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Committee on Herbal Medicinal Products (HMPC)

## Assessment report on *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch, 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>Glycyrrhiza glabra</i> L. and/or of <i>Glycyrrhiza inflata</i> Bat. and/or <i>Glycyrrhiza uralensis</i> Fisch, radix
Herbal preparation(s)	a) Comminuted herbal substance b) Soft extract (1:0.4-0.5), extraction solvent water d) Soft extract (3:1), extraction solvent water
Pharmaceutical forms	Herbal preparations in liquid dosage forms for oral use.  Comminuted herbal substance as a herbal tea for oral use.
Rapporteur	
Assessor(s)	

Note: This Assessment Report is published to support the release for public consultation of the draft Community herbal monograph on *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch, 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

- Herbal substance(s)

Liquorice root consists of the dried unpeeled or peeled, whole or cut root and stolons of *Glycyrrhiza glabra* L and/or of *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch. It contains not less than 4.0 per cent of 18  $\beta$ -glycyrrhizic acid ( $C_{42}H_{62}O_{16}$ , Mr 823), calculated with reference to the dried drug (European Pharmacopoeia 2010).

*Glycyrrhiza glabra* is a perennial herb native to central and south-western Asia and the Mediterranean region. It is cultivated in the Mediterranean basin of Africa, in southern Europe, and in India. It is a medicinal plant in China and India, and cultivated for this purpose (WHO).

The genus *Glycyrrhiza* (Leguminosae) consists of about 30 species native to Europe, Asia, North and South America as well as Australia, including *G. glabra*, *G. uralensis*, *G. inflata*, *G. aspera*, *G. korshinskyi* and *G. eurycarpa*. *G. glabra* includes three varieties: Persian and Turkish liquorices assigned to *G. glabra* var. *violacea*, Russian liquorice is *G. glabra* var. *gladulifera*, and Spanish and Italian liquorices are *G. glabra* var. *typical* (Nomura et al., 2002). *G. uralensis*, *G. inflata* and *G. glabra* are the only species mentioned in the Chinese Pharmacopoeia. The Chinese name is *gan-cao* which means "sweet herb"

The word *Liquorice* essentially derives from Old Greek *glykyrrhiza*, which is a contraction of *glykeia rhiza* "sweet root". The first part *glykys* means "sweet", the second element *rhiza* is cognate to English "root". In Latin, the Greek plant name appears as *liquiritia*, originating from *liquere*, "flow", for the liquid form of liquorice juice. Latin *liquiritia* is the source of many names for liquorice in modern European languages, e.g., German *Lakritze*, Yiddish *lakrets*, etc. Liquorice root has been used since prehistoric times. Between the end of the XV and the beginning of the XVI century, botany as a science was born, and liquorice was categorized according to taxonomic classifications. The first attempt to create a botanical nomenclature came from the German botanist Leonhard Fuchs (1501–1566) who, accurately describes and characterises the plant. Works of eminent Arabic scientists like Al Razi, continued to be translated into Latin. In the XVIII century, the Neapolitan chemist and philosopher Giuseppe Donzelli described liquorice, referring to it by its modern name. The Swedish naturalist Carl von Linné (1707–1778) proceeded to subdivide plants into genus and species and identified three different species of *Glycyrrhiza*: *Glycyrrhiza glabra*, *Glycyrrhiza echinata* and *Glycyrrhiza hirsuta* (Fiore 2005).

### Saponins

Liquorice root contains triterpenoid saponins (4–20%), mostly glycyrrhizin, a mixture of potassium and calcium salts of glycyrrhizic acid (also known as glycyrrhizic or glycyrrhizinic acid and a glycoside of glycyrrhetic acid), which is 50 times sweeter than sugar (Blumenthal et al., 2000). Other triterpenes present are liquiritic acid, glycyrrhetol, glabrolide, isoglabrolide and liquorice acid (Isbrucker 2006).

Glycyrrhizin (3-*O*-(2-*O*- $\beta$ -d-glucopyranuronosyl)- $\alpha$ -d-glucopyranurosyl)-3- $\beta$ -hydroxy-11-oxo-18 $\beta$ ,20 $\beta$ -olean-12-en-29-oic acid) is the main active compound of *Glycyrrhiza glabra* root extracts. It is a monodesmoside, which on hydrolysis releases two molecules of D-glucuronic acid and the aglycone glycyrrhetic (glycyrrhetic) acid (enoxolone) (WHO 1999). It is the major bioactive compound in the underground parts of *Glycyrrhiza* (liquorice) plants which possesses a wide range of pharmacological properties and is used worldwide as a natural sweetener. Because of its economic value, the

biosynthesis of glycyrrhizin has received considerable attention. Glycyrrhizin is most likely derived from the triterpene  $\beta$ -amyrin, an initial product of the cyclisation of 2,3 oxidosqualene. The subsequent steps in glycyrrhizin biosynthesis are believed to involve a series of oxidative reactions at the C-11 and C-30 positions, followed by glycosyl transfers to the C-3 hydroxyl group (Seki 2008).

Glycyrrhizin and its aglycon, glycyrrhetic acid, have interesting therapeutic properties. Therapeutic potential of glycyrrhizin is mainly ascribed to the action of the steroid-like structure aglycone (18- $\beta$ -glycyrrhetic acid) having immunomodulatory properties. Traces of the  $\alpha$ -form of glycyrrhetic acid are also present in liquorice roots but have no pharmacological activity (Claude 2008).

Glycyrrhizin is present in the root as potassium and calcium salts, at percentages of between 2 and 15% (w/w), depending on plant species, geographic and climatic conditions, and consists of an aglycon (a pentacyclic triterpenic structure) bound to two glucuronic acid molecules (Sabbioni 2005).

### Flavonoids

More than 300 flavonoids have been isolated from *Glycyrrhiza* species. These flavonoids belong to various types, including flavanones or flavanonols, chalcones, isoflavans, isoflavenes, flavones or flavonols, isoflavones and isoflavanones. Amongst them, flavanones and chalcones are the types in majority (Zhang 2009).

Flavonoids are responsible for the yellow colour of liquorice. They include liquiritin, liquiritigenin, rhamnoliquiritin, neoliquiritin, chalcones isoliquiritin, isoliquiritigenin, neoisoliquiritin, licuraside, glabrolide and licoflavonol (Williamson, 2003). The compounds 5,8-dihydroxy-flavone-7-O-beta-D-glucuronide, glychionide A, and 5-hydroxy-8-methoxyl-flavone-7-O-beta-D-glucuronide, glychionide B were also isolated from the roots of *G. glabra* (Li et al., 2005).

In liquorice, the isoflavones glabridin, galbrene, glabrone, shinpterocarpin, licoisoflavones A and B, formononetin, glyzarin, kumatakenin, have been found (Williamson, 2003). Other isoflavones present are hispaglabridin A, hispaglabridin B, 4'-O-methylglabridin and 3'-hydroxy-4'-O-methylglabridin (De Simone et al., 2001; Haraguchi, 2001), glabroisoflavanone A and B glabroiso-flavanone B (Kinoshita et al., 2005).

### Coumarins

Coumarins include liqcoumarin, glabrocoumarone A and B, herniarin, umbelliferone, glycyrin, glycocoumarin, licofuranocoumarin, licopyranocoumarin (De Simone et al., 2001; Haraguchi, 2001) and glabrocoumarin (Kinoshita et al., 2005).

### Other compounds

Minor components occur in proportions that vary depending on the species and geographical location (WHO). The dihydrostilbenes, dihydro-3,5-dihydroxy-4'-acetoxy-5'-isopentenylstilbene, dihydro-3,3',4'-trihydroxy-5-O-isopentenyl-6-isopentenylstilbene, dihydro-3,5,3'-trihydroxy-4'-methoxystilbene and dihydro-3,3'-dihydroxy-5beta-d-O-glucopyranosyloxy-4'-methoxystilbene were isolated from the leaves of *G. glabra* grown in Sicily (Biondi et al., 2005). Moreover, fatty acids (C2-C16) and phenols (phenol, guaiacol), together with common saturated linear  $\gamma$ -lactones (C6-C14). 4-methyl- $\gamma$ -lactones, 4-ethyl- $\gamma$ -lactones in trace amounts, as well as asparagines, glucose, sucrose, starch, polysaccharides (arabinogalactants) and sterols ( $\beta$ -sitosterol, dihydrostigmaterol) have also been found (Hayashi et al., 1998; Blumenthal et al., 2000) (Näf and Jaquier, 2006).

- Herbal preparation(s)

There are about 14 species known, although most commercial liquorice is extracted from varieties of *G. glabra* grown in southern and central Europe (var. *typica*), in central and southern Russia (var. *glandulifera*), and in Iran and Iraq (var. *violacea*). Commercial liquorice products are derived from extracts of the root (Isbrucker 2006).

The harvesting of liquorice root occurs in the autumn of its third or fourth year of growth. The roots are dug up, washed and transported to warehouses for baling, sorting and drying. The dried roots are crushed by millstones and the pulp is boiled to make the extract. After removal of the solids, the extract is vacuum dried to a dark paste, which is cast into blocks or short sticks, or may be dried to a powder (Asl 2008).

The European Pharmacopoeia reports the following liquid and dry extracts.

Liquorice ethanolic liquid extract, standardised (Liquiritiae extractum fluidum ethanolicum normatum), which is produced from the herbal drug by a suitable procedure for liquid extracts using ethanol (70 per cent V/V) and contains 3 to 5% of 18  $\beta$ -glycyrrhizic acid. The extract is a clear liquid, dark brown, with a faint characteristic odour and a sweet taste (European Pharmacopoeia 2010 under minor revision).

Liquorice dry extract for flavouring purposes (Liquiritiae extractum siccum ad saporandum) produced from the cut liquorice root by a suitable procedure using water, and contains: 5 to 7% of 18  $\beta$ -glycyrrhizic acid. The dry extract is a yellowish-brown or brown powder with a very sweet taste (European Pharmacopoeia 2008 under revision).

However, no information on products on the market containing the standardised liquorice ethanolic liquid extract mentioned in the European Pharmacopoeia, are available.

- 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 (not mandatory field)
Austria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	None as a single preparation. Several combination products.
Belgium	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only in multi-component teas (> 6 herbal substances each).
Bulgaria	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/registered HMPs/THMPs.
Cyprus	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/registered HMPs/THMPs.
Czech Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input checked="" type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	None as a single preparation. Several combination products.
Denmark	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	For 70 years.
Estonia	<input checked="" type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only combination products with more than 5 substances.
Finland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/registered HMPs/THMPs.
France	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	2 combination products.
Germany	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Soft extract (1:0.4-0.5), extraction solvent: water.
Greece	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised or registered HMPs/THMPs.
Hungary	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input checked="" type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only combination products.
Iceland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Ireland	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Italy	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised or registered HMPs/THMPs as a single preparation.
Latvia	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only combination with more than 5 substances.
Liechtenstein	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	

Member State	Regulatory Status				Comments (not mandatory field)
Lithuania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Luxemburg	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Malta	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
The Netherlands	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input checked="" type="checkbox"/> Other Specify: Food supplements	No authorised/registered HMPs/THMPs.
Norway	<input checked="" type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Poland	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Portugal	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised/registered HMPs/THMPs Only as an excipient.
Romania	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	
Slovak Republic	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only combination with more than 5 substances.
Slovenia	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	No authorised or registered HMPs/THMPs.
Spain	<input type="checkbox"/> MA	<input checked="" type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Comminuted herbal substance Also combination products.
Sweden	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	Only as an excipient.
United Kingdom	<input type="checkbox"/> MA	<input type="checkbox"/> TRAD	<input type="checkbox"/> Other TRAD	<input type="checkbox"/> Other Specify:	

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.

### 1.3. Search and assessment methodology

This assessment report reviews the scientific literature data available for *Glycyrrhiza glabra*, and from the WHO monograph, European Pharmacopoeia monograph, British Pharmacopoeia monograph, ESCOP monograph, PubMed, EMA library and the internet, as well as available information on products marketed in the European Community, including pharmaceutical forms, indications, posology and methods of administration.

The keywords "*Glycyrrhiza glabra*", "licorice", "liquorice", in all text fields were used. Only clinical studies with *Glycyrrhiza glabra* extracts were included in the assessment report. Clinical studies carried out with single active principles present in *Glycyrrhiza glabra* were not considered.

## 2. Historical data on medicinal use

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

The earliest evidence of the use of liquorice comes from the ancient tombs of Egyptian pharaohs, including the 3000-year old tomb of King Tut. References to liquorice have also been made on Assyrian tablets dating back to the second or third millennia b.c. In the ancient Greece and Rome, liquorice was commonly used as a tonic and cold remedy. Theophrastus suggested liquorice as a remedy to combat infertility, to heal wounds and ulcerations of the mouth, and to treat malaises of the throat. In the first century b.c., Pliny the Elder alleged that liquorice clears the voice and postpones hunger and thirst, and consequently used it for dropsy.

As reported by Lucas in *Nature's Medicines*, the ancient Hindus believed that liquorice, administered as concoction with milk and sugar, increased sexual vigour. The ancient Chinese thought that liquorice root gave them strength and endurance, and they prepared it most often in tea for its tonic, expectorant, rejuvenating, aperient and nutritive properties. During the Middle Ages, Arabic medical scientists like Ibn Sinna (Avicenna, 980-1037) wrote about liquorice in his "Canone". The knowledge of phytotherapy passed around XI century a.d. onwards in monasteries. Numerous medical uses of liquorice are documented by the English physician Nicholas Culpeper (1616–1654) in his work the "Complete Herbal" (1653). At the beginning of the Industrial Age, liquorice can be found again in the formulation "teriaca", in the Pharmaceutical Code established by the Republic of Venice (1790) (Fiore 2005).

