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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Peumus boldus* Molina, folium

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)		*Peumus boldus* Molina, folium
Herbal preparations		Comminuted herbal substance Dry extract (DER 5:1), extraction solvent water
Pharmaceutical forms		Comminuted herbal substance as herbal tea for oral use. Herbal preparation in solid dosage forms for oral use.
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1. Introduction

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

- Herbal substance

Peumus boldus Molina, folium

Boldo leaf (boldi folium) is defined in the European Pharmacopoeia as the whole or fragmented dried leaf of *Peumus boldus* Molina. It contains not less than 0.1% of total alkaloids, expressed as boldine ($C_{19}H_{21}NO_4$; M_r 327.4), calculated with reference to the anhydrous drug.

Boldo leaf contains 2-4% of volatile oil. Major constituents are reported as: ascaridole (16-38%), 1,8 cineole (11-39%) and p-cymene (9-29%) (Bradley, 2006). Ascaridole is highly toxic (see Section 3.3.7) and this raises concerns about the suitability of boldo leaf in traditional herbal medicinal products.

Constituents: (Barnes *et al.,* 2007; Bradley, 2006; ESCOP, 2003; Leung and Foster, 1996; Wichtl, 2004)

Alkaloids: Isoquinoline-type 0.25-0.7%. Boldine, isoboldine, 6a, 7-dehydroboldine, isocorydine, isocorydine-N-oxide, norisocorydine, laurolitsine, laurotetanine, N-methyllaurotetanine, reticuline, (-)-pronuciferine, sinoacutine. Boldine is usually the major alkaloid (reported as 14-36% of total alkaloids). However, some sources of leaf are reported to have boldine as a minor alkaloid (0.28-0.32% total alkaloids) (Bradley, 2006).

Volatile oil: 2.0-2.6% (Vogel *et al.,* 1999). Major constituents reported as: ascaridole (16-38%), 1,8 cineole (11-39%) and *p*-cymene (9-29%) (Bradley, 2006).

Vogel *et al*. (1999) have shown that the principal components of the oil are determined genetically and have reported levels: ascaridole (34.6%), p-cymene (3.9%), 1,8-cineole (0.5%). Other constituents include: α-pinene, camphene, β-pinene, sabinene, Δ3-carene, terpinolene, limonene, γ-terpinene, 2-nonanone, fenchone, 1-methyl-4-isopropenylbenzene, camphor, α-fenchol, terpinen-4-ol, α-terpineol and methyl eugenol (Opdyke, 1982).

Polyphenols & Flavonoids: Mainly proanthocyanidins and flavonol glycosides: A recent study using high-performance liquid chromatography with diode array detection (HPLC-DAD) and electrospray ionization tandem mass spectrometry (HPLC-MS*n*) has identified a range of quercetin glycosides, kaempferol derivatives, isorhamnetin glycosides, phenolic acids, caffeoylquinic acid glycoside and proanthocyanidins. Isorhamnetin glucosyl-di-rhamnoside was the most abundant flavonol glycoside in the male boldo sample, whereas isorhamnetin di-glucosyl-di-rhamnoside was the main phenolic compound in female boldo leaves infusion (Simirgiotis and Schmeda-Hirschmann, 2010)

Other constituents: coumarin, resin, tannin.

- Herbal preparations

a) Comminuted herbal substance

b) Dry extract (DER 5:1), extraction solvent water

- 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

Literature searches were carried out using the available databases – see below and additional searches were carried out on published books on herbal medicines and plant monographs.

Search engines used: Google, Google Scholar

Scientific databases: PubMed, DIMDI, SciFinder, SCOPUS, ScienceDirect

Medical databases: Medline, Cochrane Database, EMBASE, Biomed Central

Toxicological databases: Toxline

Pharmacovigilance resources: WHO Global ICSR database, VigiBase

Data from EU regulatory authorities: see information about products on the market in the EU/EEA Member States, section 2.1.1.

Other resources: Historical literature – see references

In this revision information was obtained from the following EU countries: Belgium, Croatia, Czech Republic, Denmark, Finland**,** France, Germany, Ireland, Latvia, Slovakia, Spain, Sweden, UK.

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	Mild spasmodic disorders of the gastrointestinal tract, particularly based on functional affections of the biliary system	Herbal tea 1.5 g/150 ml boiling water 2 times daily	1996, DE, Standard Marketing Authorisation according to section 36 of the German Medicinal Products Act: 71 herbal teas with single active ingredient
Comminuted herbal substance	Traditional herbal medicinal product for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract	Tablets containing 325 mg Posology for adults: 2 tablets, twice per day Duration of use: 1 to 2 weeks	2011 Spain Registered as a THMP: Jan 2011

