



EUROPEAN MEDICINES AGENCY  
SCIENCE MEDICINES HEALTH

12 July 2011  
EMA/HMPC/572844/2009  
Committee on Herbal Medicinal Products (HMPC)

## Assessment report on *Symphytum officinale* L., radix

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

Draft

|   |  |
|---|--|
| Herbal substance(s) (binomial scientific name of the plant, including plant part) | <i>Symphytum officinale</i> L., radix                            |
| Herbal preparation(s)   | Liquid extract (DER 2:1), extraction solvent 65% (V/V) ethanol   |
| Pharmaceutical forms  | Herbal preparations in semi-solid dosage forms for cutaneous use |
| Rapporteur  |  |
| Assessor(s)   |  |

Note: This Assessment Report is published to support the release for public consultation of the draft Community herbal monograph on *Symphytum officinale* L., radix. It should be noted that this document is a working document, not yet fully edited, and which shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which the Rapporteur and the MLWP will take into consideration but no 'overview of comments received during the public consultation' will be prepared in relation to the comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft monograph.



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# 1. Introduction

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

*Symphytum officinale* L. (Boraginaceae) or common comfrey is a perennial native of Europe and Asia and has been naturalised throughout North America (Longe, 2005). It is very common in all of Europe, especially in damp soils (Bruneton, 1999). There are about 25 species of the herb, including further medicinal plants apart from common comfrey (e.g. *S. asperum* Lepechin, *S. tuberosum* L., and *S. × uplandicum* Nyman (syn. *S. peregrinum*, *S. asperum* × *officinale*, according to Tutin, 1992) (Longe, 2005; De Smet et al., 1992).

Comfrey grows well in rich, moist, low meadows, or along ponds and river banks, where it may reach a height of 1.2 m (usually 0.3-1.2 m). Comfrey root is large, branching, and black on the outside with a creamy white interior containing slimy mucilage. The root is slimy and horn-like when dried. Hollow, erect stems, also containing mucilage, are covered with bristly hairs that cause itching when in contact with the skin. The thick, somewhat succulent, veined leaves are covered with rough hairs. They are alternate and lance shaped, with lower leaves as large as 25 cm in length, and dark green on top and light green underneath. The lower ones and the basal ones are ovate-lanceolate and pulled together in the petiole; the upper ones are lanceolate and broad. Small, bell shaped flowers grow from the axils of the smaller, upper leaves on red stalks. Flowers are mauve to violet and form in dense, hanging clusters, blooming in summer. They are arranged in crowded, apical, 2-fayed hanging cymes. The calyx is fused and has 5 tips. The corolla is also fused and is cylindrical-campanulate with a pentangular tube and 5-tipped border. The tips are revolute and there are 5 awl-shaped scales in the mouth of the tube. The scales are close together in a clavate form and have a glandular tipped margin. There are 5 stamens and 1 style. The ovary is 4-valved. The fruit consists of 4 smooth, glossy nutlets (Longe, 2005; PDR for Herbal Medicines, 2000).

The medicinal parts are the root and the leaves. Comfrey is occasionally used as an ingredient of soups and salads. It is listed by the Council of Europe as natural source of food flavouring (category N4). This category indicates that although comfrey is permitted for use as food flavouring, insufficient data are available to assess toxicity (Barnes et al., 2007). The herb has long been used as a cooked green vegetable in early spring, and the fresh, young leaves have been added to salads. The widespread suffering caused by the Irish potato famine of the 1840s motivated Henry Doubleday, an Englishman, to fund research into comfrey's potential as a nutritional food crop. Farmers have valued comfrey as a nutritious fodder for cattle (Longe, 2005).

### Constituents

**Alkaloids:** Pyrrolizidine alkaloids constitute a class of plant toxins associated with diseases in humans and animals. They are found in a wide variety of plant species in the world and it is estimated that ~3% of the world's flowering plants contain toxic pyrrolizidine alkaloids. These toxins are present in more than 12 higher plant families, among which three families, Asteraceae (e.g. genus *Senecio*), Boraginaceae (e.g. genera *Heliotropium*, *Cynoglossum*, and *Symphytum*), and Fabaceae (e.g. genus *Crotalaria*), contain most toxic pyrrolizidine alkaloids (Wexler, 2005) (Dewick, 2002). Over 350 of pyrrolizidine alkaloids have been identified in more than 6000 plant species (Fattorusso and Taglialatela-Scafati, 2008). The wide distribution of plants containing pyrrolizidine alkaloids around the globe makes it difficult to prevent human and animal exposure and every year animals and people suffer from acute and chronic pyrrolizidine alkaloid exposures (Wexler, 2005).

Pyrrolizidine alkaloids derive from L-ornithine or L-arginine. Further characteristics of these alkaloids are as follows: (1) they are accumulated in plants as *N*-oxides; (2) they are poisons; (3) some of them

have a bioimpact (Aniszewski, 2007). These alkaloids are based on two inclined five-membered ring with a nitrogen in position 4, a hydroxymethyl group in position 9, and a hydroxy group in position 7. This structure is called the necine base (otonecine bases contain additionally a carbonyl function in position 8 and a methyl group in position 4). Pyrrolizidine alkaloids containing a saturated necine base are nontoxic, whereas a double bond between position 1 and 2 is the premise of toxicity (De Smet *et al.*, 1992). Pyrrolizidine alkaloids are composed of esters of basic alcohols (necine bases) connected to necic acid (C<sub>5</sub>-C<sub>10</sub> chain acids) via hydroxy groups at positions 7 and 9 by an ester function. Monoesters are esterified at C-9, diesters at C-7 and C-9 by a dibasic acid (Barceloux, 2008) (Hänsel and Sticher, 2004).

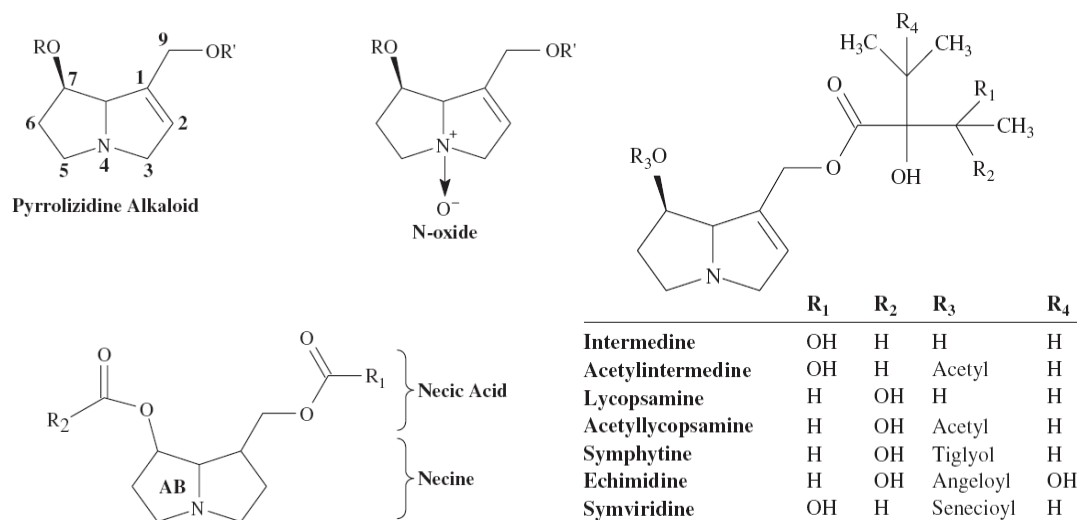


Figure 1. General structures of pyrrolizidine alkaloids and chemical structures of pyrrolizidine alkaloids in *Symphytum officinale* (Barceloux, 2008).

Comfrey roots contain 0.2-0.4% pyrrolizidine alkaloids: symphytine, lycopsamine/intermedine (diastereoisomers), acetyl-lycopsamine/acetyl-intermedine (diastereoisomers), myoscorpine, lasiocarpine, heliosupine, viridiflorine, echiumine, *symlandine* and *echimidine* (Figure 1.) (Barnes *et al.*, 2007; Coulombe, 2003). A considerable proportion of the pyrrolizidine alkaloids may be present as their *N*-oxides (De Smet *et al.*, 1992). Notable quantities of echimidine are often reported, apparently because other species that are morphologically very close, such as *S. asperum* Lepechin and *S. x uplandicum* Nyman, are mistaken for *S. officinale*, which in theory contains no echimidine at all (Blumenthal *et al.*, 1998). The presence of echimidine and symlandine may refer to the falsification of the herbal substance with *S. pergrinum* roots, since these two alkaloids are not present in the roots of *S. officinale* (Hänsel and Sticher, 2004).

The pyrrolizidine content of *Symphytum* species varies with plant part, season, natural biological variation, and species. Small young leaves early in the season possess higher total alkaloid content than older leaves, and the roots contain greater concentrations of total pyrrolizidine alkaloids than above-ground plant parts (Barceloux, 2008). In a study of commercial samples of common comfrey (*S. officinale*), analysis of the total pyrrolizidine alkaloid content demonstrated values ranging from 1380-8320 µg/g root compared with 15-55 µg/g leaf (Couet *et al.*, 1996b). The major pyrrolizidine alkaloids were symphytine and symlandine along with lesser concentrations of echimidine, lycopsamine, and acetyl-lycopsamine. Analysis of comfrey tablets indicated that total alkaloid concentrations are similar to the alkaloid content of comfrey roots (Barceloux, 2008).

**Carbohydrates:** Gum (arabinose, glucuronic acid, mannose, rhamnose, xylose); mucilage (glucose, fructose) (Barnes *et al.*, 2007).

Tannins: Pyrocatechol-type, 2.4% (Barnes *et al.*, 2007).

Triterpenes: Sitosterol and stigmasterol (phytosterols), steroidal saponins, isobauerenol, triterpene saponins symphytoxin A, cauloside D, leontoside A, leontoside B, leontoside D (Barnes *et al.*, 2007; Ahmad *et al.*, 1993; Mohammad *et al.*, 1995).

Other constituents: Allantoin 0.75–2.55%, caffeic acid, carotene 0.63%, chlorogenic acid 0.037%, caffeic acid 0.035%, choline, lithospermic acid, rosmarinic acid and silicic acid (Barnes *et al.*, 2007; Aftab *et al.*, 1996). Comfrey contains vitamins A and B12, and is high in calcium, potassium, and phosphorus (Longe, 2005).

- Herbal substance(s)

Fresh or dried root section of *Symphytum officinale* L. (Boraginaceae) (Blumenthal *et al.*, 1998).

Dried rhizome and roots of *Symphytum officinale* L. (British Herbal Pharmacopoeia, 1996).

- Herbal preparation(s)

Well established use:

Fluid extract of *S. officinale* L. radix (DER 1:2), extraction solvent: 60% (V/V) ethanol; 35% fluid extract in an ointment base, for cutaneous use.

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

## 1.2. Information about products on the market in the Member States

### Regulatory status overview

| Member State   | Regulatory Status           |                               |                                     |   | Comments   |
|----------------|-----------------------------|-------------------------------|-------------------------------------|---|--|
| Austria        | <input type="checkbox"/> MA | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products.<br>In Austria there is a special requirement for medicinal products containing Tussilago, Senecio, Symphytum: the applicant has to demonstrate that in the finished product no pyrrolizidin alkaloids are detectable using analytical techniques according state of the art. Exceptions only for homoeopathic products for external use from D4, internal use from D6. |
| Belgium        | <input type="checkbox"/> MA | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | Only combination: multi-ingredient herbal tea, containing 50 mg/g Symphyti radix; authorised 1964 but awaiting TU (if considered acceptable). The marketing authorisation of 3 herbal teas (combinations) was revoked. Symphyti radix is not allowed in food supplements.  |
| Bulgaria       | <input type="checkbox"/> MA | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No response.   |
| Cyprus         | <input type="checkbox"/> MA | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products.  |
| Czech Republic | <input type="checkbox"/> MA | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised <i>S. officinale</i> products.   |
| Denmark        | <input type="checkbox"/> MA | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products.<br>Until 30 years ago some doctors prescribed  |

| Member State    | Regulatory Status                      |                               |                                     |   | Comments   |
|-----------------|--|-------------------------------|-------------------------------------|---|--|
|                 |  |                               |                                     |   | tinctures with symphyti radix as magistrel products. They were produced at the pharmacy for the individual patient. Symphyti radix is not allowed in Denmark as food supplements due to the high content of toxic pyrrolizidine alkaloids. |
| Estonia         | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products.  |
| Finland         | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products.  |
| France          | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | One product containing <i>S. officinale</i> .  |
| Germany         | <input checked="" type="checkbox"/> MA | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | Three authorised products of <i>S. officinale</i> .  |
| Greece          | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products.  |
| Hungary         | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | Three registered traditional herbal medicinal products, 1 authorised herbal medicinal product.   |
| Iceland         | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products.  |
| Ireland         | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products.  |
| Italy           | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products   |
| Latvia          | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products   |
| Liechtenstein   | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No response  |
| Lithuania       | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No response  |
| Luxemburg       | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No response  |
| Malta           | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No response  |
| The Netherlands | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No registered or authorised products   |
| Norway          | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No response  |
| Poland          | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No response  |
| Portugal        | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify: | No response  |

| Member State    | Regulatory Status                      |  |                                     |  | Comments   |
|-----------------|--|--|-------------------------------------|--|--|
| Romania         | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD            | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify:            | No response  |
| Slovak Republic | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD            | <input type="checkbox"/> Other TRAD | <input checked="" type="checkbox"/> Other Specify: | Only in combination (1 product)  |
| Slovenia        | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD            | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify:            | No registered or authorised products   |
| Spain           | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD            | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify:            | No registered or authorised products   |
| Sweden          | <input type="checkbox"/> MA            | <input type="checkbox"/> TRAD            | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify:            | No registered or authorised products   |
| United Kingdom  | <input checked="" type="checkbox"/> MA | <input checked="" type="checkbox"/> TRAD | <input type="checkbox"/> Other TRAD | <input type="checkbox"/> Other Specify:            | Two authorised products (1 of them as a traditional remedy). Two products contain allantoin as well so they are not strictly 'herbal medicinal products. |

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'

This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

According to the information provided by the National Competent Authorities<sup>1</sup>

### Austria

No registered or authorised products.

### Czech Republic

One product containing Symphyti tincture (1:5) was registered from 1994 to 2006. The registration was refused on 22.02.2006 as the marketing authorisation holder was not able to guarantee limited content of pyrrolizidine alkaloids.

### France

#### Preparations:

Liquid extract (DER 1:2, ethanol 60% (V/V)) (100 g of cream contains 35 g of *S. officinale* extract).

#### Pharmaceutical form:

Cream.

#### Posology:

2 to 4 times daily.

<sup>1</sup> Data were collected using the template entitled 'Document for information exchange for the preparation of the assessment report for the development of Community monographs and for inclusion of herbal substance(s), preparation(s) or combinations thereof in the list' (EMA/HMPC/137093/2006)



Indication:

Traditionally used topically as a soothing and anti-pruriginous application for dermatological ailments and as a protective treatment for cracks, grazes, chapped skin and insect bites.

Legal status:

traditional use

Since when is on the market:

2007

**Germany**

Three products based on the extract of *Symphyti radix*.

Preparations (authorised products):

1-3) Liquid extract of *S. officinale* (1:2), extraction solvent: ethanol 60% (V/V), containing 0.13-0.25% sodium hydroxide and 1% PPG-1-PEG-9 Lauryl Glycol Ether.

Pharmaceutical form:

1, 2) Cream.

3) Poultice.

Posology:

1) For cutaneous use for adults and children over 3 years.

100 g cream contain 35 g liquid extract.

Depending on the size of the treated part of the body and the strength of the complaints apply 2-4 times daily approx. 1.2-6 g corresponding to a string of ointment of 4-18 cm length on the affected parts of the body and massage thoroughly.

In case of heavier complaints an ointment bandage could be applied.

Apply once daily 10-20 cm ointment and cover it with adequate bandage material.

Children from 3 to 12 years: Do not use longer than 1 week.

2) For cutaneous use for adults and adolescents over 12 years.

100 g cream contain 35 g liquid extract.