In the XIX century, the American Samuel Stearns and John Monroe asserted that the liquorice root serves as an emollient, demulcent, attenuant, expectorant, detergent and diuretic. The root 'abates thirst in dropsies', 'helps defluctions of the breast', 'softens acrimonious humours', 'temperates salt', 'allays the heat of the blood', promotes urine, and thickens the sanguinary fluid, when too thin'. Moreover, the root is 'good for pleurisy, gravel, dysury and intense pain'.

In India, liquorice is believed to ease thirst, as an antitussive and demulcent, and it serves as a treatment for influenza, uterine complaints, and biliousness. The Chinese and their Far Eastern neighbours have traditionally used liquorice most extensively. It is used in many Chinese formulas as a "guide herb" to enhance the effectiveness of the other ingredients, reduce toxicity and improve the taste and flavour.

Liquorice continues to serve as a flavouring agent, sweetening the bitter taste of many drugs, as a filler for pills, as an 'essential ingredient in ointments for treating skin diseases' and for prolonging the effects of strong tonic medicines, Addison's disease, and to potentiate glucocorticoid action.

In 1949, Costello and Lynn extracted estrogenic constituents from *Glycyrrhiza glabra*. They suggested that the plant could be used for medicinal purposes in treating hormone imbalances associated with menstruation; however the glycoside of glycyrrhetic acid has also been shown to possess anti-estrogenic activity (Sharaf 1971).

Beginning in the late 1940s and extending well into the 1950s, there was a growing interest in the metabolic activity of glycyrrhetic acid as treatment of adrenal and electrolyte disorders.

Liquiritiae radix has been a subject of the Czechoslovak Pharmacopoeia since 1947, and it is used in several combination products that are still on the Czech market.

Liquorice extracts have been commonly used in many European countries to relieve gastric and duodenal ulcers. Carbenexolone sodium, an anti-peptic ulcer drug, which is a succinate derivative of glycyrrhetic acid, has been extensively employed for the purpose of alleviating ulcers.



Molhuysen et al. (1950) reported side-effects of liquorice, which include water retention of sodium and chloride, and excretion of potassium. They also concluded that liquorice extracts exhibit effects similar to injections of deoxycorticosterone, but the effects are more persistent, even after the drug has been discontinued, until a salt-free diet is given. However, they did not observe a positive, but rather a slight negative response to liquorice extract in a patient suffering from Addison's disease who did not respond to ACTH. Card et al., in 1953, examined the effects of liquorice on normal subjects, as well as on patients who suffered from Addison's disease. They concluded that liquorice appeared to have positive results in reversing the effects of Addison's disease. In 1957 (Kumagai et al.), it was shown that glycyrrhizine has favorable effects on rheumatoid arthritis, when administered along ACTH or cortisone, but a little effect if administered alone. The results of these and other investigations suggested that the main effect of liquorice is to potentiate rather than mimic endogenous steroids (Isbrucker 2006).

In Germany, at least since 1976, a *Liquiritiae radix* soft extract (DER 1:0.4-0.5, extraction solvent water) is on the market as a traditional medicinal product, orally used to support gastric function. Several combination products, mainly with *Hederae helioides folium* and *Thymi herba*, are on the market in form of herbal teas according to the German Standard Marketing Authorisation procedure. Other traditional medicinal products are in combination with *Althaeae radix*, *Primulae radix*, *Thymi aetheroleum*.

In Denmark *Liquiritiae radix* preparations have a long history of use. Several medicinal products containing *Liquiritiae radix* preparations have been on the market, and also two herbal medicinal products containing Liquorice soft extract (DER 3:1, extraction solvent water) are authorised for more than 70 years, for their expectorant properties.

In Spain a herbal tea, as an infusion, decoction or a macerate, is authorised for more than 30 years (before 1973), in both traditional indications: as an adjuvant in gastric ulcers and as an expectorant in cough and catarrhs of the upper respiratory tract.

Since 1992, a herbal tea is authorised in Poland as an adjuvant in chronic gastric ulcer disease and as an expectorant in bronchial catarrh with cough and adjuvant in bronchi inflammations.

In many European countries (Austria, Czech Republic, Germany, Austria, Norway, Netherland, etc.), *Glycyrrhiza glabra*, radix is used as an excipient, both in herbal teas and as an extract in other medicinal products.

Various combination products containing *Liquiritiae radix* are on the European market with different indications, depending on the combination partners (used as an expectorant or in gastritis, etc).

In France, two combinations are on the market: a *Glycyrrhiza* extract (type of extract not specified), in combination with levomenthol, used to relieve throat irritations, and a herbal tea containing *liquiritiae radix* and *Melissa*, traditionally used to promote digestion.

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

### **Industrial uses**

Although glycyrrhizin is considered much sweeter than sucrose, the associated liquorice flavour limits its commercial value as a sweetener. Because glycyrrhizin also gives an undesirable brownish colour to foods, and the sweetness is lost in acidic solutions, as occurs in most beverages, glycyrrhizin remains of little value to the food and beverage industries. Primary use for liquorice products and glycyrrhizin is limited to flavouring tobacco and candy (Isbrucker 2006). Liquorice paste is the preferred form for

flavouring tobacco, whereas liquorice powder is preferred for confectionery and pharmaceuticals (Isbrucker 2006).

Some minor consumer use for liquorice root extract and glycyrrhizin has been in beer and ales, where they provide good surfactant (foaming) properties and take the edge off these potentially bitter-tasting beverages. Similarly, liquorice root extract and glycyrrhizin may be used to alleviate the bitter after taste in some saccharinated products and pharmaceutical preparations. Glycyrrhizin may be also used as a flavour enhancer for cocoa. Industrial uses for glycyrrhizin include an adhesive agent in insecticides and a wetting agent (surfactant). The residues of liquorice root after extraction serve in fire-extinguishing agents, to insulate fiberboard, compost for growing mushrooms and as feed for cattle, horses and chickens (Armanini et al., 2005; Isbrucker 2006).

### **Medicinal uses**

Liquorice presents demulcent and expectorant properties for dissolving and facilitating the discharge of mucus in catarrhs and for upper respiratory tract diseases and is currently employed in cough preparations (Blumenthal et al., 2000).

Ulcer-healing properties, anti-inflammatory and mild laxative activities have been documented. It shows mineralocorticoid properties due to the presence of glycyrrhizinic acid and its metabolite glycyrrhetic acid, which is an inhibitor of cortisol metabolism (Armanini et al., 2002).

The use of deglycyrrhized liquorice extract preparations in aphthous, stomatitis (oral ulcers) is also reported (Blumenthal 2003).

Useful applications have been described in the treatment of atopic dermatitis (Saeedi et al., 2003).

According to the British Herbal Pharmacopoeia, *Glycyrrhiza glabra* has anti-inflammatory and expectorant activities (BHP 1996).

In Germany, Liquiritiae radix soft extract (water, 1:0.4-0.5) is on the market as an oral liquid to support gastric function, for traditional use.

In Denmark, two oral liquids containing Liquorice soft extract (DER 3:1, extraction solvent water) are authorised for more than 70 years as expectorants for use in cough in shorter periods. One product contains 98.5 mg/ml soft extract corresponding to 4.64 mg glycyrrhizinic acid. The other contains 80.9 mg/ml soft extract corresponding to 3.92 mg glycyrrhizinic acid. The herbal substance used for the preparation is the unpeeled liquorice root of *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch, radix.

In summary, *Glycyrrhiza glabra* has been traditionally used in herbal medicine as an expectorant helping to relieve complaints, such as catarrhs, coughs and bronchitis (ESCOP 2003; Blumenthal et al. 2000;) and inflammatory conditions of the gastrointestinal tract, such as gastritis, gastric and duodenal ulcers in adults (ESCOP 2003).

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

Dosage: for oral administration for traditional uses as recommended in older and contemporary standard herbal literature:

- Powdered or comminuted root: 1-5 g as an infusion or a decoction three times a day.  
To prepare an infusion, use 4.5 g of finely comminuted herbal substance for 150 ml of boiling water in case of gastrointestinal disturbances, 1.5 g in 150 ml for bronchitis or cough. To be taken 2 to 3 times daily. Infusion time: 10-15 minutes (DAC 2009).

- To prepare a decoction, use the same proportion of the herbal substance and cold water. Bring it to the boil, allow it to steep for 10-15 minutes and then strain (PDR 2004).

Drink one cup of tea after meals. Not to be used for children below 4 years.

- In Poland the following posologies are used: as an expectorant: 1,5 g – 2 g up to 2 times daily; in chronic gastric ulcer disease: 4 g – 7 g daily divided in 2 – 4 doses.
- In Spain the posology of the herbal tea used as infusion, decoction or macerate for gastric ulcers or for cough and catarrhs of the upper respiratory tract is the following: 3-15 g root daily/L, divided in 2-3 cups a day.
- Recommended dosage in the last version of the Czech Pharmacopoeia (2009): single dose 1.5 g, daily dose 5 – 20 corresponding to 200 – 800 mg of glycyrrhizic acid (duration of use 4 – 6 weeks).

Average daily dose of crude plant material, 5–15 g, corresponding to 200–800 mg of glycyrrhizin.

Therefore the range of traditional posology for the herbal tea is broad and comprises also the use in ulcers, which is not acceptable for a traditional herbal medicinal product. The following posology may be considered as usual in practice:

Use as an expectorant: 1.5 g of comminuted herbal substance as a herbal infusion in 150 ml of boiling water or as a decoction 2 times daily.

Use for the relief of digestive symptoms, including burning sensation and dyspepsia: 1.5 - 2 g of comminuted herbal substance as a herbal infusion in 150 ml of boiling water or as a decoction 2 to 4 times daily.

- Glycyrrhizae radix should not be used for longer than 4 – 6 weeks without medical advice (WHO).
- Liquorice extract (DER and extraction solvent not specified): 0.6-2 g (Barnes 2002).
- Liquiritiae radix soft extract (water, 1:0.4-0.5) to support gastric function: 32 mg 2-3 times daily for oral use. Not more than 160 mg (32 mg 5 times) daily (German posology).
- Liquiritiae radix soft extract (water, DER 3:1) as an expectorant: 1.2-1.5 g 3-4 times daily.
- Magisterial preparations traditionally used in Norway as cough remedies, refer to the posology of the ESCOP monograph for cough and bronchial catarrh: 1.5 -5 g *Glycyrrhiza glabra*, radix equivalent to 60-200 mg glycyrrhizic acid.

### 3. Non-Clinical Data

A large amount of studies are published on the pharmacological effects of liquorice and liquorice derivatives.

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

##### **Liquorice derivatives**

Most of the pharmacological studies of liquorice saponins focus on the main constituent glycyrrhizic acid and its aglycone, glycyrrhetic acid. These two compounds exhibit extensive biological activities, including antiulceric, anti-inflammatory, antiallergic, antioxidative, antiviral, anticarcinogenic, antithrombotic, antidiabetic, hepatoprotective and neuroprotective activities. Recently, glycyrrhizic acid has been used as a potential therapeutic agent for several virus diseases, including chronic hepatitis B and C, as well as human acquired immunodeficiency syndrome (AIDS) (Zhang 2009).

Glycyrrhizin promotes the biosynthesis of cholesterol in rat liver. The excretion of cholesterol in the liver appears to be proportional to a subsequent decrease of cholesterol levels in the blood (Davis 1991).

Liquorice flavonoids gained popularity because of their significant biological activities including antiulceric, antioxidative, anti-inflammatory, antimicrobial, antispasmodic, antitumor, metabolic syndrome preventive activities and others (Zhang 2009).

#### Corticosteroid activity

##### **Liquorice derivatives**

Based on the similarities in the corticosteroid hormones and the glycyrrhizate structure, initial theories assumed a direct binding of glycyrrhetic acid to the mineralocorticoid and glucocorticoid receptors in various tissues. However, competitive binding assays revealed that the affinities of glycyrrhizin and glycyrrhetic acid for the corticoid receptors are 3000 – 10000 times less than that for endogenous adrenocortical hormones (Armanini 1989).

Glycyrrhetic acid, present as its glycoside in liquorice has been shown to be a potent competitive inhibitor of 11 $\beta$ -HSD (11 $\beta$ -hydroxysteroid dehydrogenase). Lowered 11 $\beta$ -HSD activity results in higher peripheral and intrarenal concentrations of corticosterone in experimental animals and cortisol in humans, which may then interact with mineralocorticoid receptors and promote Na<sup>+</sup> re-absorption. Other processes may also be involved. Souness and Morris (1993) reported that acute pretreatment of adrenalectomized male rats with carbenoxolone sodium, the water-soluble succinate derivative of glycyrrhetic acid, caused both cortisol and corticosterone to display significant mineralocorticoid-like activity, particularly Na<sup>+</sup> retention. They also showed that the same dosage of carbenoxolone sodium, which does not affect Na<sup>+</sup> or K<sup>+</sup> excretion on its own, amplifies the antinatriuretic but not the kaliuretic activity of the two mineralocorticoids, aldosterone and 11-deoxycorticosterone. The latter steroid is particularly significant since it does not possess a hydroxyl group at the c-11 position in the steroid nucleus and therefore is not a substrate for the enzyme 11 $\beta$ -HSD. Glycyrrhetic acid has also been shown by Latif (1992) to be a potent inhibitor of the important steroid metabolizing enzyme S/3-reductase, and also an inhibitor of 3 $\beta$ -hydroxysteroid dehydrogenase to a lesser extent. It does not inhibit Sa-reductase. Thus liquorice derivatives reroute the metabolism of aldosterone, deoxycorticosterone and glucocorticoids resulting in the accumulation of unmetabolised hormones and their corresponding Sa-dihydro and 3 $\alpha$ ,5-tetrahydro derivatives (as in children with the syndrome of AME: apparent mineralocorticoid excess) (Davis 1991).

Originally the structure and activity of glycyrrhetic acid were thought to be similar to adrenal steroid hormones, such as aldosterone and cortisol, since ingestion of liquorice mimicked hyperaldosteronism and was suggested as a treatment for Addison's disease. It is now thought that the presence of intact adrenals is required for liquorice ingestion to cause sodium retention, leading to subsequent hypertension (Davis 1991).