Active substance	Indication	Pharmaceutical form	Regulatory Status
Comminuted herbal substance	Traditional herbal medicinal product for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract	Capsules containing 260 mg Posology for adults: 2 capsules, twice per day Duration of use: 1 to 2 weeks	2010 Spain Registered as a THMP: Oct 2010 (marketed in Spain since 1989)
Dry extract (5:1; water)	Traditional herbal medicinal product for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract	Hard capsule Adults: 1 to 2 hard capsules, two times daily 1 hard capsule = 200 mg of extract Duration of use : 2 weeks	1987, France Product marketed in France since 1987 (THMP 2016)
Boldus, Leaf, Alcoholic Extract 1000.0 mg/g		Oral solution – Dropper container – Oral use	Belgium On the market since: 07/10/1998 – 16/06/2003

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

CZECH REPUBLIC

Herbal tea for oral use containing Agrimoniae herba 250 mg, Marrubii herba 250 mg, Boldo folium 100 mg, Frangulae cortex 100 mg, Matricariae flos 100 mg, Menthae piperitae herba 100 mg Taraxaci radix cum herba 100 mg in one tea bag.

Indication: adjuvant treatment in biliary tract disorders
Posology: 2 tea bags/250 ml of boiling water 3 times daily
On the market since 1969, switched to the traditional herbal medicinal product 27.4.2011

IRELAND

Combination products with the following: *Cynara scolymus* folium, *Silybum marianum* fructum, *Taraxacum officinalis* Weber radix cum herba, *Mentha x piperita* herba.

LATVIA

Coated tablets containing Sennae folii extractum, Frangulae corticis extractum (40 mg, equivalent to 8 ng of cascarozide A), Boldo folii pulvis (50 mg), Anisi fructus pulvis.
Indication: For the treatment of occasional constipation in adults. Posology: 1–2 tablets at night. Duration of treatment: NMT 8-10 days. On the market since: 1998.

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

Peumus boldus Molina (Monimiaceae) is an evergreen shrub or small tree indigenous to Chile and Peru. A detailed review on the pharmacognosy of *P. boldus* has been given by Schindler (Schindler, 1957).

The dried leaves have been reported in medicinal usage since the 19th century in South America against diseases of the liver and gallstones. In 1870, the leaves of *P. boldus* were reported to have been introduced in Europe and first described by Bourgoin and Verne (Lanhers *et al*., 1991).

Pharmacognostical texts, pharmacopoeias and handbooks list the therapeutic uses as cholagogue, choleretic, digestive disturbances, diuretic, hepatic stimulant, stomachic, sedative and anthelmintic (Grieve, 1931; Bradley, 2006; British Herbal Pharmacopoeia, 1976; British Pharmaceutical Codex, 1934; British Herbal Medicine Association, 2003; Blumenthal *et al*., 1998; ESCOP, 2003; Hansel, 1991; Martindale Extra Pharmacopoeia, 1924; Wren, 1971; Benedum *et al.,* 2006). Boldo leaf has also been reported as used for the treatment of headache, earache, toothache, rheumatism and urinary tract inflammation (Speisky and Cassels, 1994).

Cholagogues and choleretics are well known in traditional herbal medicine: cholagogues are reported to stimulate the release of bile that has already formed in the biliary system whilst choleretics stimulate bile production by hepatocytes (Mills and Bone, 2000; Schulz *et al*., 1998).

An overview of the historical medicinal uses of boldo leaf is presented in Table 2.

Table 2: Overview of historical data

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
Tincture 1:10 in 60% alcohol	Tonic, antiseptic, stimulant. Useful in chronic hepatic torpor. Tincture of boldo used as a diuretic. The oil in 5-drop doses has been found useful in genito-urinary inflammation.	Tincture 1:10 in 60% alcohol: 0.6-2.4 ml Duration of use: No information	A Modern Herbal (Grieve, 1931)
Tincture 1:10 in 60% alcohol	Used as a diuretic and liver stimulant	Tincture 1:10 in 60% alcohol: 0.6-2 ml Duration of use: No information	British Pharmaceutical Codex (1934)
Comminuted herbal substance	For dyspepsia, liver affections, rheumatism and as a diuretic for atony of the bladder.	Dried leaf: 0.06-0.18 g Duration of use: No information	Martindale Extra Pharmacopoeia (1924)
Tincture (1 in 5) 90% alcohol		0.6–1.2 ml Duration of use: No information	
Comminuted herbal substance	For dyspepsia, liver affections, rheumatism and as a diuretic for atony of the bladder.	Dried leaf:0.06-0.18 g Duration of use: No information	Martindale Extra Pharmacopoeia (1941)
Tincture (1:10) 60% alcohol		0.6–2 ml Duration of use: No information	
Liquid extract (1:1)	As a diuretic, for hepato-biliary disorders and for gastrointestinal disorders such as constipation.	0.5-1 ml Duration of use: No information	Martindale Extra Pharmacopoeia (1967)
Tincture (1 in 5)		0.5-2 ml Duration of use: No information	
Boldo tincture (1:5, ethanol 80% V/V)		1-3 ml	Pharmacopee Francaise 8th edition 1965; 9th edition 1979
Fluid extract (1:1 ethanol 80% V/V)		0.5-1 ml	
Comminuted herbal substance	Mild spasmodic disorders of gastrointestinal tract; dyspeptic complaints.	Average daily oral dose: 3 g. Equivalent amount of preparations. Duration of use: No information.	The Complete German Commission E Monographs (Blumenthal, 1998) Published April 23, 1987
Comminuted herbal substance Fluid extract 1:1 (solvent not stated) Tincture 1:5 (solvent not stated)	Mild dyspepsia and spastic gastrointestinal complaints. Gallstones, liver ailments, cystitis, and rheumatism.	Daily oral dose: 3 g Fluid extract 1:1: 3 ml Tincture 1:5: 15 ml Duration of use: No information.	Herbal Medicine. Expanded Commission E Monographs (Blumenthal, 2000)