Depending on the size of the treated part of the body and the strength of the complaints apply 2-4 times daily a string of ointment of 2-6 cm length on the affected parts of the body and massage thoroughly.

3) For cutaneous use for adults and adolescents over 12 years.

100 g poultice contain 35 g liquid extract.

The poultices could be applied 1-2 times daily up to 5 h (warm poultices not longer than 2 h). Normally apply cold.

For warm poultices temper the tube in a water bath accordingly.

Coat a moist piece of bandage material approximately 1 mm thick with the paste, put it on the affected part of the body and cover it with a cloth. The fixation can be carried out by a bandage.

To avoid a strong maceration of sensitive skin, a pause of approximately 2-4 h should be observed before the poultice can be renewed.

After few days of application a treatment break of 1-2 days is recommended.

Indication:

1-3) Contusion, strain, sprain.

Legal status:

1-3) Authorised product.

Since when is on the market:

1-3) At least since 1976.

**Hungary**

Preparations:

Traditional herbal medicinal products ("healing products"):

- 1) 50 g solution containing the ethanol extract of 10 g *Symphyti radix* (DER 1:5).
- 2) 30 ml liquid extract containing the ethanol extract of 3 g *Symphyti radix* (DER not known).
- 3) 100 g cream containing 10 g *Symphyti radix* tincture (DER 1:4, solvent not known) + 6 g paraffin oil extract of *Calendulae flos* and *Matricariae flos*.

Herbal medicinal product with well established use:

- 4) 100 g ointment contains 35 g comfrey root liquid extract (*Symphytum officinale*) (DER: 1:2), extraction solvent: ethanol 60% V/V.

Pharmaceutical form:

- 1) Cutaneous liquid.
- 2) Cutaneous liquid.
- 3-4) Cream.

Posology:

- 1) After cleaning the intact skin, depending on the size of the skin surface to be treated, apply 5-15 doses of the doser pump 2-3 times daily on the skin, and massage gently. Not to be applied longer than 4-6 weeks per year.
- 2) For external use only, to rub on the aching part of the body or apply as a compress. Maximum dose: 5 times 5-10 drops daily. Not to be applied longer than 4-6 weeks per year and on children under 2 years of age.
- 3) For topical application. Apply no more than 1-2 times daily. The maximum daily dose is 15 cm of cream. Not to be applied longer than 4-6 weeks per year.
- 4) For topical application. Depending on the size of the part of the body to be treated and the severity of the symptoms, apply a thread of cream of 2-6 cm in length twice to four times daily.

Indication:

- 1) For the treatment of swellings and bruising resulting from closed injuries, contusions, sprains, as an adjuvant treatment after fractures, as an antiphlogistic to treat phlebitis and local dermatitis, to relieve rheumatic pains.
- 2) To relieve the symptoms associated with bruises, sprains and dislocations, and rheumatic pain.
- 3) For the treatment of different closed injuries and sport injuries, bruises, strains, inflammations related to dislocations and sprains, swellings and haematoma, to improve motor functions after injuries. For the treatment of local skin inflammations and phlebitis.
- 4) Treatment of bruises, pulled muscles and ligaments, sprains and painful joints.

Legal status:

- 1-3) Registered traditional herbal medicinal product ("Healing product").
- 4) Authorised herbal medicinal product.

Since when is on the market:

- 1) 03.03.2006.
- 2) 02.12.1999.
- 3) 03.08.1998.
- 4) 27.02.2009.

**United Kingdom**

Preparations:

- 1) 100 g cream containing 35 g of liquid extract (DER 1:2) from comfrey (*Symphytum officinale*) root (extraction solvent: ethanol 60% (V/V)).
- 2) 100 g ointment containing 10 g liquid extract (DER 2:1) from comfrey (*Symphytum officinale*) root (extraction solvent: ethanol 65% (V/V)).

Pharmaceutical form:

- 1) Cream.
- 2) Ointment.

Posology:

- 1) For cutaneous use. Application may only be made to intact skin.

Adults, children aged 12 years and older, and the elderly: If not otherwise prescribed, depending on the size of the joint to be treated and the severity of the symptoms, apply a thread of cream of 2-6 cm in length four times daily for 8 days.

Treatment duration should not exceed 21 days. Not recommended in children under 12 years of age.

- 2) For external use only. Adults (all ages) and children: Bathe the affected areas in warm water and apply the ointment morning and night. Not to be applied over breaks in the skin. Use no longer than ten days at a time.

Indication:

- 1) For the symptomatic treatment of joint pain, sprains, inflammation and strains associated with restricted joint mobility.

2) A traditional herbal remedy used for the symptomatic relief of bruises and sprains.

Legal status:

- 1) Authorised herbal medicinal product as a traditional remedy (based on traditional use).
- 2) Registered herbal medicinal product.

Since when is on the market:

- 1) 2006.
- 2) At least since 1968.

### **1.3. Search and assessment methodology**

Databases assessed and other sources used:

Databases Science Direct, SciFinder, PubMed and Web of Science were searched using the terms [Symphytum], [comfrey] and [pyrrolizidine alkaloid]. Handbooks and textbooks on the topic were also used.

Inclusion and exclusion criteria for literature:

Data concerning other *Symphytum* species other than *S. officinale* L. were excluded.

## **2. Historical data on medicinal use**

### **2.1. Information on period of medicinal use in the Community**

*Symphytum officinale* L. root and other parts of the herb have been valued medicinally for more than 2,000 years. The specific name *officinale* designates its inclusion in early lists of official medicinal herbs. Comfrey has been prepared as a poultice or compress with healing properties for blunt injuries, fractures, swollen bruises, boils, carbuncles, varicose ulcers, and burns. Poultices were also applied to ease breast pain in breastfeeding women. Comfrey, taken internally as a tea or expressed juice, has been used to soothe ulcers, hernias, colitis, and to stop internal bleeding. As a gargle it has been used to treat mouth sores and bleeding gums. The herbal tea has also been used to treat nasal congestion and inflammation, diarrhoea, and to quiet coughing. The hot, pulped root, applied externally, was used to treat bronchitis, pleurisy, and to reduce pain and inflammation of sprains (Longe, 2005).

Comfrey use was first documented by the ancient Romans and Greeks. Around 200 AD, the Greek physician Dioscorides praised the therapeutic uses of comfrey in his book *Materia Medica*, and coined the genus name *Symphytum* from the Greek word *symphuo*, which means "to make to grow together." During the Middle Ages, comfrey in the form of an external poultice became popular for healing broken bones. As the popularity of comfrey grew over the centuries so did its indications for use. Comfrey has been used to treat respiratory problems (bronchitis, catarrh, haemoptysis, pleurisy, whooping cough), gastrointestinal diseases (cholecystitis, colitis, dysentery, diarrhoea, ulcers, haematemesis), metrorrhagia, phlebitis, and tonsillitis. Comfrey has also been touted for its nutritional value; it has been considered a good source of protein and vitamin B12, which is unusual for a plant (Cupp, 2000).

*Symphytum officinale* has been known by many names, including boneset, knitbone, bruise wort, black wort, salsify, ass ear, wall wort, slippery root, gum plant, healing herb, consound, or knit back. The common name comfrey is from the Latin *confirmare* meaning to join together. The herb is named after its traditional folk use in compress and poultice preparations to speed the healing of fractures, broken bones, bruises, and burns (Longe, 2005).

*Symphytum officinale* has a long tradition and is still applied nowadays as an external treatment for inflammatory disorders of joints, wounds, gout, bone fractures, distortions, haematomas and thrombophlebitis. It is also applied as a decoction for oral and pharyngeal gargle. For internal application, comfrey is claimed to benefit gastritis and gastroduodenal ulcers, though its effects have never been demonstrated in controlled investigations. In addition, herbal practitioners recommend comfrey capsules for the treatment of rheumatoid arthritis, bronchitis, various allergies and for diarrhoea, regardless of the pathogenic cause (Stickel and Seitz, 2000).

In the UK, the Medicine Control Agency (now the Medicines and Healthcare products Regulatory Agency (MHRA)) recently included comfrey in a list of herbs under consideration for restriction to physician prescription only (Rode, 2002).

The use of comfrey root in Germany is limited to external products. According to the Commission E, the daily dose should not exceed more than 100 µg pyrrolizidine alkaloids with 1,2 unsaturated necine structure, including its *N*-oxides. The duration of treatment should not be longer than 4-6 week per year (Blumenthal *et al.*, 1998).

In France, the only indication that may be claimed for the comfrey root is as follows: as an adjunct in the emollient and anti-pruriginous treatment of skin disorders, and as a trophic protective agent for cracks, bruises, frostbite and insect bites (Bruneton, 1998).

Outside Europe: the distribution of comfrey in Canada has been restricted; in the USA, the Food and Drug Administration has requested voluntary compliance for removal of products containing comfrey (Rode, 2002).

## **2.2. Information on traditional/current indications and specified substances/preparations**

The Commission E suggest the external use of comfrey root (crushed root, extracts, the pressed juice of the fresh plant for semi-solid preparations and poultices) in case of bruising, pulled muscles and ligaments, and sprains (Blumenthal *et al.*, 1998).

## **2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications**

Ointments or other preparations for external use are made up of 5-20% of the drug and prepared accordingly. According to Blumenthal *et al.*, the daily dose should not exceed more than 100 µg pyrrolizidine alkaloids with 1,2 unsaturated necine structure, including its *N*-oxides. The duration of treatment should not be longer than 4-6 week per year (Blumenthal *et al.*, 1998). However, according to the regulations in Germany, products for cutaneous use with less than 10 µg pyrrolizidine alkaloids in the daily dosage can be used without any limitation on the duration of use (BGA, 1992).

In the British Herbal Pharmacopoeia (1974 and 1983), fresh *Symphytum* root is indicated externally for the treatment of ulcers, wounds, fractures and hernia with the following posology:

Ointment: *Symphytum* root 10–15% root extractive in usual type ointment basis applied topically three times daily.

Although internal use of comfrey is no longer advised, dosages for oral administration for traditional uses were recommended in older standard herbal reference texts. The recommended oral (unless otherwise stated) doses of The British Herbal Pharmacopoeia (1974 and 1983) for the treatment of gastric and duodenal ulcer, colitis and hematemesis were as follows:

Dried root/rhizome: 2-4 g (in the 1983 edition) or 2-8 g (in the 1974 edition) as a decoction three times daily.

Root, liquid extract: 2-4 ml (in the 1983 edition) or 2-8 ml (in the 1974 edition) (1:1 in 25% alcohol) three times daily.

### 3. Non-Clinical Data

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

##### Pharmacodynamics

###### Anti-inflammatory effect

Anti-inflammatory properties are probably mediated through rosmarinic acid, which is also likely to account for the analgesic and astringent effects. These antiphlogistic properties are conveyed not only through inhibition of the arachidonic acid metabolism, but seem also to be the result of inhibitory actions of plant extracts upon the classical and alternative pathway of complement activation. High molecular glycoproteins (>300 kDa) so far uncharacterised have been isolated which seem to interact with the complement factors C<sub>3</sub> and C<sub>4</sub> in a dose-dependent fashion but do not affect factors C<sub>1</sub> and C<sub>2</sub> (Stickel and Seitz, 2000) (Van den Dungen *et al.*, 1991).

In a screening study evaluating 29 traditionally used European herbal drugs used for anti-inflammatory purposes, COX-1 and 2 inhibitory activities of extracts were tested. The n-hexane extract of *S. officinale* roots (50 µg/ml) inhibited markedly COX-1 and COX-2 (56.6% and 79.6%) (Lohmann *et al.*, 2000).

A bioassay-guided fractionation of the aqueous extract from *Symphytum officinale* roots led to the isolation of an antiphlogistic glycopeptide. The compound with an isoelectric point at pH 4.8 was found to contain 16 amino acids as well as galactose, fructose, arabinose and glucose. The calculated molecular mass was approximately 9,000 dalton. On carrageenan-induced rat paw oedema the isolated glycopeptide exerted a remarkable, dose dependent antiphlogistic effect. The ED<sub>50</sub> was 61 µg/kg *p. o.* (control: indomethacin 10 mg/kg). Investigations on the release of arachidonic acid, cyclooxygenase and lipoxygenase metabolites as well as on arachidonic acid induced platelet aggregation indicated that the isolated glycopeptide (0.1-1000 ng/ml) inhibited dose-dependently the release of prostaglandins and leukotriens *via* decreasing the expression of phospholipase A<sub>2</sub>. The cyclooxygenase metabolite thromboxane A<sub>2</sub> was released when arachidonic acid was given as a substrate and therefore the glycopeptide does not represent a COX inhibitor (Hiermann and Writzel, 1998).

In an experiment, the concentration of pyrrolizidine alkaloids in crude *S. officinale* extract was changed by a factor of about 0.1 using protonated cation exchangers. Using an *in vivo* model it could be shown that the reduction of pyrrolizidine alkaloids result in a small but measurable decrease in antiphlogistic efficacy. The model was based on measuring the decrease of redness and pain sensitivity of UV-B irradiated human skin after treatment with the extracts suspended in ointment base. Concomitant with the removal of pyrrolizidine alkaloids, the concentrations of Cu<sup>2+</sup> and Mn<sup>2+</sup> were also reduced. It should be taken into consideration, that these ions are important catalysts for the oxidation of *o*-substituted polyphenols (e.g. caffeic acid derivatives) to pharmacologically more active products (Andres *et al.*, 1990).

The effect of aqueous extract of comfrey leaves on prostaglandin synthesis was investigated on rats by Stamford and Tavares (1983). Dried comfrey leaves were homogenized in Krebs solution, filtered and a stock solution made equivalent to 10 mg dried leaves in 1 ml. The combined gastric corpus and

antral mucosa and muscle from male Wistar rats were cut into small pieces, mixed and washed three times in Krebs solution. Tissue samples were then suspended in Krebs solution or Krebs solution containing comfrey extract. Comfrey extract increased the release of prostaglandin-like material from gastric tissue. Radioassay and high performance liquid chromatography (HPLC) indicated greater outputs of PGF<sub>2α</sub> and 6-keto-PGF<sub>1α</sub>, which is in contradiction with previous experiments carried out with root extracts or compounds.

Rosmarinic acid isolated from *S. officinale* possessed an inhibitory activity on the formation of malondialdehyde in human platelets by the TBA method. The IC<sub>50</sub> for rosmarinic acid was 3.37 mM. Structurally related minor constituents, chlorogenic acid and caffeic acid did not show significant activity in this model (Gracza *et al.*, 1985).

Since there are no data on the human bioavailability of the compounds/extracts studied for anti-inflammatory activity in the presented articles, no conclusion can be drawn for the efficacy of cutaneously applied comfrey products.

Andres *et al.* (1989) evaluated the antiphlogistic efficacy of 10 ointments containing comfrey root extract with an *in vivo* model. Circular erythema was generated using UV-B radiation, which was then treated with the dermatics to be studied. The healing process was monitored by measuring redness and pain sensitivity. The products studied were then characterized by chemical profiles using HPLC and GC/MS including allantoin, caffeic acid derivatives, pyrrolizidine alkaloids, carbohydrates. Though Andres *et al.* state that chemometric methods were used to find correlations between clinical and analytical data, no details are given on these correlations. Similarly, the exact composition of the applied ointments were not published.

Due to the absence of relevant data (concentration of the extracts/compounds, bioavailability data) the presented articles on the anti-inflammatory effect of comfrey cannot be taken into consideration in the assessment of *in vivo* and clinical efficacy of cutaneously applied comfrey products.

#### Wound healing effect

The pharmacological mechanisms are thought to be based partly upon allantoin, which is responsible for the stimulation of connective tissue proliferation and regeneration (Stickel and Seitz, 2000).

In an animal experiment, the crude juice of the leaves of *S. officinale* afforded the cicatrization process by increasing at first (at the 7th, 11th and 14th days) the number of fibroblasts and, in a later phase (after 14 days), the number of collagen fibers in experimental lesions produced in rats. The number of blood vessels was also increased at the 7th day of treatment. On the experimental oedema induced by carrageenin in rat's paws, the crude extract at doses of 150 and 300 mg/kg *per os* showed no effect compared to 75 mg/kg phenylbutazone. Analgesic effect was seen with doses 300 mg/kg *per os* (Goldman *et al.*, 1985). Since these results were obtained with comfrey leaf preparations are not relevant for root extracts.