##### **Liquorice**

Hypertensive children with the syndrome of AME lack 11 $\beta$ -hydroxysteroid dehydrogenase (11- $\beta$ HSD) enzyme activity, a condition resulting in reduced peripheral metabolism of cortisol. Liquorice ingestion causes biological effects with changes in the pathways of adrenal steroid metabolism similar to those demonstrated in children with the syndrome of AME. Studies in normal subjects fed liquorice showed that the corticosteroid-like effects were associated with a change in cortisol metabolism, which suggests an inhibition of the 11 $\beta$ -HSD enzyme (Stewart et al., 1996). 11 $\beta$ -HSD catalyzes the oxidation of the active mineralocorticoid, cortisol, the inactive cortisone and is also responsible for the reverse, a reduction reaction. These findings collectively indicate that the metabolites of glycyrrhizin promote the development of a pseudoaldosteronism by inhibiting cortisol metabolising processes at several levels.

#### Anti-inflammatory effects

The anti-inflammatory properties of 4 liquorice extracts have been investigated. Extracts of roasted liquorice were obtained by ethanol (rLE) or water extraction (rLW) and extracts of raw liquorice obtained by ethanol (LE) or water extraction (LW). rLE demonstrated strong anti-inflammatory activity by reducing nitric oxide and prostaglandin E2 production in the LPS-stimulated mouse macrophage cell, RAW264.7. It also inhibited the production of pro-inflammatory cytokines and CD14 expression on the LPS-stimulated RAW264.7 cells. LPS-induced degradation and phosphorylation of IK-B $\alpha$ , along with DNA-binding of NF-KB, was significantly inhibited by rLE exposure in RAW264.7 cells. In the murine model, it was found that *in vivo* exposure to rLE induced an increase in the survival rate, reduced plasma levels of TNF- $\alpha$  and IL-6, and increased IL-10 production in LPS-treated mice. These data suggest that the use of rLE may be a useful therapeutic approach to various inflammatory diseases (Kim 2005).

The same group of researchers showed the anti-inflammatory activity of liquorice (LE) and roasted liquorice (rLE) extracts, determined in the murine phorbol ester-induced acute inflammation model and collagen-induced arthritis (CIA) model of human rheumatoid arthritis. The study demonstrated that rLE and LE dose-dependently inhibited phorbol ester-induced ear oedema, and rLE possesses greater activity than LE. Oral administration of LE or rLE reduced clinical arthritis score, paw swelling, and histopathological changes in a murine CIA. LE and rLE decreased the levels of proinflammatory cytokines in serum and matrix metalloproteinase-3 expression in the joints. Cell proliferation and cytokine secretion in response to type II collagen or lipopolysaccharide stimulation were suppressed in spleen cells from LE or rLE-treated CIA mice. Furthermore, LE and rLE treatment prevented oxidative damages in liver and kidney tissues of CIA mice. Taken together, LE and rLE have dose-dependent oedema reducing effects and show benefits in protecting against both acute inflammation and chronic inflammatory conditions, including rheumatoid arthritis. According to these data it appears that rLE may inhibit the acute inflammation more potently than LE (Kim 2010).

#### Antiulcer effects

The most important traditional medicinal use for liquorice has been as a demulcent for the digestive system.

Anti-ulcer effects of liquorice are due to inhibition of 15-hydroxyprostaglandin dehydrogenase and delta13-prostaglandin reductase. 15-hydroxyprostaglandin dehydrogenase converts prostaglandins E2 and F2 $\alpha$  to 15-ketoprostaglandins, which are inactive. Delta13-Prostaglandin reductase metabolises the inactive A13-prostaglandin to 13,14-dihydro,15-ketoprostaglandin, which is further metabolised and excreted in urine. Thus, liquorice has the effect of raising the local concentration of prostaglandins that promote mucous secretion and cell proliferation in the stomach, leading to healing of ulcers (Baker 1994).

The protective effect against gastric ulcer of liquorice extract was ascribed to glycyrrhizic acid-free fractions. It has been found that the flavonoids also made up part of the pharmacological activities of liquorice. Therefore, the saponins and flavonoids are both considered as the major bioactive constituents of liquorice (Zhang 2009).

Among the chemical constituents of the plant, glabridin and glabrene exhibit inhibitory activity against the growth of *Helicobacter pylori in vitro*. These flavonoids also showed anti-*H. pylori* activity against a clarithromycin and amoxicillin resistant strain (Fukai 2002).

The effects of a F<sub>M</sub>100, a fraction obtained from the methanolic extract of liquorice root by a fractional precipitation with sodium hydroxide and hydrochloric acid, on gastrin production in male rats and female mongrel dogs, were examined. In fasting rats, serum gastrin concentrations were slightly, but significantly, elevated as compared to control animals following the oral administration of 800 mg/kg of F<sub>M</sub>100, but not in those animals receiving a dose of 400 mg/kg. When entry of gastric juice from the

fundus into the antrum was physically blocked in rats prior to the intraduodenal administration of  $F_M100$  800 mg/kg, there was a significant decrease in the serum concentration of gastrin. Increase in serum gastrin was nearly completely abolished following the intraduodenal administration of 200mg  $F_M100$ . These results suggest that the anti-ulcer effects of methanolic liquorice extract may be due to reduced gastric secretions caused by an inhibition of gastrin release (Ishii and Fujii 1982).

### **Carbenoxolone**

Carbenoxolone is a compound developed as glycyrrhizate analog and has shown to be effective in clinical trials in the treatment of gastric ulcer at the medium dose of 100 mg three times a day (Horwich 1965; Turpie 1965; Fraser 1972; Langman 1973) and duodenal ulcers (Brown 1972; Doll 1968; Montgomery 1968).

The mechanism of action of carbenoxolone remains unclear. Peskar reported the inhibition of prostaglandin metabolising enzymes by carbenoxolone in human gastric tissue and inhibition of the enzyme phosphodiesterase. Moreover, it has been suggested that in the gastric mucosa both increased prostaglandin levels and effects on the nitric oxide system could contribute to the protective action of carbenoxolone (Dembiska-Kiec 1991).

### Anti-viral, anti-microbial and immunostimulatory effects

Liquorice and glycyrrhizate compounds have long been used in the treatment of chronic viral hepatitis in China and Japan, but the possible mechanism of anti-viral activity remains unknown (Van Rossum 1998).

### **Glycyrrhizin**

Glycyrrhizin inhibits *in vitro* the growth of a number of viruses, including human immunodeficiency virus. It has been suggested that glycyrrhizin has an effect on viral growth, possibly through an inhibition of viral particle to cell membrane binding, replication mechanisms, or through cellular signal transduction mechanisms (Crance 2003). Glycyrrhizin has been reported as the most active in inhibiting replication of the severe acute respiratory syndrome (SARS) associated coronavirus (Cinati 2003).

The immunostimulatory properties of glycyrrhizin were also studied by Utsunomiya et al. (1997). BALB/c mice infected with influenza virus A2 (H2N2) were unable to survive 10 times the mean lethal dose (LD50) of virus. However, a complete survival was observed when these animals were treated with 10 mg glycyrrhizin/kg, intraperitoneal, on the day prior to, the day after, and on the fourth day after infection.

This same dosing regimen conferred 70% survival in mice infected with 50 times the viral LD50. This response was demonstrated to be dose-dependent with improved survival in animals administered greater than 2.5 mg glycyrrhizin/kg.

It has been suggested that the anti-viral mechanism of glycyrrhizin could be indirect and possibly stimulating endogenous viral defence mechanisms. The administration of  $\beta$ -interferon monoclonal antibody to infected mice blocked the anti-viral activity of glycyrrhizin treatment (Utsunomiya et al., 1997), thus suggesting that the anti-viral activity of glycyrrhizin is due to its stimulating of  $\beta$ -interferon production by T-cells.

### **Liquorice extracts**

*In vitro* antiviral effects were observed for viruses causing respiratory tract infections like influenza virus and the severe acute respiratory syndrome (SARS) corona virus, the Hepatitis B virus and



Epstein Barr virus, human immunodeficiency virus (HIV), encephalitis causing viruses like herpes simplex virus and Japanese encephalitis virus (Utsunomiya et al., 1997; Cinati et al., 2003).

The therapeutic effect of the extract Stronger Neo-Minophagen C (SMNC) on liver dysfunction associated with cytomegalovirus (CMV) infection in immunocompetent individuals has been evaluated. SNMC is an intravenous solution, comprised of 0.2% glycyrrhizin (GL), 0.1% cysteine and 2.0% glycine in a saline solution. GL is an aqueous extract of liquorice root (*glycyrrhizae radix*). Liver dysfunction in 4 cases improved and CMV disappeared from urinary samples after administration of GL intravenously by the age of 12 months. SNMC treatment could be used for the treatment of patients with SARS (Numazaki 2003).

The decoction of root has been used to treat allergic inflammation and microbial infections. The antimicrobial activity of crude extracts and fractions of the *Glycyrrhiza glabra* roots traditional used as demulcents and expectorants was investigated. Extracts and their fractions were tested against six bacteria and two fungal strains using well diffusion method and microdilution method.

The air-dried roots of the plant (3.25 kg) of *Glycyrrhiza glabra* were percolated with 80% ethanol (10 L) twice at room temperature. The extract was concentrated in vacuum, yielded 385 g of crude extract. The extract (361.5 g) was suspended in water, and then fractionated successively with equal volumes of chloroform, ethyl acetate and n-BuOH, leaving a residual water soluble fraction. Each fraction was evaporated in vacuum to yield the residues of chloroform soluble fraction (101 g, 28% w/w), ethyl acetate soluble fraction (12.5 g, 3.5% w/w) and n-BuOH soluble fraction (51.5 g, 14 % w/w). The remaining water fraction was (196.5 g, 54% w/w). Each organic extract was then evaporated to dryness. Stock extract solutions were prepared at 200 mg/ml in distilled water.

All extracts and fractions showed to possess antimicrobial effect. Two fungal strains, *Candida albicans* and *Trichophyton rubrum*, showed interesting susceptibility profiles when evaluated using high concentrations of the extracts and fractions with MICs ranging from 0.8 to 200 mg/mL. In case of bacterial strains, *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli* were susceptible to the extracts and fractions with MICs ranging from 0.2 to 1.2 mg/mL. Gentamicin for bacteria and clotrimazole for fungi were used as standard antibiotics for comparison with extracts and fractions (Gopal 2009).

Antimycobacterial activity of methanolic *Glycyrrhiza glabra* root extract was observed at 500 µg/mL. Bioactivity guided phytochemical analysis identified glabridin as potentially active against both *Mycobacterium tuberculosis* H37Ra and H37Rv strains at 29mcg/ml concentration. Glabridin exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria. Results indicate the potential use of liquorice as antitubercular agent (Gupta 2008).

The *in vitro* antimicrobial activity of methanol, ethanol, chloroform, diethyl-ether and aqueous *Glycyrrhiza glabra* extracts have been tested against selected bacteria by using agar well diffusion assay against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus*. The results obtained with *P. aeruginosa* were particularly interesting since it was not inhibited by the antibiotic used, but the tested plant extracts effectively inhibited the growth of *P. aeruginosa*. Plants extract in water was effective against spore forming of *B. subtilis*, while *S. aureus* and *E. coli* were not effectively inhibited. The extract was prepared by soaking five grams of plant material in 20 ml of double distilled water and placed at ambient temperature for 48 h; 100 µl of the extract was used. The results indicated that *G. glabra* presents a noteworthy potential of antibacterial activities (Shinwari 2009).

#### Antioxidant activity

A group of neolignan lipid esters and phenolic compounds isolated from the roots and stolons of liquorice (*Glycyrrhiza glabra*) were found to have chemopreventive properties. Of these compounds, hispaglabridin B isoliquiritigenin, and paratocarpin B were found to be the most potent anti-oxidant agents (Chin 2007).

#### Antiatherogenic effects

*In vitro* and *in vivo* studies have demonstrated that liquorice extracts possess antiatherogenic effects and can inhibit LDL oxidation. The administration of small amounts (0.1 g/day for 1 month) of liquorice-root alcoholic free of glycyrrhizin extract may act as a potent antioxidant in atherosclerotic apolipoprotein E-deficient mice (Fuhrman et al., 1997) and in moderately hypercholesterolemic patients (Fuhrman et al., 2002).

#### Hepatoprotective effects

##### **Glycyrrhizin**

It was reported that glycyrrhizin (1 mg injection, intramuscular, twice per week) protected mice from the hepatocellular injuries caused by the 1–9 month administration of 10% ethanol in drinking water. Liver sections from mice receiving the concurrent administration of glycyrrhizin and 10% ethanol had no microscopic signs of alterations or injuries whereas those receiving ethanol alone had greatly changed morphological features typical of chronic ethanol exposure (Isbrucker 2006).

Glycyrrhizin, at concentrations of 25–200 µg/ml, was found to significantly inhibit the CCl<sub>4</sub>-induced release of AST and LDH. It has been speculated that this function was due to an alteration of membrane fluidity by the glycyrrhizin, or perhaps an inhibition of CCl<sub>4</sub>-induced membrane lipid peroxidation.

Kiso et al. (1984) examined the effects of glycyrrhizin and its metabolites on the CCl<sub>4</sub>-induced free radical generation and lipid peroxidation in cultured rat hepatocytes and microsomes. The authors concluded from these results that the *in vivo* hepatoprotective mechanism of glycyrrhizic acid is due to its aglycone, 18β-glycyrrhetic acid, which inhibits both free radical generation as well as lipid peroxidation (Kiso et al., 1984).

The *in vivo* protection of glycyrrhizin against CCl<sub>4</sub>-induced hepatotoxicity was illustrated by Jeong et al. (2002). Pretreatment of mice with 10–100 mg/kg, subcutaneous, significantly reduced the elevated serum ALT and AST as well as the liver lipid peroxidation caused by CCl<sub>4</sub>. The depletion of hepatic glutathione was also reduced in a dose-dependent manner by glycyrrhizin treatment.