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
Liquid extract (1:1)	Used as a cholagogue, liver stimulant and diuretic; used for the treatment of gallstones and cystitis and as an aid to slimming.	Liquid extract (1:1) 0.5–2 ml Duration of use: No information	Potter's New Cyclopedia of Botanical Drugs and Preparations (Wren, 1971)
Comminuted herbal substance	Gall-stones, Pain in liver or gall bladder. Cystitis. Rheumatism. Specific: Cholelithiasis with pain.	Dosage: 3 times daily Dried leaves. Dose 60-200 mg or by infusion Duration of use: No information.	British Herbal Pharmacopoeia (1976)
Liquid extract 1:1 (45% alcohol)		Dose 0.1-0.3 ml, 3 times daily Duration of use: No information.	
Tincture 1:10 (60% alcohol)		Dose 0.5 – 2 ml, 3 times daily Duration of use: No information.	
No information	Traditionally used to facilitate urinary and digestive elimination functions. Traditionally used as a choleretic or cholagogue.	Daily oral dose: No information. Duration of use: No information.	Médicaments à base de plantes (Ministère de la Santé et de L'action Humanitaire, 1990)
No information	Reported to have choleretic, diuretic, stomachic and cholagogic properties.	Daily oral dose: No information. Duration of use: No information.	Encyclopedia of common natural ingredients (Leung, 1980)
Comminuted herbal substance	Mild diuretic especially in liver complaints like jaundice. Used for dyspepsia, gout, hepatosis, rheumatism, syphilis and worms. Infusion of leaves used for stomach and liver troubles	Daily oral dose: 2–8 g Duration of use: No information.	Handbook of Medicinal Herbs (Duke, 1985)
Comminuted herbal substance Hydroethanolic extract	Minor hepatobiliary dysfunction, symptomatic treatment of digestive disturbances.	Daily dose: 2-5 g of the drug as a tea infusion. 0.2-0.6 g of the drug or equivalent hydroethanolic extract Duration of use: not more than 4 weeks	ESCOP Monograph (2003)
Tincture (1:5, ethanol 80% V/V)		1-3 ml Duration of use: not more than 4 weeks	

Herbal preparation	Documented use / Traditional use	Pharmaceutical form	Reference
Fluid extract (1:1 ethanol 80% V/V)		0.5-1 ml Duration of use: not more than 4 weeks	
Comminuted herbal substance	Primarily used as a cholagogue	Daily dose: 1-2 g as infusion in 150 ml water 2-3 times daily Duration of use: No information	Herbal Drugs and Phytopharmaceuticals (Wichtl, 2004)

2.3. Overall conclusions on medicinal use

Traditional medicinal use of *P. boldus* Molina leaf, in the form of comminuted herbal substance, herbal tea or ethanolic extracts is well documented in a number of bibliographic sources for mild digestive disturbances which would be suitable for self- medication. The requirement of medicinal use for at least 30 years (including at least 15 years within the European Union) according to Directive 2004/24/EC is considered fulfilled.

In view of the potential presence in herbal preparations and herbal medicinal products of the toxic ascaridole constituent (see Section 3.3.7), only herbal teas and aqueous extracts are considered acceptable. However, the levels of ascaridole in the herbal preparations and herbal medicinal products should be determined.

The use of comminuted herbal substance as such, and of ethanolic extracts of boldo leaf are not considered acceptable for traditional herbal medicinal products in view of the potential risks associated with the toxic ascaridole constituent (see Section 3.3.7). The information on the products authorised in Spain containing comminuted herbal substance is insufficient at the present time.

The following preparations are considered acceptable for use in traditional herbal medicinal products for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract.