#### Other effects

In an *in vitro* study, it was found that the total aqueous extract obtained from *S. officinale* roots precipitated human glycoproteins, agglutinated sheep red blood cells (SRBC) and stimulated lymphocyte adherence to nylon fibers. The extract precipitated human gammaglobulins. If the cells were pretreated with rabbit antibodies against SRBC, the extract agglutinated the cells. The adherence of mouse but not human lymphocytes to nylon fibers were stimulated by extract of *Symphytum officinale*. This process was neither stimulated nor inhibited by mannose (Man), galactose (Gal), glucose (Glc), N-acethyl-galactose (GalNAc) and N-acethyl-glucose (Glc-NAc). These biological effects of the extract could be the expression of a lectin-like ability to bind various sugars other than those mentioned (Lenghel *et al.*, 1995).

In an *in vitro* study, 70% ethanol extract of *S. officinale* was concentrated by tangential flow ultrafiltration. In the extract some polyphenolic compounds were identified by HPLC: chlorogenic acid, caffeic acid, ferulic acid, coumaric acid, rutin, rosmarinic acid, luteolin and quercetin. The cytostatic activity of the total plant extract was studied on HeLa cells culture. By comparison to the bystander value of 100%, it was noticed that *in vitro* treatment of HeLa neoplastic cells with the concentrated extracts determined a mitoinhibitory effect with statistical and cytostatical significant amplitude. These values were almost 57.6% for *Symphytum officinale* (Roman *et al.*, 2008).

The antibacterial activity of triterpene saponins isolated from the roots of *Symphytum officinale* was determined using an agar diffusion method (Sener, 1994).

In anaesthetised rats the ethanol extract of *S. officinale* root and the bidesmosidic triterpene glycoside symphytoxide A caused a fall in systolic as well as diastolic blood pressure in a dose-dependent manner. The hypotensive responses of both crude extract and the pure compound were quite similar, very briefly returning to normal within 1 minute while no significant decrease was observed on heart rate (Ahmad *et al.*, 1993).

### **Pharmacodynamic interactions**

No relevant pharmacodynamic interactions have been documented. According to Barnes *et al.* (2007), the potential for preparations of comfrey to interact with other medicines administered concurrently, particularly those with similar or opposing effects, should be considered.

However, regarding the toxicity of comfrey, a study involving rats showed that phenobarbital induces the metabolism of pyrrolizidine alkaloids to their lethal metabolites. In a study using perfused organs, it was shown that some of the pulmonary damage caused by monocrotaline, a pyrrolizidine alkaloid is mediated by hepatic biotransformation of the compound. This metabolism was inducible with phenobarbital (a P-450 type II monooxygenase inducer) pretreatment (Lafranconi and Huxtable, 1984).

Based on the assessed preclinical data the clinical plausibility can not be proven since it is not demonstrated that the preclinically active extracts/compounds are present at the site of action in sufficient concentration to exert their effect.

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

#### **Absorption**

In *ex vivo* experiments, permeation of rosmarinic acid across excised rat skin was about 8 times higher from alcoholic solution than from water, indicating that ethanol may act as a sorption promoter. The flux from water or alcoholic solution was 4.4 or 10 micrograms/cm<sup>2</sup>/h, and the lag time (t<sub>lag</sub>) was 7.8 or 3.7 h, respectively. Upon topical administration of rosmarinic acid in form of a W/O (water in oil) ointment (25 mg/kg, 50 cm<sup>2</sup>), the absolute bioavailability was 60% (Ritschel *et al.*, 1989).

The pharmacokinetic properties of pyrrolizidine alkaloids have not been investigated systematically, but data about single compounds which might have a representative character are available. After oral administration of tritiated senecionine and seneciphylline to lactating rats, maximum blood levels were reached in less than 1 h (De Smet *et al.*, 1992)

The absorption of comfrey pyrrolizidine alkaloids through unbroken skin is very low. An analysis of a commercial sample of *Symphyti radix* originating from Poland with a total alkaloid content of 0.07% revealed the presence of 7 pyrrolizidine alkaloid-*N*-oxides: 7-acetyl intermedine, 7-acetyl lycopsamine as the main constituents and lycopsamine, intermedine, symphytine and traces of 2 not further identified alkaloids. The percutaneous absorption of these alkaloids was investigated in rats, using a



crude alcoholic extract of the plant corresponding to a dose of 194 mg alkaloid-*N*-oxides/kg body weight. The separation and identification of the alkaloids was carried out by GC-MS from urine samples. Quantification was made by peak integration. Metabolites of para-aminosalicylic acid (Pas) were also measured. The excretion of *N*-oxides in the urine during 2 days was in the range of 0.1-0.4% of the dose. The dermally absorbed *N*-oxides were not or only to a small extent converted to the free alkaloids in the organism. The oral application led to a 20-50 times higher excretion of *N*-oxides and free alkaloids in the urine (3.4-9.4% vs. 0.08-0.41%) (Brauchli *et al.*, 1982).

### **Metabolism, elimination**

The pyrrolizidine alkaloids are not toxic until they are metabolized in the liver. Biotransformation of pyrrolizidine alkaloids involves *N*-oxidation to *N*-oxides (detoxification), hydrolysis by esterases of diesters to monoesters and finally to free necic acid and necic bases (detoxification), and dehydrogenation to toxic pyrrolic ester dehydroalkaloids (toxification) (Coulombe, 2003). Pyrrolic esters are further metabolized to toxic pyrrolic alcohols (dehydroamino alcohols). The major pyrrolizidine alkaloids in comfrey contain structural features which favour activation by oxidative dehydrogenation (Abbott, 1988). The parent pyrrolizidine alkaloids are nontoxic, and the development of hepatic damage requires oxidative metabolism of the pyrrolizidine alkaloids by the cytochrome P450 oxidase system (e.g., CYP3A4, CYP2B6) to the hepatotoxic pyrrolic-like compounds (Barceloux, 2008). Support for the cytochrome P450-mediated activation of pyrrolizidines into their corresponding toxins comes from the observation that potent microsomal enzyme inducers, such as the anticonvulsant phenobarbital, can enhance the toxicity of pyrrolizidines (Stickel and Seitz, 2000; Lafranconi and Huxtable, 1984).

In an *in vitro* experiment, two pyrrolizidine alkaloids and one pyrrolizidine alkaloid-*N*-oxide were incubated with microsomal preparations. The alkaloids were converted to dehydroretronecine, the putative toxic metabolite, by both rat and human microsomal preparations. In addition, alkaloid-*N*-oxides, the major detoxication products from pyrrolizidine alkaloids, were also formed. The pyrrolizidine alkaloid-*N*-oxide was converted to both dehydroretronecine and the parent alkaloid. This suggests that the toxicity of pyrrolizidine alkaloid-*N*-oxides could be greater than suggested hitherto as a result of conversion to the toxic metabolite via the parent alkaloid. (Couet *et al.*, 1996a) The highly reactive dehydropyrrolizidine alkaloids (pyrrolic esters) either form adducts with cell constituents or undergo further hydrolysis to dehydroamino alcohol compounds (pyrrolic alcohols) (Barceloux, 2008). Both the pyrrolic esters (or dehydroalkaloids; primary metabolites) and the pyrrolic alcohols (or dehydroamino alcohols; secondary metabolites) have antimitotic effects and are responsible for the damage to cells in the liver (Abbott, 1988) (Cupp, 2000). The latter compounds are relatively stable and can be distributed throughout the body (Abbott, 1988).

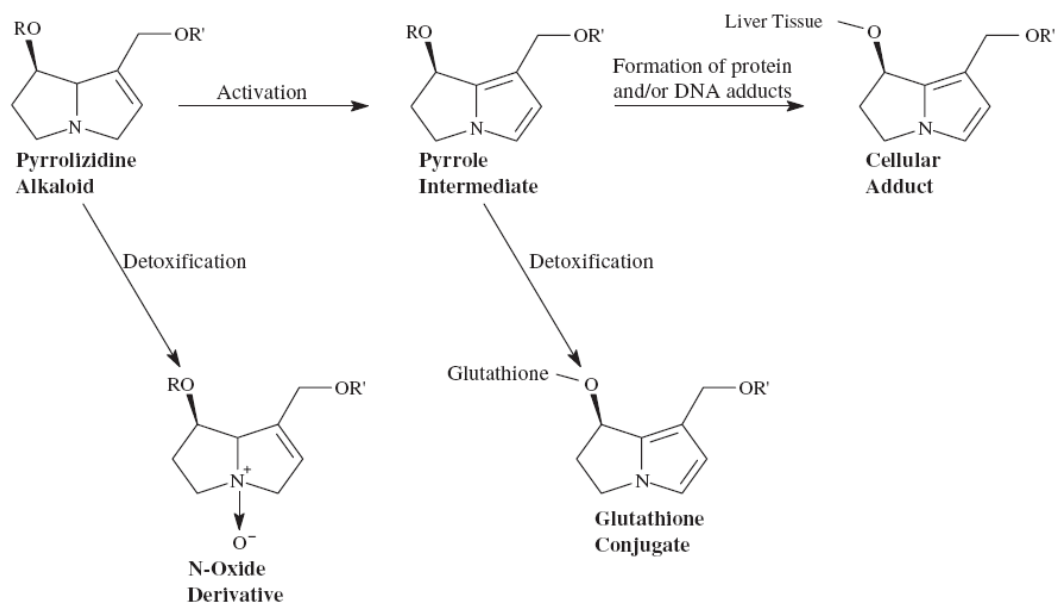


Figure 2. Activation and biotransformation of pyrrolizidine alkaloids (Barceloux, 2008).

Detoxification involves also conjugation of the pyrrole intermediate with glutathione. Cleavage of pyrrolizidine alkaloids by nonspecific blood esterases produces necine compounds and necic acids, which ultimately appear in the urine as conjugates (Barceloux, 2008). *In vitro* studies indicate that glutathione and *N*-acetylcysteine detoxify pyrrolizidine metabolites by forming water-soluble conjugates that are excreted by the kidney (Yan *et al.*, 1995). Animal studies indicate that the elimination of pyrrolizidine compounds is relatively rapid with most of the metabolites appearing in the urine within 24 h after exposure (Barceloux, 2008).

No data is available on the distribution of pyrrolizidine alkaloids into human breast milk. However, rats suckled by mothers fed lasiocarpine, a pyrrolizidine alkaloid found in comfrey, developed liver damage (Cupp, 2000). After oral administration of tritiated senecionine and seneciphylline to lactating rats, radioactivity was excreted into the milk with concentrations 50% less compared to the blood concentrations. After 6 h 83% of the radioactive necine bases remaining in the blood were not dialyzable, indicating a tight (possibly covalent) binding to macromolecules, such as albumin. Six h after administration of the pyrrolizidine alkaloids the highest concentrations were detected in the liver and lungs of the rats (De Smet *et al.*, 1992). A study performed with lactating mice, using the same pyrrolizidine alkaloids but  $^{14}\text{C}$ -labeled and injected via the intraperitoneal route, showed that 66-75% of the radioactivity was excreted in the urine, 14-18% in the faeces, 1.14% in the milk of the animals, and 0.2-0.5% was expired as  $\text{CO}_2$ . The highest concentrations of radioactivity were found in the liver (De Smet *et al.*, 1992).

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

The therapeutic application of comfrey is overshadowed by the well-recognised toxicity of pyrrolizidine alkaloids (Barnes *et al.*, 2007). Though there are no data on the acute toxic effects of *Symphytum*, the chronic ingestion of the plant material is toxic due to its pyrrolizidine alkaloids content. In the liver they are dehydrogenated to pyrrole derivatives, which then act as potent alkylating agents. They react with bases in the deoxyribonucleic acid (DNA) strand, cross-linking strands and causing strand breakage. Studies in rats have supported the hepatotoxic, carcinogenic, and teratogenic role of comfrey root. In humans, a form of Budd-Chiari syndrome known as veno-occlusive disease has been

the primary concern. Clinical manifestations are hepatomegaly and refractory ascites, often progressing to hepatic failure. Untreated, there is a high mortality rate (Kaufman *et al.*, 1999).

In general, the pyrrolizidine alkaloids accumulate in the plant as polar *N*-oxides, facilitating their transport, and above all, maintaining them in a non-toxic form. The *N*-oxides are easily changed back to the tertiary amines by mild reduction, as will occur in the gut of a herbivore (Dewick, 2002).

Despite the remarkable pyrrolizidine alkaloid content of *S. officinale*, serious livestock poisoning episodes are mentioned in literature from pyrrolizidine alkaloid-plants other than common comfrey, especially some *Senecio* species (Aniszewski, 2007).

### Hepatotoxicity

Many pyrrolizidine alkaloids are known to produce pronounced hepatic toxicity and there are many recorded cases of livestock poisoning. Although themselves non-toxic, these alkaloids are transformed by mammalian liver oxidases into reactive pyrrole structures, which are potent alkylating agents and react with suitable cell nucleophiles, e.g. nucleic acids and proteins (Dewick, 2002).

#### Pyrrolizidine alkaloids

Most hepatotoxic pyrrolizidines are cyclic diesters (e.g. retrorsine, senecionine), and the structural requirement for pyrrolizidine toxicity is the presence of an unsaturated pyrrolizidine ring. Esterification of necine compounds containing a double bond in the 1,2 position causes the formation of toxic alkaloids. All toxic pyrrolizidine alkaloids are ester derivatives of 1-hydroxymethyl-1,2-dehydropyrrolizidine with variable esterification at positions C-1 and C-7 (Barceloux, 2008). The potential of pyrrolizidine alkaloid compounds as hepatotoxins is governed by certain minimum structural features: (1) an unsaturated 3-pyrroline ring, (2) one or two hydroxyl groups each attached to the pyrroline ring, (3) one or preferably two esterified groups and (4) the presence of a branched chain on the acid moiety (Figure 3.) (Prakash *et al.*, 1999). The 1,2-double bond greatly facilitates aromatization of the B-ring by the mixed function oxidases of the liver by chemical oxidants, while the esterification of hydroxyl groups at C-9 and C-7 causes the metabolite so formed to be a powerful alkylating agent (Culvenor *et al.*, 1976).

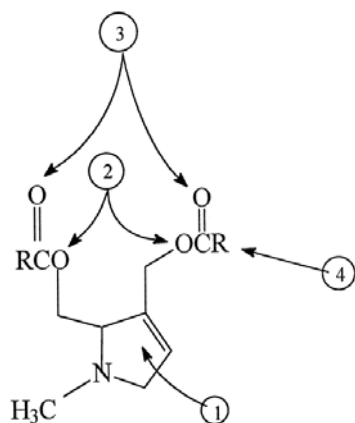


Figure 3. Structural features essential for pyrrolizidine alkaloid toxicity.

Reactive dehydroalkaloid (pyrrolic) intermediates resulting from the metabolism of pyrrolizidine compounds are potent electrophiles, bifunctional alkylating agents that can cross-link with cellular components (e.g., DNA, RNA, proteins, amino acids, glutathione). These adducts are responsible for local cytotoxic and antimetabolic effects (Barceloux, 2008). As the pyrrole intermediates are highly reactive, the majority is trapped shortly after its formation in the liver. A correlation exists between the ability of pyrrolizidine alkaloids to bind to the macromolecules of the liver and their hepatotoxicity. Most effective in this way are macrocyclic pyrrolizidine alkaloid diesters, which have been shown directly to bind covalently to liver constituents (De Smet *et al.*, 1992). The reactive pyrrole intermediates produce

nonthrombolytic obliteration of small hepatic veins that may progress to cirrhosis and liver failure. Dehydroalkaloid intermediates with longer half-lives (e.g., retrorsine, seneciphylline) may cause damage in the lungs (e.g., pulmonary endothelial cells, type II pneumocytes) similar to monocrotaline and fulvine. Upon metabolic activation, pyrrolizidine alkaloids exhibit a variety of genotoxic properties including DNA binding, DNA-protein cross-linking, sister chromatid exchange, chromosomal

aberrations, and carcinogenicity. The most potent genotoxic pyrrolizidine alkaloids are macrocyclic diesters (retronecine, heliotridine, otonecine) (Barceloux, 2008).

