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Committee on Herbal Medicinal Products (HMPC)

# Assessment report on on *Glycyrrhiza glabra* L.; *Gycyrrhiza inflata* Bat.; *Glycyrrhiza uralensis* Fisch., radix

Draft – Revision 1

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

| | | |
|---|---|---|
| Herbal substance(s) (binomial scientific name of the plant, including plant part) | Liquiritiae radix | |
| Herbal preparation(s) | a) Comminuted herbal substance<br>b) Soft extract (DER 1:0.4-0.5), extraction solvent water<br>c) Soft extract ( DER 3:1), extraction solvent water | |
| Pharmaceutical form(s) | Comminuted herbal substance as herbal tea for oral use<br>Herbal preparations in liquid dosage forms for oral use. | |
| First assessment | Rapporteur | M. Delbò / G. Calapai |
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Note: This draft assessment report is published to support the public consultation of the draft revised European Union herbal monograph on *Glycyrrhiza glabra* L.; *Gycyrrhiza inflata* Bat.; *Glycyrrhiza uralensis* Fisch., radix. It is a working document, not yet edited, and shall be further developed after the release for consultation of the revised monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but

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no ‘overview of comments received during the public consultation’ will be prepared on 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.

# *Table of contents*

# 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 18β-glycyrrhizic acid ($C_{42}H_{62}O_{16}$, $M_r$ 823), calculated with reference to the dried drug (European Pharmacopoeia 01/2012:0277).

*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. (WHO 1999).

To date, more than 200 chemical constituents have been isolated from liquorice (Jiang *et al.*, 2020).

Saponins

More than 70 triterpenic saponins have been identified in *Glycyrrhiza* (Li *et al.*, 2020), the most relevant of which is glycyrrhizin, a mixture of potassium and calcium salts of 18β-glycyrrhizic acid (also known as glycyrrhizic or glycyrrhizinic acid and a glycoside of glycyrrhetinic acid) which typically represents 5–10% of the roots and has been recognized as an efficient sweetening agent, that is 50 times sweeter than refined sugar (Isbrucker & Burdock, 2006). However, the amount of glycyrrhizin in the root varies with plant variety and climatic conditions of growing place and is between 5-25% (Bahmani *et al.*, 2015; Omar *et al.*, 2012).

Glycyrrhizin 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 and its aglycone, 18β-glycyrrhetinic acid (also known as 18β-glycyrrhetic acid or glycyrretic acid or glycyrrhetinic acid), have interesting therapeutic properties. Therapeutic potential of glycyrrhizin is mainly ascribed to the action of the steroid-like structure aglycone (18β-glycyrrhetinic acid) having immunomodulatory properties. Traces of the α-form of glycyrrhetinic acid are also present in liquorice roots but have no pharmacological activity (Claude *et al.*, 2008).

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. Among them, flavanones and chalcones are the main types (Zhang & Ye, 2009).

Flavonoids are responsible for the yellow colour of liquorice. They include liquiritin, liquiritigenin, rhamnoliquiritin, neoliquiritin, licochalcones, isoliquiritin, isoliquiritigenin, neoisoliquiritin, liquiritin apioside, isoliquiritin apioside, licuraside, glabrolide and licoflavonol (Williamson, 2003; Pastorino *et al.*, 2018; Cheng *et al.*, 2021).

In liquorice, the isoflavones glabridin, galbrene, glabrone, shinpterocarpin, licoisoflavones A and B, formononetin, glyzarin, kumatakenin, have been found. Other isoflavones present are

hispaglabridin A, hispaglabridin B, 4'-O-methylglabridin and 3'-hydroxy-4'-O-methylglabridin, glabroisoflavanone A and glabroisoflavanone B (Kinoshita *et al.*, 2005; Mamedov & Egamberdieva, 2019).

Coumarins

Coumarins include licopyranocoumarin, licoarylcoumarin, liqcoumarin, glabrocoumarone A and B, herniarin, umbelliferone, glycyrin, glycocoumarin, licofuranocoumarin, and glabrocoumarin (Kinoshita *et al.* 2005; Mamedov & Egamberdieva, 2019).

Other compounds

A significant amount of sucrose (up to 18%) can be present in liquorice roots (Bahmani *et al.*, 2015). Minor components occur in amounts that vary depending on the species and geographical location (WHO 1999). Volatile constituents of the roots include pentanol, hexanol, linalool oxide A and B, tetramethyl pyrazine, terpinen-4-ol, α-terpineol, geraniol, piperitone, thymol, estragole, anethole, eugenol, indole, cumic aldehyde, 7-nonalactone, p-vinyl guaiacole, 5-methyl furfurale (Størmer *et al.*, 1993; Roshan *et al.*, 2012; Farag & Wessjohan, 2012).

- Herbal preparation(s)

The European Pharmacopoeia reports the following dry extract:

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 β-glycyrrhizic acid. The dry extract is a yellowish-brown or brown powder with a very sweet taste (European Pharmacopoeia 01/2012:2378).

Techniques for extraction of active components from the root generally include initial comminution of the root and extraction with hot water and steam. The primary extract may be concentrated to a paste and cast into blocks or sticks or dried to a powder. This crude form contains 10–25% glycyrrhizin (Fry, 2012).

The glycyrrhizin contents of 42 samples of UK liquorice-containing confectionery, health products and raw materials ranged between 0.26 and 7.9 mg/g for confectionary, whilst contents in health products were 0.30 – 47.1 mg/g. The highest levels of glycyrrhizin were found in liquorice block (44 – 98 mg/g) and extract powder (79 - 113 mg/g). The sample of one herbal tea examined in this study had a glycyrrhizin content of 20 mg/g; according to the authors 9 g of liquorice herbal tea corresponded to an intake of approx. 200 mg of glycyrrhizin (three or four tea bags) (Spinks & Fenwick, 1990).

An analysis of 33 brands of herbal teas marketed in The Netherlands revealed that 13 contained < 10 mg/l, 16 contained 10 to 100 mg/l and 4 contained > 100 mg/l with a maximum of 450 mg/l (concentrations refer to the prepared beverage). Among these 33 brands, for 10 brands the label information stated liquorice plant material as ingredient, and for these teas the average glycyrrhizinic acid content was 126 mg/l (range 2 - 450 mg/l) (SCF, 2003). A cup of liquorice tea with a volume of 250 mL could therefore be expected to contain, on average, approximately 31.5 mg of glycyrrhizin.

A HPLC/UV analysis of 219 samples of confectionery, ice cream, and tea with liquorice on the food market in Denmark showed a mean glyryrrhizinic acid (GA) content of 1066 mg/kg (range between < LOQ and 4936 mg/kg) in confectionery made of liquorice candy (excluding products of pure liquorice with or without aroma) of 17,922 mg/kg (range between 14,935 mg/kg and 23,154 mg/kg) in pure liquorice with or without aroma, of 920 mg/kg (range between 408 and 1690

mg/kg) in ice cream, of 133 mg/L (range between < LOQ and 1203 mg/L) in brewed tea. If the high content (1203 mg/kg) from the only tea sample with liquorice, liquorice root, and aroma on the ingredient list is removed, the mean GA content is reduced to 114 mg/L with a maximum content of 534 mg/L (Ballin *et al.*, 2023).

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

Not applicable.

## *1.2. Search and assessment methodology*

This assessment report reviews the scientific literature data available for *Glycyrrhiza glabra,* and from the WHO monograph, European Pharmacopoeia monograph, British Pharmacopoeia monograph, Health Canada monograph, PubMed, Toxnet, Cochrane Database of Systematic Reviews, books and the internet, as well as available information on products marketed in the European Community, including pharmaceutical forms, indications, posology and methods of administration.

For revision of the monograph the same databases were searched in the period January 2011 – December 2023. The keywords "*Glycyrrhiza glabra*", "liquorice", "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.

A bibliographic search was also performed to retrieve older publications which could be relevant from a safety and efficacy perspective (e.g. animal toxicity studies, clinical interactions, clinical trials) not mentioned in the first assessment.

In addition, a search on EudraVigilance database has been carried out resulting in 1733 reports; 282 cases were found using terms "liquorice root extract", "*Glycyrrhiza glabra* L. Radix" and "*Glycyrrhiza uralensis* root".

**Main changes introduced in the 1st revision - for the revision of monograph**
From the bibliographic research, 2542 and 3177 new references not yet available during the first/previous assessment were identified using the search term "*Glycyrrhiza glabra*" on Pubmed and Embase, respectively. A minor number of new references was found using the other mentioned databases.
The posology of the herbal tea has been widened based on a new product on the market which fulfils the requirements for TU.
The herbal preparation d) Dry extracts that correspond to preparations mentioned under b) and c) present in the original version of the monograph has been deleted. Indeed, there is no need to specify any preparation which corresponds to the others already included in a monograph, as the evidence of traditional use for these preparations will be assessed within a marketing authorisation application.
Clinical evidence suggests a potential for interaction of liquorice with drugs metabolised by CYPs; this information has been included in the revised sections 4.4 and 4.5 of the monograph.
Information from literature that glycyrrhizin is detectable in the breastmilk of some women taking liquorice has been added to section 4.6.

# 2. Data on medicinal use

## *2.1. Information about products on the market*

### 2.1.1. Information about products on the market in the EU/EEA Member States

**Information on medicinal products marketed in the EU/EEA**

Table 1: Overview of data obtained from marketed medicinal products

| Active substance | Indication | Pharmaceutical form | Regulatory status |
|---|---|---|---|
| Liquiritiae radix | Catarrh of the upper airway; inflammatory gastrointestinal diseases | Herbal tea<br>SD: 4.5 g with 150 ml boiling water as herbal tea<br>DD: 9 – 13.5 g<br><br>No longer than 4-6 weeks without medical advice | 1986, DE, Standard Marketing Authorisation according to section 36 of the German Medicinal Products Act |
| Liquorice root | As an adjuvant in gastric ulcers and as an expectorant in cough and catarrhs of the upper respiratory tract | 3-15 g root daily/l, divided in 2-3 cups a day as infusion, decoction or macerate | Before 1973, ES, currently not more on the market |
| Liquorice root | To relieve digestive ailments,<br>such as burning sensation and indigestion | Herbal tea, as a decoction, in adults and elderly: approx. 1.5 g of liquorice roots pour over ¾ cup (approx. 150 ml) of cold water, cook slowly, covered, for 5 - 7 minutes, set aside for 15 minutes, strain. Drink the decoction prepared in this way 2 to 4 times a day after meals.<br>Duration of use: Do not use for more than 4 weeks. If symptoms last longer than 2 weeks despite the use of the medicinal product should be consulted a doctor or a pharmacist. | PL National registration 1992.08.14, TUR since 2013 |
| Liquorice root | As an expectorant in coughs accompanying the common cold. | Herbal tea, as a decoction, in adults and elderly: about 1.5 g of liquorice roots pour over ¾ cup (approx. 150 ml) of cold water, cook slowly, covered, for 5 - 7 minutes, set aside for 15 minutes, strain. Drink the decoction prepared in this way twice a day. | PL National registration 1992.08.14, TUR since 2013 |

| | | | |
|---|---|---|---|
| | | Duration of use: If symptoms persist for more than a week (7 days) despite use medicinal product, consult your doctor or pharmacist. | |
| Soft extract (1:0.4-0.5), extraction solvent water | Traditionally used to support gastric function | Oral liquid<br>SD 32 mg<br>DD 64-96 mg<br>(not more than 5 times 32 mg =160 mg)<br><br>No longer than 4-6 weeks without medical advice | At least since 1976 until 06/2012, DE, TU |
| Liquiritiae radix soft extract (DER 3:1), extraction solvent water | As an expectorant | Oral liquid<br>1.2-1.5 g 3-4 times daily for oral use<br>In Denmark, two oral liquids containing Liquorice soft extract (DER 3:1, extraction solvent water) are authorised. One product contains 98.5 mg/ml soft extract corresponding to 4.64 mg glycyrrhizic acid. The other contains 80.9 mg/ml soft extract corresponding to 3.92 mg glycyrrhizic acid. | Authorised in DK for more than 70 years |

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

**Information on relevant combination medicinal products marketed in the EU/EEA**

Not applicable

**Information on other products marketed in the EU/EEA (where relevant)**

Not applicable

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

Not applicable

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

Since the beginning of recorded history, humans have made use of liquorice (mainly the species *Glycyrrhiza glabra* L., Leguminosae) as a remedy (Fiore *et al.*, 2005). 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 ashtma and for healing wounds. 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 (Davis & Morris, 1991).

As reported by Lucas in *Nature's Medicines,* the ancient Hindus believed that liquorice, administrated 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 (Davis & Morris, 1991). 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 *et al.*, 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' (Davis & Morris, 1991).

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. In modern Chinese medicine, it is used as a tonic, an antipyretic, an antidote (e.g. counteracting mushroom poisoning), a demulcent to the lungs, an expectorant, an analgesic, to soothe sore throats and coughs, to treat asthma, and to alleviate toxic abscesses as well as acute abdominal pains (Davis & Morris, 1991).

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 (Davis & Morris, 1991).

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 18β-glycyrrhetinic acid has also been shown to possess anti-estrogenic activity (Davis & Morris 1991).

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 18β-glycyrrhetinic acid, has been extensively employed for the purpose of alleviating ulcers (Davis & Morris 1991).

Based on pharmacopoeials and traditional systems of medicine liquorice is used as a demulcent in the treatment of sore throats, and as an expectorant in the treatment of coughs and bronchial catarrh. It is also used in the prophylaxis and treatment of gastric and duodenal ulcers, and dyspepsia (WHO 1999).

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

Table 2: Overview of historical data

| **Herbal preparation** | **Documented use / Traditional use** | **Strength (where relevant) Posology Duration of use** | **Reference** |
|---|---|---|---|
| Powdered or finely cut roots | For catarrhs of the upper respiratory tract and gastric/duodenal ulcers | Daily dose: 5-15 g as an infusion or decoction, equivalent to 200 to 600 mg of glycyrrhizin | Commission E monograph, 1991 |
| Succus liquiritiae (dry extract containing about 20% glycyrrhizin) | For catarrhs of the upper respiratory tract | 0.5-1 g in solid or liquid dosage forms for oral use | |
| Succus liquiritiae (dry extract containing about 20% glycyrrhizin) | For gastric/duodenal ulcers | 1.5-3.0 g in solid or liquid dosage forms for oral use<br>Duration of treatment: not more than 4-6 weeks without medical advice | |
| Powdered roots | Bronchial catarrh, bronchitis, chronic gastritis, peptic ulcer, colic | 1-4 g as a decoction 3 times daily | British Herbal Pharmacopoeia 1983 and British Hebal Compendium 1992 (cited by Barnes, Anderson & Phillipson 2007) |
| Liquorice extract from roots | Bronchial catarrh, bronchitis, chronic gastritis, peptic ulcer, colic | 0.6-2.0 g | British Pharmaceutical Codex, 1973 (cited by Barnes, Anderson & Phillipson 2007 |

| Herbal preparation | Documented use / Traditional use | Strength (where relevant) Posology Duration of use | Reference |
|---|---|---|---|
| Liquorice roots | Cough and bronchial catarrh; gastric and duodenal ulcers and gastritis | Adult and elderly daily dose, taken in divided doses when required: 1.5-5 g, equivalent to 60-200 mg of glycyrrhizic acid; equivalent aqueous preparations or 1.5-5 ml of standardised liquorice ethanolic liquid extract (containing 4.0% m/m of glycyrrhizic acid and 52-65% V/V of ethanol).<br><br>Maximum daily dose: 15 g of liquorice root (or 600 mg of glycyrrhizin)<br><br>Children 4 years of age and older: as an expectorant only, in aqueous preparations: proportion of adult dose according to age or body weight<br><br>Duration of treatment: not more than 4-6 weeks without medical advice | ESCOP 2003 |
| Liquorice roots | Bronchitis or cough | As an infusion: 1.5 g of finely comminuted herbal susbtance in 150 ml of boiling water to be taken 2-3 times daily. Infusion time: 10-15 minutes | DAC 2009 |

| Herbal preparation | Documented use / Traditional use | Strength (where relevant) Posology Duration of use | Reference |
|---|---|---|---|
| | Gastrointestinal disturbances | As an infusion: 4.5 g of finely comminuted herbal susbtance in 150 ml of boiling water to be taken 2-3 times daily. Infusion time: 10-15 minutes<br><br>Do not use in children below 4 years of age | |
| Liquorice roots | Chronic gastritis | Herbal tea. Pour 150 mL boiling water over one teaspoon (2–4 g) liquorice root, simmer for 5 minutes and filter through a tea strainer after cooling.<br><br>One cup after ech meal. | Braun & Cohen 2015 |

## *2.3. Overall conclusions on medicinal use*

There is evidence of traditional use for liquorice roots for the relief of digestive symptoms including burning sensation and dyspepsia. Specifically, the use of the comminuted root as herbal tea has been authorized in Poland since 1980, while a soft extract (1:0.4-0.5) obtained using water as an extraction solvent has been marketed in Germany for over 30 years.

Extensive literature data and products on the market in Poland and Germany support the traditional use of the comminuted root as an herbal tea as an expectorant in cough associated with cold. In addition, a soft extract (3:1) obtained using water as an extraction solvent has been marketed in Denmark for more than 70 years.

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.

Table 3: Overview of evidence on period of medicinal use

| **Herbal preparation Pharmaceutical form** | **Indication** | **Posology, Strength** | **Period of medicinal use** |
|---|---|---|---|
| Comminuted root | Traditional herbal medicinal product for the relief of digestive symptoms including burning sensation and dyspepsia. | Herbal tea: 1.5 - 2 g of comminuted herbal substance in 150 ml of boiling water as a herbal infusion or decoction 2 to 4 times daily<br>SD: 1.5 – 2 g<br>DD: 3 – 8 g | PL, since 1992 |
| | Traditional herbal medicinal product used as an expectorant in cough associated with cold. | Herbal tea: 1.5 – 4.5 g of comminuted herbal substance in 150 ml of boiling water as a herbal infusion or decoction 2-3 times daily<br>SD: 1.5 – 4.5 g<br>DD: 3 – 13.5 g | PL, since 1992<br>DE, since 1976 |
| Soft extract (DER 1:0.4-0.5), water | Traditional herbal medicinal product for the relief of digestive symptoms including burning sensation and dyspepsia. | Oral liquid<br>32 mg 2-3 times daily, up to 5 times daily<br>SD: 32 mg<br>DD: 64 – 96 mg, not more than 160 mg | DE, since at least 1976 |
| Soft extract (DER 3:1), water | Traditional herbal medicinal product used as an expectorant in cough associated with cold. | Oral liquid<br>1.2-1.5 g 3-4 times daily<br>SD: 1.2 -1.5 g<br>DD: 3.6 – 6 g | DK, for at least 70 years |

# 3. Non-Clinical Data

Due to the large number of studies published on the pharmacological effects of liquorice and liquorice derivatives, this AR for what concerns the secondary pharmacodynamics will focus on those carried out using the powdered/comminuted roots and the water extracts.

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

### 3.1.1. Primary pharmacodynamics

*Anti-inflammatory activity*

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 incubated at a concentration of 10 µg/ml for 18 h demonstrated strong anti-inflammatory activity by reducing nitric oxide (NO) and prostaglandin E2 production in the LPS-stimulated mouse macrophage cell, RAW264.7. It also inhibited the production of pro-inflammatory cytokines (10 µg/ml for 24 h) and CD14 expression (10 and 30 µg/ml for 20 h) on the LPS-stimulated RAW264.7 cells. LPS-induced degradation and phosphorylation of IK-Bα, along with DNA-binding of NF-KB, was significantly inhibited by rLE exposure at 30 µg/ml in RAW264.7 cells. In the murine model, it was found that *in vivo* oral exposure to rLE 10 mg/kg induced an increase in the survival rate, reduced plasma levels of TNF-α and IL-6, and increased IL-10 production in LPS-treated mice (Kim *et al.*, 2006).

*Glycyrrhiza uralensis* root was water distilled, and the distillate was subsequently extracted with dichloromethane. Residual aqueous solution from the extraction was fractionated using column chromatography. A total of 127 chemicals were identified in the dichloromethane extract, which inhibited hexanal oxidation by over 90% for 45 days at the level of 50 µg/ml. A fraction eluted from the residual aqueous solution with acetone (62, 125, 250 µg/ml) exhibited potent antioxidant activities both in a thiobarbituric acid assay (76.1 ± 7.6%) and in a malonaldehyde/gas chromatography assay (83.1 ± 1.5%). The acetone fraction also exhibited strong anti-inflammatory activity (77.9% inhibition at the level of 62 µg/ml) in a lipoxygenenase inhibitor screening anti-inflammatory assay (Tanaka *et al.*, 2008).

A DMSO extract of liquorice at both 0.2 and 0.5 mg/ml concentrations showed slightly anti-inflammatory activity via reduction of IL-6 and TNF-a but also IL-10 in RAW 264.7 macrophages stimulated with LPS. In addition, the extract at 0.5 mg/ml completely inhibited iNOS expression (Mueller *et al.*, 2010).

In another experiment, RAW 264.7 cells were incubated with a hot water extract of of *G. uralensis* roots (GL) at concentrations of 300 and 1000 µg/ml. After 1 h, lipid A moiety of LPS was added to the culture. After 24 h, culture supernatants were collected, and levels of TNF-α and IL-6 in the culture supernatants were determined by using ELISA kits. The extract significantly decreased the levels of IL-6 and TNF-α induced by lipid A stimulation in a dose-dependent manner. The effects of the water extract and of the isolated constituent isoliquiritigenin (ILG) on TNF-α and IL-6 production were also investigated *in vivo*. BALB/c mice ($n$=5) were administered with GL 400 mg/kg peritoneally, 24 h and 1 h before LPS administration. Similarly, BALB/c mice ($n$=5) were administered with ILG 50 mg/kg orally, 24 h and 1 h before LPS administration. Plasma was collected at 1 h and 4 h after LPS injection. ILG significantly decreased the levels of TNF-α and IL-

6, whereas only TNF-α production was affected significantly by GL treatment. All these effects were weaker than those of dexamethasone 5 mg/kg which was used as a positive control (Honda *et al.*, 2012).

The inhibitory effect of a flavonoid rich, dry extract of *G. Glabra* (DSR 1:4, extraction solvent acetone; GutGard®) and its phytoconstituents glabridin, glycyrrhizin, and isoliquiritigenin LPS-induced prostaglandin $E_2$ ($PGE_2$), calcimycin (A23187) induced thromboxane ($TXB_2$), and leukotriene ($LTB_4$) release was studied using murine macrophages (J774A.1) and human neutrophil (HL-60) cells. Results revealed that, *G. glabra* extract at concentrations ranging from 2.5 to 40 µg/ml and glabridin in the range 1.25–10 µg/ml significantly inhibited $PGE_2$, $TXB_2$ (COX) and $LTB_4$ (LOX) in a dose-dependent manner; isoliquiritigenin exerted inhibitory effect only against COX products but failed to suppress LOX product. However, glycyrrhizin at the tested concentrations failed to exhibit inhibitory effect on both COX and LOX products (Chandrasekaran *et al.*, 2011a).

The same extract (20 and 40 µg/ml) and isoliquiritigenin (2.5, 5 and 10 µg/ml) significantly inhibited LPS (0.1 µg/ml for 24 h) stimulated NO, IL-1β and IL-6 production in J774A.1 murine macrophages. Glabridin (5 and 10 µg/ml) showed significant inhibition of NO and IL-1β release but failed to attenuate IL-6 levels. In addition, glycyrrhizin (0.62 – 10 µg/ml) did not exhibit inhibitory response towards any of the LPS-induced pro-inflammatory mediators (Thiyagarajan *et al.*, 2011).

The anti-inflammatory effects of water and ethanol extracts of liquorice were also studied *in vitro* by Yue & colleagues (2012). Both the extracts significantly inhibited NO production in LPS-activated RAW264.1 cells with the ethanol extract which resulted more effective. Specifically, the ethanol extracts (100–800 µg/mL) inhibited NO production in a concentration-dependent manner without any cytotoxicity. In addition, in the presence of the ethanol extracts (6.25–50 µg/mL), the $PGE_2$ and IL-10 concentrations were also decreased in dose-dependent manner; however, no effects on TNF-α production were observed. The proliferative response of phytohaemagglutinin (PHA)-activated PBMC was investigated by the [methyl-3H]-thymidine incorporation assay. The water extract at 200 µg/mL significantly stimulated the proliferation responses of PBMC ($p<0.05$) and decreased their viability, while the ethanol extract at 25-200 µg/mL significantly inhibited the proliferation in a concentration-dependent manner without any cytotoxicity. The ethanol extract (50–200 µg/mL) showed inhibitory effects on TNF-α, IFN-γ and IL-10 production in a concentration-dependent manner and these effects were comparable to those of dexamethasone 1 µM at the highest concentration (Yue *et al.*, 2012).

A study was carried out to determine if the anti-inflammatory effects of individual herbal constituents of an anti-ashtma herbal medicine intervention containing the Chinese plants *Ganoderma lucidum*, *Sophora flavescens* and *Glycyrrhiza uralensis* exhibited synergy to suppress Th2 cytokines and eotaxin-1 production. The effects of the herbal medicine and the aqueous extract of its single constituents on Th2 cytokine secretion by murine memory Th2 cells (D10.G4.1) and eotaxin-1 secretion by human lung fibroblast (HLF-1) cells were determined by measuring levels in culture supernatants by enzyme-linked immunosorbent assay. Individual aqueous extracts and their combination inhibited production of IL-4 and IL-5 by murine memory Th2 cells and eotaxin-1 production by HLF-1 cells. The mean 25%-inhibitory-concentration ($IC_{25}$) values (mg/mL) for the herbal medicine, aqueous extracts of *Ganoderma lucidum*, *Sophora flavescens* and *Glycyrrhiza uralensis* for IL-4 production were 30.9, 79.4, 123, and 64.6, respectively; for IL-5 production were 30.2, 263, 123.2 and 100, respectively; for eotaxin-1 were 13.2, 16.2, 30.2, and 25.1, respectively. The $IC_{50}$ values (mg/mL) for the herbal medicine, aqueous extracts of *Ganoderma lucidum*, *Sophora flavescens* and *Glycyrrhiza uralensis* for IL-4 production were 158.5,

239.9, 446.7, and 281.8, respectively; for eotaxin-1 were 38.1, 33.1, 100, and 158.5, respectively. Inhibition of IL-5 did not reach $IC_{50}$ values (Jayaprakasam *et al.*, 2013).

Both a water extract of *G. inflata* roots (0.5–2 mg/mL) and its constituents Licochalcone A (10 μM) and Liquiritigenin (10 μM) dose-dependently suppressed NO, TNF-α, IL-1β, PGE2 productions in inflammation-stimulated (LPS 1 μg/ml for 20 h) RAW 264.7 macrophage, after pre-treatment of these cells for 8 h. Similarly, pre-treatment for 24 h with *G. inflata* extract (50–500 μg/mL), licochalcone A (10–100 nM) or liquiritigenin (10–100 nM) dose-dependently effectively suppressed the NO production Iba1 expression in BV2 microglia cells stimulated with a combination of LPS (1 μg/mL) and IFN-γ (100 ng/mL) in DMEM-1% FBS medium for 24 h (Chiu *et al.*, 2018).

To determine the effect of a dried hydroalcoholic extract (ethanol 80%/$H_2O$ 20%) of *G. uralensis* (GUE) on TNF-α-induced NF-κB activation, HepG2 cells were transfected with NF-κB cis-reporter plasmid, pNF-κB-Luc, which contains five repeats of NF-κB binding element GGGGACTTTCC in the enhancer element of the plasmid. Thereafter, cells were pretreated with or without 30 min GUE and its subfractions and then incubated with TNF-α (10 ng/mL) for 6 h. TNF-α significantly increased the NF-κB activation by a factor of 2.1 over the spontaneous control. GUE (25 μg/mL) treatment clearly attenuated the NF-κB activation induced by TNF-α with an $IC_{50}$ = 18.1 ± 2.8 μg/mL. In addition, TNF-α enhanced NF-κB activity was significantly attenuated by glycyrrhetinic acid (10 – 40 μM) in a concentration-dependent manner in the NF-κB reporter gene assay. Glycyrrhetinic acid 40 μM decreased the gene expression of iNOS through inhibited IκBα phosphorylation and p65 translocation in protein level. Furthermore, NO production and iNOS expression were reduced by glycyrrhetinic acid in TNF-α-induced rat primary hepatocytes (Chen *et al.*, 2014a).

The anti-inflammatory activities of both glycerrhitinic acid (GA) and the aqueous liquorice extract (ALE) in comparison with diclofenac sodium (DS) (10 mg/kg), using the carrageenan-induced paw edema model in male albino rats. Rats were divided into the following 6 groups, each composed of 6 rats: Negative control group received 0.2 mL of sesame oil; Positive control group was injected intramuscularly (IM) with DS (10 mg/kg); Treated group 1 was injected IM with GA (100 mg/kg); Treated group 2 was injected IM with ALE (250 mg/kg); Treated group 3 was injected IM with a combination of GA and DS (100 mg/kg and 10 mg/kg, respectively); Treated group 4 was injected IM with a combination of ALE and DS (250 mg/kg and 10 mg/kg, respectively). The intraplantar injection of the hind paw by carrageenan induced a progressive edema. All agents under investigation showed an anti-inflammatory activity similar to that obtained by DS (10 mg/kg). The combination group of DS and GA acid produced the greater reduction of edema (78.3%). However, when compared with DS alone (73.9%), there was no significant difference (Aly *et al.*, 2005).

The anti-inflammatory activity of liquorice (LE) and roasted liquorice (rLE) extracts was evaluated 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 (1.0 and 2.0 mg/ear) dose-dependently inhibited phorbol ester-induced ear oedema, and rLE possesses greater activity than LE. Oral administration of LE or rLE 10 mg/kg for twenty consecutive days 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 (Kim *et al.*, 2010).

The effect of hexane/ethanol extract of roots of *Glycyrrhiza uralensis* (HEGU) on inflammatory responses in lipopolysaccharide (LPS)-treated Raw 264.7 macrophages and in mouse skin treated with 12-O-tetradecanoylphorbol-13-acetate (TPA). In the LPS-stimulated macrophages, HEGU (0–2 μg/ml) reduced nitric oxide (NO) release and the protein expression and transcriptional activity of inducible NO synthase (iNOS). HEGU reduced prostaglandin $E_2$ release and phospholipase $A_2$ transcripts. HEGU reduced the secretion and mRNA levels of tumour necrosis factor-α, interleukin (IL)-6, and IL-1β. HEGU prevented IκBα degradation, p65 nuclear translocation, NFκB DNA binding and transcriptional activities. Additionally, dehydroglyasperin C, isolated from HEGU, reduced NO production, iNOS expression, and NFκB transcriptional activity. In the mouse inflammation model, by (TPA)-induced skin swelling in male ICR mice, HEGU 0–2 mg topically administered to the shaven back 1 h prior to the topical treatment with 10 nmol of TPA, suppressed skin swelling and iNOS and cyclooxygenase-2 expression (Cho *et al.*, 2010).

The anti-inflammatory and healing effects of an aqueous liquorice extract was also investigated in acetic acid-induced ulcerative colitis (UC) in rat as an animal model. Forty-eight male Wistar rats were divided into six equal groups. Group I as normal control group received 0.5 ml/kg normal saline; group II, 0.5 ml/kg saline after induction of UC with 3% acetic acid; group III, 50 mg/kg liquorice extract orally; group IV, 100 mg/ kg liquorice extract orally; group V, 150 mg/kg liquorice extract orally; and group VI, 150 mg/kg liquorice extract intracolonic. In all animals, the distal 10-cm portion of the colon was removed after 7 days for macroscopic and histological investigation. Inflammation following acetic acid administration was characterized by edema, diffuse inflammatory cell infiltration, and necrosis. Administration of oral 100 and 150 mg/kg and intracolonic 150 mg/kg of liquorice extract significantly reduced the colonic inflammatory response and edema. Intracolonic administration of liquorice extract showed more anti-inflammatory and healing effects in comparison to other groups (Takhshid *et al.*, 2012).

*Gastro-intestinal activity*

The effect of a standardised-flavonoid rich extract of *G. glabra* on gastric emptying and gastrointestinal transit was studied *in vivo* on male albino Wistar rats, randomly allotted to eight groups each consisting of six animals. Group I was adminstered with carboxy methyl cellulose (CMC) and Group II was administered Domperidone (10 mg/kg) as a single dose on day 8, while Group III, IV, V were adminstered various dose levels of extract (6.25, 12.5 and 25 mg/kg) respectively and Group VI, VII, VIII were adminstered various dose levels of Glabridin (2.5, 5 and 10 mg/kg) respectively for 8 consecutive days. On day 8, sixteen hours fasted rats were administered phenol red meal (2 ml/animal) 2 h post the test substance or reference drug administration. Percentage gastric emptying and gastrointestinal transit were measured immediately and 20 minutes after phenol red meal administration. The percentage gastric emptying in control group was found to be 53.33% and group administered with single dose of domperidone (10 mg/kg p.o.) was found to be 87.03%. Groups administered with extract and glabridin at all dose levels exhibited statistically significant increase of gastric emptying over control group (83.11%, 86.14% and 88.13%, respectively with extract 6.25, 12.5 and 25 mg/kg; 86.52%, 87.80%, and 89.71%, respectively with glabridin 2.5, 5 and 10 mg/kg). Groups administered with domperidone and liquorice extract at all dose levels demonstrated statistically significant increase in gastrointestinal transit (%) compared to the control group. Glabridin treated groups showed non-significant increase in gastrointestinal transit (%) compared to the control group (Murugan *et al.*, 2017).

*In vivo*, on adult Balb-C mice, isoliquiritigenin at increasing doses of 0.003, 0.03, 0.3, 3 and 30 mg/kg produced a dual dose-related effect on the charcoal meal travel, inhibitory at the doses up

to 0.03 mg/kg, while prokinetic at the high doses. *In vitro*, isoliquiritigenin 0.1–10 mM showed an atropine-sensitive concentration-dependent spasmogenic effect in isolated rat stomach fundus. However, a spasmolytic effect was observed in isolated rabbit jejunums, guinea pig ileums and atropinized rat stomach fundus, either as noncompetitive inhibition of agonist concentration-response curves, inhibition of high $K^+$ (80 mM)-induced contractions, or displacement of $Ca^{2+}$ concentration-response curves to the right, indicating a calcium antagonist effect. Pretreatment with N(omega)-nitro-L-arginine methyl ester (L-NAME; 30 microM), indomethacin (10 microM), methylene blue (10 microM), tetraethylammonium chloride (0.5 mM), glibenclamide (1 microM), 4-aminopyridine (0.1 mM), or clotrimazole (1 microM) did not inhibit the spasmolytic effect (Chen *et al.*, 2009).

*Antitussive effects*

Both the water (2 mg/ml) and ethanol (125 µg/ml) extracts of *Glycyrrhiza glabra* roots showed attenuation of contractions of rat tracheal rings induced by acetylcholine and carbacol $10^{-3}$ M (Yue *et al.*, 2012).

A water-extracted polymeric fraction (WE) of *Glycyrrhiza glabra*, consisting mainly of 3- and 3,6-linked galactopyranosyl, and 5- and 3,5-linked arabinofuranosyl residues, decreases the number of citric acid induced cough efforts in guinea pigs more effectively than codeine 10 mg/kg b.w. when administered orally at 50 mg/kg b.w. to adult male guinea-pigs (n=24) (Saha *et al*., 2011).

The effect of a dry ethanol (70% V/V) extracts of *Glycyrrhiza glabra* roots on sulphur dioxide ($SO_2$) gas induced cough was studied in Albino mice. Animals were divided into five groups, containing 7 mice. Group I served as a control group and was not administered anything. Group II, Group III, and Group IV were received standard drug, i.e., Codeine sulfate 10 mg/kg, 15 mg/kg, and 20 mg/kg orally, respectively. Group IV received ethanol extract of *G. glabra* orally in a dose of 200 mg/kg. Each animal assisted as its own control and was exposed to $SO_2$ gas twice, i.e., before and 60 min after the drug treatment. In normal controls, there was no significant change in the number of cough bouts, between the two exposures, whilst the ethanol extracts significantly inhibited the cough reflex compared to the control group ($p < 0.01$). Mice showed inhibition of 41.17%, in cough on treatment with *G. glabra.* Codeine sulfate used as a standard drug for suppression of cough, produced 25.29%, 33.33%, and 47.13% inhibition in cough at a dose of 10 mg/kg, 15 mg/kg, and 20 mg/kg, respectively. Moreover, codeine sulfate (20 mg/kg) showed a maximum of 47.13% ($p < 0.001$) inhibition at 60 min of the experiment. The extract also has significant ($p < 0.05$) effects in inhibiting the cough reflex at a dose of and 200 mg/kg bw in comparison with the standard group (Shitole & Pawar, 2019).