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	**Indication**	**Posology, Strength**	**Period of medicinal use**
Comminuted herbal substance Herbal tea for oral use	Traditional herbal medicinal product for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract.	1–2 g of herbal substance To be taken 2-3 times daily	Traditional use since 1976 (British Herbal Pharmacopoeia, 1976; Commission E Monograph, published 1987, Blumenthal *et al.* 1998; ESCOP, 2003; Duke, 1985, Wichtl, 2004)
Dry extract (5:1, aqueous) Solid dosage forms for oral use	Traditional herbal medicinal product for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract.	200-400 mg, 2 times daily	Traditional use since 1987 (marketed in France)

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

Choleretic effects

Studies with boldo leaf extracts

Early studies in rats have reported choleretic effects with extracts of boldo (full details not available) (Bohm, 1959; Pirtkien *et al*., 1960; Borkowski *et al*., 1966; Levy-Appert-Collin and Levy, 1977).

Subsequent experiments in rats, however, failed to demonstrate choleretic activity after oral administration of 400 or 800 mg/kg aqueous ethanolic extract, intraduodenal administration of 200 or

800 mg/kg, or after intravenous administration of a dry ethanolic extract (4:1) corresponding to boldo leaf at 125-500 mg/kg (Lanhers *et al*., 1991).

Studies with boldine

The physiology of bile secretion has been reviewed; two principal pathways are involved: bile acid-dependent bile flow (BADF), and bile acid-independent bile flow (BAIF) (Esteller, 2008). Key to BAIF is the function of Mrp2 transporter (multidrug resistance-associated protein 2) which mediates biliary secretion of osmotically active glutathione (GSH).

The choleretic potential of boldine has been investigated in rats following intravenous infusion or after 28-day oral treatment. The aim of the study was to identify conditions required for the choleretic effect of boldine to occur in healthy rats as well as in animals with impaired BADF and/or BAIF mechanisms either induced by ethinylestradiol (EE) administration or by congenital deficit of Mrp2 transporter.

Infusion of boldine instantly increased the bile flow 1.4-fold in healthy rats as well as in animals with Mrp2 deficiency or ethinylestradiol induced cholestasis. This effect was not associated with a corresponding increase in bile acid or glutathione biliary excretion, indicating that the effect is not related to stimulation of either BADF or BAIF mechanisms of bile formation and points to the osmotic activity of boldine itself. Administration of 50 mg/kg/day boldine by gastric gavage for 28 days induced a slight but significant sustained choleresis. This effect was not dependent on plasma or bile concentrations of boldine. The study confirms that boldine increases bile production by direct osmotic activity. In addition, boldine may induce sustained mild choleresis on the basis of Farnesoid X receptor (FXR)-mediated up-regulation of Bile Salt Export Pump (Bsep) transporter with consequent stimulation of bile acid biliary secretion. This study provides a possible basis for a mechanism of action for the choleretic effect of boldine (Cermanova *et al*., 2015).

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

Herbal preparation tested	**Posology**	**Experimental model**	**Reference**	**Main non-clinical conclusions**
Boldo extracts	No details available	*In vivo:* in rats	Bohm, 1959; Pirtkien *et al*., 1960; Borkowski *et al*., 1966; Levy-Appert-Collin and Levy, 1977	Choleretic effect
Boldo aqueous ethanolic extract (no further details)	400 or 800 mg/kg oral; ad 200 or 800 mg/kg intraduodenal	*In vivo:* in rats	Lanhers *et al*., 1991	No choleretic activity
Boldo dry ethanolic extract (4:1)	corresponding to boldo leaf at 125-500 mg/kg, intravenous	*In vivo:* in rats	Lanhers *et al*., 1991	No choleretic activity
Boldine	50 mg/kg/day boldine by gastric gavage for 28 days	*In vivo:* in rats	Cermanova *et al*., 2015	Induction of a slight but significant sustained choleresis
Boldine	intravenous infusion	*In vivo:* in rats	Cermanova *et al*., 2015	1.4-fold increased bile flow, by direct osmotic activity, in healthy rats and with Mrp2 deficiency or ethinylestradiol induced cholestasis

3.1.2. Secondary pharmacodynamics

Boldine, the major alkaloid of boldo leaf has been shown to have a number of pharmacological activities including anti-inflammatory, antipyretic, antiatherogenic, antiplatelet, antitumor promoting, cytoprotective and tyrosinase inhibitory properties (O'Brien *et al.,* 2006; Si *et al*., 2013). Recent studies have also reported that boldine has an antiproliferative effect on glioma cell lines by G2/M arrest (Gerhardt *et al.,* 2009), in human bladder cell lines (Gerhardt *et al.,* 2014) and beneficial antitumor properties against glioma in a mouse model (Gerhardt *et al.,* 2011). A recent study has also reported anti-tumor effects of boldine by stimulation of apoptosis *in vitro* and its feasible application by intraperitoneal injection (50 or 100 mg/kg) in an animal model of breast cancer (Paydar *et al*., 2014). In addition, boldine has been shown to inhibit telomerase in cells treated with sub-cytotoxic concentrations (Noureini and Tanavar, 2015).

Laxative effect: studies with boldo leaf extracts

A laxative effect has been reported in rats following oral administration of a hydroethanolic extract at 400 or 800 mg/kg daily for 8 weeks (Magistretti, 1980).

