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COMMITTEE ON HERBAL MEDICINAL PRODUCTS (HMPC)

ASSESSMENT REPORT ON *GENTIANA LUTEA* L., RADIX

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I. REGULATORY STATUS OVERVIEW[1]

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
Other: If known, it should be specified or otherwise add 'Not Known'

Member State	Regulatory Status				Comments[2]
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	400mg G. Radix has been assessed as safe in food very old products
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Germany	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Since 30 years on the market
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No licensed products
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No products
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Poland	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations In 1999 there were two cases of

[1] This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.
[2] Not mandatory field

Member State	Regulatory Status				Comments[2]
					acute poisoning with *Veratrum album* L. because of adulteration of *Gentiana pannonica* Scop. with *Veratrum album* L.
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No response
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only combinations as natural remedies
United Kingdom	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	And combinations

II. ASSESSMENT REPORT

GENTIANA LUTEA L., RADIX

BASED ON ARTICLE 10A OF DIRECTIVE 2001/83/EC AS AMENDED

(WELL-ESTABLISHED USE)

BASED ON ARTICLE 16D(1) AND ARTICLE 16F AND 16H OF DIRECTIVE 2001/83/EC AS AMENDED

(TRADITIONAL USE)

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 (4.5-5.5:1) ethanol 53% v/v c) Tincture (1:5) ethanol 70% v/v d) Liquid extract (1:1) ethanol 45% v/v
Pharmaceutical forms	Comminuted herbal substance as herbal tea for oral use. Herbal preparation in solid or liquid dosage forms for oral use The pharmaceutical form should be described by the European Pharmacopoeia full standard term
Rapporteur	Dr. Werner Knöss
Assessor	Dr. Friederike Stolte

II.1 INTRODUCTION

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

- Herbal substance(s) [3]:

Gentianae radix [European Pharmacopoeia]

Dried, fragmented underground organs of *Gentiana lut*ea 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.

Gentianae radix is known under the synonyms: German: Großer Enzian, (Berg)-Fieberwurzel, Hochwurzel; Engl.: Bitter wort, Common gentian, Great yellow gentian, Yellow gentian; French: Gentiane jaune, Grande gentiane; Ital.: Genziana maggiore.

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 will be cultivated for plant production mostly in Germany and France [HagerROM 2006, Hänsel-Sticher 2007] .

The composition of the constituents (carbohydrates and essential bitters) is depending on the time of harvesting. The content of sugars, decrease in spring and increase to their maximun 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 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 [HagerROM 2006] .

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.

However, Gentianae radix distillate is also used as an ingredient of a strong liqueur called “Enzian”.

Constituents: [HAGERROM 2006, Wichtl 2002, Hänsel-Sticher 2007, Seitz 2005]

<u>Bitter constituents</u>:

Bitter constituents (2-8%) are located mostly in the cortex of the root. The most bitter constituents belong to the class of secoiridoid glycosides, with gentiopicroside (also known as gentiamarine and gentiopicrine) as main component 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 mill., 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.

<u>Xanthones</u>:
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-

3 According to the ‘Procedure for the preparation of Community monographs for traditional herbal medicinal products’ (EMEA/HMPC/182320/2005 Rev.2) and the ‘Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use (EMEA/HMPC/182352/2005 Rev.2)

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), trisaccharide (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):
  - i) Comminuted herbal substance
  - ii) Dry extract (4.5-5.5:1) ethanol 53% v/v
  - iii) Tincture (1:5) ethanol 70% v/v
  - iv) Liquid extract (1:1) ethanol 45% v/v

- Combinations of herbal substance(s) and/or herbal preparation(s)[4]
  Gentianae radix is used in combinations with many other bitter and/or aromatic herbal substances / herbal preparations, usually for treatment of dyspeptic or choleretic complaints, Typical examples of such combination partners are the following: Rumicis herba pulvis, Sambuci flos pulvis, Primulae flos pulvis, Verbenae herba pulvis, Plantaginis folium, *Curcuma zedoaria* Rosc. Angelicae radix, *Fraxinus ornus* L. (radix), Myrrha exudatum, *Carlina acaulis* L. (radix).
  This monograph refers exclusively to Gentianae radix, although the use of such combinations seems to be more traditional than the use of the mono-preparations.
- Vitamin(s)[5] not applicable
- Mineral(s) not applicable