The antitussive effects were evaluated using a classical mice cough model induced by ammonia liquor. Mice were orally administered with water liquorice extract (50 or 200 mg/kg) or several isolated compounds (20 or 50 mg/kg). Pentoxyverine citrate (P, 50 mg/kg) was the positive control. Ammonia treatment in the vehicle group (0.5% CMC-Na) induced 10–12 coughs/3 min, and the latent period was 66–68 s. The positive drug, pentoxyverine citrate at 50 mg/kg significantly reduced cough frequency by 58%, 53% and 50% at 1 h, 2.5 h and 5 h, and enhanced the latent period at 1 h, compared with the data before drug administration ($p < 0.001$). Liquorice extract had dose-dependent anti-tussive effects, with a significant reduction in cough frequency at all the timepoints, although only a weak effect was seen on latent period. Among liquorice constituents, liquiritin apioside, liquiritin, and liquiritigenin exhibited potent antitussive activities. At 50 mg/kg, liquiritin apioside could reduce cough frequency by 30%-78% ($p < 0.01$) and enhance the latent period to 1.3–1.9 folds ($p < 0.05$). Liquiritin apioside exhibited remarkably stronger

effects at 1 h than at 2.5–5 h, for both cough frequency ($p < 0.01$) and latent period ($p < 0.001$). Liquiritin and liquiritigenin showed increased effects at 2.5–5 h, with lower cough frequency ($p < 0.001$) and longer latent period ($p < 0.05$) than at 1 h. Glycyrrhetinic acid exhibited moderate activities, and decreased cough frequency by 27–40% at 50 mg/kg ($p < 0.05$). Glycyrrhizic acid showed no significant activities. Glabidrin exhibited moderate antitussive effects, decreasing the cough frequency at 2.5 h ($p < 0.05$). The expectorant activities of isolated constiturnts of liquorice were evaluated using the phenol red secretion mice model. Compared with the control group, the positive drug guaifenesin exhibited significant increase in phenol red secretion to 3.0 folds ($p < 0.001$). Liquiritin apioside and liquiritin at 50 mg/kg exhibited potent expectorant activities (increased to 1.97 and 2.50 folds, respectively, $p < 0.001$). Liquiritigenin showed moderate effects, increasing the phenol red secretion to 1.57 folds ($p < 0.01$). The antitussive activities of different extracts of liquorice were evaluated, including the water extract (LWE), the ethanol extract (LEE), the *n*-butanol extract (LBE), and the ethyl acetate extract (LEAE). LWE and LEE, which contain abundant liquiritin apioside and liquiritin, exhibited potent antitussive effects, reducing the cough frequency by 25–59% at 1–5 h ($p < 0.05$), and remarkably enhancing the latent period at 2.5 h and 5 h, respectively ($p < 0.05$). LWE and LEE at 200 mg/kg could also significantly increase the phenol red secretion to 1.45 and 1.62 folds ($p <0 .05$, $p < 0.01$). LBW possessed moderate antitussive effects. LEAE, which contains very low amounts of liquiritin apioside, liquiritin and liquiritigenin, exhibited no antitussive effects (Kuang *et al.*, 2018).

Table 4: Overview of the main non-clinical data/conclusions on anti-inflammatory effects

| **Herbal preparation tested** | **Posology** | **Experimental model** | **Reference** | **Main non-clinical conclusions** |
|---|---|---|---|---|
| Water extract | 400 mg/kg peritoneally | LPS-stimulated mice | Honda *et al.*, 2012 | ↑ plasma levels of TNF-α |
| Water extract | 250 mg/kg i.m. alone or combined with diclofenac sodium (DS) 10 mg/kg i.m. | carrageenan-induced paw edema model in male albino rats | Aly *et al.*, 2005 | ↓ of edema, no significant difference with DS |
| Water extract | 50, 100, 150 mg/kg orally and 150 mg/kg intracolonic | acetic acid-induced ulcerative colitis (UC) in rat | Takhshid *et al.*, 2012 | oral 100 and 150 mg/kg and intracolonic 150 mg/kg of liquorice extract significantly ↓ the colonic inflammatory redponse and edema |
| roasted liquorice ethanolic extract | 10 mg/kg orally | LPS-stimulated mice | Kim *et al.*, 2006 | ↑ survival rate, ↓ plasma levels of TNF-α and IL-6, and ↑ IL-10 production |
| Isoliquiritigenin | 50 mg/kg orally | LPS-stimulated mice | Honda *et al.*, 2012 | ↓ plasma levels of TNF-α and IL-6 |

| Herbal preparation tested | Posology | Experimental model | Reference | Main non-clinical conclusions |
|---|---|---|---|---|
| Glycerrhitinic acid | 100 mg/kg i.m. alone or combined with diclofenac sodium (DS) 10 mg/kg i.m. | carrageenan-induced paw edema model in male albino rats | Aly *et al.*, 2005 | ↓ of edema, no significant difference with DS |

Table 5: Overview of the main non-clinical data/conclusions on gastro-intestinal activity

| Herbal preparation tested | Posology | Experimental model | Reference | Main non-clinical conclusions |
|---|---|---|---|---|
| Flavonoid rich, dry extract of *G. Glabra* (DSR 1:4, extraction solvent acetone) | 6.25, 12.5 and 25 mg/kg orally | Rats | Murugan *et al.*, 2017 | significant ↑ of gastric emptying and transit over control group |
| Glabridin | 2.5, 5 and 10 mg/kg orally | Rats | Murugan *et al.*, 2017 | significant ↑ of gastric emptying over control group |
| Isoliquiritigenin | 0.003, 0.03, 0.3, 3 and 30 mg/kg | Mice | Chen *et al.*, 2009 | inhibitory effect on charcoal meal travel at the doses up to 0.03 mg/kg, while prokinetic at the high doses |

Table 6: Overview of the main non-clinical data/conclusions on antitussive effects

| Herbal preparation tested | Posology | Experimental model | Reference | Main non-clinical conclusions |
|---|---|---|---|---|
| Water extract | 50 and 200 mg/kg orally | Mice cough model induced by ammonia liquor | Kuang *et al.*, 2018 | Dose-dependent significant ↓ in cough frequency and ↑ the latent period at 1 h, 2.5 h and 5 h after induction<br><br>Significant ↑ in secretions |

| Herbal preparation tested | Posology | Experimental model | Reference | Main non-clinical conclusions |
|---|---|---|---|---|
| Water-extracted polymeric fraction | 50 mg/kg orally | Expectorant activity assessed by the phenol red secretion mice model<br><br>Guinea pig | Saha *et al.*, 2011 | ↓ the number of citric acid induced cough efforts |
| Ethanol extract | 50 and 200 mg/kg orally | Mice cough model induced by ammonia liquor<br><br>Expectorant activity assessed by the phenol red secretion mice model | Kuang *et al.*, 2018 | Significant ↓ in cough frequency and ↑ the latent period at 1 h, 2.5 h and 5 h after induction<br><br>Significant ↑ in secretions |
| Liquiritin apioside, liquiritin, and liquiritigenin | 20 and 50 mg/kg orally | Mice cough model induced by ammonia liquor<br><br>Expectorant activity assessed by the phenol red secretion mice model | Kuang *et al.*, 2018 | Significant ↓ in cough frequency and ↑ the latent period<br><br>Significant ↑ in secretions |
| Glycyrrhetinic acid, glycyrrhizic acid glabridin | 20 and 50 mg/kg orally | Mice cough model induced by ammonia liquor | Kuang *et al.*, 2018 | Glycyrrhetinic acid and glabridin: significant ↓ in cough frequency at 2.5 h after induction.<br><br>Glycyrrhizic acid: no significant activities |

## 3.1.2. Secondary pharmacodynamics

*Anti-ulcer and gastro-protective effects*

Thirty-five healthy beagle dogs (15 male and 20 female) were randomly assigned to one of seven treatment groups (n = 5 in each group). Group 1 received 2 mg/kg b.w. carprofen orally (PO) every 24 h (q 24 h). Group 2 received 2 mg/kg b.w. carprofen, PO, q 24 h and 0.5 mg/kg b.w. lansoprazole, PO, q 24 h. Group 3 received 2 mg/kg BW carprofen, PO, q 24 h; 50 mg/dog liquorice extract, PO, q 24 h10; and 0.1 ml/kg BW herbal solution (liquid alcohol extract that consists of thyme, icelandic lichen, hyssop, and saponariae root), PO, q 24 h, diluted 1:5 with warm water. Group 4 received 2 mg/kg b.w. robenacoxib, PO, q 24 h. Group 5 received 2 mg/kg b.w. robenacoxib, PO, q 24 h and 0.5 mg/kg b.w. lansoprazole, PO, q 24 h. Group 6 received 2 mg/kg b.w. robenacoxib, PO, q 24 h; 50 mg/dog liquorice extract, PO, q 24 h; and 0.1 ml/kg b.w. herbal solution, PO, q 24 h, diluted 1:5 with warm water. The dogs in control Group 7 received an empty gelatin capsule. Endoscopy and biopsy of the caudal gastrointestinal tract were performed pretreatment and on the last day of a 21-day treatment period. Both carprofen and robenacoxib tested damaged the colonic mucosa with most severe microscopic lesions following administration of robenacoxib with lansoprazole. The risk of histopathological lesions in the colon increased most rapidly in robenacoxib with lansoprazole (absolute risk increase — 0.85) similar to robenacoxib only (— 0.75), whereas the best result was recorded following the plant remedies together with carprofen (— 0.15) and the plant remedies together with robenacoxib (— 0.2) (Szweda *et al.*, 2014).

The protective effect of the total flavonoids of *G. uralensis* (TFGU) on irinotecan–induced colitis mice from the perspective of gut microbiota and fecal metabolism was investigated by Yue *et al*. (2021). The purity of TFGU expressed as liquiritin equivalents was 80.12%. Oral administration of TFGU 135 mg/kg to C57BL/6 mice for 10 days significantly attenuated the loss of body weight and the shortening of colon length induced by irinotecan (40 mg/kg i.p.). The elevated disease activity index and histological score of colon as well as the up-regulated mRNA and protein levels of TNF-α, IL-1β, and IL-6 in the colonic tissue of irinotecan–treated mice were significantly decreased by TFGU. Meanwhile, TFGU restored the perturbed gut microbial structure and function in irinotecan–treated mice to near normal level. TFGU also effectively reversed the irinotecan–induced fecal metabolic disorders in mice, mainly call backing the hypoxanthine and uric acid in purine metabolism (Yue *et al.*, 2021).

Finally, different preparations of liquorice have shown activity against *Helicobacter pylori in vitro* (Krausse *et al.*, 2004; Asha *et al.*, 2013); in addition, an ethanolic extract of liquorice attenuated either *H. pylori*-induced gastritis or tumorigenesis *in vivo* (Park *et al.*, 2014a).

*Anti-emetic effects*

An ethanolic tincture of *Glycyrrhiza uralensis* Fisch. inhibited heterologously expressed human 5-HT3A receptors responses (5-HT 2.5 µM, approximately $EC_{50}$) at a concentration of 1 Vol.-%, using the two-electrode voltage-clamp technique. A strong inhibition was observed (84.6 ± 1.4%), whilst at lower concentration of 0.1 Vol.-% the inhibitory efect decreased (26.6 ± 4.4%). Inhibition was reversible. Among the single constituents, Glycyrrhizin 1 mM exhibited no modulatory effect (−3.1 ± 4.3%), whereas glabridin 100 µM revealed an inhibition of 62.8 ± 2.1%. Licochalcone A 1 mM and (-)-liquiritigenin 1 mM were the most effective antagonists, showing inhibitions of 70.6 ± 3.1% and 92.8 ± 2.2%, respectively (Herbrechter *et al.*, 2015).

*Anti-viral effects*

An ethanol extract of *Glycyrrhiza uralensis* roots tested at concentration of 200 µg/ml possessed more than 50% suppressing effect on RANTES (regulated on activation, normal T cell expressed and secreted) secretion by $H_1N_1$-infected A549 bronchial epithelial cells. RANTES production, determined at 72 h after virus inoculation, in the presence of 20, 100 and 200 µg/ml of *Glycyrrhiza uralensis* were, respectively, 75.5 ± 11.9, 33.7 ± 3.5 and 3.0 ± 1.8%, as compared with cells infected with $H_1N_1$ alone (Ko *et al.*, 2006).

This antiviral activity was confirmed in colostrums-deprived piglets inoculated with porcine rotavirus K85 (G5P[7]) strain. On the onset of diarrhea, piglets were treated with different concentration of a methanolic extract of dried roots of *G. uralensis* (GUE). To evaluate the antiviral efficacy of GUE, fecal consistency score, fecal virus shedding and histological changes of the small intestine, mRNA expression levels of inflammation-related cytokines (IL8, IL10, IFN-β, IFN-γ and TNF-α), signaling molecules (p38 and JNK), and transcription factor (NFκB) in the small intestine and spleen were determined. Among the dosages (100-400 mg/ml) administrated to animals, 400 mg/ml of GUE cured diarrhea, and markedly improved small intestinal lesion score and fecal virus shedding. mRNA expression levels of IL8, IL10, IFN-β, IFN-, TNF-α, p3, JNK and NFκB in the small intestine and spleen were markedly increased in animals with Group A rotavirus (RVA)-induced diarrhea, but dose-dependently decreased in GUE treated animals after RVA-induced diarrhea (Alfajaro *et al.*, 2012).

Angiotensin converting enzyme 2 (ACE2) is a key entry point of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus known to induce Coronavirus disease 2019 (COVID-19). Male Sprague Dawley rats were left undisturbed or exposed to chronic mild stress for five weeks. For the last two weeks, animals continued with a placebo diet or received a diet containing water extract of *Glycyrrhiza glabra* root at a dose of 150 mg/kg b.w./day. Quantitative PCR measurements showed a significant decrease in gene expression of ACE2 in the small intestine of rats fed with diet containing *Glycyrrhiza glabra* extract. This effect was independent of the stress condition and failed to be observed in non-target tissues, namely the heart and the brain cortex. In the small intestine the reduction of ACE2 at the protein level was also observed (Jezova *et al.*, 2021).

An aqueous extract obtained from dried liquorice roots and glycyrrhizin acid ammonium-nitrate were investigated for their antiviral activity against SARS-CoV-2 *in vitro* using Vero E6 cells. Serial dilutions of liquorice root extract or glycyrrhizin (0.004–4 mg/mL) were pre-incubated with 100 50% tissue culture infective dose ($TCID_{50}$) of SARS-CoV-2 for 1 h at 37°C and subsequently incubated on confluent Vero E6 cells grown in 96-well microtiter plates and analyzed for cytopathic effects (CPE) (combined pre- and post-entry approach). The neutralizing titer was determined as the concentration required for reducing virus-induced CPE by 100%. Moreover, Vero E6 cells were infected with 100 $TCID_{50}$ SARS-CoV-2 for 4 h and subsequently treated with various glycyrrhizin concentrations ranging from 0.002–4 mg/mL. After 2 days of incubation, the cells were analyzed for CPE (post-entry conditions). Aqueous liquorice root extract showed antiviral effects even at a subtoxic concentration of 2 mg/mL. of 0.5 mg/mL (combined pre- and post-entry conditions) or 1 mg/mL (post-entry conditions). The $EC_{50}$ of glycyrrhizin was calculated with 0.44 mg/mL. Glycyrrhizin treatment significantly reduced SARS-CoV-2 RNA levels in cell culture supernatants. Finally, glycyrrhizin completely inhibited protease $M^{pro}$ activity of SARS-CoV-2 at a concentration of 2000 µM (1.6 mg/mL) and reduced its activity by 70.3% at a concentration of 30 µM (0.024 mg/mL) (van de Sand *et al.*, 2021).

*Antimicrobial and antifungal activity*

Liquorice extract either by itself or in combination with $Ca(OH)_2$ had a significant inhibitory effect against *Enterococcus faecalis* compared with that of $Ca(OH)_2$ alone when determined by agar-well diffusion methods (liquorice extract: mean inhibition zone 3.97 ± 0.24 mm; liquorice/$Ca(OH)_2$ mixture: mean 2.56 ± 0.015 mm; $Ca(OH)_2$ mean 0.97 ± 0.017 mm: $P<0.05$), broth microdilution tests (minimal bactericidal concentration of liquorice extract was 12.3 µg/mL) and biofilm susceptibility assays (100% kill with liquorice extract and liquorice/$Ca(OH)_2$ mixture vs approx. $10^6$ CFUs/disc with $Ca(OH)_2$) (Badr *et al.*, 2011).

The antimicrobial activity of aquo-alcoholic extract of *Glycyrrhiza glabra* was assessed against *P. aeruginosa* causing lung infection in Swiss albino mice. Total 18 mice were taken for this study and divided (n = 6 per group) randomly in three groups (control, D1 and D2). Lung infection was given by administration via intratracheal route of 100 mL of a suspension of bacterial cells ($10^5$ CFU/mL). The herbal treatment commenced on the same day of infection and given twice a day at the interval of 2 and 4 h of post-inoculation. The treatment includes oral doses of D1 = 20 and D2 = 80 mg/kg b.w. and continued for 7 days. At the time of commencement of herbal treatment, the bacterial burdens were 3.38 ± 1.2, 3.38 ± 0.2 and 3.32 ± 0.1 $Log_{10}$ CFU/mL for control (infected), D1, and D2, respectively. In the control group the bacterial load increases persistently and reaches upto 4.69 ± 0.001 $Log_{10}$ CFU/mL. D1 have shown significant decline of the bacterial load in blood and reduces by 3.00 ± 0.02 $Log_{10}$ CFU/mL at $7^{th}$ day. D2 have shown a sharp reduction in bacteremia with 2.30 ± 0.02 $Log_{10}$ CFU/mL at day 7. Radiological analysis represents that the control (infected) group exhibited severe soft tissue damage of lungs. The radiological imaging of soft tissue damage showed significant differences between the herbal treated and infected mice. Moreover, the effect of herbal shown to be capable in arresting and reducing the tissue damage. Histopathological results showed more diffuse and patchy accumulation of inflammatory cells within the alveolar space also the infiltrates were noted in all the lung section of infected mice. In treated animal group improved lung histology was seen with the exudates were less seen in D1 dose (20 mg/kg) and disappeared in D2 dose (80 mg/kg) (Chakotiya *et al.*, 2017).

An aqueous extract of *Glycyrrhiza glabra* rhizome showed a MIC 1.56 mg/ml against *Candida albicans* clinical isolates from a 5-month-old South African baby affected by AIDS; conversely, MIC increased to 12.5 mg/ml against *Candida albicans* clinical isolates from an HIV-infected adult patient and against a standard strain (ATCC 10231). Higher antifungal activity was observed with ethanol and ethyl acetate extracts. MICs were 0.52 mg/ml and 2.09 mg/ml against *Candida albicans* clinical isolates from a 5-month-old baby, respectively; MICs were 2.09 mg/ml and 1.03 mg/ml against *Candida albicans* clinical isolates from an adult, respectively; finally, for both the extracts a MIC of 2.09 mg/ml was determined against the standard strain (Motsei *et al.*, 2003).

Different preprations of liquorice have shown activity against several strains of *Mycobacterium tuberculosis* both *in vitro* and *in vivo* (Gupta *et al.* 2008; Gupta *et al.*, 2018; Viswanathan *et al.*, 2018).

*Antioxidant activity*

The antioxidant, free radical-scavenging and immunostimulating effects of a liquorice infusion (LI) were investigated, and its constituents liquiritin and glycyrrhizin. LI in a concentration range of 10–100 µg/ml weakly scavenged DPPH in a dose-dependent manner, and both the compounds (10–200 µg/ml) showed negligible effects. Both LI and glycyrrhizin (10–200 µg/ml) substantially scavenged superoxide radicals. The β-carotene bleaching was inhibited by LI (at 200 µg/ml about 83% of the initial β-carotene after 1 h retained), but liquiritin and glycyrrhizin (5–200 µg/ml) showed no effect. The LI (100–300 µg/ml), liquiritin and glycyrrhizin (at 12 and 25 µg/ml)

exhibited no meaningful activities against hypochlorous acid, and they showed pro-oxidant effects in the myeloperoxidase (MPO)-chlorinating system. Granulocytes and NK (Natural Killer) cells were markedly activated by LI (measured by CD69 expression on cells), whereas the compounds were inactive. The LI substantially stimulated the expression of CD69 on granulocytes in a concentration-independent manner in the range 100–800 µg/ml; LI showed a similar effect on NK cells, but on a lower scale. The LI, liquiritin and glycyrrhizin showed no effects on the lymphocyte cell cycle (Cheel *et al.*, 2010).

A *Glycyrrhiza glabra* root and rhizome aqueous ethanolic extract in microemulsion carrier systems intended for transdermal delivery of incorporated antioxidant actives, flavonoids and polyphenols showed approximately 13-fold higher *ex vivo* antioxidant capacity compared with the liquorice extract solution. Indeed, in the thiobarbituric acid assay on rat liver homogenates, the $IC_{50}$ values of lipid peroxidation for rutin, liquorice extract in microemulsion carrier and liquorice extract were 6.22 µg/ml, 21.74 µg/ml and 287.44 µg/ml, respectively. In the DPPH assay, the scavenging concentration 50 ($SC_{50}$) value for the liquorice extract is 125.32 µg/ml and 109.58 µg/ml for the extract in microemulsion carrierand is 15.53 µg/ml for the standard antioxidant, rutin (Mostafa *et al.*, 2014).

An ethanolic extract from the roots of *G. glabra* with a glycyrrhizin content of 36 mg/g and a polyphenol content of ~91.2 mg expressed as mg equivalents of gallic acid per g of extract, was incorporated in liposomes and hyalurosomes, which have previously shown optimal features for skin delivery. *In vitro* antioxidant activity (DPPH assay) of these preparations was compared to that of both liquorice extract (LQC) and glycyrrhizin (GLZ) solutions. The liquorice extract in ethanol displayed a moderate antioxidant activity (55%). The incorporation of the extract in the vesicles strengthened the antioxidant activity up to 83%. As a comparison, quercetin, one of the most potent natural antioxidants present in numerous plants, was also tested. The antioxidant power of the LQC was ~7% of quercetin inhibitory activity, and increased thanks to the incorporation in the vesicles (~9.5%). By contrast, the antioxidant power of GLZ (in solution) was very low (~6% corresponding to <1% of quercetin inhibitory activity). The ability of the prepared formulations to protect the cells against $H_2O_2$-induced oxidative stress was tested using 3T3 fibroblasts. The viability of $H_2O_2$-stressed cells was ~64%; it increased slightly upon treatment with the LQC in ethanolic solution (~80%) and reached ~98% when the extract was loaded in vesicles. On the other hand, the treatment with GLZ was not able to efficiently counteract the oxidative effect of $H_2O_2$ (no statistical difference with $H_2O_2$-stressed control cells). In addition, the incorporation of the LQC into the vesicular systems promoted the proliferation and migration of 3T3 fibroblasts, favouring the closure of the scratched area. *In vivo* anti-inflammatory tests on mice confirmed the ability of the proposed nanosystems to improve the local efficacy of the extract, favouring the re-epitelization process. (Castangia *et al.*, 2015).

A dry methanol fraction obtained from fractionation of a crude methanolic extract of *G. glabra* L. roots exhibited maximum scavenging activity against DPPH and nitric oxide free radicals and compared to hexane, chloroform, ethyl acetate, and water fractions. Activity was higher than that of standard antioxidant L-ascorbic acid (L-AA) ($IC_{50}$ value of 71.93 ± 4.62 µg/ml vs 35.58 ±0.93 µg/ml for DPPH assay and $IC_{50}$ value of 38.9 ± 3.4 µg/ml vs 72.6 ± 5.6 µg/ml for nitric oxide radical scavenging assay; $p < 0.0001$). At the highest concentration of 800 µg/ml ($p > 0.005$), methanol fraction exhibited scavenging activity lesser than standard compound L-AA (75.5 ± 0.04% of inhibition vs 88.77 ± 0.22%) in superoxide radical scavenging assay. At 800 µg/ml concentration, L-AA and methanol fraction showed significant hydrogen peroxide radical scavenging activity (91.77 ± 3.04 and 70.96 ± 3.04%, respectively; $p < 0.001$). Administration of methanol

fraction also considerably reduced the malondialdehyde produced due to lipid peroxidation in mammalian liver tissues. Moreover, the levels of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione *s* transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) in the oxidative stress induced tissues were refurbished significantly after treatment with plant's methanol fraction (Hejazi *et al.*, 2017).

More recently, the detoxification effect of a water extract of dried roots and stems of liquorice was assessed in KM mice after induction of paraquat (PQ)- induced pulmonary edema and fibrosis. Animals were randomly divided into 6 groups (n = 8) as follows: (1) control group, (2) PQ group, (3) dexamethasone group, (4) liquorice extract (20 mg/kg), (5) liquorice extract (40 mg/kg), and (6) liquorice extract (60 mg/kg). Liquorice extract (20, 40, and 60 mg/kg) groups were treated with liquorice extract (20, 40, and 60 mg/kg/d, i.g.) 1 h before mice received PQ. The dexamethasone (DXMS) group was treated with DXMS (0.5 mg/kg/d, ip) 1 h before mice received PQ. The PQ group, PQ+liquorice extract group, and DXMS group were treated once with an intragastric infusion of PQ solution (40 mg/kg). Pulmonary damages in each group of mice were evaluated by H&E staining. Liquorice extract alleviated pulmonary edema and fibrosis, decrease malondialdehyde (MDA) contents and increase SOD activity in PQ-induced ALI mice, protect the morphologic appearance of lung tissues, induce cytochrome 3A4 (CYA3A4) and nuclear factor erythroid 2-related factor 2 (Nrf2) expression to active detoxification pathways, reduce the accumulation of PQ *in vivo*, protect or improve the liver and renal function of mice, and increase the survival rate (Liu *et al.*, 2019).

*Immunomodulatory effects*

The daily consecutive oral administration of *G. uralensis* decoction at a dosage of 2 g/kg per day reduced the carrageenan-induced decrease in immune complexes (IC) clearance in male C3H/He mice (Matsumoto *et al.*, 1996).

The effect of polysaccharides obtained from an ethanolic extract of *Glycyrrhiza glabra* roots (GGP) on immune and antioxidant activities was studied in high-fat (HF) mice. Forty 10-week-old Kunming mice were randomly divided into four groups: control group, HF group, and polysaccharides-treated (100, 300 mg/kg) groups. Mice of the control group (n = 10) were given a basic diet. Mice of the HF group (n = 10) were given a HF diet. Mice of the polysaccharides' groups were allowed free access to high-fat feed, water and treated by oral infusion with polysaccharides at a dose of 100 mg/kg bw/day dissolved in physiological saline for that same period. Compared with the normal group, the proliferation indexes of spleen lymphocytes of the HF group were significantly decreased ($P < 0.01$). The administration of GGP dose-dependently significantly enhanced proliferation index of spleen lymphocytes in the GGP-treated groups when compared with the HF group ($P < 0.01$). Compared with the normal group, the serum IgA, IgG and IgM levels of the HF group were significantly decreased ($P < 0.01$). The administration of GGP dose-dependently significantly enhanced serum IgA, IgG and IgM levels in the GGP-treated groups when compared with the HF group ($P < 0.01$). Compared with the normal group, the blood MDA levels of the high-fat group were significantly increased ($P < 0.01$). The administration of GGP significantly decreased blood MDA levels in mice when compared with the HF group ($P < 0.01$). Compared with the normal group, the serum TC, TG, and LDL-c levels of the HF group were significantly increased ($P < 0.01$). In addition, the serum HDL-c level of the HF group was significantly reduced ($P < 0.01$). The administration of GGP significantly decreased serum TC, TG, and LDL-c levels in mice when compared with the HF group ($P < 0.01$). The serum HDL-c level of polysaccharides-treated groups was slightly enhanced ($P > 0.01$). Compared with the normal group, the serum SOD, CAT, GSH-Px and total antioxidant capacity (TAOC) activities of the HF group were significantly decreased (P <

0.01). The administration of GGP significantly increased serum SOD, CAT, GSH-Px and TAOC activities in mice when compared with the high-fat group ($P < 0.01$) (Hong YK *et al.*, 2009).

A polysaccharide fraction isolated via ion-exchange chromatography from the water extract significantly enhanced the maturation of dendritic cells. This fraction had a molecular weight of 29100 Da and contained 93.5 ± 0.68% of carbohydrates and 8.7 ± 0.23 of sulphates. Monosaccharyde composition consisted of Rha, Ara, Man, Glc, and Gal at molar ratios of 1:13.87:1.59:16.76:15.72 (Aipire *et al.*, 2020).

A concentrated extract of liquorice (GR; DER 11,25:1, extraction solvent ethanol 95%) was used *in vitro* with primary mouse splenocytes (SPLC) in the condition of anti-CD3/CD28 stimulation and interferon (IFN)-γ-producing $CD4^+$ (TH1)/$CD8^+$ (TC1) polarization as well as IFN-γ-stimulated BV2 cells. For experimental autoimmune encephalomyelitis (EAE) induction, female C57BL/6 mice were immunized with 200 µg of myelin oligodendrocyte glycoprotein (MOG)35-55 without pertussis toxin. EAE SPLC (*ex vivo*) and EAE mice (*in vivo*) were treated with GR extract to evaluate the changes in antigen-specific responses. SPLC media containing antigenspecific responses were used to stimulate BV2 cells. GR extract effectively modulated the responses of reactive splenic T cells through the reduction in IFN-γ+T cell populations at concentration of 50 µg/ml, the expressions of cell adhesion molecules (CAMs) at concentration of 75 µg/ml, and secretions of cytokines containing IFN-γ and a chemokine IFN-γ-induced protein 10 (IP-10) at concentration of 75 µg/ml *in vitro*. In addition, GR extract 50 µg/ml significantly decreased nitric oxide production and secretion of tumor necrosis factor (TNF)-α and IP-10 in IFN-γ-stimulated BV2 cells. The antigen-specific TH1 and TC1 populations were decreased following administration of 100 mg/kg of GR extract, whereas CD8+IL-17A+ (TC17) population was increased on day 36 after EAE induction. Moreover, IFN-γ, which showed the highest secretion among examined cytokines, and IP-10 decreased on day 36. SPLC media derived from 100 mg/kg GR extract-administered EAE mice revealed the ameliorative effects on BV2 cell stimulation (Yang *et al.*, 2019).

*Anti-atherogenic effects*

Sixty apolipoprotein E-deficient ($E^0$) transgenic mice aged 6 wk were divided into three groups. 20 mice in each, and fed the following via their drinking water: 1) placebo, 2% alcohol in water (control group): 2) 200 µg of a liquorice antioxidant alcoholic extract free of glycyrrhizinic acid daily in 2% alcoholized water; or 3) 20 µg purified glabridin/d in 2% alcoholized water. Administration per mouse of 200 µg liquorice/d or 20 µg glabridin/d, for a period of 6 wk resulted in a significant ($P < 0.01$) reduction in the susceptibility of the LDL of the $E^0$ mice to copper ion-induced oxidation as measured by a 68% and 22% inhibition in thiobarbituric acid reactive-substances (TBARS) formation, and by a prolongation of the lag phase by 50 and 35 mm, respectively. Light microscopy revealed histopathologic atherosclerotic lesions in the aortic arch of both groups of mice, although the incidence of the lesions was far greater in the placebo-treated mice (Fuhrman *et al.*, 1997).

*Hepatoprotective effects*

A monolayer culture of primary rat hepatocytes was used as an *in vitro* model to examine the protective effects of a water extract of liquorice and glycyrrhizic acid (GA) against hepatotoxicity of azathioprine. Liquorice (5 g/L or 25 g/L) or GA (0–0.3 g/L) was added 1 h before the addition of 1 µM azathioprine for a further 48 h treatment. It was found that both liquorice and GA showed substantial protection on azathioprine-injured primary rat hepatocytesaccording to assays of cell viability and intracellular GSH, while neither GSH nor N-acetylcysteine (NAC) had such a protective

function. Similarly, GA protected human hepatocytes from intracellular GSH depletion on exposure to 1 microm azathioprine (Wu *et al.*, 2006).

In a pre-clinical study 8–10-week-old ovariectomized female animals were randomized to normal and high fat diets plus various botanical estrogens (BEs) supplemented in the food and monitored over several weeks. The BEs were administered to laboratory animals through pelleted AIN76 - based rodent diet into which the liquorice root components have been incorporated either root powder (LRP), methanolic extracts (LRE) or isolated isoliquiritegenin (ILQ). The dietary level chosen to evaluate biological effects was 5% LRP, which is equivalent to approximately 50 mg/kg bw/d based on consumption of diet equivalent to 10% of mouse bw/d. The LRE diet (0.5% of a 10-fold concentrated extract) was prepared to deliver the same dose of whole root equivalents (50 mg/kg bw/d). The ILQ diet (0.05%) was chosen to deliver a similar dose of purified ILQ (50 mg/kg bw/d). Although LRE and ILQ provided some benefit, LRP was the most effective in reducing body weight gain, overall fat deposition, liver steatosis, and expression of hepatic lipid synthesis genes following ovariectomy (Erdogan *et al.*, 2016).

To evaluate the protective effects of *Glycyrrhiza* polysaccharide (GPS) against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced hepatotoxicity in Jian carp, the fish were fed diets containing GPS at doses of 0.1, 0.5 and 1.0 g/kg for 60 days before an intraperitoneal injection of 0.6 μg/kg TCDD at a volume of 0.05 mL/10 g body weight. At 72 hr post-injection, blood and liver samples were taken for biochemical analysis and the fish liver samples were used for the preparation of pathological slices. The results showed that increases in alanine aminotransferase (GPT), aspartate aminotransferase (GOT), lactate dehydrogenase (LDH), and alkaline phosphatase (AKP) in serum induced by TCDD were significantly inhibited by pre-treatment with 1.0 g/kg GPS. Following the 1.0 g/kg GPS pre-treatment, TP, albumin (Alb), CAT, GSH-Px, TAOC and SOD activities in liver tissue increased significantly, MDA formation ($P < 0.05$ or $P < 0.01$) was significantly inhibited, and the expression of CYP1A, aryl hydrocarbon receptor 2 (AHR2) and aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) mRNA ($P < 0.05$) was significantly enhanced. Histological observations on fish liver were obtained by preparing paraffin tissue sections via HE stains, and the results showed that histological changes were obviously reduced by 0.5 and 1.0 g/kg GPS. GPS significantly reduced liver tissue damage caused by TCDD (Du *et al.*, 2017).

In another study, the protective effects of a liquorice root extract (LE) and magnesium isoglycyrrhizinate (MIG) against triptolide-induced hepatotoxicity, as well as the regulatory effects of LE and MIG on the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, were investigated in male Wistar rats with acute liver injury induced by intragastric administration of triptolide (TP). Forty-two rats were randomly assigned to the following seven groups: (1) control; (2) TP 0.6 mg/kg; (3) MIG 13.5mg/kg + TP; (4) rifampicin (RIF) 50mg/kg + TP; (5) low dose of LE (LLE) (120 mg/kg) + TP; (6) medium dose of LE (MLE) (240 mg/kg) + TP; and (7) high dose of LE (HLE) (480 mg/kg) + TP. Compounds were given via i.g. administration. Rat received MIG, RIF, or LE once daily for 7 consecutive days. TP was administered on the eighth day. The serum activities of AST and ALT in the TP-treated group increased significantly ($p<0.01$) compared with the blank group. Administration of LLE and HLE significantly reduced the elevation in serum AST and ALT activity induced by TP ($p<0.01$). MIG also diminished the increase in serum ALT activity ($p<.05$). TP significantly inhibited the serum activities of SOD ($p<.05$) and GSH-Px ($p<0.01$) and increased the serum level of MDA ($p<0.01$). Conversely, treatment with LLE resulted in a significant increase in the amount of GSH-Px ($p<0.05$) and a significant decrease in the level of MDA ($p<0.01$); HLE showed a significant effect on MDA only ($p<0.05$). Livers of rats in the TP group showed significant histopathological changes, including apparent severe hepatocellular hydropic degeneration and

necrosis. The levels of hepatocellular hydropic degeneration and necrosis were slightly decreased in the MIG + TP, RIF + TP, and LLE + TP groups and were significantly decreased in the MLE + TP and HLE + TP groups. In addition, *in vitro* studies showed that triptolide decreased the mRNA and protein levels of nuclear factor erythroid 2-related factor 2 (Nrf2) and down-regulated Nrf2 target genes, including uridine diphosphate glucuronosyltransferase 1A (UGT1A), bile salt export pump (BSEP), and multidrug resistance-associated protein 2 (MRP2), while pretreatment with LE and MIG reversed these effects (Tan *et al.*, 2018).