Spasmolytic effect: studies with boldine

Boldine had a concentration-dependent smooth muscle relaxing effect on the acetylcholine induced contraction of isolated rat ileum via a competitive antagonist mechanism (Speisky *et al*., 1991a).

Antioxidant activity: studies with boldo leaf extracts and boldine

A large number of studies have been carried out on boldo extracts and isolated boldine showing potent free radical-scavenger and antioxidant activity. These studies have been reviewed in detail (O'Brien *et al.,* 2006).

A hydroethanolic extract (corresponding to 0.5 and 1 mg of dried leaf per ml) and boldine (33 µg/ml) showed a hepatoprotective effect against *tert*-butyl hydroperoxide-induced hepatotoxicity in isolated rat hepatocytes (Lanhers *et al*., 1991).

A recent study has investigated the effects of boldo leaf aqueous extract and boldine in reducing the oxidative damage induced by Fe^{2+}/citrate in liver mitochondria of rats. The boldo leaf aqueous extract was more efficient as an antioxidant than boldine, when mitochondria were used (hepatic mitochondrial swelling, 2′,7′-dichlorofluorescin (DCFH) oxidation and lipid peroxidation). The authors concluded that the antioxidant effects of the extract can be attributed to its polyphenolic compounds and thus can have synergic antioxidant properties which may contribute to the protective effect of boldo against liver diseases associated with oxidative stress and free iron (Klimaczewski *et al*., 2014).

Antioxidant activity: studies with boldine

Boldine inhibited rat liver microsomal lipid peroxidation by 50% at a concentration of 0.015 mM (Cederbaum *et al*., 1992).

Boldine inhibited the peroxidative (accumulation of thiobarbituric acid reactive substances) and lytic damage (trypan blue exclusion and lactate dehydrogenase leakage) to isolated rat hepatocytes induced by *tert*-butyl hydroperoxide (Bannach *et al*., 1996).

Boldine concentration-dependently prevented the haemolytic damage induced by the free radical initiator 2,2'-azobis-(2-amidinopropane)(AAPH) (Jimenez *et al*., 2000).

Boldine reduced the lethal effect induced by stannous chloride on the survival of *Escherichia coli* cultures. In addition, the structural confirmation of the plasmid pUC 9.1 was not modified by stannous

chloride in the presence of boldine. These effects were considered to be due to the antioxidant activity of boldine (Reiniger *et al*., 1999).

The antioxidant properties of boldine have been demonstrated by the prevention of rat brain homogenate autoxidation, the 2,2'-azobis-(2-amidinopropane)(AAPH)-induced lipid peroxidation of red cell plasma membranes and the AAP-induced inactivation of lysozyme.

According to the authors, these results indicated that boldine acted as an antioxidant in biological systems susceptible to free radical-mediated reactions (Speisky *et al*., 1991b).

Boldine prevented the ferric-ATP catalysed peroxidation of human liver microsomes and inactivation of cytochrome P450E1 (Kringstein and Cederbaum, 1995).

Anti-inflammatory effects: studies with boldo extracts

An aqueous alcoholic extract showed anti-inflammatory activity in the rat-paw carrageenan induced oedema test following intraperitoneal administration, but boldine was negative in the test at doses of 10 and 20 mg/kg (Lanhers *et al*., 1991, 1992).

Anti-inflammatory effects: studies with boldine

Oral administration of boldine was shown to exhibit a dose-dependent anti-inflammatory activity in the rat-paw carrageenann-induced oedema test with an ED_{50} of 34 mg/kg (Backhouse *et al*., 1994).

Intrarectal administration of boldine (100 mg/kg) to rats with colitis resulted in significantly reduced colonic neutrophil infiltration. Boldine also protected against acid-induced oedema as shown by decreased total colon weight (Gotteland *et al*., 1997).

Antipyretic effects: studies with boldine

Oral administration of boldine (60 mg/kg) was shown to reduce bacterial pyrogen-induced hyperthermia in rabbit (Backhouse *et al*., 1994).

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

Most investigations have been carried out using the isolated alkaloid, boldine. Limited information is available on herbal preparations of boldo leaf and where studies have been reported, details of the preparations are usually lacking. The choleretic effects of boldo leaf have not been confirmed but recent studies have indicated for the first time that boldine enhances bile production in rats via osmotic and Farnesoid X receptor dependent mechanisms.

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

Studies with boldo extracts

Boldine was found in the urine of rats after oral administration of a hydroethanolic extract (no further details recorded) of boldo at 400 and 800 mg/kg (Magistretti, 1980).

Studies with boldine

In vitro experiments

Addition of boldine at 200 µM to a suspension of isolated rat hepatocytes was followed by a time-dependent disappearance of boldine from the extracellular medium and accumulation within the cells. Boldine was also concentration-dependently removed from the extracellular medium when boldine was portally perfused through isolated rat livers (Jimenez and Speisky, 2000).

