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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Gentiana lutea* L., radix

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

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Gentiana lutea* L., radix (gentian root)
Herbal preparation(s)	a) Comminuted herbal substance b) Dry extract (DER 4.5-5.5:1) ethanol 53% V/V c) Liquid extract (DER 1:1) ethanol 45% V/V d) Tincture (ratio of herbal substance to extraction solvent 1:5) ethanol 70% V/V
Pharmaceutical form(s)	Comminuted herbal substance as herbal tea for oral use. Herbal preparation in solid or liquid 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

Gentianae radix is described in the European Pharmacopoeia (1380) as follows:

"Dried, fragmented underground organs of *Gentiana lutea* L., with a characteristic odour and a strong and persistent bitter taste. Gentian root occurs as single or branched subcylindrical pieces of various lengths and usually 10-40 mm thick but occasionally up to 80 mm thick at the crown."

Gentiana lutea is a species of the Gentianaceae family, growing to 1-2 m tall, with broad lanceolate to elliptic leaves 10-30 cm long and 4-12 cm broad. The flowers are yellow, with the corolla separated nearly to the base into 5-7 narrow petals. The main root can be over 1 meter in length and can weigh up to 7 kg (fresh). It grows in grassy alpine and sub-alpine pastures, usually on calcareous soils native to the mountains of central and southern Europe. It grows naturally on uncultivated ground in France, Spain and the Balkan mountains. The plant is under wildlife protection; therefore it is cultivated for plant production mostly in Germany and France (Blaschek *et al*., 2016, Hänsel and Sticher, 2007).

The composition of the constituents (carbohydrates and essential bitters) is depending on the time of harvesting. The content of sugars decreases in spring and increases to their maximum content in July. In contrast, the bitter substances reach their maximum content in spring and decrease according to the growth of the sugar content (Franz *et al*., 1985).

It is important that the plant is dried directly after the harvesting to avoid fermentative processes, which reduce the extract content extremely and lead to changes in the colour (Blaschek *et al*., 2016).

Plants of the species *Veratrum album* have often been taken by mistake for *Gentiana lutea*. The main attribute to differentiate between these two genera is that the leaves of *Veratrum* are alternate in contrast to the opposite leaves of *Gentiana*. The medicinal use of Gentianae radix has a very long tradition.

- *Constituents:* (Blaschek *et al*., 2016; Wichtl, 2002; Hänsel and Sticher, 2007; Seitz *et al*., 2005)

Bitter constituents: (2-8%) are located mostly in the cortex of the root. Most of the bitter constituents belong to the class of secoiridoid glycosides with gentiopicroside (also known as gentiamarine and gentiopicrine) as main components and a lower amount of amarogentine (0.025 – 0.4%). The occurrence of swertiamarine and sweroside has been reported occasionally. The bitter value of gentiopicroside is 12000; that of amarogentine is 58 million, the most bitter substance known. The quantity of the bitter constituents depends on the season as well as the age of the roots and the altitude. The total content increases with the altitude and reaches its maximum in spring.

Xanthones: Up to 1% xanthones: gentisine, isogentisine, methylgentisine, gentiseine, 1-hydroxy-3,7-dimethoxyxanthone, 1,3,7-trimethoxyxanthone, dihydroxy-1,3-dimethoxy-2,7-xanthone and gentisine-1-O-primveroside and gentioside-7-O-primveroside. Xanthones are also responsible for the yellow colour of the root.

Carbohydrates: 30-55% carbohydrates in the dried root including monosaccharides (glucose and fructose), disaccharides (saccharose and gentiobiose), trisaccharides (gentianose) and polysaccharides (e.g. pectins). During the drying process the bitter disaccharide gentiobiose or the sweeter disaccharide saccharose arise due to the degradation of gentianose.

Volatile oil: 0.1–0.2% volatile oil; important mainly in the liqueur-production for giving its characteristic flavour.

Other constituents: phytosterols, triterpenes

- Herbal preparation(s)

As herbal preparations containing gentian root have been on the European market for a period of at least 30 years a monograph on traditional use has been established by the Committee on Herbal Medicinal Products (HMPC) in 2009. Herbal preparations available on the European market are listed in section 2.1.1.

- 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.

See section 2.1.1.

1.2. Search and assessment methodology

The revision of this assessment report is based on the literature on *Gentiana lutea,* radix which was obtained in response to the EMA HMPC call for data of 10.07.2017 and 28.7.2017 and on the results of a literature search in PubMed and DIMDI in medical and scientific databases as MEDLINE, National Center for Biotechnology Information (NCBI), Cochrane Database of Systematic Reviews, which was performed in July 2017 using the following terms: Gentiana, human, clinical, pharmacokinetic, toxicology, safety.

Several publications have been found which were published in Asian countries investigating different species of *Gentiana*, mainly focusing on single constituents (secondary metabolites) of the plants such as gentiopricoside. Only the articles considered as relevant for the establishment of this assessment report on a traditional use of *Gentiana lutea*, radix within the European Union were included in the reference list.

