2 February 2016
EMA/HMPC/278089/2015
Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Equisetum arvense* L., herba

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Equisetum arvense* L., herba
Herbal preparation(s)	a) Comminuted herbal substance b) Expressed juice from fresh herbal substance (DER 1:1.6-2.0) c) Liquid extract from fresh herbal substance (DER 1:9), extraction solvent: water d) Dry extract (DER 4-7:1), extraction solvent: water e) Liquid extract (DER 1:5), extraction solvent: ethanol 96% (V/V):water: sweet wine 16.5% (V/V) (16.5:13.5:70) (m/m) f) Liquid extract (DER 1:4.5-5.0), extraction solvent: sweet wine 16% (V/V):ethanol 96% (V/V) (91:9) (m/m) g) Liquid extract (DER 1:1), extraction solvent: 25% ethanol h) Liquid extract (DER 1:4-5), extraction solvent: ethanol 31.5% (V/V) i) Dry extract (DER 7.5-10.5:1), extraction solvent: ethanol 70% (V/V)
Pharmaceutical form(s)	Comminuted herbal substance as herbal tea for oral use. Herbal preparations in liquid or solid dosage forms for oral use. Comminuted herbal substance for decoction preparation for cutaneous use. Herbal preparations in liquid dosage forms for cutaneous use.
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1.

Introduction

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

- Herbal substance(s)

Diverse national monographs for Equiseti herba have been replaced by the current European Pharmacopoeia 8th edition 2015 (1825) Equisetum stem - Equiseti herba. Equiseti herba is the whole or cut, dried sterile aerials parts of *Equisetum arvense* L. Content: minimum 0.3% of total flavonoids, expressed as isoquercitroside (Ph. Eur., 14/2012:1825).

Equisetum is widely distributed throughout the temperate zones of the northern hemisphere, Canada, the USA, Europe and Asia south to Turkey, Iran, the Himalayas and across China, Korea and Japan (Sandhu *et al*., 2010). The material of commerce is imported from China, as well as from eastern and south-eastern European countries.

Name

The species *Equisetum arvense* L. is known under the synonyms: *Allosites arvense* Brongn.; *Equisetum boreale* (L.) Börner.

Adulteration and contamination

Adulteration with *Equisetum palustre*, *Equisetum hyemale* L., *Equisetum fluviatile* L., *Equisetum sylvaticum* L. is possible. Some *Equisetum* species such as *Equisetum palustre* contains potentially toxic palustrin (Blaschek *et al*., 2013). The European Pharmacopoeia 8th edition (2015) contains a test of thin-layer chromatography for identification of the herb and specification of the absence of *Equisetum palustre*. Only quality controlled horsetail (from pharmacies) should be consumed by the patients, as adulteration with *Equisetum palustre* is possible, when it is harvested by unqualified persons.

Principal constituents of the herbal substance

The drug consists of the whole or cut dried sterile aerials parts of *Equisetum arvense* L. According to Blaschek *et al*. (2013) it contains about

- Inorganic constituents: with 5-7.7% silicic acid (or silicates respectively) of witch 10% are water-soluble, 1.5% aluminium chloride, potassium chloride and manganese
- Flavonoids: mostly kaempferol- and quercetin glycosides and their malonyl esters (kaempferol-3-*O*-β-D-glucoside, kaempferol-7-*O*-β-D-glucoside, kaempferol-7-*O*-β-D-diglucoside (= equisetrin), kaempferol-3-*O*,7-*O*-β-D-diglucoside und quercetin-3-*O*-β-D-glucoside), luteolin-5-*O*-β-D-glucoside, apigenin-5-*O*-β-D-glucoside und 6-chloroapigenin; the pharmacopoeial standard is minimum 0.3% of total flavonoids.

  There are apparently two chemotypes. Asian and North American varieties contain luteolin-5-glycoside, which is absent from European plants (Wichtl, 2004; Wichtl & Anton, 2003)
- Alkaloids: small amounts of nicotine, 3-methoxypyridine, traces of palustrin are possible (contradictory information); equisetonin, a saponin-complex, is mentioned in some sources, but this has been suggested to be a mixture of sugars and flavonoids (Wichtl, 2004; Wichtl & Anton, 2003); Schneider & Kubelka (1989) suggested, equisetonin should be cancelled from the list of compounds of horsetail, as it does not exist

Lovkova *et al*. (1993) reported a selenium content of 0.60 µg/g. Based on plant and soil analyses, the coefficient of biological accumulation, defined as the ratio of plant selenium content versus soil selenium content, was calculated to 30.

Saint-Paul (1980) analysed *Equisetum arvense* for its mineral content. He found that the Equisetaceae is rich in silica, potassium, calcium, manganese and phosphor.

A high presence of thiaminase in the fresh and dried plant could be shown by Fabre *et al*. (1993). The thermo labile enzyme was inactivated totally at 80°C and the temperature for a 50% denaturation was near 70°C. There was no thiaminase activity observed in the industrial aqueous dry extracts an in the fluid (ethanol 30%) extract.

- 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

A literature search was performed using the DIMDI (Deutsches Institut für Medizinische Dokumentation und Information)-database information system (in ZT00; CC00; CCTR93; CDSR93; DAHTA; CDAR94; AR96; GA03; GM03; INAHTA; MK77; NHSEED; ED93; ME60; CV72; CB85; AZ72; IA70; BA70; EM47; DH64; EA08; DD83; II78; IS74), the database of the division for Complementary and Alternative Medicines of the Federal Institute for Drugs and Medical Devices (BfArM) and information received from other member states or submitted as response to the call for scientific data from the EMA. Additional hand searches were performed in books on herbal medicines and plant monographs in the BfArM own library. The bibliographies of included trials and other relevant reviews were searched to identify further potential trials.

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
comminuted herbal substance	to increase of the amount of urine in case of minor complaints of lower urinary tract	herbal tea SD: 2 g DD: 6 g	THMP; 2013; AT
		herbal tea SD: 1.3 g DD: 3.9-5.2 g	THMP; 2011; AT
comminuted herbal substance	oral use: adjuvant in treatment of kidney and urinary tract inflammation and infections cutaneous use:	oral use: SD: 1 tea spoon/250 ml of boiling water DD: 2-3 times cutaneous use:	THMP; 1997; CZ

Active substance	Indication	Pharmaceutical form	Regulatory Status
	for compresses and ablution of superficial wounds with tendency to poor healing, for lavage in case of nose bleeding	1 tablespoon/250 ml of boiling water; several times daily	
comminuted herbal substance	diuretic	oral use: SD: 1 tea spoon/250 ml of boiling water DD: 3 times	TRAD; at least since 1991; HR
comminuted herbal substance	for improvement diuresis	oral use: SD: 3-5 g DD: 3 times	THMP; LT
comminuted herbal substance	traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints	solid dosage form SD: 0.57 g DD: 1.71 g	THMP; at least since 1978 TU registration 2010; SP
comminuted herbal substance (powder)	traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints	oral use: SD: 500 mg DD: 1500 mg	THMP; at least since 1981 TU registration 2015; FR
expressed juice from fresh herbal substance (DER 1:1.6-2.0)	oral use: traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints cutaneous use: supportive treatment for poorly healing wounds	oral use: SD: 10-20 ml DD: 30-60 ml (DD corresponding to appr. 22-33 g fresh herbal substance) cutaneous use: 40 ml in 500 ml water (DD corresponding to appr. 22 g fresh herbal substance)	WEU; at least since 1978-2011; DE THMP; 2011; DE
liquid extract from fresh herbal substance (DER 1:9), extraction solvent: water	traditionally used to promote the renal elimination function	oral liquid SD: 10 ml DD: 30-40 ml (DD corresponding to appr. 3.3-4.4 g fresh herbal substance)	TRAD; at least since 1978-2014; DE
dry extract (DER 4-7:1), extraction solvent: water	irrigation therapy for bacterial and inflammatory diseases of the lower urinary tract and renal gravel	capsules/tablets SD: 370-540 mg DD: 1080-1110 mg (SD corresponding to 2-3 g herbal substance; DD corresponding to 6 g herbal substance)	WEU; at least since 1978; DE
	traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract	oral use: capsules SD: 185 mg DD: 555 mg	THMP; at least since 1987 TU registration 2015; FR

Active substance	Indication	Pharmaceutical form	Regulatory Status
	as an adjuvant in minor urinary complaints		
liquid extract (DER 1:4.5-5.0), extraction solvent: sweet wine 16% (V/V):ethanol 96% (V/V) (91:9) (m/m)	traditionally used to promote the renal elimination function	oral liquid SD: 1.1 ml DD: 3.3 ml (DD corresponding to appr. 0.6 g herbal substance)	THMP; at least since 1978; DE
liquid extract (DER 1:5), extraction solvent: ethanol 96% (V/V):water:sweet wine 16.5% (V/V) (16.5:13.5:70) (m/m)	traditionally used to promote the renal elimination function	oral liquid SD: 0.96-1.23 ml DD: 2.88-4.92 ml (DD corresponding to appr. 0.6-1.04 g herbal substance)	THMP; at least since 1978; DE
liquid extract (DER 1:4-5), extraction solvent: ethanol 31.5% (V/V)	traditionally used to promote the renal elimination function	oral liquid SD: 0.7 ml DD: 2.1 ml (DD corresponding to appr. 0.45 g herbal substance)	THMP; at least since 1978; DE
dry extract (DER 7.5-10.5:1), extraction solvent: ethanol 70% (V/V)	irrigation therapy for bacterial and inflammatory diseases of the lower urinary tract and renal gravel	capsule SD: 225 mg DD: 675 mg	WEU; at least since 1978; DE
dry extract (DER 8-10:1); extraction solvent: ethanol 70% (V/V)	irrigation therapy for bacterial and inflammatory diseases of the lower urinary tract and renal gravel	tablet SD: 200 mg DD: 600 mg	WEU; at least since 1978; DE

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

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

For combination products there is often lack of 30 years of tradition, unclear composition (unclear number of composition partners, unclear content of composition partner) or unclear declaration of the extracts. Because of the number of composition partners an extrapolation to a plausible dosage for a single preparation is not possible, so they are not proposed for the monograph/list.

Numerous combination partners, such as *Arctostaphylos uva ursi*, *Betula* ssp., *Juniperus communis, Ononis spinos*a, *Orthosiphon stamineus*, *Solidago virgaurea*, *Taraxacum officinale* and *Urtica* ssp., are reported.

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

Not applicable

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

Not applicable

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

Horsetail has an old tradition in the European context. Madaus (1976 – reprint from 1938) describes that an "additional important area of this medicine are diseases of the urinary organs". He notes, that horsetail was mentioned in phytotherapeutical books since the 16th century, had fallen into oblivion somehow after the 18th century and was brought back into phytotherapy by Kneipp (19th century).