Enzymatically converted alkaloid metabolites may be highly reactive electrophilic compounds capable of reacting with cellular structures, forming adducts which may cause acute or chronic toxicity. Some of these adducts may be persistent in tissues and may re-induce damage long after the initial time point of ingestion (Prakash *et al.*, 1999).

Culvenor *et al.* (Culvenor *et al.*, 1976) reported that diesters of heliotridine and retronecine are four times as toxic as the respective monoesters, and heliotridine esters are 2-4 times as toxic as retronecine esters. These structure-toxicity relationships place comfrey pyrrolizidine alkaloids (retronecine mono and diesters) in a class of lower toxicity compared with the pyrrolizidine alkaloids implicated in human poisonings that have occurred worldwide owing to *Senecio*, *Heliotropium* and *Crotalaria* (heliotridine diesters and macrocyclic diesters of retronecine) (Rode, 2002).

The mechanism of hepatotoxic activity of pyrrolizidine alkaloids is connected to metabolism in the parenchymal cells, where pyrrolizidine alkaloids change to pyrroles acting on hepatocytes and blood vessels in the liver or lungs (McLean, 1970). It has been reported that as a consequence of this, disaggregation of polyribosomes, absence of pyruvate oxidation and lysosomal activity and necrosis occur (Aniszewski, 2007).

The primary toxicity of pyrrolizidine alkaloids is a sinusoidal obstruction syndrome similar to the hepatotoxicity associated with bone marrow transplantation. During the acute phase of pyrrolizidine-induced hepatotoxicity, sinusoidal endothelial cells, central venular endothelial cells, and hepatic parenchymal cells undergo degeneration followed by a subacute phase consisting of fibrotic occlusion of central and sublobular veins along with sinusoidal fibrosis (Copple *et al.*, 2003).

Based on animal studies, the toxicity of pyrrolizidine alkaloids varies with individual compounds and animal species (e.g., pigs, chickens, and rats are much more sensitive than mice and sheep) (Barceloux, 2008). The acute toxicity of pyrrolizidine alkaloids varies widely; it is recognized by the International Programme on Chemical Safety (IPCS) that for rats the LD<sub>50</sub> of most alkaloids is 34-300 mg/kg. Lasiocarpine doses equivalent to 0.2 mg/kg body weight per day lead to the development of tumours in rats. Some pyrrolizidine alkaloids are thought to cause lung damage, affect blood pressure and lead to secondary effects of the functioning of the right side of the heart. Moreover, according to the WHO data, pyrrolizidine alkaloids produce chromosomal aberrations in mammalian cells (Aniszewski, 2007).

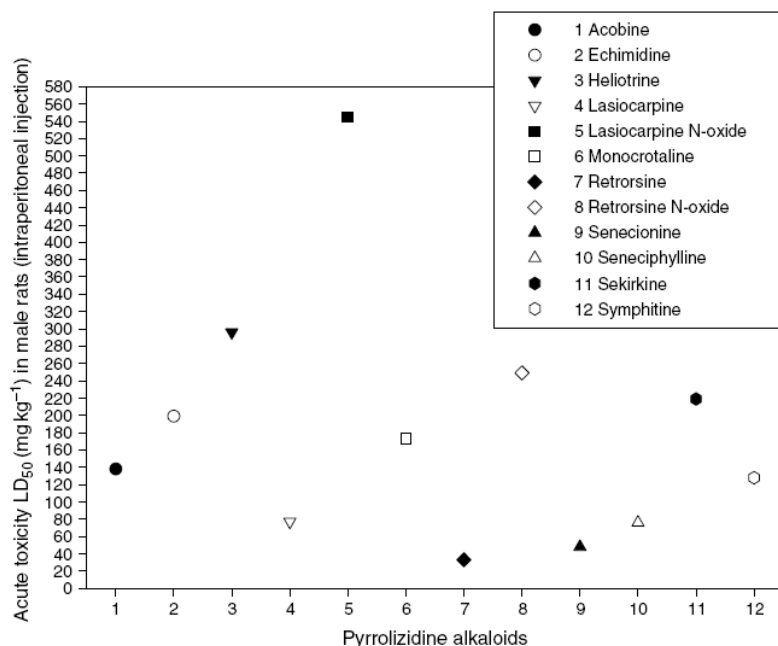


Figure 4. Acute toxicity (LD<sub>50</sub>) of some pyrrolizidine alkaloids in male rats (Aniszewski, 2007).

Even in the 1940s, it was reported that senecionine produces necrosis in the periportal and midzonal areas of liver lobules of monkeys. However, other species, including guinea pigs are resistant to pyrrolizidine alkaloid toxicity. Esterase hydrolysis was observed in the metabolism of the guinea pig, and in the case of rats, there was no esterase activity. This explains the guinea pig's resistance to pyrrolizidine alkaloid toxicity (Aniszewski, 2007).

#### Herbal substance

In one study, the activity of various hepatic drug-metabolising enzymes in liver homogenates was examined. Three groups of six male Long-Evans rats were fed a 5%, 10%, or 30% comfrey diet ad libitum for 3 weeks (Garrett *et al.*, 1982). The activity of aminopyrine *N*-demethylase was found to be increased, but the activity of glutathione S-transferase and epoxide hydrolase was not affected by comfrey. Epoxide hydrolase activity has previously been reported to be increased by carcinogens, and is thought to play a role in the neoplastic process (Cupp, 2000).

Yeong *et al.* studied the effect of high and lower doses of pyrrolizidine alkaloids on rat liver. In their initial study (Yeong *et al.*, 1991) three groups of rats were fed with pyrrolizidine alkaloid fraction derived from *Symphytum × uplandicum* roots and leaves. The presence of pyrrolizidine alkaloids was confirmed by thin layer chromatography; however, no data were published on the composition of the extract. Group I animals received a single dose of 200 mg/kg, group II 100 mg/kg 3 times a week for 3 weeks and group III 50 mg/kg 3 times a week for 3 weeks. All rats showed light and electron-microscopic evidence of liver damage, the severity of which was dose dependent. There was swelling of hepatocytes and hemorrhagic necrosis of perivenular cells. There was a concomitant loss of sinusoidal lining cells with disruption of sinusoidal wall and the sinusoids were filled with cellular debris, hepatocyte organelles and red blood cells. Extravasation of red blood cells was evident. Terminal hepatic venules were narrowed by intimal proliferation, and in groups II and III, reticulin fibres radiated from these vessels.

In their subsequent study, Yeong *et al.* (1993) applied relative lower doses of the same extract. Eight young adult rats were dosed weekly for six weeks with 50 mg/kg of comfrey derived alkaloids. The animals were dissected one week after the last dose and the livers examined by light and electron microscopy. Changes at the light microscopic level showed vascular congestion, mild zone 3 necrosis

and loss of definition of hepatocyte cellular membranes. Extensive ultrastructural abnormalities were identified in the form of endothelial sloughing and the loss of hepatocyte microvilli. A striking finding was florid bleb formation on the sinusoidal borders of hepatocytes. Many blebs were shed into the space of Disse and extruded to fill, and sometimes occlude, sinusoidal lumina. Platelets were frequently found in areas of bleb formation. There was evidence of late damage in collagenisation of Disse's space. Hepatocyte bleb formation is known to occur under a variety of pathological conditions but there is little to no information in the literature on the effects, if any, of bleb formation on fibrogenesis and the microcirculation and its role in the pathogenesis of liver disease.

Despite the remarkable pyrrolizidine alkaloid content of *S. officinale*, serious livestock poisoning episodes are mentioned in literature from pyrrolizidine alkaloid-plants other than common comfrey, especially some *Senecio* species (*Senecio riddellii*, *Senecio douglasii* and *Senecio jacobaea*). There are also known cases of animal poisoning from pyrrolizidine alkaloids found in *Cynoglossum officinale* (Boraginaceae) (Aniszewski, 2007).

## **Mutagenicity**

### Pyrrolizidine alkaloids

Mutagenic and carcinogenic effects of pyrrolizidine alkaloids are attributable to their electrophilic feature and reaction with DNA. Mutagenicity has been demonstrated by many pyrrolizidine alkaloids using several standard tests. Positive results have been obtained in assays in *Drosophila melanogaster* and *Salmonella typhimurium*, as well as in mammalian cell culture. Pyrrolizidine alkaloids have been shown to induce both point mutations and chromosomal aberrations, as well as being able to induce sister chromatid exchange and "unscheduled" DNA synthesis in mammalian cell culture (Mattocks, 1986; Cupp, 2000; Abbott, 1988). A variety of pyrrolizidine alkaloids (clivorine, petasitenine, heliotrine, lasiocarpine, ligularidine, and senkirinine) have been demonstrated to be mutagenic in the Ames assay. However, lycopsamine, monocrotaline, retronecine, senecionine, and seneciphylline were negative in this assay, even though some of these compounds were demonstrated to be carcinogenic in rodents. In addition, in V79 Chinese hamster fibroblasts in culture, heliotrine, lasiocarpine, petasitenine, and senkirinine induced chromosomal aberrations and mutations; the effect was increased after addition of rat liver microsomes (De Smet *et al.*, 1992). In primary rat hepatocytes, monocrotaline, senecionine, seneciphylline, epoxyseneciphylline, jacobine, senecicannabinine, senkirinine, petasitenine, acetylpetasitenine, syneilesine, clivorine, dihydroclivorine, neoligularidine, ligularidine, and ligularizine induced DNA repair, which indicates a DNA damaging effect. In this experiment, the saturated ligularinine and retronecine (missing the activating necic acid moiety) were negative (De Smet *et al.*, 1992).

Mutagenic effects of certain pyrrolizidine alkaloids (heliotrine, lasiocarpine, monocrotaline, senecionine, jacobine, fulvine, retrorsine, and isatidine) have also been demonstrated *in vivo* using *Drosophila melanogaster* (De Smet *et al.*, 1992).

### Herbal substance

Mei *et al.* (2005) evaluated the mutagenicity of comfrey in the liver *cII* gene of Big Blue rats. To determine an appropriate dose for treatment, a preliminary experiment was conducted by feeding diets containing 2, 4 and 8% comfrey. Based on a minimum effect on weight gain, lack of overt toxicity to the liver, and a maximum effect on mutagenicity, a diet containing 2% comfrey root was chosen for the mutagenesis experiment. Groups of six 6-week-old male Big Blue rats were fed either a basal diet or the comfrey diet. The animals were killed after 12 weeks of treatment. Mutant frequencies (MFs) were determined for the liver *cII* gene of the rats treated with comfrey. The MF for rats fed comfrey was  $146 \pm 15 \times 10^{-6}$ , which was significantly greater than the MF for control rats,  $30 \pm 16 \times 10^{-6}$ . This result suggests that comfrey induces liver tumours by a genotoxic mechanism. The mutational spectrum from

comfrey-treated rats suggests that pyrrolizidine alkaloids in the plant are responsible for mutation induction and tumour initiation in rat liver.

In a subsequent study, Mei *et al.* (2006) identified comfrey-induced gene expression profile in the livers of rats. Groups of 6 male transgenic Big Blue rats were fed a basal diet and a diet containing 8% comfrey roots, a dose that resulted in liver tumours in a previous carcinogenicity bioassay. The animals were treated for 12 weeks and sacrificed one day after the final treatment. A rat microarray containing 26,857 genes was used to perform genome-wide gene expression studies. Dietary comfrey resulted in marked changes in liver gene expression, as well as in significant decreases in the body weight and increases in liver mutant frequency. When a two-fold cut off value and a P-value less than 0.01 were selected, 2,726 genes were identified as differentially expressed in comfrey-fed rats compared to control animals. Among these genes, there were 1,617 genes associated by Ingenuity Pathway Analysis with particular functions, and the differentially expressed genes in comfrey-fed rat livers were involved in metabolism, injury of endothelial cells, and liver injury and abnormalities, including liver fibrosis and cancer development.

The methanol extract of *S. officinale* roots was investigated by Behninger *et al.* (1989) for its chromosome-damaging effect in human lymphocytes *in vitro*. In concentrations of 1.4 µg/ml and 14 µg/ml the extract had no effect; in concentrations of 140 µg/ml and 1400 µg/ml sister chromatid exchanges (SCE) as well as chromosome aberrations occurred. Additionally, the influence of rat liver enzymes (S9) was tested. The SCE-inducing capacity and the clastogenic effect of the *Symphytum* extract was increased by simultaneous application of S9-mix.

Furmanowa *et al.* (1983) investigated the mutagenic effect of 3 alkaloidal fractions of *S. officinale* roots, aqueous extract of roots and the alkaloid lasiocarpine. Mitotic index and chromosomal aberrations were measured on the lateral roots of *Vicia faba* L. var. *minor*. Alkaloid fraction I had antimutagenic and mutagenic effects, fraction II showed no such effects, fraction III had only antimutagenic action. The alkaloid fraction I containing lasiocarpine had a stronger mutagenic effect than lasiocarpine alone (metaphases with chromosomal aberrations: 18.9% and 4.9-7.5%, respectively; the concentration of lasiocarpine was of the same magnitude:  $10^{-3}$  M). Aqueous extracts had similar mutagenic effects (metaphases with chromosomal aberrations: 4.2-6.1%).

Acetone extract of common comfrey herb was evaluated for mutagenic activity with Ames test utilising tester strains TA98 and TA100 and in the presence and absence of induced liver microsomes. The extract produced toxic responses that were abolished in the presence of the microsomal bioactivation system S-9 mix (White *et al.*, 1983).

Chou and Fu (2006) determined that the metabolism of tumorigenic pyrrolizidine alkaloids (riddeliine, intermedine, symphytine, lycopsamine, senecionine, lasiocarpine, heliotrine, senkirkine, clivorine) resulted in the formation of a set of 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizidine (DHP)-derived DNA adducts. From the compounds listed above, symphytine, intermedine, lycopsamine, lasiocarpine can be found in common comfrey. It was also shown that DHP-derived DNA adducts were also formed in the liver of rats treated orally with comfrey root extract, comfrey compound oil and comfrey leaves (all in forms of dietary supplements). Rats were treated by oral gavage with dietary supplements for three consecutive days. The doses were ten-fold greater than the recommended human daily oral dose of the commercial products based upon an estimated 60-kg human body weight. As positive control, riddeliine at 1.0 mg/kg/day was applied for 3 consecutive days. DHP is a reactive metabolite of pyrrolizidine alkaloids. The quantity of pyrrolizidine alkaloids and the quantity of formed DHP is proportional. Compared with the positive control group ( $1350 \pm 127$  adducts/ $10^8$  nucleotides), the livers of rats gavaged with the commercial comfrey root extract and comfrey compound oil contained  $22.0 \pm 3.8$  and  $31.9 \pm 5.1$  adducts/ $10^8$  nucleotides, respectively. In case of pyrrolizidine alkaloid-free comfrey leaf products, DHP-derived DNA adducts were not detected. It was also found

that the levels of DHP-derived DNA adducts in liver cells, hepatocytes, and endothelial cells of rats treated with riddelliine correlated with the incidence of hepatocellular sarcoma and haemangiosarcoma (Chou and Fu, 2006).

Comfrey root liquid extract (liquid extract from fresh *S. officinale* root; extraction solvent: ethanol 60% (V/V), DER 1:2, pyrrolizidine alkaloid content <1 ppm) was investigated for its ability to induce gene mutations in the bacterial reverse mutation assay (Ames test) in *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 with and without metabolic activation using the mammalian microsomal fraction S9 mix (liver microsomal fraction derived from male Wistar rats) and plated on selective medium according to the direct plate incorporation and the pre-incubation method. Reference mutagens (4-NOPD, 2-AA, NaN<sub>3</sub>, MMS) were used to check the validity of the experiments. Comfrey root fluid extract showed no biologically relevant increases in revertant colony numbers of any of the five tester strains in 6 different concentrations (0.0306-5 µl/plate), neither in the presence nor in the absence of metabolic activation. The reference mutagens induced a distinct increase of revertant colonies indicating the validity of the experiments. In conclusion, the comfrey root fluid extract contained in Kytta-Salbe® f and Kytta-Plasma® f was not mutagenic in the bacterial reverse mutation assay (Benedek *et al.*, 2010).