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

The request for information from 08.02.2017 on drug preparations containing gentian root as single active ingredient on the market in the European Union showed the following results:

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status**
Tincture of Gentianae radix (ratio of herbal substance to extraction solvent 1:5) Extraction solvent: ethanol 70% (V/V)	Dyspeptic symptoms (e.g. loss of appetite, flatulence, bloating)	Oral liquid Adults and adolescents >12 years: 2-3 times 0.94 ml per day	WEU (from 1976 to 2008), DE
Tincture of Gentianae radix (ratio of herbal substance to extraction solvent 1:5) Extraction solvent: ethanol 70% (V/V)	Dyspeptic symptoms (e.g. loss of appetite, flatulence, bloating	Oral liquid Adults and adolescents >12 years: 3 times 1 ml tincture per day	WEU (since 1978), DE

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status
Tincture of Gentianae radix (DER 1:3-5) extraction solvent: ethanol 70% (V/V)	Traditional herbal medicinal product used in mild Dyspeptic/ gastrointestinal complains and in temporary loss of appetite	Oral liquid Adults: 2-3 times 1 ml per day to be taken ½ hour before meal (for the indication loss of appetite) resp. directly after meal (for the indication mild dyspeptic disorders)	TUR (2015), DE
Comminuted herbal substance	Loss of appetite digestive complains (e.g. bloating, flatulence)	Herbal tea for oral use 1 g/150 ml of boiling water 2-4 times daily to be taken ½ hour before meal (for the indication loss of appetite) resp. directly after meal (for the indication mild dyspeptic disorders)	Standardzulassung Standard marketing authorisation (since 1986), DE
Dry extract from Gentianae radix (DER 4.5-5.5:1) ethanol 53% V/V	Digestive disorders (dyspeptic complains) like loss of appetite, feeling of fullness and bloating	Hard capsule Adults and adolescents >12 years: single dose: 240 ml dry extract daily dose: 480-729 mg dry extract	WEU (from 1978-2016), 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 other products marketed in the EU/EEA (where relevant)

Not applicable

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

Gentiana is a plant which is widely used in traditional Chinese medicine. However, for this purpose different species than *Gentiana lutea* are used. Radix gentianae ('Long Dan') consists of the dried root and rhizome of *Gentiana manshurica, Gentiana scabra, Gentiana triflora or Gentiana rigescens*. The major bitter principle of all four *Gentiana* species is gentiopicroside. According to its traditional description it has a bitter taste and a cold property, acting in the liver and gallbladder channels (Zhu, 1998). Radix Gentianae macrophyllae (Largeleaf gentian root, 'Qin Jiao') is the dried root of *Gentiana macrophylla*, *Gentiana straminea*, *Gentiana crassicaulis*, and *Gentiana dahurica*. The roots of all four *Gentiana* plants contain mainly iridoid glycosides such as: gentiopricoside and swertiamarine. In

traditional Chines medicine it is pungent and bitter in taste and neutral in property, acting on the stomach, liver and gallbladder channels (Zhu, 1998).

Although in traditional Chinese medicine varieties of *Gentiana* are administered differing from *Gentiana lutea* L. which is used as herbal preparation in Europe this non-European tradition is mentioned here. A lot of research has been performed in Asian countries investigating the pharmacological effects of the main constituents of the plant (e.g. gentiopricoside) which are also contained in *Gentiana lutea* L. These publications are not mentioned here, as gentiopricoside is just one of the compounds of *Gentiana lutea* radix. In the European tradition the entire herbal preparation with all its constituents is regarded as the active principle. Thus, these data on single compounds do not have special importance for this assessment.

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

According to Madaus (1938) gentian root was used as a bitter stomachic and stimulant and for the treatment of intermittent fever attacks. Gentian root is also known as a bitter ingredient of many beverages. The usage of gentian root was also mentioned in the Cahier de l'Agence (1998) for stimulation of appetite and referenced in Martindale (2004) because of the bitter principle. Haffner (2008) reported the use of herbal tea and herbal preparations.

In 1985 the German Kommission E published a monograph on gentian root which was revised in 1990. According to this monograph the drug consists of the dried, unfermented roots and rhizome of *Gentiana lutea* L. as well as its preparations in effective dosage. The drug contains the bitter substances amarogentin, gentiopricoside and the bitter tasting gentiobiose and has a bitter value of 10,000 at least. The indication is digestive disorders such as loss of appetite, fullness and flatulence. In Table 2 the data available from literature on documented historical medicinal use of *Gentiana lutea* L. are listed.

Table 2: Overview of historical data

Herbal preparation	**Documented use / Traditional use**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Reference**
Fluid extract (1:1); ethanol 45% V/V	Digestive disorders such as loss of appetite, fullness, flatulence	Daily dose: 2-4 g (DAB EB6)	Kommission E (1985, 1990)
	Dyspeptic complaints, loss of appetite, flatulence	Daily dose: 2-4 g	PDR (2004)
	Gastric complaints, stimulation of appetite, digestive complaints such as loss of appetite, fullness, flatulence	Single dose: 1 g Daily dose: 2-4 g	Blaschek *et al.* (2016)
Comminuted herbal	Digestive disorders	Single dose: 1 g	Kommission E (1985, 1990)