#### Anti-carcinogenic effects

##### **Glycyrrhizin and glycyrrhetic acid**

Glycyrrhizin has been shown to reduce hepatocellular carcinomas in mice induced with diethylnitrosamine (Shiota et al., 1999). The intramuscular administration of 2 mg glycyrrhizin/mouse, 3 days per week, reduced the incidence and total number of tumours or hepatocellular carcinomas per liver at week 32 of treatment.

Similarly, glycyrrhizin (10 mg/kg, intraperitoneal, on days 1, 3, 5, and 7) significantly reduced the incidence of B16F10 melanoma metastases in the lungs of mice (Kobayashi et al., 2002). Pulmonary metastasis by this cell line is strongly influenced by interleukin-4 (IL-4) produced by tumour-associated Th2 cells. The authors found that isolated CD4<sup>+</sup> T cells from treated mice produced significantly less IL-4 than those isolated from control animals, suggesting that this may be a mechanism by which glycyrrhizin reduces this specific form of tumourigenesis.



Isoliquiritigenin was demonstrated to prevent the incidence of 1,2-dimethylhydrazine-induced colon and lung tumours in mice when administered at a dose of 300 mg/kg (Chin 2007).

The effects of glycyrrhetic acid induce anoikis-like death and cytoskeletal disruption in the central nervous system (CNS) tumourigenic cells. Anoikis is a form of apoptosis, or programmed cell death that occurs when a cell becomes dislodged from its matrix. Glycyrrhetic acid was cytotoxic in a time- and dose-dependent manner, and the tumourigenic cells shed floating cells upon glycyrrhetic acid treatment and even some of the adherent cells were easily detached from the fibronectin-coated culture dish by gentle shaking and aspiration. Reculture of the detached cells revealed that the longer the duration of glycyrrhetic acid exposure, the less the number of the proliferatable cells. These results indicate that glycyrrhetic acid perturbs cell adhesion and induces anoikis-like cell death. Further, glycyrrhetic acid also induced morphologic changes and disturbed cytoskeletal proteins. The study provides evidence that glycyrrhetic acid is capable of effectively killing tumour cells in the CNS, that adhesion loss caused by glycyrrhetic acid is fatal and irreversible, and that glycyrrhetic acid induces cytoskeletal disruption and anoikis-like death. Glycyrrhetic acid content in liquorice has been reported to be 5.8 to 11.4%, and plasma glycyrrhetic acid levels reached 10 µM in humans ingesting liquorice, which is exactly the same concentration that affected the tumour cells effectively in the study. Although validation studies supporting the utilization in clinical practice are warranted, a liquorice compound glycyrrhetic acid, that could disturb cytoskeletons and adhesion, may potentiate anticancer effects on the anoikis-prone cells (Yamaguchi 2010).

### **Liquorice extracts**

Hepatocellular carcinoma (HCC) occurs in patients with hepatitis C virus-RNA positive chronic liver disease. A retrospective study was carried out to evaluate the long term preventive effect of Stronger Neo-Minophagen C (SNMC) on HCC development. SNMC is a Japanese medicine that is commonly administered to patients with chronic hepatitis C to improve the serum alanine aminotransferase (ALT) level. Out of 453 patients diagnosed with chronic hepatitis C retrospectively in the study hospital between January 1979 and April 1984, 84 patients (Group A) had been treated with SNMC. SNMC was given at a dose of 100 mL daily for 8 weeks, then 2–7 times a week for 2–16 years (median, 10.1 years). Another group of 109 patients (Group B) could not be treated with SNMC or interferon for a long period of time (median, 9.2 years) and were given other medicine (such as vitamin K). The patients were retrospectively monitored, and the cumulative incidence of HCC and risk factors for HCC were examined.

The 10th year rates of cumulative HCC incidence for Groups A and B were 7% and 12%, respectively, and the 15th year rates were 12% and 25%. By Cox regression analysis, the relative risk of HCC incidence in patients not treated with SNMC (Group B) was 2.49 compared with that of patients treated with SNMC (Group A). The authors concluded that long term administration of SNMC in the treatment of chronic hepatitis C was effective in preventing liver carcinogenesis (Arase 1994).

### Antimutagenic effects

#### **Glycyrrhizin and glycyrrhetic acid**

A study on the anti-mutagenic effects of glycyrrhizin and glycyrrhetic acid demonstrated, using a modified Ames test, that both of these compounds inhibited the mutagenicities of 3-amino-1-methyl-5*H*-pyrido [2,3-*b*]indol (Trp-p-2), 2-acetyl aminofluorene, and benzo(*a*)pyrene, in the presence of S9 fraction hepatic enzymes. When the assay was repeated using mutagens not requiring metabolic activation, such as methyl glyoxal, glyceraldehyde and glucose pyrolysate, glycyrrhizin inhibited the number of induced *Salmonella typhimurium* TA98 revertants, whereas glycyrrhetic acid promoted the number of revertants per plate. These results prompted the authors to speculate that glycyrrhetic acid may act by inhibiting the metabolic activation of some mutagens (Isbrucker 2006).

## Liquorice extracts

The inhibitory effect of liquorice ethanolic extract against the mutagenicity of N-nitrosodimethylamine (NDMA), N-nitrosopyrrolidine (NPYR), N-nitrosodibutylamine (NDBA), and N-nitrosopiperidine (NPIP) has been evaluated in the Ames test. Liquorice ethanolic extract showed an inhibitory effect (ranging from moderate to strong) against mutagenicity of all N-nitrosamines tested. The ethanolic extract showed the strongest inhibitory effect against NPIP (72%), NDMA (45%), and NPYR (39%). The mutagenicity of NDBA was markedly reduced (25-46%) by concentrations of 500 g/plate of liquorice ethanolic extract (Ikken et al., 1999).

To elucidate the inhibition mechanism of chemically induced mutagenicity by liquorice extract, glycyrrhizin, 18 $\alpha$ - and 18 $\beta$ -glycyrrhetic acid, Zani et al. (1993) studied their desmutagenic and antimutagenic effects on the activity of the direct-acting mutagens ethyl methanesulfonate (EMS), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), and ribose-lysine browning system. None of the compounds tested showed desmutagenic or antimutagenic activity against MNNG-induced reversions in *S. typhimurium* TA100. Studies on EMS-induced mutations showed no detectable desmutagenic activity of any compound tested, and only the liquorice extract demonstrated a true antimutagenic activity at sub-toxic concentrations. All four test compounds were desmutagenic against the ribose-lysine induced mutants, with 18 $\beta$ -glycyrrhetic acid the most effective. Only the liquorice extract was antimutagenic towards ribose-lysine, suggesting that a non-glycyrrhizin compound is the active antimutagenic component of this extract.

Mitscher et al. (1986) had also reported that liquorice extracts were highly effective against the mutagenic effects of EMS in the Ames test. The antimutagenic activity of liquorice extract was confirmed in the *rec*-assay in *Bacillus subtilis* strain M45, which is deficient in the genetic recombination function. However, liquorice extract was not antimutagenic to the activities of the frameshift mutagens 9-aminoacridine or acriflavine, suggesting specificity in its mechanism of action. These results led to the hypothesis that the root extract might be acting as an antimutagen either by enhancing a DNA repair response or by directly interfering with the mutagen. Results in *Escherichia coli* K-12 AB1157, which contains a transposon within the *ada* locus, show that liquorice extract improves the survival of the bacteria when applied prior to exposure of the cells to EMS. Authors concluded that liquorice extract exerts an antimutagenic effect by inducing the adaptive response in bacterial cells.

### Anti-cariogenic studies

Several studies have been conducted on the effects of liquorice and glycyrrhizin on the growth and acid production of oral bacteria associated with the development of dental caries.

## Glycyrrhizin

Segal et al. (1985) reported that neither liquorice "juice," nor glycyrrhizin inhibited the growth of seven *Streptococcus mutans* strains. In the presence of sucrose, 0.5–1% glycyrrhizin had no effect on growth, but significantly inhibited bacterial adherence to glass by nearly 100% at the highest concentration tested.

### Expectorant activity

*Glycyrrhiza* has been shown to decrease irritations in the throat and to produce expectorant effects. It is assumed that *Glycyrrhiza* is able to stimulate tracheal mucus secretions and hence produce demulcent and expectorant effects (Davis 1991; Alternative Medicine Review 2005).

### Behavioural studies

The effects of aqueous extract of *Glycyrrhiza glabra* in mice subjected to the forced swimming test (FST) and tail suspension test (TST) were studied. The extract of *G. glabra* (75, 150 and 300 mg/kg)

was administered orally for 7 successive days in separate groups of Swiss young male albino mice. The dose of 150 mg/kg of the extract significantly reduced the immobility times of mice in both FST and TST, without any significant effect on locomotor activity of mice.

The efficacy of extract was found to be comparable to that of imipramine (15 mg/kg, intraperitoneal) and fluoxetine (20 mg/kg, intraperitoneal). Liquorice extract reversed the reserpine-induced extension of immobility period of mice in FST and TST. Sulpiride (50 mg/kg, intraperitoneal; a selective D2 receptor antagonist) and prazosin (62.5 µg/kg i.p.; an α1-adrenoceptor antagonist) significantly attenuated the extract-induced antidepressant-like effect in TST. On the other hand, p-chlorophenylalanine (100 mg/kg, intraperitoneal; an inhibitor of serotonin synthesis) did not reverse the antidepressant-like effect of liquorice extract. This suggests that the antidepressant-like effect of liquorice extract is mediated by an increase of brain norepinephrine and dopamine, but not by an increase of serotonin. The monoamine oxidase inhibiting effect of liquorice may be contributing favourably to the antidepressant-like activity. Liquorice extract may possess an antidepressant-like effect (Dhingra 2006).

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

Pharmacokinetic studies have been performed on glycyrrhizin, either singly or in aqueous liquorice root extract.

In humans, Glycyrrhizin has a poor oral bioavailability. Glycyrrhizin was detected at very low levels after a single oral dose in the range of 100–1600mg/kg. Glycyrrhetic acid, the aglycone of glycyrrhizin, is readily detected in plasma following the ingestion of glycyrrhizin or liquorice extract (Isbrucker 2006).

After oral administration, glycyrrhizin is metabolized to glycyrrhetic acid by intestinal bacteria which contain β-D-glucuronidase. Intravenously administered glycyrrhizin is metabolized in the liver by lysosomal β-D-glucuronidase to 3-mono-glucuronide glycyrrhetic acid. This metabolite is excreted with bile into the intestine, where it is metabolized by bacteria into glycyrrhetic acid, which can be reabsorbed. The enterohepatic circulation of glycyrrhetic acid can be expected in humans because glycyrrhetic acid metabolites can be hydrolyzed by human gastrointestinal bacteria (Asl 2008; Ploeger et al., 2000).

The time to maximum glycyrrhetic acid plasma concentration is 10 h for glycyrrhizin and 2 h longer with liquorice extract in healthy adult volunteers fed 800 or 1600 mg glycyrrhizin as its ammoniated salt or in liquorice extract (Cantelli-Forti et al., 1994).

Interindividual differences in glycyrrhizin response, metabolism and kinetics are influenced, at least in part, by the intestinal microflora profile. Neither glycyrrhizin nor glycyrrhetic acid accumulate in tissues. However, both compounds adhere extensively to human and rat serum albumin through a saturable process (Ishida 1992; Ploeger et al., 2000).

The pharmacokinetics of glycyrrhizin is nonlinear. After bolus intravenous administration at a dose of 20, 50, or 100 mg/kg in rat, the decline in the concentration of glycyrrhizin in plasma, is generally biexponential at each dose, but the terminal disposition became much slower as the dose was increased. In addition, the apparent total body clearance decreased significantly with increases in the dose. The apparent distribution volume after intravenous administration is unaffected by the dose. Glycyrrhetic acid has a large volume of distribution, a long biological half-life, and undergoes substantial enterohepatic circulation. Thus large doses of KCl supplementation for weeks are necessary because of the long half-life of glycyrrhetic acid (Asl 2008).

The plasma clearance of glycyrrhizin and glycyrrhetic acid is dose dependent when administered at levels which exceed the saturation of serum protein binding, and not dose dependent at doses below 120 mg in healthy volunteers. The plasma clearance is in the range of 38–64ml/h/kg and the volume of distribution at steady state (38–64ml/kg) was close to the mean serum volume for humans, 43ml/kg (Cantelli-Forti 1994).

Other components contained in liquorice extracts could affect the pharmacokinetics of glycyrrhizin and glycyrrhetic acid, the main metabolite of glycyrrhizin. It has been observed that after administration of aqueous liquorice root extract (LE) to rats and humans, glycyrrhizin and glycyrrhetic acid levels are lower compared with glycyrrhizin alone, and the pharmacokinetic curves showed significant differences in the areas under the plasma-time curve (AUC),  $C_{max}$ , and  $T_{max}$  parameters. Also, the data obtained from urine samples confirmed a reduced bioavailability of glycyrrhizin present in LE compared with pure glycyrrhizin (Isbrucker 2006).

It has been also suggested that interaction between the glycyrrhizin and other components in LE during intestinal absorption causing modified bioavailability could explain the various clinical adverse effects resulting from the chronic oral administration of glycyrrhizin alone as opposed to LE (Cantelli-Forti et al., 1994).

Plasma clearance of glycyrrhetic acid is significantly decreased in patients with chronic hepatitis C and liver cirrhosis (Ploeger et al., 2000 van Rossum et al., 1998). Together, these data suggest a hepatic related capacity-limited process in metabolism and/or excretion in the bile.

Glycyrrhetic acid is able to cross the placental barrier and can be detected in the rat foetuses (Hundertmark et al., 2002). In this study, dams were fed 100 mg glycyrrhetic acid/kg/day commencing on the 13th day of gestation. On the 17th, 19th and 21st days of gestation the maternal plasma glycyrrhetic acid concentrations were approximately 100 mg/ml, whereas the foetal concentrations were 5, 18 and 32 mcg/ml, respectively.