II.1.2 Information on period of medicinal use in the Community regarding the specified indication

The following herbal substances and herbal preparations have been on the European market for a period of30 years and are proposed for the monograph on traditional use. The following data derive from the overview of marketed products and from literature.

i) Comminuted herbal substance
ii) Dry extract (4.5-5.5:1) ethanol 53% v/v
iii) Tincture (1:5) 70% v/v
iv) Liquid extract (1:1) ethanol 45% v/v (Deutsches Arzneibuch – Ergänzungsband 6 - EB6)

Posology and indications of the traditional herbal substance and preparations of Gentianae radix:

[4] According to the 'Guideline on the clinical assessment of fixed combinations of herbal substances/herbal preparations' (EMEA/HMPC/166326/2005)
[5] Only applicable to traditional use

- comminuted herbal substance as a herbal tea

  Indication: digestive disorders such as loss of appetite, dyspepsia.

  Posology: oral use 2-4 g comminuted herbal substance daily

- dry extract from Gentianae radix (4.5-5.5:1) ethanol 53% v/v

  Indication: digestive disorders (dyspeptic complaints) like loss of appetite, feeling of fullness and bloating

  Posology: 2 -3 times daily 2 capsules
  Single dose: corresponding to approx. 1.2 g herbal substance

  Daily dose: corresponding to approx. 2.4 -3.6 g herbal substance

- tincture (1:5); extraction solvent: ethanol 70% v/v

  Indication: digestive disorders (dyspeptic complaints) like loss of appetite, feeling of fullness, bloating

  Posology: 3 times daily 20 drops (= 1 ml)

  Single dose: corresponding to approx. 1 ml (= 1 – 1.5 g) tincture (1:5)

  Daily dose: corresponding to approx. 3 ml (= 3 – 4.5 g) tincture (1:5)

- fluid extract (1:1); ethanol 45% v/v (EB6)

  Indication:

  Posology: 2-4 times daily 1g liquid extract

  Single dose: corresponding to approx. 1 g fluid extract (1:1) [Haffner, Schulz]

  Daily dose: corresponding to approx. 2-4 g fluid extract (1:1)

II.2 NON-CLINICAL DATA

II.2.1 Pharmacology

II.2.1.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

<u>Note</u>: There is no precise declaration of the Gentiana herbal preparations used in the different experimental studies. There is no 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.

There are some data which could support the hypothesis that the extract of Gentianae radix increases gastric secretion due to effects in mouth and stomach [Leslie 1978, Borrissow 1903 and 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].

- [Borrissow 1903]
  After direct application on the tongue, bitters increase the secretion of gastric fluid during *in vivo* experiments in dogs.

- [Moorhead 1915]
  The experiments should demonstrate whether the so-called stomachic or bitter tonics, acting in the mouth or in stomach, could affect at first the appetite and than the quantity and quality of gastric secretion and cachexia.

- [Leslie 1978]
  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.

More recent investigations are the following:

in vitro:

- [Gebhart 1997]
  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 h. Determination of the cellular accumulation of radiolabelled 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 min. 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 postulate that an aqueous dry *Gentiana* extract is able to directly stimulate acid production by the gastric mucosa.

in vivo:

- [Öztürk,1998]
  Effects of an ethanolic extract prepared from fermented *Gentiana lutea* roots (with ethanol, DER 2:1) on the bile production and liver in rats were investigated. The extract contained 21% gentiopricroside, 5.2% swertiamarine and 2.55% sweroside. Bile flows of rats which were treated by a single i.p. dose of CCI4, 24 h prior to experiments were measured after the cannulation of bile duct under urethane anaesthesia. After an equilibration period of 1 h, the lyophilized extract were administered intraduodenally (500 mg/kg i.p.), while control animals received physiological saline only. To monitor the effect of multiple dose therapy, rats received the same dose of *G. lutea* extract for 3 days (2 days prior to CC14 administration) and their bile flows were measured after the cannulation. In all groups, bile samples were collected for 3 h with 15 min intervals. After the completion of bile flow experiments, rat livers were removed and put in neutral formaldehyde solution (10Y0) for the histological examination. According to the results obtained, multiple dose treatment of rats with the plant extract normalized the decreased bile flow due to CCI4 whereas single dose therapy was ineffective on the impaired bile flow. The authors declaimed, that these data indicate that the studied extract has a potential hepatoprotective activity.