Herbal preparation	Documented use / Traditional use	Pharmaceutical form Strength Posology Duration of use	Reference
substance (tea)	such as loss of appetite, fullness, flatulence	Daily dose: 2-4 g	
	Gastric complaints, stimulation of appetite, digestive complaints such as loss of appetite, fullness, flatulence	Single dose: 1-2 g comminuted drug as herbal tea (infusion) Daily dose: 2-4 g comminuted drug as herbal tea (infusion) several times a day ½ hour before meal	Blaschek *et al.* (2016)
	Strong bitter as an appetite stimulant, roburant and tonic; gastrointestinal disorders and loss of appetite; aromatic bitter and stomachic	Single dose: 1-2 g comminuted drug as herbal tea (infusion)	Wichtl (2002)
	Bitter stomachic and stimulant	Single dose: 2.1 g comminuted drug as herbal tea (infusion) 1 tablet with 0.125 g comminuted drug Daily dose: 2.1 g comminuted drug as herbal tea (infusion) 3x1 tablet with 0.125 g comminuted drug	Madaus (1938)
	Bitter; gastric stimulant; sielagogue; cholagogue	Single dose: 0.6–2 g drug, also as herbal tea (infusion or decoction) Daily dose: 0.6–2 g 1-3 times daily drug, also as herbal tea (infusion or decoction)	BHP (1976)
	Dyspeptic complaints, loss of appetite, flatulence	Single dose: 1–2 g comminuted drug as herbal tea (infusion)	PDR (2004)

Herbal preparation	Documented use / Traditional use	Pharmaceutical form Strength Posology Duration of use	Reference
		Daily dose: 2–4 g comminuted drug as herbal tea	
	Anorexia e.g. after illness, dyspeptic complaints	Single dose: 0.1–2 g comminuted drug as herbal tea (infusion) 1-3 times daily	ESCOP (2003)
Tincture (1:5); extraction solvent: ethanol 70% V/V	Digestive disorders such as loss of appetite, fullness, flatulence	Daily dose: 1-3 g (EB6)	German Kommission E (1985, 1990)
	Gastric complaints, stimulation of appetite, digestive complaints such as loss of appetite, fullness, flatulence	Single dose: 1 ml Daily dose: 1-3 g	Blaschek *et al*. (2016)
	Anorexia e.g. after illness, dyspeptic complaints	1 ml up to 3 times daily hydroethanolic extract equivalent bitterness value	ESCOP (2003)
	Dyspeptic complaints, loss of appetite, flatulence	Daily dose: 1–4 ml 1-3 times daily	PDR (2004)
	Bitter; gastric stimulant; sielagogue; cholagogue	Single dose: 1–4 ml 1-3 times daily	BHP (1976)
	Bitter stomachic and stimulant	Single dose: 10 drops (=1ml) tincture; 2-3 times daily	Madaus (1938)

2.3. Overall conclusions on medicinal use

The traditional use of *Gentiana lutea*, radix is sufficiently documented. A monograph for gentian root has been established by the German Kommission E in 1985 which was revised in 1990. This monograph includes the following preparations: tincture, fluid extract, dry extract and the comminuted herbal substance for tea preparation.

The overview of marketed products containing gentian root in Europe shows that preparations of the comminuted herbal substance or tincture are available for more than 30 years and thus confirm their traditional use. Assessment of existing data for revision of the monograph led to the conclusion that the daily dose for comminuted herbal substance is within a range of 0.6 to 6 g. The minimum and maximum are especially supported by BHP (1976). This range includes posologies that are cited in other references. The former maximum daily dose of 8 g comminuted herbal substance of the European Union monograph on Gentianae radix is not substantiated by existing references. Moreover, because of the extremely bitter taste, this maximum does not favour compliance. In order to ensure the compliance, the maximum daily dose is adapted to 6 g. The traditional use of the liquid extract is sufficiently described in literature, corresponding medicinal products, however, have not been marketed so far. The posology of the tincture (1:5; ethanol 70% (V/V)) in the monograph was derived from the data available; because of the bitterness higher single doses were not taken into account. A dry extract from Gentianae radix (4.5-5.5:1) ethanol 53% (V/V) has been on the German market since 1978 and was authorized in 2003 for a well-established use in digestive disorders (e.g. loss of appetite, fullness, flatulence). Although it was withdrawn in 2009, a tradition of 30 years of medicinal use of at least 30 years in the EU is fulfilled. The indication for traditional use was compiled as "Traditional herbal medicinal product used in mild dyspeptic/gastrointestinal disorders, and/or in temporary loss of appetite". During the revision it was decided to align the wording of the indication following the example of the European Union monograph Absinthii herba: Indication 1) Traditional herbal medicinal product for temporary loss of appetite. Indication 2) Traditional herbal medicinal product for mild dyspeptic/gastrointestinal disorders.

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	**Indication**	**Posology, Strength**	**Period of medicinal use**
Comminuted herbal substance as herbal tea	a) loss of appetite, b) digestive complaints (e.g. bloating, flatulence)	0.6–2 g 1-3 times daily	Since 1976 (BHP)
Tincture of Gentianae radix (ratio of herbal substance to extraction solvent 1:5) extraction solvent: ethanol 70% (V/V)	Dyspeptic symptoms (e.g. loss of appetite, flatulence, bloating)	Adults: 1-3 times 1 ml per day	Since 1976 (data from market overview, DE)
Fluid extract (DER 1:1); ethanol 45% (V/V)	Digestive disorders such as loss of appetite, fullness, flatulence	Single dose: 1 g 2-4 times daily 1 g liquid extract Daily dose: 2-4 g	Since 1985 (Kommission E)
Dry extract from Gentianae radix (DER 4.5-5.5:1) ethanol 53% (V/V)	Digestive disorders (dyspeptic complaints) like loss of appetite, feeling of fullness and bloating	2-3 times daily 2 capsules corresponding to 120 mg extract per capsule Single dose: 240	From 1978 to 2009 (data from market overview, DE)

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
		mg dry extract Daily dose: 480-720 mg dry extract	

3. Non-Clinical Data

Note: There is no precise declaration of the *Gentiana* herbal preparations used in the different experimental studies. Information about the use of fresh or dry herbal substance and about the precise extraction solvent used or definition of the ratio of herbal substance to genuine herbal preparation is not available.

