 mg/kg the authors observed a mean Cmax values 340.3 and 316.5 ng/mL and the AUC last values 377.3 and 1276.5 h ng/mL for esculin and esculetin respectively. The bioavailability of esculin was calculated to be 0.62%.

Wang et al. (2016) studied metabolic profile of esculin after oral administration to rats. Esculin was administered by intragastric gavage to male Sprague-Dawley rats (100 mg/kg) randomly divided into two animal groups (n=6). Group I was kept in the metabolic cages to collect urine and feces samples. Blood samples were collected at: 0.5h, 1h, 3h, 6h, and 24 h after administration. Rats in a group II were cannulated in bile duct to collect bile for 12h. Blank samples of plasma, bile, urine and feces were collected from rats without administration. The authors found 10 types of Phase I metabolites. Deglycosylation metabolite (esculetin) was found in urine, plasma, bile and feces. Urine samples contained hydroxylated metabolites (M3, M4), sulphated metabolites after hydroxylation (M5), greater polarity and methylated metabolites (M6, M7) and hydrolysed (M8). Among Phase II (conjugation) metabolites were glucuronide conjugate (M9), sulphated conjugate (M12), dehydrogenated metabolite (M16) and sulphated metabolite (M17). A glucuronide conjugated metabolite (M19) was found only in plasma and bile samples. Apart for the metabolic profile (pathways) qualitative data are not yet available.

Esculin, although is soluble in water, on a base of in silico experiment was found to be blood brain barrier negative (Varier, 2017).

Metabolism of horse chestnut bark water extract ellagitannines was not studied. Ellagitannins and ellagic acid are constituents of food which are metabolized by a gut microbiota of the mammals with production of urolithins, excreted with feces and, after conjugation with glucuronides and sulphates coming to plasma and are excreted with urine (Gonzalez-Barrio R 2011, Garcia-Vitalba 2016). Of 15 different tested ellagitannines those possessing hexahydroxydiphenoyl moieties were metabolized to urolithins (Piwowarski J et al. 2016). Important part of ellagitannins in diet comes with herbal teas (Yang 2019).

Metabolism of procyanidins from horse chestnut bark was not studied. However, procyanidins from other sources was studied for absorption and metabolism in from small intestine in rats by Appeldoorn et al.(2009). The procyanidines A1, A2 , B2 (from peanut skin), A trimers (from cranberries, purified and fractions) and monomeric epicatechin (administered in a form of DMSO solution) were compared by in situ perfusion of the rats small intestine for 0–30 min. The rats had their bile duct, portal vein, and small intestine cannulated. Procyanidin dimers A1, A2, and B2 were only slightly absorbed (of the experimental solution) from the small intestine, were not conjugated or methylated, thus conserving their biological activity after absorption. A1 and A2 dimers were better absorbed than B2. Absorption of the A-type dimers was only 5–10% of that of monomeric epicatechin. Dimers were not conjugated or methylated in contrast to epicatechin, which was partly methylated and 100% conjugated. A-type trimers were not absorbed.

Bioavailability of procyanidin A2 after oral use was studied on a rat model by Schötz et al. (2015) but was negligible.

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

There are no toxicological data on herbal preparations which are on the market, powdered herbal substance and water extracts (presented in Table 1). Therefore, data are presented for isolated components.

3.3.1. Single dose toxicity

Pencheva I et al. (1998) tested toxicity of esculin after single i.p. application to H-albino mice. Esculin was found to be of low toxicity. Doses 5.50g/kg caused death with signs of convulsion and paralysis within a few hours after application. At doses 0.05-0.15 g/kg no lethality was observed until day 30. LD_{50} was calculated 1.90-2.22 g/kg. This value is referred by the European Chemicals Agency (ECHA report, 2018).

Kostova and Iossifova (2002) reported on tests of esculin after oral administration to white Wistar rats, in doses 50 – 8000 mg/kg and found it "practically nontoxic". No lethality was observed up to 21st day with highest dose used. No significant changes were found in behaviour and the reflexes of the animals. No pathological deviations from physiological values were found in haematological and clinical indices.

Niu (2015) tested esculin (isolated from Cortex Fraxini) for oral acute toxicity on male and female Kunming mice (18-22g) in doses 5, 10, 20 mg/kg. The animals were observed for 14 days after administration and no toxicity signs were found. There were no significant differences in the body weight between esculin and the control group.

Acute toxicity of esculetin (6,7-dihydroxycoumarin) extracted from the bark of *Aesculus hippocastanum* was tested in mouse after an intraperitoneal and oral administration. The intraperitoneal LD_{50} was 1450 mg/kg and the oral LD_{50} was > 2000 mg/kg (Tubaro *et al.* 1988).