Pharmaceutical text books such as Hoppe (1975) and Hänsel *et al*. (1993) describe a broad traditional use, for example to promote the elimination of water in catarrhs of kidneys and bladder, as an haemostyptic at nose, stomach, lung and a strong menstruation, as an adjuvant in the treatment of tuberculosis, fissured nails and loss of hair, in form of a bath at gynaecological diseases, rheumatic diseases, gout, and treatment of poorly healing wounds, tumescence and break of bones. The BHMA (2003) indicates the use for prostatic disease, cystitis with haematuria and urethritis, urinary incontinence, enuresis in children.

Strobili are boiled and eaten in Japan and roots are used as food by Indians in New Mexico (Duke, 1985). Duke (2002) summarises about 80 worldwide traditional indications. Martindale (2013) reports the traditional use in respiratory disorders. According to Bézanger-Beauquesne *et al*. (1986, 1990) the plant is used for remineralisation because of the high potassium and silicon content. In Canada horsetail is used for silicon remineralisation (Health Canada, 2007).

The EFSA Panel on Dietetic Products and Allergies considered in 2009, that *Equisetum arvense* had not been sufficiently characterised for invigoration of the body, maintenance of skin, maintenance of hair, maintenance of bone and maintenance or achievement of normal body weight.

Only a few of these traditional uses of horsetail have been introduced into current Pharmacopoeias or accepted collections in the European countries:

Table 2: Overview of historical data from pharmacopoeias and textbooks

Herbal preparation	Documented Use/Traditional Use	Pharmaceutical form	Reference
comminuted herbal substance	enuresis, prostatic disease, cystitis with haematuria, urethritis. Specific indications: Inflammation or benign enlargement of the prostate gland; urinary incontinence; enuresis of children	as infusion or decoction SD: 1-4 g DD: 3-12 g	British Herbal Pharmacopoeia (BHP) (1976)
comminuted herbal substance	diuretic, astringent		BHP (1996)
comminuted herbal substance	weak diuretic		Martindale (1977)
comminuted herbal substance	oral use: posttraumatic and static oedema; for irrigation therapy in bacterial and inflammatory diseases of the lower urinary tract and the renal gravel cutaneous use: supportive treatment for poorly healing wounds	as tea preparation or other oral galenic preparations Oral use: SD: 1.5-2 g DD: 6 g cutaneous use: decoct of 10 g herbal substance in 1 l water	Kommission E (1986)

Herbal preparation	Documented Use/Traditional Use	Pharmaceutical form	Reference
comminuted herbal substance		tea preparation SD: 1.5 g for one cup of decoct	EB 6 (1953)
comminuted herbal substance	oral use: post-traumatic and static oedema; for irrigation therapy in bacterial and inflammatory diseases of the lower urinary tract and the renal gravel. cutaneous use: supportive treatment for poorly healing wounds	tea preparation SD: 2 g in 150 ml water as infusion DD: 6 g cutaneous use: decoct of 10 g herbal substance in 1 l water	Braun (2004)
comminuted herbal substance		tea preparation SD: 2-4 g boiling 5-15 min in 150 ml hot water DD: 6 g cutaneous use: decoct of 10 g herbal substance in 1 l water	Hänsel *et al*. (1993) Hager *et al*. (2003) Blaschek *et al*. (2013)
comminuted herbal substance	Traditionally used to promote renal and digestive elimination functions. Traditionally used as an adjuvant in slimming diets/to assist loss of weight, complementary to dietary measures. Traditionally used to promote renal elimination of water		Cahier N°3 de l'Agence du Médicament (1998)
comminuted herbal substance	"Species diureticae"	SD: 2 g as infusion	Pharmacopoea Helvetica (1971)
comminuted herbal substance			Garnier *et al*. (1961)
comminuted/powdered herbal substance	diuretic, haemostatic, remineralisant	powder: 1-2 g tea preparation: 10 g/l	Paris & Moyse (1976)
comminuted/powdered herbal substance	infections of the renal, bronchial, genital tract, mineralisation	powder: 1-2 g per day tea preparation: 15 g/l, 3 cups per day	Van Hellemont (1986)
comminuted herbal substance	internal: inflammation or mild infections of the genito-urogenital tract external: badly healing wounds	internal: SD: 1-4 g DD: 3-12 g by infusion or decoction external: as infusion or decoction in compresses	Bradley (1992)
liquid extract (DER 1:1); extraction solvent: ethanol 25%	internal: inflammation or mild infections of the genito-urogenital tract external: badly healing wounds	3 x 1-4 ml daily	Bradley (1992); Herbal Medicines (2015)

Herbal preparation	Documented Use/Traditional Use	Pharmaceutical form	Reference
tincture (1:5); extraction solvent: ethanol 25%	internal: inflammation or mild infections of the genito-urogenital tract external: badly healing wounds	3 x 2-6 ml daily	Bradley (1992); Herbal Medicines (2015)
liquid extract (DER 1:1); extraction solvent: ethanol 25%	enuresis, prostatic disease, cystitis with haematuria, urethritis specific indications: inflammation or benign enlargement of the prostate gland; urinary incontinence; enuresis of children	3 x 1-4 ml daily	BHP (1976)
Extract; extraction solvent: propylene glycol; E/D=2:1	in products of massage, skin restoring elasticity (striae, wrinkles), antiperspirants, hair lotions (hair loss), greasy skins; remineralisating agent, antihaemorrhagic, disinfiltrating agent, tissue drainage	up to 10%	Patri & Silano (1989); Anton *et al*. (2001)

Tea - method of preparation

According Blaschek *et al*. (2013), the tea should prepared by boiling the herb 5-15 minutes. Bye *et al.* (2010) demonstrated in experimental studies, that the level of silicon in the whole plant is approximately 5%, whereas the maximum water-extractable silicon was only 0.3% of the plant (horsetail tea, prepared without boiling). Meyer *et al.* (2012) also assumes, the high silica content cannot really be extracted by making tea in the usual way. Soaking the dried plant and then boiling it for several hours will considerably increase the yield. The preparation as infusion is also traditionally documented. In conclusion, considering the traditional method of preparation and the experimental data, for the monograph it is recommended, the tea should prepared by infusion or boiling the herb 5-15 minutes.

2.3. Overall conclusions on medicinal use

For horsetail herb a period of at least 30 years in medical use as requested by Directive 2004/24/EC for qualification as a traditional herbal medicinal product is fulfilled. Altogether the overview of data obtained from marketed medicinal products and the literature data support the traditional use for the following indications

1. for oral use
    i) traditionally used to promote renal elimination function
    ii) posttraumatic and static oedema
    iii) for irrigation therapy in bacterial and inflammatory diseases of the lower urinary tract and the renal gravel/kidney and bladder stones

There are no new data witch indicate a change of the indication for internal use of the document Community herbal monograph on *Equisetum arvense* L., herba, 3 July 2008 (Doc. Ref. EMEA/HMPC/394894/2007) in this review.

Diuretics are a group of drugs that block normal solute reabsorption (not water reabsorption directly) along the nephron, inducing solute diuresis. They decrease the extra cellular fluid volume, and are primarily used to produce a negative extra cellular fluid balance. The data for efficacy of horsetail are not appropriate to document a diuretic mechanism of activity with a negative extracellular fluid balance (Veit, 1994). From the above mentioned indications, only the indication as traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints is appropriate for use without the supervision of a medical practitioner for diagnostic purposes or for prescription or monitoring of treatment.

According to the actual recommendations of the HMPC, preparations with a 30 year old tradition in pharmacopoeias should also be introduced in the monograph. Therefore for the revised horsetail HMPC monograph, based on the BHP (1976) the liquid extract (1:1), extraction solvent: ethanol 25% is recommended. For this preparation no marketed preparations are known.

2. cutaneous use:

The traditional use for the supportive treatment of poorly healing wounds as compresses and irrigation is documented for adecoction of the herb and the expressed juice.

The topical use of phytotherapeutics should be cautious (Willuhn, 1995) with a critical benefit-risk assessment. A positive benefit- risk relation is considered only for the supportive treatment of superficial wounds for self-medication and is recommended for the monograph.

All together qualification as a traditional herbal medicinal product is fulfilled for the following preparations listed in Table 3:

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
comminuted herbal substance for tea preparation by infusion or decoction	oral use: Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints cutaneous use (decoction): supportive treatment for poorly healing wounds	oral use: SD: 1-4 g DD: 3-12 g cutaneous use: 10 g in 1 l water	at least since 1976
comminuted herbal substance (powder)	oral use: Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.	oral use: solid dosage form SD: 500-570 mg DD: 1500-1710 mg	at least since 1981
expressed juice from fresh herbal substance (1:1.6-2.0)	oral use: Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints. cutaneous use: supportive treatment for poorly healing wounds	oral use: ED: 10-20 ml DD: 30-60 ml cutaneous use: 40 ml in 500 ml water	at least since 1978

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
liquid extract from fresh herbal substance (DER 1:9); extraction solvent: water	Traditionally used to promote the renal elimination function.	SD: 10 ml DD: 30-40 ml	at least since 1978
dry extract (DER 4-7:1); extraction solvent: water	Irrigation therapy for bacterial and inflammatory diseases of the lower urinary tract and renal gravel.	SD: 370-540 mg DD: 1180-1110 mg	at least since 1978
liquid extract (DER 1:5) extraction solvent: ethanol 96% (V/V): water:sweet wine 16.5% (V/V) (16.5:13.5:70) (m/m)	Traditionally used to promote the renal elimination function.	SD: 0.96-1.23 ml DD: 2.88-4.92 ml	at least since 1978
liquid extract (DER 1:4.5-5.0) extraction solvent: sweet wine 16% (V/V): ethanol 96% (V/V) (91:9) (m/m))	Traditionally used to promote the renal elimination function.	SD: 1.1 ml DD: 3.3 ml	at least since 1978
liquid extract (DER 1:1); extraction solvent: ethanol 25%	enuresis, prostatic disease, cystitis with haematuria, urethritis	SD: 1-4 ml DD: 3-12 ml	BHP (1976)
liquid extract (DER 1:4-5); extraction solvent: ethanol 31.5% (V/V)	Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.	SD: 0.7 ml DD: 2.1 ml	at least since 1978
dry extract (DER 7.5-10.5:1); extraction solvent: ethanol 70% (V/V)	see above	SD: 200-225 mg DD: 600-675 mg	at least since 1978

Traditional use in children

A posology for the topical use as decoction in children and adolescents is described in "Kinderdosierungen von Phytopharmaka" (Dorsch *et al*., 1998):

0-1 year	1-4 years	4-10 years	10-16 years
-	2-5 g/liter	10 g/liter	10 g/liter

The traditional use was shown for adolescents and adults from the marketed preparations. Therefore the use in children under 12 years of age is not recommended.

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

Diuretic effect

Aqueous extracts

In vivo

The diuretic effect of *Equisetum arvense* was tested in older studies. In a study published in 1912 an *Equisetum arvense* preparation (no further information) showed a diuretic effect in dogs about 15-20% compared with water (Cow, 1912).