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

3.1.1. Primary pharmacodynamics

There are some older data which could support the hypothesis that the extract of Gentianae radix increases gastric secretion due to effects in mouth and stomach (Leslie, 1978; Borissow, 1903; Moorhead 1915). It is also supposed that that bitter substances may increase appetite independent of their effects in mouth and stomach (Moorhead, 1915; Gebhardt, 1997; Wegner, 1997).

- Effects on gastric secretion

In-vitro: Isolated and enriched parietal cells from rat gastric mucosa were cultured in the presence of EGF (epidermal growth factor) and insulin, expanding the cell population by 170% within 48 hours. Determination of the cellular accumulation of radio-labelled aminopyrine was used for indirectly measuring acid production by parietal cells. Addition of 10^4 M histamine rose the aminopyrine ratio more than 2-fold within 20 minutes. When an aqueous dry extract of *Gentiana lutea* L. root was added, a concentration dependent rise of the aminopyrine ratio was observed leading to a 1.7-fold stimulation at 100 µg/ml, while cytotoxic effects occurred above 5 mM only. No stimulatory effect was exerted by an artichoke extract. The authors postulated that an aqueous dry Gentiana extract is able to directly stimulate acid production by the gastric mucosa (Gebhardt, 1997).

In-vivo: After direct application on the tongue, bitters increase the secretion of gastric fluid during *in vivo* experiments in dogs (Borissow, 1903). The experiments of Moorhead (1915) in dogs should demonstrate whether the so-called stomachic or bitter tonics, acting in the mouth or in stomach, could affect first the appetite and second the quantity and quality of gastric secretion and cachexia.
In rats, gentian extract increased gastric secretion in a dose-dependent way after direct ingestion in the stomach. Only at the highest concentration of 4% the extract showed an influence on pH: increasing it from 4.25 to 4.85 (Leslie, 1978).

- Secretolytic effects

In-vivo: Gentian root infusion (no further information available), administered orally to sheep at a daily dose of 5 g, before feeding produced a stimulant effect on secretion of enzymes in the small intestine ESCOP (2003).

As compared to control animals *in vivo* experiments in rabbits demonstrated elevated broncho-secretion after administration of gentian root extract (0.2 g Gentianae radix 100 g ethanol 19% (V/V))

directly in the stomach by gavage, for 3 days (the equivalent of 12.6 mg/kg per day of dried root). Concerning secretolytic effects significantly increased activity was shown with production rate levels of 37.7% and 104%, respectively, above the control group (Chibanguza *et al*., 1984).

3.1.2. Secondary pharmacodynamics

Antioxidant, antimicrobial (antibacterial and antifungal), hepatoprotective and immunological effects have been described for extracts and isolated components from gentian root.

Kusar *et al*. (2006): Free-radical scavenging activity of methanolic extracts of gentian leaves and roots (without further particulars) were tested in two different systems using electron spin resonance (ESR) spectrometry. Assays were based on the stable free radical 1.1-diphenyl-2-picrylhydrazyl (DPPH) and the superoxide radicals (O_2^+) generated by the xanthine/xanthine oxidase (X/XO) system. The results of gentian methanolic extracts were compared with the antioxidant capacity of synthetic antioxidant butylated hydroxyanisole (BHA). This study proves that gentian leaves and roots exhibit considerable antioxidant properties expressed either by their capability to scavenge DPPH or superoxide radicals. Definite data are not given from these experiments. The authors postulated further studies to prove the above mentioned thesis.

Amin (2008): Ketoconazole (KET) is an antifungal drug with a broad spectrum of activity that also induces reproductive toxicity in humans and animals. The protective effect of Gentiana (GEN) extract (*Gentiana lutea*) (without further particulars) against KET-induced testicular damage was evaluated in male Wistar rats. GEN extract was administered orally (1 g/kg bw per day) for 26 days. Three weeks after extract's administration KET was co-administered *i.p*. at a dose of 100 mg/kg once a day for 5 days. KET-induced reproductive toxicity was associated with clear reductions of the weights of testes and epididymides, sperm indices and serum testosterone levels. KET also induced severe testicular histopathological lesions such as degeneration of the seminiferous tubules and depletion of germ cells. In addition, marked oxidative damage to testicular lipids and alterations of natural antioxidants (catalase (CAT) and superoxide dismutase (SOD)) were reported in association with KET toxicity. Most of the KET-induced effects were greatly decreased with the concomitant application of GEN extract. The authors indicated a protective role of GEN extract that could be attributed to its antioxidant properties.

Van der Sluis *et al*. (1983); Guérin and Réveillère (1985): Furthermore *Gentiana lutea* extracts (aqueous extract 1:4) and gentiopicroside showed *in vitro* fungitoxic effects.

Kumarasamy *et al*. (2003): Gentiopicroside, a secoiridoid glycoside isolated from the methanol extract of the aerial parts of *Centaurium erythraea*, has been assessed for its antibacterial activities as now the results were given for the antioxidative activities). General toxicity of gentiopicroside has also been determined by brine shrimp lethality bioassay. Gentiopicroside inhibited the growth of 12 of 17 pathogenic bacterial species tested. The minimum inhibitory concentrations (MICs) were between $6.3x10^{-3}$ and $1.0x10^{-1}$ mg/ml.