Further studies confirmed the hepatoprotective activity of different preparations of liquorice in different models of liver injury *in vivo* (Huo *et al.*, 2011; Wang *et al.*, 2012; Man *et al.*, 2020; Chauhan *et al.*, 2020; Wang *et al.*, 2022).

*Anti-carcinogenic effects*

To determine the effect of liquorice root on mitosis Rafi & Colleagues (2002) measured the effect of liquorice root on the anti-apoptotic protein Bcl-2 and cell cycle in breast tumor cells by immunoblotting. Liquorice root extracted with ethyl acetate, DMSO, or ethanol induced Bcl-2 phosphorylation, as demonstrated by a slower migrating band, in contrast to the vehicle control (ethanol alone) or a water extraction (Rafi *et al.*, 2002).

To analyze the inhibition of trichloromethane, ethyl acetate, 70% methanol, and hexane extracts of *Glycyrrhiza uralensis* Fisch root on the growth of human breast cancer MCF-7 cells, DNA synthesis in the presence of liquorice root was measured. Trichloromethane and ethyl acetate extracts of liquorice root inhibited the proliferation of MCF-7 cells in a dose- and time-dependent manner. After 72 h of treatment, the 50 µg/mL of trichloromethane extract nearly caused a 63% inhibition and the 50 µg/mL of ethyl acetate extract nearly appeared an 83% inhibition of cell growth as compared with control. Also, 70% methanol and hexane extracts of liquorice root inhibited the proliferation of MCF-7 cells in a dose- and time-dependent manner. After 72 h of treatment, at the same dose, this 70% methanol extract caused nearly a 62% inhibition, and the hexane extract appeared nearly an 80% inhibition of cell growth as compared with control. When MCF-7 cells were treated with 50 µg/mL liquorice root extracts for 48 h, cells exhibited typical morphological changes of apoptosis. Flow cytometry showed that cells accumulated in the subG1 phase gradually from 24 to 72 h after treatment with the test compound, whereas the number of cells in G1 phase decreased in the same manner (Jo EH *et al.*, 2004).

The cytotoxicity of the methanol extracts of nine samples of the roots of *G. glabra*, collected from various geographical origins (Italy, Dagestan, Uzbekistan, Afghanistan, Syria and Turkey), exhibited different levels of cytotoxicity against immortal human keratinocyte (HaCaT), lung adenocarcinoma (A549) and liver carcinoma (HepG2) cell lines using the *in vitro* 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT) cell toxicity/viability assay. The HepG2 cells were the least susceptible to the test extracts (8 out of 9 extracts), while the HaCaT cells were the most susceptible to the extracts at the concentration of 250 µg/mL. A commercial sample (selected yellow tip medium width) obtained from Afghanistan was the most active extract and showed considerable cytotoxicity against all three cell lines, particularly against HaCaT cell line ($IC_{50}$ = 158.8 µg/mL); however, a second sample from Afghanistan (unpeeled cut pieces) was only cytotoxic to A549 cell line ($IC_{50}$ = 205.6 µg/mL). A commercial sample obtained from Calabria, Italy was the least cytotoxic against all cell lines, whereas another sample collected from the same geographical origin showed cytotoxicity against A549 and HaCaT cell lines ($IC_{50}$ = 189.1 and 241.9 µg/mL, respectively) (Basar *et al.*, 2015).

Dichloromethane extract from liquorice roots inhibited the proliferation of B16-F10 melanoma cells with an $IC_{50}$ value of 48.7 ± 2.5 µg/mL, whereas water end ethanol extracts exhibited little effects ($IC_{50}$ > 100 µg/mL) (Zheng *et al.*, 2018).

Anti-carcinogenic effects of liquorice have been observed also in several in vivo studies (Sheela *et al.*, 2006; Seon *et al.*, 2012; Park *et al.*, 2016; Huo *et al.*, 2016).

*Spasmolytic activity on smooth muscles*

A 50% ethanolic extract of *Glycyrrhizae uralensis* Fisch prepared by maceration of dried powdered root showed vasorelaxant effect on thoracic aortic rings isolated from SD rats with efficiency concentration of 50% ($EC_{50}$) and maximal relaxation ($R_{max}$) of 0.016 ± 0.007 mg/ml and 116 ± 6.18%, respectively. The extract caused the relaxation of the aortic rings pre-contracted with phenylephrine either in the presence or absence of endothelium and pre-contracted with potassium chloride in endothelium-intact aortic ring. Nω-nitro-L-arginine methyl ester, methylene blue, or 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one inhibited the vasorelaxation effect of the extract in the presence of endothelium. On the other hand, in the presence of the potassium channel blockers (tetraethylammonium and barium chloride), the vasorelaxation effect of the extract was not affected, but glibenclamide and 4-aminopyridine did inhibit the vasorelaxation effect of the extract. With indomethacin, atropine and propranolol, the vasorelaxation effect by the liquorice extract was significantly reduced. The extract was also found to be effective in reducing $Ca^{2+}$ release from sarcoplasmic reticulum and the blocking of calcium channels (Tan *et al.*, 2017).

*Spasmolytic activity on skeletal muscles*

The powdered aqueous extract of *G. radix* (0.5 and 1.0 g/kg, i.d.) significantly inhibited tetanic contractions ($P<0.01$) in an isolated tibial nerve of rat electricaly stimulated. At a higher dose (1.0 g/kg, i.d.), *G. radix* exhibited a stronger inhibition (15–34% of tetanic amplitude), particularly between 20 and 60 min after administration. Among the single constituents of *G. radix*, isoliquiritigenin, liquiritigenin, and glycyrrhetinic acid showed early and prominent inhibitory effects on the tetanic contractions during the initial 30 min, followed by significant and longer lasting inhibitory effects of liquiritin apioside and glycycoumarin. Glycyrrhetinic acid demonstrated significant inhibition throughout the entire test period. Liquiritin alone showed a sporadic inhibitory effect 60 min after administration (Lee *et al.*, 2013a).

*Other effects on the skeletal muscles*

A crude water extract (CWE) of *G. uralensis* at concentrations of 50 and 100 µg/mL enhanced proliferation and differentiation of myoblast C2C12 cells cultured in growth media for one day (Lee *et al.*, 2021).

A dose of 1 or 1.5 g/kg b.w. of a concentrated liquorice flavonoid oil (LFO) solution containing 30% liquorice ethanolic extract and 70% medium chain tryglycerides (MCT) enhanced muscle mass when administered by mouth once daily for 4 weeks to male genetically type II diabetic KK-$A^y$/Ta mice (Yoshioka *et al.*, 2018).

*Anti-allergic effect*

A dried decoction of *Glycyrrhiza glabra* root (LE) inhibited calcium ionophore-stimulated histamine release from mast cells in a dose-dependent manner (reduction of released histamine by the LE of 500 µg/ml: 19.5%, $p = 0.04$; 1000 µg/ml: 32.6%, $p = 0.01$; 1500 µg/ ml: 37.2%, $p < 0.01$). Liquorice suspension (1, 3, and 5 mg/ml) inhibited the dilatation of blood vessels in the ear of Balb/c mice in response to ovalbumin-induced local anaphylaxis, decreasing the degree of blue

hyperpigmentation in the ear. The absorption of Evans blue in the left ear subcutaneously injected with the liquorice suspension was significantly reduced in a dose-dependent manner (reduction of Evans blue extravasation by the liquorice suspension of 1 mg/ml: 42.6%, $p < 0.01$; 3 mg/ml: 55.1%, $p < 0.001$; 5 mg/ml: 62.0%, $p < 0.001$) (Chang *et al.*, 2021).

*Anti-diabetic effects*

*Glycyrrhiza uralensis* Fisch. showed a concentration –dependent inhibitory effect on α-glucosidase by a spectrophotometric method with percentages of inhibition ranging from 12.4 ± 3.7% (5.0 mg/ml) to 67.4 ± 4.6% (40 mg/ml). The $IC_{50}$ was 20.1 mg/ml (Li *et al.*, 2010a).

Another study investigated whether liquorice extracts inhibited mesangial cell (MC) proliferation and matrix accumulation induced by high glucose (HG). Human renal MC were cultured in media containing 5.5 mM glucose plus 27.5 mM mannitol as an osmotic control or 33 mM glucose for 3 days in the presence of water or ethanol extracts from raw liquorice (LW, LE) or roasted liquorice (RLW, RLE). Non-polar components including glycyrrhetic acid were elevated during liquorice roasting, whereas polar components soluble in water extracts were diminished. Exposure of cells to HG caused significant increases in collagen IV secretion and connective tissue growth factor (CTGF) expression, which was appeased by RLW and RLE at transcriptional levels. The inhibitory potency was high in the order of RLE > or = RLW > or = LE > > LW. Non-polar glycyrrhetic acid but not glycyrrhizin retarded HG-stimulated mesangial matrix deposition through diminishing CTGF expression. In addition, RLW and RLE but not LW modulated membrane type matrix metalloproteinase-1 (MT-1 MMP) expression, MMP-2 activity and tissue inhibitor of MMP-2 (TIMP-2), which facilitated the degradation of mesangial matrix. Furthermore, the augmented expression of CTGF and TIMP-2 in HG-exposed cells was mediated by Akt activation and TGF-β/Smad signaling through PKCβ2-responsive signaling pathways. However, HG-down-regulated MT-1 MMP expression was independent of activation of ERK1/2 and Akt (Li *et al.*, 2010b).

The ethanol extract of *Glycyrrhiza uralensis* Fisch., radix and rhizoma, dried was studied at 200 μg/mL was discovered to significantly reduce glucose transport across the Caco-2 cell monolayer by 43.7±0.5%. When the initial glucose concentration on the apical side was 25 mM (the simulated fed state), treatment with the aforementioned ethanol extract reduced glucose transport across the cell monolayer by 31.8±5.0%. However, treatment with the crude polysaccharide extract did not have a potent inhibitory effect on glucose transport (Wang *et al.*, 2018).

A dry liquorice extract at a dose of 20, 40, and 80 mg/kg/day improved the levels of fasting blood glucose, insulin resistance, serum lipids, and endotoxemia-related colonic inflammation in diabetic mice in a dose-dependent manner when administered orally. Western blots also suggested that a high-dose liquorice extract could effectively decrease the levels of nuclear factor kappa-B (NF-κB), toll-like receptor 4 (TLR4), and TNF-α in colon of diabetic mice. More importantly, all the doses of liquorice extract reshaped the gut microbiota by decreasing the contents of *Lachnospiraceae_NK4A136_group* at the genus level and increasing the contents of *Alloprevotella*, *Bacteroides*, and *Akkermansia*, especially for the high dose of liquorice extract. These results indicated that the anti-diabetic effect of liquorice extract might be attributed to the regulation of the gut microbiota and the colon TLR4/NF-κB signaling pathway in diabetic mice (Zhang *et al.*, 2022).

*Anti-obesity effects*

The hypocholesterolaemic and antioxidant effects of *Glycyrrhiza glabra* (GG) root powder were examined in hypercholesterolaemic male albino rats. The root powder was extracted in petroleum

ether to remove fat and subjected to acid and alkaline treatment. A 4-week administration of GG root powder (5 and 10 gm% in diet) to hypercholesterolaemic rats resulted in significant reduction in plasma, hepatic total lipids, cholesterol, TG and plasma LDL and VLDL-cholesterol accompanied by significant increases in HDL-cholesterol levels. Furthermore, significant increases in fecal cholesterol, neutral sterols and bile acid excretion along with an increase in hepatic HMGCoA reductase activity and bile acid production were observed in these animals. The root powder administration to hypercholesterolaemic rats also decreased hepatic lipid peroxidation with a concomitant increase in SOD and CAT activities and total ascorbic acid content. The normo-cholesterolaemic animals when fed with GG root powder at 10 gm% level, registered a significant decline in plasma lipid profiles and an increase in HDL-cholesterol content. The antioxidant status of these animals also was improved upon treatment (Visavadiya & Narasimhacharya, 2006).

In adipocytes, the expression of cannabinoid receptors type 1 (CB1R) increases during differentiation, and CB1R stimulation accelerates the differentiation of pre-adipocytes through transcriptional activation of peroxisome proliferator-activated receptor gamma (PPAR-γ). An *in vitro* study was carried out to investigate the inhibitory activity of an ethanol/water (30:70, v/v) extract of liquorice root (0.1, 1, 3, 10, 30, and 100 µg/mL) and its main ingredients in human CB1R-expressing Chem-1 cells, determining wheter they may mitigate the effects of anandamide (AEA), an endogenous cannabinoid receptor ligand, on CB1R signaling. AEA activated $Ca^{2+}$ flux with an $EC_{50}$ value of 0.91 ± 0.08 µM in CB1R-expressing Chem-1 cells; extract of liquorice roots inhibited the $Ca^{2+}$ flux in a concentration-dependent manner, with $IC_{50}$ value of 9.17 ± 1.62 µg/mL against 3 µM AEA. The supplementation of 18β-GA 30 mg/kg, per oral once a day for 8 weeks, significantly lowered body weight, fat weight, and plasma lipids levels in obese male C57BL/6J mice (Park M *et al.*, 2014b).

A study investigated the *in vivo* effects of MCT-coconut oil (MCO) and its combination with a dry aqueous extract of *Glycyrrhiza uralensis* roots (LE-MCO) on serum lipid profile, hepatic steatosis, and local fat pad proteins in diet-induced obese C57BL/6J mice. Each group of HFD mice were administrated for 12 weeks with vehicle (HFD-alone) and 37.5 mg/kg liquorice aqueous extract (LE), 337.6 µl/kg MCO, or 337.6 µl/kg MCO containing 37.5 mg/kg LE (LE-MCO) daily via gavage. No liver toxicity was observed in 45% fat diet (HFD)-fed mice orally treated with LE, MCO, and LE-MCO. Their supplementation reduced HFD-enhanced body weight, blood glucose, and insulin in mice. Plasma levels of both phospholipid transfer protein (PLTP) and lecithin–cholesterol acyltransferase (LCAT) were boosted in LE-MCO-administered mice. Supplementation of LE-MCO diminished plasma levels of TG and TC with concomitant reduction of the LDL-C level and tended to raise blood HDL-C level compared to that of HFD alone-mice. Treatment of LE-MCO encumbered the hepatic induction of hepatosteatosis-related proteins of sterol regulatory element-binding protein-(SREBP)2, (SREBP)1c, fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), and fatty acid translocase (CD36) in HFD-fed mice. Substantial suppression of this induction was also observed in the liver of mice treated with MCO. Oral administration of LE-MCO to HFD mice boosted hepatic activation of 5'-adenosine monophosphate activated protein kinase (AMPK) and the induction of uncoupling protein 1 (UCP-1) and fatty acid transport protein 1 (FATP1) in brown fat. Conversely, LE-MCO disturbed hepatic PPAR-liver X receptor (LXR)-retinoid X receptor (RXR) signaling in HFD-fed animals and reversed HFD-elevated epididymal PPARγ (Lee *et al*., 2018).

*Effects on lipid and glucose metabolism*

Compared to mice fed the control diet, mice supplemented with long-term administration of a glavonoid-rich oil (GRO) derived from ethanol extraction of liquorice (*Glycyrrhiza glabra Linne*) root (0.3% or 0.8% GRO (w/w) for 4–12 weeks) had reduced body and white adipose tissue weights,

serum levels of TG and cholesterol, and improved glucose tolerance, while food intake was not affected. Reductions in the gene expression levels of stearoyl-coenzyme A desaturase 1 (Scd1) and pyruvate dehydrogenase kinase isoenzyme 4 (Pdk4) in the liver, in addition to decreased expression of FAS in inguinal white adipose tissue (iWAT) were observed (Igarashi *et al.*, 2021).

*Cardioprotective effects*

A dry aqueous extract of *Glycyrrhiza glabra* (*Gg*) root (20 $\mu$g/ml to 200 $\mu$g/ml) alleviated the cardiotoxicity of doxorubicin (DOX 5 µM) when evaluated *in vitro* using H9c2 cardiomyocytes increasing the survival rate by 24%. The Gg extracts maintained the membrane integrity and improved the lipid homeostasis and stabilized cytoskeletal element actin. Re-treatment of cells with Gg extract at a dose of 40 $\mu$g/ml significantly reduced the level of total reactive oxygen species (ROS) in H9c2 cardiomyocytes when compared with DOX treatment (252 ± 31 vs 348.43 ± 71.55) (Upadhyay *et al.*, 2020).

*Anti-depressive effects*

The effects of chloroform water (0.1%) extract of powdered root of *Glycyrrhiza glabra* L. on depression was evaluated in mice using forced swim test (FST) and tail suspension test (TST). 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 i.p.) and fluoxetine (20 mg/kg i.p.). Liquorice extracts reversed reserpine-induced extension of immobility period of mice in FST and TST. Sulpiride (50 mg/kg i.p.; a selective $D_2$ receptor antagonist) and prazosin (62.5 µg/kg i.p.; an $\alpha_1$-adrenoceptor antagonist) significantly attenuated the extract-induced antidepressant-like effect in TST. On the other hand, p-chlorophenylalanine (100 mg/kg i.p.; an inhibitor of serotonin synthesis) did not reverse antidepressant-like effect of liquorice extract (Dhingra & Sharma, 2006).

*Nephroprotective effects*

Epithelial-to-mesenchymal transition (EMT) of renal proximal tubular cells plays a crucial role in tubulointerstitial fibrosis. Herein, an *in vitro* study was carried out to elucidate the detailed mechanism of EMT in renal proximal tubular epithelial cells (NRK-52E) under high glucose (HG) conditions, and to investigate the potential of liquorice to inhibit HG-induced EMT. A methanolic dry extract of liquorice root powder (LE) and a de-glycyrrhizinated extract (LE/GC-KO) were tested. To prepare glycyrrhizin (GC)-knockout liquorice extract, the dry methanol extract was dissolved in loading buffer (5% methanol) and then applied to an immunoaffinity column that was conjugated with the anti-GC monoclonal antibody. Cells exposed to HG resulted in EMT induction characterized by increased fibronectin and α-SMA (alpha-smooth muscle actin) but decreased E-cadherin. Elevated levels of cleaved Notch2 were also concomitantly detected in HG-cultured cells. LE and LE/GC-KO treatments substantially inhibited HG-induced changes in α-SMA, fibronectin and E-cadherin in a dose-dependent manner, with a minimum effective concentration of 100 ng/ml. Noteworthily, increased levels of Notch intracellular domain 2 (NICD2) in HG-cultured NRK-52E cells were also remarkably reduced by both LE (110 ng/ml) and LE/GC-KO (100 ng/ml), suggesting that Notch2 signaling activation was positively associated with HG-induced EMT. On the other hand, GC exhibited only weak or moderate anti-EMT activity even at high concentrations (100 ng/mL and 300 ng/mL) (Hsu *et al.*, 2020).

The protective effects of a dry hydroalcoholic (70% methanol) *Glycyrrhiza glabra* rhizome extract (GGE) on methotrexate (MTX)-induced hepato-renal damage was investigated in Wistar albino rats. Rats were pre-treated with GGE (100, 200 or 400 mg/kg) from day 1 to 15 and administered MTX (20 mg/kg) on day 4. Treatment with MTX (20 mg/kg) alone induced a significant ($p < 0.001$) increase in weight of kidney, whereas co-treatment with GGE (100, 200 or 400 mg/kg) dose-dependently reversed this effect ($p < 0.001$). MTX induced renal damage resulted in elevated serum levels of blood urea nitrogen (BUN) and creatinine, increased pro-inflammatory cytokines concentration and oxidative stress. Conversely, co-treatment with GGE dose-dependently ameliorated oxidative stress, serum interleukins, renal toxicity biomarkers ($p < 0.001$), and downregulated both caspase-3 and NFkB expression in renal tissue. Renal tissue from MTX-group rats exhibited distortion of basement membranes, dilatation of the peritubular region, tubular degeneration and vascular congestion of red blood cells. Co-treatment with GGE dose-dependently improved these cyto-architectural changes and decreased peritubular dilatation, haemorrhage and congestion (Chauhan *et al.*, 2020).

*Neuroprotective effects*

A methanolic extract of liquorice roots inhibited monoamine oxidase B (MAO-B) with an $IC_{50} < 0.07$ mg/ml, as determined by an enzyme microarray format (Mazzio *et al.*, 2013).

The protective effects of *G. glabra* root water extracts were investigated on HypoE22 cells and isolated rat striatum specimens challenged with 6-hydroxydopamine (6-OH-DA). The extract effects against LDH, nitrites, and 8-iso-prostaglandin(PG)F2α were evaluated using either single-extract treatments or a treatment with a pharmacological association. The liquorice extract at a concentration of 10 µg/ml significantly reduced the LDH release induced by 6-OH-DA on Hypo-E22 cells, whereas it had any significant effect in isolated rat striatum specimens. Liquorice significantly lowered 6-OH-DA-induced nitrite, 8-iso-prostaglandin(PG)F2α levels and dihydroxyphenilacetic acid/dopamine (DOPAC/DA) ratio in isolated rat striatum specimens (Orlando *et al.*, 2019).

The neuroprotective mechanism of the Indian traditional medicine Yashtimadhu, prepared from the dried roots of *Glycyrrhiza glabra* L. was studied in the rotenone-induced cellular model of PD. Retinoic acid–differentiated IMR-32 cells were treated with rotenone (PD model) and Yashtimadhu aqueous extract. The co-treatment of Yashtimadhu at 200 µg/ml with $IC_{50}$ of rotenone at 100 nM prevented cell death; indeed, Yashtimadhu co-treatment reduced the activation of cleaved caspase-3, expression of apoptosis initiation factor (AIF), and decreased ERK-1/2 hyper-phosphorylation. Alteration in the citric acid cycle is well reported in PD. The metabolites such as α-ketoglutarate and succinate were significantly reduced with rotenone treatment and eventually restored by Yashtimadhu co-treatment. Citrate, isocitrate, fumarate, and malate showed reduced levels with rotenone treatment compared to control and were not restored by the Yashtimadhu treatment. The dysregulation of the citric acid cycle by rotenone-induced energetic stress via dysregulation of the mTORC1-AMPK1 axis was prevented by Yashtimadhu. Yashtimadhu co-treatment restored rotenone-induced ATG7-dependent autophagy and eventually caspasesmediated cell death (Karthikkeyan *et al.*, 2022).

The same group of authors, using the same the rotenone-induced cellular model of PD, showed that the aqueous extract of Yashtimadhu at concentration of 200 µg/ml conferred protection against rotenone-induced cytotoxicity, countered cell death, reduced expression of pro-apoptotic proteins such as cleaved-caspases-9, and 3, cleaved-Poly-ADP-ribose-polymerase (PARP), BCL2-associated-X protein (BAX), BCL2-antagonist/killer (BAK) and increased BCL-2. Rotenone-induced cell cycle re-entry (G2/M transition), was negated by Yashtimadhu and was confirmed with

proliferating cell nuclear antigen (PCNA) levels. Yashtimadhu countered rotenone-mediated activation of mitochondrial proteins involved in oxidative stress, cytochrome-C, pyruvate dehydrogenase-E1 alpha-1 (PDHA1), and HSP60. Inhibition of rotenone-induced ERK-1/2 hyperphosphorylation prevented activation of apoptosis, which was confirmed with MEK-inhibitor, highlighted the action of Yashtimadhu via ERK-1/2 modulation (Karthikkeyan *et al.*, 2021).

Spinocerebellar ataxia (SCA) types 1, 2, 3, 6, 7, and 17 and dentatorubropallidoluysian atrophy, as well as Huntington disease, are a group of neurodegenerative disorders caused by a CAG triplet-repeat expansion encoding a long polyglutamine (polyQ) tract in the respective mutant proteins. The cytoplasmic and nuclear aggregate formation, a pathological hallmark of polyQ diseases, is probably the initial process triggering the subsequent pathological events. Compromised oxidative stress defense capacity and mitochondrial dysfunction have emerged as contributing factors to the pathogenesis of polyQ diseases. The aggregate-inhibitory effect of *Glycyrrhiza inflata* herb water extract and its constituents licochalcone A and ammonium glycyrrhizinate (AMGZ) was studied *in vitro* in both 293 and SH-SY5Y ATXN3/$Q_{75}$ cells, SCA3 cell models. The reporter assay showed that *G. inflata* herb extract 3–15 mg/ml, licochalcone A 1.2–3 μM, and AMGZ 160–400 μM could enhance the promoter activity of PPARγ, coactivator 1α (PPARGC1A), a known regulator of mitochondrial biogenesis and antioxidative response genes. *G. inflata* extract 50 μg/ml, licochalcone A 10 nM, and AMGZ 1 μM upregulated PPARGC1A expression and its downstream target genes, SOD2 and CYCS, in the 293 ATXN3/$Q_{75}$ cell model. The expression of nuclear factor erythroid 2-related factor 2 (NFE2L2), the principal transcription factor that binds to antioxidant- responsive elements (AREs) to promote ARE-dependent gene expression when the cells respond to oxidative stress, and its downstream genes, HMOX1, NQO1, GCLC, and GSTP1, was also increased by *G. inflata* herb extract, licochalcone A, and AMGZ. Knockdown of PPARGC1A increased aggregates in ATXN3/$Q_{75}$ cells and also attenuated the aggregate-inhibiting effect of the tested compounds. *G. inflata* extract and its constituents significantly elevated GSH/GSSG ratio and reduced reactive oxidative species in ATXN3/$Q_{75}$ cells (Chen *et al.*, 2014b).

A study investigated an 95% ethanol extract from liquorice roots (GR), for possible neuroprotective effects on neurotoxicity induced by amyloid β protein (Aβ) (25-35) in cultured rat cortical neurons. Exposure of cultured cortical neurons to 10 μM Aβ (25-35) for 36 h induced neuronal apoptotic death. GR (10-50 μg/mL) dose-dependently prevented the Aβ (25-35)-induced neuronal apoptotic death, as assessed by a MTT assay and Hoechst 33342 staining. Furthermore, GR decreased the expression of Bax and active caspase-3, proapoptotic proteins, and increased Bcl-2. GR also significantly inhibited Aβ (25-35)-induced elevation of the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]i$) and generation of ROS measured by fluorescent dyes. Isoliquiritigenin (1-20 μM), isolated from GR as an active component, inhibited Aβ (25-35)-induced neuronal apoptotic death, elevation of $[Ca^{2+}]_i$, ROS generation, and the change of apoptosis-associated proteins in cultured cortical neurons, suggesting that the neuroprotective effect of GR may be, at least partly, attributable to this compound (Lee *et al.*, 2012).

Further *in vitro* studies have shown that liquorice could protect against the neurotoxicity of Aβ oligomers (Kanno *et al.*, 2013) and inhibit the aggregation of misfolded Aβ (Chiu *et al.*, 2018).

*In vivo*, the effects of *G. glabra* on learning and memory were evaluated by the elevated plus-maze and passive avoidance paradigm. Three doses (75, 150, and 300 mg/kg p.o.) of aqueous extract of *G. glabra* were administered for 7 successive days in separate groups of mice. The dose of 150 mg/kg of the aqueous extract of liquorice significantly improved learning and memory of mice, whilst no improvementy was observed with the other doses. Furthermore, the dose of 150 mg/kg

reversed the amnesia induced by diazepam (1 mg/kg i.p.), scopolamine (0.4 mg/kg i.p.), and ethanol (1 g/kg i.p.) (Dhingra *et al.*, 2004).

The neuroprotective effect of raw and roasted liquorice was evaluated in term of the LDH release using PC12 cells after hypoxia in an *in vitro* study and after transient forebrain ischemia in an *in vivo* study on Mongolian gerbils. Raw and roasted liquorice significantly reduced LDH release from PC12 cells exposed to a hypoxic chamber for 1 h. In the raw liquorice-treated group, LDH release was decreased by 9%–33% at concentrations of 10– 1000 μmol/L, while in the roasted liquorice-treated group, LDH release was decreased by 17%–49% at concentrations of 10–1000 μmol/L. In roasted liquorice-treated animals (50 and 100 mg/kg), approximately 66%–71% of CA1 pyramidal cells in the ischemic hippocampus were stained with cresyl violet compared to the control group. However, in the raw liquorice-treated animals, no significant neuroprotection against ischemic damage was shown. In addition, ischemic animals in roasted liquorice-treated group maintained the Cu, Zn- SOD1 activity and protein levels compared to the control group, while in raw liquorice-treated group SOD1 activity and protein levels were reduced significantly. HPLC analysis showed that non-polar compounds containing glycyrrhizin-degraded products, such as glycyrrhetinic acid (GA) and glycyrrhetinic acid monoglucuronide (GM), were increased in roasted liquorice (Hwang *et al.*, 2006).

Duan & colleagues (2022) evaluated the detoxification of a water extract of liquorice (LWE) and its three active monomers – glycyrrhizic acid (GA), liquiritigenin (LIQ), isoliquiritigenin (ISL) on the acute toxicity induced by *Semen Strychni* (STR) in rats. LWE was orally administrated at 6 g/kg/day, whilst GA/ LIQ/ISL at 50 mg/kg/day following an i.p. injection of 0.2 g/kg/day STR. During the one-week injection cycle, there was an increased level in LWE group as well as liquorice active components groups (GA, LIQ, ISL group) compared with STR group in motor coordination assessment (beam walking test). In addition, H&E staining of rat hippocampus showed liquefactive necrosis and karyopyknosis of neurons in the STR group but not in the LWE and monomer detoxification groups. Among the three monomers of liquorice, the effect of liquiritigenin was relatively weak. According to the TUNEL staining, LWE and active components groups showed a very low apoptotic fraction of hippocampal neurons compared to STR. Furthermore, treatment with LWE and monomers could also reduce the level of Bax, a pro-apoptotic factor ($p < 0.05$) and MDA content which were significantly increased in the STR group ($p < 0.05$). Among the monomer groups, ISL showed the strongest effect on reducing oxidative stress and neuronal apoptosis induced by *Semen Strychni*. Differently from LWE, both LIQ and ISL alleviated the increase caused by STR of the TNF-α and IL-6 to a certain extent ($p < 0.05$). GA showed the strongest effect on decreasing TNF-α level ($p < 0.01$), but it had no significant effect on the reduction of IL-6. According to the ELISA result, there was a robust increase in serum concentration of neuron-specific enolase (NSE), a specific and sensitive biomarker of neuronal damage, in the STR group compared with the control group ($p < 0.01$), and treatment with liquorice and monomers reduced this effect significantly ($p < 0.05$). LWE and its three constituents caused a significant increase in nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) levels compared with the STR group ($p < 0.001$). The nucleus-to-cytoplasm translocation of the high mobility group box 1 (HMGB1) was increased in the STR group, but it was inhibited by LWE and the active components. GA could inhibit the translocation of HMGB1 effectively ($p < 0.05$) (Duan *et al.*, 2022).

The protective effect of liquorice was further studied *in vivo* on copper-induced neurotoxic and genotoxic effect (Mostafa *et al.*, 2017).

*Osteoprotective effect*

A roasted liquorice ethanolic extract (rLE) at concentrations in the range 2.5–10 mg/mL inhibited expression and secretion of receptor activator of nuclear factor kB ligand (RANKL) as well as the mRNA and protein expression of cyclooxygenase-2 in osteoblastic cells exposed to the conditioned medium of breast cancer cells (prepared by sequential incubation of MDA-MB-231 human metastatic breast cancer cells with DMEM/F-12 (1:1) supplemented with 10% FBS and with serum-free DMEM/F-12 (1:1)). rLE at concentrations in the range 5.0–7.5 mg/mL dramatically inhibited RANKL-induced osteoclastogenesis in murine bone marrow-derived macrophages (BMMs), thereby reducing osteoclast-mediated pit formation. The activity of rLE on breast cancer-mediated bone destruction was also evaluated *in vivo*. The subcutaneous injection of MDA-MB-231 metastatic human breast cancer cells into the tibia of mice induced serious bone destruction. Twenty-four hours later, rLE at 0.5, 1, or 2 mg/kg b.w. was administrated by oral gavage once per day for five consecutive days, followed by further administration once weekly for 44 days. rLE substantially blocked tumor growth and bone destruction. Serum levels of tartrate-resistant acid phosphatase and C-terminal cross-linking telopeptide of type I collagen and trabecular bone morphometric parameters were reversed to almost the same levels as the control mice by the rLE treatment (Lee *et al.*, 2013b).

*Effects on healing of wounds*

The effect on wound healing of microcapsules containing *Glycyrrhiza* soluble polysaccharide (GP) was evaluated using a rat trauma model. The dorsal skin of rats was excised in the forming of 2.0 cm × 2.0 cm. Forty SD rats were randomly divided into four groups with 10 rats in each group. The wounds of rats were treated with four combinations including Model group (only with sterile collagen sponge), Negative group (only with sterile gauze), Ferulic acid group (collagen sponge loading with 12.5 μg Ferulic acid microcapsule), and polysaccharide microcapsule group (collagen sponge loading with 72 μg GP in microcapsule). All treatment groups were visually inspected every 5 days for the wound healing of rats. The results showed that after the administration of GP combined with microcapsules, the content of hydroxyproline in granulation tissue increased, the proliferation of capillaries and fibroblasts in granulation tissue became active, and the number of microvessels in wound increased. The formation density of collagen fibers was uniform and orderly. GP combined with microcapsules could activate the expression of phospho-signal transducer and activator of transcription 3 (p-STAT3) and vascular endothelial growth factor (VEGF) proteins and up-regulate the transcription level of VEGF mRNA and miRNA-21 genes. Furthermore, GP combined with microcapsules could accelerate wound healing and promote neovascularization (Hao *et al.*, 2020).

### 3.1.3. Safety pharmacology

No data available.

### 3.1.4. Pharmacodynamic interactions

No data available.

### 3.1.5. Conclusions

Liquorice roots have been traditional used to treat digestive complaints. Pre-clinical studies have shown that different liquorice preparations and isolated compounds can affect gastro-intestinal motility, although with opposite effects depending on the tested concentrations.

The expectorant activity of liquorice has been investigated in several animal studies. *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 & Morris 1991; *Glycyrrhiza glabra* monograph in Alternative Medicine Review 2005). Saponins in *Glycyrrhiza* could contribute to the expectorant effect, although the exact mechanism of action is not clear (Hoffmann, 2003).

## *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 the rat, plasma levels of glycyrrhizin after oral administration at doses of 40, 80, and 120 mg/kg bw or at the proportional dosages of liquorice extract (523, 1046, 1569 mg/kg/bw) were lower than the limit of sensivity of HPLC (0.2 µg/ml). However, it was possible to determine glycyrrhizin concentration in the plasma of the rats treated with 160 mg/kg/bw while, on the contrary, glycyrrhizin concentrations were below or near to the limit of sensitivity (0.2 µg/ml) in the samples treated with a corresponding dose of liquorice extract of 2092 mg/kg/bw, that contains the same amount of glycyrrhizin as in the pure compound. Following the administration of a single dose of glycyrrhizin 480 mg/kg/bw and of 6276 mg/kg/bw liquorice extract the $AUC_{36h}$ were 53.4 ± 6.3 µgxhr/ml and 20.9 ± 3.2 µgxhr/ml, respectively; $C_{max}$ were 4.8 ± 0.5 µg/ml and 1.5 ± 0.2 µg/ml, respectively. The time required for a maximum concentration ($T_{max}$) of glycyrrhizin was 12 hr after administration of liquorice extract, while glycyrrhizin reaches $T_{max}$ at 10 hr after administration of glycyrrhizin alone. If compared with administered dosage, the glycyrrhizin and glycyrrhizic acid excreted is very low reflecting the low plasma concentration, but both glycyrrhizin and glycyrrhizic acid excretions are significantly higher in animals treated with glycyrrhizin alone than in those treated with liquorice extract. (Cantelli-Forti *et al.*, 1994).

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. 18β-Glycyrrhetinic 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 18β-glycyrrhetinic acid (Asl & Hosseinzadeh, 2008).

Intravenously administered glycyrrhizin is metabolized in the rat liver by lysosomal β-D-glucuronidase to 3-mono-glucuronide 18β-glycyrrhetinic acid. This metabolite is excreted with bile into the intestine, where it is metabolized by bacteria into glycyrrhetinic acid, which can be reabsorbed (Asl & Hosseinzadeh, 2008).

A further study compared the PK behaviors of a water extract of liquorice and nine single compounds in male SD rats. The results indicated that interactions among liquorice compounds altered their PK behaviors in 4 aspects: improvement in bioavailability for aglycones (133- and 109-fold increase for liquiritigenin and isoliquiritigenin, respectively), prolongation in system circulation for glycosides (0.3 h delay in $T_{max}$ for liquiritin apioside and isoliquiritin apioside),

decrease of potential toxicity for saponins such as glycyrrhizic acid, and shift in plasma distribution for phase II metabolites (Qiao *et al.*, 2012).