Secretolytic effect

- [ESCOP, Kazakov 2003]
  Gentian root infusion, 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.

- [Chibanguza et al.1984]
  During *in vivo* experiments in rabbits, bronchosecretion was elevated in comparison with control animals after administration of gentian root extract (0.2 g Gentianae radix/ 100g ethanol 19% v/v) directly in the stomach by gavage, for 3 days (the equivalent of 12.6 mg/kg/day of dried root). Concerning secretolytic effects, a significantly increased activity was shown with production rate levels of 37.7% and 104%, respectively, above the control group.

Other pharmacological activities:

- [Kusar 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 (O2 +) 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 provides 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 postulate further studies to prove the above mentioned thesis.

- [Kumarasamy 2003]
  Gentiopicroside (1), a secoiridoid glycoside isolated from the methanol extract of the aerial parts of Centaurium erythraea, has been assessed for its antibacterial and free radical scavenging activities. General toxicity of 1 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 6.3 x10-3 and 1.0 x10-1 mg/ml.

- [Kondo 1994]
  The hepatoprotective activity of gentiopicroside was evaluated in the chemically and immunologically induced acute liver injury rnodels in mice, after treatment with CCI4, and LPS/BCG, respectively. When mice were given gentiopicroside for 5 days before treatment with CCl4, or lipopolysaccharide (LPS)/Bacillus calmette-Guerin (BCC), liver injuries were significantly suppressed at doses of 30- 60 mg/kg/day.

- [Mahady 2005]
  As part of an ongoing screening program, the study assessed the *in vitro* susceptibility of 15 Helicobacter pylori (HP) 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.

- [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 (1g/kgbwt/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 1983; Guérin 1985]
  Furthermore *Gentiana lutea* extracts (aqueous extract 1:4) and gentiopicroside showed *in vitro* fungitoxic effects.

- [Zimmermann 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-level of the patients treated with Gentianae radix were decreased, while the treatment with Chinae cortex caused an increased sIgA-level in the saliva. The author postulated a potential immunological influence of bitters.

II.2.1.2 Overall conclusions on pharmacology

It is well-known that the bitter constituents stimulate the gustatory nerves in mouth and possibly 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 also been documented in many well-known handbooks dating since 1938 [Madaus 1938, Martindale 1977, Schulz et al. 1999, HagerRom 2006]

There are more recent publications that postulate an additional direct local effect in the gastrointestinal tract.

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

Other possible pharmacodynamic actions as antibacterial, antifungal, antioxidant, immunological and hepatoprotective properties are also described. However, they do not seem to play a role for the known traditional use.

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

Note: There is no precise declaration of the Gentiana herbal preparations used in the different experimental studies. There is no information about the use of fresh or dry herbal substance, the precise extraction solvent used, nor the ratio of herbal substance to genuine herbal preparation.

II.2.2 Pharmacokinetics

II.2.2.1 Overview of available data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

- [Wang 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 rats with oral administration of GPS alone, using doses adjusted to deliver 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 h and 6.21 +/- 3.07 h, respectively. Kinetics of plasma GPS following oral administration of LXT could be fitted to a two compartments open model with an elimination half time f 3.83 & 1.54 h. 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 h/ml (DG), 32.7 +/- 12.9 µg h/ml (GPS) and 19.1 +/- 5.9 µg h/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 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 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.

II.2.2.2 Overall conclusions on pharmacokinetics

Limited data are available on pharmacokinetics. For the herbal substance or the herbal preparation no data are available.
For gentiopicroside (the secoiridoid glucoside isolated from *Gentiana lutea*) some more data exist but they are not relevant for the herbal substance or herbal preparations.