The diuretic effect of a 6% infusion was tested by Wachter (1938) in 210 rats. Compared with water, after 30 minutes the effect was about 196% higher, after 45 minutes about 79% higher and after 60 minutes about 39% higher. The speed of the elimination of urine was much higher in the tea group compared with the water group. A negative fluid balance after 4 hours could not be determined. The author concluded, that horsetail is a fast and good diuretic. Similar were the experiments of Herre (1937) and Kreitmair (1936, 1953). Kreitmair (1953) reported, that a 5 ml of an oral applicated infusion (DER 1:2) increased the urine approximately in 185% in dogs.

Lower diuretic effects of an *Equisetum arvense* decoct were shown by Vollmer & Hindemith (1937), Vollmer & Hübner (1937) and Vollmer (1941), on mice (39% diuretic effect), rats (13% diuretic effect) and rabbits (23-50% diuretic effect and about 30% chloride). The authors compared the collected urine of 24 hours of the tea group with the water group. The content of 0.948% chloride in *Equisetum arvense* was considered.

No diuretic effect was detected by Jaretzky *et al*. (1938) using aqueous preparations in rats and Breitwieser (1939) using different preparations in rats.

The intragastrical application of an infusion in rats (2.5 g herb/100 ml water; 2 ml/100 g bw) increased the urine after 5 hours approximately by 60%; methanolic extracts by 62%. (Rebuelta & San Roman, 1978) (see table 4).

Table 4: Increase of urine (in %) rats after application of different preparations from *Equisetum arvense*

time (h)	aqueous extract (2 ml/100 g bw)	theophylline control (5 mg/kg bw)	infusion (2 ml/100 g bw)	methanolic extract (2 ml/100 g bw)
1	9.7	30.20	32.29	28.75
2	11.7	42.66	43.91	43.00
3	20.83	45.29	47.95	49.90
4	22.58	50.29	53.75	54.83
5	33.95	54.94	59.91	62.08

Other Equisetum spp. (different to that covered by the monograph) – chloroform extracts

Pérez Gutiérrez *et al.* (1985): Chloroform extracts of 4 *Equisetum* spp. (20 g herb/250 ml chloroform, vacuum evaporated, suspended in water; single dose 50 mg extract/kg mice, corresponding ca. 4 mg herb/kg mice) were compared for activity with standard diuretics in studies with mice. The most active was *Equisetum hiemale* [hyemale] var. affine, followed by *Equisetum fluviatile*, *Equisetum giganteum* and *Equisetum myriochaetum*. *Equisetum hiemale* was a more effective diuretic than any of the three standards. The tested dosage showed significant increase in sodium, potassium and chloride excretion, as well as a rise in the urine pH level. The authors concluded that the mechanism of action may be similar to that of hydrochlorothiazide.

Antiurolithiasic effect

Aqueous extracts

In vivo

Grases *et al*. (1994) studied the effects of *Equisetum arvense* infusion on prevention and treatment of kidney stone formation in female Wistar rats. The infusion was prepared from 3 g herb/l water. Variations of the main urolithiasis risk factors (citraturia, calciuria, phosphaturia, pH and diuresis) were evaluated. No relevant difference was found in diuresis. Calciuria and citraturia values were not affected. The infusions do not increase the crystallisation inhibitory capacity of the urine.

Wound healing

Aqueous extracts

In vivo

Hayat *et al*. (2011) investigated the effect of a 5% water decoction of *Equisetum arvense* on wound healing in rabbits. The epithelisation was completed on post-operative day 14 on 5% decoction gauze-applied wounds, whereas it was not completed on 0.9% sodium chloride gauze-applied wounds, where ulceration in central wounds was seen. The authors concluded, the positive effect on wound contraction may have resulted from silicea, silicic acid, silicon and saponins in the *Equisetum arvense* extract.

Powdered herbal substance

In vivo

Ozay *et al.* (2013) evaluated the effect of *Equisetum arvense* ointment (1:1 mixture of petroleum and lanoline, and 5 or 10% herbal powder) on dermal diabetic wound healing in 56 streptozotocin-induced diabetic rats. On day 14, groups to which *Equisetum arvense* 5-10% ointment was applied, showed 99.71% and 99.93% wound closure ratio and higher dermal and epidermal regeneration, angiogenesis, and granulation tissue thickness than the other groups ($p<0.05$).

Ethanolic extracts (not traditionally used for wound healing)

In vitro

Alexandru *et al.* (2011) evaluated the potential of *Equisetum arvense* to stimulate the wound healing process by Sirco assay. The rate of soluble collagen produced in cell culture medium by fibroblasts (cell line L-929) treated with various concentrations from 35-140 µg/ml of herbal ethanolic extract (no further information) was evaluated. Collagen synthesis was observed which was almost 2 times higher as compared to the synthesis of untreated cells. The authors concluded, the results explain the medicinal utility of accelerating the wound healing process.

3.1.2. Secondary pharmacodynamics

Antimicrobial effects

Aqueous and ethanolic extracts

In vitro

Kumar & Kaushik (2011) studied the antibacterial potential of *Equisetum arvense* (no information on DER) against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* which was compared to 1 unit strength of antibiotic tetracycline. The alcoholic extract was found to be more effective than chloroform extracts and both extracts were less effective than tetracycline. The extract prepared from plant from the summer season was more effective than from winter and rain season.

Ceyhan *et al*. (2012) observed antibacterial effects (agar dilution assay) of ethanolic extracts of *Equisetum arvense* (40 mg/0.1 ml, DER 400:1) against *Staphylococcus aureus* (MIC = 0.78 mg/ml), *Escherichia coli* (MIC=3.12 mg/ml) and *Klebsiella pneumonia* (MIC=1.56 mg/ml). The water extracts showed only low effects against *S. aureus* (MIC=200 mg/ml), *E. coli* (MIC=100 mg/ml) and *K. pneumonia* (MIC=400 mg/ml). Ethyl acetate and hexane extracts showed no activity. The tested ethanolic extracts showed MIC values, comparable with standard antibiotics, which ranged from 0.78-6.25 mg/ml.

Geetha *et al.* (2011) evaluated the antibacterial activity of *Equisetum arvense* on selected urinary tract pathogens *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* using disc diffusion technic. Amoxicillin and Ciprofloxacin were used as positive control. The ethanolic and water extract (no further information) in concentrations of 250, 500 and 1000 µg/disc exhibited antibacterial activity against the bacterial species tested.

Wojnicz *et al.* (2012) determined the influence of 6 different plant extracts (dried water extracts 0.125-20.0 mg/ml) traditionally used in urinary tract infections (UTIs) on bacterial survival and virulence factors involved in biofilm formation of the uropathogenic *Escherichia coli* rods. For *Equisetum arvense* a moderate antimicrobial activity was reported while it showed the most effective inhibition of biofilm formation. The maximum amount of biofilm mass was only 21.4% of the control sample (8^{th} day).

Samoilova *et al.* (2014) observed that the water extract of *Equisetum arvense* (1 g boiled in 30 ml water for 30 minutes) modifies biofilm formation in *Escherichia coli* (compared to cefotaxime).

Equisetum arvense dry extract (20 g herbal substance extracted three times with 200 ml ethanol 50% and evaporated) had a strong antibacterial activity on *Staphylococcus aureus* with MIC and MBC of 11.14 and 22.28 mg/ml respectively and a weak effect on *Bacillus cereus* with MIC of 89.10 mg/ml. The authors considered the antimicrobial effect is directly related to the high total phenolic content in herbal material amounted to 355.80±17.8 mg GAE/g of the dried extract (Kukric *et al*., 2013).

Ten gram of freshly powdered *Equisetum arvense* was extracted with 100 ml of ethanol 50% (DER unknown) and tested against uropathogenic *Escherichia coli*. No activity was found. The authors concluded, that the data on absence of direct cytotoxicity indicate that bactericide effects are not responsible for the potential clinical activity, antiadhesive effects may be an explanation for the traditional use (Rafsanjany *et al.*, 2013).

Sinha (2012) analysed the ethanolic dry extract (no further information) of *Equisetum arvense* to find out the antibacterial activity against six different bacteria measured by zone inhibition. Gram positive bacteria *Bacilis subtilis*, *Micrococcus luteus* and the Gram negative bacteria *Escherischia coli*, *Shigella flexneri* were found to be very sensitive to the extract at all tested concentrations (50, 100, 200 and 400 µg/ml). *Shigella dysenteriae* and *Vibrio cholera* were found to be insensitive.

Uslu *et al.* (2013) demonstrated antimicrobial activity against *Staphylococcus epidermidis* and *Escheria coli* bacteria and no effects against *Candida albicans*. The zone diameters from 0-21 mm were formed by 32 different *Equisetum arvense* extracts in 150 mg/ml. The differences was attributed to the varying extraction parameters as temperature, stirring speed, ethanol content of 10, 50 or 90%, extraction time (2, 7, 12 hours) and solid-liquid ratio (1:10 or 1:30). Significant higher cytotoxic activity was processed with extraction medium containing 90% ethanol for 12 hours, while extracts obtained with 10% ethanol for 2 hours did not decrease the viability upon exposure to fibroblast cells.

An ethanol extract (ethanol 80%) of *Equisetum arvense* was evaluated for its antimicrobial activity capacity. The antimicrobial activity of the extract (5 µg/disk) was comparable to the activity of the positive controls (ampicillin and nystatine, 30 µg per disk) (Milovanović *et al*., 2007).

Other preparations (different to those covered by the monograph)

Heisey & Gorham (1992) tested an extract (1:1) (V/V), extraction solvent methanol/dichlormethan (1:10) (g/ml) from *Equisetum arvense*, for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans*, *Candida albicans*, *Fusarium oxysporum* and *Trichophyton rubrum*. No antimicrobial activity was found.

Aswal *et al*. (1984) tested *Equisetum arvense* (no further information) for antibacterial, antifungal, antiprozoal and antiviral activity. It showed no activity. The LD_{50} was >1000 mg/kg i.p. in mice.

Dried aerial components of *Equisetum arvense* were extracted using a mixture of methanol: water (1:1) continuously until exhaustion of the plant material. The extracts were evaluated with regard to antibacterial activity at 10-1000 µg/ml, concentration range that elicited dose-dependent effects of proliferation of human bone marrow cells. The extracts caused dose-dependent population growth inhibition of *Staphylococcus aureus*, but were not active against the tested Gram-negative bacteria (Bessa Pereira *et al*., 2012).

Canadanovic-Brunet *et al*. (2009) tested the values of antimicrobial activity of petroleum ether, chloroform, ethyl acetate, *n*-butanol and water extracts of horsetail and standards (DER unknown). None of the horsetail extracts inhibited the growth of *Escherichia coli*. For *Pseudomonas aeruginosa* the MIC with ethyl acetate was 50 mg/ml, and for *n*-butanol extract 75 mg/ml. The ethyl acetate extract showed a strong antibacterial activity against *Staphylococcus aureus* (MIC=25 mg/ml). For water extracts only weak antimicrobial activity (MIC>100 mg/ml) was found against the tested bacteria.