18β-Glycyrrhetinic acid is able to cross the placental barrier and can be detected in the rat foetuses. In one study, dams were fed 100 mg 18β-glycyrrhetinic acid/kg/day commencing on the 13$^{th}$ day of gestation. On the 17$^{th}$, 19$^{th}$ and 21$^{st}$ days of gestation the maternal plasma 18β-glycyrrhetinic acid concentrations were approximately 100 µg/ml, whereas the foetal concentrations were 5, 18 and 32 µg/ml, respectively (Isbrucker & Burdock, 2006).

*Drug interactions*

The extracts of *Glycyrrhiza glabra* L., *Glycyrrhiza uralensis* Fish. ex DC. and *Glycyrrhiza inflata* Batalin were tested for inhibition of 9 cytochrome P450 enzymes using a UHPLC-MS/MS cocktail assay. The extracts were prepared by maceration at room temperature of root powders from each species with a solvent mixture consisting of ethanol, isopropanol and water (90:5:5, v/v/v) and a plant powder/volume of solvent ratio of 1:15. *G. glabra* showed moderate inhibitory effects against CYP2B6, CYP2C8, CYP2C9, and CYP2C19, and weak inhibition against CYP3A4. In contrast, *G. uralensis* strongly inhibited CYP2B6 and moderately inhibited CYP2C8, CYP2C9 and CYP2C19, and *G. inflata* strongly inhibited CYP2C enzymes and moderately inhibited CYP1A2, CYP2B6, CYP2D6, and CYP3A4. None of the three species inhibited CYP2E1 and 2A6. (Li *et al*., 2017).

Inhibition assays by the fluorescent product formation method showed that a dried lyophilized hydro-alcoholic extract of *G. glabra* containing glycyrrhizin 6.51% (w/w) possessed higher $IC_{50}$ values than their positive inhibitors, ketoconazole and quinidine for CYP3A4 (129.47 ± 2.41 µg/ml vs 4.54 ± 1.34 µg/ml) and CYP2D6 (125.16 ± 0.88 µg/ml vs 2.23 ± 0.79 µg/ml), respectively. Glycyrrhizin indicated a less interaction potential with CYP3A4 and CYP2D6 than the extract ($IC_{50}$ = 172.33 ± 1.92 µg/ml and 153.38 ± 1.98 µg/ml, respectively) (Pandit *et al.*, 2011).

Prolonged intake of high liquorice extract or glycyrrhizin doses may result in accelerated metabolism of co-administered drugs. Daily oral doses of liquorice extract (LE, 3138 or 6276 mg/kg body weight per os) or glycyrrhizin (240 or 480 mg/kg body weight per os) for 1, 4 or 10 consecutive days in mice, were able to induce significantly hepatic CYP3A- and, to a lesser extent, 2B1- and 1A2-dependent activities, as well as 6-β- (mainly associated to CYP3A), 2-α-, 6-α-(CYP2A1, 2B1), 7-α-, 16-α-(CYP2B9) and 16-β-testosterone hydroxylase activities. (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 bw per os) or its natural constituent glycyrrhizin (240 or 480 mg/kg bw per os) on SD 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 6β- (CYP3A1/2, CYP1A1/2), 7α- (CYP1A1/2, CYP2A1), 16α- (CYP2B1, CYP2C11), 2α- (CYP2C11) and 2β-(CYP3A1, CYP1A1) –dependent oxidases as well as on androst-4-ene-3,17-dione- (CYP3A1/2) -supported monooxygenases were also achieved (Paolini *et al.* 1999).

The effect of *Glycyrrhiza uralensis* Fisch (GRZ) aqueous extract and one of its main constituents Glycyrrhetinic acid (GRT) on hepatic cytochrome P450 in mice were investigated. Oral administration of GRZ at 10 g/kg/d or GRT at 50 mg/kg/d for 7 days was found to increase the P450 contents up to 4.6-fold compared with the controls. The activities of aryl hydrocarbon hydroxylase (AHH, 3.1 and 3.3-fold), aminopyrine N-demethylase (ADM, 4.2 and 3.2 folds), and 7-

ethoxycumarin O-deethylase (ECOD, 2.8 and 2.5-fold) were also shown to be increased. Western blot analysis showed that the subtypes of P450 isoforms induced selectively by GRZ and GRT included CYP1A1 (1.8 and 1.5-fold over that of the control, respectively), CYP2B1 (both 1.3-fold), and CYP2C11 (3.2 and 3.0-fold). Moreover, significant positive correlation between the P450 content or the isoforms and the corresponding enzyme activities mentioned above was observed (Hu *et al.*, 1999 only abstract available).

In the liver microsomes prepared from rats pretreated with *Glycyrrhiza uralensis* decoction (3 g/kg) and phenobarbital (0.12 g/kg), lidocaine was completely metabolized at 30 min regardless of the initial lidocaine concentration. Oral pre-treatment of male SD rats (n=5) with *Glycyrrhiza uralensis* decoction (3 g/kg) for 6 days changed substantially the PK of lidocaine when administered i.v. at doses of 10 mg/kg compared to control group (n=5). The terminal elimination half-life decreased by 39% ($p < 0.01$), the total clearance increased by 1.6-fold ($p < 0.05$) and the AUC value reduced by 41% ($p < 0.05$). The apparent volume of distribution and urinary excretion remained unchanged (Tang *et al.*, 2009).

A single oral dose of an aqueous extract of 900 mg/kg *Glycyrrhiza uralensis* Fisch significantly induced the levels of total $P_{450}$ in SD rats, consistent with the activation of pregnane X receptor (PXR) and induction of CYP3As and CYP2Cs. In addition, five days of treatment with the liquorice extract significantly decreased the AUC ($p < 0.01$) and significantly increased the clearance ($p < 0.01$) of 2 mg/kg intravenous warfarin in SD rats, suggesting that liquorice can increase the metabolism of co-administered warfarin *in vivo*, through the induction of CYP2C9 (Mu *et al.*, 2006).

The effects of glycyrrhizin (GZ) and of a liquorice root decoction (LD) on the PK of methotrexate (MTX) was evaluated in male SD rats. Animals were divided in eight group: group 1 (n=6) did not received any dose of liquorice decoction or glycyrrhizin (control group), group 2 (n=6) received a single dose of 75 mg/kg GZ, group 3 (n=6) received a single dose of 150 mg/kg GZ, group 4 (n=6) received a single dose of LD containing 75 mg/kg GZ, group 5 (n=6) received a single dose of LD containing 150 mg/kg GZ, group 6 (n=6) received a dose of 150 mg/kg GZ twice daily for seven consecutive days, group 7 (n=6) received a single dose of LD containing 150 mg/kg GZ twice daily for seven consecutive days, and group 8 (n=5) received diclofenac 25 mg/kg (positive control group). Rats in groups 1-7 were given MTX (5 mg/kg) orally after the administration of a single dose or after the seventh dose of GZ and LD. Rats in group 8 were given MTX (5 mg/kg) orally after the administration of diclofenac. In the control group, the MTX concentration fell below the lower limit of quantification (LLOQ) after 12 h post-dosing. In contrast, when MTX was coadministered with diclofenac, GZ, or LD, the serum MTX concentration could be quantitated up to 33 h. When MTX was co-administered with diclofenac, the $C_{max}$, $AUC_{0-t}$, and mean residence time (MRT) of MTX were significantly enhanced by 64%, 324%, and 185%, respectively. Following co-administrations of 75 and 150 mg/kg of GZ, the $AUC_{0-t}$ of MTX were significantly increased by 161% and 207%, respectively, whereas the MRT was significantly prolonged by 177% at the dose of 75 mg/kg of GZ. Following co-administration with LD containing 75 and 150 mg/kg of GZ, the $AUC_{0-t}$ of MTX were significantly increased by 264% at the higher dose, whereas the MRT were significantly prolonged by 283% and 172%, respectively. After pretreatment with seven doses of GZ (150 mg/kg), the $C_{max}$, $AUC_{0-t}$, and MRT of MTX were significantly increased by 176%, 480%, and 193%, respectively. The magnitude of the increase of $C_{max}$ and $AUC_{0-t}$ after a seven-dose pretreatment of GZ was more pronounced than that caused by a single dose. With regard to the seven-dose pretreatment of LD containing 150 mg/kg of GZ, the $AUC_{0-t}$ and MRT of MTX were significantly increased by 399% and 280%, respectively (Lin *et al.*, 2009).

A PK study investigated the effects of a water extract prepared from root of liquorice (LE) and its major ingredient, glycyrrhizin (GZ), on ciclosporin A (CsA) in male SD rats. Control rats (n = 6) were orally given CsA (2.5 mg/kg) with water (12.0 mL/kg). Two treatment groups of rats (n = 6 in each group) received oral CsA (2.5 mg/kg) with GZ (150 mg/12.0 mL/kg), and LE (12.0 mL/kg containing 150 mg/kg of GZ), respectively. In addition, in order to reach steady state of blood level, another group of rats (n = 5) were pretreated with seven doses of LE (12 mL/kg containing 150 mg/kg of GZ) twice daily before CsA dosing. The four treatments were performed in parallel design and drugs were administered with gastric gavage. The CsA concentrations in blood were measured by a fluorescence polarisation immunoassay. When CsA was coadministered with single dose of GZ (150 mg/kg), the $C_{max}$ and $AUC_{0-t}$ of CsA were significantly decreased by 49.0% and 45.6%, respectively. In the second group of rats, coadministration with single dose of LE significantly decreased the $C_{max}$ and $AUC_{0-t}$ of CsA by 81.3% and 78.2%, respectively. In the last group, pretreatment with seven doses of LE significantly decreased the $C_{max}$ and $AUC_{0-t}$ of CsA by 91.4% and 89.9%, respectively. In addition, the elimination half-life of CsA after pretreatment with seven doses of LE was significantly prolonged by 80.9%. *In vitro* transport assay carried out with human colon adenocarcinoma cell line LS 180 and studies with commercially available CYP450 screening kits revealed that glycyrrhetic acid (GA), the major metabolite of GZ, significantly activated the functions of P-gp and CYP3A4 (Hou *et al.*, 2012).

Oral pre-administration of a water extract of liquorice roots to SD rats (n=10) did not alter $AUC_{last}$ or $C_{max}$ but it significantly delayed the $T_{max}$ of oral metformin 20 mg/kg. Indeed, $T_{max}$ was 3.1±0.1 h compared to the $T_{max}$ of 2.3±0.3 h in the control group (n=9) which received only metformin (Awad *et al.*, 2016).

Seven and fourteen days of oral treatment with a water extract of *G. glabra* (4 ml/kg bw) showed significant reduction of $C_{max}$, $AUC_{(0-24)}$ and $AUC_{(0-\infty)}$ of verapamil (30 mg/kg) in rabbits (Al-Deeb *et al.*, 2010).

A freshly prepared liquorice beverage once daily for three days significantly increased $C_{max}$ of three statins (80 mg/kg) given to rats in the morning of the day of the experiment after 12 hrs fasting (atorvastatin: from 25.5±13.77 ng/ml to 31.80±19.23 ng/ml; simvastatin: from 28.6±14.99 to 80.4±12.76 ng/ml; lovastatin: from 32.40±10.93 to 93.3±11.22 ng/ml). The same trend was observed for the $AUC_{(0-last)}$ (atorvastatin: from 63.6 to 77.45 ng.hr/ml; simvastatin: from 114.25 ng/ml to 285.25 ng.hr/ml; lovastatin: from 140.25 to 409.5 ng.hr/ml) (Dayyih *et al.*, 2016).

A 14 days pretreatment of rats with liquorice decoction (3 g/kg) could decrease the $AUC_{0-t}$ from 7483.08 ± 528.78 to 6679.12 ± 266.56 mg/L × h ($P < 0.01$) and increase the total clearance (CL) from 0.36 ± 0.02 to 0.39 ± 0.02 L/h/kg of intravenous paclitaxel 3 mg/kg ($P < 0.01$). However, a single co-administration of liquorice did not significantly alter the pharmacokinetic parameters of paclitaxel, such as $AUC_{0-t}$ (from 7483.08 ± 528.78 to 7201.24 ± 292.76 mg/L × h; $P > 0.05$) and CL (from 0.36 ± 0.02 to 0.36 ± 0.01 L/h/kg; $P > 0.05$) (Ha *et al.*, 2019).

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

### 3.3.1. Single dose toxicity

In mice oral $LD_{50}$ values of *Glycyrrhiza* extract is > 7.5 g/kg. In rats oral $LD_{50}$ values range between 14.2 and 18.0 g/kg. The intraperitoneal administration of 2 g/kg of 18α-glycyrrhetic acid is lethal to adult female SD 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 & Burdock, 2006).

A dose of 1000 mg/kg of the aqueous and ethanol extracts of *Glycyrrhiza glabra* by intra-gastric tube root did not cause any mortality to two set of female albino rats (n = 3 per set). There were slight gross behavioral changes such as reduced alertness, spontaneous locomotor activity and reactivity to touch (Chowdhury *et al.*, 2013).

The hydroalcoholic (ethanol 70% V/V) extract of *G. glabra* roots and rhizomes (2900-5000 mg/kg, p.o.) induced hypoactivity, mild depression and ataxia in Swiss mice of both sexes during the first 30 min and for a period of up to 6 h after administration. However, in the animals treated with dose lower than 1600 mg/kg, it produced no signs of acute toxicity or death. There were no significant changes in food and water intake and or body weight during the 14 days of observation (data not shown). The $LD_{50}$ was estimated about 2950 mg/kg when administered orally in mice (Jalilzadeh-Amin *et al.*, 2015).

A single dose of a standardised-flavonoid rich extract of *G. glabra*, at 5000 mg/kg b.w. did not produce treatment related clinical signs of toxicity or mortality in three SD rats tested during the 14-day observation period. Therefore, the median lethal dose was estimated by the authors to be more than 5000 mg/kg (Bhide *et al.*, 2022).

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 & Burdock, 2006).

### 3.3.2. 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 (Isbrucker & Burdock, 2006).

A non-polar extract (NERG) of liquorice roots (extraction solvent: mixture of hexane/ethanol 9:1) was used in subchronic toxicity study in male ICR mice. Animals were separated into four groups by dose treatment (50, 100, 500, and 1,000 mg/kg b.w.) and were treated orally for 120 days.

There were no abnormal changes in body weight, no signs of adverse effects (data not shown) and no death occurred during the 120 days of treatment (data not shown). While the NERG-treated group showed increased liver weight in all dose groups when compared to the control group, there was no significant difference in the weight of any other organ in the NERG-treated group as compared to the control group. Biochemical parameters showed a significant increase compared to control groups in albumin (500 mg/kg and 1000 mg/kg dose groups), calcium (1000 mg/kg dose group), cholesterol (1000 mg/kg dose group), HDL-cholesterol (500 mg/kg and 1000 mg/kg dose groups), iron (1000 mg/kg dose group) and total protein (500 mg/kg and 1000 mg/kg dose groups), whilst a significant decrease in alkaline phosphatase (100 mg/kg, 500 mg/kg and 1000 mg/kg dose groups) was seen. Among haematological parameters a significant increase in lymphocytes and a significant decrease in monocytes was observed in all dose groups. No histopathological changes of the liver, kidney, and spleen was observed in the high dose group (Kim *et al.*, 2019).

A subchronic oral toxicity study of a standardised-flavonoid rich extract of *G. glabra* for 90 days in SD rats at the dose levels of 250, 500, and 1000 mg/kg did not show any treatment related adverse clinical signs. The treated animals exhibited normal weight gain and comparable feed intake. Ophthalmoscope examination did not reveal any abnormalities. Few haematological investigations were found to be statistically significant when compared with those of respective controls. In male rats 1000 mg/kg dose group there was a significant increase in MCV, MCH, WBC and platelets values on day 91, whereas in female rats 1000 mg/kg dose group only a significant increase in WBC was observed. Among the biochemistry parameters, a significant increase in phosphorus in all dose groups and in sodium only in 1000 mg/kg dose group were observed in female rats, but not in male rats. Urine parameters did not change compared to control group in all treated rats. The relative organ weight of vital organs did not differ significantly as compared to control. On day 91, organ weight of male animals of 1000 mg/kg dose group showed increased relative weights of liver and kidneys whereas 500 mg/kg and 1000 mg/kg dose groups showed increased relative weight of adrenals. In comparison with controls on day 91, female animals of 500 mg/kg and 1000 mg/kg dose groups showed increased relative weight of liver; increased in relative weights of heart was observed with 250 mg/kg, 500 mg/kg, and 1000 mg/kg dose groups. Gross and histopathological findings did not show any remarkable and treatment related changes. Reversal groups which were included to study the reversibility/delayed occurrence of symptoms, showed that all the observed changes were reversible after stopping the treatment. Based on the current experimental study findings, the authors considered that the median lethal dose ($LD_{50}$) of the extract was >5000 mg/kg b.wt and the NOAEL was 1000 mg/kg rat b.w. (Bhide *et al.*, 2022).

The oral administration of a 5% glycyrrhizin solution (~1600 mg/kg) for five days to male Slc:Wistar/K4 rats altered renal functions by the third day of treatment, but the effects were terminated upon withdrawal for four 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 & Burdock, 2006).

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% (~375 mg/kg) for male mice and 0.3% (~750 mg/kg) for female mice. Glycyrrhizin was administered in drinking water for 96 weeks at concentrations of 0.04, 0.08, and 0.15 or 0.3%, delivering an approximate daily dose of 71, 166, or 229 mg/kg to the male mice and 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 (Kobuke *at al.*, 1985).

Table 7. Overview of repeat dose toxicity studies.

| **Study (reference)** | **Herbal substance/ preparation/isolated compounds** | **Species/Sex/Number/ Group** | **Dose/ Route/ Duration** | **NOEL/NOAEL (mg/kg/day) according to the authors** | **Major findings according to the authors** |
|---|---|---|---|---|---|
| Isbrucker & Burdock, 2006 | Extract (not described) containing 53% of glycyrrhizin | Male and female rats (no other information available) | 0.31, 0.63, 1.25, or 2.5 g/kg/day orally for 90 days | 0.31–0.63 g extract/kg (approximately 165–334 mg glycyrrhizin/kg) | Alteration of red blood, (≥ 1.25 g/kg) lymphocyte and neutrophil cell counts (2.5 g/kg); alteration of total protein, albumin, AST, ALT and serum cholesterol (2.5 g/kg) |
| Kim *et al.*, 2019 | Extract of roots (mixture of hexane and ethanol 9:1) | Male mice, n=40 (10 each dose group) | 50, 100, 500, and 1,000 mg/kg b.w. orally for 120 days | Not reported | ↑ Albumin, HDL-cholesterol and total protein (500 and 1000 mg/kg); ↑ calcium, cholesterol and iron (1000 mg/kg); ↓ alkaline phosphatase (100, 500 and 1000 mg/kg; ↑ lymphocytes and ↓ monocytes (all dose groups) |
| Bhide *et al.*, 2022 | Standardised-flavonoid rich extract of *G. glabra* root containing ≥ 10% w/w total flavonoids including ≥ 3.5% w/w of glabridin | Rats (n=100, 20 animal/sex each main group (0, 250, 500, and 1000 mg/kg) plus 5 animal/sex for reversal groups (0, 1000 mg/kg) | 250, 500, and 1000 mg/kg orally for 90 days | 1000 mg/kg | ↑ MCV, MCH, WBC and platelets in male rats (1000 mg/kg);↑ WBC (1000 mg/kg) in female rats; ↑ P (all dose groups) and Na (1000 mg/kg) in female rats; ↑ relative weights of liver and kidneys (1000 mg/kg) and adrenals (500 and 1000 mg/kg) in male rats; ↑ relative weights of liver (500 and 1000 mg/kg) and heart (all dose groups) in female rats |
| Kobuke *at al.*, 1985 | Glycyrrhizin in drinking water | Mice (n=330, 180 male and 150 female), divided in | Oral administration of 71, 166, or 229 | Not reported | Glycyrrhizin treatment did not significantly affect average body weights, cumulative |

| | | | | | |
|---|---|---|---|---|---|
| | | the following dose groups: 0.04% (n=60), 0.08% (n=70) or 0.15% (n=50) glycyrrhizin to male mice and 0.08%, 0.15% or 0.3% (N=50, each group) glycyrrhizin to female mice. | mg/kg to the male mice and 117, 217, or 407 mg/kg to the female animals for 96 weeks | | mortality rates and mean time to death, incidence of tumours, or types or distribution of tumours |

### 3.3.3. Genotoxicity

Mitscher *et al.* (1986) had reported that liquorice extracts were highly effective against the mutagenic effects of ethyl methanesulfonate (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 (Mitscher *et al.* 1986).

Zani *et al*. (1993) studied the desmutagenic and antimutagenic effects on the activity of the direct-acting mutagens EMS, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and the ribose-lysine browning system of *Glycyrrhiza glabra* ethanolic extract, glycyrrhizinic acid, 18α-glycyrrhetinic acid, and 18β-glycyrrhetinic acid. The desmutagenic and antimutagenic activities were evaluated by measuring the inhibition of TA 100 $His^+$ revertants induced by EMS. Studies on EMS-induced mutations showed no detectable desmutagenic activity of all the compounds tested (data not shown). On the contrary, a true antimutagenic activity of liquorice extract was demonstrated at doses of 25 and 50 µg/plate, whereas at doses higher than 50 µg/plate the antimutagenic activity is in part due to toxicity. None of the compounds tested showed desmutagenic or antimutagenic activity against MNNG-induced reversion (data not shown). The *Glycyrrhiza glabra* extract, glycyrrhizinic acid, 18a-glycyrrhetinic acid, and 18/3-glycyrrhetinic acid showed desmutagenic activity against ribose-lysine induced mutants. In no case did the tested compounds produce a complete suppression of the induced revertants. 18β-Glycyrrhetinic acid was the most effective among these substances in inhibiting ribose-lysine mutagenicity. At a concentration of 1000 µg/plate, 18β-GA resulted in 79% inhibition of mutagenicity (Zani *et al.*, 1993).

Liquorice ethanolic extract showed an inhibitory effect (ranging from moderate to strong) in the Ames test against mutagenicity of several N-nitrosamines such as N-nitrosodimethylamine (NDMA), N-nitrosopyrrolidine (NPYR), N-nitrosodibutylamine (NDBA), and N-nitrosopiperidine (NPIP) in strain of *Salmonella typhimurium* TA100 using metabolic activation. This ethanolic extract

inhibited the mutagenicity of NPIP from 56% to 72%. The mutagenicity of NDBA was markedly reduced (25-46%) by concentrations ≥500 µg/plate of liquorice ethanolic extract. The antimutagenicity effect against NPIP and NDBA increased with increasing concentration of the extract up to 2000 µg/plate and decreased at concentrations >2000 µg/plate (data not shown). Liquorice ethanolic extract showed a strong antimutagenic effect against NDMA (50 µg/plate; 45%) but only a moderate inhibitory effect against NPYR (500-1000 µg/plate; 29-39%) (Ikken *et al.*, 1999).

In an Ames test a standardized de-glycyrrhizinated extract of *G. glabra* root did not show significant increase in number of revertant colonies in *Salmonella typhimurium* strains (TA98 and TAMix) with/without S9 fraction. In chromosome aberration and micronucleus studies carried out using Chinese Hamster Ovary cells (CHO-K1 cells), the extract did not show clastogenic effect at 4 and 18 h treatments with and without S9 fraction (Chandrasekaran CV *et al.*, 2011b).

Water, chloroform, and methanol extracts obtained from ten herbs, including *G. glabra* were screened for their genotoxic effects at concentrations of 0.78, 1.56, 3.13, 6.25, 12.50, and 25.00 mg/mL in a bacterial mutation assays with *S. typhimurium* TA98 and TA100 strains, with or without the S9 metabolic activating system. All the *G. glabra* extract gave negative results except the water extract at the highest concentration of 25 mg/ml in presence of metabolic activation which displayed mutagenic activity against the TA100 strain (Abudayyak *et al*., 2015).

The genetic safety of a water extract from *G. glabra* and its effects on cadmium (as $CdCl_2$) induced genotoxicity was investigated by Dirican & Turkez (2014). $CdCl_2$ (5 ppm) and *G. glabra* extracts (5, 10 and 20 ppm) were added into human peripheral blood lymphocyte culture tubes separately and together. After supplementation of $CdCl_2$ and plant extracts, the blood samples were incubated for 72 h at 37°C to adjust to body conditions. Each individual whole blood culture without $CdCl_2$ or *G. glabra* extract was used as a control group. There were significant increases ($P<0.05$) in both sister chromatid exchange (SCE) and micronucleus (MN) frequencies of cultures treated with $CdCl_2$ (5 ppm) as compared to controls. However, co-application of *G. glabra* extract (5, 10 and 20 ppm) and $CdCl_2$ resulted in decreases of MN and SCE rates as compared to the group treated with $CdCl_2$ alone. The authors concluded that *G. glabra* extracts provided increased resistance of DNA against $CdCl_2$-induced genetic and oxidative damage in human lymphocytes (Dirican & Turkez, 2014).

Antimutagenic and anti-genotixic activity was observed in vitro for several constituents of liquorice such as glycyrrhizin, 18β-glycyrrhetinic acid, glycyrrhizic acid, glabridin, licochalcone A and liquiritigenin (Isbrucker & Burdock 2006; Kaur *et al.*, 2012; Inami *et al.*, 2016).

Table 8. Overview of genotoxicity studies.

| **Type of test/reference** | **Test system** | **Herbal substance/ preparation/ isolated compound** | **Concentrations/ Concentration range/ Metabolising system** | **Results positive/negative/ equivocal** |
|---|---|---|---|---|
| Gene mutations in bacteria: Chandrasekaran CV *et al.*, 2011b | TAMix and TA98. TAMix is a mixture of the *Salmonella typhimurium* strains viz., TA7001, TA7002, | Licorice extract (not described) | 1.6, 5, 16, 50, 158, 501 µg/mL; +/- S9 | Negative |

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|---|---|---|---|---|
| Abudayyak *et al*., 2015 | TA7003,TA7004, TA7005 and TA7006<br><br>*Salmonella typhimurium* strains TA98 and TA100 | Water, methanol, and chloroform extracts | 0.78, 1.56, 3.13, 6.25, 12.50, and 25.00 mg/mL; +/- S9 | Negative except 25 mg/ml in presence of metabolic activation which was positive in TA100 strain only |
| Gene mutations in mammalian cells: Chandrasekaran CV *et al.*, 2011b | CHO-cells | Licorice extract (not described) | 4, 12.6 and 40 µg/mL for 4 h with or without S9 and 1.46, 4.6 and 14.6 µg/mL for 18 h without S9 | Negative |

### 3.3.4. Carcinogenicity

No studies on carcinogenicity were available.

### 3.3.5. Reproductive and developmental toxicity

In 1971, the Food and Drug Research Labs conducted a 4-species teratologic evaluation of glycyrrhizin (ammonium salt) 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 6$^{th}$ day of gestation. CD-1 mice and Wistar rats were dosed for 10 consecutive days whereas the golden hamsters and Dutchbelted 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 & Burdock, 2006).

The potential teratogenic effects of disodium glycyrrhizin in pregnant Wistar rats was examinated by Itami & Colleagues (1985). 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 (Itami *et al.*, 1985).

A similar study, evaluating the teratogenicity of glycyrrhizin (ammonium salt) in pregnant SD rats, was conducted by Mantovani and colleagues (1988). Commencing on the 7th day of gestation, dams were provided with 0, 10, 100, or 250 mg ammonium glycyrrhizin/100 ml drinking water (groups 0, 1, 2, and 3 equal to 0, 21, 239 and 680 mg/kg bw/day) and maintained up to the 17th 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 per litter. No significant differences were found in the number of pre-implantation losses. The prevalence of resorptions was significantly related to the dose ($P < 0.03$) when tested by means of the Armitage-Cochran test. No significant differences were found in the number of dead foetuses. The prevalence of runts was similar for groups 0, 1 and 3; the higher prevalence in group 2 was due to a litter with a particularly low average weight. Skeletal abnormalities were significantly elevated in the two highest treatment groups. These abnormalities included misaligned, asymmetric and bipartic sternebrae and hemisternebrae. Soft-tissue abnormalities were mostly renal and their prevalence were dose-dependent. The presence of foetuses with renal ectopy and the rate of litters showing at least one foetus with this anomaly were significantly increased in the 250 mg/100ml dose test group. External haemorrhages were also observed in some foetuses. According to the study authors, the data indicate a slight adverse effect of ammonium glycyrrhizinate on foetal rats. (Mantovani *et al.*, 1988).

Since 11β-hydroxysteroid dehydrogenase (11β-HSD) is important in the regulation of pulmonary surfactant synthesis during development, the effects of maternal 18β-glycyrrhetinic acid consumption on rat foetal lung development were studied. Pregnant Wistar rats were fed a daily diet delivering 0, 10, 100, or 1000 mg/kg 18β-glycyrrhetinic 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 11β-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 18β-glycyrrhetinic acid exposure; neither was there any abnormal behaviour observed in the neonatal rats (Hundertmark *et al.*, 2002).

Effects of liquorice extract on foetal abnormalities induced by cyclophosphamide were investigated in rats. Pregnant SD 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 *et al.*, 2007; only abstract available).

The findings by Jeon and colleagues were confirmed by a further study on the effects of a water extract of liquorice on the fetal defects induced by cyclophosphamide. Pregnant SD rats were daily

administered with liquorice (100 mg/kg) by gavage for 7 days, from the 6th to 12th day of gestation, and intraperitoneally administered with cyclophosphamide (11 mg/kg) 1 hr after the final liquorice treatment. On the 20th day of gestation, maternal and fetal abnormalities were determined by Cesarian section. Cyclophosphamide was found to reduce fetal and placental weights without increasing resorption or death. In addition, it induced malformations in live fetuses; 93.8, 41.1, and 100% of the external (skull and limb defects), visceral (cleft palate and ureteric dilatation), and skeletal (acrania, vertebral/costal malformations, and delayed ossification) abnormalities, respectively. When pre-treated with liquorice, cyclophosphamide-induced body weight loss and abnormalities of fetuses were remarkably aggravated. Moreover, repeated treatment with liquorice greatly increased mRNA expression and activity of hepatic CYP2B, thus enhancing the metabolic conversion of cyclophosphamide to teratogenic acrolein and cytotoxic phosphoramide mustard as concluded by the authors (Park *et al.*, 2011).

The extract of *Glycyrrhizae radi*x decreased total serum testosterone (T) levels slightly in 11.5 and 22.5 mg/kg doses but showed the dose-dependent increase in 22.5 to 90 mg/kg doses, and a significantly higher value in the highest dose (284 ± 8.0 pg/ml) than 0 mg/kg dose (257 ±10 pg/ml) when orally administered daily for 2 weeks to androgen-sterilized rats. Serum estradiol/T (E2/T) ratios were significantly elevated with 11.25 to 90 mg/kg doses of *Glycyrrhizae radi*x. Serum LH and FSH levels were not changed by the treatments. There were no changes in serum T, LH and FSH levels when the extract was orally administered for 2 weeks at doses of 45 and 90 mg/kg b.w. to oophorectomized rats (Takeuschi *et al.*, 1989).

To examine the effects of liquorice on fertilization success in mice, an aqueous extract of liquorice was added to the culture medium used for insemination (0.12 mg/ml). The fertilization rate was improved by the aqueous liquorice extract, but not specifically by glycyrrhizin (Tung *et al.*, 2014). The same group of authors suggested that isoliquiritigenin and formononetin could contribute to the improved rate of fertilization *in vitro* (Tung *et al.*, 2015).

The effects of a liquorice extract (LE) on spermatogonial proliferation and spermatocyte differentiation during neonatal mice spermatogenesis was investigated in an organ culture model of testis tissue. The proliferation activity of spermatogonia in twenty neonatal C57BL/6N mice was identified with the positive rate quantitative analysis of 5-bromo-2-deoxyuridine (BrdU) and anti-proliferating cell nuclearantigen (PCNA) antibody by immunohistochemical staining. The results showed that, compared to the control group, the percentage of positive cells by BrdU staining enhanced dramatically and that the expression of PCNA protein increased significantly in the spermatogonia from the LE group (0.2, 2, 20 µmol/L) and showed a concentration-dependent manner ($P<0.05$). The proliferation rate of spermatogonia was similar in 20 µmol/L LE group and 200 µg/L porcine follicle stimulating hormone (FSH) group (positive control group) (Wang *et al.*, 2016).

A water extract of liquorice roots (containing 3.06% of glycyrrhizin on average) orally administered at doses of 500, 1000 and 2000 mg/kg to 6-weeks-old SD mice for 9 weeks neither induced clinical signs, nor affected the daily feed consumption and body weight gain. There were no significant changes in testicular weights, gross and macroscopic findings, and daily sperm production between vehicle- and liquorice-treated animals, in spite of slight decreases in prostate weight and daily sperm production at 2000 mg/kg. In addition, liquorice did not affect the motility and morphology of sperm, although the serum testosterone level tended to decrease without significant difference compared to the vehicle-treated group, showing a 28.6% reduction in the 2000 mg/kg group. The authors concluded that the NOAEL of the water extract was higher than 2000 mg/kg (Shin *et al.*, 2008).

The effect of liquorice aqueous extract on carbendazim-induced testicular toxicity was studied in albino rats. Animals were divided into four groups: Rats in group 1 (n=10) served as controls and were given corn oil additionally to their food; rats in Groups 2 (n=25) and 3 (n=25) received the liquorice aqueous extract 50 mg/kg b.w. and 100 mg/kg/b.w. orally for 3 days weekly for 8 weeks, respectively; rats in Group 4 (n=30) were treated orally with 100 mg/kg b.w. carbendazim for 3 days weekly for 8 weeks. Administration of carbendazim induced significant decrease in testis weight, diameter, and germinal epithelial height of the seminiferous tubules. Histological results revealed degeneration of seminiferous tubules, loss of spermatogenic cells, and apoptosis. Moreover, carbendazim caused elevation of testicular MDA, and reduced the activity of the SOD and CAT. Co-administration of liquorice extract with carbendazim improved the histomorphological and histopathological changes observed in animals treated with carbendazim. In addition, liquorice treatment leads to a significant decrease in the level of MDA and increase in the activities of SOD and CAT (Sakr & Shalaby, 2014).

Table 9. Overview of reproductive and developmental toxicity studies.

| **Study** | **Species; Number Female/group** | **Herbal preparation/ Route & dose** | **Dosing period** | **NOAEL according to the authors** | **Major findings according to the authors** |
|---|---|---|---|---|---|
| **Male fertility** | | | | | |
| Shin *et al.*, 2008 | Sixty 6-weeks-old mice (n=15 each dose group) | Water extract of roots (containing 3.06% of glycyrrhizin on average) | 0, 500, 1000 and 2000 mg/kg orally for 9 weeks | 2000 mg/kg | No significant changes in testicular weights, gross and macroscopic findings, daily sperm production, and the motility and morphology of sperm; non significant decrease in T serum level in the 2000 mg/kg dose group |
| Sakr & Shalaby, 2014 | Ninety sexually mature rats (n=10 in control group; n=25 in licorice 50 mg/kg group; n=25 in licorice 100 mg/Kg group); n=30 in carbendazim 100 mg/kg+licorice 100 mg/kg group) | Water extract | licorice 50 mg/kg or 100 mg/kg three days weekly for 8 weeks and carbendazim 100 mg/kg oral single dose followed by licorice 100 mg/kg three days weekly for 8 weeks | Not reported | Co-administration of liquorice extract with carbendazim improved the histomorphological and histopathological changes induced by carbendazim. Liquorice treatment leads to a significant decrease in the level of MDA and increase in the activities of SOD and CAT |
| **Female fertility** | | | | | The extract decreased total T levels slightly in |

| | | | | | |
|---|---|---|---|---|---|
| Takeuschi *et al.*, 1989 | Experiment I: fifty androgen-sterilised rats (n=10 each group) | Extract of *Glycyrrhizae radix* (no further detail available) | Experiment I: 0, 11.25, 22.5, 45 and 90 mg/kg) daily for 2 weeks | Not reported | 11.5 and 22.5 mg/kg doses but showed the dose-dependent increase in 22.5 to 90 mg/kg doses; serum E2/T ratios were significantly elevated with 11.25 to 90 mg/kg doses |
| | Experiment II: thirty-three oophorectomized rats (n=11 each group) | Extract of *Glycyrrhizae radix* (no further detail available) | Experiment II: 0, 45 and 90 mg/kg daily for 2 weeks | Not reported | No significant changes in T, LH and FSH levels |
| **Embryo-fœtal development**<br>Jeon et al., 2007 | Pregnant rats (no further information available) | Water extract | 100 mg/kg orally for 7 days, from days 6 to 12 of gestation | Not reported | Licorice enhanced the dcerase in body weight and the fetal malformations induced by cyclophosphamide (11 mg/kg i.p.) |
| Park *et al.*, 2011 | Pregnant rats (n=12–14/group) | Water extract | 100 mg/kg orally for 7 days, from days 6 to 12 of gestation | Not reported | Licorice enhanced the decrease in body weight and the fetal malformations induced by cyclophosphamide (11 mg/kg i.p.); licorice increased mRNA expression and activity of hepatic CYP2B, thus enhancing the conversion of cyclophosphamide in acrolein |

## 3.3.6. Local tolerance

No data available.