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

#### **Acute toxicity**

In mice oral LD50 values of Glycyrrhiza extract is > 7.5 g/kg. In rats oral LD50 values range between 14.2 and 18.0 g/kg. The intraperitoneal administration of 2 g/kg of 18 $\alpha$ -glycyrrhetic acid is lethal to adult female Sprague–Dawley rats. This dose led to the progressive impairment of cardiac function. The histopathology of rats revealed brain, cerebellum and lung oedema with renal haematic stasis. Focal changes of the papillary muscles as well as swollen cardiomyocytes were noted (Isbrucker 2006).

Acute toxic effects of convulsions and slight haemolysis in mice administered 70 mg/kg glycyrrhizin intravenously have been reported. There were no toxic effects seen at lower doses of glycyrrhizin (Isbrucker 2006).

#### **Short-term studies (repeat dose toxicity)**

Toxic effects of short-term liquorice extract administration to Wistar rats have been examined. Rats were orally administered 0.31, 0.63, 1.25, or 2.5 g liquorice extract/kg/day for 90 days with liquorice extract estimated to contain 53% glycyrrhizin. Body weight gain was slightly inhibited in animals that received 2.5 g/kg/day. Haematological evaluation revealed a significant decrease in the red blood cell counts with decrease in haematocrit of the male, but not female, rats receiving the two highest doses of liquorice extract. Male rats had a slightly, but significantly, elevated neutrophil and decreased lymphocyte count at the highest dose. Total protein, albumin, AST and ALT were significantly elevated

in the male rats receiving the highest doses, whereas the same parameters were significantly decreased in the female rats administered the highest doses. Serum cholesterol was also decreased in both male and female rats with a 40% decrease in the female rats administered 2.5 g liquorice extract/kg/day. Although the average liver and kidney weights increased in the 1.25 and 2.5 g/kg/day dose groups, there were no significant histological changes observed in these organs. Histology performed on the highest dosed group revealed a slight atrophy of the thymus medulla, along with some lymphofollicular formations, as well as some atrophy and catarrh of the stomach mucosa. These changes were not considered significant, because recovery was seen upon withdrawal of the liquorice extract. The authors considered the no observable/observed effect level to be 0.31–0.63 g extract/kg (approximately 165–334 mg glycyrrhizin/kg) for 90 days of treatment. Glycyrrhizin altered renal functions by the third day of treatment, but the effects were terminated upon withdrawal for four days. Male SLC: Wistar/K4 rats were administered 4ml/day by oral gavage of a 5% glycyrrhizin solution (~1600mg/kg) for five days. Glycyrrhizin administration significantly inhibited urine production, as well as urine sodium and potassium excretion, during the five day treatment. These effects were reversible as all measured parameters returned to control levels following four days removal from the glycyrrhizin (Isbrucker 2006).

### **Genotoxicity**

No genotoxicity studies were available.

### **Long-term studies (chronic toxicity and carcinogenicity)**

Kobuke et al. (1985) studied the chronic effects of disodium glycyrrhizin consumption in male and female B6C3F1 mice. A preliminary, sub-chronic, range-finding study had determined the maximum tolerated doses to be 0.15% (~375mg/kg) for male mice and 0.3% (~750mg/kg) for female mice. Glycyrrhizin was administered in drinking water for 96 weeks at concentrations of 0, 0.04, 0.08, 0.15 or 0.3%, delivering an approximate daily dose of 0, 71, 166, or 229 mg/kg to the male mice and 0, 117, 217, or 407 mg/kg to the female animals. Glycyrrhizin treatment did not significantly affect average body weights, cumulative mortality rates and mean time to death, incidence of tumours, or types or distribution of tumours. The authors concluded that the long-term daily administration of glycyrrhizin to these mice did not provide any evidence of chronic toxicity or tumourigenicity.

No studies on carcinogenicity were available.

### **Reproductive and developmental toxicity**

In 1971, the Food and Drug Research Labs conducted a 4-species teratologic evaluation of ammoniated glycyrrhizin for the FDA. Mice, rats, hamsters and rabbits were orally gavaged with 0, 27, 90, 300, or 1000 mg/kg/day of ammonium glycyrrhizin commencing on their 6th day of gestation. CD-1 mice and Wistar rats were dosed for 10 consecutive days whereas the golden hamsters and Dutch-belted rabbits were dosed for 5 and 13 days, respectively. There were no reported effects of glycyrrhizin treatment on nidation or on maternal or fetal survival in any of the species. Gross and histological examination revealed no treatment related effects in either the soft or skeletal tissues as compared to untreated animals (Isbrucker 2006).

Itami et al. (1985) examined the potential teratogenic effects of disodium glycyrrhizin in pregnant Wistar rats. Rats were administered 0, 0.08, 0.4, or 2% disodium glycyrrhizin in their diet (80, 400 or 2000 mg/kg) during days 0–20 of gestation. Rats were either sacrificed and the foetuses examined, or brought to term and monitored for up to eight weeks post-partum. There were no significant effects of glycyrrhizin administration on food intake, number of implants, number of corpora lutea, incidence of intrauterine deaths, number of live foetuses, sex ratios, foetal body weights, placental weights, degrees of ossification, live birth index or body weight gain after birth. One foetus in the 0.08%

treatment group was found with dilatation of the renal pelvis, but no other malformations or anomalies were noted in the treatment groups. There was a significant reduction in the maternal weight gain following delivery in the 0.4 and 2% dose groups. The authors concluded that disodium glycyrrhizin is not teratogenic in rats under the conditions of this study.

A similar study, evaluating the teratogenicity of ammoniated glycyrrhizin in pregnant Sprague–Dawley rats, was conducted by Mantovani et al. (1988). Commencing on the seventh day of gestation, dams were provided with 0, 10, 100, or 250 mg ammoniated glycyrrhizin/100 ml drinking water (10, 100 or 250 mg/kg) and maintained up to the 20th day of pregnancy. No deaths or clinical signs attributable to the treatment were observed in any dams of any treatment group. Although the embryotoxicity score was significantly dose related when measured using the Armitage–Cochran test, there were no significant differences in number of corpora lutea, implants or live foetuses. Skeletal abnormalities were significantly elevated in the two highest treatment groups but the authors noted that similar anomalies were also found in high numbers of control litters. These abnormalities included misaligned, asymmetric and bipartite sternebrae and hemisternebrae. Soft-tissue abnormalities were mostly renal and were significantly elevated in the 100 and 250 mg/100ml (100 and 250 mg/kg) dose test groups. External haemorrhages were also observed in some foetuses. From these data, the authors concluded that ammoniated glycyrrhizin exhibited some embryotoxicity to the developing rat foetus, but no toxicity to the mother, and that the foetal effects were minor.

Effects of liquorice extract on foetal abnormalities induced by cyclophosphamide were investigated in rats. Pregnant Sprague–Dawley rats were orally administered green tea or liquorice extract (100 mg/kg) for 7 days, from days 6 to 12 of gestation, and intraperitoneally exposed to cyclophosphamide (11 mg/kg) 1 h after the final treatment. Cyclophosphamide reduced foetal and placental weights and induced malformations in live fetuses; 94.6%, 41.5% and 100% of external, visceral and skeletal defects, respectively. Liquorice extract further decreased the foetal body weights and markedly enhanced foetal defects, resulting in 76.4% of cranial defect and exencephaly, 22.7% of micrognathia and tongue extrusion, 85.5% of vertebral defects, 85.5% of costal defects, and 100% of delayed skeletal ossification. The results suggest that repeated pretreatment with green tea or liquorice extract may aggravate body weight loss and malformations of foetuses, induced by intrauterine exposure to cyclophosphamide (Jeon 2007).

A study examined more closely the effects of glycyrrhetic acid on rat foetal lung development (Hundertmark et al., 2002). Since 11 $\beta$ -HSD is important in the regulation of pulmonary surfactant synthesis during development, the authors explored the foetal implications of maternal glycyrrhetic acid consumption. Pregnant Wistar rats were fed a daily diet delivering 0, 10, 100, or 1000 mg/kg glycyrrhetic acid commencing on the 13th day of gestation. Foetuses were examined on days 17, 19 and 21 of gestation as well as on the 1st post partum day. Foetal lung 11 $\beta$ -HSD activity was moderately, but significantly, reduced in the highest dosed group as compared to controls. Foetal and maternal plasma corticosteroid, sodium and potassium levels were not affected by the treatment at any doses but there was a significant decrease in the foetal lung surfactant protein A mRNA levels in the 1000 mg/kg group. Histological examination of foetal lungs in this dose group showed a significant reduction in lamellar body content and a reduced number alveolar lamellar body and surfactant clusters as compared to controls. Despite these effects, there was no apparent increase in malformation or foetal death rate associated with glycyrrhetic acid exposure; neither was there any abnormal behaviour observed in the neonatal rats.

### **Regulatory status of glycyrrhizin-containing products**

Liquorice and liquorice derivatives, including ammoniated glycyrrhizin, are considered as 'Generally Recognized as Safe' (GRAS) for use in foods by the U.S. FDA (21 CFR 184.1408).



FDA assumes that glycyrrhizin levels in foods do not pose a health hazard, provided that these foods are not consumed in excess or by individuals who are sensitive to low levels of glycyrrhizin (Anon, 1983). Liquorice extract and its derivatives are also approved for use in some over-the-counter drugs (21 CFR 310.528; 310.544; 310.545), and liquorice is included as a GRAS ingredient in animal feeds (21 CFR 582.10; 582.20).

Liquorice is used as a flavouring agent in many brands of chewing tobacco in the United States. Because glycoside derivatives of glycyrrhetic acid induce symptoms of mineralocorticoid excess through the inhibition of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -OHSD), the continuous use of chewing tobacco can result in hypertension, sodium retention, and hypokalaemia (Isbrucker 2006).

Glycyrrhizinic acid was evaluated during a Joint FAO/WHO Expert Committee on Food Additives (JECFA) meeting (WHO, 2005). Although a formal acceptable daily intake (ADI) was not established, the committee indicated that consumption of 100 mg/day would be unlikely to cause adverse effects in the majority of adults, and recognized that a subset of the population may be more susceptible to its physiological effects even at lower doses.

Council of Europe and the UK Food Additive and Contaminants Committee consider liquorice as a natural plant product intended for use in small quantities as a food additive, with the intention that its consumption is to be limited by the glycyrrhizin levels (Fenwick et al., 1990). A limit of less than 50 ppm glycyrrhizin was established by these organizations.

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

Liquorice derivatives and liquorice extracts effects were widely investigated.

Liquorice extracts showed corticosteroid-like activity, anti-inflammatory properties (ethanolic extracts), anti-ulcer effects, anticarcinogenic effects. In particular, ethanolic and methanolic extracts of liquorice root have been shown to have antimicrobial activity and ethanolic extracts showed to have antimutagenic effects.

In vitro and in vivo studies have also demonstrated that liquorice extracts possess antiatherogenic effects and can inhibit LDL oxidation.

*Glycyrrhiza* has been shown to decrease irritations in the throat and to produce expectorant effects, thus supporting its traditional uses.

Moreover, both liquorice and glycyrrhizin showed to have effects on the growth and acid production of oral bacteria associated with the development of dental caries.

In humans, the bioavailability of the main constituent glycyrrhizin is poor after oral administration. It is lower when measured after liquorice root extract administration. After oral administration, glycyrrhizin is metabolised to glycyrrhetic acid by intestinal bacteria. Clearance of glycyrrhetic acid is via a capacity-limited hepatic metabolism and/or biliary excretion. Enterohepatic circulation of glycyrrhetic acid can be expected in humans. Glycyrrhetic acid crosses through the placental barrier and can be detected in the rat fetuses.

The acute oral toxicity of *Glycyrrhiza* extract in mice and rats is very low. The no observable/observed effect level has been evaluated to be 0.31–0.63 g extract/kg (approximately 165–334 mg glycyrrhizin/kg) for 90 days of treatment.

Long-term daily administration of glycyrrhizin (0.3 %) to mice does not provide any evidence of chronic toxicity or tumourigenicity.

In several developmental toxicity studies, glycyrrhizin exhibited some embryotoxicity to the developing rat foetus, but the foetal effects were considered as minor. No toxicity to the mother was observed.

Liquorice and liquorice derivatives, including ammoniated glycyrrhizin, are considered as 'Generally Recognized as Safe' (GRAS) for use in foods by the U.S. FDA (21 CFR 184.1408). Council of Europe and the UK Food Additive and Contaminants Committee consider liquorice as a natural plant product intended for use in small quantities as a food additive with the intention that its consumption is to be limited by the glycyrrhizin levels. A limit of less than 50 ppm glycyrrhizin was established by these organizations.

## 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

Liquorice has been widely used as a demulcent for the digestive system, for the treatment for gastric ulcers. Glycyrrhizin was the component considered to be the active anti-ulcerogenic agent. Despite its historical use, studies in humans have not provided positive results.

Carbenoxolone was developed as glycyrrhizate analog and has shown success in clinical trials for gastric and duodenal ulcers. Tolerable hypermineralocorticoid-like side effects were detected.

The mechanism of action of carbenoxolone remains unknown. It has been suggested that anti-ulcer effects of liquorice extract may be due to reduced gastric secretions caused by an inhibition of gastrin release.

Other studies carried out with deglycyrrhizinated liquorice indicate that other components exist in the extract, which promote gastric healing.

The anti-inflammatory and antiallergic actions of the drug have been attributed to the corticosteroid-like activity of glycyrrhizin and glycyrrhetic acid (WHO 1999).

Dry cough and chronic obstructive lung diseases have been treated with liquorice for a number of years. The antitussive and expectorant properties of the drug have also been attributed to glycyrrhizin, which accelerates tracheal mucus secretion (WHO 1999). It also seems that mucilage present in it, or secretion produced under the influence of the active substances covers the oral and throat mucosa soothing its irritability and relieving dry cough (Asl 2008).