A propylene glycol extract of *Equisetum arvense* (200 mg/ml) was effective against oral microorganisms of interest to dentistry as *Staphylococcus* spp., *Streptococcus* spp., and *Candida* spp. at concentrations of 100 mg/ml (de Oliveira *et al*., 2013).

Antifungal activity

Aqueous extracts

In vitro

Guerin & Reveillere (1984) tested the aqueous extract of *Equisetum arvense* herba (DER 1:6) against 9 fungi species. *Equisetum arvense* showed no antifungal activity against *Saccharomyces pastorianus*, *Candida albicans*, *Rhizopus nigricans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Botrytis cinerea*, *Penicillium digitatum*, *Fusarium oxysporum* and *Trichophyton mentagrophytes*.

Antiviral effect

Ethanolic extracts

In vitro

Husson *et al*. (1986) tested a series of plant extracts *in vitro* for antiviral properties using cell cultures. An alcoholic extract of fresh plant of *Equisetum arvense* (DER 5:100, no further information) showed an antiviral effect.

Suganda *et al*. (1983) screened the ethanolic extract (no further information) of *Equisetum arvense* for antiviral activity (human polio virus type 2 and human herpes virus type 1). *Equisetum arvense* had no antiviral effect.

Antinociceptive and anti-inflammatory properties

Ethanolic extracts

In vivo

Do Monte *et al*. (2004) evaluated antinociceptive and anti-inflammatory effects of hydroalcoholic extract of stems from *Equisetum arvense* ("the dried stems were extracted with 50% ethanol-water, the ethanol was evaporated and the extract was stored in the concentration of 5%") in mice. The extract (10, 25, 50 and 100 mg/kg, i.p.), reduced the writhing induced by acetic acid in 49, 57, 93 and 98%, respectively, compared with the control group treated with saline. In the formalin test, 50 and 100 mg/kg (i.p.) extract reduced the licking activity in 80 and 95% of the animals in the first phase, but in the second phase only the latter dose diminished the licking time (in 35% of the animals). In both phases, naloxone failed to revert the analgesic effect of the extract.

In the hot-plate test, the extract (100 and 200 mg/kg) did not change the latency to licking or jumping. In the carrageenan-induced rat paw oedema, the extract (50 mg/kg) reduced the paw oedema 2 hours (25%) and 4 hours (30%) after carrageenan administration. The dose of 100 mg/kg caused reduction of the paw oedema (29%) only 4 hours after carrageenan administration. The authors concluded that this extract exhibits an antinociceptive (analgesic) effect in chemical models of nociception which is not related to the opioid system, as well as significant anti-inflammatory properties.

Assessor's comments

As the extracts ware administered with intraperitoneal injection the results cannot be per se transferred to the oral applicated preparations. The clinical relevance of the analgesic effect is unclear.

Sedative and anticonvulsant effects

Ethanolic extracts

In vivo

Dos Santos *et al*. (2005a): The ethanol extract of *Equisetum arvense* (extraction solvent: ethanol 50%; extraction time: 15 days, DER unclear) tested i.p. at doses of 200 and 400 mg/kg in rats showed a significant activity on activity in the open-field test, enhanced the number of falls in the rota-rod test and reduced the time permanence in the bar and increased (46% and 74%) barbiturate-induced sleeping time, while lower doses showed no effect. In the elevated plus maze, the doses of 50, 100 and 150 mg/kg did not affect the evaluated parameters. The extract presented anticonvulsant and sedative effects. In the pentylenetetrazole-seizure test the extract increased the first convulsion latency, diminished the severity of convulsions, reduced the percentage of animals which developed convulsion (50% and 25%) and protected animals from death.

Rezaie *et al*. (2011) found after i.p. injection of extracts of *Equisetum arvense* (no further information) in rats at the dose of 200 mg/kg bw a significant better effect than 1.2 mg diazepam. The extract had lower induction time and higher sleeping time, better sedative and pre-anaesthetic effects than diazepam.

Singh *et al*. (2011) showed, that ethanol extracts of *Equisetum arvense* (50 and 100 mg/kg, i.p.) significantly increased the time-spent and the percentage of the open arm entries in the elevated plus-maze model which was compared to diazepam (2 mg/kg, i.p.). At 100 mg/kg the extract prolonged the ketamine-induced total sleeping time and decreased the locomotor activity in mice. The results suggest that the ethanolic extract possess anxiolytic effect with lower sedative activity than that of diazepam.

Assessor´s comment

The results suggest anticonvulsant, anxiolytic and sedative effects of ethanolic extracts. The relevance of intraperitoneal injection to the clinical use is not known.

Cognitive enhancement effects

Ethanolic extracts

In vivo

Dos Santos *et al*. (2005b) investigated if the chronic administration of the ethanolic extract of stems from *Equisetum arvense* reverses the cognitive impairment in aged rats; moreover the *in vitro* antioxidant properties were evaluated. The chronic administration of the extract (no further information) at dose of 50 mg/kg (i.p.) improved both short- and long-term retention of inhibitory avoidance task and ameliorated the cognitive performance in reference and working memory version of the Morris Water Maze. No differences were found between all three groups of young controls, aged controls and extract-treated animals with regard to the open field and elevated plus maze tests. No toxicity manifestations were observed during the treatment for eight weeks. *In vitro* assays revealed that the extract diminished the thiobarbituric acid reactive substances as well as the nitrite formation, but did not alter the catalase activity. Thus, the cognitive enhancement effect of the extract was attributed, at least in part, to the antioxidant action.

Assessor's comments

Because of the intraperitoneal injection the impact on the oral human use is unclear. The tested dosage is unknown, as the extract is not described in detail. Equisetum arvense has not been traditionally used in context of cognitive enhancement.

Tumor-damaging capacity

Aqueous and ethanolic extracts

In vivo

Belkin *et al*. (1952) tested 4 extracts of *Equisetum arvense* a single subcutaneous dose for necrotising capacity against Sarcoma 37 implanted in CAF1 mice: an aqueous suspension (1 mg/g bw), olive-oil suspension (1 mg/g bw), ethanol extract (1 mg/g bw) and an acid extract (0.02 ml/g bw, 0.01 ml acid extract represents about 5 mg of the original plant material). The aqueous suspension, the olive-oil suspension and the ethanol extract showed no tumour damaging capacity. The acid extract showed "a lesser degree of induced effect".

Assessor's comments

The results for Equisetum arvense aqueous suspension and alcohol extract are negative regarding tumour damaging capacity. Because of the subcutaneous administration, no conclusions can be drawn for the oral use. The results do not highlight any specific activity for the relevant indication or safety concerns for the oral use.

Anticancer and antithrombin activity

Aqueous and ethanolic extracts

In vitro

Alexandru *et al*. (2007) shows, that a water extract (DER 1:20) in low concentrations (124 and 248 µg/ml) does not influence the apoptotic process in human leukaemia cells (U 937 cells) *in vitro*, while the highest concentration (496 µg/ml) induce early and late apoptosis, as compared to the control.

Other preparations (different to those covered by the monograph)

Goun *et al*. (2002): A chromogenic bioassay was utilised to determine the anti-thrombin activity of the methylene chloride and methanol extract prepared *Equisetum arvense*. Mouse leukaemia cells (L1210) were utilised to screen the extracts ("200 g plants, dry weight extracted in sequence with methylene chloride (24 hours) and ethanol"), for activity against cancer. The methylene chloride extract of *Equisetum arvense* demonstrated high activity against both thrombin and cancer (84/99%). The methanol extract of *Equisetum arvense* demonstrated 45/38% activity against thrombin and mouse leukaemia L1210 cells.

Assessor's comments

For in vitro data, a physiological correlation is not possible. Furthermore the results by Goun et al. (2002) show a strong dependence of the effect from the used vehicle. The methylene chloride extract had approximately the double potency in the anticancer activity as compared with the methanolic fraction. The tested dose is unclear, as the DER of the extracts and the extraction solvent is not described clearly. The used lipophilic extracts are not part of the preparations discussed in this AR. The results do not highlight any specific activity for the relevant indication or safety concerns for the oral use.

Hypoglycaemic effect

Other preparations (different to those covered by the monograph)

In vivo

Andrade Cetto *et al*. (2000) examined the hypoglycaemic effect of aqueous extracts as well as of butanol extracts prepared from the aerial parts of *Equisetum myriochaetum* in streptozotocin-induced diabetic rats. A single oral administration of the aqueous extract at doses of 7 and 13 mg/kg and of the butanol extract at doses of 8 and 16 mg/kg significantly ($p<0.001$) lowered the plasma glucose levels within 3 hours of administration. As a reference drug, glibenclamide was used and showed, at a dose of 3 mg/kg, similar hypoglycaemic effects to the tested extracts.

Soleimani *et al*. (2007b) found that oral administration of a methanol extract of *Equisetum arvense* (no further information) in streptozotocin-induced diabetes in male rats (50, 100, 250 and 500 mg/kg daily for 5 weeks) lowers the level of serum glucose, urinary creatinine and microalbuminuria. The dose of 100 mg/kg bw did not show significant activity of plasma glucose level compared with glibenclamide (5 mg/kg bw).

Soleimani *et al*. (2007a) showed that the methanol extract of *Equisetum arvense* (no further information) produced a significant anti-diabetic activity at doses of 50 and 250 mg/kg bw daily when given for five weeks. Histological studies of the pancreas of streptozotocin-induced diabetic rats showed comparable regeneration by methanolic extracts.

Hepatoprotective activity

Other preparations (different to those covered by the monograph)

In vitro

Oh *et al*. (2004) showed that the hepatoprotective activity-guided fractionation of the methanol extract of *Equisetum arvense* resulted in the isolation of two phenolic petrosins, onitin and onitin-9-*O*-glucoside, along with four flavonoids, apigenin, luteolin, kaempferol-3-*O*-glucoside and quercetin-3-*O*-glucoside. Among these, onitin and luteolin exhibited hepatoprotective activities on tacrine-induced cytotoxicity in human liver-derived Hep G2 cells, displaying EC_{50} values of 85.8±9.3 µM and 20.2±1.4 µM, respectively. Silybin, used as a positive control, showed the EC_{50} value of 69.0±3.3 µM.

Onitin and luteolin also showed superoxide scavenging effects (IC_{50}=35.3±0.2 µM and 5.9±0.3 µM, respectively) and DPPH free radical scavenging effect (IC_{50} of 35.8±0.4 µM and 22.7±2.8 µM, respectively). The authors concluded that these results support the use of this plant for the treatment of hepatitis in oriental traditional medicine.

Assessor's comment

The results of the preclinical studies on the hepatoprotective activity do not highlight any specific activity for the relevant indication or safety concerns.