## 3.3.7. Other studies

No data available.

## 3.3.8. Conclusions

The PK profile of the most studied constituent of liquorice root, glycyrrhizin, has been well characterised. Interestingly, the PK of a water extract of liquorice root appears to be different form that of single constituents with a decrease of potential toxicity for saponins such as glycyrrhizic acid.

Liquorice root extracts and glycyrrhizin have been demonstrated to inhibit or to stimulate the activity of several isoforms of P450 hepatic enzymes both *in vitro* and *in vivo*; in addition, also interference with drug transporters such as organic anion transporters (OATs) and multidrug resistent proteins (MRPs) including P-glicoprotein has been suggested. (Al-Deeb *et al.*, 2010; Hou *et al.*, 2012; Lin *et al.*, 2009; Dayyih *et al.*, 2016; Ha *et al.*, 2019).

Through the above reported mechanisms, liquorice affected in *in vivo* studies the systemic exposure of several drugs (i.e. lidocaine, warfarin, verapamil, metothrexate, paclitaxel, ciclosporin A and statins). Drastic increase of systemic exposure to methotrexate was observed using high doses of liquorice water extracts and pure glycyrrhizin up to 7 days of treatment.

Based on the outcome of single- and repeated-dose toxicity studies in rats and mice no toxicity concerns are expected for liquorice when based on the administered oral doses.

A number of old pre-clinical studies investigated the teratogenic potential of liquorice and possible effect on foetal development of glycyrrhizin and 18β-glycyrrhetinic acid. Glycyrrhizin or 18β-glycyrrhetinic acid when administered orally at high doses exhibited some embryotoxicity to the developing rat foetus, but the foetal effects were considered as minor. Of note, in a couple of studies, a water extract of liquorice significantly aggravated the cyclophosphamide-induced body weight loss and abnormalities of fetuses; the clinical implications of this observed interaction in animals should be further investigated. There is no evidence that a water extract liquorice could impair fertility in male rats when administered orally as repeated doses up to 2000 mg/kg bw for 9 weeks.

The majority of bacterial genotoxicity studies have reported an absence of genotoxic effects or an anti-mutagenic effect from liquorice extracts or glycyrrhizinate compounds.

Liquorice has a long history of use in medicine and as a flavouring substance, and glycyrrhizic acid and its ammonium salt (ammonium glycyrrhizinate) are widely used as sweeteners and flavourings in confectionary, sweets, drugs, beverages, chewing gum, tobacco products and toothpastes.

Liquorice and liquorice derivatives, including ammonium 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 (Isbrucker & Burdock 2006).

In its scientific evaluation on glycyrrhizinic acid and its ammonium salt, the EC Scientific Committee on Food concluded that an ADI for glycyrrhizinic acid and ammonium glycyrrhizinate cannot be derived, however an upper limit for regular ingestion of 100 mg/day (found in about 60 g of liquorice confectionery) provides a sufficient level of protection for the majority of the population. At the same time, the Committee realised that within the human population there are subgroups for which this upper limit might not offer sufficient protection. These subgroups comprise people with decreased 11-beta-hydroxysteroid dehydrogenase-2 activity, people with

prolonged gastrointestinal transit time, and people with hypertension or electrolyte-related or water homeostasis-related medical conditions (SCF/CS/ADD/EDUL/225 Final, 2003).

According to the JEFCA, available data suggest that an intake of 100 mg/day would be unlikely to cause adverse effects in the majority of adults. In certain highly susceptible individuals, physiological effects could occur at exposure levels somewhat below this figure. The intake data indicate that consumers with a high intake of liquorice confectionery or herbal tea containing liquorice may be exposed to glycyrrhizinic acid at more than 100 mg/day (Joint FAO/WHO Expert Committee on Food Additives, 2005).

The 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.

In 2011 EFSA has delivered a a scientific opinion on the safety of „Glavonoid“, an extract derived from the root or rootstock of Glycyrrhiza glabra L. by extraction with ethanol followed by further extraction with medium-chain triglycerides, as a food ingredient in the context of Regulation (EC) No 258/97. Glavonoid was considered safe for the general adult population up to 120 mg/day (EFSA Journal 2011;9(7):2287).

The WHO defines safe range of liquorice consumption levels between 5 and 15 g/d (which is equivalent to 200-800 mg per day of glycyrrhizinic acid) for no longer than 4 to 6 weeks without medical advice. Excessive consumption according to the WHO is liquorice consumption of more than 1.75 oz (50 g) for more than 6 weeks (WHO. Liquorice. Medicine plants in newly independent states, 2010).

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

Non-clinical data on gastro-intestinal activity supports the traditional use of liquorice root for the relief of digestive symptoms including burning sensation and dyspepsia. Results from relevant experimental studies on the anti-tussive activity supports the traditional use of liquorice root as an expectorant in cough associated with cold.

In developmental toxicity studies, glycyrrhizin (ammonium salt) and 18β-glycyrrhetinic acid exhibited some embryotoxicity to the developing rat foetus when given orally at high doses up to two weeks, but the foetal effects were considered as minor. Finally, a couple of studies suggested that 100 mg/kg of liquorice extract repeated for 7 days may also aggravate body weight loss and malformations of foetuses, induced by intrauterine exposure to cyclophosphamide. These pre-clinical observations, taking into account that there is evidence that 18β-glycyrrhetinic acid crosses through the placental barrier and can be detected in the rat foetuses, have been reported in section 5.3 of the monograph.

An *in vivo* study has suggested that concurrent administration of methotrexate and high daily doses of either GL (HED of 700 mg/day and 1500 mg/day) or liquorice decoction administrered up to 7 consecutive days can drastically increase MRT and AUC of methotrexate (Lin et al., 2009), but the clinical relevance of this interaction has not yet studied.

Oral administration of liquorice preparations included in the monograph can be regarded as safe at traditionally used doses for a short period of treatment with the exception of patients with severe renal or cardiac disease e.g. renal and heart failure due to the mineralcorticoid effects.

Adequate tests on reproductive toxicity and genotoxicity have not been performed. Test on carcinogenicity have not been performed.

# 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.

There is also evidence that aqueous extract (1 mg/mL) of *Glycyrrhiza glabra* could inhibit the adhesion of *Helicobacter pylori* to human stomach tissue. This effect was related to the polysaccharides isolated from the extract, with one purified acidic fraction (0.25 SPB) as main active polymer. Purified polysaccharides did not exhibit direct cytotoxic effects against *Helicobacter pylori* and did not influence hemagglutination (Wittschier *et al.*, 2009).

The anti-inflammatory and antiallergic actions of the drug have been attributed to the corticosteroid-like activity of glycyrrhizin and 18β-glycyrrhetinic 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 & Hosseinzadeh 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 glycerrhitinic acid. Anti-inflammatory action of liquorice is partially due to 11β-HSD inhibition and increased local glycocorticoid activity (Schleimer, 1991).

Liquorice ethanolic extract and its constituent glabridin were shown to inhibit LDL oxidation by a mechanism involving scavenging of free radicals (Fuhrman *et al.*, 1997).

The effect of a hot water extract of liquorice in antagonizing oxytocin-, $PGF_{2\alpha}$-, and high-KCl-induced uterine smooth muscle contractions was studied using myometrial strips from uterine tissues of pregnant women who underwent cesarean section at 37 weeks' gestation or cesarean section for pregnancy following myomectomy at 37 weeks' gestation. The extract (250 mg/mL)

inhibited oxytocin-induced (final concentration 50 mU/mL), $PGF_{2\alpha}$-induced ($10^{-7}$ M) and high-KCl-induced (72.7 mM) contractions (Tsuji *et al.*, 2012).

A phase 0, double-blind, repeated within subject, randomized pilot study showed that a tincture of *Glycyrrhiza glabra* greatly increased the number of activated CD25 + CD4 T cells in human subjects within 24 h of ingestion (Zwickey *et al.*, 2007).

In another study the tincture 0.87 g (as dry herb) stimulated immune cells as quantified by CD69 expression on CD4 and CD8 T cells. This activation took place within 24 h of ingestion, and continued for at least 7 days (Brush *et al.*, 2006).

Anti-human respiratory syncytial virus (HRSV) activities of hot water extracts of air-dried root of *Glycyrrhiza uralensis* (*Radix Glycyrrhizae*), glycyrrhizin and 18β-glycyrrhetinic acid (18β-GA) were examined by plaque reduction assay in both human upper (HEp-2) and low (A549) respiratory tract cell lines. In addition, a hot water extract of a preparation obtained following by slicing crude liquorice, mixed well with honey, and heated on a small fire until the color turns golden or deep yellow (*Radix Glycyrrhizae Preparata*) was tested. Abilities of crude liquorice to inhibit viral replication and to stimulate IFN-β were evaluated by reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. *Radix Glycyrrhizae* and *Radix Glycyrrhizae Preparata* dose-dependently inhibited HRSV-induced plaque formation in both HEp-2 and A549 cell lines ($p<0.0001$). The effect of *Radix Glycyrrhizae* was better than that of *Radix Glycyrrhizae Preparata* on HEp-2 cells. However, there was no difference of their anti-HRSV effects on A549 cells. Besides, glycyrrhizin was ineffective at all. Nevertheless, 18β-GA showed a potent anti-HRSV activity. *Radix Glycyrrhizae* was more effective when given before viral inoculation ($p<0.0001$) which may be due to its inhibition of viral attachment on ($p<0.0001$) and penetration ($p<0.0001$) into the host cells. The anti-HRSV activity of *Radix Glycyrrhizae* was further confirmed by RT-PCR and qRT-PCR. 300 μg/ml *Radix Glycyrrhizae* markedly decreased the viral amounts within the cells and in the suspension. *Radix Glycyrrhizae* might further stimulate mucosal cells to secrete IFN-β to counteract viral infection (Yeh *et al.*, 2013).

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

When *Glycyrrhizae Radix* is ingested orally, glycyrrhizin only slightly penetrates through the gastrointestinal tract epithelium due to its highly hydrophilic sugar moiety. It is absorbed as glycyrrhetinic acid after the sugar moiety is hydrolyzed and converted from glycyrrhizin to glycyrrhetinic acid by enterobacteria in the large intestine (Hattori *et al.*, 1983 and 1985; Størmer *et al.*, 1993; Shimada *et al.*, 2019).

Therefore, 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. 18β-glycyrrhetinic acid is readily detected in the plasma following the ingestion of glycyrrhizin or liquorice extract (Isbrucker & Burdock 2006).

The content of glycyrrhizin in plasma samples was not detectable in healthy adult volunteers up to 36 h after the administration of a single dose of 800 glycyrrhizin as ammonium salt or as a liquorice extract (LE) 10.5 g. Only from analysis of one volunteer, who had been given a single dose of 1600 glycyrrhizin as ammonium salt, the levels of glycyrrhizin were detectable in plasma; however, a week later and in the same volunteer, after LE administration (21 g) no detectable glycyrrhizin level was found. In another volunteer group the amounts of glycyrrhizin or

glicyrrhizinic acid excreted were also very low in urine at 4, 8, 12, 24, and 36 hr after either glycyrrhizin or LE administration at the highest dosage. However, at all the time intervals considered, the glycyrrhizin excretion averaged a lower value with LE than with glycyrrhizin administration, suggestisting a reduced bioavailability of glycyrrhizin present in LE compared to pure glycyrrhizin. The authors explained his finding to the interaction during intestinal absorption between the glycyrrhizin constituent and the several components in LE. (Cantelli-Forti *et al*., 1994).

After oral administration, glycyrrhizin is metabolized to 18β-glycyrrhetinic acid by intestinal bacteria which contain β-D-glucuronidase. Intravenously administered glycyrrhizin is metabolized in the rat liver by lysosomal β-D-glucuronidase to 3-mono-glucuronide 18β-glycyrrhetinic acid. This metabolite is excreted with the bile into the intestine, where it is metabolized by bacteria into 18β-glycyrrhetinic acid, which can be reabsorbed. The enterohepatic circulation of 18β-glycyrrhetinic acid can be expected in humans because 18β-glycyrrhetinic acid metabolites can be hydrolyzed by human gastrointestinal bacteria (Asl & Hosseinzadeh 2008; Ploeger *et al.*, 2000).

The plasma concentration of 3-monoglucuronyl glycyrrhetinic acid (3MGA) was reported to be significantly higher in patients with hypokalemia than in those with normokalemia and chronic hepatitis who had been orally treated with glycyrrhizin for more than four weeks, even though the plasma concentration of 18β-glycyrrhetinic acid did not differ between the two groups (Kato *et al.*, 1995)

However, in a more recent study, 3MGA has been rarely detected by LC-MS/MS in patients with symptoms/signs of pseudoaldosteronism after liquorice intake, whereas 18β-glycyrrhetyl-3-O-sulfate was found as a major GL metabolite in human blood; sulfotransferase 2A1 catalysed the metabolic reaction of glycyrrhetinic acid to this compound. Thus, it was assumed that Kato *et al.* might have also detected 18β-glycyrrhetyl-3-O-sulfate, not 3MGA, in their investigation (Takahashi *et al.*, 2019). Glycyrrhetinic acid and 18β-glycyrrhetyl-3-O-sulfate are highly bound to albumin in blood circulation and are predominantly excreted into bile via multidrug resistance-associated protein 2 (Mrp-2) (Yoshino *et al.*, 2021). When bile excretion of 18β-glycyrrhetyl-3-O-sulfate is suppressed due to Mrp2-dysfunction, it is transferred into blood circulation. Since glycyrrhetyl-3-O-sulfate has existed in blood circulation with the binding-form to serum albumin, it is not excreted into urine by glomerular filtration. However, it can be transported from blood circulation into tubular cells via organic anion transporter-1 (OAT-1) and 3, and can inhibit 11βHSD2 to develop pseudoaldosteronism (Makino, 2021).

Inter-individual differences in glycyrrhizin response, metabolism and kinetics are influenced, at least in part, by the intestinal microflora profile. Neither glycyrrhizin nor 18β-glycyrrhetinic acid accumulate in tissues (Isbrucker & Burdock 2006). Glycyrrhizin and 18β-glycyrrhetinic acid adhere extensively to human and rat serum albumin through a saturable process (Ishida *et al.*, 1992; Ploeger *et al.*, 2000).

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).

The plasma clearance of glycyrrhizin and 18β-glycyrrhetinic acid is dose dependent when administered at levels which exceed the saturation of serum protein binding. It is 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 (Isbrucker & Burdock, 2006). The plasma clearance of 18β-

glycyrrhetinic acid is significantly decreased in patients with chronic hepatitis C and liver cirrhosis (Ploeger *et al.*, 2001, van Rossum *et al.*, 1998). Together, these data suggest a hepatic related capacity-limited process in metabolism and/or excretion in the bile (Isbrucker & Burdock 2006).

*Drug-drug interactions in humans*

Alcoholic liquorice root extract (1.4 to 69 µg/mL) and glabridin (0.625 to 40 µM) inactivated human CYP3A4 in a time- and concentration-dependent manner. The inactivation was NADPH-dependent and not reversible by extensive dialysis, which was also demonstrated to be correlated with the loss of the P450-reduced CO spectrum and the intact heme moiety (Kent *et al.*, 2002).

The effects of 32 g liquorice alone (containing glycyrrhizin 0.13% w/w) or in combination with 25 mg hydrochlorothiazide a day for 2 weeks was studied in 10 healthy volunteers aged between 18 and 40 years using a randomized, open and crossover design. The sequential treatments were separated by at least a 3-week washout phase. During the liquorice phase, there were no changes in plasma potassium, sodium, creatinine, renin activity, serum aldosterone, blood pressure or heart rate. Weight tended to increase by 0.4 kg (70.2 to 70.6 kg; p = 0.056). During the liquorice–hydrochlorothiazide phase, the plasma potassium decreased by 0.32 mmol/l (p = 0.0015), plasma renin activity increased by 1.6 µg/l/h (p = 0.0064) and the weight decreased by 0.9 kg (70.5 to 69.6 kg, p = 0.0065). Twenty per cent of the subjects (2/10) became hypokalaemic during the combined liquorice–hydrochlorothiazide treatment. Furthermore, both subjects developed hypokalaemia within the first week of the combined treatment leading to premature discontinuation (Hukkanen *et al.*, 2009).

11β-dehydrogenase converts endogenous cortisol to cortisone; orally administered glycyrrhizin is metabolised mainly to 18β-glycyrrhetinic acid. Glycyrrhetinic acid is a more potent inhibitor of 5α-, 5β-reductase and 11β-dehydrogenase than is glycyrrhizin.

The effect of glycyrrhizin on the metabolism of prednisolone was investigated on 6 male subjects with an average age of 25.0 (range 21-33) years. Each subject was injected with 200 ml of Ringer solution and 0.096 mg/kg of prednisolone hemisuccinate (PSL-HS, equivalent to 0.075 mg/kg of prednisolone), first as a control, and then, two weeks later, each one was injected with 200 mg of glycyrrhizin, and the same dose of PSL-HS. The pharmacokinetic parameters of PSL were determined, using non-compartmental analysis. GL was found to increase significantly the concentration of total PSL at 6, 8 h, and of free PSL at 4, 6, and 8 h after PSL-HS infusion. Glycyrrhizin was also found to modify the pharmacokinetics of PSL. After the administration of glycyrrhizin, the AUC increased, total plasma clearance (CL) decreased, and the mean residence time (MRT) was prolonged. However, only those of AUC, CL, and MRT of free PSL were significantly different. The volume of distribution at a steady state of both total and free PSL showed no evident change (Chen *et al.* 1990).

By means of the skin vasoconstrictor assay carried out on 23 healthy volunteers with no previous exposure to exogenous corticosteroids (12 women and 11 men of mean age 29 years), glycyrrhetinic acid was shown to potentiate the action of hydrocortisone through potent inhibition of 11β-hydroxysteroid dehydrogenase (11β-OHSD) in the skin (Teelucksingh *et al.*, 1990).

In a parallel group study, 6 patients were given glycyrrhizin 225 mg daily for 7 days, and 6 patients were given the same dose of glycyrrhizin and dexamethasone 1.5 mg daily for 7 days. The mineralcorticoid effects of glycyrrhizin were significantly reduced by dexamethasone; cortisol plasma concentrations and urinary excretions were reduced by up to 70% (Kageyama *et al.*, 1992).

The influence of of liquorice on the absorption and metabolism of cortisone acetate (CA) was assessed on 17 patients with Addison's disease who were on stable CA replacement therapy. Liquorice was added as tablet at a dose of 24 g/day (150 mg/day of glycyrrhetinic acid) for 3 consecutive days on standard treatment. Time series of glucocorticoids (GCs) in serum and saliva were obtained, and GCs in 24 h urine samples were determined. The main outcome measure was the AUC for serum cortisol in the first 2.6 h after orally administered CA. Liquorice treatment increased the median AUC for serum cortisol compared with the ordinary treatment (53 783 vs 50 882, P<0.05). Median cortisol levels in serum were also elevated 2.6 h after tablet ingestion (liquorice 223 vs 186 nmol/l, P<0.05). Finally, liquorice increased the median urinary cortisol/cortisone ratio (0.43 vs 0.21, P<0.00001) (Methlie *et al.*, 2011).

Glycyrrhizin caused a significant reduction in midazolam AUC compared to placebo when given as a monopotassium glycyrrhizinate tablets at doses of 150 mg twice daily to 16 healthy adult male subjects for 14 days in a randomised crossover bioequivalence study. A 4-week washout period between phases was applied. All subjects refrained from use of any medication 2 weeks before and throughout the study. They also abstained from taking grapefruit juice, herbal dietary supplements, and caffeine-containing beverages including coffee and green tea for 2 weeks before the study and during the study period. The volunteers were served standard meals during the experimental period. On the 15$^{th}$ day, after an overnight fast, each subject received an oral dose of midazolam (7.5 mg). Blood samples (5 ml) were collected before (0 min) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 h after midazolam administration The 90% CI for $AUC_{0-\infty}$ and $C_{max}$ for the geometric mean ratio of glycyrrhizin over placebo were both out of the no-effect boundaries of 0.80–1.25. The median $T_{max}$ and mean $t_{1/2}$ values were similar for both treatments. The administration of glycyrrhizin resulted in a modest induction of CYP3A that was clinically relevant according to the bioequivalence analysis (Tu *et al*. 2010a).

The same group of authors carried out a bioequivalence crossover trial on 18 healthy subjects with different CYP2C19 genotype (six CYP2C19*1/*1, five CYP2C19*1/*2, one CYP2C19*1/*3, five CYP2C19*2/*2 and one CYP2C19*2/*3). The study was carried out in a two-phase crossover manner with a 4-week washout period between phases. In each phase, volunteers received placebo or glycyrrhizin salt tablet 150 mg two times daily for 14 days. On the 15th day, all subjects were given a single oral dose of omeprazole (20-mg capsule) with 250 ml warm water, after an overnight fast. The Cmax decreased by 39.89% ± 18.93% (p = 0.032), 25.50% ± 19.10% (p = 0.039), and 15.40% ± 11.64% (p = 0.041) in the CYP2C19*1/*1, CYP2C19*1/*2 or *3 and CYP2C19*2/*2 subjects, respectively; AUC0-∞ of omeprazole decreased by 14.72% ± 12.60% (p = 0.037), 21.97% ± 18.93% (p = 0.034) and 16.99% ± 13.28% (p = 0.024) in subjects with CYP2C19*1/*1, CYP2C19*1/*2 or *3 and CYP2C19*2/*2, respectively. No significant difference in t1/2 and tmax were observed for omeprazole between placebo and glycyrrhizin treatment phases. On the other hand, glycyrrhizin treatment produced marked decrease in $AUC_{omeprazole}/AUC_{omeprazole\ sulfone}$ ratios in all subjects, indicating that glycyrrhizin can stimulate the CYP 3A4 activity (Tu *et al*. 2010b).

A decrease of the pharmacokinetic parameters of 18β-glycyrrhetinic 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).

*Assessor's comment: the potential of liquorice root extracts and glycyrrhizin to inhibit or to stimulate the activity of several isoforms of P450 hepatic enzymes both in vitro and in vivo was clear in pre-clinical studies. There is clinical evidence that the oral assumption of 300 mg/day of glycyrrhizin for 14 days can significantly decrease the systemic exposure to midazolam and omeprazole, through*

*induction of CYP3A4-mediated metabolism. Since, based on literature data, there is a high variability of glycyrrhizin intake with different liquorice products, a definite daily intake of glycyrrhizin cannot be predicted according to the posology reported in the monograph; therefore, caution is adviced when the herbal preparations described in the monograph are taken concomitanly with drug known to be substrate of CYP3A4 (e.g. midazolam, ciclosporin A, omeprazole).*

*In addition, inhibition of 11β-hydroxysteroid dehydrogenase by glycyrrhetinic acid may slightly delay the clearance of hydrocortisone and prednisolone and thereby enhance their effects. However, whether a mineralocorticoid or glucocorticoid is a substrate for this enzyme system depends on its chemical structure. Therefore, it cannot be assumed that liquorice will inhibit the inactivation of all corticosteroids (e.g dexamethasone seems to attenuate the mineralocorticoid effects of glycyrrhizin). Precautionally, concomitant use of liquorice preparations reported in the monograph with corticosteroids, which may aggravate electrolyte imbalance is not recommended.*

## *4.2. Clinical efficacy*

### 4.2.1. Dose response studies

Not available.

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

Clinical studies have been carried out on:

- functional dyspepsia

- aphthous stomatitis

- gastric and duodenal ulcers

- postoperative sore throat

- other indications (hyperlipidemia, antiatherogenic effects etc.)

**Clinical studies on functional dyspepsia**

A randomised, double-blind, placebo-controlled study was conducted to evaluate the efficacy of a deglycyrrhizinated acetone extract of *Glycyrrhiza glabra* root, in patients with functional dyspepsia. The patients received either placebo (n = 25) or 75 mg twice daily of extract (n = 25) for 30 days. The primary outcome variables of the study were the change in the severity symptoms (as measured by 7-point Likert scale) and the global assessment of efficacy. The quality of life was evaluated as a secondary outcome measure using the short- form Nepean Dyspepsia Index. In comparison with placebo, the extract showed a significant decrease ($P \leq 0.05$) in total symptom scores on day 15 and day 30, respectively. Similarly, the extract showed marked improvement in the global assessment of efficacy in comparison to the placebo. The verum group also showed a significant decrease ($P \leq 0.05$) in the Nepean dyspepsia index on day 15 and 30, respectively, when compared to placebo (Raveendra *et al.*, 2012).

*Assessor's comment: clinical study carried out with a deglycyrrhizinated acetone extract with a too low number of patients to support the well-established use.*

**Clinical studies on gastric ulcers**

A double-blind, randomized, placebo-controlled therapeutic trial of glycyrrhizinic-acid-reduced liquorice was carried out on 130 men with relapse of chronic duodenal ulcer. Each capsule in the active group contained 380 mg of glycyrrhizinic-acid-reduced liquorice, while the dummy capsules contained lactose coloured with caramel. The dose was two capsules thrice daily after meals for six weeks. In addition to the treatment capsules, each patient was given an alkali. The mean age in verum group was 41 (20-70) y vs 39 (18-63) y in placebo group. The patients were seen at two-weekly intervals. The results were analysed by chi-square test on the basis of the doctors' and the patients' own assessments of symptoms and also with regard to the frequency and intensity of the pain and the quantity of alkali consumed in the three successive two-weekly periods. Only 90 patients (n = 45 each group) completed the six-weeks course of treatment for analysis of the results; 20 patients in each group dropped out for several reasons (mainly pain worsened, missing capsules, dietary card incomplete, defaullted). Analysis of the data culled from the diaries kept by the patients on the frequency and severity of the pain and with regard to the quantity of antiacid consumed showed no significant differences between the active and placebo treatments (British Medical Journal, 1971).

Other two old small clinical studies investigated the effects of deglycyrrhizinated liquorice (DGL) on duodenal or gastric ulcers but failed to show any clinical superiority against placebo in the variations of healing patterns, symptomatic response or relapse rates (Larkworthy *et al.*, 1977; Hollanders *et al.*, 1978).

Ninety-six patients (n = 48 per group; 61M/35 F) with a benign looking gastric ulcer were 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, each 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. 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 (Bardhan KD *et al.*, 1978).

A randomized, double blind placebo-controlled study was conducted to evaluate the efficacy of a dry extract of *G. Glabra* roots (DSR 1:4, extraction solvent acetone) in the management of *H. pylori* gastric load. Indian subjects diagnosed with *H. pylori* infection were randomly assigned to two groups to orally receive 150 mg of extract (n = 55) or placebo (n = 52) once daily for 60 days. Mean age was 32.86 ± 6.50 y in verum group and 33.10 ± 5.59 in placebo group. *H. pylori* infection was assessed using $^{13}$C-urea breath test ($^{13}$C-UBT) at days 0, 30, and 60. Stool Antigen test (HpSA) was also performed on days 0, 30, and 60. Finally, 100 subjects per protocol were analyzed. The proportion of subjects with initial positive $^{13}$C-UBT and HpSA test results found to be negative at day 30 and day 60 was measured. Significant difference in mean Delta Over Baseline values was observed between liquorice extract (n = 50) and placebo (n = 50) treated groups after intervention period (after 60 days, gastric load decreased from 7.12 ± 1.36 to 4.21 ± 1.15 in the liquorice group and from 6.88 ± 1.34 to 6.10 ± 1.30 in the placebo group). At day 0 and day 30 all the subjects in placebo and liquorice treated groups showed positive response to HpSA test and $^{13}$C-UBT. On day 60, the results of HpSA test were negative in 28 subjects (56%) in liquorice treated group and 2 subjects (4%) in placebo treated group; the difference between the groups was statistically significant. On day 60, the results of $^{13}$C-UBT were negative in 24 (48%) in

liquorice and one (2%) in placebo consumed subjects; the difference was statistically significant (Puram *et al.*, 2013).

In a further small clinical study, a standardised dry extract of liquorice was used together with omeprazole, amoxillin and metronidazole in a quadruple regimen for the eradication of *H. pylori* infection in Iranian patients with peptic ulcer. Its effect was compared with the same treatment regimen where liquorice was replaced by bismuth (as salycilate). The use of of liquorice (380 mg bid as tablets) instead of bismuth gave comparable responses to treatment (Momeni *et al.*, 2014).

A further study evaluated the effect of liquorice in *H. pylori* eradication in Iranian patients suffering from dyspepsia either with peptic ulcer disease (PUD) or non-ulcer dyspepsia (NUD) in comparison to the clarithromycin-based standard triple regimen. In this randomized controlled clinical trial, 120 patients who had positive rapid urease test were included. Sixty patients were randomly assigned to receive one of two treatment regimens: triple regimen consisting of clarithromycin (500 mg BID) + Amoxicillin (1 gr qd) + 20 mg BID of Omeprazole (control group) or the same regimen supplemented by liquorice (380 mg BID) for two weeks (LR group). Both groups received at least four weeks of treatment with omeprazole 20 mg daily subsequently. Two weeks after completing the treatment, *H. pylori* eradication was assessed with HpSA test. Out of 120 eligible patients, 110 patients (n = 54 per group) completed the study complying with the prescribed medications and follow-up. HpSA negative was 83.3% in the LR group compared to 62.5% in the control group (p = 0.018). Response rate based on endoscopic findings was significantly higher for the LR group to treat PUD (85% in LR group vs 50% in control group), but not for NUD (82.4% in LR group vs 67.5% in control group) (Hajiaghamohammadi *et al.*, 2016).

*Assessor's comment: liquorice has been traditionally used for the treatment of gastric ulcers; however, the evidence of clinical efficacy are conflicting. Old clinical studies failed to show any benefit compared to placebo, whereas only a limited number of trials carried out in the last thirty years showed some positive results for liquorice, mainly when used as add-on to a therapeutic regime for the eradication of H. pylori (i.e. Puram et al., 2013; Momeni et al., 2014; Hajiaghamohammadi et al., 2016). These clinical studies cannot support a well-established use indication, due to severe methodological deficiences, such as small sample size, lack of blinding/randomisation or lack of information on statistical analysis. In addition, in most studies the herbal preparation was not fully described.*

**Clinical studies on postoperative sore throat**

Post-Operative Sore Throat (POST) is an undesirable side effect of endotracheal intubation. Liquorice has been used as an expectorant in cough and cold preparations.

Adult Austrian patients (n=236; aged 18 to 90 years) undergoing elective thoracic surgery requiring a double-lumen endotracheal tube were randomly assigned to gargle, 5 minutes before induction of anesthesia for 1 minute, with: (1) Extractum Liquiritiae Fluidum (liquorice 0.5 g); or (2) Sirupus Simplex (sugar 5 g); each diluted in 30 mL water. Sore throat was evaluated 30 minutes, 90 minutes, and 4 hours after arrival in the postanesthesia care unit, and the first postoperative morning using an 11-point Likert scale (0 = no pain; 10 = worst pain) by an investigator blinded to treatment. The overall treatment effect across the 3 measurements was the primary analysis. The incidence of POST was significantly reduced in patients who gargled with liquorice rather than sugar-water. The corresponding estimated treatment effects (relative risks) were 0.54 (95% CI, 0.30–0.99, liquorice versus sugar-water; P = 0.005), 0.31 (0.14–0.68) (P < 0.001), and 0.48 (0.28–0.83) (P < 0.001). The overall RR of experiencing sore throat across the 3 measurements during the first 4 hours after surgery was estimated as 0.46 (95% CI, 0.29–0.72,

liquorice versus sugar-water; P < 0.001), as analyzed with repeated-measures analysis of variance (Ruetzler *et al.*, 2013).

The positive results of the above-mentioned clinical study were confirmed by a subsequent meta-analysis of 5 randomized controlled trials (Agarwal 2009; Gupta 2013; Ruetzler 2013; Honarmand 2015; Ibrahim 2017) which assessed the effects of the topical application for prevention of postoperative sore throat. Clinical studies that included 574 participants provided data on the incidence of sore throat at 24 h after surgery/extubation. Topical application of liquorice was associated with a reduced incidence of post-operative sore throat (RR, 0.44; 95% CI, 0.28–0.69; P < 0.001; I2=63.6). The number needed to prevent postoperative sore throat was 4 (95% CI, 3–7). Three trials involving 330 participants reported the severity of sore throat at 24 h after surgery/extubation. One study suggested that topical application of liquorice was associated with decreased severity of post-operative sore throat (SMD, −0.69; 95% CI, −0.96, −0.43; P < 0.001). The two other studies also reported that the severity scores were significantly lower in the liquorice group than in the control; however, there were no data that could be pooled. Subgroup analysis by liquorice dose found that there was a significant dose-response relationship (P=0.010). As the liquorice dose increased, the effect size became larger (Fig. 4). The subgroup using 1 g of liquorice showed a significantly larger effect size than that using 0.25 g or 97 mg of liquorice. Although the trial sequential analysis suggested that the evidence of liquorice for prevention of POST was adequate, the sensitivity analysis using Hartung-Knapp-Sidik-Jonkman method suggested that topical application of liquorice might not significantly prevent POST. (Kuriyama & Maeda, 2019).

*Assessor's comment: In a well-designed double-blind, randomised, placebo-controlled clinical trial, a single water gargle of a fluid extract of liquorice was effective in reducing the incidence of post-operative sore throat when applied to adult patients undergoing elective thoracic surgery requiring a double-lumen endotracheal tube (Ruetzler et al., 2013). However, the fluid extract is not fully described and there are no medicinal products on the market containing liquorice authorised with this therapeutic indication. In addition, the effectiveness of a single liquorice water gargle applied before other types of surgery with different patient's positions is still to be demonstrated. Other clinical studies present severe methodological shortcomings (e.g. small sample size and lack of blinding), thus limiting the clinical relevance of the meta-analysis by Kuriyama & Maeda, 2019 to justify a WEU.*

**Clinical studies on oral mucositis (OM)**

Mucositis is a major complication of irradiation in head and neck tumors, the addition of chemotherapy to irradiation may enhance this dose-limiting problem. The therapeutic effects of liquorice in OM was investigated in small clinical studies carried out on Iranian patients with head and neck cancer (HNC) undergoing postoperative radiotherapy (RT). In the first study (Ghalayani *et al*., 2017), triamcinolone acetonide (T) and liquorice root extract (L) mucoadhesive films were applied to patients with mucositis of WHO scales 2 and 3 for 4 weeks. Comparison of incidence, severity and duration of OM between two groups demonstrated no meaningful difference in any consecutive week. In the second study (Najafi et al., 2017), patients received 20 cc aqueous extract of liquorice root applied topiccaly twice a day or the same amount of placebo from the first day of starting radiotherapy until the end of the second week. After 14 days, the difference in the grade of OM was significant between the two groups and favoured liquorice group (P<0.001). In addition, liquorice extract after 14 days of treatment reduced significantly mucosal irritation and mucosal wound size compared to placebo.

The effect of liquorice root extract mouthwash with combined mouthwash on the incidence and severity of chemotherapy-induced mucositis symptoms was also investigated in colon cancer

Iranian patients treated with chemotherapy (patients who had undergone radiotherapy in addition to chemotherapy were excluded). Patients in the control group received routinely used combined mouthwash. Both mouthwash solutions were administered every eight hours daily at a dose of 10 cc from the first day of chemotherapy for one week. The intervention and control groups had no significant difference on the first, third, and seventh days of treatment in the incidence and the severity of mucositis (Sattari *et al.*, 2019).