Furthermore, human lung converts cortisol to cortisone mainly in the parenchyma, less in the trachea and pleura and not at all in either the small airways or pulmonary vessels. This conversion is inhibited by glycyrrhizinic acid. Anti-inflammatory action of liquorice is partially due to 11 $\beta$ -HSD inhibition and increased local glycocorticoid activity. Glycyrrhizin decreases trachea spasm induced by histamine and promotes up-regulation of the beta2-adrenergic receptors after long-term treatment with beta agonists. Liquorice also promotes phagocytosis, IL-1 and INF secretion by human macrophages and has a mitogenic action on B lymphocytes (Bouras 2001).

Mechanisms for antiviral activity of liquorice includes reduced transport to the membrane and sialylation of hepatitis B virus surface antigen, reduction of membrane fluidity leading to inhibition of fusion of the viral membrane of HIV-1 with the cell, induction of interferon gamma in T-cells, inhibition of phosphorylating enzymes in vesicular stomatitis virus infection and reduction of viral latency (Fiore 2008).



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

In humans, glycyrrhizin has a poor bioavailability after oral administration; it is detected at very low levels after a single oral dose in the range of 100–1600 mg/kg. Glycyrrhetic acid is readily detected in plasma following the ingestion of glycyrrhizin or liquorice extract (Isbrucker 2006).

The time to maximum plasma concentration of glycyrrhetic acid (the aglycone of glycyrrhizin) is 10 h for glycyrrhizin and 2 h longer for liquorice extract in healthy adult volunteers after 800 or 1600 mg glycyrrhizin as ammoniated salt or in liquorice extract are administered (Cantelli-Forti et al., 1994).

Glycyrrhizin and glycyrrhetic acid adhere extensively to human and rat serum albumin through a saturable process (Ishida 1992; Ploeger et al., 2000). Glycyrrhetic acid crosses the placental barrier and can be detected in the rat fetuses (Hundertmark et al., 2002).

After oral administration, glycyrrhizin is metabolized to glycyrrhetic acid by intestinal bacteria which contain  $\beta$ -D-glucuronidase. Intravenously administered glycyrrhizin is metabolized in the liver by lysosomal  $\beta$ -D-glucuronidase to 3-mono-glucuronide glycyrrhetic acid. This metabolite is excreted with bile into the intestine, where it is metabolized by bacteria into glycyrrhetic acid, which can be reabsorbed (Asl 2008; Ploeger et al., 2000).

After administration of aqueous liquorice root extract (LE) to rats and humans, glycyrrhizin and glycyrrhetic acid levels were lower compared to glycyrrhizin alone, and the pharmacokinetic curves showed significant differences in the areas under the plasma-time curve (AUC),  $C_{max}$ , and  $T_{max}$  parameters. Also, the data obtained from urine samples confirmed a reduced bioavailability of glycyrrhizin present in LE compared with pure glycyrrhizin (Isbrucker 2006).

It has been also suggested that interaction between the glycyrrhizin and other components in LE during intestinal absorption causing modified bioavailability could explain the various clinical adverse effects resulting from the chronic oral administration of glycyrrhizin alone as opposed to LE (Cantelli-Forti et al., 1994).

Pharmacokinetics of glycyrrhizin is nonlinear. Glycyrrhetic acid has a large volume of distribution, a long biological half-life, and undergoes substantial enterohepatic circulation. Thus large doses of potassium chloride supplementation for weeks are necessary because of the long half-life of glycyrrhetic acid (Asl 2008).

The plasma clearance of glycyrrhizin and glycyrrhetic acid is dose dependent when administered at levels which exceed the saturation of serum protein binding. Plasma clearance is significantly decreased in patients with chronic hepatitis C and liver cirrhosis (Ploeger et al., 2001; van Rossum et al., 1999b).

### **4.2. Clinical Efficacy**

#### **4.2.1. Dose response studies**

See section 4.2.2.

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

Clinical studies have been carried out on:

- aphthous stomatitis
- gastric and duodenal ulcers
- postoperative sore throat

## Studies on aphthous stomatitis

### The Efficacy of the Bioadhesive Patches Containing Liquorice Extract in the Management of Recurrent Aphthous Stomatitis (Moghadamnia 2009)

A study to evaluate the efficacy of liquorice bioadhesive hydrogel patches to control the pain and reduce the healing time of recurrent aphthous ulcer was conducted.

The study was carried out in three episodes of ulcers: in the first episode of ulcer, all 15 patients were asked to record their baseline individual pain level by a visual analog scale. In the second and third episodes, comparative and consecutive subjective and objective evaluations of the bioadhesive were done. The effects of the following variables were investigated: (1) VAS pain score for 5 consecutive days, (2) profile of aphthous ulcers on days 3 and 5, (3) time to complete relief of pain and healing of the ulcers, (4) diameter of the lesions and necrotic zone.

A total of 15 patients, 5 women and 10 men (age 22–35 years, mean  $\pm$  SD,  $26.27 \pm 4.28$  years) with a history of recurrent aphthous stomatitis (RAS) and currently suffering from ulceration located in the anterior region of the mouth were selected from patients referred to the School of Dentistry in Babol (North of Iran). Excluding criteria were: iron deficiency, inflammatory and allergic conditions, history of medication, smoking, pregnancy, wearing denture, receiving antibiotics for RAS and those who were unable to apply patches. The clinical diagnosis of minor RAS was made by the presence of a well demarcated, painful ulcer on the non-keratinized oral mucosa. The previous history of their RAS for the duration and periods between episodes were recorded.

Two series of patches, a base and patches containing liquorice were used in this study. The patches were made from a pharmaceuticals grade tragacanth gum and were prepared in the Department of Pharmacology of Babol Medical University. Liquorice extracts were isolated by maceration of *Glycyrrhiza glabra* with chloroform in 48 h. After filtering and drying the extract, patches containing liquorice 1% were made. The patches were sealed in foil sachets. Contact with saliva at the site hydrated the gel, forming an adhesive hydrogel which attaches to the mucosa.

This was a placebo-controlled, observer blind, consecutive-group clinical trial. The study was performed over three clinical visits along three episodes of RAS. The first episode of RAS was used to gather some baseline data of the subjects. The subjects completed this step and were designated the no-treatment group. The second and third episodes were assigned to bioadhesive without liquorice and bioadhesive with liquorice, respectively. A detailed personal and ulcer assessment questionnaire was filled out including: site of the ulcer, size of the ulcer, duration, frequency, pain, previous treatment and received medications, and the healing time of the ulcer episodes.

All 15 patients (5 female and 10 male mean  $\pm$  SD age  $26.27 \pm 4.28$ ) recorded three consecutive RAS episodes. The first episodes of RAS were investigated to obtain some baseline information on the subjects. The second and the third episodes were assigned to bioadhesive with or without liquorice.

A significant reduction in VAS was recorded following application of the liquorice patches on days 2, 3, 4 and 5 compared with the no-treatment group ( $p < 0.001$ ). Liquorice patches caused a significant reduction in the diameter of the inflammatory halo and necrotic center compared with the placebo group ( $p < 0.03$ ).

The authors concluded that according with the results of the study liquorice bioadhesive can be effective in the reduction of pain and of the inflammatory halo and necrotic center of aphthous ulcers.

*Assessor's comment: the number of patients recruited is small; there is no randomization, not double blind, use of questionnaire.*

## Studies on gastric and duodenal ulcers

#### A trial of deglycyrrhizinated liquorice in the treatment of duodenal ulcer (H. Feldman 1971)

A double-blind trial of deglycyrrhizinated liquorice (Caved-S) was performed in 47 patients with active duodenal ulcer.

Included in the trial were patients from the clinic suffering from duodenal ulcer for at least six months. The patients had recent radiological proof of a deformed bulb or ulcer niche and had ulcer pains at the start of the study. The following tests were performed before and after the trial: blood count, urine analysis, blood urea, GO-transaminase, bicarbonate, sodium, potassium, and chloride. Patients were seen before and then one week, two weeks, and 30 days after the start of therapy. Blood pressure and weight were also recorded. Patients received two tablets three times a day after meals for 30 days and were instructed to chew them before swallowing.

Each Caved-S tablet contained 380 mg deglycyrrhizinated powdered block liquorice, 100 mg bismuth subnitrate, 100 mg aluminium hydroxide gel, 200 mg magnesii carbonas levis, 100 mg sodium bicarbonate and 30 mg powdered frangula bark. The placebo tablets were inert tablets of the same composition except that each contained lactose flavoured with aniseed to simulate the taste of liquorice.

Twenty-four patients received a placebo and 23 the trial medication (Caved-S) for one month. Both groups were clinically similar. No advantage of deglycyrrhizinated liquorice over placebo was found. There were no side effects attributable to treatment and, in particular, no fluid retention and no effects on blood pressure and electrolytes.

*Assessor's comment: Trial carried out with a combination product. No randomization, small number of patients.*

#### Double-blind trial of deglycyrrhizinated liquorice in gastric ulcer (Engqvist 1973)

In order to investigate the reported beneficial effect of deglycyrrhizinated liquorice in gastric ulcer, a trial with a double-blind, cross-over design was performed. The patients were treated during two consecutive periods of four weeks each with either liquorice extract during the first period and placebo during the second, or placebo during the first period and liquorice during the second. Only patients with chronic ulcer disease were accepted for the trial. The dosage of the liquorice extract was 760 mg three times daily. During the first period 38 patients with 47 ulcers in the ventricle and during the second period 30 patients with 36 ulcers took part in the trial. The patients' sex, age, site of the ulcer in the ventricle, and ulcer size in the groups treated with liquorice and placebo during periods I and II were similar. The liquorice extract and placebo were distributed in identical capsules, which both contained 380 mg deglycyrrhizinated liquorice. The medicine (Caved-S) was administered in bottles with 42 capsules each (one week's consumption). The dose was two capsules three times daily. No difference could be shown between the groups treated with liquorice and placebo with respect to heredity, duration of ulcer disease, alcohol consumption, smoking, or the use of drugs.

There was no tendency to quicker healing in either group with regards to the change of ulcer area or complete healing. Small ulcers healed more quickly than big ones. Ulcers at the angulus healed very poorly. No side effects of treatment were observed.

The study was not able to demonstrate any healing effect of the liquorice extract (Caved-S) on gastric ulcer.

*Assessor's comment: random, double-blind, cross-over, small number of patients, no information on the extract.*

#### Prophylaxis with deglycyrrhizinated liquorice in patients with healed gastric ulcer (Hollanders 1977)

A trial to evaluate prophylaxis with deglycyrrhizinised liquorice (DGL) in patients with healed gastric ulcer has been conducted.

Forty-one patients (23 men, 18 women) with benign chronic gastric ulceration, in whom complete ulcer healing had been shown both radiologically and endoscopically within the previous four weeks, were selected. All were under the age of 75. The trial was a double-blind controlled study, in which participants received five capsules a day, each containing either 450 mg of DGL (Ulcedal) or an identical placebo. Antacids were taken as required. The patients were reviewed monthly for recurrence of symptoms, and a full haematological and biochemical profile was taken at each visit. Gastroscopy and barium meal examinations were performed at six-monthly intervals. Patients were followed up for at least two years or until the ulcer recurred.

On completion of the study eight patients had withdrawn, leaving 33 patients for analysis, of whom 22 had received placebo and 11 DGL. Eighteen patients developed a further gastric ulcer: five were receiving DGL and 13 placebo. This represents a relapse rate during follow-up of 45 % for DGL and 59 % for placebo. This difference is not significant. Seventy-eight per cent of the total recurrences occurred within the first 10 months of prophylaxis. Reasons for withdrawal from the trial were defection from follow-up (five patients) and intercurrent illness (three patients). No clinical, biochemical, or haematological abnormality was detected during treatment and no evidence of long-term toxicity was found.

*Assessor's comment: placebo, double-blind, no randomization, no information on the extract.*

#### Deglycyrrhizinised liquorice in duodenal ulcer (Larkworthy 1977)

A controlled endoscopic trial of DGL given as a chewing gum or in capsules in duodenal ulcer was carried out. Patients with endoscopically active duodenal ulcers who agreed to take part were randomly allocated treatment with DGL (Ulcedal capsules) or a placebo (lactose coloured with caramel), given either as a capsule or in a chewing-gum base. They took two capsules or two blocks of gum five times daily on an empty stomach for eight weeks, each dose containing 900 mg of DGL or the placebo. They swallowed the capsules whole and chewed the gum for 30 minutes. They were advised to eat small frequent meals, avoiding fatty foods, and were given a supply of magnesium trisilicate compound or aluminium hydroxide compound tablets to take as required and asked to record their symptoms. A second endoscopy was performed after eight weeks. Thirty-four patients were treated, 17 receiving DGL and 17 the placebo, while 20 were given the chewing-gum base and 14 the capsule treatment.

The factorial trial design allowed assessing the value of mastication as an accessory treatment. Patients chewed the gum preparations for over two hours a day. No evidence was detected suggesting that chewing had beneficial effects, for instance by increasing salivation. Results gave no support to the concept that DGL will accelerate the healing of duodenal ulcers, and corroborate the findings of others in large controlled simple symptomatic studies.

*Assessor's comment: randomization, placebo, no blind, small number of patients, no information on the extract.*

#### Clinical trial of deglycyrrhizinised liquorice in gastric ulcer (Bardhan, 1978)

Ninety-six patients with gastric ulcer were randomly allocated to treatment either with deglycyrrhizinised liquorice (DGL) or placebo.

All patients had a benign-looking gastric ulcer, verified by endoscopy on entry to the trial. Radiographs were taken showing the ulcer in maximum profile. Ulcer areas in mm<sup>2</sup> were standardised with radiographic image. Physical examination and blood tests were done within 24 hours of the admission endoscopy.

Patients were then randomly allocated under double blind conditions, and stratified for age, sex and the presence or absence of either cardiovascular disease or hypertension, to take two capsules five times daily for four weeks, containing either 500 mg DGL or 200 mg sucrose.

Measured amounts of antacid tablets and mixture were supplied and patients recorded daily their consumption of each type, and any pain experienced.