Effect on lipid components

Powder

In vivo

In a cholesterol rich diet *Equisetum arvense* 4% caused dermatitis in the neck, head and back in about 20-65% of the rats. No dermatitis was caused by *Equisetum* when a normal diet was administered to the rats. There were no apparent effects on serum or liver lipids in the rats. *Equisetum arvense* is used in Japan not only as medicine but also in cooking materials. The authors suggest, ingestion of large amounts of *Equisetum arvense* as cooking material is not recommended for those with a cholesterol rich diet (Maeda *et al*., 1997).

Antioxidant and antiproliferative effect

Aqueous and ethanolic extracts

Katalinic *et al*. (2006) analysed the total phenolic content and related total antioxidant capacity of an aqueous extract of *Equisetum arvense* (infusion, 3 g of the herb/200 ml boiled water). The total phenolics were measured by Folin-Ciocalteau assay and the total antioxidant capacity was estimated by Ferric Reducing/Antioxidant Power (FRAP) assay. To make a practical comparison of the relative antioxidant potential of phenolics extracted from the plant, the phenol antioxidant coefficient (PAC) was calculated. There was a significant linear correlation between total phenolic content and FRAP. The best results were obtained for Melissae folium infusions. Equiseti herba had a PAC of 2.5.

Nagai *et al*. (2005) investigated the antioxidative activity of aqueous extracts and ethanol extracts from top and body portions of *Equisetum arvense*, using four different methods (5 g herb were extracted by 5 volumes water or ethanol (unknown concentration), evaporated and solved in 1 ml ethanol (unknown concentration, 0.1 and 1% sample solutions were used.) The contents of total phenolic components were richer in the ethanol extract fractions of each portion than in the aqueous extracts, while the protein contents were much lower in ethanol extract fractions than in aqueous

extract fractions. The ethanolic fractions had antioxidative activities, similar to that of 5 mM ascorbic acid. Aqueous extracts of both portions showed high superoxide anion radical-scavenging activities. Hydroxyl radicals were effectively scavenged by ethanol extracts. The authors concluded that *Equisetum arvense* is rich in vitamins C and E. Moreover it contains high levels of copper and zinc. These are essential elements for superoxide dismutase to act against active oxygen species.

Stajner *et al*. (2006) evaluated the scavenger activities of *Equisetum arvense* above ground parts. Phosphate buffered (pH 7) aqueous extract (1 g of fresh plant material in 5 ml 0.1 mol/l K_2HPO_4) was evaluated using three different methods: DPPH assay, ESR and NO radical inhibition assay. The total reducing power was determined by FRAP assay. ESR signal of DMPO-OH radical adducts in the presence of *Equisetum arvense* phosphate buffered aqueous extract was reduced to 73.5%.

Trouillas *et al*. (2003) evaluated the antioxidant, anti-inflammatory and anti-proliferative capability of *Equisetum arvense*. The biological properties of the water-soluble fraction ("5 g of ethanol extract were extracted by 50 ml water addition") were measured. Antioxidant properties were evaluated by the electron spin resonance (ESR) method in order to visualise the inhibition of the DPPH, superoxide and hydroxyl radicals. *Equisetum arvense* had by comparison with reference molecules, e.g. vitamin E and quercetin, only low antioxidant properties. Antioxidant effects were correlated with the total amount of phenolic compounds contained in the extracts. Anti-inflammatory activity was measured by evaluating inhibition of lipoxygenase activity. *Equisetum arvense* showed a high activity (IC_{50}=1.5 mg/ml) and in high concentrations (>0.5 mg/ml) a significant anti-proliferative effect on the proliferation of melanoma B16 cells could be shown.

Kukric *et al*. (2013) evaluated the antioxidative effect of an ethanol dry extract of *Equisetum arvense* (20 g herb was extracted three times with 200 ml of ethanol 50% and evaporated). It had an IC_{50} and AAI (antioxidant activity index) of 13.5 and 3.9, respectively (vitamin C = 5.4 and 9.6, respectively). The authors considered the antioxidant effect is directly related to the high total phenolic content in herbal material which amounted to be 355.80±17.8 mg/g of the dried extract.

For the ethanol extract (ethanol 80%; 62.5 µg/ml) of *Equisetum arvense* a high antioxidant capacity was reported (Milovanović *et al*., 2007).

Other preparations (different to those covered by the monograph)

Cetojević-Simin *et al*. (2010) investigated antioxidative and anti-proliferative activity of different *Equisetum arvense* extracts. A methanolic extract (methanol 70%) was dried and divided in different fractions. The antioxidative activity, measured by the electron spin resonance (ESR) spectroscopy-spin trapping method. The results indicated, that *n*-butanol, methanol, ethyl acetate and water fractions of the methanol extract had significant peroxyl radical scavenging activity. Anti-proliferative activity was measured using the sulforhodamine B colorimetric assay on the human cancer cell lines HeLa, HT-29, and MCF7. For low concentrations (below 0.25 mg/ml) stimulation of HeLA and HT-29 cells, but not in MCF-7 cells was observed. At high concentrations the opposite effect (inhibition) was seen. No extract reduced the growth by 50%, not even in the highest concentration of 1 mg/ml, but the effects were significantly different from the control. Anti-proliferative activity of high dosed fractions based on IC_{50} values decreased in the following order: ethyl acetate, chloroform, petroleum ether.

3.1.3. Safety pharmacology

No data found/available

3.1.4. Pharmacodynamic interactions

No data found/available

3.1.5. Conclusions

The pharmacological data are not appropriate to document a diuretic action with a negative extracellular fluid balance. While in some studies a low diuretic effect was reported, other studies showed no effect or high dosages compared to the dosages traditionally used were needed to see diuretic activity. However, the results of the pharmacological studies of diuretic activity can be seen as support of the traditional indication of the monograph. The flavonoids and the high potassium content may contribute to the effects described. Some antibacterial effects (*in vitro*) could be shown for preparations of *Equisetum arvense*, even if such effects were not always reproducible.

A wound healing effect was observed in different pharmacological studies with comparable/similar preparations compared to preparations of the monograph. The authors of the studies discussed that the positive effects on wound contraction may have resulted from silicea, silicic acid, silicon and saponin content in the extracts. The results support the traditional topical use of the water decoct in wound healing.

Even though that some hypoglycaemic effects of orally administered extracts could be shown, clinical cases of an antidiabetic effect of therapeutic dose are not reported, so at present no warning is recommended for the monograph.

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

Absorption, distribution, metabolism, elimination

No data found/available

Pharmacokinetic interactions

Aqueous extracts

Sevior *et al*. (2010) used the N-in-one cocktail method for rapid screening of ten commercially available herbal products for the inhibition of nine major human hepatic cytochrome P450 enzymes. The methanol and aqueous extracts (100 mg of product/ml) were tested. The aqueous extract significantly inhibited CYP2A6 and CYP2C8 with IC_{50}=18.3 and 93.0 µg/ml, respectively. Clinically the inhibition of CYP2C8 was considered to be relevant, despite the low IC_{50}=3.9/dose unit (capsule of 360 mg Equiseti herba). The authors concluded, a potential interaction could be in patients with urinary tract infections who use antibiotics such as trimethoprim, which is also an inhibitor of CYP2C8. The increased concentration of trimethoprim in the serum could lead to increased adverse effects.

Aqueous extract of *Equisetum arvense* (800 mg herb boiled in 40 ml water, evaporated to dry extract, solved in 60% ethanol) was investigated for its *in vitro* CYP1A2, 2D6, and 3A4 inhibition potential. IC_{50} inhibition constants were estimated from CYP activity inhibition plots using non-linear regression. The extract had a diverse inhibition profile. The IC_{50} constants ranged from 27±1 µg/ml for CYP1A2, 103±0 µg/ml for CYP2D6 and 2064±155 µg/ml for CYP3A4. The authors concluded the calculated human single dose of 97 mg/l exceeds the respective IC_{50} concentration for CYP1A2 of 27 by 3.6 times and could be clinically relevant. *In vivo* potentials should be investigated (Langhammer & Nilsen, 2014).

Ethanolic extracts

High-throughput enzyme inhibition screening assays were used to quantify the effect of ethanol extract (1:5); extraction solvent ethanol 55% (V/V)) of 2 accessions against the activity of the human cytochrome P450s: CYP3A4, CYP19, and CYP2C19. The fluorescence readings were measured by a Cytofluor 4000 Fluorescence Measurement System plate reader with excitation and emission at 485/20 and 535/25 with two gains (50 and 75). In addition, phytochemical biomarkers within the extract were identified and quantified using HPLC-MS or GC. *Equisetum arvense* had a low effect on CYP3A4 and CYP19 activity. Furthermore, the concentration of certain phytochemical markers varied significantly between accessions (i.e., rosarin and essential oils), suggesting that the extent of metabolic inhibition is directly dependent upon the concentration of bioactive constituents in an extract (Scott *et al*., 2006).

Other preparations (different to those covered by the monograph)

Schauss & Steels (2006) investigated if a dietary supplement containing *Crateva nurvala* bark extract and standardised *Equisetum arvense* (composition unknown) is a potential inducer of human cytochrome P450 (CYP1A2 and CYP3A4). An assay using immortalised human hepatocytes (Fa2N-4 cells) found a lack of interference of P450 cytochromes.

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

3.3.1. Single dose toxicity

In an acute toxicity study in rats the ethanol extract of *Equisetum arvense* (extraction solvent: ethanol 50%, DER unclear) induced at doses of 2 and 5 g/kg i.p. mortality in 12% and 37.5% of the animals. Doses of 1 g/kg i.p. did not influence mortality. Because the LD_{50} values were higher than 5 g/kg, the extract was considered as non-toxic (Dos Santos *et al*., 2005a).

3.3.2. Repeat dose toxicity

The chronic administration of the ethanol extract of *Equisetum arvense* (no further information) at dose of 50 mg/kg (i.p.) did no show toxicity manifestations during the treatment for eight weeks (Dos Santos *et al*., 2005b).

Tago *et al*. (2010) evaluated the influence of administration of an aqueous extract of *Equisetum arvense* (no further information) in diet at doses of 0, 0.3, 1 and 3% for 13 weeks in male and female F344 rats (1% was thought to mirrow an proximate dosage level of 500 mg/kg). No death or obvious clinical signs were noted in any of the animals.The NOAEL was determined to be >1.79 g/kg/day (males) and >1.85 g/kg/day (females) under the condition of the study.

Fifty Wistar rats were divided in four groups, one control group (receiving water) and the other groups received oral by gavage *Equisetum arvense* (30, 50, and 100 mg/kg, respectively) for 14 days. The anatomo-pathologic exam of the hepatic tissue showed organs with preserved lobular structure. In the same way, there was no significant change in the seric activities of the hepatic enzymes when compared to control group. No hepatic changes were produced by the plant (Baracho *et al*., 2009).