Mahady *et al*. (2005): As part of an ongoing screening program the study assessed the *in vitro* susceptibility of 15 *Helicobacter pylori* strains to botanical extracts which historically are known for their traditional use in the treatment of gastrointestinal disorders. Among the methanolic extracts (without further particulars) with a minimum inhibitory concentration (MIC) of 100 µg/ml was that of *Gentiana lutea* roots.

Kondo *et al*. (1994): The hepatoprotective activity of gentiopicroside was evaluated in the chemically and immunologically induced acute liver injury rnodels in mice, after treatment with CCI_4, and LPS/BCG, respectively. When mice were given gentiopicroside for 5 days before treatment with CCI_4 or

lipopolysaccharide (LPS)/*Bacillus* calmette-Guerin (BCC), liver injuries were significantly suppressed at doses of 30-60 mg/kg per day.

Zimmermann *et al*. (1986): It was shown that the concentration of the secretory immunoglobulin A (sIgA-level) in saliva, which is increased by patients with inflammable gastro-intestinal diseases, was decreased with Gentianae radix D1 (ethanolic tincture, 3 times daily 20 drops). For comparison healthy patients were treated with the same dose of China D1 (Chinae cortex ethanolic tincture D1) as well as pure ethanol as the control group. The sIgA-levels of the patients treated with Gentianae radix were decreased, while the treatment with Chinae cortex caused an increased sIgA-level in the saliva. The authors postulated a potential immunological influence of bitters.

Kesavan *et al*. (2016): Having investigated the protective mechanism of *Gentiana lutea* aqueous root extract and one of its constituents isovitexin on endothelial inflammation, smooth muscle cell migration, and on the onset and progression of atherosclerosis in streptocin-induced diabetic rats the authors assume that the extract and isovitexin exhibited anti-atherosclerotic activities.

3.1.3. Safety pharmacology

No data available.

3.1.4. Pharmacodynamic interactions

No data available.

3.1.5. Conclusions

It is well-known that bitter constituents stimulate the gustatory nerves in mouth and potentially increase the secretion of gastric fluid and bile, thereby enhancing appetite and digestion, while the detailed molecular mechanism of such activities is still to be investigated.

Bitter constituents are typical in many plant families, nevertheless, their chemical structures are very heterogeneous. In many cases, bitter constituents have a lactone or -CO-CH=CH- chemical structure which is also typical for bitters from gentian root.

The medicinal use of such bitter constituents has been documented in many well-known handbooks dating since 1938 (Madaus, 1938; Martindale, 2004; Schulz and Hänsel, 1999; Blaschek *et al*., 2016).

Results from *in vitro* and *in vivo* studies in animals with Gentianae radix extracts support the traditional use as appetite and digestion stimulant.

Other possible pharmacodynamic actions such as antibacterial, antifungal, antioxidant, immunological and hepatoprotective properties have also been described. However, they do not seem to support the known and proposed traditional use.

The traditional use of Gentianae radix for the treatment of loss of appetite and for the symptomatic treatment of dyspepsia is supported by the long standing use and the above mentioned pharmacological data.

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

Data on pharmacokinetics are very limited. For the herbal substance or the herbal preparation data have not been found in literature.

For gentiopicroside (the secoiridoid glucoside isolated from *Gentiana lutea*) some more data are available which, however, are not relevant for the herbal substance or herbal preparations.

Wang *et al*. (2007): The pharmacokinetics and bioavailability of gentiopicroside (GPS), an active component of the gentian plant species, from orally administered decoctions of *Gentiana* (DG) or in combination with other plants in the prescription of Longdan Xiegan Tang (LXT) was compared *in vivo* in rats with oral administration of GPS alone, using doses adjusted to equivalent amounts of GPS (150 mg/kg). Changes in plasma levels of GPS following oral administration of GPS could be fitted to a one compartment open model with elimination half times of 3.35±0.76 hours and 6.21±3.07 hours, respectively. Kinetics of plasma GPS following oral administration of LXT could be fitted to a two compartments open model with an elimination half time of 3.83±1.54 hours. The bioavailability of GPS was markedly better and that from LXT markedly worse compared with GPS alone, as judged by the area under concentration-time curve (AUC) values of 70.0± 13.9 µg hour per ml (DG), 32.7± 12.9 µg hours per ml (GPS) and 19.1±5.9 µg hours per ml (LXT). The study demonstrated the marked variability in pharmacokinetics and bioavailability of gentiopicroside (GPS) as the active component from different herbal preparations.

El-Sedawy *et al*. (1989): As a part of the studies on the metabolism of crude drug components by intestinal bacteria gentiopicroside (the secoiridoid glucoside isolated from *Gentiana lutea*) was anaerobically incubated with various defined strains of human intestinal bacteria. Many species had the ability to transform it to a series of metabolites. Among them, *Veillonella parvula* subsp. *parvula* produced five metabolites which were identified as erythrocentaurine, gentiopicral, 5-hydroxymethylisochroman-1-one,5-hydroxymethylisochromen-1-one and trans-5,6-dihydro-5-hydroxymethyl-6-methyl-1H,3H-pyrano[3,4-c]pyran-1-one.

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

3.3.1. Single dose toxicity

The LD_{50} of the herbal drug Gentianae radix is unknown (Blaschek *et al*., 2016).

3.3.2. Repeat dose toxicity

Chibanguza *et al*. (1984): Rabbits treated with 12.6 mg per day of gentian extract (no details available) for 3 days did not exhibit symptoms of toxicity nor abnormal clinical serum parameters, with the exception of slightly lower erythrocyte levels in the treatment group, compared to a control group.