There are no available data on horse chestnut bark ellagitannins single dose toxicity. Ellagitannin geraniin acute oral toxicity, tested in Sprague Dawley rats, following a single oral dose (according to the guidelines of the OECD 423 test) was established to be 2000 mg/kg (Moorthy et al. 2019).

3.3.2. Repeat dose toxicity

There is no available data on herbal preparations, water extract and powdered herbal substance available on the market.

There is no available data on the hydroxycoumarine glycosides.

There is no data on horse chestnut bark ellagitannines. Ellagitannins are usually contained in our diet, in a tea or in fruits, in bigger amounts than in the 200 mg capsules or the water extract.

There are no data for ellagitannines obtained from horse chestnut bark. Available are data on repeat dose toxicity on Sprague-Dawley rats which were fed with a diet containing 6% ellagitannines of pomegranate, for 37 days. Punicalagin, the pomegranate ellagitannine and related metabolites were identified in plasma, liver, and kidney. Five punicalagin-related metabolites were detected in liver and kidney, that is, two ellagic acid derivatives, gallagic acid 3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one glucuronide, and 3,8,10-trihydroxy-6H-dibenzo[b,d]pyran-6-one. Feedstuff intake, food utility index, and growth rate were lower in treated rats during the first 15 days without significant adverse effects. No significant differences were found in treated rats blood parameter analysed, in the antioxidant enzymes glutathione peroxidase and superoxide dismutase. Blood urea and triglycerides level, were observed to have low values throughout the experiment. The authors suspected that the decrease could be due to the lower nutritional value of the punicalagin-enriched diet than the standard rat food. Histopathological analysis of liver and kidney corroborated the absence of toxicity. The results reported, together with the large safety margin considered, indicated the lack of toxic effect of punicalagin in rats during the 37 day period investigated (Cerda B, 2003).

3.3.3. Genotoxicity

There is no genotoxicity data available on water extracts which are authorised/registered in the EU countries.

Esculin was screened on 6 Ames strains (TA92, TA94, TA97, TA98, TA100 and TA102) at 4 concentrations ranging from 0.2 to 500 µg/plate with or without S9. It was not mutagenic (Uwaifo 1984).

Esculin was not genotoxic at doses 20 and 40 mg/kg in Comet assay on kidney and liver cells of Bulb /C mice. Esculin and its oligomers significantly decreased DNA damages induced by mitomycin in this test (Bzeouich et al. 2016).

There are no genotoxicity data on horse chestnut bark ellagitannines.

Gallic acid was not mutagenic in the Ames Salmonella tester strains TA98 and TA100 without ant with activation (Chen & Chunk, 2000).

3.3.4. Carcinogenicity

No carcinogenicity study is available for the horse chestnut bark preparations nor for individual compounds.

3.3.5. Reproductive and developmental toxicity

No studies available

3.3.6. Local tolerance

No data available on tolerance of products containing horse chestnut preparations which have been used on the EU markets over last three decades.

Comaish & Kersey (1980) reported on a case of contact dermatitis a result of use two products (ointment and suppositoria) containing esculin and cinchocaine. The patient gave strong positive reactions for tests with 1% cinchocaine + esculin and weak reaction for wool fat. Control tests on esculin among 12 other patients gave negative reactions.

Ellagitannins present in a tea are important component of human diet, there are no known objections for tolerance in normal conditions of use.

3.3.7. Other special studies

No available special studies.

3.3.8. Conclusions

Very few studies about horse chestnut bark preparations were found in the literature.

There was no adverse reactions over review period and no constituent with safety concern was discovered.

3.4. Overall conclusions on non-clinical data

Results from relevant experimental studies on the pharmacological activity of horse chestnut bark, powdered herbal substance and water extract, to support the proposed indications are limited but in line with traditional uses.

Effects on vessels, anti-oxidant and anti-enzymatic activity

Horse chestnut bark water extract and its constituents: esculetin, esculin, ellagitannin, ellagic acid, are reported to have anti-oxidant properties through inhibition of the peroxidation and some enzymes activity (cycloxygenase, 5-lipoxygenase), inhibition of elastase release, protective effects on vessel endothelium and in several tissue experiments (on gastrointestinal tract, pancreas and nephroprotective effects).