3.3.3. Genotoxicity

The ethanol extract (ethanol 80%; 62.5 µg/ml) of *Equisetum arvense* was evaluated for its genotoxic properties. The extract showed higher incidence of micronucleus formation than that of the control (21% expressed as percentage relative to the control). The results from *Equisetum arvense* was

comparable with that caused by quercetin alone (ca. 20% at concentration of 1.3 µg/ml) (Milovanović *et al*., 2007).

Joksić *et al*. (2003) analysed an ethanolic extract of Equiseti herba (extraction with ethanol 70%; no further information) in a cytochlasin block micronucleus test using blood lymphocytes from healthy donors. Equiseti herba had weak clastogenic properties, increasing the yield of micronuclei in unirradiated samples and reducing the level of radiation-induced micronuclei in a concentration-dependent manner. It was discussed by the authors, that weak toxicity of the flavonoids of Equiseti herba could modulate the competition between misrepair and legitimate repair, favouring legitimate repair that results in a reduced level of radiation-induced micronuclei.

3.3.4. Carcinogenicity

No data available

3.3.5. Reproductive and developmental toxicity

No data available

3.3.6. Local tolerance

No data available

3.3.7. Other special studies

No data available

3.3.8. Conclusions

Only scarce toxicological data are available from literature. Data from the studies Dos Santos *et al*. (2005a; 2005b) on single and repeated dose toxicity (i.p.) give no reason for safety concerns of an ethanol extract of *Equisetum arvense* for human therapeutic doses. Tago *et al*. (2010) determined in rats the NOAEL (p.o.) to be >1.79 g/kg bw/day (males) and >1.85 g/kg bw/day (females) for Equiseti herba for repeated dosages.

Tests on carcinogenicity, reproductive and developmental toxicity and local tolerance are not available. Adequate tests on genotoxicity have not been performed.

3.4. Overall conclusions on non-clinical data

The preclinical pharmacological data are not appropriate to document a diuretic action with a negative extracellular fluid balance. A low diuretic effect was reported by the authors in different animal species, however the mechanism of action is not known.

Influence on wound healing was observed in pharmacological studies. The authors of the studies discussed that the positive effects on wound contraction may have resulted from silicea, silicic acid, silicon and saponins in the *Equisetum arvense* extract. The results support the traditional topical use of the water decoction in healing of minor wounds.

From the secondary pharmacological studies some antibacterial effects for ethanolic extracts and for aqueous extracts could be shown (*in vitro*), even though such effects were not always reproducible.

No data were found to pharmacological interactions.

Data on absorption, distribution, metabolism and elimination are not available. From the available pharmacokinetic interaction studies (aqueous extracts) an inhibition of CYP2C8 and CYP1A2 was seen. The results should be considered in the clinical assessment.

The scarce data available on single and repeated dose toxicity reveal no suspicion of safety concerns regarding human therapeutic doses (ethanol extracts). Test on carcinogenicity, reproductive and developmental toxicity and local tolerance are not available.

As adequate tests on genotoxicity are not available, a list entry is not recommended.

4. Clinical Data

4.1. Clinical pharmacology

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

Clinical studies conducted with other preparations (different to those covered by the monograph)

Effects on water balance and urinary biochemical parameters

Lemus *et al*. (1996): A 10% infusion of *Equisetum bogotense* was administered to 25 healthy volunteers at a single daily dose equivalent to 0.75 g plant/person for 2 consecutive days during a 6-day study. Effects on water balance and urinary biochemical parameters were determined. The infusion showed a significant diuretic effect. The analysis of urinary electrolytes showed a significant increase in sodium, potassium and chloride excretion with respect to the control group, but within normal physiological limits. No adverse effects were noted.

Reducing the blood glucose levels

Revilla *et al*. (2002): The hypoglycaemic effect of a water extract from aerial parts (0.33 g/kg) of *Equisetum myriochaetum* was analysed in 11 recently diagnosed type 2 diabetic patients. A single dose of this extract was orally administered. Glucose and insulin were determined at 0, 30, 60, 90,120 and 180 min after administration. The same patients served as the control group and received only coloured water as placebo. The administration of the extract significantly reduced the blood glucose levels of the type 2 diabetic patients within 90, 120 and 180 minutes. There were no significant changes in the insulin levels.

Nail alterations

Sparavigna *et al*. (2006): Two clinical trials were carried out with a new formulation based on *Equisetum arvense* and a sulfur donor in a hydro-alcoholic solution, with the aim to evaluate the efficacy and preventive activity of this new formulation on nail alterations. For the first study, 36 women with nail plate alterations applied the test product every night on the nails of one hand, randomly assigned for 28 days. The results demonstrated a significant reduction in longitudinal grooves as well as an 85% reduction in patients reporting lamellar splitting of treated nails, while no significant change was observed in untreated controls. In the second study, 22 women with nail plate alterations applied the test product randomly on the nails of one hand only, on alternating days, preferably in the evening, for 14 days. After drying, a common nail polish was applied on the finger nails of both hands and removed by an organic solvent every other day before the application of the

next product. The results from this study showed a significant decrease (p<.001) of lamellar splitting compared to baseline with the test product (82% of cases).

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

Graefe & Veit (1999): In order to examine the metabolism and renal excretion of flavonoids and hydroxycinnamic acids a standardised extract from *Equisetum arvense* was administered to 11 volunteers following a flavonoid-free diet for 8 days. Twenty four-hour urine samples were collected and analysed by HPLC-DAD. The putative quercetin metabolites, 3,4-dihydroxyphenylacetic acid or 3,4-dihydroxytoluene could not be detected in urine in any sample. The endogenous amount of homovanillic acid, generally regarded as one of the main quercetin metabolites, was 4±1 mg/day and did not increase significantly. Hippuric acid, the glycine conjugate of benzoic acid, increased twofold after drug intake. Thus, the degradation to benzoic acid derivatives rather than phenylacetic acid derivatives seems to be a predominant route of metabolism.

4.2. Clinical efficacy

4.2.1. Dose response studies

Not available

4.2.2. Clinical studies (case studies and clinical trials)

In a double-blind, randomised clinical trial, 36 healthy male volunteers were randomly distributed into three groups (n=12) that underwent a three-step treatment. For four consecutive days, alternately a standardised dried extract of *Equisetum arvense* (900 mg/day; dry extract with 0.026% total flavonoids; no further information), placebo (corn starch, 900 mg/day), or hydrochlorothiazide (25 mg/day) were administered, separated by a 10-day washout period. The daily dose was divided into three single doses. Each volunteer served as his own control, and the groups' results were compared. The diuretic effect of the extract was assessed by monitoring the volunteers' water balance over a 24-hour period. In the assessment of the intragroup final fluid balance the negative final fluid balance (FB) of hydrochlorothiazide was similar to that of *Equisetum arvense*.
Group A (*Equisetum arvense*) exhibited a negative final FB of −321.81±481.02 ml (p<0.001).
Group B (hydrochlorothiazide) exhibited a final FB of −231.84±726.60 ml (p=0.067) and group C (placebo) exhibited a positive final FB of 130.27±534.30 ml (p=0.164).
The assessment of the intergroup final fluid balance comparison revealed significant differences between group A (*Equisetum arvense*) and group C (placebo) and between group B (hydrochlorothiazide) and group C (placebo). No difference between group A and B was observed. A negative final FB indicated the induction of a diuretic effect by *Equisetum arvense* and hydrochlorothiazide and the absence of this effect with placebo (Carneiro *et al*., 2014).

Table : Clinical studies on humans

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Carneiro *et al*., 2014	double-blind, placebo controlled, randomised clinical trial study duration 32 days: for four consecutive days therapy, separated by a 10-day washout period (0-1,2,3,4-10-1,2,3,4-10-1,2,3,4)	three treatment groups: *group A:* *E. arvense* dry extract (with 0.026% total flavonoids) (3 x 300 mg) *group B:* hydrochlorothiazide (25 mg/day) *group C:* placebo (corn starch, 900 mg/day	36 male patients 20-55 years	healthy volunteers biochemical screening tests three month before the onset of the study had been normal	diuretic effect was assessed by monitoring the volunteers' water balance over a 24-hour period during the four days of treatment (final FB= posttreatment FB-FB0) a) intragroup final fluid balance (FFB): *group A:* negative FFB of −321.81±481.02 ml (p<0.001) *group B:* FFB of −231.84±726.60 ml, (p=0.067) *group C:* positive FFB of 130.27±534.30 ml, (p=0.164) b) Intergroup final fluid balance (FFB): significant FFBs between *group A* and *group C* (p<0.001) and *group B* and *group C* (p<0.026) no difference between *group A* and *group B* (p=0.056) *E. arvense* did not exert significant effects on the urinary excretion of electrolytes as sodium and potassium Safety: no signs of liver, kidney, haematological or electrolyte toxicity laboratory tests remained within the normal range rare minor adverse events: headache in all groups	Student´s t-test	negative FFB indicates the induction of a diuretic effect by *E. arvense* and hydrochlorothiazide and the absence of this effect with placebo

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

Not available

4.4. Overall conclusions on clinical pharmacology and efficacy

Clinical pharmacological data of *Equisetum arvense* preparations are not available.

Clinical pharmacokinetic data on absorption, distribution and pharmacokinetic interactions are scarce. In a clinical pharmacokinetic study the putative quercetin metabolites, 3,4-dihydroxyphenylacetic acid or 3,4-dihydroxytoluene could not be detected in the urine. Hippuric acid, the glycine conjugate of benzoic acid, increased twofold after drug intake. Thus, the degradation to benzoic acid derivatives rather than phenylacetic acid derivatives seems to be a predominant route of metabolism.

In the double-blind, randomised clinical trial the diuretic effect of a dry extract of *Equisetum arvense* (no further information) was assessed in comparison to hydrochlorothiazide and placebo in 36 healthy volunteers. The negative final fluid balance of hydrochlorothiazide was similar to that of *Equisetum arvense*, indicating a diuretic effect. *Equisetum arvense* did not exert significant effects on the urinary excretion of electrolytes as sodium and potassium. As the study was conducted only in 36 healthy volunteers and the information on the dry extract is not precise, the results are not sufficient to demonstrate a well-established-use of the marketed preparations.

Data of the study with *Equisetum myriochaetum* showed a hypoglycaemic effect in diabetic patients. The results are consistent with non-clinical *in vivo* data on *Equisetum myriochaetum*. This *Equisetum* species grew in Mexico, and the relevance of these findings to *Equisetum arvense* is uncertain. Generally data on the base of other plant species are not considered in the labelling. For *Equisetum arvense* no clinical hypoglycaemic effects are reported.

In summary the clinical data do not fulfil the requirements of a well-established medicinal use with recognised efficacy and are not eligible for a marketing authorisation. The results of a study in 36 healthy volunteers support the traditional use of *Equisetum arvense* products as mild diuretic. The efficacy of the herbal preparation(s)/medicinal product is only plausible on the basis of long-standing use.