II.2.3 Toxicology

II.2.3.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

- [Hager 2006]
  For the herbal drug Gentianae radix the LD50 value is unknown.
- [Chibanguza 1984]
  Rabbits treated with 12.6 mg/day of gentian extract 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 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 LD50 in mice of 25 ml/kg of gentian extract (37% ethanol and a bitterness value of 200 Ph.Helv. units/g) was the same of that of 30% ethanol.
- [Morimoto 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, and 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 micrograms, S1 (gentisine) and S2 (isogentisine) had similar specific mutagenic activities. At doses of over 10 to 50 micrograms, the mutagenic activities of S2 and S1 were 19.1 and 6.94 revertants per microgram, respectively. This much 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 1985]
  The mutagenicity of naturally occurring xanthones were 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.

II.2.3.2 Overall conclusions on toxicology

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 typhomorum* 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. Because of the existing data from the above mentioned AMES test, it is to start with *in vitro* test on mammalian cells (e.g. mouse lymphoma assay), according to the "Guideline on the assessment of genotoxicity of herbal medicinal substances/preparations" (EMEA/HMPC/107079/2007. Additionally, 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 should be excluded due to the insufficient data presented.

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

II.3 CLINICAL DATA

II.3.1 Clinical Pharmacology

II.3.1.1 Pharmacodynamics

II.3.1.1.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

Bitters stimulate the gustatory nerves in the mouth.

- In two studies [Blumenberger 1966, Glatzel 1967] it could be shown 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).
- [Zimmermann 1986]
  It was shown 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) as well as pure ethanol as the control group. The sIgA-level of the patients treated with Gentianae radix were decreased, while the treatment with Chinae cortex caused an increased sIgA-level in the saliva. The author postulated a potential immunological influence of bitters.
- [Borgia 1981]
  A controlled clinical study was performed 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 6 x 6 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 with ethanol had no such effect. No differences were observed at later measurement times.

Bitters stimulate the gastrointestinal tract.

- The postulated thesis of the direct effect of bitters on the gastrointestinal tract was confirmed in previous studies, tested the influence of bitters on the secretion of the gastric fluid and of the gastric mucosa [Ivancevic 1938, Amann 1988].

More recent studies show that bitter taste receptors can not 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 generate integrated responses as secretion, motility or absorption [Sternini 2006].

II.3.1.1.2 Overall conclusions on pharmacodynamics

Long standing use of preparations of Gentianae radix, pharmacological studies and current findings of physiological properties establish the use of Gentianae radix for the treatment of loss of appetite and for the symptomatic treatment of dyspepsia and mild spasmodic disorders of the gastrointestinal tract.

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 direct stimulation in the stomach.

Clinical pharmacological data of *Gentiana lutea* preparations according to the level of the current scientific knowledge do not exist.

II.3.1.2 Pharmacokinetics

No data available.

II.3.1.2.1 Overview of available data regarding the herbal substance(s)/herbal preparation(s) including data on constituents with known therapeutic activity.

II.3.1.2.2 Overall conclusions on pharmacokinetics

Due to lack of data no conclusions can be drawn.

II.3.2 Clinical Efficacy[6]

- [Wegner 1997]
  In an open study, 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.

[6] In case of traditional use the long-standing use and experience should be assessed.

Note: The uncontrolled observational study supports the plausibility of the application of gentian root dry extract in a solid dosage form and not only in a liquid form for the treatment of dyspeptic symptoms.

- [Borgia 1981]
  A controlled clinical study was performed 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 6 x 6 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 with ethanol had no such effect. No differences were observed at later measurement times.

  In a second approach, patients were subdivided into four groups of 20 patients each, which where randomly allocated to receive one of the following treatments: (1) complete herbal preparation, preparation, two pairs of its components: (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.

II.3.2.1 Dose response studies

No data available.

II.3.2.2 Clinical studies (case studies and clinical trials)

No data available.

II.3.2.3 Clinical studies in special populations (e.g. elderly and children)

None reported.