Leslie and Salmon (1979): No treatment-related adverse effects were observed in rats treated orally for 13 weeks with 1.6 ml/kg of a combination product containing alcoholic extracts of gentian root, chamomile and liquorice. No effects on reproduction, fertility or mating performance in female rats were observed and no teratogenic ones in rabbits. The acute oral LD_{50} in mice of 25 ml/kg of gentian extract (37% ethanol and a bitterness value of 200 Ph. Helv. units per g) was the same as that of 30% ethanol.

3.3.3. Genotoxicity

Morimoto *et al*. (1983): The mutagenic activities of 2 hydroxyxanthones, gentisine and isogentisine obtained from the methanol extract of Gentianae radix were investigated. The methanol extract of Gentianae radix which showed mutagenicity in the Ames test in *Salmonella typhimurium* strain TA100 with S9 mix was fractionated by column chromatography on Sephadex LH-20. The fractions were purified by preparative TLC and column chromatography on polyamide. Two mutagenic materials thus

obtained, S1 and S2, each gave a single band on TLC. Identification of S1 and S2 was accomplished by comparing the analytical (mps, elementary analyses) and spectral (UV, IR, mass, NMR) results for S1 and S2 with literature data for gentisine and isogentisine. At doses below 10 µg S1 (gentisine) and S2 (isogentisine) had similar specific mutagenic activities. At doses of over 10-50 µg the mutagenic activities of S2 and S1 were 19.1 and 6.94 revertants per µg, respectively. Such substantially lower activity of S1 than S2 could be attributed to its poor solubility - possibly due to the presence of the OMe group at C-3. The combined yield of S1 and S2 was about 76 mg (40 mg of S1 and 36 mg of S2) which accounted for 76% of the content of mutagenic compounds (100 mg) estimated roughly from the total mutagenic activity in the extract of the starting materials (100 g).

Matsushima *et al*. (1985): The mutagenicity of naturally occurring xanthones was tested in *Salmonella typhimurium* TA100, TA98, TA97, and TA2637 by the pre-incubation method. Xanthydrole, gentiseine, gentisine, isogentisine, 1-hydroxy-3,7-dimethoxyxanthone, 1,3,7-trimethoxyxanthone, desmethylbellidifoline, bellidifoline and dimethylbellidifoline were mutagenic, but unsubstituted xanthones were not mutagenic to TA100, TA98, TA97 and TA2637. The β-O-glucosides, nor-swertianoline and swertianoline, were only mutagenic when a metabolic activation system containing beta-glucosidase was used while the C-glucoside mangiferine was not mutagenic even by using this system.

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

The above mentioned toxicological data are very limited referring to the studied extract and have not been obtained according to current scientific guidance. There are some data for pure gentiopicroside, but they cannot be transferred to the herbal preparation of gentian root, as it is a mixture of various different chemical constituents.

There seems to be a potential mutagenicity (Ames-test in *Salmonella typhimurium* TA100, TA98, TA97, and TA2637 tested with isolated xanthones) possibly caused by the content of gentiopicroside and other xanthones. More data are required for the different herbal preparations of gentian root according to the current guidelines. The average amount of xanthone derivatives in the extracts in use or in herbal tea preparations shall be given (as range) and the test should be done (repeated) with extracts for which the amount on xanthone derivatives content is at the upper end of the particular range.

The use in pregnancy and lactation is not recommended due to the insufficient data presented.

Due to the lack of preclinical safety (especially genotoxicity) data, a list entry for *Gentianae luteae,* radix cannot be recommended.

3.4. Overall conclusions on non-clinical data

Results from relevant experimental studies on *Gentiana lutea*, radix to support the proposed indications are very limited. The reported pharmacological effects are consistent with the traditional use.

Specific data on pharmacokinetics and interactions are not available.

Non-clinical information on the safety of *Gentiana lutea*, radix is scarce. Tests on reproductive toxicity and carcinogenicity have not been performed.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

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

According to the monograph of Kommission E the essential active principle are the bitter substances contained in the herb which bring about a reflex excitation of the taste receptors leading to increased salivary and gastric secretion. Gentian root is therefore considered not to be simply a pure bitter, but also a roborant and tonic (Kommission E, 1990).

Some clinical studies have been performed with gentian root which demonstrate the influence of bitters on the secretion of the gastric fluid and of the gastric mucosa. Exact descriptions of the extract administered are missing.

First studies were published by Ivancevic and Kadrnka (1938). Following the administration of refined extracts from *Gentiana* to 5 healthy probands radiological examinations of the gastric mucosa using contrast agents were performed. On the images obtained a thickening of gastric mucosa and increased secretion of gastric mucus were observed indicating direct local effects of bitters on the stomach.

In two clinical studies Blumberger and Glatzel (1966), Glatzel and Hackenberg (1967) showed that the secretion of saliva and gastric fluid was stimulated after an oral dose of an ethanolic gentian root extract. In addition, a direct effect on the gastrointestinal tract could be demonstrated (cholagogic effect).

A controlled clinical study was performed by Borgia *et al*. (1981) in order to test the activity of an herbal preparation containing as one component gentian tincture. Salivary secretion was measured in 24 healthy volunteers at 0-time and during 120 minutes after six different treatments (complete herbal preparation, gentian tincture 2%, rhubarb fluid extract 2%, placebo, placebo with 7% ethanol, 4% citric acid as active control) administered according to a 6x6 Latin square design replied 4 times. The complete preparation and its components alone (including gentian tincture 2%) induced a significant increase of salivary secretion over 30 minutes similar to the active control, while placebo and placebo/ethanol did not have such effect. No differences were observed at later measurement times.