### **Carcinogenicity**

While there is no evidence of cancer in the literature concerning domestic animals exposed to pyrrolizidine alkaloids, studies carried out under laboratory conditions have been able to produce pyrrolizidine alkaloid-induced cancer in rodents. Some of the plant species known to cause cancer in rodents are *Symphytum officinale*, *Senecio longilobus*, *Petasites japonicus*, *Tussilago farfara* *Farfugium japonicum*, *Ligularia dentata* and *Senecio cannabifolius*. Further, individual pyrrolizidine alkaloid compounds such as monocrotaline, heliotrine, lasiocarpine, clivorine, petasitenine and riddelliine have also been shown to be carcinogenic in experimental animals (Prakash *et al.*, 1999).

#### Herbal substance

Long-term studies in animals (usually rats) have shown that comfrey is carcinogenic (Cupp, 2000). Guo *et al.* (2007) suggested that the carcinogenicity of comfrey results from pyrrolizidine alkaloid. To confirm this hypothesis, the expression of genes and processes of biological functions that were altered by comfrey (mixture of the plant with pyrrolizidine alkaloids) and riddelliine (a prototype of carcinogenic pyrrolizidine alkaloid) in rat liver for carcinogenesis was compared in their study. Groups of 6 Big Blue Fisher 344 rats were treated with riddelliine at 1 mg/kg body weight by gavage five times a week for 12 weeks or fed a diet containing 8% comfrey root for 12 weeks. Animals were sacrificed one day after the last treatment and the livers were isolated for gene expression analysis. The gene expressions were investigated using Applied Biosystems Rat Whole Genome Survey Microarrays and the biological functions were analysed with Ingenuity Analysis Pathway software. Although there were large differences between the significant genes and between the biological processes that were altered by comfrey and riddelliine, there were a number of common genes and function processes that were related to carcinogenesis. There was a strong correlation between the two treatments for fold-change alterations in expression of drug metabolising and cancer-related genes. These results suggest that the carcinogenesis-related gene expression patterns resulting from the treatments of comfrey and riddelliine are very similar, and pyrrolizidine alkaloids contained in comfrey are the main active components responsible for carcinogenicity of the plant.

In a study published in 1978 (Hirono *et al.*, 1978a), seven groups of inbred strain ACI rats were fed dried comfrey leaves (*S. officinale*) or dried comfrey roots (*S. officinale*) over a 480 to 600 day period. Three groups of rats consisting of 19-28 rats were fed comfrey leaves as 8-33% of their diet, and four groups consisting of 15-24 rats were fed comfrey roots as 0.5-8% of their diets. A control group was fed a normal diet. All groups of rats fed comfrey roots or leaves developed hepatocellular adenomas



(metastatic foci were not observed), while the rats in the control groups did not develop liver tumours. The results also showed that the highest incidence of liver tumours occurred in those rats being fed comfrey roots (Hirono *et al.*, 1978a).

Gomes *et al.* (2007) investigated the effects of chronic oral treatment of rats with 10% comfrey ethanolic extract in a 'resistant hepatocyte model' (RHM). In this model, it is possible to observe easily the phenomena related to the early phases of tumour development, since pre-neoplastic lesions (PNLs) rise in about 1-2 months of chemical induction. Wistar rats were sequentially treated with *N*-nitrosodiethylamine (intraperitoneal) and 2-acetilaminofluorene (*per os*), and submitted to hepatectomy to induce carcinogenesis promotion. Macroscopic/microscopic quantitative analysis of PNL was performed. Comfrey treatment reduced the number of pre-neoplastic macroscopic lesions up to 1 mm, the percentage of oval cells and mitotic figures, as well as the number of Proliferating Cell Nuclear Antigen (PCNA) positive cells and acidophilic pre-neoplastic nodules. On the other hand, the percentage of cells presenting megalocytosis and vacuolar degeneration was increased. Scores of fibrosis, glycogen stores and the number of nucleolus organizing regions were not altered. The study indicated that oral treatment of rats with 10% comfrey alcoholic extract reduced cell proliferation in this model.

Hirono *et al.* (1978b) concluded that the highest incidence of tumours may be attributed to the regimen of normal and 0.5% comfrey root diets alternately administered at 3-week intervals after a 1% comfrey diet was given for a long period. Their finding was in accordance with the observation of McLean (1970) that pyrrolizidine alkaloids produce tumours most frequently when doses were interrupted or ceased altogether several months before the death of the animal.

#### Pyrrolizidine alkaloids

A study published almost 1 year later showed very similar results. Twenty rats were injected with 13 mg/kg of symphytine (a pyrrolizidine alkaloid common in comfrey) (10% of the LD<sub>50</sub>) extracted from dried comfrey roots, while a control group received intraperitoneal injections of 0.9% sodium chloride (Hirono *et al.*, 1978b). Of the rats injected with symphytine, four developed liver tumours; three developed hemangioendothelial sarcomas, and one developed liver cell adenoma. The rats in the control group developed no liver tumours. This pattern of carcinogenicity was similar to that seen in the previous study using comfrey leaves.

Lasiocarpine is another carcinogenic pyrrolizidine alkaloid of *S. officinale*. An experiment conducted by Rao and Reddy (1978), involved feeding 20 male inbred strain F-344 rats lasiocarpine at a concentration of 50 ppm over 55 weeks. Ten control rats were fed a diet without lasiocarpine. At the end of 59 weeks, necropsies were performed on all animals. None of the control rats had any abnormalities based on light and electron microscopy. Of the 20 experimental rats, 17 developed malignancies, 45% (nine) developed angiosarcomas while 35% (seven) developed hepatocellular carcinomas. One rat developed malignant adnexal tumour of the skin and one developed lymphoma. Four rats with angiosarcoma developed lung metastases while one rat with hepatocellular carcinoma developed lung metastases.

In the study carried out by Rao and Reddy (1978), lasiocarpine, a compound having both antimetabolic and carcinogenic properties was applied. Although it is claimed that treatment of lasiocarpine has to be interrupted for the expression of carcinogenic properties, in the experiment of Rao and Reddy tumours were developed even when the animals were on continuous treatment with lasiocarpine.

#### **Pregnancy, lactation**

A study with heliotrine injected at doses 15-300 mg/kg to pregnant rats in the second week of gestation produced abnormalities in the litters only at doses which affected the dams as well. Similar results were obtained in another study with heliotrine and its toxic metabolite dehydroheliotrine,

moreover the pyrrole metabolite was five times as effective as the corresponding alkaloid. Teratogenic effects of heliotrine have also been demonstrated in *Drosophila* larvae fed with low doses of the pyrrolizidine alkaloid (De Smet *et al.*, 1992).

Conflicting data are available regarding the toxicity of pyrrolizidine alkaloids on embryonic livers. On the one hand, foetal liver seems to be more resistant to toxic pyrrolizidine alkaloids than maternal ones; on the other hand, however, lasiocarpine given at doses of 35 mg/kg to pregnant rats on days 13 and 17 of pregnancy was harmful for the foetal livers without affecting the mother (De Smet *et al.*, 1992).

### **3.4. Overall conclusions on non-clinical data**

The non-clinical data concerning the pharmacology of *S. officinale*, including the studies on the anti-inflammatory effects of the extracts, probably account at least in the supposed therapeutic value of the preparations in indications specified in section 2. However, little is known about the pharmacologically active constituents of comfrey and the mechanism of action.

Although *Symphyti radix* contains pyrrolizidine alkaloids that (either in pure form or in an alkaloid rich comfrey extract) exhibited hepatotoxic, carcinogenic and mutagenic activities in preclinical studies, pharmacokinetic studies suggest that the cutaneous application of the *S. officinale* extracts result in very low absorption of these compounds through the skin. Based on these results, the therapeutic cutaneous application of the *Symphyti radix* extracts with low pyrrolizidine alkaloid content is expected to be safe. Moreover, the special extract (liquid extract from fresh *S. officinale* root; extraction solvent: ethanol 60% (V/V), DER 1:2, pyrrolizidine alkaloid content <1 ppm) contained in the products applied in the clinical studies showed no biologically relevant increase in revertant colony numbers and consequently was absent of mutagenic effect in Ames tests carried out according the Organisation for Economic Co-operation and Development (OECD) and European Medicines Agency (EMA) guidelines (Benedek *et al.*, 2010; OECD, 1997; EMA, 2008).

## **4. Clinical Data**

### **4.1. Clinical Pharmacology**

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

No relevant data available.

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

Upon ingestion, pyrrolizidine alkaloids are absorbed mainly through the small intestine and are carried to the liver. The most hydrophobic pyrrolizidine alkaloids are readily excreted unchanged in the urine within a 24 hour period while less hydrophobic pyrrolizidine alkaloids are metabolized by cytochrome P450s and flavin monooxygenase enzymes. The metabolites are eliminated as soluble glutathione and other conjugates in the bile and urine (Wexler, 2005).

## 4.2. Clinical Efficacy

### 4.2.1. Dose response studies

According to the available literature, no dose-finding studies have been conducted with *Symphyti radix*.

### 4.2.2. Clinical studies (case studies and clinical trials)

Study 1.: randomized, controlled.

Koll *et al.* (2004) investigated the percutaneous efficacy of an ointment of comfrey extract in a double-blind, multicenter, randomized, placebo-controlled, group comparison study on patients suffering from unilateral acute ankle sprains (n=142, 18-60 years of age, mean age 31.8 years, 78.9% male). Out of 143, 80 (55.9%) received verum and 63 received placebo (44.1%). The inclusion criterion was an uncomplicated, acute unilateral ankle distortion that had been endured no longer than 6 hours previously.

Kytta-Salbe® f served as the verum: 100 g ointment contained 35 g pyrrolizidine alkaloid reduced liquid extract (1:2) produced from fresh Comfrey root with ethanol/water (ethanol 60% (V/V)). The extract specification allows an allantoin content of 0.2–0.5% (m/m). For the placebo, the ointment basis without active principles was used (equivalent appearance to verum).

The duration of treatment was 8 days (9 days, if recruitment occurred on a Sunday, and 7 days if on a Saturday). The measurement times were on days 0, 4, 7. Local treatment of the afflicted ankle was performed with ca. 2 g (a 6 cm strand of ointment) of either verum or placebo.

The tonometrically recorded pressure pain was defined as the primary target variable. Secondary target variables included the girth of the ankle (swelling) (recorded by the figure-of-eight method; a tape measure is wrapped around the ankle in a figure-of-eight pattern), pain scaling using a visual analog scale (VAS), evaluation of the limitation of movement by the neutral zero method, the use of emergency medication (up to 4 g paracetamol), the final global evaluation of efficacy (physician's and patient's judgment, 4-step scale), and evaluation of cosmetic properties (patient's judgment).

Concerning the tonometrically reported pain criterion (MC), in the verum group, there was a significantly stronger alleviation of pain during the course of the study compared to the placebo group. At visit 3, the reduction compared to initial measurements was 2.44 kp/cm<sup>2</sup> under verum, compared to only 0.95 kp/cm<sup>2</sup> under placebo (Figure 5).

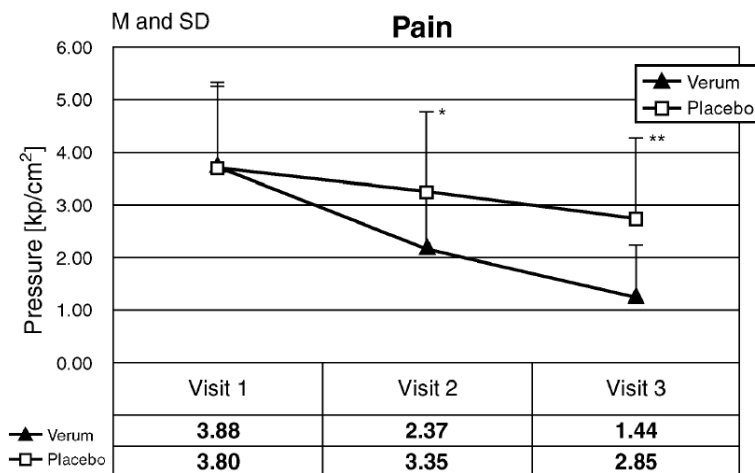


Figure 5. Tonometrically registered pain criteria (pressure difference between the injured and the healthy foot) (M=mean, SD=standard deviation)); \*p=0.001; \*\*p=0.0001 (Koll *et al.*, 2004).

The area under the concentration curve (AUC) for the pressure differences between the injured and healthy foot between visits 1 and 3 amounted to 5.03 visit\* kp/cm<sup>2</sup> for verum, and was significantly lower (p<0.0001) by 1.65 visit\* kp/cm<sup>2</sup> than the corresponding value from the placebo group (6.68 visit\* kp/cm<sup>2</sup>) (t-test, one-tailed). The AUC of the pressure differences between visits 1 and 2 was also significantly different between the study groups (p=0.0116). Consistent with the confirmative analysis, the results of repeated measures ANOVA for this variable were also found to be significantly different (visit 2: p=0.0001 and visit 3: p=0.0001). The analysis of variance also showed that the results were comparable amongst the study centers.

The time course of the differences in swelling between the injured and contralateral feet confirmed the results of the pain measurement. Reduction of swelling was also accomplished significantly more rapidly under verum than under placebo. The difference between the treatments was also significant for visit 2 (p=0.0011) and visit 3 (p=0.0001) with an explorative repeated measures ANOVA. The AUC of the difference in swelling between the injured and healthy feet between visits 1 and 3 was 6.38±4.23 cm for the verum (mean±SEM) and 2.58 cm lower than the corresponding value for the placebo group (3.8±5.05 cm) (t-test, one-tailed, explorative, p=0.0012).

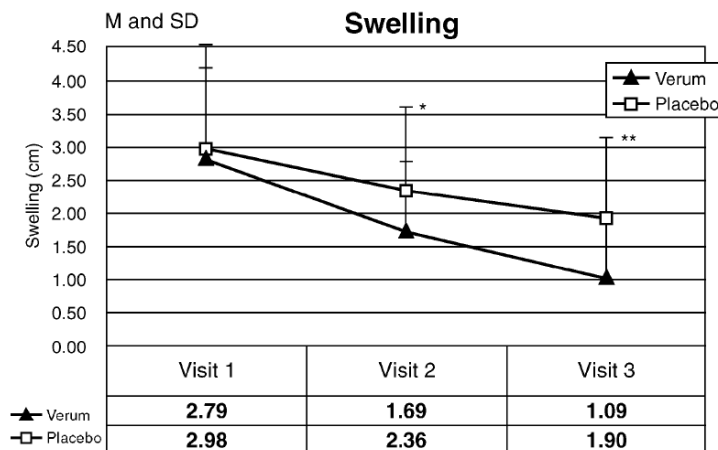


Figure 6. Swelling progress of the difference in swelling between the injured and the healthy feet (M=mean, SD=standard deviation)); \*p=0.0011; \*\*p=0.0001 (Koll, 2004).

Compared to initial values, the VAS value for subjective pain sensation (resting pain) was reduced on average by 3.42 cm in the verum group and 3.25 cm in the placebo group, while the movement pain was reduced by 4.46 and 3.72 cm, respectively.

The joint mobility was increased. Average dorsiflexion and plantar flexion were both improved in a significantly quicker fashion under verum treatment (p=0.002 and 0.0116, respectively).

An intake of paracetamol as emergency medication was registered only in two (placebo) patients. The end evaluations of global efficacy were significantly better in the verum group compared to placebo for both the physicians and the patients (p<0.0001 and p=0.0009, respectively, Fisher's exact test, explorative) (physician's judgement excellent or good in the verum group and the placebo group: 86.3% and 22.6%, respectively; patient's judgement excellent or good in the verum group and the placebo group: 81.3% and 50.0%, respectively). No major differences in the evaluations of efficacy could be determined between the patients' and the physicians' evaluations.

At the end of the study, overall tolerance regarding the whole body was judged as either good or excellent in 91.3% (physicians' evaluation) and 82.6% (patients' evaluation) of the patients. Group differences were statistically insignificant.