*Assessor's comment: the evidence of clinical efficacy of liquorice is conflicting and the relevance of the clinical studies is poor due to the small sample size of the studies. Finally, the herbal preparations have not been fully described. therefore, a WEU in oral mucositis is not supported.*

**Clinical studies on apthous stomatitis**

Recurrent aphthous stomatitis (RAS) are a common and painful condition. Six clinical studies (Martin *et al*., 2008, Moghadamnia *et al.*, 2009, Galal *et al.*, 2012, Raeesi *et al.*, 2015, Salehi *et al.*, 2017, Akbari *et al.*, 2020) have investigated the effect of liquorice in the treatment of RAS. Different extracts of liquorice roots obtained by extraction with water, chloroform, methanol/water 7:3, ethanol/water 7:3 or deglycyrrhizinated liquorice dry extract (prepared by roots defatted with n-hexane and then macerated with 50% methanol solution; deglycyrrhinization was done in acidic medium) were used in these studies. The extracts were applied from one to four times daily on the lesions in the form of patches, mucoadhesive pastes or mucoadhesive tablets with treatment periods ranging from five to eight days or until the complete healing of the oral lesions. Liquorice effect was analysed against active comparators, placebo or no-treatment groups. An active placebo group, which received a patch with powdered star anise fruit (which tastes like liquorice) was used in one clinical study (Martin *et al*., 2008). The placebo patch was designated as an "active" placebo since star anise has been shown to have a very mild antibiotic effect, which may have affected the course of the lesions in some way. In another study (Galal *et al.*, 2012), *Acacia nilotica*, Acacia & Liquorice, and control group were included together with the liquorice group. In the study by Raeesi *et al.*, 2015 the effect of a bioadhesive patch containing liquorice was compared to dephenhydramine mouth wash or to a bioadhesive patch containing placebo. A diphenhydramine elixir was also used as an active comparator in the study by Akbari *et al.*, 2020, whereas only comparison versus placebo was carried out in the study by Salehi *et al.*, 2017. Finally, in the sixth study 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 patch without liquorice and bioadhesive with liquorice, respectively (Moghadamnia *et al.*, 2009). Ulcer size and pain together with healing time were the efficacy measures. Liquorice was superior to star anise in the reduction of ulcer size and pain; licorcie also significantly reduced pain and the diameter of the inflammatory halo and necrotic center compared with the placebo group. Liquorice alone and in combination with acacia significant increased the salivary epidermal growth factor (EGF) levels and significantly reduced mean pain score and mean ulcer size compared to acacia or placebo. Liquorice also significantly shortened healing times compared to placebo. Finally, licorcice was superior to diphenhydramine in reducing pain score and in improving the average duration of wound healing.

*Assessor's comment: all six clinical studies showed that liquorice in different dosage forms could reduce the pain and the inflammation associated to RAS, thus accelerating the healing. However, due to the reduced number of patients enrolled and the use of different liquorice extracts (in most cases*

*not fully described) a well-established use is not supported. The promising results obtained in these small studies should be confirmed in larger clinical trials.*

**Clinical studies on dental caries and gengivitis**

Dental caries (tooth decay) is caused by a specific group of cariogenic bacteria, *like Streptococcus mutans*, which convert dietary sugars into acids that dissolve the mineral in tooth structure. Killing cariogenic bacteria is an effective way to control or prevent tooth decay.

Conflicting results were obtained from pilot clinical study ivestigating the ability of lollipops containing ethanolic dry extracts of liquorice roots or liquorice gel on reducing the count of salivary *S. mutans* (Hu et al., 2011, Söderling *et al.*, 2006).

The anti-inflammatory effect of two mouthwashes of liquorice and chlorhexidine (CHX) in 75 Iranian patients aged from 20 to 40 years old with plaque-induced gingivitis was compared in a randomized double-blind clinical study. Subjects were randomly divided into 3 groups (n = 25 each), the first group received 1% liquorice mouthwash, the second group received CHX mouthwash at a concentration of 0.2% and the third group received a placebo mouthwash. Patients were instructed to have a mouthwash of 10 ml twice a day (morning and evening) for 30 seconds in combination with daily oral hygiene (brushing using the Bass method with whitening toothpastefor 3 weeks. Follow-up sessions were performed in the first week and the third week after using mouthwash to record plaque (plaque index = loe and silness index) and gingival (loose and silness index) index. In intra-group comparison, plaque index significantly decreased in all three groups (P= 0.001). There was no significant statistical difference between the liquorice group and the CHX group compared to placebo group at the beginning of the study and the first week, but the difference became statistically significant after three weeks. The maximum effect for reduction of plaque index was in the CHX group but this was not statistically different compared to liquorice group. The same rend was observed for gingival index (Molania *et al.*, 2019).

Recently, a further study assessed the effectiveness of a mouth rinse with 20% (w/v) of aqueous liquorice root extract against dental plaque and gingivitis and compared it with 0.2% CHX gluconate mouth rinse. In this double-blind, concurrent parallel randomized controlled clinical trial 44 Indian volunteers 18-40 years old with moderate gingivitis, having at least 20 natural functional teeth without any restorations, orthodontic appliances, and habitual mouth breathing were randomized into two groups (n = 22, each group) through the computer-generated random sequence. Both the groups were asked to rinse with their respective mouthwash twice daily for 15 days. Gingivitis was evaluated using gingival index (GI), and dental plaque was evaluated using the Turesky modification of the Quigely Hein Plaque Index (PI). The evaluation was carried out at day zero, 8$^{th}$ and 23$^{rd}$ (15 days after intervention). There was a statistically significant (P = 0.000) reduction in mean PI and GI scores for both the groups after a follow up of 15 days; however, the reduction was considerable in CHX group as compared to liquorice group and their differences was statistically significant (P < 0.001) for both the groups after 15 days (Sharma *et al.*, 2022).

*Assessor's comment: Very small studies were carried out with different liquorice preparations (not fully described), therefore the potential efficacy of liquorice in reducing dental caries and gengivitis in adults appears uncertain and needs to be investigated in larger randomised, double-blinded clinical studies.*

**Clinical studies on periodontitis**

The efficacy of *G. glabra* in the prevention and cure of periodontitis was assessed in a randomized clinical control trial on 104 Indian patients with mild to moderate periodontitis. For measuring

periodontal status, gingival bleeding, periodontal pocket, and loss of attachment parameters were used. Patients were randomly divided into two groups – experimental group (patients who received treatment *G. glabra* gum paint) and control group (patients who received no treatment). The gums contained of a dry hydroalcoholic extract prepared by maceration of dried grounded roots of *G. glabra* with a mixture of ethanol in water (30:70). Concentration of glycyrrhetic acid in the extract was 28% as determined by a UV spectrophotometer. The crude extract was dissolved in glycerine and was present in the gum at a concentration of 10% V/V. Patients were followed for 1 month. Patients of the experimental group were instructed to massage the gums by *G. glabra* gum paint two times a day after brushing (morning and night). All patients (experimental and control) were given toothpaste and brush to exclude biases. Pre-intervention and post-intervention scores were taken. Gingival bleeding, depth of periodontal pocket, and loss of attachment significantly reduced in patients of the experimental group compared to the pre-intervention, whereas, in patients of the control group, mean scores were improved as they were following good oral hygiene as instructed by a specialist, but improvement was not significant (Madan *et al.*, 2019).

*Assessor's comment: study not blinded; no information on statistical analysis. Poor clinical relevance.*

**Clinical studies on xerostomia**

Dry mouth (xerostomia) is a common symptom in hemodialysis patients, which is associated with a reduced salivary flow. A single-blind randomized controlled trial was carried out in hemodialysis patients to assess the effect of a liquorice mouthwash in reducing xerostomia and improve saliva flow rates. Taiwanese patients aged ≥ 20 years old (average age was 60.79 y) who felt mouth dryness in the past 4 weeks were randomly assigned to one of three groups: pure water mouthwash; n = 41, liquorice mouthwash (8.34 g of liquorice concentrate in 500 cc of pure water); n = 48, or no mouthwash (control); n = 37. Participants were instructed to use the mouthwash 30–60 min after each meal (thrice daily). The Summated Xerostomia Inventory, and unstimulated whole salivary flow rate measured dry mouth and salivary flow, respectively. Data was collected at baseline, dialysis Day 5 and Day 10. There was no significant reduction in xerostomia compared to the baseline or the control group with pure water mouthwash after 5 (15.7 ± 3.7) and 10 days (15.5 ± 3.8) of treatment. However, the liquorice mouthwash resulted in a significant reduction in SXI scores compared to baseline at Day 5 (12.0 ± 4.7; $p < 0.001$) and Day 10 (11.2 ± 4.6; $p < 0.001$) compared to the control and the pure water mouthwash group. There was no changed in the unstimulated flow rate for the control group at Day 5 (0.06 ± 0.06) or Day 10 (0.05 ± 0.05) compared to baseline. However, a small improvement was seen in participants using the pure water mouthwash compared to controls at both time points (0.08 ± 0.07, 0.08 ± 0.06, Day 5 and Day 10, respectively; $p < 0.05$). A more significant improvement in unstimulated salivary flow rate ($p < 0.001$) was seen in the participants using the liquorice mouthwash: Day 5 = 0.18 ± 0.12, Day 10 = 0.19 ± 0.13) (Yu *et al.*, 2016).

*Assessor's comment: clinical relevance of this study is limited due to the sample size of the groups and the lack of a double-blind design.*

**Clinical studies on asthma**

In a clinical study 80 Egyptian patients with chronic stable moderate bronchial asthma were randomised in two groups (n = 40, each group), group 1 (Placebo group) maintained on inhaled corticosteroids (ICs, fluticasone or budesonide in moderate to high doses) and long-acting beta agonist (LABA, salmeterol or formoterol) and received starch capsule (500 mg starch) twice daily as placebo and group 2 (active treatment group) maintained on the same asthma treatment as group 1 in addition to 500 mg aqueous liquorice extract capsule (equivalent to 100 mg

glycyrrhizin) taken twice daily. The median for age in group 1 was 26.5 (25-34), while in group 2 was 30 (23.25-40).The efficacy of liquorice was measured by estimation of pulmonary function and percentage of blood eosinophils. The addition of liquorice capsules to ICs and LABA resulted in a non-significant improvement in blood eosinophils (P = 0.754). However, it resulted in a highly significant improvement in Forced Vital Capacity % and Forced Expiratory Volume1 % when compared to group 1 (P = 0.031 and 0.040, respectively) after 3 weeks of treatment (Sadek *et al.*, 2019).

*Assessor's comment: clinical relevance of this study is limited due to the sample size of the groups and the lack of blinding.*

**Clinical study on non-alcoholic fatty liver disease (NAFLD)**

The effects of liquorice on NAFLD were assessed in a double blind randomized clinical trial involving 66 Iranian patients, aged 21 to 56 years, with pathology confirmed by confirmed by sonography and presence of elevated levels of AST and ALT. Patients were divided into two groups (n = 33, each group) randomly (by using a random number table). The case group was treated with one capsule containing 2 g aqueous liquorice root extract alone (20% glycyrrhizin) per day for 2 months, while the control group was treated in the same manner with capsules containing placebo (2 g starch). In the case group, the mean ALT level decreased from 64.09 to 51.27 IU/mL and the AST level decreased from 58.18 to 49.45 IU/mL, which were statistically significant ($p < 0.001$ for both). In the control group, the mean ALT level decreased from 66.90 to 62.77 IU/mL and the AST level decreased from 57.86 to 54.81 IU/mL ($p > 0.05$ for both). The BMI difference before and after the study was not statistically significant in both groups (Hajiaghamohammadi *et al.*, 2012).

The effects of liquorice root supplementation on liver enzymes, hepatic steatosis, metabolic and oxidative stress parameters in Iranian women with NAFLD was also evaluated more recently in a further randomized double-blind, placebo-controlled trial. Sixty women, aged 18 to 65 years, with NAFLD were selected and randomly assigned into two groups (n = 30 each group) to take 1000 mg/day of liquorice root extract powder (24% monoammonium glycyrrhizinate) as orally two capsules daily (each containing 500 mg) before breakfast and at bedtime or placebo for 12 weeks. In addition, all the patients were advised to follow a weight loss diet and healthy lifestyle. The diagnosis of NAFLD was made on the basis of the presence of steatosis, on ultrasound examination, associated with a persistently elevated ALT concentration of >60 U/L for 6 month before the study and at the time of randomization. Through the 12-weeks period of supplementation, women who received powder of liquorice root experienced a statistically significant improvement in ALT ($p < 0.001$), insulin ($p = 0.002$), insulin resistance ($p = 0.003$), MDA ($p < 0.001$) serum levels, and ultrasonographic findings of liver steatosis ($p < 0.001$), compared to the placebo group. No significant changes compared to the control group were observed for TG, GGT, AST, TC, LDL-C, HDL-C, and fasting blood sugar. Finally, weight decreased significantly in the liquorice and control groups but the difference was not significant between the groups (Rostamizadeh *et al.*, 2022).

*Assessor's comment: liquorice in a couple of clinical trials significantly reduced liver enzymes, but histopathological examination and the assessment of inflammatory factors were missing. In addition, the the diagnosis of liver disease was not confirmed by liver biopsy in both studies. Therefore, further studies are needed to ascertain the role of liquorice in NAFLD.*

**Clinical studies on obesity and prevention of atherosclerosis**

The effect of an extract of liquorice roots in lowering body fat mass was studied in 15 normal-weight subjects (7 males, age 22-26 yr, and 8 females, age 21-26 yr), who consumed for 2

months 3.5 g a day of a commercial preparation of liquorice. Body fat mass (BFM, expressed as percentage of total body weight, by skinfold thickness and by bioelectrical impedance analysis, BIA) and extracellular water (ECW, percentage of total body water, by BIA) were measured. BMI did not change. ECW increased (males: 41.8±2.0 before vs 47.0±2.3 after, $p<0.001$; females: 48.2±1.4 before vs 49.4±2.1 after, $p<0.05$). BFM was reduced by liquorice: (male: before 12.0±2.1 vs after 10.8±2.9%, $p<0.02$; female: before 24.9±5.1 vs after 22.1±5.4, $p<0.02$); plasma renin activity (PRA) and aldosterone were suppressed. Liquorice was able to reduce body fat mass and to suppress aldosterone, without any change in BMI (Armanini et al., 2003a).

The effect of ingestion of a single 600 mg dose of liquorice flavonoid oil (LFO) on energy metabolism, including fat oxidation, was investigated by measuring body surface temperature under resting conditions and respiratory gas analysis under exercise conditions in 34 healthy Japanese females. The ingestion of LFO elevated body trunk skin temperature when measured in a slightly cooled air-conditioned room, and increased oxygen consumption and decreased the respiratory exchange ratio as measured by gas analysis during 40% maximal oxygen uptake ($Vo_{2max}$) exercise with a cycle ergometer. Furthermore, repeated ingestion of 300 mg of LFO for 8 days decreased respiratory exchange during the recovery period following 40 minutes of 30% $Vo_{2max}$ exercise on a treadmill. The authors concluded that LFO enhanced fat oxidation in humans during light exercise (Mori et al., 2015).

The effect of liquorice-root extract on carotid intima-media thickness (CIMT) was investigated in a randomized longitudinal cohort placebo-controlled study on 110 Israeli individuals with hypercholesterolemia (TC $\geq$ 6.18 mmol/L [240 mg/dL]) and without significant stenosis, hypertension, diabetes mellitus, and ischemic heart disease. Subjects were randomly allocated to two groups: an experimental group (n = 59; mean age 61.5 ± 16.3 years) that consumed 0.2 g/day of a deglycyrrhizinated ethanolic extract of liquorice root for 12 months, and a control group (n = 51; mean age 60.4 ± 15.7 years) that received a placebo. Ninety-four subjects completed the study. CIMT is defined as the distance between the luminal intima and medial adventitia interfaces at the distal 10 mm of the common carotid artery. Mean CIMT was 0.92 ± 0.25 mm at baseline in the experimental group and was significantly lower after one year (0.84 ± 0.21 mm, $p = 0.000$). In the control group, mean CIMT at baseline was 0.85 ± 0.17 and was significantly higher after one year (88 ± 0.19). Mean plasma TC levels and LDL cholesterol decreased, at the range baseline to 1 year, from 284 ± 32 mg/dl to 262 ± 25 mg/dl and from 183 ± 8.5 mg/dl to 174 ± 9.1 mg/dl, respectively, for the experimental group ($p < 0.001$) and from 291 ± 35 to 289 ± 31 mg/dl and from 177.6 ± 10.7 to 179.3 ± 9.6 ($p = 0.08$), respectively, for the control group. Mean HDL did not change significantly in either group. In the experimental group, SBP decreased from 138 ± 12 mmHg to 125 ± 13 mmHg after 1 year ($p=0.01$) and increased from 136 ± 15 mmHg to 137 ± 13 mmHg in the control group. Diastolic blood pressure (DBP) decreased from 92 ± 9 mmHg to 84±10 mmHg ($p=0.01$) in the experimental group and increased from 89 ± 11 mmHg to 90 ± 8 mmHg in the control group (Fogelman *et al.*, 2016).

The metabolic changes after liquorice consumption were analysed through a systematic review with meta-analysis and Trial Sequential Analysis (TSA) of 15 clinical trials (Aoki *et al*. 2007; Armanini *et al.* 2005; Armanini *et al*. 2007; Bell *et al*. 2011; Chigurupati *et al*. 2016; Fuhrman *et al*. 2002; Kinoshita *et al*. 2016; Leskinen *et al*. 2014; Mirtaheri *et al*. 2015; Namazi *et al*. 2017; Panda *et al*. 2017; Raveendra *et al*. 2012; Serra *et al*. 2002; Tominaga *et al*. 2006; Tominaga *et al*. 2009). To be included in this systematic review, studies were to accomplish the following criteria: to be clinical trials in humans, to present a true control group, to show the results of the outcomes at the baseline and at the end of the trial, and to indicate the standard deviation of the measurements.

Some studies were divided into different trials based on different dosages of liquorice used, different durations of treatment, different type of patients enrolled or different genetic polymorphism (Pro/Ala or Pro/Pro genotypes): this implied that finally a total of 26 clinical trials were considered for the quantitative synthesis of the data, totalizing 985 patients enrolled. The patients subjected to the intervention with liquorice were mainly healthy and overweight volunteers. Other types of patients were also enrolled, namely women with PCOS, hypercholesterolemic patients and elderly people. The intervention duration ranged from 2 to 16-weeks, but 4 and 8-weeks were more usual. Liquorice was administered to the patients in several ways, being the capsules of LFO the most common option. Many of the included studies were performed in Japan, but other countries around the world were also considered. Threee studies (Armanini *et al*., 2007; Fuhrman *et al*., 2002; Leskinen *et al*., 2014) were non-randomizedOnly 7 trials detailed the randomization process and so were classified as "Low risk" in the random sequence generation domain and several studies were classified as "unclear" with respect to selection, performance and detection bias. In addition, other sources of bias were found, namely because of some funding enterprises that belonging to the researchers, and which can skew the results obtained. The primary outcomes considered in this meta-analysis included the body weight, BMI, lipid profile (TC, TG, HDL and LDL) and blood pressure (SBP and DBP) of the participants in the clinical trials. Overall, liquorice consumption significantly reduced the body weight (weighted mean differences, WMD: −0.433 kg; 95% CI: −0.683 to −0.183; p= 0.001) and consequently the BMI of patients (WMD: −0.150 kg/m$^2$; 95% CI: −0.241 to −0.058; p =0.001); nevertheless, moderate heterogeneity ($I^2$=58.373%) was observed among the trials for body weight, contrariwise to the high heterogeneity observed for the BMI ($I^2$=89.555%). Another obtained result with statistical significance was the increase in DBP (1.737 mmHg; 95% CI: 0.835 to 2.621; p <0.0001) observed in the group which was subjected to liquorice consumption, although high heterogeneity ($I^2$=96.189%) was found among the results of the different clinical trials considered. Results of TSA indicated that the evidence needed to reach a conclusion was sufficient and no further trials were needed; thus, additional trials are not required and are unlikely to alter the conclusions obtained in this work. Subgroup analysis showed that only for 4 and 8-weeks of treatment with liquorice, a significant reduction of body weight was observed, wherease a significant reduction of BMI was obtained only with a treatment duration of 8 weeks. In addition, subgroup analysis also showed that the reduction in body weight and BMI was significant only in overweight patients. A significant increase in SBP and DBP was also verified for 2 and 8-weeks. In what concerns to the type of patient, a significant reduction of BMI was observed for overweight people. Moreover, in healthy patients and in women with polycystic ovary syndrome a significant increase of both SBP and DBP was verified. Considering the dosage of liquorice/day, in general, for the different concentrations, there was a significant increase of SBP and DBP, accompanied by a significant reduction of body weight and BMI (Luis *et al.*, 2018).

*Assessor's comment: a number of clinical trials have been carried out, mainly in overweight/obese subjects, to assess the effects of liquorice on biochemical parameters and lipid profile; their relevance is limited by the small size. A meta-analysis including most of these studies showed that liquorice consumption significantly reduced the body weight and consequently the BMI of overweight patients, but the moderate/high heterogeneity of the results based on $I^2$ testing does not allow any definitive conclusion on the beneficial effects of liquorice.*

**Clinical studies on ischemic stroke**

The benefit of liquorice treatment in patients with ischemic stroke was evaluated in a randomized double-blind placebo-controlled trial. Ninety-two adult Iranian patients were prescribed oral 450

mg (n = 52) or 900 mg (n = 57) liquorice extract or placebo capsules (n = 50) three times daily for 7 days. Capsules contained a dry water extract obtained from dried, roasted and grounded liquorice root, with a final mean value of 7.85% by mass glycyrrhizic acid. National institute of Health stroke scale (NIHSS) and Modified Rankin Scale (MRS) scores were assessed before initiation of therapy and 3 months after treatment for evaluation of neurologic deficits in patients with stroke. The study population with data and follow-up available for the analysis consisted of 75 patients (mean age of 65.7 years) who were equally distributed in 3 medication groups. Mean NIHSS scores in 450 mg and 900 mg groups decreased from an initial score of 10.68 and 10.44 to 6.4 and 5.48 after 3 months, respectively, while in the control group changed from 8.36 to 5.64. The decline in NIHSS scores were significantly greater in liquorice treated groups than the control group (P = 0.003). Similarly, the decrease in MRS was greater in the liquorice treated groups (4.2–2.9 in 450 mg liquorice group, and 4.4–2.8 in 900 mg liquorice group) versus the control group (3.9–2.8), but it was not statistically significant (P = 0.524) (Ravanfar *et al.*, 2016).

*Assessor's comment: the small number of patients included in the study, the high number of drop-out and the lack of radiological imaging studies significantly limit the clinical evidence for the beneficial effect of whole liquorice extract in neurologic improvement of patients with acute ischemic stroke.*

**Clinical studies on primary dismenorrhea**

In a clinical study with a randomized, active controlled and triple-blind design, 60 patients aged 18-25 years old with moderate and severe dysmenorrhea were randomly divided into two groups; one group received 400 mg Ibuprofen tablets every 8 h and placebo syrup and the other received 5 cc *G. glabra* syrup (150 mg/mL) two times a day and placebo tablets. The grade of dysmenorrhea was considered moderate in case of painful menstruation with influence on daily activity and use of analgesics for pain relief, and severe in case of painful menstruation with significant limitation on daily activity, poor effect of analgesics, and systemic symptoms such as headache, tenderness, nausea, vomiting, and diarrhea. Patients took the drugs from the first day of menstruation to fifth for two consequent cycles. The primary pain intensity and its changes were evaluated by 10 cm VAS in each group and compared between two groups. A total of 26 students in the *G. glabra* group and 24 in the Ibuprofen group completed the treatment process. The results showed a significant difference in the pain score before and after treatment in both groups of *G. glabra* (mean difference 5.85 ± 3.11; $p < 0.001$) and Ibuprofen (mean difference 6.92 ± 1.87; $p < 0.001$), but there was no significant difference in the pain relief between *G. glabra* and Ibuprofen groups (p = 0.151) (Jafari *et al.*, 2019).

*Assessor's comment: study with poor clinical relevance due to the small sample size, high rate of drop-out and limited duration. The herbal preparation used in this study was not described.*

**Clinical studies on polycystic ovary syndrome (PCOS)**

The effect of spironolactone (SP) versus spironolactone plus liquorice on plasma renin activity, aldosterone and androgen levels was assessed in 32 hirsute women (median age 24, range 21–28 years) with PCOS. Women were alternately assigned to receive 100 mg a day of SP (n = 16) or 100 mg of SP plus 3.5 g a day of a dried extract of boiled liquorice root containing 7.6% (w/w) of glycyrrhetinic acid corresponding to a daily dose of 265 mg. Serum potassium, total and free testosterone, androstenediol glucuronide (a metabolite of di-hydrotestosterone), plasma renin activity (PRA), aldosterone, cortisol and sex hormone binding protein (SHBG) were measured before therapy and after 4, 7, 30, and 60 days of therapy. Before and at the end of treatment 24-h urine was collected to measure tetrahydrocortisol (THF), allo-tetrahydrocortisol (allo-THF), and

allo-THF/cortiosol ratio (THE) to have an indirect index of liquorice effect on 11b-hydroxysteroid dehydrogenase type 2. Serum potassium did not change during either of the treatments. Twenty percent of women treated with SP and none treated with the addition of liquorice complained of symptoms related to volume depletion. Percentage increases in both PRA and aldosterone were significantly higher in the SP group than in SP plus liquorice. The prevalence of metrorrhagia was lower in the combined therapy. There were reduced concentrations of testosterone during the first four days of treatment at 103 ± 29 ng/d in the SP group compared to 91 ng/d (±19) in the SP + liquorice group. Plasma cortisol increased significantly after 1 and 2 months with both therapies. Urinary THF and allo-THF/THE) did not change significantly with the two treatments. The authors concluded that the mineralocorticoid properties of liquorice can reduce the prevalence of side effects related to the diuretic activity of SP in patients with PCOS (Armanini *et al.*, 2007).

*Assessor's comment: no conclusion can be drawn of the ability of liquorice to reduce the side effects of spironolactone in patients with PCOS due to the severe methodological limitation of this study (i.e. low number of patients studied; the study was neither randomised nor blinded; lack of a placebo arm).*

**Clinical studies on idiopathic hirsutism**

A double-blind, randomized placebo-controlled study was performed on 90 female subjects to compare the effect of 755 nm alexandrite hair removal laser with that of alexandrite laser plus topical gel containing liquorice on the improvement of idiopathic hirsutism. AThe patients were divided into two randomization groups: alexandrite laser plus gel containing 15% of an ethanol (80%) extract of liquorice root (n = 45) (group designated as A) and alexandrite laser plus placebo (n = 45) (group designated as B). Each subject received one of both products over one side of the face, twice daily for 24 weeks on the hirsute locations. Each group underwent five sessions of alexandrite laser at 6-week intervals. The primary outcome was the changes in terminal hair density. The terminal hair was counted by the investigator using manual magnification on the treatment and control sites at baseline and on each follow-up visit. The counting was assessed by marking each counted hair with a pen to ensure that each hair was only registered once. To minimize the effects of confounding variables, the test is performed on two separate zones of patients' skin (in center and periphery of hirsute area of the face). The mean ± SD numbers of terminal hairs in group A were 7.05 ± 4.55 for zone 1 and 6.06 ± 3.70 for zone 2. In group B, they were 3.18 ± 1.75 for zone 1 and 2.49 ± 1.63 for zone 2. The difference in the mean number of terminal hairs was statistically significant ($p < 0.001$) between the two groups (Faghini *et al.*, 2015).

*Assessor's comment: clinical study with limited clinical relevance due to the small number of patients in each group. The DER of the ethanol extract of liquorice root was not reported.*

**Clinical studies on (post)-menopausal women**

Liquorice root is known to contain phytoestrogens, which bind the oestrogen receptor (ER) and activate a downstream cascade of oestrogendriven cellular changes, including cell proliferation and growth. For a long time, liquorice has been used for the symptomatic control of menopause because of these oestrogenic effects (Harding & Stebbing, 2017).

The effectiveness of a liquorice extracts on menopausal symptomatology was assessed in a couple of double-blind, placebo controlled clinical studies. In the first trial, carried out on 51 symptomatic peri- and menopausal Iranian women, treatment with liquorice (50 mg or 100 mg) showed not statistically significantly difference in reducing moderate hot flushes compared to placebo after 12

months ((Baker *et al*., 2011, only abstract available). In the second study, 90 menopausal women complaining of hot flashes received 3 capsules daily containing 330 mg liquorice extract or placebo over 8 weeks of intervention with 4 weeks of follow-up. Differences in the frequency of hot flushes were statistically significant between liquorice group and placebo group for the whole treatment period and up to 2 weeks after the intervention. Hot flashes severity decreased in liquorice and in placebo groups before and 1 week after the intervention. However, the decrease was continued until 2 weeks after the therapy in the liquorice group while it was only significant in the placebo group in the 1st week of therapy and from the 2nd week to the end of follow-up stage, no significant difference was found compared to the beginning of the study (Nahidi *et al.*, 2012).

The effects of liquorice on hot flash symptoms in menopausal women were also evaluated in a further randomized, double blind clinical study on 52 menopausal Iranian women aged from 45 to 60 years old, who were in the first 5 years post-menopause. Each patient was allocated randomly to liquorice 1140 mg/day or HRT (conjugated estrogen 0.312 mg/day and Medroxyprogesterone 2.5 mg/day) groups for a treatment period of 90 days. Liquorice was administered in the tablet form containing 650 mg liquorice root extract (Glycyrrhizin level was 3%). There was a significant reduction in the duration of hot flashes in liquorice and HRT groups ($p = 0.018$, $p = 0.062$, respectively), while the duration of hot flashes had a greater reduction in the liquorice group. No significant reduction was observed in the duration of hot flashes in the liquorice group compared with the HRT group after treatment ($p = 0.153$). The number of hot flashes significantly decreased in the HRT group ($p = 0.008$), but no significant reduction was observed in the liquorice group ($p = 0.157$). Nevertheless, there was no significant difference in reduction of number of hot flashes between the two groups ($p = 0.134$). The severity of hot flashes in the liquorice group was not significantly reduced, but there was a significant reduction in the HRT group ($p = 0.698$, $p = 0.031$, respectively). In addition, a significant difference was observed in reduction of the severity of hot flashes between two groups ($p = 0.019$) (Menati *et al.*, 2014).

In a randomised controlled trial, 120 menopausal Iranian women aged 48-52 years with a minimum of 1 year and a maximum of 3 years since their last menstrual cycle were divided into 4 groups of 30 subjects. Group 1 participants were administered 3 tablets each containing 380 mg *Glycyrrhiza glabra* extract daily. Group 2 participants had a regular exercise program like regular walking 3 sessions (even days) per week at 9 am for 4 weeks. Group 3 participants were simultaneously administered *Glycyrrhiza glabra* tablets like group 1 and had an exercise program like group 2. Group 4 received no intervention. The participants' QOL was investigated before and 1 month after the intervention using the Menopause-Specific Quality of Life (MENQOL) Questionnaire. Kruskal-Wallis test, Mann-Whitney test was applied to compare the mean scores of the groups. Significant differences were observed between groups 1 and 3 after the intervention in terms of the sexual dimension ($P = 0.004$) and QOL ($P = 0.001$). The results showed a significant difference between *Glycyrrhiza glabra* and controls after the intervention in terms of vasomotor ($P = 0.001$), emotional-social ($P = 0.001$), and physical dimensions ($P = 0.001$), and QOL ($P = 0.001$). Moreover, the results showed a significant difference between groups 2 and 3 after the intervention in terms of vasomotor dimension ($P = 0.001$), and QOL ($P = 0.001$). A significant difference was observed between group 2 and the control group in exercise and vasomotor control ($P = 0.005$), psychosocial ($P = 0.001$), physical ($P = 0.001$), and QOL ($P = 0.001$) scores. Furthermore, in group 2 and the control group, a significant difference was observed in vasomotor ($P = 0.001$), psychosocial ($P = 0.001$), physical ($P = 0.001$), and QOL ($P = 0.001$) scores (Asgari *et al.*, 2015).

Seventy postmenopausal Iranian women for at least 1 year, having symptoms of vaginal atrophy (dryness, soreness, and burning or itching of the vagina and dyspareunia), gynecology

examination, vaginal pH>5, and with a score of 0–49 in maturation vaginal index (MVI) were included in a double-blind, randomized controlled trial. Participants were randomly assigned into two groups. One of the groups received liquorice 2% vaginal cream (n = 35; mean age 56.40 ± 4.29 y) while the other was given placebo (n = 35; mean age 56.17 ± 4.73) over a period of 8 weeks. Vaginal atrophy signs including maturation vaginal index and pH were measured by vaginal smears at the baseline and 8 weeks after the intervention. The results indicated that at the baseline, none of the subjects in either group had a MVI within 65–100 (, corresponding to a great effect of estrogens. However, after 8 weeks of therapy, it improved significantly to 82.9% in Liquorice group and 11.4% in the placebo group ($p < 0.001$). As a primary outcome, the vaginal dryness, vaginal itching, vaginal soreness, and dyspareunia were improved after 2 weeks of treatment in liquorice group compared to the placebo group, which continued in 4 and 8 weeks after the treatment. Also, the vaginal mucus cells changed from the baseline cells to intermediate and superficial cells within and between the two groups after the treatment ($p < 0.001$). Finally, the pH level significantly decreased in liquorice group over time ($p < 0.001$) (Sadeghi *et al.*, 2020).

Most recently, a clinical study was carried out to compare the effect of liquorice vaginal cream and estrogen vaginal cream on the sexual function of postmenopausal Iranian women. Eighty-two postmenopausal were randomly divided into 2 groups (n = 41/each). One group was given estrogen vaginal cream 2%, and the other vaginal liquorice cream 2%. Participants first applied 5 g of cream for 2 weeks and after 2 weeks, rested for 10 days, and then used vaginal cream for another 2 weeks. Data collection method included gynecological examinations and a female sexual function index (FSFI) questionnaire. At the end of the study, the mean score of total FSFI was significantly increased compared to the baseline in both groups. At the end of the study the score for each domain increased compared to baseline in both the groups, but this was signidicantly higher in the estrogen gruop for sexual desire ($p < 0.01$), orgasm ($p < 0.01$) and sexual satisfaction ($p < 0.01$) compared to liquorice group (Ahmadizad *et al.*, 2022).

*Assessor's comment: the clinical efficacy of liquorice in reducing the frequency, duration and hot flashes in menopausal women should be further investigated; indeed, clinical trials cited in this assessment report present several shortcomings, e.g. small sample size (in the study by Nahidi et al., 2012), use of self-reporting surveys, lack of placebo group (in the study by Menati et al., 2014), limited study duration (in the study by Menati et al., 2014). In addition, the extracts used in these studies were not fully described.*

*A clinical trial assessing the efficacy of a vaginal cream containing liquorice for the treatment of vaginal atrophy in menopausal women showed positive results that should be confirmed in a larger well-designed clinical study. A further study showed that the positive effect of a liquorice-based vaginal cream on sexual function was lower than the same cream containing an estrogen (not reported); in both studies, no detail on the herbal preparations was provided.*

**Clinical studies on allergic rhinitis**

In a non-blinded clinical study 60 participants were randomly assigned to one of three interventions: LNI (n = 20), corticosteroid nasal irrigation (CNI, n = 20), and saline nasal irrigation (SNI, n = 20). Patients included had a diagnosed AR with intermittent or persistent symptoms ranging from mild to severe. In the experimental group a suspension with a concentration of 3 mg/ml of dry water extract of liquorice roots was used for nasal irrigation; in the positive comparison group, 2 mg mometasone, 3 g iodine-free salt, and 300 ml warm water were added to the bottle to prepare the corticosteroid solution for nasal irrigation. Patients performed nasal irrigation once a day for 1 month. Subjective questionnaires such as (22-item Sino-Nasal Outcome

Test [SNOT-22] and VAS) were used as primary outcome measures; in addition, objective examinations (acoustic rhinometry and nasal endoscopy) were used for effectiveness assessments. All three interventions could improve SNOT-22 scores, but the effects of LNI and CNI were more significant (before vs after treatment of SNOT-22 in LNI [mean ± SD] = 27.9 ± 15.5 vs 13.2 ± 9.6, $p < 0.001$; CNI = 39.8 ± 19.3 vs 23.6 ± 17.7, $p < 0.001$; SNI = 27.8 ± 13.9 vs 22.2 ± 19.4, $p = 0.04$). According to VAS scores for nasal blockage, rhinorrhea, sneezing, nasal pruritus, postnasal discharge, and olfactory disturbance, the effect of LNI was superior to those of CNI and SNI. The results of rhinometry revealed that LNI significantly improved nasal resistance. Endoscopic analysis showed that both LNI and CNI, but not SNI, could significantly improve turbinate hypertrophy (Chang et al., 2021).