Anti-inflammatory activity

The anti-inflammatory activity of the powdered herbal substance and a water extract may be due to the inhibiting properties of esculetin and esculin and it could be linked to the inhibition of lipoxygenase. Esculetin extracted from horse chestnut bark demonstrated an activity in the inflammatory ear test in mice and the acetylcholine-writhing test in mice. Esculin and esculetin in different pharmacological models showed inhibition of cytokines release. It was compared with dexamethasone. Although the detailed mechanism remains unclear.

No safety pharmacology data for horse chestnut bark preparations are available.

Pharmacokinetics

No pharmacokinetics data about horse chestnut bark preparations are available. Basic pharmacokinetic parameters and a metabolic pathways of esculin have been identified and ellagitannins metabolism is roughly known. The quantitative pharmacokinetic data for horse chestnut bark preparations were not studied.

Toxicology

There are no toxicity data for horse chestnut bark preparations.

Data are available on individual compounds which are present in water extracts of horse chestnut bark or chemical substances similar to those found in horse chestnut bark water extract. The acute toxicity of esculin and esculetin is low. Available data on ellagitannins single dose indicate low toxicity. Repeat dose toxicity study Sprague-Dawley rats were fed with a diet containing 6% ellagitannines (similar, from pomegranate) for 37 days, shown decrease of feedstuff intake; growth rate was lower in treated rats during the first 15 days with a decrease in food intake but without significant adverse effects.

No other toxicity data for horse chestnut bark preparations was available. 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.

In conclusion, the literature about non-clinical studies with horse chestnut bark preparation is sparse.

Results from relevant experimental studies on Hippocastani cortex to support the proposed indications:

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

are limited but in line with traditional uses.

Specific data on pharmacokinetics and interactions of herbal medicinal products are not available.

Non-clinical information on a safety of Hippocastani cortex and its water extracts is scarce.

Oral administration of Hippocastani cortex in powdered form or water extract in capsules can be regarded as safe at traditionally used doses.

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

No clinical pharmacodynamic data are available.

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

No clinical pharmacokinetic data are available.

4.2. Clinical efficacy

No clinical study or case study reports can be found to illustrate the clinical efficacy of the preparations. The medicinal use in patients is supported by the tradition.

Hippocastani corticis extractum siccum is commonly used as an anti-inflammatory constituent in combinations venous diseases and esculin, isolated from horse chestnut bark, is used in combination products commonly used in haemorrhoids.

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

No clinical data on mono-component horse chestnut bark preparations are available.

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

No information available.

4.4. Overall conclusions on clinical pharmacology and efficacy

The clinical efficacy of *Aesculus hippocastanum* bark preparation relies only on the traditional use. No pertinent clinical efficacy data can be found in the literature to support the claimed indications. Only few publications mention the use of horse chestnut bark in traditional medicine and contribute to demonstrate the plausibility of the traditional use given the link between its constituents and the attributed indications in mild venous complaints.

5. Clinical Safety/Pharmacovigilance

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

No data available.

5.2. Patient exposure

No data available.

5.3. Adverse events, serious adverse events and deaths

There are no reports for one-component products containing horse chestnut bark preparations in the EudraVigilance system.

There was one short letter report (Nagy, 1973) on a possibility of poisoning in humans with horse chestnut seed, leaves and twigs, manifested by muscle twitching, weakness, lack of coordination, dilated pupils, vomiting diarrhea, paralysis and stupor. The author cited the old encyclopedia where were described poisonings "by eating the seed or drinking 'tea' made from leaves and twigs". The information on intoxication symptoms was attributed to esculin while the mentioned parts, seeds and leaves contains mainly escin. The signal had a low relevance.

There was one documented allergic reaction in person after a rectal administration of a combination product for the treatment of haemorrhoids (the product contained hydrocortisone, dibucaine hydrochloride, neomycin sulfate and esculine) (Comaish & Kersay 1980; see also second product below the p. 21). The patient was tested to be allergic in 1% cinchocain (dibucaine hydrochloride) and also 1% esculin. The same case was cited by Calapai et al. (2014). Lee (1998) reported similar contact dermatitis to the same combination product. Patch tests with its ingredients showed strong positive reaction to dibucaine hydrochloride. Cross-sensitivity for other topical anaesthetics were checked (for lidocaine, lidocaine with prilocaine, bupivacaine, tertracaine) but the results were negative. The strongest reaction was to the dibucaine hydrochloride. Two further cases of contact dermatitis after use of the same combination product and tests for particular constituents of the combination reported Hughes and Pratt (2018). The authors found, apart from dibucaine hydrochloride, also for neomycin allergic reaction. The authors observed cross sensitization between constituents of creams used for haemorrhoids (Proctosedyl cream, Proctomyxin cream, Anusol HC, and Proctozone). Esculin was not found among allergens (what excludes its participation in cross-sensitization with local anaesthetics in the products observed).