In vivo experiments

Absorption of boldine was rapid following oral administration to rats at 25, 50 or 75 mg/kg with maximum plasma concentration reached within 15-30 minutes. Boldine was found to be preferentially concentrated in the liver (Jimenez and Speisky, 2000).

Overall conclusions on pharmacokinetics

Limited data are available on pharmacokinetics.

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

3.3.1. Single dose toxicity

Studies with boldo extracts

Oral administration of a hydroethanolic extract (no further details recorded) of boldo to rats in single doses up to 3 g/kg body weight caused no deaths or toxic symptoms (Magistretti, 1980).

The intraperitoneal LD_{50} in mice of an ethanolic extract of boldo (80%; no further details recorded) was found to be equivalent to 6 g/kg (Levy-Appert-Collin and Levy, 1977).

Studies with boldine/total alkaloids

When boldine was administered orally, doses of 500 and 1000 mg/kg were required to cause death of mice and guinea pigs, respectively (Kreitmair, 1952). The intraperitoneal LD_{50} values of total alkaloids and of boldine in mice were reported to be 420 and 250 mg/kg, respectively (Levy-Appert-Collin and Levy, 1977).

Total alkaloids from boldo administered by sub-cutaneous injection to dogs produced vomiting, diarrhoea and epileptic symptoms with recovery after 50 minutes (Kreitmair, 1952).

3.3.2. Repeat dose toxicity

Oral administration of a dry ethanolic extract of boldo (92.8%; no further details recorded) or of boldine to rats daily for 90 days at 200 mg/kg/day caused significant reductions in blood levels of cholesterol, aspartate aminotransferase (AST), total bilirubin, glucose and urea, although cholesterol and AST were raised after 30 and 60 days. There were no significant changes in creatinine levels. Doses of 50 mg/kg/day did not produce any significant changes over the 90-day period. Neither the boldo extract nor boldine caused any overt sign of toxicity in the heart or kidneys but steatosis was observed in two animals at doses of 800 mg/kg (De Almeida *et al.*, 2000).

Neurotoxicity

Oral administration of an aqueous extract of boldo (no further details recorded) to rats daily for 21 days caused significant decrease in the latency time of the rotarod test compared to control group

which received 6-hydroxydopamine intracranially; a neurotoxic effect was concluded for the aqueous extract of boldo (Mejia-Dolores *et al.*, 2014).

3.3.3. Genotoxicity

Studies with boldo extracts

No studies were located using herbal preparations of boldo leaf.

Studies with boldine

Boldine did not show genotoxic activity in the SOS chromotest with *Escherichia coli* or in the Ames test using *Salmonella typhimurium* strains TA100, TA98 and TA102. Furthermore, it did not induce point and frameshift mutations in haploid *Saccharomyces cerevisiae* cells.

Boldine did, however, induce mitotic recombinational events in diploid yeast cells and cytoplasmic 'petite' mutation in haploid yeast cells (Moreno *et al.*, 1991).

Boldine did not induce a statistically significant increase in the frequency of chromosome aberrations or sister-chromatid exchanges when tested *in vitro* on human peripheral blood lymphocytes or *in vivo* using mouse bone marrow cells (Tavares and Takahashi, 1994).

Boldine administered intra-peritoneally at sub-lethal doses induced no signs of genotoxicity in mouse bone marrow as assessed by the micronucleus test (Speisky and Cassels, 1994).

3.3.4. Carcinogenicity

No studies were located using herbal preparations of boldo leaf or boldine.

3.3.5. Reproductive and developmental toxicity

Pregnant rats treated orally with a dry ethanolic extract of boldo (92.8%; no further details recorded) or with boldine at 500 or 800 mg/kg body weight on days 1-5 or days 7-12 of pregnancy showed anatomical alterations in the fetus at the higher dose. Incidents of blastocystotoxic-antizygotic action and a few cases of abortion were also observed in both treated groups at 800 mg/kg body weight. Daily doses of 500 mg/kg body weight did not produce teratogenic or abortifacient activity but reduced fetal weight by 28-40%. The authors concluded at 800 mg/kg/day the boldo extract and boldine had adverse effects at the beginning of egg production and also during implantation (De Almeida *et al.*, 2000).

3.3.6. Local tolerance

No studies on boldo extracts were available.

3.3.7. Other special studies

Toxicity of boldo leaf volatile oil

Boldo leaf oil is stated to be one of the most toxic oils due to the presence of ascaridole (Tisserand and Balacs, 1995) and the oil should not be used internally or externally.

Ascaridole is a bicyclic monoterpene with a bridging peroxide functional group. It is the main constituent (90%) of Chenopodium oil (*Chenopodium ambrosioides* L., American Wormseed oil) and renders this oil one of the most toxic known. Chenopodium oil has been used in the past as an

anthelmintic but has been superseded by safer treatments. The anthelmintic activity is due to the ascaridole content.