Compared to placebo, the active treatment was clearly superior regarding the reduction of pain (tonometric measurement,  $p < 0.0001$ , as the primary efficacy variable) and ankle oedema (figure-of-eight method,  $p = 0.0001$ ). Statistically significant differences between active treatment and placebo could also be shown for ankle mobility (neutral zero method), and global efficacy. Under active treatment, no adverse drug reactions were reported. The good local and global tolerance of the trial medication could also be confirmed.

Study 2.: randomized, controlled.

Predel *et al.* (2005) carried out a single-blind, controlled, randomised, parallel group multicentric study to compare the efficacy and tolerability of an ointment of *Symphytum officinale* root extract (Kyttasalbe® f: 100 g contained 35 g 99%-pyrrolizidine alkaloid reduced Rad. Symphyti fluid extract (1:2, ethanol 60% (V/V))) with that of diclofenac gel (Voltaren® Schmerzgel: 100 g contained 1.16 g diclofenac, diethyldiamine sodium salt) in the treatment of acute unilateral ankle sprain (distortion).

The study was designed to show non-inferiority of the herbal preparation. A double-blind design was not possible due to the differences between the two products (cream versus (vs.) gel, colour, odour). Therefore, the study was carried out as an "investigator-blind" trial: at no moment in time during patient treatment did the investigator see or come into contact with the treatment drugs.

Treatment period was  $7 \pm 1$  days. Study variables were measured at days 0, 4 and  $7 \pm 1$ . A total of 164 patients (mainly young patients with sport injuries, mean age 29 years, 47.6% female) were included, suffering from acute unilateral ankle sprain distortion for no longer than 6 hours prior to inclusion. Difference in foot swelling compared to contralateral non-traumatised foot was greater than 12 mm; sensitivity to pain on contralateral, not injured side was at least  $2.5 \text{ N/cm}^2$ . Basic value of the tonometric measurement on the injured foot should not have exceeded 50% of the respective value of the contralateral side.

Groups were advised to treat the affected area with 6 cm of the preparations four times daily: Kyttasalbe f ointment (containing about 7 mg allantoin,  $n = 82$ ) or Voltaren gel (containing about 23 mg diclofenac,  $n = 82$ ). Primary outcome measure was the area under curve (AUC) of the pain reaction to pressure on the injured area measured by tonometer. Secondary outcome measures were the (1) circumference (centimetres, swelling of the joint), (2) pain at rest and movement (VAS), (3) judgement of movements of the injured joint (neutral-zero method), (4) consumption of the rescue medication paracetamol and (5) global efficacy and global tolerance evaluation (physician and patient; four ranks).

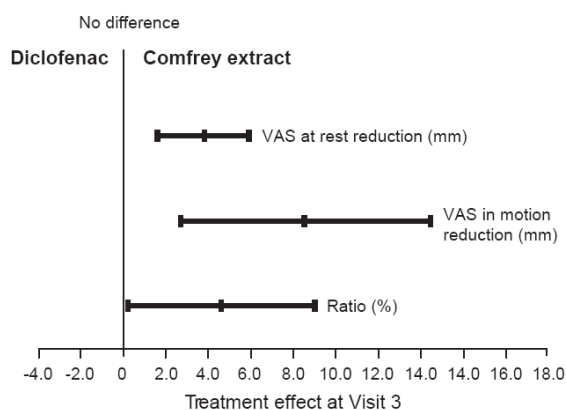


Figure 7. Treatment effect after  $7 \pm$  days.

The 95% CI AUC (*S. officinale* extract minus diclofenac gel) was 19-104  $\text{h} \cdot \text{N/cm}^2$ , above the margin of non-inferiority. After 7 days of treatment, pain at rest and at movement improved by 92/83%

(comfrey) and 85/72% (diclofenac). Tenderness values improved by 86% (comfrey) and 80% (diclofenac), ankle circumference by 3.7% (comfrey) and 3.2% (diclofenac) and the reduction of swelling by 80% (comfrey) and 69% (diclofenac). Seventy-eight percent of the comfrey group and 61% of the diclofenac patients rated the treatment as good or excellent. None of the patients took any rescue medication.

Both the investigators and the patients rated both treatments as "excellent" (n=80 and n=81, respectively) or "good" (n=2 and n=1, respectively).

These results confirmed that the comfrey root extract ointment is not inferior to diclofenac gel (Chrubasik, 2006; Predel *et al.*, 2005).

The data deriving from the study of Predel *et al.* (2005), showing non-inferiority of the comfrey extract was re-evaluated by D'Anchise *et al.* (2007) for superiority according to the guidelines of the Committee for Proprietary Medicinal Products (CPMP) (now known as Committee for Medicinal Products for Human Use). CPMP guidelines (CPMP/EWP/482/99) state that in a trial planned for non-inferiority it is acceptable to calculate the p-value associated with a test of superiority if the 95% CI for the treatment effect not only lies entirely above the non-inferiority margin  $-\Delta$  but also above zero. This appeared to be the fact in the case of the study of Predel *et al.* (2005). On average (mean difference comfrey extract minus diclofenac), the AUC was +61.1 h\*N/cm<sup>2</sup> greater for patients treated with comfrey extract compared to diclofenac treated patients. The difference between the two treatment groups was statistically significant (ANOVAs with factors "study drug", "centre" and "drug-centre interaction" p=0.0012, 0.0759 and 0.2945, respectively). The changes of the tenderness values from baseline to Visit 2 and Visit 3 were statistically significant between the treatment groups (V2-1: p=0.0035, V3-1: p=0.0070). The ratio of tenderness of the injured/contralateral site changed statistically significantly from baseline to Visit 2 (V2-1: p=0.0449).

Concerning the VAS values at rest and movement, in both groups relative reductions were found from baseline to Visit 2 and 3, respectively. The changes from Visit 1 to 2 and 1 to 3 were not statistically significantly different for the VAS at rest (P=0.3611 and 0.2949, respectively) between the two treatment groups. The mean relative reductions in VAS in motion from baseline to Visit 3 were virtually of the same magnitude (83.20% for comfrey and 72.37% for diclofenac).

Both treatments reduced significantly the ankle swelling; although the patients in the comfrey group experienced a faster decrease of the swelling, the difference to the diclofenac group was not statistically significant.

Concerning the global assessment of efficacy, the treatment effect was statistically significant (Mann-Whitney-Wilcoxon test: p=0.0130) showing that the investigators rated the comfrey extract superior to the diclofenac preparation. A good or excellent global efficacy was documented for 78% of the patients in the comfrey extract group compared to 61% in the diclofenac group in the intention-to-treat analysis.

Both treatments showed a relevant and clear effect in the treatment of ankle sprains, the reduction of pain is impressive in both cases. The authors concluded that the re-evaluation of the data showed superiority of the plant based ointment over the diclofenac gel in the treatment of distortions in the key parameters like AUC of tenderness values, VAS at movement (*however, no statistical analysis supports this statement in the article!*) and global assessment of efficacy by investigators and patients. Interestingly, the difference between the two treatment groups was more pronounced at the earlier measurement point.

Study 3.: randomized, controlled.

Grube *et al.* (2007) investigated the effect of a daily application of 6 g Kytta-Salbe® f (comfrey root liquid extract (1:2, ethanol 60% V/V, 35%), allantoin content of 0.2–0.5% (m/m), <0.35 ppm pyrrolizidine alkaloids) (3×2 g) over a 3 week period with patients suffering from osteoarthritis of the knee in a randomised, double-blind, bicenter, placebo-controlled clinical trial.

The included 220 patients examined consisted of 153 women and 67 men of an average age of 57.9 years. On average, the complaints relating to osteoarthritis of the knee had persisted for 6.5 years. However, no information was given in the article about the stage of osteoarthritis or the baseline characteristics, which would guarantee the homogeneity of the groups. Two hundred and twenty patients were included in the Full Analysis Set (FAS) and safety collective, 186 (84.5%) in the Valid Case Analysis Set (VCAS) collective.

The patients applied a 6 cm long thread of verum or placebo ointment on the skin covering the knee joint three times a day and massaged this in. In the event of osteoarthritis of both knees, both knee joints were treated but only the knee that was more severely affected on admission to the trial was assessed. Treatment continued for a period of 21 days. Following a thorough initial examination during the first visit, clinical controls were performed after 6–8, 13–15 and 20–22 days (visits 2–4). In addition to the visits, patients logged the course of pain in a diary.

In the course of the trial, the visual analog scale (VAS) total score (primary target value) in the verum group dropped by 51.6 mm (54.7%) and in the placebo group by 10.1 mm (10.7%). The average difference between the groups of 41.5 mm (95% confidence interval=34.8 to 48.2 mm) or 44% was significant ( $p<0.001$ ). In accordance with the primary target value the intensity of pain was reduced considerably in the verum group from an initial average of “moderate pain” (47.1%) to “mild pain” (21.3%). The difference between the treatment groups increased systematically, in parallel with the term of the treatment.

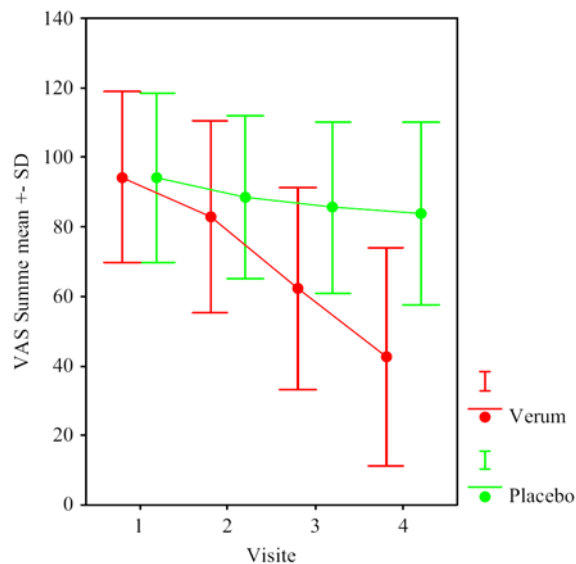


Figure 7. VAS total score (pain at rest and on movement) (Grube *et al.*, 2007).

With regard to pain at rest, a significant decline of pain of 20.9 mm (56.6%) was recorded in the verum group, while the placebo group recorded a decline of only 4.6 mm (12.2%). The difference between the groups of 44.4% is significant ( $p<0.001$ ). With regard to pain on movement, the verum group had undergone a reduction of pain on movement of 30.7 mm (53.5%) while the placebo group had only undergone a reduction of 5.6 mm (9.9%).

The significance is confirmed through the evaluation of the diary, the VCAS evaluation and the separate assessment of the two centres. This also applies to the separate assessment of the VAS total

score following pain at rest and on movement. The WOMAC (Western Ontario and McMaster Universities) total score (secondary target value) also improved similar to the VAS total score. At the end of the trial, a reduction by 60.4 mm (58.0%) was recorded for the verum group and a reduction of 14.7 mm (14.1%) for the placebo group. The average group difference of 45.7 mm (95% confidence interval=37.1 to 54.3 mm) or 43.9% was significant ( $p<0.001$ ). The difference between the treatment groups increased systematically and significantly, in parallel with the duration of the treatment. Thus, the superiority of the treatment with Kytta-Salbe® f over that with the placebo was proven, even by means of the multi-factorial multivariate analysis for repetitive measurements. The significance is also confirmed by the VCAS assessment and the separate assessment of the two centres. The division of the WOMAC by pain, stiffness and physical function also resulted in a significant superiority ( $p<0.001$  each) of the verum over the placebo.

In respect of the explorative secondary target values SF-36 (quality of life), angle measurement (mobility of the knee), CGI (clinical global impression) and global assessment of efficacy by the physician and the patient, a significant superiority ( $p<0.001$  each) of the verum group over the placebo group was also proven. Considerable improvement of the quality of life (SF-36) of the verum group over the placebo group was observed. The significant group difference was 33.9% ( $p<0.001$ ) for physical function and 7% ( $p=0.006$ ) for mental function. Patients in the verum group experienced a significant improvement ( $p<0.001$ ) of knee flexion – on an average by 7.5 (7%). In the VCAS collective the group difference was 7.8. The neutral-zero scaling of the knee showed that patients, on an average, were unable to fully extend their knee (upper and lower leg form a straight line) at the outset of the trial. At the end of the trial, the verum group had undergone an average improvement of 2, while the placebo group had undergone an average deterioration of 0.4. The group difference is significant ( $p<0.001$ ), in the VCAS collective as well. The Clinical Global Impression (CGI) on the severity of the disease and on the change of the condition also resulted from the significant superiority ( $p<0.001$ ) of the verum over the placebo. In the verum group 29.1% of patients exhibited a “slight”, 52.7% a “clear” and 10.9% a “comprehensive” improvement, in compliance with the change of the condition, as expected. Seven patients no longer required treatment. In the placebo group 82.6% did not experience any improvement.

Table 1. Summary of the changes achieved after three weeks and the group difference (Grube *et al.*, 2007).

| Efficacy variable                    | Verum group |              | Placebo group |              | Group difference |      |
|--------------------------------------|-------------|--------------|---------------|--------------|------------------|------|
|                                      | Absolute    | Relative (%) | Absolute      | Relative (%) | Absolute         | %    |
| <i>VAS (mm)</i>                      |             |              |               |              |                  |      |
| Total score                          | 51.6        | 54.7         | 10.1          | 10.7         | 41.5             | 44.0 |
| Pain at rest score                   | 20.9        | 56.6         | 4.6           | 12.2         | 16.4             | 44.4 |
| Pain on movement score               | 30.7        | 53.5         | 5.6           | 9.9          | 25.1             | 43.6 |
| <i>WOMAC (mm)</i>                    |             |              |               |              |                  |      |
| Total score                          | 60.4        | 58.0         | 14.7          | 14.1         | 45.7             | 43.9 |
| Pain score                           | 12.1        | 58.2         | 2.7           | 12.9         | 9.4              | 45.3 |
| Stiffness score                      | 4.8         | 55.8         | 1.2           | 13.2         | 3.6              | 42.6 |
| Function score                       | 43.4        | 58.2         | 10.7          | 14.4         | 32.7             | 43.8 |
| <i>SF-36 (points)</i>                |             |              |               |              |                  |      |
| Physical function score              | 11.9        | 38.1         | 1.3           | 4.2          | 10.6             | 33.9 |
| Mental function score                | 4.2         | 9.5          | 1.1           | 2.5          | 3.1              | 7.0  |
| <i>Angle measurement (°)</i>         |             |              |               |              |                  |      |
| Knee flexion                         | 7.5         | 7.0          | 0             | 0            | 7.5              | 7.0  |
| Extension of the knee (neutral-zero) | -2.0        | -65.8        | 0.4           | 19.6         | 2.4              | 85.4 |

At the end of the trial (visit 4), a global assessment of tolerance was performed by the physician and the patient using a scale. Values ranging from “very good”, “good”, “moderate” and “bad” could be given. Only “good” and “very good” ratings were issued. In the verum group the “very good” ratings



predominated (73.6%, physician and patient) and in the placebo group, the "good" ratings (50.9% physician and 53.6% patient).

The results suggest that the comfrey root extract ointment is useful in the short-term treatment of osteoarthritis of the knee. Pain is reduced, mobility of the knee improved and quality of life increased (Chrubasik, 2007, Grube *et al.*, 2007).

Study 4.: randomized, controlled.

The objective of the study carried out by Giannetti *et al.* (2009) was to show the superiority of comfrey root extract ointment (Kytta-Salbe® f) to placebo ointment in patients with acute upper or low back pain. The study was conducted as a double-blind, multi-centre, randomised clinical trial with parallel group design over a period of 5±1 days. The patients (n=120, mean age 36.9 years) were treated with verum or placebo ointment three times a day, 4 g ointment per application. The trial included four visits. The primary efficacy variable was the area under the curve (AUC) of the visual analogue scale (VAS) on active standardised movement values at visits 1 to 4. The secondary efficacy variables were back pain at rest using assessment by patient on VAS, pressure algometry (pain-time curve; AUC over 5 days), global assessment of efficacy by the patient and the investigator, consumption of analgesic medication, and functional impairment measured with the Oswestry Disability Index. There was a significant treatment difference between comfrey extract and placebo regarding the primary variable. In the course of the trial the pain intensity on active standardised movement decreased on average (medians) about 95.2% in the verum group and 37.8% in the placebo group. The results of this clinical trial were clear-cut and consistent across all primary and secondary efficacy variables (Giannetti *et al.*, 2009).