5. Clinical Safety/Pharmacovigilance

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

In a double-blind, randomised clinical trial in 36 healthy male volunteers (900 mg dry extract on four consecutive days) possible acute toxic effects related to liver, kidney and heart functions were investigated during the clinical and laboratory examinations. ECGs were performed at the onset and conclusion of the study. The clinical examinations and laboratory tests (liver, kidney and haematological function tests) showed no changes before or after the experiment, suggesting that the preparation is safe for acute use. *Equisetum arvense* did not exert significant effects on the urinary excretion of electrolytes as sodium and potassium. Rare minor adverse events were reported. Headache was the most frequently reported symptom, occurring in all treatment groups (Carneiro *et al*., 2014).

5.2. Patient exposure

Aside from market presence and data from the clinical study, there are no concrete data concerning patient exposure. If patients with known intolerance to *Equisetum arvense*, are excluded, a traditional use is possible if administration follows the instructions as specified in the monograph.

5.3. Adverse events, serious adverse events and deaths

From the textbooks and monographs the following adverse events are described:

Commission E monographs (1998)	No known side effects.
Blaschek *et al*. (2013)	Not known.
Mills & Bone (2005)	Allergic reaction is possible to patients susceptible to nicotine.
Bradley (1992)	Data unavailable. A related species *Equisetum palustre* is toxic, due to the presence of palustrine, hence correct plant identification is important.
Sandhu *et al*. (2010)	Silicates produce digestive problems, especially when used for long.

Clinical data

Allergic reactions

The case 91009533 of the BfArM database refers to a local allergic reaction after horsetail bath.

The case 03015301 of the BfArM database refers to an allergic reactions/rash after oral consumption. Because of negative rechallenged the rash was not related to the consumption of the *Equisetum arvense* product.

The case 10032351 refers to hand and face swelling in a 28-year old female patient after two days, oral consumption of Prodiuret (3 times daily 225 mg dry extract of *Equisetum arvense* (DER 7.5-10.5:1); extraction solvent: ethanol 70% (V/V). The patient recovered after treatment of symptoms.

Agustìn-Ubide *et al*. (2004): A patient reported contact dermatitis when preparing a meal from carrot. She tolerated food ingestion. While she used *Equisetum arvense* for loss of weight, she showed a sensation of cough, breathing difficulty and itching after the ingestion of cooked carrots. The authors concluded that *Equisetum arvense* (with a similar protein as carrots) possibly increased the symptoms.

Sudan (1985) reported, after passive inhalation of tobacco smoke, a patient regularly in contact with horsetail developed dermatitis on his hand and face which resembled seborrheic dermatitis. A fresh exposure to horsetail induced a more rapid reaction which necessitated local application of adrenaline and oral antihistamines. It was assumed, his history of atopic reactions to nicotine as a hapten in tobacco smoke correlated with the possible presence of nicotine in horsetail.

Assessor´s comment

From the information to allergic reactions it can be concluded, that people with unknown hypersensitivity can develop local and systemic reactions.

Gastrointestinal reactions

One BfArM case refers to an adverse event in two (elder) people, who developed gastrointestinal reactions as nausea, diarrhoea, sleep disorder and weariness after horsetail tea consumption (BfArM case nr.97001924). The patients had diverse unknown “complaints of old age”. No information is given to concomitant medication for these complaints. There is no hint on none-drug related explanations as infectious diseases. The patients considered the adverse reactions as toxic reactions. Adulteration with *Equisetum palustre* was ruled out. The intake of horsetail was not related to the adverse event, as the case is insufficiently documented.

Assessor´s comment

Gastrointestinal reactions are listed as adverse reactions in the informational texts of the marketed products. According Sandhu et al. (2010) silicates of horsetail can produce digestive problems, especially when used for long.

Cardiac conduction disorder

Kolettis *et al*. (2005): The authors report a case of a transient complete atrioventricular block in a 38-year-old man, after intake of a mixture of herbs (no trade name, *Ribes nigrum*, *Helicrysum italicum*, *Taraxacum officinale*, *Uncaria tomentosa*, vitamin C, vitamin E, *Fumaria officinalis*, *Melissa officinalis*, *Equisetum arvense* in unclear composition and DER) for two days, intended to aid cigarette smoking cessation. Since all other causes of conduction disturbances were excluded, a side effect of the herbal remedy was suspected as the most likely explanation.

A 38-year-old female suddenly collapsed and went into cardiopulmonary arrest. Cardiac catherisation performed, revealed normal coronary anatomy. Serum potassium was 2.8 mEq/l. A thiamine level was sent at that point and was found to be low. Electrolyte and thiamine replacements eventually lead to complete resolution of twitching. Clinical status failed to improve due to anoxic brain injury. The patient had been using an “herbal medication sent from Mexico” (with unknown plants), that acted as a weight loss agent which helped her urinate (no information on composition and dose). The authors suggested horsetail used in high doses could be responsible for the event (Boulos *et al*., 2011).

Assessor´s comment

Cardiac conduction disorder cannot be concluded from the available information. In the case report Kolettis et al. (2005) the patient has a consumption of various other plants preparations. From Uncaria tomentosa it is well known that it contains oxindol alkaloids which are associated with a negative chronotropic and inotropic cardiac effect (Länger, 2002). Because of the concomitant plants taken by the patient, unknown dosages, a causal relationship between the ingestion of Equisetum arvense and the transient complete atrioventricular cannot be concluded. In the case report of Boulos et al. (2011) the only information of the drug product is “an herbal medication sent from Mexico”. Due to the uncertainty in defining the species, it is not clear whether the report relates to Equisetum arvense. From safety aspect it is to note, the use of preparations of Equisetum arvense is contraindicated in patients with cardiac dysfunction (see section 5.5.2).

Hepatotoxicity

Klnçalp *et al*. (2012) reported a case of severe acute mixed-type hepatitis associated with the use of *Equisetum arvense*. A 52-year-old man was examined in an outpatient clinic for the slight elevation of liver enzymes. On the basis of the biochemical tests on preadmission time, it was concluded that the patient had Child-Pugh A chronic liver disease due to hepatitis B virus. Then he had drunk the boiled *Equisetum arvense* juice (500 ml per day) for two weeks and developed sever hepatitis. The increase

in liver enzymes was ascribed to the *Equisetum arvense* juice due to the temporal relationship. Other aetiologies of hepatitis (alcohol, steatohepatitis, autoimmune hepatitis) were ruled out. Liver function tests completely returned to the preadmission levels 8 weeks after withdrawal of the juice. The authors concluded that possibly the presence of the underlying chronic liver disease secondary to hepatitis B infection may have predisposed the patient to the acute hepatotoxicity. Patients with abnormal liver tests should be queried in terms of herbal use.

Whiting *et al*. (2002): Six patients have been presented with clinical, biochemical and histological evidence of severe hepatitis after taking different herbal remedies. One of the six patients took a horsetail containing combination (Chaparral, Dandelion, *Witania somnifera*, Horsetail and Echinacea) and presented with jaundice, fatigue and pruritus. Chaparral is known to cause subacute hepatitis. In 1992 the FDA issued a warning about the potential danger of its use. The authors concluded that healthcare providers and members of the public should be aware of the potential adverse effects of remedies such as Chaparal, Comfrey, Germander, Kombucha tea, Mistletoe, Sassafras, Senna, White chameleon.

Assessor´s comment

From the report of Whiting et al. (2002) in five cases no information of the used preparation is given. In one case a combination was used which contained other hepatotoxic plants and no information was given to the exact composition, dosages and to comedication and illness. In the case-report Klnçalp *et al. (2012) the patient drunk 500 ml Equisetum arvense juice per day, which corresponds to the 8-fold traditionally used daily dose (60 ml juice per day). From the preclinical safety pharmacology, in an acute hepatotoxicity study in rats (Baracho et al., 2009), oral doses of 30, 50, and 100 mg/kg of the plant administered for 14 days, produced no hepatic changes. Oh et al. (2004) reported that phenolic compounds isolated from Equisetum arvense were hepatoprotective in human hepatoma Hep G2 cell lines. The examinations and laboratory tests (liver, kidney and haematological function tests) performed in the clinical study Carneiro et al. (2014) give no hint to hepatotoxicity for the short term use for low doses. (see also sections 5.2; 5.4). From the information available no labelling of hepatotoxicity is recommended for the monograph at present.*

Other information

Thiaminase content

Henderson *et al.* (1952) reported occurrence of *Equisetum* poisoning in 3 horses. Two of these responded favourably to daily subcutaneous injections of 100 mg of thiamine hydrochloride for 4 days. Similar symptoms were produced in a 2-year old colt fed for 35 days on a ration consisting solely of *Equisetum*-containing hay from the same field. Thiamine injections which were begun after the animal was unable to rise, failed to bring about recovery. *In vitro* experiments demonstrated that the *Equisetum* caused an almost complete destruction of thiamine and of the thiamine content of oats and dried brewer's yeast. The enzymatic nature of this destruction was indicated.

Assessor´s comment

In the literature there are reports on a "thiamine like factor" which is discussed to be responsible for toxicity in animals, particularly in horses, as muscle weakness, weight loss, abnormal pulse rate, cold extremities and fever, symptoms similar to nicotine poisoning (Pohl, 1955; Hamon & Awang, 1992; Jean-Blain & Grisvard, 1973). The Canadian government department decided that, the manufacturers must prove, that their Equisetum arvense products are free of thiaminase activity, because the "thiamine like factor" destroys thiamine in the stomach of monogastric animals, including man, which can lead to irreversible brain damage in thiamine deficient people (Hamon & Awang, 1992; HC, 2007, 2014).

In traditional medicinal use in Europe adverse events such as brain damage in thiamine deficient people, or toxic reactions similar to nicotine poisoning have not been observed in humans. Knowing, enzymes are inactivated by preparing ethanolic extracts or at high temperatures, which are used at the preparation of tea or commercial expressed juice (Fabre et al., 1993), no labelling is suggested for the preparations of the monograph. For the plant powder (not heated, no extraction process with ethanol) a potent toxicity cannot be excluded, but is not expected from quantities used traditionally.

Others

For the toxicity in horses and cows the presence of aconitic acid and the presence of one or more alkaloids is discussed by Rapp (1954). Palustrin, an ingredient of *Equisetum palustre*, is also discussed (Frohne & Pfänder, 1984). Veit (1987) noted that 1% of *Equisetum palustre* in the hay can cause heavy damages in animals.

Perazella (2002) recommends patients with known impaired renal potassium handling to be cautious in using herbs as horsetail, that contain large amounts of potassium, as they could lead to severe hyperkalaemia.

Assessor´s comment

The European Pharmacopoeia excludes the presence of palustrin by a test.

The use in patients with renal diseases is contraindicated in the monograph.

Conclusion

Patients with known hypersensitivity to *Equisetum arvense* and conditions where a reduced fluid intake is recommended (e.g. severe cardiac or renal diseases) are ruled out under contraindications of the monograph.