According to the results of a clinical study conducted by Amann and Maiwald (1988) who administered a bitter concentrate (multiple herbal combination preparation-no exact description given in the

publication) to healthy probands the bitter substances improved the production of gastric acid and increased gastric proteolysis leading to an optimisation of gastrointestinal regulation.

Zimmermann *et al*. (1986) showed that the concentration of the sIgA-level in saliva, which is increased by patients with inflammable gastro-intestinal diseases, was decreased with Gentianae radix D1 (ethanolic tincture, 3 times daily 20 drops). For comparison healthy patients were treated with the same dose of China D1 (Chinae cortex ethanolic tincture D1) or pure ethanol. The sIgA-level of the patients treated with Gentianae radix was decreased while treatment with Chinae cortex caused an increased sIgA-level in the saliva. The author postulated a potential immunological influence of bitters.

In other studies it was shown that bitter taste receptors cannot only be found in the lingual epithelium but also in the gastrointestinal tract of animals (Rozengurt, 2006). It is postulated that activation of bitter taste receptors generates integrated responses as secretion, motility or absorption (Sternini, 2007).

More recently, McMullen *et al*. (2014) investigated, if the bitter tastants, gentian root (*Gentiana lutea* L.) and wormwood herb (*Artemisia absinthium* L.), stimulate cephalic and/or gut receptors to alter postprandial haemodynamics during the gastric-phase of digestion. Normal participants ingested (1) 100 ml water plus capsules containing either cellulose (placebo-control) or 1000 mg of each tastant (n=14); or (2) 100 ml of water flavoured with 500 or 1500 mg of each tastant (a) gentian (n=12) and (b) wormwood (n=12). A single beat-to-beat cardiovascular recording was obtained for the entire session. Pre/post-ingestion contrasts with the control were analysed for (1) the encapsulated tastants, in the "10 to 15" minute post-ingestion period, and (2) the flavoured water in the "5 to 10" minute post-ingestion period. Water, the placebo-control, increased cardiac contraction force and blood pressure whereas heart rate decreased. Encapsulated tastants did not further alter postprandial haemodynamics. In contrast gentian (500 and 1500 mg) and wormwood (1500 mg) flavoured water elicited increased peripheral vascular resistance and decreased cardiac output, primarily by reducing stroke volume rather than heart rate. The authors' conclusion from this study is that drinking 100 ml water elicits a pressor effect during the gastric-phase of digestion due to increased cardiac contraction force. The addition of bitter tastants to water elicits an additional and parallel pressor effect due to increased peripheral vascular resistance; yet the extent of the post-prandial blood pressure increases are unchanged, presumably due to baroreflex buffering. According to the authors the vascular response elicited by bitter tastants can be categorised as a sympathetically-mediated cephalic-phase response. A possible mechanism by which bitter tastants could positively influence digestion is altering gastric-phase postprandial haemodynamics and supporting postprandial hyperaemia.

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

4.2.1. Dose response studies

There are no dose response studies available.

4.2.2. Clinical studies (case studies and clinical trials)

So far, only a few clinical studies have been performed with *Gentiana lutea*, radix.

Borgia *et al*. (1981) published the results of a controlled clinical study which investigated the activity of a herbal extract widely used to treat various mild disturbances of the gastrointestinal tract such as loss of appetite, dyspepsia and constipation. The herbal extract contains rhubarb, cascara, boldo and gentian tincture.

After, in a first step Borgia *et al*. (1981) had demonstrated increased salivary secretion for gentian tincture 2% (see 4.1.1.), in a second approach patients were subdivided into four groups of 20 patients each, who were randomly allocated to receive one of the following treatments: (1) complete herbal preparation, (2) a preparation with rhubarb (2% fluid extract) and gentian (2% tincture) and (3) a preparation with boldo (1% tincture) and cascara (2% fluid extract) (4) placebo. The therapeutic activity was evaluated in a double-blind, double controlled trial considering 30 different symptoms divided into four groups (loss of appetite, dyspepsia, constipation and non-target symptoms). The results were significantly better with the complete test preparation both when compared with placebo and with the two different pairs of its components.

The clinical study of Borgia *et al*. (1981) was not included into the tabular presentation, since in the second part of the study including patients with mild gastrointestinal complaints gentian root was administered in combination with rhubarb (2% fluid extract).

An open, non-interventional study in 205 patients with mild gastrointestinal complaints was performed by Wegner (1997). The aim of this study was the assessment of therapeutic effects and tolerability of a dry extract of gentian root in patients with dyspeptic symptoms under conditions of clinical practice. 205 patients (mean age 53.3 years, 65% female) with various dyspeptic symptoms (heartburn, vomiting, stomach aches, nausea, loss of appetite, constipation, flatulence) were treated with capsules containing 120 mg dry extract of gentian root (4.4-5.5:1) ethanol 53% V/V at a dosage of 2-3 times daily. The average dosage was 4.8 capsules per day which is equivalent to 2.9 g *Gentianae radix*. The duration of the application was 15 days. Improvements in symptoms were evident after 5 days in most cases and by the end of the study the average level of improvement was 68%. The efficacy of the preparation was assessed by the doctors as excellent (symptoms eliminated) in 31% of patients, good in a further 55%, moderate in 9% and inadequate in 5% of cases.