Acute toxicity boldo oil

An acute oral LD_{50} value for boldo oil has been given as 0.13 g/kg body weight in rats, with doses of 0.07 g/kg causing convulsions (Opdyke, 1982). The acute dermal LD_{50} in rabbits was between 0.625 and 1.25 g/kg.

Toxicity of Chenopodium oil

Human toxicity and fatal poisoning have been reported with Chenopodium oil (Opdyke, 1976). Toxic effects include skin and mucous membrane irritation, headache, vertigo, nausea, vomiting, constipation, tinnitus, temporary deafness, diplopia and blindness, transient stimulation followed by depression of the CNS leading to delirium and coma, occasional convulsions, circulatory collapse due to vasomotor paralysis and sometimes pulmonary oedema. Chenopodium oil is also toxic to the kidneys, liver and haematuria, albuminuria and jaundice have been observed. Several cases of fatal poisoning have been reported in children.

Studies of the mechanism of toxicity of Chenopodium oil and have shown that the major ingredients, carvacrol, caryophyllene oxide and ascaridole inhibited the mitochondrial electron transport chain. The toxicity of ascaridole on mitochondrial oxidative phosphorylation strongly depended on the availability of redox-active Fe^{2+} (Monzote *et al*., 2009).

In view of the known toxicity, boldo oil should not be used internally or externally. Where boldo leaf is used, the total exposure to ascaridole should be considered from a safety standpoint. The levels of ascaridole in herbal preparations and herbal medicinal products should be determined.

3.3.8. Conclusions

Most investigations have been carried out using boldine. Limited information is available on herbal preparations of boldo leaf and where studies have been reported, details of the preparations are usually lacking. There are no reported genotoxicity or carcinogenicity studies with herbal preparations of boldo leaf. Abortifacient and teratogenic effects in rats were observed with high doses of a dry ethanolic extract and boldine.

In view of the known toxicity, boldo oil should not be used internally or externally. Where boldo leaf is used, the total exposure to ascaridole should be considered from a safety standpoint. The levels in herbal medicinal products should be determined. No data was located comparing levels of ascaridole in preparations derived from boldo leaf. In view of the low solubility of ascaridole in water the use of aqueous extracts including herbal tea could be accepted. However, the levels of ascaridole in the herbal preparations and herbal medicinal products should be determined. The use of comminuted herbal substance as such, and of ethanolic extracts of boldo leaf is not considered acceptable for traditional herbal medicinal products in view of the potentially higher levels of the toxic ascaridole constituent.

3.4. Overall conclusions on non-clinical data

The non-clinical data on boldo leaf are limited. Whilst results from relevant experimental studies on boldo preparations to support the proposed indications are very limited there are some data showing effects on bile production which supports the plausibility of the traditional use. The reported pharmacological effects are not considered contradictory to the traditional uses.

Specific data on pharmacokinetics and interactions are not available. Non-clinical information on the safety of boldo preparations is scarce.

In view of the findings in the studies on reproductive toxicity, use during pregnancy and lactation should be avoided. Tests on genotoxicity and carcinogenicity have not been performed.

Oral administration of boldo leaf water extracts can be regarded as safe at traditionally used doses.

4. Clinical Data

4.1. Clinical pharmacology

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

Twelve healthy volunteers treated daily with either 2.5 g of a dry extract of boldo (ethanol 60% V/V) containing 0.4% of total alkaloids and 0.12% of boldine showed prolongation of intestinal transit time compared to placebo (Gotteland *et al*., 1995).

Due to lack of data no conclusions can be drawn.

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

No data available.

4.2. Clinical efficacy

No clinical studies were located with mono-preparations containing boldo. Therefore it is concluded that there are no data to support boldo as a well-established medicinal product with recognised efficacy and acceptable safety.

4.2.1. Dose response studies

No data available.

4.2.2. Clinical studies (case studies and clinical trials)

No data available.

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

No data available.

4.4. Overall conclusions on clinical pharmacology and efficacy

Due to lack of data no conclusions can be drawn.

5. Clinical Safety/Pharmacovigilance

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

No data available.

5.2. Patient exposure

No data available.

5.3. Adverse events, serious adverse events and deaths

No data available.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

No data available.

5.5.1. Use in children and adolescents

No data available. Use in children and adolescents is not recommended because data are not sufficient and medical advice should be sought.

5.5.2. Contraindications

Boldo preparations should not be used by patients with known hypersensitivity to boldo.

One case report of anaphylaxis following intake of a boldo leaf infusion has been located (Monzón *et al*., 2004). The patient, who had a history of allergic rhinoconjunctivitis related to grass pollen suffered an acute and generalized urticaria, facial angioedema, dysphagia, dysphonia and dyspnea. The reaction was confirmed by positive oral challenge. Hypersensitivity to boldo leaf should be included as a contraindication.