Study 5. – open, observational.

Koll and Klingenburg (2002) conducted a prospective open multicentric observational study involving 162 general practitioners to analyse the anti-inflammatory and analgetic properties of the topical comfrey preparations Kytta-Salbe® f (100 g contains 35 g Symphyti radix fluid extract (1:2)), Kytta-Plasma® f (100 g contains 30 g Symphyti radix fluid extract (1:2)) and Kytta-Balsam®f (100 g contains 35 g Symphyti radix fluid extract (1:2) and 1.2 g methylnicotinate) applied to bruises, sprains and distortions and painful conditions of the muscles and joints. (Kytta products contain extracts of fresh herbal drug; 98% of the pyrrolizidine alkaloids are removed; end products contain pyrrolizidine alkaloids less than 35 ppm.) During the 2 weeks of observation the patients received an average of 1-3 applications of the comfrey preparation per day. Altogether 492 questionnaires were evaluated. Efficacy and tolerability were assessed by both physician and patient. Pain at rest and movement, as well as tenderness have improved in the overall observation group by an average of 45-47%. The duration of morning joint stiffness decreased from 20 minutes initially to 3 minutes. There were only slight differences between the global efficacy scores of the three Kytta products: 1.76 for Plasma, 1.73 for Balsam, 1.69 for Salbe. During the course of the treatment with comfrees, more than 2/3 of the patients were able to reduce or even discontinue their intake of non-steroidal anti-inflammatory drugs and other specific concomitant medication. In most of the cases, both effectiveness and tolerability were assessed to be excellent or good.

Study 6. – randomised, controlled with combination product.

In a randomised, double-blind, placebo-controlled study lasting 8 weeks with 61 participants suffering from non-activated osteoarthrosis of the knee, the efficacy of a cream containing comfrey and mistletoe extract. At the end of the study, 65.6% of the patients in the verum group and 60% in the placebo group rated the treatment good or excellent. The active treatment was not superior to placebo. Moreover, no conclusion can be drawn concerning the efficacy of comfrey in this poorly

designed trial using a combination product without specified composition (Schmidtke-Schrezenmeier *et al.*, 1992).

Table 2. Randomized, controlled clinical studies with *Symphytum officinale* preparations.

| Study   | Design   | Study population   | Treatment  | Endpoints   | Results (comfrey vs. placebo/comparator)   |
|---|--|--|--|---|--|
| Koll et al., 2004   | double-blind multicenter randomized placebo-controlled | unilateral ankle sprain<br>n=143, 78,9% male<br>mean age: 31.8 years (18-60) | 80 patients Kytta-Salbe® f ointment<br>60 patients placebo ointment<br>~ 2 g (~6 cm) ointment<br>duration: 8 days              | 1 pressure pain AUC for pressure differences<br>2 swelling AUC of the difference<br>2 resting pain VAS<br>2 movement pain VAS<br>2 limitation of movement<br>2 use of emergency medication<br>2 final global evaluation of efficacy (physician)<br>2 final global evaluation of efficacy (patient)<br>2 evaluation of cosmetic properties (patient) | -2.44 kp/cm <sup>2</sup> vs. -0.95 kp/cm <sup>2</sup> (p=0.0001)<br>5.03 visit*kp/cm <sup>2</sup> vs. 6.68 visit*kp/cm <sup>2</sup> (p < 0.0001)<br>-1.70 cm vs. -1.08 cm (p=0.0001)<br>6.38±4.23 cm vs. 3.8±5.05 cm (p = 0.0012)<br>-3.42 cm vs. -3.25 cm<br>-4.46 cm vs. -3.72 cm<br>dorsiflexion and plantar flexion: p = 0.002 and 0.0116<br>0 vs. 2<br>excellent or good: 86.3% vs. 22.6% (p<0.0001)<br>excellent or good: 81.3% vs. 50% (p=0.0009) |
| Predel et al., 2005   | single blind multicentric randomized controlled        | acute unilateral ankle sprain<br>n=164, 47.6% female<br>mean age: 29 years   | 82 patients Kytta-Salbe® f ointment<br>82 patients Voltaren gel<br>~6 cm of the preparations 4 times daily<br>duration: 7±days | 1 pressure pain AUC<br>2 swelling<br>2 resting pain VAS<br>2 movement pain VAS<br>2 limitation of movement<br>2 use of emergency medication<br>2 final global evaluation of efficacy (physician)<br>2 final global evaluation of efficacy (patient)   | 95% CI AUC (comfrey minus diclofenac): 19-104 h*N/cm <sup>2</sup><br>-3.67% vs. -3.19%<br>92.01% vs. 84.96%<br>83.2% vs. 72.37%<br>data not shown<br>0 vs. 0<br>excellent or good: 78% vs. 61%<br>excellent or good: 84.2% vs. 70.8%   |
| Reassessment of the data of the Predel 2005 study by D'Anchise et al., 2007 |  |  |  | 1 pressure pain AUC<br>2 swelling<br>2 resting pain VAS<br>2 movement pain VAS  | +61.1 h*N/cm <sup>2</sup> greater in comfrey group (p=0.0012)<br>difference not significant<br>difference not significant (p=0.2949)   |

|                        |   |   |  |  |   |
|------------------------|---|---|--|--|---|
|                        |   |   |  | 2 final global evaluation of efficacy (physician)  | difference not significant<br>comfrey superior to diclofenac  |
| Grube et al., 2007     | double-blind<br>bicenter<br>randomized<br>placebo-controlled    | painful osteoarthritis in knee<br>n=220, 69.5% female<br>mean age: 57.9 years | 110 patients Kytta-Salbe® ointment<br>110 patients placebo<br>~6 cm of the preparations 3 times daily<br>duration: 21 days | 1 VAS total score<br>pain at rest VAS<br>pain at movement VAS<br>2 WOMAC total score<br>pain score<br>stiffness score<br>function score<br>2 quality of life (SF-36)<br>physical function score<br>mental function score<br>2 angle measurement<br>knee flexion<br>extension of knee<br>2 Clinical Global Impression | 51.6 mm (54.7%) vs. 10.1 mm (10.7%) (p<0.001)<br>20.9 mm (56.6%) vs. 4.6 mm (12.2%) (p<0.001)<br>30.7 mm (53.5%) vs. 5.6 mm (9.9%)<br>60.4 mm (58.0%) vs. 14.7 mm (14.1%) (p<0.001)<br>12.1 mm (58.2%) vs. 2.7 mm (12.9%) (p<0.001)<br>4.8 mm (55.8%) vs. 1.2 mm (13.2%) (p<0.001)<br>43.4 mm (58.2%) vs. 10.7 mm (14.4%) (p<0.001)<br>11.9 pts (38.1%) vs. 1.3 pts (4.2%) (p<0.001)<br>4.2 pts (9.5%) vs. 1.1 pts (2.5%) (p = 0.006)<br>7.5 (7.0%) vs. 0 (p<0.001)<br>-2.0 (-65.8%) vs. 6.4 (19.6) (p<0.001)<br>improvement: 92.7% of the patients vs. 17.4% (p<0.001) |
| Giannetti et al., 2009 | double-blind<br>multicenter<br>randomized<br>placebo-controlled | acute upper or low back pain<br>n=120<br>mean age: 36.9 years                 | 3 times 4 g ointment<br>duration: 5±days   | 1 AUC of the VAS on active standardized movement values<br>2 back pain at rest VAS<br>2 pressure algometry AUC<br>2 global assessment of efficacy (investigator)<br>2 global assessment of efficacy (patient)<br>2 consumption of analgesic medication<br>2 Oswestry Disability Index                                | significant treatment difference  |

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

Staiger and Wegener (2008) carried out a multicentric, prospective post-marketing surveillance study on the tolerability and efficacy of an ointment containing comfrey root fluid extract. Three hundred and six children (148 girls, 158 boys) aged between 3 and 12 years (average age 7.7 years) were included in the study. Altogether 31 paediatricians applied Kytta-Salbe® to treat bruises (61.4%), luxations (30.4%) and sprains (14.1%) or similar injuries (6.9%) of children until recovery or significant improvement of the symptoms or for a maximal interval of 1 week. For treatment, 4-6 cm ointment was applied 2-4 times daily. At the end of the treatment the tolerability was assessed and the adverse events were recorded. To assess the efficacy, pain, restriction of movement, haematoma and the decrease of the general health state were recorded (each on a 5-grade scale). Children assessed the pain on a 5-grade Smiley-scale. Pain sensitivity, restriction of movement, general health state, pain at rest, pain at movement, pain at night improved until the end of the treatment with an average of 61.4%, 62%, 55.8%, 62.6%, 60.3%, and 59.3%, respectively, as assessed by the patients and their parents. Paediatricians assessed the improvement of tenderness, restriction of movement, haematoma, general health state, swelling to 59.7%, 59.8%, 53.2%, 54.9%, and 8.4%, respectively. Eighty-eight point four percent of the paediatricians assessed the tolerability of the treatment as very good or good. In case of the patients/their parents the same values was 98.7%. The authors concluded that the topical application of the studied comfrey preparation is effective and safe also for children. However, due to the study design (open study, heterogenous study population) the efficacy of comfrey in children in special indications cannot be confirmed from this study.

### 4.3. Overall conclusions on clinical pharmacology and efficacy

No relevant information is available with regard to the pharmacodynamic properties of common comfrey and no dose-response studies are available. However, based on the available clinical evidence, cutaneous application of *S. officinale* extracts is well-established in the treatment of sprains. To date 2 placebo-controlled, randomized studies confirm the superiority of comfrey over placebo, and in one trial it was verified that *S. officinale* was not inferior (and even superior) to diclofenac. However, the preparations applied in the studies presented in this assessment report are different from the herbal preparation included in the monograph. No appropriate clinical studies have been carried out in special populations, e.g. elderly and children.

Since the exact production steps due to several steps to remove the pyrrolizidine alkaloids and therefore the final composition of the product is not known, these clinical studies cannot be taken into consideration for the preparation of a well-established use monograph.

## 5. Clinical Safety/Pharmacovigilance

Common comfrey has been a traditional medicinal herb, which earlier was used both externally and internally. While comfrey is the most widely-recognized source of dietary pyrrolizidine alkaloids in developed countries, other herbal preparations that contain pyrrolizidine alkaloids also have been implicated in liver disease in humans. The oral application of comfrey is not part of the rational phytotherapy and occurs only sparsely in Europe. The intake from root-tea has been estimated at between 8 and 26 mg per cup (Abbott, 1988). The estimated daily intakes of certain pyrrolizidine alkaloids leading to fatal intoxications range from 0.5 to 3.3 mg/kg (De Smet *et al.*, 1992). However, little is known about the chronic ingestion of smaller doses of pyrrolizidine alkaloids.

There is no doubt that some pyrrolizidine alkaloids are toxic to man, and many cases have been reported in which single persons as well as hundreds or even thousands of people have been intoxicated by the intake of pyrrolizidine alkaloids in the form of herbal remedies or foods (De Smet *et*

*al.*, 1992). In 1920, there was a large outbreak of food poisoning in South Africa. This incident was due to the contamination of wheat flour with toxic pyrrolizidine alkaloids containing plants. Also, large outbreaks have been reported in Afghanistan, India, and the former USSR. These large outbreaks were possible because pyrrolizidine alkaloid-containing plants grow in climatic conditions in which food sources such as wheat are usually grown (Wexler, 2005). However, none of these toxicity cases were related to *S. officinale*. Human hepatotoxicity with pyrrolizidine-containing plants is well documented, particularly following the ingestion of *Crotalaria*, *Heliotropium* and *Senecio* species (Barnes *et al.*, 2007).

The main route of exposure of humans and animals to pyrrolizidine alkaloids is the oral pathway. Human exposure occurs through consumption of food contaminated by toxic plant products or by the ingestion of herbal medicines containing the toxin. Pyrrolizidine alkaloids have been found in wheat, milk, honey, herbal medicines, and herbal teas at different concentrations. Livestock exposure to pyrrolizidine alkaloids is attributed to the consumption of pyrrolizidine alkaloid containing plants while grazing (Wexler, 2005). In case of *S. officinale*, the appropriate therapeutic application of good quality medicinal products (with low pyrrolizidine alkaloid content) bears no danger to today's knowledge due to the poor absorption of pyrrolizidine alkaloids through the skin.

Percutaneous absorption of pyrrolizidine alkaloids present in comfrey is reported to be low, although application of comfrey preparations to the broken skin should be avoided. The inclusion of comfrey in products intended for topical application is permitted, provided the preparation is only applied to the unbroken skin and that its use is restricted to ten days or less at any one time (Barnes *et al.*, 2007). There are no exact data on the absorption of paraaminosalicylic acid through human skin in the literature.

The conclusion that comfrey is not safe for internal use in humans is primarily based on studies in which high levels of purified pyrrolizidine alkaloids were administered to rodents. Systematic toxicity testing or clinical trials have not been performed. Although pyrrolizidine alkaloid poisoning in humans can occur, this is most commonly a consequence of consumption of plants other than comfrey. Heavy reliance on data obtained from experiments conducted using rodents or from human poisonings by other plants, is probably not an accurate reflection of the risk and/or therapeutic benefit of comfrey in humans (Rode, 2002). However, to date there is no rational scientific reason or evidence for the internal application of comfrey.

In the last decade, dietary supplement consumption has increased in Europe and the United States. This has prompted regulatory agencies to enact regulations to protect the health of consumers. In 1992, the Federal Health Department of Germany restricted the manufacture and use of pharmaceuticals containing pyrrolizidine alkaloids with an unsaturated necine skeleton. Also, in 1994, the US Congress passed the Dietary Supplement Health Education Act (DSHEA), which amended the US Federal Food, Drug, and Cosmetic Act (FFDCA) and created a new regulatory category for the Food and Drug Administration (FDA) to regulate dietary supplements. Furthermore, in 1997, the US FDA published Good Manufacturing Practice (GMP) regulations that manufacturers of herbal products must follow. These regulations are meant to improve the quality of dietary supplements and minimize the risk of poisoning due to the presence of pyrrolizidine alkaloids in dietary supplements (Wexler, 2005).

As a result of a 1993 report by the Committee on Toxicity of Chemicals in Food to the Food Advisory Committee and the Ministry of Agriculture, Fisheries and Food (UK), the health food trade voluntarily withdrew all products, such as tablets and capsules, and advice was issued that the root and leaves should be labelled with warnings against ingestion. It was considered that comfrey teas contained relatively low concentrations of pyrrolizidine alkaloids and did not need any warning labels (Barnes *et al.*, 2007).

In Germany, authorised products are on the market at least since 1976. In 1992, a graduated plan (grade II) concerning medicinal products containing pyrrolizidine alkaloids with a necine system unsaturated in 1,2 position came into force. The maximal daily dose of pyrrolizidine alkaloids in case of cutaneous application is 100 µg. In this dose, the maximal duration of application is 6 weeks per year. According to the German regulation, products for cutaneous use with less than 10 µg pyrrolizidine alkaloids in the daily dosage can be used without any limitation in the duration of use (BGA, 1992).

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

In the clinical trial carried out by Koll *et al.* (2004) (altogether 143 participants) three minor adverse events were observed during the course of the study, whereby in each (active treated and placebo) group a single case of minor skin reddening was noticed at the site of application which regressed without treatment. A causal relationship with the ointment treatment was considered possible in one of these cases (placebo), whereas in the other case this was considered as improbable (*verum*), since a reddening was also seen on the contralateral foot. Serious adverse effects were never registered during the course of the study. Local tolerance was evaluated as either good or excellent by 92.5% of the *verum* patients and 88.9% of the placebo patients. A bad local tolerance was only registered in one case (placebo).

In the study of Predel *et al.* (2005) (altogether 164 participants) the tolerability of both the comfrey and diclofenac treatment was excellent, only minor adverse events occurred. A total of five patients (3%) experienced five adverse events in the course of the clinical trial (four patients (4.8%) in the comfrey extract group and one patient (1.2%) in the diclofenac group. The total number of adverse events was 4 in the comfrey extract group (headache, heartburn, bronchitis, redness of skin) and 1 in the diclofenac group (bronchitis). At visit 3, the patients and the investigators assessed the tolerability of the study drugs by means of a four-point scale with the categorisations "poor", "fair", "good", and "excellent" under blind conditions.