At the end of four weeks, the physical examination, blood testing, gastroscopy, and (in most cases) barium meal were repeated, usually all within a 24-hour period.

Ulcer healing on gastroscopy was defined as reepithelialisation with or without a scar; the radiological definition was disappearance of the crater. After four weeks no differences were found between the treatment groups in the proportions with complete healing, whether assessed by gastroscopy or radiology, or in the percentage reduction in ulcer area, or in clinical improvement.

*Assessor's comment: no information on the extract.*

### **Studies on postoperative sore throat**

#### An Evaluation of the Efficacy of Liquorice Gargle for Attenuating Postoperative Sore Throat: A Prospective, Randomized, Single-Blind Study (Agarwal 2009)

Postoperative sore throat (POST) contributes to postoperative morbidity. Liquorice has been used as an expectorant in cough and cold preparations. In this randomized, single-blind, placebo-controlled study the evaluation of the efficacy of liquorice gargle for attenuating POST was performed.

Forty adults (18–60 years), ASA (American Society of Anesthesiologists) physical status I and II of either sex, undergoing elective lumbar laminectomy were randomized into two groups of 20. Group C: received water; Group L: received 0.5 g liquorice in water. Both groups received a 30 mL mixture for 30 s, 5 min before anaesthesia which was standardised. The gargle solution was prepared by the process of decoction, which involves boiling the liquorice powder (5 g) in 300 mL of water and filtering the decoction. This decoction was used within 24 h of its preparation and was used for gargling at room temperature. The solution was not compounded with any other additives like sugar or alcohol.

The primary end point was POST (incidence and severity at rest and on swallowing); secondary end points were occurrence of postextubation cough and side effects, if any.

The incidence and severity of POST at rest and on swallowing and side effects were assessed at 0, 2, 4, and 24 h, postoperatively. Severity of POST was assessed by visual analog scale (between 0 and 100 mm; where 0 means no sore throat and 100 means worst imaginable sore throat). Postextubation cough was assessed immediately after tracheal extubation. Data were analyzed by Z test and Fisher's exact test.  $P < 0.05$  was considered as significant.

POST (incidence and severity) was reduced in the Group L compared with Group C at rest and on swallowing for all time points ( $P < 0.05$ ), except that the severity of POST at rest at 24 h, was similar in both groups ( $P < 0.05$ ). Postextubation cough was reduced in Group L compared with Group C ( $P < 0.05$ ).

There was no difference in side effects between groups ( $P < 0.05$ ).

Conclusion: Liquorice gargle performed 5 min before anaesthesia is effective in attenuating the incidence and severity of POST.

*Assessor's comment: randomization, placebo, single-blind, available information on the extract.*

### **Studies on hyperlipidemia**

In this study, the antiatherogenic effects of liquorice-root extract consumption in moderately hypercholesterolemic patients were investigated.

Supplementation of liquorice root extract (0.1 g/d) to patients for 1 month was followed by an additional 1 month of placebo consumption. Twelve hypercholesterolemic patients (45 to 55 years old, plasma cholesterol level of 220 to 260 mg/dL, and LDL cholesterol level of 120 to 170 mg/dL) were selected. All patients were non-smokers and none had ever been treated with hypolipidemic drugs. To eliminate possible analytical drift or any other potentially confounding results, the patients and the laboratory technicians did not know which capsule (the liquorice or the placebo) was consumed for the first month because both capsule types appeared the same. Therefore, this study was considered blind for all participants. All patients continued their habitual diets during the study.

Liquorice ethanolic extract free of glycyrrhizinic acid was used. Powdered roots of commercial *Glycyrrhiza glabra* were extracted in ethanol to obtain, after solvent evaporation, a brown solid extract. The powder was encapsulated in a softgel capsule. Placebo capsules without liquorice contained inert gelatinous material that were included in softgel capsules.

Liquorice consumption: 1) reduced patients' plasma susceptibility to oxidation (by 19%); 2) increased resistance of plasma LDL against three major atherogenic modifications: oxidation (by 55%), aggregation (by 28%), and retention, estimated as chondroitin sulfate binding ability (by 25%); 3) reduced plasma cholesterol levels (by 5%), which was due to a 9% reduction in plasma LDL cholesterol levels; and 4) reduced (by 14%) plasma triacylglycerol levels. After the 1 month of placebo consumption, these parameters reversed towards baseline levels. Liquorice extract supplementation also reduced systolic blood pressure by 10%, which was sustained during the placebo consumption.

The authors concluded that dietary consumption of liquorice-root extract by hypercholesterolemic patients may act as a moderate hypocholesterolemic nutrient and a potent antioxidant agent and, hence against cardiovascular disease (Fuhrman 2002).

#### **Antiatherogenic effects**

Ten healthy men received liquorice (0.1 g/d) in a softgel capsule for 2 weeks. Ten normal subjects who received placebo capsules without liquorice served as a placebo control group. Liquorice ethanolic extract as well as a pure material, which was identified by gas chromatography-mass spectroscopy as the isoflavan glabridin, were shown to inhibit LDL oxidation by a mechanism involving scavenging of free radicals. These findings could be related to the absorption and binding of glabridin to the LDL particle and subsequent protection of the LDL from oxidation (Fuhrman et al., 2002).

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

A sample of 1049 Finnish women and their healthy singleton infants was studied. Glycyrrhizin intake was calculated from detailed questionnaires on liquorice consumption. Glycyrrhizin exposure was grouped into three levels: low (<250 mg/week; n = 751), moderate (250–499 mg/week; n = 145), and heavy (≥500 mg/week; n = 110). Babies with heavy exposure to glycyrrhizin were not significantly lighter at birth, but they were significantly more likely to be born earlier. The odds ratio for being born before 38 weeks' gestation was 2.5 (95% confidence interval: 1.1, 5.5; p = 0.03). Although the effect of heavy glycyrrhizin intake on mean duration of gestation was small (2.52 days) when expressed as an effect on the mean, this shift to the left of the distribution of duration of gestation was sufficient to double the risk of being born before 38 weeks. The association remained in multivariate analyses. In conclusion, heavy glycyrrhizin exposure during pregnancy did not significantly affect birth weight or maternal blood pressure, but it was significantly associated with lower gestational age (Strandberg 2001).



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

Clinical studies have been carried out on:

- aphthous stomatitis
- gastric and duodenal ulcers
- postoperative sore throat

#### **Studies on aphthous stomatitis**

Efficacy of bioadhesive patches containing liquorice extract was evaluated in the management of recurrent aphthous stomatitis. The authors concluded that liquorice bioadhesive can be effective in the reduction of pain and of the inflammatory halo, and necrotic center of aphthous ulcers. However, some critical points are to be noted: a chloroform dry extract was used (DER not specified), the number of patients recruited is small; there is no randomization, double blind was not performed and endpoint were measured through the use of questionnaire.

#### **Studies on gastric and duodenal ulcers**

Several studies evaluating the efficacy of deglycyrrhizinated on duodenal and gastric ulcers were carried out. Taken together, these do not show any efficacy of deglycyrrhizinated liquorice extracts.

#### **Studies on postoperative sore throat**

A prospective, randomized, single-blind study on the evaluation of the efficacy of a decoction of 5 g of liquorice powder in 300 mL of water in form of gargle for attenuating postoperative sore throat has been published. Postoperative sore throat (POST) contributes to postoperative morbidity. The primary end point was POST (incidence and severity at rest and on swallowing). The authors concluded that liquorice gargle performed 5 min before anaesthesia is effective in attenuating the incidence and severity of POST. Limits of the study were the small number of patients and single-blinding.

*Assessor's comment:*

*Glycyrrhiza glabra has been traditionally used as an expectorant to help relieve chest complaints, such as catarrhs, coughs and bronchitis and to help relieve inflammatory conditions of the gastrointestinal tract, such as gastritis.*

*There are no clinical data to support any "well-established medicinal use" in the scientific literature.*

*Several clinical studies evaluated the efficacy of deglycyrrhizinated extract, administered orally, to treat gastric and duodenal ulcers, but the lack of evidence of efficacy resulted in a loss of interest for this indication.*

*A clinical trial evaluated the effect of a decoction of liquorice used as gargle to treat postoperative sore throat (POST), a condition of postoperative morbidity, because Glycyrrhiza extract has been shown to decrease irritations and cough in the throat with expectorant effects.*

*The topical oromucosal use of patches containing 1 % of a chloroform extract of liquorice showed good results in a recent clinical study on the treatment of oral aphthous ulcer without relevant side effects.*

## 5. Clinical Safety/Pharmacovigilance

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

Hypermineralocorticoid-like side effects were noted in all clinical studies, although most authors considered these effects to be tolerable.

### 5.2. Patient exposure

Not to be used in patients with hypokalaemia, high blood pressure, or a kidney or cardiovascular disorder (ESCOP 2003).

Not to be used in patients with taking thiazide diuretics, cardiac glycosides, corticosteroids, stimulant laxatives or other medications which may aggravate electrolyte imbalance (ESCOP 2003; Ghielmini 1990; Blumenthal et al. 2000).

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

A 70 year old woman experienced flaccid quadriplegia due to profound hypokalaemia. Hypokalaemia and myoglobinuria were caused by the ingestion for 2-3 years intermittently of small amounts of liquorice contained in a laxative preparation. The patient admitted having taken no more than 2 or 3 teaspoonfuls of this preparation a week. Five ml was calculated to contain 47 mg of the calcium and potassium salts of glycyrrhizic acid. Subsequent controlled administration of small amounts of this preparation induced marked hypokalaemia, which was associated with sodium retention and potassium loss suggesting a mineralocorticoid-like action. The sodium retention was associated with suppression of plasma levels of renin and aldosterone (Cumming 1980).

A 15-year-old healthy boy has been reported to develop hypertension encephalopathy after ingestion of 0.5 kg liquorice candy. About 3 h later he developed a serious headache, nausea, vomiting, and right-sided weakness. His general practitioner measured a blood pressure of 200-130 mm Hg. The next morning the hemiparesis was increased and the patient was admitted to the hospital. He recovered completely in the course of 5 months (van der Zwan 1993).

A case has been described of a 40-year-old female with severe hypertension and hypokalaemic metabolic alkalosis due to prolonged ingestion of liquorice candies (Heikens 1995).

Two cases showing hypokalaemia induced by glycyrrhizic acid in patients with hypertension or oedema induced by liquorice flavoured chewing gums have been reported. In the first case a 21 year old woman presented headache and hypertension. She declared an intake of liquorice corresponding to about 100 g of liquorice daily. She used an oral contraceptive. Clinical examination was unremarkable except that her blood pressure was 190/120 mm Hg. She was advised to stop eating liquorice and taking the oral contraceptive. Despite these measures, her blood pressure remained raised even after treatment with a combination of atenolol, lisinopril, hydrochlorothiazide and amlodipine.

The clinical picture was compatible with exogenously induced hypermineralocorticoidism. She admitted that she had replaced her liquorice daily intake by chewing gum. Her daily intake of glycyrrhizic acid was calculated to be about 120 mg. Three weeks after she stopped using the gum her blood pressure was 110/80 mm Hg and plasma potassium concentration 5.3 mmol/l.

In the second case a 35 year old woman with profound hypokalaemia of 2.2 mmol/l was described. She denied eating liquorice. She used an oral contraceptive. Because she had pretibial oedema, she took chlorothiazide 500 mg twice daily. Clinical examination showed that her blood pressure was



140/80 mm Hg and that she had pitting oedema. Plasma potassium and bicarbonate concentrations were 2.2 mmol/l and 30.8 mmol/l, respectively.

The clinical picture was suggestive of exogenous mineralocorticoid administration. She frequently used chewing gum. The gum was liquorice flavoured and contains 160 mg liquorice, of which 10% is glycyrrhizic acid, in each 16 g packet. She used about three packets a day (50 mg glycyrrhizic acid). She was advised to stop using the gum. Intravenous and oral potassium supplementation was able to be stopped after 2 and 15 days, respectively. Three weeks after she stopped using the chewing gum her oedema had disappeared completely, her blood pressure had fallen to 110/80 mm Hg, and her plasma potassium had risen to 4.2 mmol/l in association with normalisation of the other electrolyte concentrations (de Klerk 1997).

Russo et al. described two case reports of hypertension encephalopathy associated to high blood pressure as a result of regular daily intake of 40 – 50 g of liquorice candies, corresponding to 80 - 100 mg of glycyrrhizic acid. Since, further a specific study conducted with the purpose to assess the safety of liquorice intake, 100 mg of glycyrrhizic acid was previously considered as the lowest level at which adverse effects consisting of hypertension with no complications are observed, the authors suggest that the higher susceptibility of some people to lower doses could be caused by a 11 $\beta$ -hydroxysteroid dehydrogenase deficiency. This suggestion should be confirmed by additional studies (Russo et al. 2000) and is in line with the outcome of the JECFA meeting (WHO, 2005).

A 65-year-old man was complaining of difficulty walking and maintaining seated position. He had a 13-year history of diabetes mellitus and a several year history of alcoholic liver cirrhosis and chronic gastritis. For a few years he had been taking a very small dose of liquorice containing stomachics (0.06 g liquorice extract in total granules of 3.0 g/day). For 2 months, due to worsened liver dysfunction, he had been receiving 40 mg/day of glycyrrhizin (total 800 mg) intravenously. Diagnosis of liquorice-induced pseudoaldosteronism with cardiac muscle failure was made (Hasegawa 1998).

A patient suffered life-threatening hypokalaemic paralysis caused by consumption of liquorice in the form of a tea sweetener superimposed on long-term consumption of liquorice candy. The patient admitted that during the previous year he and his 3 sons had consumed a half bag (25 g) of liquorice candy daily. During the 2 weeks before his admission he drank large amounts of tea. Patient consumed an additional 100 mg of glycyrrhizic acid daily with the tea, which aggravated his hypokalaemia and resulted in the development of the progressive life-threatening paralysis. Aggressive fluid and potassium replenishment produced complete and lasting recovery (Elinav 2003).