II.3.2.4 Longstanding use and experience

Gentianae luteae radix has been used as a medicinal product in different preparations for a long time in various indications. The medicinal use, primarily based on the content of bitters have been reported in many European handbooks and Pharmacopoeia. The claimed indications are listed in the following table:

Bitter stomachic and stimulant; Intermittent fever attacks	Madaus (1938)
Strong bitter as an appetite stimulant, roburant and tonic; gastrointestinal disorders and loss of appetite; aromatic bitters and stomachic	Wichtl (2002): Herbal drugs and Phytopharmaceuticals.
Bitter	Martindale. 34th edition (2004)
Digestive disorders, such as loss of appetite, fullness, flatulence	German Commission E (1985)
Bitter; Gastric stimulant; Sielagogue; Cholagogue	British herbal Pharmacopoeia (1983)
Loss of appetite; digestive disorders, such as fullness, flatulence	Standardzulassung Nr.: 9199.99.99 (2004)
Stimulate appetite	Cahier de l'Agence n°3:Médicaments à base de plantes Ed. Agence du Médicament, Paris, 1998.

Loss of appetite, fullness and flatulence; tonic to stimulate bile secretion	PDR for Herbal Medicines 3rd edition; Ed. Thomson PDR, 2004.
Anorexia e.g. after illness; dyspeptic complaints	ESCOP Monographs, 2nd ed. (2003)
Stomach trouble, e.g. due to a lack of gastric juice digestive disorders, such as loss of appetite, fullness, flatulence	HagerRom (2006)

There are no dose response studies available. The following posology is described in literature.

	Single dose	Daily dose
Madaus (1938)	10 drops (=1ml) tincture; 2.1 g comminuted drug as herbal tea (infusion) 1 tablet with 0.125 g comminuted drug	1 ml 2-3 times daily tincture; 2.1 g comminuted drug as herbal tea (infusion) 3 x 1 tablet with 0.125 g comminuted drug
Wichtl (2004)	1-2 g comminuted drug as herbal tea (infusion)	
Haffner-Schulz 12. Auflage (2008)	1 g as herbal tea (infusion) 0.2 g extractum siccum 1.0 g extractum fluidum 1.0 g tinctura	1 g 2-4 times daily as herbal tea (infusion) 1.0 g 2-4 times daily extractum fluidum 1.0 g 1-3 times daily tinctura
Com E (1990)		1 g – 3 g tincture (DAB6) 2 g – 4 g extractum fluidum (DAB6) 2 g – 4 g drug
BHP (1979)	0.6 g – 2 g drug, also as herbal tea (infusion or decoction) 1 ml-4 ml tincture	0.6 g – 2 g 1-3 times daily drug, also as herbal tea (infusion or decoction) 1-4 ml 1-3 times daily tincture
Standardzulassung (2004)	1 g comminuted drug as herbal tea (infusion) ½ hour before meal for appetite and after meal for digestive disorders	1 g 2-4 times daily comminuted drug as herbal tea (infusion); ½ hour before meal for appetite and after meal for digestive disorders
PDR for Herbal Medicines (2004)	1 g – 2 g comminuted drug as herbal tea (infusion) 1 ml – 4 ml tincture	2 g – 4 g comminuted drug as herbal tea (infusion) several times a day including ½ hour before meal 1 ml – 4 ml 1-3 times daily tincture 2 g – 4 g liquid extract
ESCOP (2003)	0.1 g – 2 g comminuted drug as herbal tea (infusion) 1 ml tincture hydroethanolic extract equivalent bitterness value	0.1 g – 2 g 1-3 times daily comminuted drug as herbal tea (infusion) 1 ml 1-3 times daily tincture
HagerROM (2006)	1 g comminuted drug as herbal tea (infusion) 1 ml tincture 1.0 g extractum fluidum	2 g-4 g comminuted drug as herbal tea (infusion) several times a day including ½ hour before meal 2 g-4 g extractum fluidum 1 g-3 g tinctura
Post-marketing-authorithy study [Wegner 1998]	2 capsule a 120 mg dry-extract (4.4-5.5:1) extraction solvent ethanol 53% v/v (corresponding to 1.1 g – 1.3 g = 1.2 g drug)	2 capsule a 120 mg extract 2-3 times daily (corresponding to 2.2 g – 4.0 g = 3.0 g drug)

The proposed posology for the specified preparation on the basis of the long-standing use and the data given in the literature is summarized in the following table:

Specified preparation	**Dosage**
A) comminuted herbal substance	1 g - 2 g, several times daily
B) dry extract (DER 4.5-5.5:1) extraction solvent ethanol 53% v/v	2-capsules of 120 mg dry-extract, 2-3 times daily
C) tincture (1:5); extraction solvent ethanol 70% v/v	1 ml 1-3 times daily
D) fluid extract (DER 1:1); EB6	1.0 g 2-4 times daily

For the indication "loss of appetite" it is described in the literature that the liquid preparations A), C) and D) are supposed to be taken ½ hour before meal. Correspondingly, the solid dosage form B) is supposed to be taken 1 hour before meal due to the additional mechanism of disintegration of the solid form.