*Assessor's comment: study not clinically relevant due to the small sample size and the open-label design.*

**Clinical studies on atopic dermatitis**

Two topical preparations containing 1% and 2% of a methanol extract of liquorice root were studied in a double–blind clinical trial in comparison with base gel on patients with clinically diagnosed mild to moderate degrees of atopic dermatitis. The topical preparations were administered to patients, in three groups (n = 30 each, mean age 32.7 y in 1% liquorice group, 34.1 y in liquorice 2% group and 35.3 y in the placebo group), three times a day for two weeks. The primary endpoint was severity in oedema, itching and erythema. The overall clinical response was assessed by the investigator based on effect on oedema, itching, erythema and scaling, according to the following 4-point scale: absent=0, mild=1, moderate=2, and severe=3. Follow up of patients ceased after two weeks. All of the 90 evaluable subjects complete two weeks treatment. Two percent liquorice topical gel was more effective than 1% in reducing the scores for erythema, oedema and itching over two weeks ($p < 0.05$); the effect of 1% and 2% liquorice gel in reducing in reducing the scores for erythema, oedema and itching were significantly more than placebo after one and two weeks ($p < 0.01$). Treatment with liquorice extract was not significantly effective in reducing the scores of scaling ($p > 0.05$) (Saeedi *et al.*, 2003).

*Assessor's comment: the results of this study are considered preliminary due to the limited sample size and they should be confirmed in larger clinical trials. DER of the methanol extract was not reported.*

**Clinical studies on wound healing**

The healing effect of hydroalcoholic extract of liquorice root on the wound healing process caused by second-degree burns was investigated in a double-blinded randomized clinical trial. The participants were divided into two groups (n = 41, each): the first receiving topical hydrogel containing 5% dry hydroalcoholic extract (extraction solvent ethanol 80%) of powdered dried liquorice roots and the other receiving topical hydrogel (placebo). The dry hydroalcoholic extract was obtained at a ratio of 16% from the used root powder. The patients in both groups used the medication topically twice a day for 15 days without dressing. The burned area was evaluated for pain intensity, burning, redness, and inflammation on days 1, 3, 6, 10, and 15 of the onset of the burn. Inflammation and redness were assessed using scales (no inflammation = 0, mild = 1, moderate = 2 and severe = 3; no redness = 0, less than 25% of the burn area = 1, bteween 25% and 50% of the burn area = 2, more than 50% of the burn area = 3). Pain and burning were scored based on the VAS scale. Five patients in the placebo group and 7 patients in liquorice group did not receive the allocated intervention; in addition, 10 patients in placebo group and 6 patients in liquorice group discontinued the study. Thus, only 26 patients in placebo group and 28 patients

in liquorice group completed the study and were analysed. At baseline, the experimental group had a higher burn percentage ($p < 0.005$). The rate of inflammation (from the 3rd day to the 10th day), redness (from the 6th day to the 15th day), pain (on the 3rd day), and burning (from the 3rd day to the 15th day) of the wound in the experimental group was significantly lower than in the control group ($P < 0.05$), and the healing process was significantly faster than the control group. Findings from the evaluation of the general appearance of the wound show that over time the general appearance of the injury has improved in both groups, while until the tenth day, there is no significant difference between the two groups. But on the 15th day, participants who used the gel containing liquorice extract had a significantly improved wound appearance (Zabihi *et al.*, 2023).

*Assessor's comment: the positive results showed by the hydroalcoholic extract of liquorice roots in this study needs to be confirmed in further trials, since the clinical relevance is strongly limited by the small number of patients completing the study.*

**Clinical studies on Parkinson's disease**

The effectiveness of liquorice as an adjunct treatment in the management of Parkinson's disease (PD) was investigated in a double-blind, randomised clinical trial. Thirty-nine patients aged 30-80 years, with idiopathic PD whose symptoms initiated in the last 6 years, YAHR staging (Hoehn and Yahr scale) $\leq 3$ and without treatment changes within 4 weeks before the intervention starting were assigned into two groups by random either to receive 5 cc of liquorice or placebo syrups as an adjunct therapy twice a day for the time duration of 6 months. To prepare the liquorice syrup, the extract of liquorice was obtained by root powder maceration in ethanol: water (52:26 v/v) followed filtration and dryng. The mean age of the patients was 59.53 ± 8.7 years old and 64.66 ± 9.4 years old in the treatment group and the placebo group, respectively. All patients in placebo and liquorice groups were receiving standard treatment for PD, which were divided doses of a dopamine agonist pramipexol (0.360 mg/day) and/or levodopa-B (220–1000 mg/day). Ten patients in the placebo group and eleven patents in the liquorice group were receiving amantadine (100 mg twice a day) in addition to levodopa-B and/or pramipexol. The primary outcome was considered as those changes in total Unified Parkinson's rating scale (UPDRS) part I + II + III score from the baseline to the final visit. UPDRS was assessed every 6 weeks for the duration of six months. Finally, 30 patients were retained for evaluation (n = 15 in each group). The total UPDRS score was significantly different in the liquorice and the placebo groups at the end of the trial ($p \leq 0.001$, statistical power = 0.9). The total UPDRS score was improved in the liquorice group from 17.73 ± 6.31 at 1st visit to 15.80 ± 6.08 at 4th visit, on the contrary with the placebo group (from 25.00 ± 8.39 to 26.73 ± 9.12). The motor test scores were significantly lower in the liquorice group in comparison with the placebo group in the 3rd, and 4th visit sessions ($p < 0.05$). In addition, the score for daily activities was significantly lower in the liquorice group compared to the placebo group at 2nd, 3rd, and 4th visit sessions ($p < 0.01$). There was no significant difference between the two groups participants in intellectual activity, thought, and behavior (mentation UPDRS). The score for rigidity in the liquorice group was significantly lower at the 4th visit session in comparison with the placebo group ($p < 0.05$). A considerable effect size for total UPDRS and daily activities in the liquorice group was observed after 4 weeks of treatment; furthermore, the motor test improvement was observed about 4 months after liquorice intake, which was continued to the end of the study. (Petramfar *et al.*, 2020).

*Assessor's comment: the results of this study are considered preliminary due to the limited sample size and they should be confirmed in larger clinical trials.*

**Clinical studies on depression**

Mineralocorticoid-receptor (MR) dysfunction as expressed by low systolic blood pressure and a high salivary aldosterone/cortisol ratio predicts less favorable antidepressant treatment outcome. Inhibition of peripheral 11-β-hydroxysteroid-dehydrogenase type 2 (11βHSD2) reverses these markers. A pilot clinical study was carried out to check if patients treated with antidepressants could benefit from 11βHSD2 inhibitor glycyrrhizin. *Glycyrrhiza glabra* (GG) extract containing 7–8 % of glycyrrhizin at a dose of 2 × 700mg daily adjunct to standard antidepressants was administered in hospitalized patients with major unipolar depressive disorder, according to ICD-10 diagnostic criteria. These subjects were compared in an open-label fashion with patients, who did not receive GG (treatment as usual, TAU). Assessments were done at baseline and approximately 2 weeks after. Twelve subjects were treated with GG and compared to 55 subjects with TAU. At week 2, the Hamilton Depression Rating Scale (HAMD-21) change from baseline as well as the CGI-S change showed a significant time × treatment interaction ($p < 0.03$), indicating a possible therapeutic benefit of GG. The effect seems to be more pronounced in subjects with lower systolic blood pressure and significantly correlated with reduced sleep duration in the GG group (Murck *et al.*, 2020).

Different liquorice preparations have been investigated in a number of clinical studies in many different therapeutic areas. To improve the readability of this assessment report, only studies carried out in line with the therapeutic indications (i.e. functional dyspepsia) reported in the monograph have been summarised in the following table. In addition, the study by Ruetzler et al. 2013 on post-operative sore throat has been also included in the table, being the only study carried out in EU with an acceptable methodological quality.

Table 10: Clinical studies on humans, in functional dyspepsia

| **Type** | **Study** | **Test Product(s)** | **Number of subjects** | **Type of subjects** | **Outcomes** | **Statistical analysis** | **Clinical relevance** |
|---|---|---|---|---|---|---|---|
| To evaluate the efficacy of GutGard® in patients with functional dyspepsia.<br><br>Raveendra *et al.* 2012 | Randomised, double-blind, placebo-controlled study | Capsules containing dry extract of *G. Glabra* roots (DSR 1:4, extraction solvent acetone; GutGard®) 75 mg or placebo twice daily for 30 days | 50 (n = 25 per group); 31 M and 19 F; mean age in verum group 38.12 ± 1.84 y vs 45.16 ± 2.06 y in placebo group ($p<0.05$).<br><br>No drop-out | Indian patients with functional dyspepsia according to Rome-III criteria | Primary endpoints: the extract showed a significant decrease vs placebo in total symptom scores (TSS) on day 15 (−11.32 ± 0.77 vs −5.08 ± 0.57) and day 30 (−15.20 ± 0.71 vs −8.24 ± 0.76; $P \leq 0.05$); marked improvement in the global assessment of efficacy patients vs the placebo ($P \leq 0.05$); significant decrease ($P \leq 0.05$) in Nepean dyspepsia index on day 15 and day 30 versus placebo group | p value of 0.05 and $1 - \beta = 0.90$<br><br>TSS and Nepean dyspepsia index (change from the baseline) analyzed by independentsamples t-test. The global assessment of efficacy analyzed by proportion Z test. | Poor, due to the small sample size |

Table 11: Clinical study on humans, in post-operative sore-throat

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Outcomes | Statistical analysis | Clinical relevance |
|---|---|---|---|---|---|---|---|
| To check if gargling with liquorice solution immediately before induction of anesthesia prevents sore throat and postextubation coughing in patients intubated with double-lumen tubes (DLT).<br>Ruetzler *et al.* 2013 | Randomized, double-blind, placebo-controlled study | L group: 30 ml of a fluid extract of liquorice in water (0.5 g of extract)<br>P group: 30 ml of sugar diluted in water<br><br>Single gargle 5 min before anaesthesia (gargle for 1 minute) | 236; aged 18 to 90 years (mean age 57 ± 15 y in liquorice group; 58 ± 16 y in placebo group)<br><br>2 patients lost to follow-up (one in each group) | Austrian patients aged 18-90 y, ASA physical status I to III, undergoing elective thoracic surgery requiring a double-lumen endotracheal tube | Primary endpoint: incidences of POST at rest in the L and P groups were 19% and 36% at 0.5 hours after arrival in the postanesthesia care unit (PACU), 10% and 35% at 1.5 hours after arrival, and 21% and 45% at 4 hours after surgery, respectively. RR on reducing the incidence of sore throat at rest were 0.54 (95% CI, 0.30–0.99), 0.31 (0.14–0.68), and 0.48 (0.28–0.83), at 0.5 and 1.5 hours after arrival in the PACU and 4 hours after surgery, respectively. Overall RR was 0.46 (95% CI, 0.29–0.72) | Primary analysis:<br>α value of 0.05 and 1 − β = 0.90<br>Analysis ITT<br>The overall treatment effect across the 3 measurements was analysed with use of the modified Mantel-Haenszel test adjusting for the within-patient correction. | Study of good quality.<br>Preparation of the extract not described.<br>Proof of effectiveness on reduction of incidence of POST in other types of surgery with different positions of patients is lacking. |

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

**Clinical studies on dental caries and gengivitis**

The clinical effect of a lollipop containing an extract of liquorice root in the prevention of dental caries by reducing *S. mutans* (SM) colonies was evaluated in a pilot study in 16 young children (2-5 years old) in a pre-school setting. Children were grouped in high, medium and low caries-risk using baseline *S. mutans* levels as risk indicator. Each child was given a sugar-free lollipop containing 15 mg of liquorice extract, every morning and afternoon of each school day for 3 weeks. High-risk children showed the steepest early decrease in mean log-SM ($p<0.001$). At end of a follow-up period, the log-SM decrease moved the high-risk group down to moderate-risk level. High-risk children showed a decrease in fitted mean SM% not seen in other groups ($p<0.001$). The decrease reached a nadir around 22-days post-intervention. Twice-daily use of herbal lollipop significantly reduced both number and relative percent of SM in high-risk children. SM numbers were reduced for 22 days after the last lollipop, stabilized and then began to rebound (Peters *et al.*, 2010).

Sixty Indian children 7-14 years old with at least five active decayed tooth surfaces (caries status determined by Decayed, Missing, and Filled Surfaces) were included in subject- and observer-blind, randomized, chlorhexidine-controlled clinical study. Children were randomised to three groups (n=20 each): Group 1: Aqueous liquorice extract-1.5 g/10 ml saline; Group 2: Ethanolic liquorice extract-375 mg/10 ml; Group 3: Chlorhexidine mouthwash was used in a concentration of 0.0156%, such that 10 ml was dispensed at one time. For each patient, four saliva samples were collected to evaluate changes in pH and *S. mutans* colony counts, i.e. pre-rinse sample that was collected before the child performed oral rinsing, and three post-rinse samples collected immediately, 15 min, and 30 min after the intervention. The mean *S. mutans* colony counts at baseline (pre-treatment) did not show significant difference between the three groups. The mean colony counts decreased significantly immediately after rinsing in children treated with liquorice ($p < 0.001$) compared to the baseline, whilst the decrease in chlorexidine group did not reach the statistical significance. The reduction in colony count was significant in ethanolic and aqueous liquorice groups as compared to the control group ($p < 0.01$ and $p < 0.05$, respectively). The mean colony counts in all three groups decreased significantly ($P < 0.001$) compared to the baseline after 15 and 30 minutes; at the final evaluation, the colony counts in all three groups were found to be equivalent. In the control group, there was a drop in pH at the immediate post-rinse interval following which the pH continued rising till the end point. In both the test groups, immediate post-rinse samples showed a rise in pH, which decreased subsequently, with the end point pH well above the baseline pH in both the groups (Jain *et al.*, 2013).

A clinical study on 104 Indian children (58 females and 46 males), 12–15 years of age diagnosed with chronic generalized gingivitis evaluated the short-term clinical effects of a liquorice oral rinse in the reduction of plaque and gingival inflammation. Selected children were randomly divided into two groups: Group 1: 0.2% chlorhexidine mouthwash and Group 2: liquorice mouthwash. An amount of 10 ml of the two mouthwashes were applied twice daily for 4 weeks. Clinical evaluation was undertaken using the gingival index, the plaque index, and bleeding on probing at baseline, 1$^{st}$, 2$^{nd}$, and 4$^{th}$ week. Both chlorhexidine and liquorice mouthwash were helpful in reducing mean plaque accumulation from baseline to 4 weeks. The mean plaque index scores reduced from 3.8 ± 0.7 to 1.24 ± 0.92 in chlorhexidine group and from 3.88 ± 0.83 to 2.28 ± 0.93 in the liquorice group. The reduction in gingival index scores in chlorhexidine and liquorice mouthwash group was statistically significant and this was from 2.0 ± 0.00 to 0.28 ± 0.45 and from 1.96 ± 0.20 to 0.6 ± 0.5 (from baseline to 4 weeks), respectively. However, chloredixine was more persuasive in reduction of plaque accumulation and gingivitis as compared to liquorice. Although results do not reach to level of significance when

intergroup comparisons were made, individually both chlorhexidine and liquorice mouthwash were effective in reducing bleeding sites. The mean percentage of bleeding sites reduced from 84.0% ± 37.2% to 44.2% ± 50.3% in chlorhexidine group and from 87.4% ± 48.23% to 56.1 ± 33.5% in liquorice mouthwash group at 4 week (Jain *et al.*, 2017).

A randomized, double-blind, controlled study with parallel groups study was conducted in caries-free and high-caries-risk Turkish children, aged 5–11 years (n=108) to evaluate the efficacy of lollipops containing an extract of liquorice root on salivary *S. mutans* compared with a placebo control group. The groups were caries-free children (group A); high-caries risk children (decayed surfaces ≥10 and salivary *S. mutans* levels $>10^5$ CFU/ml) whose dental treatment was completed before lollipop use (group B); and high-caries-risk children who did not comply with dental treatment (group C). The groups were divided by simple randomisation into two subgroups (A-1, A-2, B-1, B-2, C-1, and C-2) according to lollipop type (herbal and placebo lollipops). All children consumed lollipops twice daily, one at morning and one at evening, for 10 days. Saliva samples were taken before and after consuming lollipops and at the end of the third month in all groups. A total sample size of 84 (14 per subgroup) was required to detect 0.8 estimated effect size that allowed for power calculation (with a power of 80% at the 5% significance level). A total of 97 children were included in the analysis. In the groups of children treated with liquorice lollipop, during the 10-day intervention, *S. mutans* levels showed no significant difference in groups A-1 and B-1 ($p>0.05$) compared to the baseline. Only in group C-1 was a significant reduction observed in salivary *S. mutans* levels after lollipop use ($p=0.033$). There were no significant differences at the third month, for groups A-1 and B-1; however, in group C-1 *S. mutans* levels increased significantly ($p=0.006$) compared to the level after lollipop use. In the placebo lollipop groups (A-2, B-2, and C-2), the salivary *S. mutans* levels showed no significant difference after lollipop use compared to the baseline ($p>0.05$) and after 3 months compared to the end of lollipop use ($p>0.05$) (Almaz *et al.*, 2017).

The effectiveness of a simple herbal caries-prevention protocol for reducing salivary *S. mutans* (SM) levels in Turkish children. A total of 90 individuals (mean age: 11.33 y) with a clinical picture of simple gingivitis were randomly divided into three groups (n=30). Mouthwashes including chlorhexidine (CHX) 0.2% solution, 15 ml of an hydroalcoholic extract of liquorice (extraction solvent ethanol 70% V/V) and saline were used as tested antimicrobial agents, and saliva samples were collected before rinsing, at the end of 5 min (T1) and 60 min (T2) following rinsing, and the differences were calculated within 5-60 min (T3). At T1 and T2 CHX showed significantly different decreases in SM count when compared with the other groups, but there were no significant differences in T3 between CHX and Liquorice ($p= 0.167$). Liquorice (at T2) and CHX (at T1 and T2) decreased SM count 100% in some samples (Öznurhan *et al.*, 2019).

The daily use of two liquorice-containing lollipops for 3 weeks significantly reduced salivary *S. mutans* levels (>80% reduction in bacterial count) in high caries-risk children (n=23) aged 3–6 with salivary *S. mutans* levels $>5x10^5$ cells/ml compared to control (n=14, no treatment or placebo, only oral health care counseling), where no decrease was observed (Chen *et al.*, 2019).

In a further small study 30 schoolchildren of 6–12-year-old having at least 5 D in the decayed missed and filled surfaces (DMFS) score were randomised into three groups, i.e., group I: gel containing aqueous liquorice root extract 1.75 g/10 mL saline, group II: gel containing ethanolic liquorice root extract 350 mg/10 mL, and group III: 0.2% chlorhexidine (CHX). The subjects were asked to rinse with 10 mL of their respective solutions for 1 minute. One prerinse and three postrinse saliva samples were collected immediately, 15 minutes, and 30 minutes after the oral rinse. The mean *S. mutans* colony counts at baseline (pre-treatment) did not show significant difference between the three groups. The mean colony counts in all three groups did not decrease significantly ($p \geq 0.001$)

immediately after rinsing and there was no difference among groups after 15 and 30 minutes post-rinse (Kumar *et al.*, 2020).

Finally, the efficacy of liquorice lollipops in decreasing caries in children was evaluated in a systematic review which included three clinical trials (Peters *et al.*, 2010; Hu *et al.*, 2011; Almaz *et al.*, 2017) for a total of 200 patients (children 2-11 years old n=174; age not reported n=26). Only the study by Almaz *et al.* had a low risk of bias according to the Cochrane Collaboration's tool for assessing risk of bias; the other two studied were rated as low quality. Randomisation and the inclusion of a control group was declared only in the study by Almaz *et al*. Liquorice lollipops twice daily for ten days and three weeks were the intervention products used in studies performed by Hu *et al* and Peters *et al*; the study by Almaz *et al* used placebo lollipops in addition to the liquorice lollipops twice daily for three months. The outcome measure in all three studies was a reduction of salivary *S. mutans* count, with positive results in each trial. Only Almaz *et al* reported *a priori* justification for the sample size. The authors concluded that although liquorice lollipops showed a promising effect in reducing caries, further research using randomised controlled clinical trial designs with larger samples sizes are needed (Nuvvula *et al.*, 2020).

*Assessor's comment: due to its antimicrobial activity, liquorice was able to reduce the level of S. mutans in children as showed in a number of clinical trials. Although its efficacy appears to be similar to that of chlorexidine, short treatment duration (single doses of liquorice mouthwashes) and limited sample size of the studies strongly limit the clinical relevance; furthermore, the herbal preparations were not fully dscribed in these clinical trials.*

*Liquorice in the form of mouthwashes significantly reduced plaque accumulation and gengivial inflammation after 4 weeks of treatment in a clinical trial in children aged 12–15 years (n=104) diagnosed with chronic generalized gingivitis; its efficacy appeared to be slightly lower when compared to chlorhexidine 0.2% mouthwash, although any clear conclusion could not be drawn due to the methodological limitations of the study (lack of blinding and information on statistical power).*

**Clinical studies on elderly**

The effects of liquorice flavonoid oil (LFO), prepared by mixing an ethanol extract of root with MCT, on the muscle mass of elderly populations was investigated in a randomized, double-blind, placebo-controlled study. Fifty Japanese participants aged 54–90 years (seven men, 43 women), who underwent rehabilitation treatment for osteoarthritis of the knee, were examined and assigned to either the LFO group (n = 26, mean age 74.6 ± 9.8 y) or the placebo group (n = 24, mean age 73.3 ± 9.7 y). The LFO group consumed 300 mg LFO per day after meal, whereas the placebo group consumed one placebo capsule every day for 16 weeks. The muscle mass, body fat percentage and the Japanese Knee Osteoarthritis Measure (JKOM) score were measured at baseline and every 4 weeks thereafter. In the LFO group, muscle mass in the body trunk increased significantly after 16 weeks of LFO intake (+0.38 kg, P = 0.02) whereas weight, BMI, total muscle mass, total body fat and trunk body fat showed no significant changes compared to the baseline. The trunk muscle mass weight of the LFO group increased significantly compared to that of the placebo group after 16 weeks (P < 0.01). Furthermore, the body fat percentage and body trunk fat percentage of the LFO group were significantly suppressed compared to that of the placebo group after 16 weeks (P = 0.03 and P < 0.01, respectively). In terms of weight and BMI, significant differences were not found between the two groups after 16 weeks. In the LFO group, from baseline to week 16, the VAS tended to improve (–6.2 mm; *P* = 0.08) and the JKOM score improved significantly (–6.8; *P* < 0.02). In the placebo group, from baseline to week 16, the VAS improved significantly (–14.8 mm; *P* < 0.01), as did the JKOM

score (−6.2; $P$ = 0.03). Between the two groups, both of the changes in the VAS and JKOM scores showed no significant differences at any measurement point. (Kinoshita *et al.*, 2017).

*Assessor's comment: Positive effects of LFO supplementation were observed in a small clinical trial with respect to increasing muscle mass and suppressing the body fat percentage of elderly populations with knee osteoarthritis, especially in the body trunk. No blood measurement were taken; in addition, neither JKOM score nor knee pain improved after LFO supplementation compared to placebo, which should be the main primary targets to support the clinical efficacy of liquorice in this setting.*

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

There is clinical evidence of interaction of 300 mg/day of glycyrrhizin with midazolam and omeprazole which should raise awareness on the potential of liquorice to inferfere with metabolism of drugs mediated by P450 hepatic enzymes, mainly CYP 3A4. Sections 4.4 and 4.5 of the monograph have been updated to include relevant information on the potential for interactions.

Liquorice have been investigated in several clinical studies covering many different therapeutic areas. The most promising evidence of efficacy derives from a randomised, placebo-controlled clinical study where a fluid extract of liquorice significantly reduced the incidence of post-operative sore throat; unfortunately, evidence is limited to a very specific setting (adult patients undergoing elective thoracic surgery requiring a double-lumen endotracheal tube) and no sufficient detail of the herbal preparation studied was provided, thus impeding the possibility to justify a WEU.

Liquorice preparation have been used also in a clinical study in patients with functional dyspepsia; but the number of patiets enrolled in this trial was too small to substantiate a WEU.

Liquorice has been traditionally used for the treatment of gastric ulcers and several clinical studies with deglycyrrhizinated liquorice have been carried out, but evidence of its efficacy is conflicting and affected by severe methodological deficiencies to support a WEU.

Clinical trials carried out in other therapeutic area cannot support any therapeutic indication based on a WEU due to the limited number of patients included in the studies and to several methodological deficiencies.

## 5. Clinical Safety/Pharmacovigilance

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

Safety data from clinical pharmacology and clinical efficacy studies carried out with liquorice are summarised in the table below.

Several clinical safety studies have been also conducted, which have been repoted hereafter.

*Haemodynamic changes*

Liquorice ingestion often elevates blood pressure, thus the haemodynamic changes induced by liquorice consumption was assessed in an open label study in 52 normotensive subjects. Subjects in the treatment group ingested liquorice daily for two weeks with an estimated daily dose of glycyrrhizin of 290–370 mg. Two weeks of daily liquorice consumption increased extracellular volume, amplified

pressure wave reflection from the periphery, and elevated central SBP and DBP (Leskinen *et al.*, 2014).

The haemodynamic effects of two-week liquorice exposure (glycyrrhizin dose 290–370 mg/day) was also evaluated in the same subjects recruited in the study of Leskinen & colleagues (2014) during orthostatic challenge. However, the present analyses included data from two additional subjects in the liquorice group not included in the previous study. Liquorice ingestion elevated radial systolic and diastolic blood pressure and systemic vascular resistance. During orthostatic challenge, heart rate increased less after the liquorice versus control diet and low frequency power of heart rate variability decreased within the liquorice group. Liquorice intake increased central pulse pressure and augmentation index (AIx) supine and upright, but in the upright position the elevation of augmentation index was accentuated. Liquorice diet also increased extracellular fluid volume and aortic to popliteal pulse wave velocity, and aortic characteristic impedance in the upright position. The authors concluded that measurements performed only at rest may result in underestimation of the haemodynamic effects of liquorice ingestion, since reduced chronotropic response and enhanced central wave reflection were especially observed in the upright position (Hautaniemi *et al.*, 2017).

The effect of liquorice ingestion on blood pressure to exogenous nitric oxide donor (nitroglycerin 0.25 mg) and $\beta_2$-adrenoceptor agonist (inhaled salbutamol 400 µg), and 11β-hydroxysteroid dehydrogenase activity, was assessed in an open-label study involving 21 volunteers and 21 reference subjects. The recordings were performed at baseline and following two weeks of liquorice intake (290–370 mg/d glycyrrhizin). Liquorice intake elevated aortic SBP and DBP and systemic vascular resistance when compared with the reference group. The liquorice-induced increase in systemic vascular resistance was observed in the presence of nitroglycerin ($p<0.05$) but no longer in the presence of salbutamol. The authors suggested that liquorice exposure impaired vasodilatation *in vivo* that was induced by exogenous nitric oxide donor but not that induced by $\beta_2$-adrenoceptor stimulation (Hautaniemi *et al.*, 2019).

The hypertensinogenic properties of liquorice were the subject of three separate studies completed among 64 healthy volunteers. The volunteers received liquorice 50 g (study 3, n = 24, 12 men and 12 women) vs 100 g (study 2, n = 30, 19 women and 11 men) vs 200 g/day (study 1, n = 10, one man and nine women), corresponding to a daily intake of 75–540 mg glycyrrhetinic acid, for 2–4 weeks. In the third study, where 75 mg of glycyrrhetinic acid was consumed, the women started the consumption on day 1–4 of the menstrual cycle. At the end of these interventions, an increase in mean systolic blood pressure by 3.3, 5.2, and 14.4 mmHg, respectively, was observed. A significant increase in mean diastlolic blood pressure (+ 9.3 mmHg) was observed only in subjects included in study 1. A significant decrease in plasma potassium levels was observed in studies 1 and 2, whilst heart rate did not change in any of the studies. A positive correlation between amount of consumed glycyrrhizic acid and blood pressure increase was found (Sigurjónsdóttir *et al.*, 2001).

In another interventional study 25 healthy subjects and 11 patients with hypertension consumed liquorice 100 g/day (i.e., 150 mg glycyrrhetinic acid) for 4 weeks. Office, 24-h ambulatory blood pressure (BP) and blood samples were measured before, during and after liquorice consumption The mean rise in BP with office measurements after 4 weeks of liquorice consumption was 3.5 mmHg ($p < 0.06$) in normotensive and 15.3 mmHg ($p = 0.003$) in hypertensive subjects, the response being different ($p = 0.004$). The mean rise in DBP was 3.6 mmHg ($p = 0.01$) in normotensive and 9.3 mmHg ($p < 0.001$) in hypertensive subjects, the response also being different ($p = 0.03$). Liquorice induced more pronounced clinical symptoms in women than in men ($p = 0.0008$), although the difference in the effect on the BP was not significant (Sigurjónsdóttir *et al.*, 2003).

*Mineralcorticoid effect*

The first clinical evidence of the mineralocorticoid effect dates back to the 1948, when Revers reported his observations that approximately one out of five patients who were treated with liquorice paste for peptic ulcers developed edema (Davis & Morris, 1991). A couple of years later, Molhuysen *et al.* (1950) showed that a dry aqueous extract of *Glycyrrhiza glabra* roots given orally at doses of 20-45 g divided into 8 equal parts daily to patients suffering from peptic ulcer caused retention of sodium, excretion of potassium, and hypertension (Molhuysen *et al.*, 1950).

Following short-term (1-4 weeks) ingestion of 100 and 200 g/liquorice day (equivalent to 0.7 and 1.4 g glycyrrhizic acid/day, respectively) in 13 healthy volunteers, urinary excretion of cortisol remained elevated for at least 1 wk after liquorice was withdrawn (Epstein *et al.,* 1978).

In addition, the renin-angiotensin-aldosterone system was suppressed for several months after cessation of liquorice ingestion in four ill women aged 38 to 55 years admitted to hospital with chronic liquorice intoxication. They had consumed 25-200 g liquorice daily for six months to five years. (Epstein *et al.,* 1977a).

The same group of authors evaluated the effect of liquorice confectionery on electrolyte status and the renin-angiotensin-aldosterone system in 14 healthy volunteers who consumed liquorice at daily doses of 100 or 200 g (equivalent to 0.7 and 1.4 g glycyrrhizic acid) for 1 to 4 weeks. Plasma potassium levels fell by more than 0.3 mmol/litre in 11 individuals, including four who were withdrawn from the study because of hypokaliaemia. One or more values of the renin angiotensin-aldosterone system, especially plasma renin activity and urinary aldosterone concentrations, were considerably depressed in all subjects (Epstein *et al.,* 1977b).

Ingestion of liquorice, 100 g daily for 8 weeks, corresponding to 0.7 g glycyrrhizic acid, caused a rise in 81% in plasma atrial natriuretic peptide (ANP) concentration in 12 healthy subjects. Mean body weight increment (1.6 kg) correlated with the increase in plasma ANP (r = 0.59; P less than 0.01). The plasma concentrations of antidiuretic hormone, aldosterone, and plasma renin activity decreased. All these hormonal effects, reflecting retention of sodium and fluid volume, were probably due to the known mineralocorticoid properties of liquorice. Blood pressure increased transiently and two subjects developed reversible hypertension. The rise in plasma ANP concentration during ingestion of liquorice may be considered a physiological response to prevent fluid retention and development of hypertension (Forslund *et al.*, 1989).

In another study, graded daily doses of dried, aqueous extract of liquorice root, containing 108, 217, 380 and 814 mg of glycyrrhizin, were administered as pills to 4 groups of 6 healthy volunteers of both sexes (12 males and 12 females) for 4 weeks. The glycyrrhizin content, as assayed by HPLC, ws 7.64% w/w. None of the subjects were taking drugs, with the exception of two women using oral contraceptives. No significant effects occurred in groups 1 and 2. After 2 weeks, side effects leading to withdrawal from the protocol occurred in a female in group 3 (headache), a male with a family history of hypertension in group 4 (arterial hypertension), and a female also taking oral contraceptives in group 4 (hypertension, hypokalaemia, increase in body weight and peripheral edema). In groups 3 and 4, the changes in renal sodium excretion did not reach statistical significance, although an isolated increase occurred at week 4 in group 3. In group 4, transient reduction in kalaemia and increase in body weight were found after 1 and 2 weeks, respectively. A statistically significant depression of plasma renin activity (PRA) occurred in groups 3 and 4 (Bernardi et al., 1994).

The mineralcorticoid effect of glycyrrhizic acid was studied in 39 healthy female volunteers aged 19-40 years. The volunteers were not allowed to smoke, to use glycyrrhizic acid containing products and to

use drugs during the experiment. The experiment lasted 12 weeks: an administration period of 8 weeks, preceded by a period of 2 weeks to get used to the restrictions and followed by a 2-week wash-out period. The volunteers were randomly divided into four dose groups: 0 (n=10), 1 (n=9), 2 (n=9) and 4 mg/kg body weight (n=11) glycyrrhinizic acid per day given orally in capsules. One volunteer in the 2 mg/kg group withdrew after 2 weeks of ingestion, because the plasma $K^+$ concentration decreased below 3.0 mmol/l; $K^+$ concentration returned to a normal value 1 week after withdrawal. A subject in 4 mg/kg group was withdrawn after 6 weeks of ingestion because of concentration difficulties and general discomfort. The aldosterone concentration in the serum and PRA of the 4 mg/kg group were significantly lower ($p < 0.001$) than those of the control group after 2, 4, 6 and 8 weeks of ingestion. The concentration of ANP decreased significantly in the 4 mg/kg group ($p < 0.001$) after the wash-out period of 2 weeks, as compared with the end of the ingestion period. Blood pressure in the 2 and 4 mg/kg group during the administration period of glycyrrhizic acid was relatively increased compared to the control group; the changes were significant in the 4 mg/kg group only ($p=0.018$). The plasma concentration of $K^+$ showed a significant decrease in the 4 mg/kg group as compared with the control group in weeks 2 to 4 of the ingestion period ($p < 0.01$), but returned to baseline values during the experiment. The daily questionnaire on physical condition filled by the participants during the study revealed a dose-related increase in headache, nausea and vomiting but only the 4 mg/kg group differed from the control group. Regarding change of defecation pattern, a swollen face and tickling in the arms and legs, the 4 mg/kg group differed from the other groups; however, no clear dose-effect relationship was found. The authors considered the dosage of 2 mg/kg bw glycyrrhizic acid to be the no-effect level of glycyrrhizic acid. Starting from this NOEL the authors derived an ADI of 0.2 mg/kg bw by using a safety factor of 100 (van Gelderen *et al.*, 2000).

The clinical relevance of the association between consistent liquorice ingestion, hypertension and hypokalaemia was assessed in a systematic review and meta-analysis of 18 clinical studies (Armanini 1996; Armanini 2003; Armanini 2004; Bernardi 1994; Epstein 1977; Ferrari 2001; Forslund 1989; Kageyama 1992; Mac Kenzie 1990; Sigurjónsdóttir 1995; Sigurjónsdóttir 2001; Sigurjónsdóttir 2003; Sigurjónsdóttir 2006; Leskinen 2014; Sobieszczyk 2010; Tu 2010; van Gelderen 2000; Yan 2013) which met the following inclusion criteria: (1) liquorice or gylcyrrhizic acid taken orally; (2) prospective study design; (3) change from baseline characteristics in relevant outcomes either presented or able to be calculated; (4) at least 100 mg of glycyrrhizic acid consumed daily; (5) at least five subjects; (6) healthy subjects. Studies involving the ingestion of deglycyrrizinated liquorice extracts such as LFO were excluded. A total of 337 participants was involved in the selected studies. Pooled change from baseline and 95% confidence intervals were calculated for SBP, DBP, plasma $K^+$, PRA and plasma aldosterone using a random effects model. A sub-group analyses stratifying by study duration, with studies being assigned to either a '4 weeks or longer' or 'less than 4 weeks' duration group was performed. The shortest study was 6 days, and 10/18 studies (55.6%) were at least 4 weeks in duration. Seven out of eighteen studies (38.9%) used liquorice confectionery, 4/18 (22.2%) used liquorice concentrate and 7/18 (38.9%) used a glycyrrhizinic acid supplement. Seven out of eighteen studies (38.9%) involved ingestion of 500 mg per day or more of glycyrrhizic acid, while 11/18 studies (61.1%) involved less than that amount. In 7/18 studies the method for calculation of glycyrrhizic acid content was either an estimate or the methods used were not clear. The mean daily dose of glycyrrhizic acid across the studies was 377.9 mg. Mean sample size of the studies was 18.7 (range 6-40). The pooled difference in means between pre- and post-liquorice ingestion for SBP was 5.45 mm Hg (95% CI 3.51–7.39), which was statistically significant ($P < 0.001$). When stratifying by study duration, the effect was higher in magnitude for studies of <4 weeks duration (+7.83 mm Hg, 95% CI 3.69–11.98) than for 4 or more weeks duration (+4.44, 95% CI 3.20–5.68). There was moderate heterogeneity in the results, which was statistically significant ($I^2 = 50.71$; $P = 0.03$). For DBP, the

pooled difference in means between pre- and post-liquorice ingestion was statistically significant at 3.19 mm Hg (95% CI 0.10–6.29; P = 0.04). When stratifying by study duration, only studies of 4 or more weeks duration showed a significant difference (+2.80, 95% CI 2.61–3.00). There was a high level of heterogeneity, which was statistically significant ($I^2$ = 99.37; P<0.001). Plasma potassium levels decreased significantly after chronic liquorice ingestion in the pooled analysis (P < 0.001), with the pooled difference in means between pre- and post-liquorice ingestion being −0.33 mmol/L (95% CI − 0.42 to 0.23). When stratifying by study duration, there was a greater magnitude change for studies of <4 weeks (−0.37, 95% CI − 0.49 to − 0.26) than for 4 weeks or more (−0.26, 95% CI − 0.42 to − 0.11). There was a moderate level of heterogeneity, which was statistically significant ($I^2$ = 60.59; P = 0.009). The pooled difference in means between pre- and post-liquorice ingestion for plasma renin activity was −0.82 ng/ml per hour (95% CI − 1.27 to − 0.37), which was statistically significant (P < 0.001). These results were consistent for both studies of 4 or more weeks duration and 4 or fewer weeks duration. There was a high level of heterogeneity, which was statistically significant ($I^2$ = 88.12; P < 0.001). For plasma aldosterone, the pooled difference in means between pre- and post-liquorice ingestion was statistically significant at − 173.24 pmol/l (95% CI − 231.65 to − 114.83; P < 0.001). These results were consistent when stratified by study duration. There was a high level of heterogeneity, which was statistically significant ($I^2$ = 84.96; P < 0.001) (Penninkilampi *et al.*, 2017).