A 74-year-old woman presented isolated weakness of the extensor muscles of the neck, a relatively rare condition, known as "dropped head syndrome" (DHS) was reported by a patient. A hypokalaemia was evident; the symptoms were resolved rapidly on supplementation with potassium and discontinuation of liquorice consumption. The patient had been taking two kinds of Chinese herbal medicines (the content was not precisely specified in the case report) that included liquorice for over 10 years. Following discontinuation of these medications, potassium levels normalised and potassium supplementation was discontinued (Yoshida 2003).

Hypokalaemic paralysis is a medical emergency due to the risks of cardiac arrhythmia, respiratory failure, and rhabdomyolysis. An elderly Korean man presented with marked limb paralysis, myalgias, and mild hypertension. He had prostate cancer treated with orchiectomy and hormone therapy 2 years previously.

The major biochemical abnormalities were hypokalaemia (K<sup>+</sup>: 1.7 mmol/l) associated with high renal K<sup>+</sup> wasting and metabolic alkalosis. Low plasma renin activity, low aldosterone concentration, and normal cortisol concentration pointed to a state of pseudohyperaldosteronism.

The patient revealed he had been consuming eight packs (100 ml/pack) of a Korean herbal tonic daily to treat his prostate cancer for the past 2 months. A significant amount of the liquorice active constituent glycyrrhizic acid (0.23 mg/ml) was detected in the tonic. Discontinuation of the herbal tonic along with potassium chloride supplementation achieved recovery in 2 weeks (Cheng 2004).

A 90-year-old woman with hypertension developed metabolic alkalosis and myoclonus. Her medications included diltiazem hydrochloride, benidipine hydrochloride, kallidinogenase, procaterol hydrochloride, sennoside, dihydrocodeine phosphate, and KM powder® antacid that contained 354 mg of liquorice and 900 mg of sodium bicarbonate per 3.9 g of powder. Endocrinological studies showed slightly reduced plasma renin activity and normal plasma aldosterone concentration. A provisional diagnosis of liquorice-induced metabolic alkalosis was established and the patient was successfully treated after correction of serum pH and cessation of the medications (Ishiguchi 2004).

Chronic ingestion of liquorice induces a syndrome with findings similar to those in primary hyperaldosteronism. Van Den Bosch (2005) described a patient who, with a plasma K<sup>+</sup> of 1.8 mmol/l, showed a paralysis and severe rhabdomyolysis after the habitual consumption of natural liquorice.

An elderly male patient experienced progressive muscle weakness and paralysis for one week following intake of liquorice. Profound hypokalaemia (K<sup>+</sup> 1.4 mmol/L) associated with renal K<sup>+</sup> wasting and hypochloremic metabolic alkalosis was noted. Low plasma renin activity and aldosterone concentration, and normal cortisol concentration implied a state of pseudoaldosteronism. A detailed history revealed that he had been taking an over-the-counter medication (six capsules daily) for three months to control his atopic dermatitis. These capsules contained a large amount of monoammonium glycyrrhizinate (51 mg/capsule), an ammonium salt of glycyrrhizic acid extracted from liquorice. His plasma K<sup>+</sup> concentration returned to 4.0 mmol/L two weeks after cessation of this drug, coupled with potassium chloride supplementation and spironolactone. This case shows that an unassuming liquorice-containing drug used to treat dermatitis can induce profound life-threatening hypokalaemia and paralysis (Chang 2006).

A 93-year-old woman showed severe pain and swelling of both knees, inability to walk, fever of 38.4 °C, and anorexia. A diagnosis of pseudo-gout has been made. She received transfusion of a fluid containing 35 mEq of Na and 20 mEq of K with concomitant oral administration of an antibiotic and a non-steroidal anti-inflammatory drug. Her knee pain and fever were ameliorated within 4 days. However, she noticed gradual muscular weakness which progressed to paralysis in the lower extremities and then to all extremities 7 days after admission. She was referred to the neurological division and subsequently to the cardiovascular department because of hypertension and severe hypokalaemia. She denied vomiting, diarrhea or the use of any other drugs including diuretics. On physical examination, her blood pressure was 180/80 mmHg, heart rate 78 beats/min and body temperature 37.4 C°. There was symmetric flaccid paralysis with areflexia in the lower and upper extremities. She had been taking herbal medicines, ninjinto and saikokeishito, each containing liquorice, ingesting 5 grams of liquorice per day for the last 7 years (Yasue 2007).

A 55-year-old man was admitted to hospital because of a one-month history of progressive fatigue and occasional leg cramps. He appeared tired with muscle weakness. The laboratory data were normal except for severe hypokalaemia (1.7 mmol per l) and metabolic alkalosis (pH, 7.55; pCO<sub>2</sub>, 30 mm Hg; HCO<sub>3</sub>, 36 mmol per l). Further investigations revealed low plasma renin concentration (upright) [4 mE per l; (normal 5–75)] with low plasma aldosterone [0.06 nmol per l; (normal 0.08–0.69)]. Urinary study revealed high excretion of potassium (transtubular potassium gradient of 9). The patient admitted that during the last year he had consumed a packet (25 g) of natural liquorice root (containing 2.3% glycyrrhizic acid) daily after quitting smoking (Mumoli 2008).

A woman showed headache, weakness, upper-limb oedema and a generalised convulsive seizure after chronic ingestion of a commercial preparation of liquorice for 3 months containing glycyrrhizin 4.9% at dose of 27 g daily, equivalent to 1.3 g of glycyrrhizin.

She was also taking oral contraceptives which can predispose to liquorice toxicity. Plasma potassium, aldosterone, renin activity and albumin were below the normal level. The abdominal echography and computerised tomography scan demonstrated a perihepatic and perisplenic thin liquid layer with liquid collection in the pelvis. The bioelectrical impedance suggested a hyperhydration state. After stopping the liquorice, the laboratory and bioelectrical values normalised and clinical upper-limb oedema and the liquid in the abdomen disappeared in a few days (Francini-Pesenti 2008).

A 50-year-old woman with confusion, generalised weakness, chest pain and sweating for four hours was recovered after liquorice ingestion. Blood pressure was 170/100 mmHg and body temperature was 37.1 °C, systolic murmur was present. Electrocardiography showed alterations according to the diagnosis of Brugada Syndrome. Brugada syndrome is characterized with electrocardiogram findings of right bundle branch block and ST segment elevation in the right precordial leads in the absence of long QT intervals and any structural heart disease and increased risk for sudden cardiac death due to malignant ventricular dysrhythmias. Serum electrolytes disclosed hypokalaemia (1.77 mEq/L). Detailed history revealed that she was drinking two cups of liquorice extract for three months to relieve generalised weakness. The report illustrates the potential role of hypokalaemia induced by liquorice consumption for the induction of malignant dysrhythmias in Brugada syndrome. Oral potassium was given for one week and after normal serum levels were reached, the patient was discharged from hospital uneventfully. An implantable cardioverter-defibrillator was inserted before the discharge for secondary prevention of sudden cardiac death (Yorgun 2009).

A 71 year old woman was admitted with hypotension and bradycardia. A electrocardiogram showed flattened T waves and increased U wave prominence, resulting in a long QT(U) syndrome. Her initial serum potassium level was 1.6 mMol/L (all other electrolytes were normal). She consumed large quantities of liquorice daily for months for laxative effects (Crean 2009).

A 44-year-old previously healthy woman experienced severe liquorice-induced hypokalaemia resulting in ventricular fibrillation. The resolution of most of the symptoms after liquorice cessation suggested that liquorice was the major culprit. She chronically consumed 250 – 500 g of liquorice daily for several years (Gerritsen 2009).

A case of bilateral carpal tunnel syndrome following excessive liquorice intake has been reported. A 44-year-old anaesthesiologist tried to abruptly stop cigarette smoking. She chewed both at work and at home a large number of pure liquorice sticks. After 3 days, a colleague noted ankle oedema; she stopped eating liquorice and the ankle swelling resolved rapidly. At the same time, she felt bilateral nocturnal hand pain and paresthesias in median-innervated fingers and a paroxysmal electric-shock-like pain radiating along the third finger of the right and left hands. Nerve conduction studies confirmed bilateral median neuropathies at the wrists (Tacconi 2009).

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

##### **Herb-drug interactions**

Glycyrrhizin decreases plasma clearance, increases areas under the plasma-time curve (AUC), increases plasma concentrations of prednisolone. 11 $\beta$ -dehydrogenase converts endogenous cortisol to cortisone; orally administered glycyrrhizin is metabolised mainly to glycyrrhetic acid.

Glycyrrhetic acid potentiates cutaneous vasoconstrictor response of hydrocortisone. Glycyrrhetic acid is a more potent inhibitor of 5 $\alpha$ -, 5 $\beta$ -reductase and 11 $\beta$ -dehydrogenase than is glycyrrhizin.

Oral contraceptive use may increase sensitivity to glycyrrhizin acid. Women are reportedly more sensitive than men to adverse effects of liquorice (Fugh-Berman 2000).

Glabridin was found to inactivate the enzymatic activities of CYP 3A4 and 2B6 and competitively inhibited 2C9 (Kent et al., 2002).

Prolonged intake of high LE or glycyrrhizin (G) doses may result in accelerated metabolism of coadministered drugs. Daily oral doses of LE or glycyrrhizin for 1, 4 or 10 consecutive days in mice, were able significantly to induce hepatic CYP3A- and, to a lesser extent, 2B1- and 1A2-dependent activities, as well as 6-beta- (mainly associated to CYP3A), 2-alpha-, 6-alpha- (CYP2A1, 2B1), 7-alpha-, 16-alpha- (CYP2B9) and 16-beta-testosterone hydroxylase activities. Thus, the induction of cytochrome P450-dependent activities by long-term ingestion of liquorice may have clinical consequences for patients taking drugs metabolized by the same CYP enzymes (Paolini et al., 1998).

The effects of single or repeated intake of conspicuous amounts of aqueous liquorice root extract (LE, 3138 or 6276 mg/kg body weight per os) or its natural constituent glycyrrhizin (G, 240 or 480 mg/kg body weight per os) on Sprague-Dawley rat liver monooxygenases has been investigated. Aqueous liquorice root extract glycyrrhizin content, assayed by HPLC, was 7.64% w/w. Whereas a single LE or glycyrrhizin dose was unable to affect CYP superfamily, four daily doses induced CYP3A, CYP1A2 and to varying extents CYP2B1-linked monooxygenases. A boosting effect on testosterone 6b- (CYP3A1/2, CYP1A1/2), 7a- (CYP1A1/2, CYP2A1), 16a- (CYP2B1, CYP2C11), 2a- (CYP2C11) and 2b- (CYP3A1, CYP1A1) -dependent oxidases as well as on androst-4-ene-3,17-dione- (CYP3A1/2) -supported monooxygenases were also achieved. Harmful outcomes associated to CYP changes (e.g. cotoxicity, cocarcinogenicity and promotion) may be of concern (Paolini 1999).

High doses of LE and glycyrrhizin could cause significant adverse effects. Thus, it seems that routine liquorice consumers under CYP3A induction might therefore be predisposed to associated adverse effects. Consumption of liquorice is contraindicated for patients with liver disorders, hypokalaemia, for example those taking cardiac glycosides. The aldosterone effects of liquorice root may counteract antihypertensive action of prescribed medications (Cassileth and Barazzuol, 2001).

A direct interaction of glycyrrhetic acid absorption with sennosides and its derivatives has been studied in humans. A decrease of the pharmacokinetic parameters of glycyrrhetic acid in human plasma has been observed and it is attributable to an interactive action of absorption from the intestinal tract by anthraquinones (Mizuhara et al., 2005).

Following drug interaction caused by intake of Glycyrrhetic acid the following adverse reactions have been reported: hypertension, oedema, hypokalaemia, increase sensitivity to glycyrrhizin, sensitivity to adverse effects in women (Asl 2008).

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

No data available.

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

Clinical studies show that short-term use (not more than 4 weeks) is safe. However, chronic use can cause hypokalaemia, hypertension and, more rarely, cardiac rhythm disorders.

## **6. Overall conclusions**

There are no clinical data in the scientific literature to support a "well-established medicinal use".

*Glycyrrhiza glabra* has been traditionally used as an expectorant to help relieve chest complaints, such as catarrhs, coughs and bronchitis and to help relieve inflammatory conditions of the gastrointestinal tract, such as gastritis.

Several clinical studies evaluated the efficacy of deglycyrrhized extract, administered orally, to treat gastric and duodenal ulcers, but the lack of evidence of efficacy resulted in a loss of interest for this indication.

A clinical trial evaluated the effect of a decoction of 5 g of liquorice powder in 300 mL of water used as a gargle to treat postoperative sore throat (POST), a condition of postoperative morbidity, because *Glycyrrhiza* extract has been shown to decrease irritations and cough in the throat with expectorant effects.

In a recent clinical study the topical oromucosal use of patches containing 1 % of a chloroform extract showed promising results on the treatment of oral aphthous ulcer without relevant side effects. This supports the use of liquorice extract preparations in aphthous, stomatitis (oral ulcers) reported by Blumenthal (2003), but there are not enough data to support the traditional use in the reduction of pain and inflammation of aphthous ulcers.

Overall, a monograph on *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch, radix is recommended with the following therapeutic indications:

- 1) Traditional herbal medicinal product used as an expectorant in cough associated with cold.
- 2) Traditional herbal medicinal product used for the relief of digestive symptoms including burning sensation and dyspepsia.

Short-term use (not more than 4-6 weeks) is safe. Serious side effects reported following chronic use of liquorice root are: hypokalaemia and hypertension. More rarely cardiac rhythm disorders can occur.

Two cases of hypertension encephalopathy have been reported in susceptible people with prolonged daily intake of low doses of liquorice, corresponding to 80-100 mg of glycyrryzic acid.

Liquorice root is contraindicated during pregnancy and lactation and not recommended in children and adolescents under 18 years.

## **Annex**

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