Duration of use

No information could be found on the recommended duration of use.

II.3.3 Clinical Safety/Pharmacovigilance

II.3.3.1 Patient exposure

II.3.3.2 Adverse events

As a result of the observational study [Wegner 1998] the following adverse reactions have to be declared: diarrhoea, spasmodic stomach-ache, tachycardia and pruritus.
The frequency of the adverse effects is based on this finished observational study. Therefore the reported adverse reactions are ranked in the following frequency categories:

Diarrhoea	uncommon	(≥ 1/1.000 bis < 1/100)
spasmodic stomach-ache	uncommon	(≥ 1/1.000 bis < 1/100)
tachycardia	rare	(≥ 1/10.000 bis < 1/1.000)
pruritus	rare	(≥ 1/10.000 bis < 1/1.000)

Additionally, reactions like headaches in especially sensitive patients were classified as "uncommon" [Wegner 1998, HagerROM 2006, ESCOP 2003].

II.3.3.3 Serious adverse events and deaths

- [HagerRom, *Gentiana*]
  Different cases of poisoning in humans are described. The most cases were due to an adulteration or mistaken use of *Veratrum album*.
- One case of hypertension after ingestion of ENZIAGIL (a solid *Gentiana* preparation) was reported to the German agency. 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.

II.3.3.4 Laboratory findings

No data available.

II.3.3.5 Safety in special populations and situations

No data available.

II.3.3.5.1 Intrinsic (including elderly and children) /extrinsic factors

No data available. Use in children and adolescents under 18 years of age is not recommended because data are not sufficient and medical advice should be sought.

II.3.3.5.2 Drug interactions

No data available.

II.3.3.5.3 Use in pregnancy and lactation

No data available.
There are hints on mutagenicity in the literature probably based on the content of xanthones. Even if such data were not obtained corresponding to the current scientific knowledge, they support the conclusion that the use during pregnancy and lactation is not recommended.

II.3.3.5.4 Overdose

No data available.

II.3.3.5.5 Drug abuse

No data available.

II.3.3.5.6 Withdrawal and rebound

No data available.

II.3.3.5.7 Effects on ability to drive or operate machinery or impairment of mental ability

No data available.

II.3.3.6 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.
Because of the increase of the gastric fluid secretion after the administration of bitters, the use in patients with gastric ulcer and duodenal ulcer is contraindicated.

II.4 OVERALL CONCLUSIONS

Gentianae luteae radix is a well known and traditional herbal medicinal product 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 above mentioned herbal preparations, 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 used in mild dyspeptic/gastrointestinal disorders, and in temporary loss of appetite.
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 the 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 1998] 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.

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 and patients with gastric and duodenal ulcer.
As the minimum required data on mutagenicity (Ames' test) are not available for herbal preparations of Gentianae radix, an inclusion into the Community list of traditional herbal substances and preparations is not recommended.
In ancient times, gentian root was used for other indications as an antipyretic and anthelminthic agent [Madaus 1938, HagerRom 2006].
Gentianae radix is often used in combination with other bitters or other herbal substances preparations, which are also used in dyspeptic/gastrointestinal disorders.

III. ANNEXES

III.1 COMMUNITY HERBAL MONOGRAPH ON *GENTIANA LUTEA* L., RADIX[7][8]

III.2 LITERATURE REFERENCES

[7] According to the 'Procedure for the preparation of Community monographs for traditional herbal medicinal products' (EMEA/HMPC/182320/2005 Rev.2)

[8] According to the 'Procedure for the preparation of Community monographs for herbal medicinal products with well-established medicinal use' (EMEA/HMPC/182352/2005 Rev.2)