Reports on adverse events for a period 2011-2021

In 2018 MAH reported on two cases. One spontaneous non serious case was reported on 14-year-old male patient taken 5 capsules of the herbal product at a time (by a confusion, instead of the homeopathic product), on a next day experienced slight diarrhoea and at a day after have no symptoms (recovered from the event). MAH assessed the case as possible and non-serious overdose. Other case classified as serious was a female patient who was being applied cream and suppositories with *Chondrus crispus*, titanium dioxide and zinc oxide, for haemorrhoids, took 1-2 capsules of horse chestnut bark preparation (orally) and next day started to complain for headache behind one eye. The use of suspected product was withdrawn, and the suppositories and cream were discontinued but the pain not recovered; the patient was diagnosed scleritis. The company assessed that although scleritis as adverse reaction is serious and potentially blinding inflammation but in this case the suspension of the orally used product with horse chestnut bark for eye infection was doubtful.

Assessor's comments

Although the cases of allergic reactions were reported for combination products containing dibucaine hydrochloride and horse chestnut bark extract there was no reports for allergic reactions to herbal medicinal products containing only esculin or horse chestnut bark preparation, without dibucaine.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

No specific data are available on use in pregnancy and lactation, overdose, effects on ability to drive or operate machinery or impairment of mental ability. Safety during pregnancy and lactation, and in children and adolescents is not established.

5.5.1. Contraindications

Hypersensitivity for the *Aesculus hippocastanum* L., cortex and its preparations.

5.5.2. Special warnings and precautions for use

Because safety during pregnancy and lactation, and in children and adolescents has not been established, the use of horse chestnut bark in these special populations is not advised.

5.5.3. Drug interactions and other forms of interaction

Interactions of horse chestnut bark preparations with other medicines are not known nor described.

Several interactions of esculin with other molecules and proteins were described but its relevance to the medicinal use is not known

5.5.4. Fertility, pregnancy and lactation

Safety during pregnancy and lactation, has not been established, the use of horse chestnut bark is not advised.

5.5.5. Overdose

Accidental overdosing of solid pharmaceutical forms (capsules) caused a slightly laxative effect.

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

Not known.

5.5.7. Safety in other special situations

No specific data are available.

5.6. Overall conclusions on clinical safety

The specific safety of *Aesculus hippocastanum* bark preparation cannot be established due to the lack of published data. Due to the presence of esculin in the bark, it could be assumed that the described adverse event with other similar products could apply. However, the long term traditional use of oral forms is in favour of their good tolerance in the target population and in the recommended range of dose.

Clinical safety data are very limited. No security issues concerning the traditional use of horse chestnut or its preparations have been reported.

Due to lack of data, the use during pregnancy and lactation, and in children and adolescents under 18 years of age is not recommended.

The referred products containing horse chestnut bark preparations have been present on the pharmaceutical market for at least 30 years and are not harmful when used in the recommended dosages for the specified indications.

6. Overall conclusions (benefit-risk assessment)

In conclusion, the traditional use of horse chestnut bark preparations is considered established in the following indications:

Indication 1)

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

Indication 2)

Traditional herbal medicinal product for symptomatic relief of itching and burning associated with haemorrhoids, after serious conditions have been excluded by a medical doctor.

The traditional use for both indications has been established for two preparations: One preparation, containing 275 mg of powdered herbal substance in capsules, which has been traditionally used for at least 30 years in Spain and one preparation, containing 200 mg of the dry extract with a genuine DER 7.0-8.5:1, extracted with water, which has been used traditionally for 28 years and was registered as traditional herbal medicinal product in France in 2017.

Pre-clinical data about a water extract of horse chestnut bark and several components that are present in horse chestnut bark support the pharmacodynamical plausibility of the use.

As there are no clinical studies conducted with horse chestnut bark in children under the age of 18 years and there was no documented experience with traditional use of the herbal preparations in children and adolescents in the upper mentioned indications, horse chestnut bark is not proposed to be used in the younger subpopulations. The use will be focused on adults patients, the target population for the indications.

Given that no reproductive toxicity studies have been conducted and there are no data from the use of horse chestnut bark in pregnant women, section 4.6 of the monograph is adapted accordingly and in compliance with the wording validated in other monographs.

On the basis of the available information esculin is considered by the HMPC in the *Aesculus hippocastanum* L., cortex, herbal substance/herbal preparation(s) as an analytical marker.

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

Annex

List of references