Table 4: Clinical studies

Type	Study	Test Product(s):	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Observational, non-interventional Aim of study: to investigate efficacy and tolerability of a dry extract from gentian root (Wegner, 1997)	Open, un-controlled	dry extract of gentian root (4.4-5.5:1) ethanol 53%: capsules 120 mg; 2-3 times daily, mean daily dose: 4.8 capsules (corresponding to 576 mg dry extract); duration of treatment: 15 days	N=205, mean age: 53.3 years, 65% female	Patients with dyspeptic symptoms: heartburn, vomiting, stomach pain, nausea, loss of appetite, constipation, flatulence	Improvements in symptoms evident after 5 days: at the end of the study the average level of improvement was 68%. Efficacy was rated by the doctor as excellent: 31% good: 55% moderate: 9% inadequate: 5%	Descriptive analysis only	Due to the open study design the study only supports the plausibility of the application of gentian root dry extract in a solid dosage form for the treatment of dyspeptic symptoms

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

No data available.

4.4. Overall conclusions on clinical pharmacology and efficacy

Long standing use of preparations of Gentianae radix, pharmacological studies and current findings of physiological properties establish the use of Gentianae radix.

Clinical pharmacological data of *Gentiana lutea* preparations according to the level of the current scientific knowledge do not exist, controlled clinical trials, so far, have not been performed.

In consequence, the plausibility of efficacy is based on the traditional use and experimental data mentioned above. Results from these experimental data support the long known action of bitters which increase the secretion of gastric juice and bile due to the stimulation of gustatory nerves in the mouth and possibly by direct stimulation in the stomach. The findings of recent investigations indicate the existence of a chemosensory pharmacological mechanism that is consistent with the traditional use of these bitter tastants to treat digestive disorders (McMullen *et al*. 2014).

5. Clinical Safety/Pharmacovigilance

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

During the open clinical study of Wegner (1997) adverse events occurred in 2.4% of the 205 patients. The adverse reactions observed were flatulence, soft faeces, stomach cramps and nausea/spasm of the stomach, and headache in one patient each.

As in the clinical study gentian root was used in patients with dyspeptic complaints, a decision cannot be made, if the adverse reactions observed have to be classified as adverse drug reactions because they were caused by the study medication or if they were part of the patients' basic symptoms. Thus, they do not have to be listed.

5.2. Patient exposure

See 5.1.

During the period of traditional use for more than 30 years only one case of hypertension after ingestion of a solid *Gentiana* preparation was reported to the German agency (German pharmacovigilance data base: results from 2.1.2017). Hypertension was known in the medical history of the patient. Further details are missing so that a definite assessment of a causal relationship is impossible.

5.3. Adverse events, serious adverse events and deaths

In 5.1 the adverse events which were observed during clinical trials are reported.

In addition, different cases of poisoning in humans are described. Most of the cases were due to an adulteration or mistaken use of *Veratrum album* (Blaschek *et al*., 2016).

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.

5.5.2. Contraindications

The administration is contraindicated with hypersensitivity to gentian root.

5.5.3. Special warnings and precautions for use

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

5.5.4. Drug interactions and other forms of interaction

No data available.

5.5.5. Fertility, pregnancy and lactation

As no data are available the use is not recommended.

5.5.6. Overdose

No data available.

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

Clinical safety data are based on the long standing use and the observational study mentioned above.

As there is no information on reproductive and developmental toxicity the use during pregnancy and lactation cannot be recommended.

Data on use in children or adolescents are not available. Thus, the use in the paediatric age group is not recommended.

The use is contraindicated in patients who are hypersensitive to gentian root.

6. Overall conclusions (benefit-risk assessment)

Gentianae luteae radix is a well-known traditional herbal substance that is medically used for centuries in European countries. The medicinal use has been documented continuously in a lot of well-known textbooks.

For *Gentiana lutea* L., radix used in herbal preparations as listed in the monograph, a period of at least 30 years in medicinal use as requested by Directive 2004/24/EC for qualification as a traditional herbal medicinal product is fulfilled.

All existing literature data support its traditional use for the following indications suitable for self-medication:

Traditional herbal medicinal product 1) for temporary loss of appetite and 2) for mild dyspeptic/gastrointestinal disorders. The duration of administration is limited to two weeks, if symptoms persist during treatment.

The pharmacological studies *in vitro* and *in vivo* indicate the stimulation of the gustatory nerves in mouth and stimulating effects on the gastric, intestinal and biliary secretion. The specific mechanism of the mode of action of bitters is not finally known. It is fact that the bitter constituents stimulate the gustatory nerves in the mouth and give rise to an increase in the secretion of gastric fluid and bile. In different experiments it could be demonstrated that these effects enhance appetite and digestion.

There are additional experimental data that support the use of Gentianae radix preparations in a solid pharmaceutical dosage form. The data of an observational study (Wegner, 1997) support the traditional use of the encapsulated bitters and show that the reflex effect stimulating the gustatory nerves in the mouth is not the only mechanism of action for bitters. The data indicate a local gastric effect of the extract and support the use of the solid pharmaceutical form.

The use of Gentianae radix is not recommended during pregnancy and lactation and Gentianae radix should not be taken by children and adolescents under 18 years of age.

As the minimum required data on mutagenicity (Ames' test) are not available for herbal preparations of Gentianae radix, an inclusion into the European Union's list of traditional herbal substances and preparations is not recommended.

Gentianae radix is often used in combination with other bitters or preparations from other herbal substances which are also used in dyspeptic/gastrointestinal disorders.

Annex

List of references