In the course of the clinical trial of Grube *et al.* (2007) (altogether 220 participants) 22 patients (10%) experienced a total of 22 adverse events. These related to 7 patients (6.4%) in the *verum* group and 15 patients (13.6%) in the placebo group. One patient in the placebo group discontinued the clinical trial at the third visit at her/his own request, due to ineffectiveness. All adverse events were not serious and did not represent an adverse drug reaction. There was no significant difference between the groups ( $p=0.072$ ).

In the surveillance study of Staiger and Wegener (2008) (altogether 306 patients) two adverse effects (pruritus) were recorded. In one case, the causal relationship with the treatment was probable, in the other case improbable. This is the only study in children, and due to the short observation period it is not sufficient to confirm the safety of comfrey in this age group.

In the study of Schmidtke-Schrezenmeier *et al.* (1992) one adverse event occurred. In the placebo group one patient reported allergic reaction after the local application of an alcohol rub.

In the observational study of Koll and Klingenburg (2002) (altogether 492 questionnaires) adverse effects were reported from 5 patients: 2 cases of burning sensation on the skin and 1 on the mucous layer, 2 skin reddening, 2 itching and in 1 case common cold. In the latter case no causal connection with the medication could be established. All the local symptoms were mild and occurred in case of application of the methylnicotinate containing product (Kyttä-Balsam® f). The tolerability of the comfrey products was according to the 98% of the patients excellent (64%) or good (34%).

## 5.2. Patient exposure

The four controlled clinical trials evaluated in the assessment report comprised altogether 647 patients (Knoll *et al.*, 2004; Predel *et al.*, 2005; Grube *et al.*, 2007; Giannetti *et al.*, 2009). In one multicentric, prospective post-marketing surveillance study 306 children were exposed to comfrey treatment (Staiger and Wegener, 2008).

Products containing comfrey extracts are on the European market at least since 1968.

## 5.3. Adverse events and serious adverse events and deaths

Although there are human data on the central nervous system and pulmonary system affecting effects of certain pyrrolizidine alkaloid-containing plants, common comfrey is toxic primarily on the liver. In almost all cases of severe or fatal *Symphytum* intoxications, the patients developed liver damage with cirrhosis and ascites.

### Liver toxicity

Although there have been no recent reports of adverse reaction to comfrey, over a decade ago, several cases of veno-occlusive disease associated with comfrey ingestion were reported. These case studies support that underlying illness, nutritional status and the concurrent use of hepatotoxic drugs increase the likelihood of veno-occlusive disease development when using pyrrolizidine alkaloid-containing drugs (Rode, 2002; Stickel and Seitz, 2000).

Because the free base and the *N*-oxide forms of pyrrolizidine alkaloids demonstrate similar toxicity, the total alkaloid content of a plant or extract probably correlates reasonably well to severity of the hepatotoxicity (Barceloux, 2008).

The symptoms of pyrrolizidine-induced veno-occlusive disease resemble those of Budd-Chiari syndrome but 10% may be asymptomatic (Stickel and Seitz, 2000). *Acute* forms of veno-occlusive disease involve the rapid progression of hepatic necrosis, cirrhosis, portal hypertension, marked elevation of serum hepatic aminotransferases, and hepatic failure characterised clinically by vomiting, progressive hyperpnea, and encephalopathy. The *subacute* form involves the gradual development of ascites, hepatomegaly, and gastrointestinal symptoms (abdominal pain, vomiting, diarrhoea, pedal oedema) that may resolve following the cessation of exposure to pyrrolizidine alkaloids. Features of *chronic* forms of pyrrolizidine poisoning include the insidious onset of fatigue, right upper quadrant abdominal pain, anorexia, generalized weakness, weight loss, gastrointestinal symptoms, ascites, hepatosplenomegaly, and progressive cirrhosis. Fever, jaundice, and bleeding are uncommon in this form of liver disease. Prolonged pyrrolizidine alkaloid consumption can also cause pulmonary artery hypertension, leading to cor pulmonale and right ventricular hypertrophy (Barceloux, 2008).

Liver biopsies of patients with veno-occlusive disease demonstrate histological changes of a centrilobular hemorrhagic congestion and necrosis affecting primarily zones II and III along with reticulin fibers within the lumen of central and sublobular veins. Fibrosis occurs predominately in the perivenular areas rather than the portal tracts. Thickening of the venules produces a functional outflow obstruction and congestion. Liver angiography demonstrates narrowing of the lumen of the small hepatic veins along with nonhomogeneous filling of the hepatic sinusoids. The main hepatic veins and the portal veins typically remain patent. Electromicroscopy demonstrates the occurrence of hepatocyte blebs (clasmatosis) in sinusoidal borders, but these changes are not pathognomonic for pyrrolizidine-induced liver disease. The hepatic lobular architecture usually remains intact until late in the course of the disease. Histological signs of inflammation are typically absent.

Laboratory abnormalities associated with veno-occlusive disease include hypoglycemia, leukocytosis, mild hyperbilirubinemia (primarily indirect), variable increases of serum hepatic aminotransferase



concentrations, thrombocytopenia, and hemolytic anemia. Ultrasound and imaging studies of the liver do not usually demonstrate significant abnormalities until portal hypertension becomes clinically significant (Barceloux, 2008).

#### Case studies

The daily consumption of comfrey root-based food supplement containing an estimated ~15 µg total pyrrolizidine alkaloids/kg body weight (total estimated minimal dose = 85 mg) over 6 months along with camomile tea and vitamin supplements was associated with the development of veno-occlusive disease in a 49-years old woman. Budd-Chiari syndrome was diagnosed on the basis of a liver biopsy specimen that showed centrilobular necrosis. The patient had portal hypertension associated with obliteration of the smaller hepatic venules. A liver biopsy specimen showed centrilobular necrosis and congestion. The clinical and analytic findings were consistent with chronic pyrrolizidine intoxication, indicating that low-level, chronic exposure to such alkaloids can cause venoocclusive disease (Ridker *et al.*, 1985).

A 13 year old boy was admitted in July 1986 for investigation of hepatomegaly and ascites. Three years earlier Crohn's disease had been diagnosed. He was treated with prednisolone and sulphasalazine with benefit. At his parents' request these drugs were discontinued and he was treated with acupuncture and comfrey root, prescribed by a naturopath. Up to 1986 he had been regularly given a herbal tea containing comfrey leaf. The exact quantities of leaves given and frequency of administration are unknown. An exacerbation of his inflammatory bowel disease in 1984 required a further course of prednisolone. In June 1986 he presented with fatigue, diarrhoea, and weight loss and a few weeks later developed fever, abdominal pain, and swelling. He was taking prednisolone and sulphasalazine. On examination, he had ascites and tender hepatomegaly but no dehydration, jaundice, or heart failure and no stigmata of chronic liver disease. He had raised serum bilirubin concentration and aspartate aminotransferase activity. Percutaneous liver biopsy showed the thrombotic variant of hepatic veno-occlusive disease. He was treated with spironolactone, salt restriction, and bed rest with a good response. The authors supposed that the only possible causal factor for hepatic veno-occlusive disease in our patient was comfrey (Weston *et al.*, 1987).

There are several case studies on the hepatotoxic effect of pyrrolizidine alkaloid- containing herbal drugs other than *S. officinale* roots.

Four young Chinese women took daily doses of an unidentified 'Indian' herbal tea as treatment for psoriasis. Three (one of whom died), developed ascites, hepatomegaly and biochemical abnormalities within 19-45 days. The fourth patient discontinued herbal tea after 21 days when she developed a skin rash. Two patients had portal hypertension, while all had liver histology showing features of veno-occlusive disease. Pyrrolizidine alkaloids were identified spectrophotometrically in the brewed tea, and in the chopped leaves of the herbal mixture; the mean dose in the tea prepared for consumption being 12 mg/day of alkaloid base and 18 mg/day of *N*-oxide. The mean cumulative dose of alkaloids (base + *N*-oxide) before onset of symptoms (three patients), was estimated to be 18 mg/kg. In the asymptomatic patient with histological liver disease only, the corresponding dose was 15 mg/kg. The source of the pyrrolizidine alkaloids could not be identified from the tea (Kumana *et al.*, 1985).

Following a 2-year period of severe drought (1970-72) a very large number of patients with massive ascites and emaciation were observed in 1974 in north-western Afghanistan. Clinicopathological study showed that these were typical cases of hepatic veno-occlusive disease. The disease was usually fatal, death occurring within 3 to 9 months from the onset of the abdominal tension. The outbreak was caused by consumption of bread made from wheat contaminated with seeds of *Heliotropium* plants, which were shown to contain pyrrolizidine alkaloids. Examination of 7200 inhabitants from the affected villages showed evidence of liver disease in 22.6%, in 15% damage was advanced. From 21 patients admitted to hospital, clinical improvement was observed in thirteen cases after 3 to 9 months of

supportive treatment, and in three cases liver biopsies showed almost complete disappearance of initial abnormalities. It was calculated that severe liver disease resulted from the daily ingestion of total pyrrolizidine alkaloids from *Heliotropium* alkaloids at an estimated dose of 30–40 µg/kg body weight (Mohabbat *et al.*, 1976).

An estimated dose of 70-147 mg of total pyrrolizidine alkaloids from *Senecio* tea (gordolobo yerba) was associated with liver cirrhosis in a 6-month-old infant (Barceloux, 2008).

The estimated daily ingestion of 60 µg total pyrrolizidine alkaloids (primarily seneciphylline)/kg/day over 15 months produced reversible veno-occlusive disease in an infant. An 18-month-old boy, who had regularly consumed a herbal tea mixture since the 3rd month of life, developed portal hypertension with severe ascites. Histology of the liver showed centrilobular sinusoidal congestion with perivenular bleeding and parenchymal necrosis without cirrhosis. The tea contained peppermint and what the mother thought was coltsfoot (*Tussilago farfara*), in fact *Adenostyles alliariae*. Pharmacological analysis of the tea compounds revealed high amounts of pyrrolizidine alkaloids. Seneciphylline and the corresponding *N*-oxide were identified as the major components (Sperl *et al.*, 1995).

According to the case report published by Yeong *et al.* (1990) a 23 year old man presented with hepatic veno-occlusive disease and severe portal hypertension and subsequently died from liver failure. Laboratory investigations showed hypoalbuminaemia, elevated bilirubin, alkaline phosphatase, glutamyl transferase and aspartate transferase. Light microscopy and hepatic angiography showed occlusion of sublobular veins and small venous radicles of the liver, associated with widespread haemorrhagic necrosis of hepatocytes. The patient had been on a predominantly vegetarian diet and, prior to his illness, took comfrey leaves (4-5 leaves every day for 1-2 weeks before the onset of symptoms). A possible causal association of comfrey and this patient's veno-occlusive disease was suggested by the temporal relationship of the ingestion of comfrey to his presentation, the histological changes in the liver and the exclusion of other known causes of the disease.

Bach *et al.* (1989) report a case of comfrey herb tea-induced hepatic veno-occlusive disease. A 47-year old non-alcoholic woman consulted a homeopathic doctor who recommended comfrey tea for the treatment of her symptoms (abdominal pain, fatigue, allergy). The patient began consuming as many as 10 cups of tea per day in addition to taking comfrey pills (not specified) by the handful, which continued for more than one year. Four years later (in 1982), her aminotransferase activities were noted to be twice the normal values. By 1986, she has developed ascites. By that time, her liver enzyme and bilirubin levels were in the normal range. Liver biopsies revealed fibrosis of portal tracts.

A case of veno-occlusive disease in a newborn of a mother consuming a herbal cough tea containing 0.6 mg/kg senecionine during pregnancy was reported. The tea contained *Tussilago farfara* and roots of *Petasites hybridus* (De Smet *et al.*, 1992).

### **Carcinogenicity**

The wide variety of pyrrolizidine compounds and the sparse carcinogenic testing of these compounds limit conclusions regarding the carcinogenic risk from ingesting comfrey and other plant materials containing pyrrolizidine compounds. The International Agency for Research on Cancer (IARC) lists lasiocarpine, riddelliine, and monocrotaline as possible human carcinogens (Group 2B), whereas retrorsine, hydroxysenkirkine, isatidine, jacobine, seneciphylline, and senkirkine are not classifiable as to carcinogenicity (Group 3). From the compounds listed above, lasiocarpine can be found in common comfrey. The large doses of pyrrolizidine used in animal studies (Hirono, 1978a) suggest that these compounds are weak carcinogens in rats. Although some pyrrolizidine alkaloids (e.g., symphytine, riddelliine) are positive in 2-year rodent studies, there is no direct evidence that the ingestion of pyrrolizidine alkaloids causes cancer in humans (Barceloux, 2008).

#### **5.4. Laboratory findings**

In the clinical setting, hepatic function is commonly assessed by monitoring the serum concentrations of proteins of hepatic origin. For example, elevations in aspartate aminotransferase (AST) might reflect liver pathology,  $\gamma$ -glutamyltransferase (GGT) and bilirubin are elevated with cholestasis, and  $\alpha$ -fetoprotein (AFP) is a specific marker for liver cancer. Although these markers are not necessarily elevated in every case of veno-occlusive disease, Anderson and McLean determined the serum concentration of AST, GGT and bilirubin in 29 long-term comfrey users, and AFP in a subgroup of seven comfrey leaf users. Although this cohort is too small to ascertain risk, it is interesting that AST, GGT, bilirubin and AFP were considered normal, and there was no evidence of liver injury even after prolonged consumption of comfrey leaf (0.5–25 g day<sup>-1</sup> for 1–30 years) (Anderson and McLean, 1989; Rode, 2002).

#### **5.5. Safety in special populations and situations**

According to Barnes *et al.* (2007), in view of the toxicity associated with the alkaloid constituents, comfrey should not be taken during pregnancy or lactation. However, Commission E suggests that during pregnancy comfrey should be used only after consultation with a physician (Blumenthal *et al.*, 1998).

Though no information is available concerning the application of comfrey on neonates, two neonatal deaths were reported in Canada after mothers used comfrey as a cream on the nipples; after this it was banned in Canada (Schaefer *et al.*, 2007).

No information is available on drug interactions, overdose, abuse and effects on ability to drive or operate machinery or impairment of mental ability.

#### **5.6. Overall conclusions on clinical safety**

There are no specific indications for the internal use of pyrrolizidine alkaloids in the modern therapy.

Because of potential toxicity, the internal use of pyrrolizidine-containing compounds is not recommended (Barceloux, 2008). However, the cutaneous use of the comfrey products with limited pyrrolizidine alkaloid content seems to be clinically safe in view of the available clinical data.

The German regulations limit the daily external application of total pyrrolizidine alkaloids to 0.1 mg for no more than 6 weeks per year. Products for cutaneous use with less than 10  $\mu$ g pyrrolizidine alkaloids in the daily dosage can be used without any limitation in the duration of use (BGA, 1992).

Due to lack of official position papers and scientific guidelines in the Community, the content of pyrrolizidine alkaloids should be limited according to national provisions.

### **6. Overall conclusions**

Based on the provided data on comfrey products, the traditional use of *S. officinale* is confirmed in the treatment of bruises and sprains. Although the efficacy in bruises, sprains, osteoarthritis and back pain is supported by good quality clinical trials, and the application in the treatment of bruises is suggested by the Commission E (Blumenthal *et al.*, 1998), a well established monograph cannot be granted since the exact composition of preparations applied in clinical trials are not known. In view of the hepatotoxic properties documented for the pyrrolizidine alkaloid constituents, comfrey should be used only externally. The topical application of comfrey-containing preparations to broken skin should be avoided (Barnes *et al.*, 2007). The benefit/risk balance of the appropriate medicinal application of good quality, pyrrolizidine-alkaloid free (or with limited content) products is positive.

There are no rational objections (self medication character, plausibility and safety) to the traditional use of comfrey products with limited pyrrolizidine alkaloid content, and the data provided support the long standing use of special *S. officinale* preparations.

## **Annex**

### ***List of references***