Boldo leaf is contraindicated where there is obstruction of the bile duct, cholangitis, liver disease, gallstones and any other biliary disorders that require medical supervision and advice.

5.5.3. Special warnings and precautions for use

The following warnings and precautions for use are recommended:

The use in children and adolescents under 18 years of age is not recommended because data are not sufficient and medical advice should be sought.

If symptoms worsen during the use of the medicinal product, a doctor or a qualified health practitioner should be consulted

5.5.4. Drug interactions and other forms of interaction

Limited data are available.

A single case report has been located of a potential interaction between warfarin and two herbal products resulting in an increase in INR (Lambert and Cormier, 2001). The herbal products were a liquid preparation of boldo leaf (no details provided) and capsules containing fenugreek (no details provided). The INR returned to normal once the patient stopped taking the herbal products. No information is available using the individual products thus it is not possible to conclude if only one or both products contributed to the increased bleeding time.

A single case report has been located of a potential interaction between tacrolimus and a boldo preparation resulting in decreased levels of tacrolimus (Carbajal *et al.,* 2014). A 78-year-old renal transplant patient presented with significantly sub-therapeutic levels of tacrolimus (<3 ng/ml); the patient recovered therapeutic levels one week after discontinuing the boldo preparation without alteration to the tacrolimus dose. Details of the boldo product are limited; the product is described as 300 mg capsules but it is not clear if this refers to comminuted herbal substance or an extract.

5.5.5. Fertility, pregnancy and lactation

No data available. Most sources recommend contraindication in pregnancy and lactation.

Tests on reproductive toxicity have been performed with a dry ethanolic extract of boldo leaf and boldine administered orally to pregnant rats. Results showed anatomical alterations in the fetus and a few cases of abortion at high doses (see section 3.3.5).

Safety during pregnancy and lactation has not been established. In view of the findings in the studies on reproductive toxicity, use during pregnancy and lactation should be avoided.

No fertility data are available.

5.5.6. Overdose

Limited data are available. One source reports emetic effect and spasms with very high doses (Braun, 1981). As the data is limited and details of the herbal preparation are not provided this is not included in the monograph.

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

No data available.

5.5.8. Safety in other special situations

Not applicable.

5.6. Overall conclusions on clinical safety

Limited data are available. Use in children and adolescents is not recommended because the available data are not sufficient and medical advice should be sought. Boldo leaf should be avoided during pregnancy or lactation. Use is contraindicated where there is obstruction of the bile duct, cholangitis, or liver disease. Medical advice is needed in cases of gall-stones or other biliary disorders. Duration of use should be limited to 2 weeks and if symptoms persist medical advice should be sought.

6. Overall conclusions (benefit-risk assessment)

Well-established use

The available data do not support boldo leaf as a well-established medicinal product with recognised efficacy and acceptable safety.

Traditional use

The traditional uses of *P. boldus* Molina, folium are accepted within some Member States, are well documented in literature including standard herbal reference books and the herbal substance/herbal preparations are included in a number of European pharmacopoeias.

The efficacy of boldo leaf preparations is considered plausible on the basis of long-standing use and experience with the administration to adults and the elderly for indications considered within the scope of Directive 2004/24/EC. Sufficient data are available to develop a European Union herbal monograph on the traditional use of *P. boldus* Molina, folium.

The proposed indication for the aqueous preparations of boldo leaf is: Traditional herbal medicinal product for symptomatic relief of dyspepsia and mild spasmodic disorders of the gastrointestinal tract.

The indication fulfils the requirements for traditional use in that it is suitable for self-medication by a route of administration acceptable for traditional herbal medicinal products. In addition, the specified preparations for traditional use have specified strength and posology.

Duration of use should be limited to 2 weeks.

Use of boldo leaf is not recommended in children and adolescents and should be avoided during pregnancy and lactation. Boldo leaf is contra-indicated where there is obstruction of the bile duct, cholangitis liver disease, gallstones or any other biliary disorder that would require medical supervision.

The use of comminuted herbal substance as such, and of ethanolic extracts of boldo leaf are not considered acceptable for traditional herbal medicinal products in view of the potential risks associated with the toxic ascaridole constituent. Where herbal preparations from boldo leaf are used, the total exposure to ascaridole should be considered from a safety standpoint; the levels of ascaridole in the herbal preparations and herbal medicinal products should be determined.

An HMPC monograph can therefore be adopted based on traditional use only.

However, because the minimum required data on mutagenicity (Ames test) are not available for the herbal preparations of boldo leaf covered by the monograph, inclusion in the European Union list of herbal substances, herbal preparations and combinations thereof for use in traditional herbal medicinal products is not recommended.

The HMPC has concluded that: No constituent with known therapeutic activity could be defined for the boldo leaf preparations listed in the monograph. Boldine serves as a characteristic constituent for assay of the herbal substance (Ph. Eur.) and there is evidence that it may contribute to the therapeutic effects of boldo leaf.

Annex

List of references