Allergic and gastrointestinal reactions are already included in the monograph. The examples in the monograph referring to allergic reactions (e.g. rash) should be supplemented by swellings (e.g. rash, swelling of the face).

Horsetail was traditionally used over a period of 2 to 4 weeks. If the symptoms persist longer than 1 week during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

5.4. Laboratory findings

In a double-blind, randomised clinical trial in 36 healthy persons using a dry extract of *Equisetum arvense* (900 mg/day) for four days, laboratory examinations were performed. Laboratory tests (liver, kidney and haematological function tests) showed no changes before or after the experiment, suggesting that the drug is safe for short term use. *Equisetum arvense* did not exert significant effects on the urinary excretion of electrolytes as sodium and potassium.
The results revealed significant variations in some of the assessed values, but none of these results exhibited values outside of the normal range. During stage 1, the extract induced significant reductions in the creatinine ($p=0.003$) and uric acid ($p=0.010$) levels, while during stage 2, it induced significant reductions in ALT ($p=0.022$), GGT ($p=0.007$), and phosphorus ($p<0.001$) levels and significant increases in urea ($p=0.025$) and creatinine ($p=0.006$) levels. During stage 3, the extract induced significant reductions in GGT ($p=0.006$), chloride ($p=0.042$), magnesium ion ($p=0.044$), and phosphorus ($p=0.032$) levels. The volunteers in the extract group exhibited consistent reductions in GGT during the second and third stages ($p=0.007$ and 0.006, resp.), which hints to a possible liver

protective action. Reductions in ALT during the second treatment stage were also observed in the extract group (Carneiro *et al*., 2014).

5.5. Safety in special populations and situations

No data exist for safe dosages in patients with hepatic and renal impairment, elderly or other special populations.

5.5.1. Use in children and adolescents

Oral use as diuretic

No special studies about the use in children under 12 years and adolescents exist. The traditional use as diuretic was shown for adolescents over 12 years of age and adults from the marketed preparations. The dosages for adolescents are the same as for adults and elderly. In accordance with the traditional use, the use in children under 12 years of age is not recommended, as medical advice is necessary.

Topical use for supportive treatment of superficial wounds

No special studies about the use in children under 12 years and adolescents exist. For topical use tradition was shown for adolescents over 12 years of age and adults from the marketed preparations. The dosages for adolescents are the same as for adults and elderly. Because of lack of available traditional clinical experience the use is not recommended in children under 12 years of age.

5.5.2. Contraindications

The products should be contraindicated in patients with hypersensitivity to the active substance.

Oral use as diuretic

In phytotherapeutic monographs as Commission E monograph contraindication is recommended in patients with conditions where a reduced fluid intake is recommended (e.g. severe cardiac or renal diseases) (Blumenthal *et al*., 1998).

5.5.3. Special Warnings and precautions for use

If complaints or symptoms such as fever, dysuria, spasm or blood in urine occur during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

No clinical interaction studies with horsetail preparations were performed. Clinical interactions of horsetail with other drugs have not been reported in monographs and handbooks (Mills & Bone, 2005; Kommission E, 1986).

Case reports

Pyevich & Bogenschütz (2001) reported a case of a 26-year-old woman on lithium therapy, presented to the emergency room with complaining of grogginess, coarse tremor, unsteady gait and nystagmus. Her lithium level was 4.5 mmol/l. She had used 2-3 weeks a herbal combination (unknown dose and composition, containing, buchu, corn silk, *Equisetum hyemale*, juniper, parsley, uva ursi) to exert

diuretic effect. The author concluded, individuals on lithium therapy who use diuretic herbs may experience dehydration and resulting toxicity.

In a review of interactions between herbal and conventional medicines, Williamson (2005) reported no cases for horsetail. The author recommends from general observations that transplant patients and patients on cancer chemotherapy should avoid concurrent use of all herbal medicinal products, as the dose of these products is critical and blood levels should be kept as predictable as possible.

Assessor`s comment
As there were several herbal diuretics of unknown dose in the combination-preparation, no conclusion for Equisetum arvense is possible from this case. The case was discussed also by Harkness & Bratman (2002). The authors recommend, in general individuals on lithium therapy, who use diuretic herbs may experience dehydration and resulting lithium toxicity.
From the available non-clinical pharmacokinetic interaction studies the inhibition of CYP2C8 and the inhibition of CYP1A2 by Equisetum arvense aqueous extracts was considered to be clinical relevant. As no good documented cases exist, no recommendation is made for the monograph.

5.5.5. Fertility, pregnancy and lactation

No clinical studies are available.

Ortega García *et al*. (2011) describes a case report of prenatal exposure of a girl with autism spectrum disorder to horsetail and alcohol. A year prior conception the mother began a weight loss diet and ingested 1200 mg/day of horsetail herbal remedies (no further information on the preparation) up to three years after birth. The mother ingested approximately 20 to 40 g of ethanol per day during the first nine days of embryonic development. Occupational exposures during pregnancy were to phosphoric acid, alkylbenzenesulfonic acid, hydroxide and sodium hypochlorite, nitric acid, sodium hydroxide and alkyl alcohol ethoxylate. The authors concluded a possible mechanism of developmental neurotoxicity could be a vitamin deficiency (thiamine/vitamin B1) potentiated by herbal supplementation and alcohol exposure.

Studies on reproductive and developmental toxicity are not available. In the cytochalasin block micronucleus test an ethanol extract of Equiseti herba had weak clastogenic properties (Joksić *et al*., 2003).

In the Commission E monograph (Kommission E, 1986) no contraindication for pregnancy and lactation is recommended. Mills & Bone (2005) indicate the use in pregnancy as category B2 (“No increase in frequency of malformation or other harmful effects on the foetus from limited use in woman. Animal studies are lacking.”) and use in lactation as category C (“Compatible with breast feeding.”).

Assessor´s comment
Indication 1: Safety during pregnancy and lactation has not been established. In absence of sufficient data, the use during pregnancy and lactation is not recommended for oral use.
Indication 2: No effects during pregnancy and lactation are anticipated for cutaneous use for superficial wounds. However, in the absence of sufficient data, the use during pregnancy is not recommended. Products containing Equiseti herba should not be applied to the breast of breastfeeding women. No fertility data are available.

5.5.6. Overdose

No case of overdose has been reported for *Equisetum arvense* preparations. According Mills & Bone (2005) toxicity is possible from eating large amounts, what occurred in children who used the stems as blowguns and whistles.

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

No studies on the ability to drive or operate machinery have been performed. There are no reports on impairment of mental ability. No concerns arise caused by known ingredients of *Equisetum arvense*.

5.5.8. Safety in other special situations

Not applicable

5.6. Overall conclusions on clinical safety

The traditional medicinal use over a long period has shown that *Equisetum arvense* is not harmful when it is used in the specified conditions. The herbal substance is traditionally used over a period of two to four weeks. If complaints or symptoms such as fever, dysuria, spasm or blood in urine occur during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted. If the symptoms persist after one week during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

It is contraindicated in patients suffering from conditions where a reduced fluid intake is recommended (e.g. cardiac or renal diseases) or in patients with known hypersensitivity to horsetail.

Equisetum arvense preparations should not be used in children under 12 years.

No interactions are reported for *Equisetum arvense* preparations. Mild gastrointestinal complaints and allergic reactions have been reported. The frequency is not known.

There are no data on reproductive and developmental toxicity, therefore the use during pregnancy and lactation cannot be recommended.

6. Overall conclusions (benefit-risk assessment)

Based on the data documented in the assessment report, a European Union herbal monograph is established on the traditional uses of several preparations from *Equisetum arvense* L., herba. The traditional uses of Equiseti herba preparations fulfil the requirement for at least 30 years of medicinal use at a specified strength and specified posology, according to Directive 2001/83/EC as amended. Only a small clinical study with poor quality was performed with an *Equisetum* dry extract. None of the data fulfils the requirements to demonstrate a well-established medicinal use with recognised efficacy, thus the monograph is restricted to traditional uses. The efficacy is plausible on the basis of long-standing use and experience for the following indications:

Indication 1)
Oral use: Traditional herbal medicinal product to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

Indication 2)
Cutaneous use: Traditional herbal medicinal product for supportive treatment for superficial wounds.

The licensing of herbal medicinal product is subject to compliance with the requirements of a European Pharmacopoeia monograph. As an unambiguous macroscopic, microscopic, chemical identification of the herbal material is possible, adulteration/contamination of the herbal substance therefore is not expected. The European Pharmacopoeia excludes adulteration with other *Equisetum* species including the potentially toxic *Equisetum palustre* with a test.

The herbal preparations should not be used in patients with hypersensitivity to the active substance and conditions where a reduced fluid intake is recommended (e.g. severe cardiac or renal diseases).

The herbal substance is traditionally used over a period of two to four weeks. If the symptoms persist longer than 1 week during the use of the medicinal product, if urinary complaints or symptoms such as fever, dysuria, spasm or blood in urine occur or if symptoms worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted. In case of cutaneous use for wound healing, if the symptoms persist longer than 1 week during the use, a doctor or a qualified health care practitioner should be consulted.

Mild gastrointestinal complaints and allergic reactions (e.g. rash, swelling) have been reported. The frequency is not known. No serious adverse events with therapeutic doses of the herbal preparations are reported in the literature/reference sources with a well-documented history.

Intoxications due to the herbal preparations are not reported in the literature/reference sources for human use. No cases of overdose have been documented in the past 30 years for herbal preparations. There are no reports on drug interactions, drug abuse, withdrawal and rebound, effects on ability to drive or operate machinery or impairment of mental ability. In a clinical study in 36 patients the clinical examinations and laboratory tests (liver, kidney and haematological function tests) showed no changes before or after the experiment, suggesting that *Equisetum arvense* preparations are safe for short-term use.

Due to lack of data, the oral use is not recommended during pregnancy and lactation. For the use of decoction or diluted juice as compresses or irrigation no effects during pregnancy and lactation are anticipated, since systemic exposure is negligible. However, in the absence of sufficient data, the use during pregnancy is not recommended.

Marketed preparations are used at specified dosages in adults and adolescents above 12 years of age. Therefore the monograph establishes the use in these age groups as well as in elderly in the absence of safety concerns for the latter. The use is not recommended in children under 12 years of age.

No data from investigations of single- and repeat-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, local tolerance or other special studies of preparations from Equiseti herba in animals, according to current state-of–the-art standards are available.

There are therapeutic alternatives for the indication 1) from other herbal preparations (e.g. *Ononis spinosa* L., radix). For the indications 2) there are therapeutic alternatives like other herbal preparations e.g. *Matricaria recutita* flos.

It can be concluded that the benefit-risk assessment for *Equisetum arvense* preparations included in the monograph is positive under the specified conditions of use and at the therapeutic dosages.

No constituent with known therapeutic activity or active marker can be recognised by the HMPC.

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

Annex

List of references