*Assessor's comment: clinical safety studies in humans confirm the effects on blood pressure and the mineralcorticoid activity of liquorice which are well documented in literature. These effects were observed with high daily doses of liquorice or glycyrrhizic acid taken for at least 4 consecutive weeks, although their occurrence even with lower intakes for shorter period, especially in patients with history of hypertension cannot be ruled out, as documented by some studies reported above and by a number of published case-reports.*

Table 12: Data from clinical safety trials on haemodynamic effects

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
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| To assess the dose-response and the time-response relationship between liquorice consumption and rise in blood pressure<br><br>Sigurjónsdóttir *et al.*, 2001 | Three open-label studies. | Study 1: 200 g of sweet liquorice, (540 mg of glycyrrhetinic acid) daily for 2 weeks.<br>Study 2: 100 g of sweet liquorice, (270 mg of glycyrrhetinic acid) daily for 4 weeks.<br>Study 3: 50 g of sweet liquorice, (75 mg of glycyrrhetinic acid) daily for 4 weeks. | Study 1: n=10, (1 M/9 F; mean age 30 y)<br><br>Study 2: n=30, (11 M/19 F; mean age 27.6 y)<br><br>Study 3: n=24, (12 M/12 F; mean age 31.7 y). Women started the consumption on day 1–4 of the menstrual cycle. | Healthy volunteers | Liquorice consumption significantly increased the SBP in all studies, apart in study 3 after 4 weeks. DBP significantly increased only in study 1.<br>No changes in HR.<br>Plasma potassium levels significantly decreased in studies 1 and 2, but not in 3.<br><br>Regression analysis revealed a linear relationship between dose and response using data from 2 weeks of liquorice consumption for SBP. | This study showed a dose-response relationship between high daily intakes of liquorice and the increase in SBP; however, clinical relevance in terms of SBP increase is limited by the lack of a control group and by the small sample size of the single studies. |
| To assess if hypertensive (HT) patients are more sensitive to | Open-label clinical study | One run-in week with definition of baseline values, one 4-week period with a daily intake of 100 g of | 25 healthy volunteers (M/F 13:12, mean age 31.2 y, and 11 subjects with hypertension (M/F | Healthy volunteers and individuals with hypertension treated with β-receptor inhibitors (n = 9) or with the combination of | 13 participants reported headache and 9 reported oedema. Few reported diarrhoea, increased abdominal gas, slight | Data from this small trial suggested that patients with essential HT are more sensitive to the inhibition of |

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| liquorice-induced inhibition of 11β-HSD type 2 than normotensive subjects (NT)<br><br>Sigurjónsdóttir *et al.*, 2003 | | liquorice (equivalent to 150 mg GA) and finally a wash-out period of 4 weeks. Women started the consumption at day 1–4 in the menstrual cycle | 8:3, mean age 40.7 y | a β-receptor inhibitor and a $Ca^{2+}$-antagonist (n = 2) | dizziness or joint pain in fingers and wrists. One woman with HT quitted liquorice within 2 weeks because of extreme rise in SBP and DBP (35.7 and 22.2 mmHg, respectively, after 14 days), two NT women because of oedema and headache, one NT woman because of oedema, shortness of breath and tiredness, one NT woman because of headache.<br><br>Significant increase in mean SBD (3.5 mmHg in NT and 15.3 mmHg in HT subjects) and DBP (3.6 mmHg in NT and 9.3 mmHg in HT subjects) was observed. | 11β-HSD by liquorice than NT subjects. |
| To examine the haemodynamic changes at | Open label study | Subjects ingested liquorice corresponding to an estimated daily dose | 52 (n = 22 in liquorice group and n = 30 in the aged-matched control | Finnish normotensive subjects with a consumption of liquorice >300 grams | Liquorice elevated peripheral and central SBP and DBP (by 7/4 and 8/4 mmHg, 95% | SBP and DBP did not exceeded values of 140 and 90 mmHg, respectively; however, |

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| rest induced by liquorice ingestion in healthy volunteers.<br><br>Leskinen *et al.* 2014 | | of glycyrrhizin was 290–370 mg (120-300 g of liquorice) for two consecutive weeks.<br><br>An age-matched group of 30 people who were advised to maintain their normal diet was recruited as a control group. | group); mean age 33±662 y in liquorice group; M/F 8:12<br><br>Drop-out: one subject in liquorice group due to infectious diarrhoea; a further subject in the liquorice group was excluded from analysis due to incomplete haemodynamic data. | per week, without blood pressure over 140/<br>90 mmHg, any cardiovascular disease with regular medication, or pregnancy. | CI: 2-11/1-8 and 3-13/1-8, respectively, P<0.05), and increased extracellular volume by 0.5 litres (P<0.05 vs controls). Also, AIx adjusted to heart rate 75/min (from 7% to 11%, 95% CI for change 0.3-7.5, P<0.05) and aortic pulse pressure (by 4 mmHg, 95% CI 1-7, P<0.05) were elevated. No subject taking liquorice gained weight over 4 kgs or experienced oedema in the lower extremities. | elevation in blood pressure was close to the efficacy of antihypertensive monotherapy in meta-analysis on pharmacotherapy of hypertension (average reduction 9/6 mmHg). The relevance of these observations is limited by the small number of patients.<br>Daily doses of liquorice in this study were very high. |
| To examine the haemodynamic changes during orthostatic challenge induced by liquorice ingestion in healthy volunteers. | As above | As above | 52 (n = 22 in liquorice group and n = 30 in the aged-matched control group); mean age 34.9±9.2 y in liquorice group; M/F 8:14<br><br>All subjects were included in the analysis. | As above | Liquorice elevated radial SBP (p < 0.001) and DBP (p = 0.018) and systemic vascular resistance (p = 0.037). During orthostatic challenge, HR increased less after the liquorice versus control diet (p = 0.003) and low frequency power of HR variability | During orthostatic challenge, liquorice ingestion resulted in a further increase of AIx indicating enhanced pressure wave reflection from the periphery, while a decreased cardiac chronotropic response was also observed. Also SVRI |

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| Hautaniemi *et al*. 2017 | | | | | decreased within the liquorice group (p = 0.034). Liquorice increased central pulse pressure (p < 0.001) and AIx, mainly in the upright position (p = 0.007). Liquorice increased extracellular fluid volume (p = 0.024) and aortic to popliteal pulse wave velocity (p = 0.027 ), and aortic characteristic impedance in the upright position (p = 0.002). | was elevated after liquorice ingestion. The relevance of these observations is limited by the small number of patients. Daily doses of liquorice in this study were very high. |

Table 13: Data from clinical safety trials on mineralcorticoid effects

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
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| To determine the association between consistent liquorice ingestion, hypertension and hypokalaemia<br><br>Penninkilampi *et al.* 2017 | Systematic review and meta-analysis of 18 clinical studies | Liquorice confectionery, liquorice concentrate or glycyrrhizinic acid supplement. The mean daily dose of glycyrrhizic acid was 377.9 (range 65 – 1400 mg). Intervention duration ranged from 6 days to 2 months. Follow-up ranged from 3 days to 1 month. | 337 in total (medium sample size n=18.7, range 6-40) | Haelthy subjects | Chronic ingestion of liquorice or GA caused a statistically significant increase in mean SBP (5.45 mm Hg, 95% CI 3.51–7.39) and DBP (3.19 mm Hg, 95% CI 0.10–6.29). Plasma $K^+$ (−0.33 mmol/l, 95% CI − 0.42 to 0.23), plasma renin activity (−0.82 ng/ml per hour, 95% CI − 1.27 to − 0.37) and plasma aldosterone (−173.24 pmol/l, 95% CI − 231.65 to − 114.83) were all significantly decreased. A significant correlation was noted between daily dose of glycyrrhizic acid and SBP ($r^2$ = 0.55) and DBP ($r^2$ = 0.65), but not for the other outcome measures. | Mineralcorticoid effects are well known in literature. Clinical implications are limited due to small sample size of the studies, most of the studies were uncontrolled, there was a significant heterogeneity in all outcome measures. However, the results of this study confirmed that the use of liquorice is not recommended in patients with hypertension and hypokaliemia. |

Table 14: Clinical safety data from clinical trials

| Type | Study | Test Product(s) | Number of subjects | Type of subjects | Adverse reactions | Comments |
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| To evaluate the efficacy of an acetone extract of liquorice root in patients with functional dyspepsia.<br><br>Raveendra *et al.* 2012 | Randomised, double-blind, placebo-controlled study | Capsules containing dry extract of *G. Glabra* roots (DSR 1:4, extraction solvent acetone) 75 mg or placebo twice daily for 30 days | 50 (n = 25 per group); 31 M and 19 F; mean age in verum group 38.12 ± 1.84 y vs 45.16 ± 2.06 y in placebo group ($p<0.05$).<br><br>No drop-out | Indian patients with functional dyspepsia according to Rome-III criteria | No changes in haemoglobin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase in *G. Glabra* group compared to placebo; significant increase of random blood sugar since day 0 up to day 30 compared to placebo ($P<0.05$); significant decrease in serum creatinine compared to placebo only at day 0 | Slight increase in blood sugar observed in patients treated with liquorice (level up to 103.6 ± 2.08 mg/dL) |
| To assess the efficacy of an acetone extract of liquorice root, in the management of *H. pylori*<br><br>Puram *et al.* 2013 | Randomized, double blind placebo-controlled trial. | 150 mg of dry extract of *G. Glabra* roots (DSR 1:4, extraction solvent acetone) or placebo capsule once daily for 60 days per os | 107 (n = 55 in liquorice group; n = 52 in placebo group); mean age 32.86 ± 6.50 y in verum group and 33.10 ± 5.59 in placebo group.<br><br>7 drop-out (5 in liquorice group and 2 in placebo group) | Indian patients aged 18–45 years with positive response in *H. pylori* stool antigen test (HpSA) and $^{13}$C-urea breath test ($^{13}$C-UBT) | The statistical analysis of side effects was performed with the chi-square analysis.<br><br>22 subjects (22%) showed at least one side-effect. One subject (1%) experienced moderate side-effect (fever); 21 subjects (21%) | Diarrohea was the most frequent side-effect (10% in liquorice group) |

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| | | | due to protocol deviation | | experienced mild side-effects, but none stopped the treatment, and all have completed the study. The incidences of side-effects were considered to be not related to treatment. The frequencies of side effects between liquorice and placebo treated groups were non-significant | |
| To evaluate the effectiveness and safety of liquorice extract in asthmatic patients.<br><br>Sadek *et al.* 2019 | Randomised, placebo-controlled clinical trial | Group 1: inhaled corticosteroids (ICs, fluticasone or budesonide in moderate to high doses) and long-acting beta agonist (LABA, salmeterol or formoterol) and 500 mg aqueous liquorice extract capsule (equivalent to 100 mg glycyrrhizin) taken twice daily<br><br>Group 2: same asthma treatment as | 80; median age 30 y (23.25-40 y) in liquorice group and 26.5 y (25-34 y); 16:24 M/F in liquorice group and 24:16 M/F in placebo group | Egyptian patients with chronic stable moderate bronchial asthma and maintained on moderate to high doses ICs/LABA combination | After 3 weeks of treatment, liquorice added on ICs and LABA therapy determined a non-significant increase in both SBP and DBP (P value= 0.236 and 0.113 respectively), a non-significant change in serum $K^+$ level (P-value= 0.105) | The low dose of liquorice used in this study did not cause a significant increase of blood pressure and decrease in serum $K^+$ level.<br><br>Patients with hypertension or heart disease were excluded from the trial. |

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| | | group 1 but patients received starch capsule (500 mg starch) instead of liquorice twice daily<br><br>Study duration: 4 weeks | | | | |
| To evaluate effects of LFO on total body fat and visceral fat together with body weight, BMI in overweight subjects.<br><br>Tominaga *et al.* 2009 | Randomized, double-blind, placebo-controlled study | One capsule containing 300 (low-dose group), two capsules containing 600 (middle-dose group), three capsules containing 900 mg (high-dose group) of LFO or 3 capsules containing placebo to be ingested just before supper with a cup of water daily for 8 weeks.<br><br>The LFO concentrate solution contained 30% liquorice ethanolic extract and 70% MCT | 84 (n = 21 per group, four groups); mean age 49.3±1.2 y in placebo group, 49.4±1.2 y in low-dose group, 50.0±1.3 y in medium-dose group and 48.9±1.4 y in the high-dose group; 56:28 M/F<br><br>2 M in the placebo group and 1 M in the 300-mg group dropped out of the study. | Healthy Japanese subjects between 40 and 60 years of age, moderately overweight with BMI between 24 and 30 kg/$m^2$ | No problematic adverse effects were observed in any LFO group. No significant changes in blood pressure compared to the baseline in liquorice and placebo groups. After 8 weeks, a statistically significant decrease in haemoglobin level was observed in 300 mg/day and 900 mg/day groups, but not in 600 mg/day and placebo groups. A significant decrease in serum γ-GTP, total proteins, albumin was seen in 900 mg/day only. | Statistically significant changes were not clinically meaningful.<br><br>A case of lumbago was observed in 300 mg/day group and in placebo group.<br><br>Subjects who had a history of serious disease (e.g., diabetes, liver, kidney, or heart disease) were not included in this study. Subjects were normotensive at baseline. |

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| To compare effects of liquorice extract concurrent with low-calorie diet with low calorie diet alone on the lipid profile and atherogenic indices in overweight and obese subjects<br><br>Mirtaheri *et al.* 2015 | Double blind randomized placebo-controlled clinical trial | Low calorie diet with either capsules containing 500 mg/day of hydroalcoholic extract (ethanol 70: water 30% v/v) of liquorice root or placebo (capsules containing 500 mg corn starch) three times daily before each meal for 8 consecutive weeks. The extract contained lowered Glycyrrhizin (<0.01%). | 64 (n = 32 per group); mean age 36.0 ± 11.97 y in liquorice group and 33.6 ± 4.8 y in placebo group; 27:37 M/F<br><br>Three subjects in each group dropped out | Overweight and obese Iranian volunteers, aged 30–60years old and with BMI > 25 $kg/m^2$.<br><br>Exclusioncriteria were cardiovascular diseases, liver, thyroid and kidney disorders, diabetes mellitus. | Participants did not report any side effects for liquorice supplementation. | Although the authors reported that liquorice did not cause any side effects, two subjects in treatment group discontinued intervention due to "gastric complication with problem". No further details have been provided. |
| To investigate liquorice potential to decrease transaminase activities in NAFLD, its effects on lipid profile and other | Systematic review, followed by a meta-analysis and TSA of 15 clinical trials in humans. Three studies were futher splitted to | Liquorice was administered to the patients in several ways, being the capsules of LFO the most common option (15 out of 26 clinical studies). All the studies included a control group with placebo used in 22 | 985 | The patients subjected to the intervention with liquorice were mainly healthy and overweight volunteers. Other types of patients were also enrolled, namely women with polycystic ovary syndrome, hypercholesterolemic | Liquorice consumption increased the DBP (WMD: 1.737 mmHg; 95% CI: 0.835 to 2.621; p<0.0001), but not the SBP (WMD: 0.779 mmHg.<br><br>As a secondary outcome measure, it was also observed that | The results of this meta-analysis showed the increase of blood pressure of patients associated with the hypernatremia caused by liquorice. |

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| cardiovascular diseases biomarkers of patients; to study the metabolic changes after liquorice consumption<br><br>Luis *et al.* 2018 | achieve a total of 26 different clinical studies. | out of 26 clinical studies.<br><br>Intervention duration ranged from 2 to 16-weeks, but 4 and 8-weeks were more usual. | | patients and elderly people. | liquorice significantly increased $Na^+$ (WMD: 0.650; 95% IC: 0.000; 1.299 ; p=0.050; WMD adjusted: 0.32590; 95% IC: −0.25820; 0.91010) | |
| To investigate the benefit of liquorice root in the treatment of acute ischemic stroke<br><br>Ravanfar *et al.* 2016 | Randomized double-blind placebo-controlled trial. | Oral 450 mg or 900 mg liquorice aqueous extract or placebo capsules three times daily for 7 days. Capsules contained a mean value of 7.85% by mass glycyrrhizic acid. | 92 (n = 29 in 450 mg liquorice group, n = 33 in liquorice 900 mg group and n = 30 in placebo group); M/F 48:27; mean age of 65.7 y.<br><br>14 patients dropped out (10 lost to follow-up, 2 died, 2 incomplete laboratory data and vital sign chart during their hospital course). Further 3 patients were excluded form the | Iranian patients aged between 18 and 85 years with signs and symptoms of acute ischemic stroke and Recognition of Stroke in the Emergency Room scale higher than 2 and with National Institute of Health Stroke Scale scores between 5 and 20 with a motor deficit of 2 or more (for either one arm or leg). | One patient passed away due to cardiac arrest 2 month after treatment in 900 mg liquorice group. No morbidity or mortality was detected due to intervention or stroke itself. No significant changes in serum $Na^+$, $K^+$, BUN, sugar, and blood pressure were observed between groups. Adverse effects of high dose liquorice including hyperglycemia, hypertension, | Strict exclusion criteria were applied, including among others, previous cerebrovascular accident, SBP >160 mmHg, DBP >110 at onset of stroke, atrial fibrillation or other tachy/bradyarrythmias , ejection fraction less than 45%, myocardial infarction in previous month, significant kidney disease (creatinine higher than 1.8 mg/dl), significant liver disease (Bilirubin |

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| | | | analysis to obtain equal groups of 25 each. | | hypokalemia and hypernatremia did not occur in any of the patients. | >20 mmoll/L), significant lung disease. |
| To examine the effects of a product based on *G. glabra* in relieving the menstrual pain.<br><br>Jafari *et al.* 2019 | Study having two parallel interventional arms with a randomized, active controlled and triple-blind design. | 400 mg Ibuprofen tablets every 8 h and and 5 cc of placebo syrup two times a day or 5 cc of a syrup containing 15% of an aqueous extract of *G. glabra* roots (DER 6.6:1) two times a day and placebo tablets every 8 h. The patients took the drug from the first day of menstruation to the fifth and for two consecutive cycles. | 60 (n = 30 per group); mean age 22.60 ± 1.84 y.<br><br>Drop-out: 10 patients, 4 in liquorice group and 6 in ibuprofen group; two were lost to follow-up and 8 discontinued interventions for personal reasons | Females between the ages of 18-25 years old with moderate to severe primary dysmenorrhea based on the Verbal Multidimensional Scoring System<br><br>Chronic hepatitis, cholestatic liver disease, cirrhosis, severe renal insufficiency, diabetes mellitus, arrhythmias, hypertension, hypertonia and hypokalemia were exclusion criteria. | Side effects were reported in 6 participants (25%) in the Ibuprofen group, including heartburn (n = 5, 20.8%) and stomachache (n = 2, 8.3%). There were no side effects in the *G. glabra* group and the syrup was well tolerated by the participants. | |
| To determine the effects of liquorice roots on the relief and recurrence of hot flash in | Double-blind, randomised, placebo-controlled clinical trial | Capsules containing 330 mg liquorice root extract or placebo capsules containing 330 mg starch 3 times a day (morning, noon and night) for 8 weeks. | 90 (n = 45 per group); mean age 53 ± 3.19 y in liquorice group and 52.69 ± 2.80 y in placebo groupo | Iranian women aged between 45-60 years old, with a BMI < 29 $kg/m^2$; experiencing amenorrhea for at least 1 year or at most 3 year; suffering from hot flash and using no | No side effect related to liquorice was reported by the subjects. Three women in the intervention group reported blotting in the $2^{nd}$ 4 week of therapy, which was | No further information on the blotting experienced by women. The authors attributed blotting to to the estrogenic effect of liquorice root. |

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| menopausal women<br><br>Nahidi *et al.* 2012 | | To assess recurrence, the subjects were followed up for 4 weeks after the capsule administration. | | drug or hormone to relieve it | relieved by discontinuation of the capsules. | |
| To evaluate the effects of liquorice on hot flash symptoms in menopausal women.<br><br>Menati *et al.* 2014 | Randomized, double blind, active comparator, clinical trial | Tablets containing 650 mg liquorice root extract (1140 mg/day of liquorice extract) or a conjugated estrogen 0.312 mg/day and Medroxyprogesterone 2.5 mg/day for 90 days (HRT).<br><br>The content of Glycyrrhizin in the liquorice extract was reduced to 3%. | 60 women treated with liquorice or HRT, however in the flow chart of the trial only 52 women were reported as enrolled, randomised and treated" (n = 26 per group). Mean ages 50.08 ± 3.01 y and 51.27 ± 2.22 y in liquorice and HRT groups, respectively. | Menopausal Iranian women aged from 45 to 60 years old, who were in the first 5 years post-menopause. | Few side effects were reported (in the liquorice group two cases of nausea and in the hormone therapy group one case of headache and another of mastodinia). | |
| To investigate the impact of a vaginal cream containing liquorice on vaginal signs and symptoms of | Randomized, double-blind, placebo-controlled trial | Vaginal cream containing 2% of hydroalcoholic extract of liquorice or placebo as a full applicator over a period of 8 weeks at bedtime.<br><br>Maturation vaginal index (MVI) and pH | 70 (n = 35 per group); mean age 56.40 ± 4.29 y in liquorice group and 56.17 ± 4.73 in placebo group.<br><br>Drop-out: 2 in liquorice group due to side effects | Iranian women who were postmenopausal for at least 1 year, having symptoms of vaginal atrophy (dryness, soreness, and burning or itching of the vagina and dyspareunia), gynecology | Two women dropped out in liquorice group due to side effects such as vulvo vaginal itching and burning (n = 1) and erythema of face skin (n=1). However, there was not any significant difference between the two | |

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| vaginal atrophy in postmenopausal women.<br><br>Sadeghi *et al.* 2020 | | measured at baseline, and after 2, 4, and 8 weeks. | Women included in the final analysis: 35 in placebo group and 33 in liquorice group. | examination, vaginal pH>5, and the percentage of cellular maturation of 0-49 in vaginal smear | groups with regards to side effects (p = 0.49). | |
| To evaluate the effectiveness, of liquorice nasal irrigation (LNI) for AR.<br><br>Chang *et al.* 2021 | Randomised, open label, controlled clinical trial | Once daily treatment with a suspension containing 3 mg/ml of dry water extract of liquorice roots (LNI), or corticosteroid solution containing 2 mg/300 ml mometasone (CNI) or isotonic saline solution (SNI) in a squeeze bottle for nasal irrigation.<br>Treatment duration: 1 month.<br><br>The glycyrrhizic acid concentration in the LNI was calculated as 249.6 μg/ml | 60 (n = 20 per group, three groups); mean age of 32.7 years (range: 20–80 years); 28:32 M/F<br><br>Drop out: 3 subjects in the CNI group for unwillingness to continue using steroids or because of antihistamine use; 2 subjects in the SNI group due to personal business or because of antihistamine use.<br>55 patients included in the final analysis. | Taiwanese outpatients aged more than 20 years who had medically diagnosed AR with intermittent or persistent symptoms ranging from mild to severe. The diagnosis of AR was based on patient's clinical symptoms and blood tests. The results of blood tests should reveal a total IgE level greater than 120 KU/L and<br>the presence of at least one specific inhaled allergen (i.e. grass pollen, house dust mite, animal dander, and mold). | None of the patients developed redness, swelling, or infection of the nasal cavity or pharynx after the therapies, and no nasal irrigation-associated complications were found.<br>Some participants experienced slight ear tingling or stuffiness when they started to practice the procedure in initially, but most of them did not experience the problem after 2–3 days of treatment. This occurrence of ear discomfort was similar in the three groups. | Liquorice was well tolerated when applied in the nasal cavity. |
| To determine the liquorice | Double-blind, randomised, | Oral treatment with 5 cc of a syrup | 39; mean age: 59.53 ± 8.7 y in | Iranian patients aged between 30 and 80 | Three participants of the liquorice group left | |

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| effectiveness as an adjunct treatment in the PD management.<br><br>Standard treatment for PD: divided doses of pramipexol 0.360 mg/day and/or levodopa-B 220–1000 mg/day.<br><br>Petramfar *et al.* 2020 | placebo-controlled clinical trial | containing a dry hydroalchoholic extract of liquorice roots (extraction solvent ethanol:water 52:26 v/v) or with placebo syrups as an adjunct therapy twice a day for the time duration of 6 months.<br><br>Each 5cc of syrup contained 136 mg of polyphenol-rich extract of liquorice with 12.14 mg of glycyrrhizic acid, 136 µg of polyphenols, and 2.6 µg of flavonoids. | liquorice group and 64.66 ± 9.4 y in placebo group; 19:11 M/F<br><br>Drop-out: 5 in liquorice group (lost to follow-up, side effects and changes in PD standard treatment) and 4 in placebo group (lost to follow-up, changes in PD standard treatment). 30 patients included in the final analysis. | years old with idiopathic PD, initiation of PD symptoms in recent 6 years, YAHR staging ≤ 3 and no treatment changes within 4 weeks before the intervention starting.<br><br>Patients with a background of diabetes, stroke, myocardial infarction, heart failure, renal failure, cardiac arrhythmia, liver diseases, uncontrolled hypertension, and hypokalemia were excluded. | the trial because of nausea, diarrhea or urticaria and their tolerability was calculated as 85%. Additionally, one patient from the liquorice group with complains of nausea, vomiting, and anorexia reported a recovery. Finally, orthostatic hypotension was reported in no patients of two groups. No significant changes were observed in blood pressure or blood glucose levels of the patients during this study. | |

## 5.2. Patient exposure

In clinical pharmacology and clinical efficacy studies mentioned in this assessment report more than 2000 individuals were exposed to several pharmaceutical forms containing different liquorice preparations and dosages for durations of treatment ranging from few days to two years. Topical or oral administration of liquorice appeared to be well tolerated, with gastro-intestinal disturbances as the more frequent adverse events, although several clinical studies did not report any information on safety outcomes or did not carried out a complete assessment of the safety profile (e.g. blood or urine analyses, histological examinations etc.).

Moreover, several liquorice preparations can be found as medicines in the EU for more than 30 years. This implies a very high number of patients taking these kinds of preparations.

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

Although the exact number of severe liquorice-induced hypertensive cases is unknown, case reports describing severe hypertension as a result of excess liquorice consumption have been documented since the late 1940s (Rachman-Elbaum & Johnson, 2014). The pseudoalstosteronism induced by an excessive intake of liquorice was firstly described in 1968 (Conn *et al.*, 1968).

Liquorice or its constituent glycyrrhetinic acid may cause hypertension and hypokalemia due to cortisol-mediated activation of the mineral corticoid receptors by decreasing 11β-HSD2 activity. Indeed, in pseudoaldosteronism, it is thought that glycyrrhetinic acid inhibits 11β -HSD2 in renal tubular cells, resulting in an accumulation of cortisol that activates mineral corticoid receptors (Shimada *et al.*, 2019).

Cortisol has an equivalent effect on mineralocorticoid receptors in the distal renal tubules as aldosterone. Mimicking the aldosterone action, cortisol activates the mineralocorticoid receptors in the distal renal tubules, causing $K^+$ excretion and $Na^+$ retention (Rachman-Elbaum & Johnson, 2014).

Two additional mechanisms of action by which glycyrrhetinic acid activates the mineralocorticoid receptors have been also described. In the first pathway, glycyrrhetinic acid inhibits 5-*β*-reductase enzyme causing aldosterone accumulation (Latif *et al.*, 1990). In the second pathway, glycyrrhetinic acid directly activates the mineralocorticoid receptors (Calò *et al.*, 2004). However, the main activity of glycyrrhetinic acid is through inhibition of 11-*β*-HSD2 causing excess cortisol subsequently inhibiting mineralocorticoid activity (Rachman-Elbaum & Johnson, 2014).

There is apparently a great individual variation in the susceptibility to liquorice. Most individuals who consume 400 mg glycyrrhizic acid daily experience adverse effects. It can also occur by taking a small dose of glycyrrhizic acid 100 mg (Størmer *et al*., 1993).

The mechanism of this difference in susceptibility has not been clarified but is thought to be due to genetic mutation. Genetic mutation in the 11-*β*-HSD2 gene may compromise its protective action against cortisol access to the mineralocorticoid receptor, and this might lead to greater sensitivity to liquorice (Kim & Kim, 2019). Liquorice shouold not be taken by people with a deficiency in 11HSD or with genetic mutation in the 11-*β*-HSD2 gene (Braun & Cohen, 2015), although clinical evidence is limited to a single case-report of a 51-year-old female with serious hypertension who had been taking herbal medicine containing liquorice for more than one year (Harahap et al., 2011).

Elimination of liquorice intake (usually along with potassium supplementation) alleviates hypertension and hypokalemia in glycyrrhetinic acid-induced hypertension. Full recovery is expected within a few weeks (Rachman-Elbaum & Johnson, 2014). However, this altered physiological state may persist for

several weeks depending upon the quantity of liquorice consumed and individual susceptibility (Omar *et al.*, 2012).

Several investigators have reported that excessive and/or long-term administration of liquorice-containing medicines, crude drug products, or glycyrrhizin frequently leads to pseudoaldosteronism with symptoms such as peripheral edema, hypokalemia, and hypertension. In addition, as a result of pseudoaldosteronism-associated hypokalemia, muscle injury (such as myopathy or rhabdomyolysis), heart injury (such as heart failure or arrhythmia), or glucose intolerance could develop (Shimada *et al.*, 2019).

High dosage and long-term use of liquorice are constitutional risk factors for pseudoaldosteronism. In humans, glycyrrhetinic acid and other metabolites of glycyrrhizin are highly bound to albumin in blood circulation and are predominantly excreted into bile via multidrug resistance-associated protein 2 (Mrp2). In constipated patients the bioavailability of glycyrrhizin metabolites is enhanced. Indeed, the hydrolyzation ratio also depends on the residence time of glycyrrhizin in the intestinal tract. The longer glicyrrhizin stays in the intestinal tract, the more it is hydrolyzed by bacterial β-glucuronidase, resulting in a higher serum concentration of glycyrrhetinic acid. Under hypoalbuminemic conditions, the unbound metabolite fractions can reach 11HSD2 at the distal nephron. Hyper direct bilirubin could be a surrogate marker of Mrp2 dysfunction, which results in metabolite accumulation. Older age is associated with reduced 11HSD2 function, and several concomitant medications, such as diuretics, have been reported to affect the phenotype. Therefore, constipation, hypoalbuminemia, hyper direct-bilirubin, older age, and concomitant medications are considered risk factors for liquorice-induced pseudoaldosteronism, together with high dosage and long-term use (Yoshino *et al.*, 2021).

The transit rate of gastrointestinal contents through the small and large intestines predominantly determines to what extent glycyrrhetic acid conjugates will be reabsorbed. Therefore in subjects with prolonged gastrointestinal transit times, glycyrrhetic acid might accumulate causing toxicity after repeated intake (Omar *et al.*, 2012).

Adverse reactions related to glycyrrhetinic acid include cardiovascular complications (hypertension, hypertension encephalopathy arrhythmias, heart failure, pulmonary edema, generalized edema, cardiac arrhythmias, embolic ischemia), neurological complications (hypokalemic myopathy, stroke, rhabdomyolysis, carpal tunnel syndrome, myoclonus, ocular deficits), electrolytes abnormalities (hypokalemia, metabolic alkalosis, elevated cardiac enzymes), renal abnormalities (acute tubular necrosis), liver disease (increased symptoms in alcoholic and nonalcoholic chronic liver disease, increased symptoms with cirrhosis, and bile duct obstruction), allergic reactions (asthma, contact dermatitis), dental care (dental carries, brownish/black tongue) (Omar *et al.*, 2012; Rachman-Elbaum & Johnson, 2014). In addition, adverse reactions during pregnancy such as pre-eclampsia and risk for miscarriage are reported (Rachman-Elbaum & Johnson, 2014).

Bibliographic research of case-reports on pseudoaldosteronism due to liquorice ingestion identified almost 70 publications since 2011. A vast majority of cases were related to an excessive intake of liquorice (more than 100 mg/day of glycyrrhizic acid) in several forms (candies, syrup, tea, powder, tablets, jelly, beverages etc.) for a long period (one month to several years). Almost half of the cases of hyperaldosteronism affected adult women and men with hypertension and, less frequently, with other pathologies such as dyslipidemia and diabetes; in a small number of these subjects mineralcorticod effects was also observed with lower amounts of liquorice (< 100 mg/day of glycyrrhizic acid) for short duration of intake (less than one month). All cases but one were reversible, in most cases with potassium supplementation and diuretics (spironolactone, canreonate) and/or hypertensive drugs when necessary. An old patient with low body weight and a history of of old cerebral infarction and Alzheimer-type dementia died due to Takotsubo cardiomyopathy-related

arrhythmia; therapy with yokukansan, a liquorice-containing (1.5 g/day) Kampo drug, for over 10 years and presence of other risk factors (older age, anorexia, intake of drug for Alzhimer-type dementia) for pseudoalsosteronism contributed to the fatal outcome (Yoshida *et al.*, 2022).

Intestingly, there is one case report showing that liquorice may cause cardiac arrhythmia without hypokalemia (Öztürk *et al.*, 2013). Hypokalemia and severe ventricular tachycardia of torsades de pointes type reported with liquorice consumption may be potentiated with concomitant use of anti-arrythmic agents (PDR, 2000).

A case-report has been published describing an episode of severe hypokaliemia in a 18-y-old female student with anorexia nervosa taking only 20 g of liquorice sweets daily, corresponding to approximately 70 mg of glycyrrhizic acid per day (based on manufacturer's data). It has been hypothesed that liquorice ingestion could potentially aggravate hypokalemia in patients with chronic laxative abuse such as those affected by anorexia nervosa (Støving *et al.*, 2011).

Ten cases of posterior reversible encephalopathy syndrome (PRES), involving also children, following ingestion of liquorice have been reported (McNicholl & Kilroy, 1969; van der Zwan, 1993; Russo *et al.*, 2000; Kim *et al.*, 2003; Chatterjee *et al.*, 2010; van Beers *et al.*, 2011; Morgan *et al.*, 2011; Tassinari *et al.*, 2015; O'Connell *et al.*, 2016), but a clear correlation in most cases was not established; in addition, the daily intake of liquorice and the duration was indicated only in three published case-reports.

Single case-reports of carpal tunnel syndrome (Tacconi *et al.*, 2009), thrombocytopenia (Celik *et al.*, 2012), angina pectoris (Machalke *et al.*, 2015), idiopathic mesenteric phlebosclerosis (Jin *et al.*, 2022), melanosis coli (Correia-Varela-Almeida *et al.*, 2018) after liquorice intake have been also published.

Adverse reactions during pregnancy such as pre-eclampsia and risk for miscarriage are reported (Rachman-Elbaum & Johnson, 2014). One case-report of severe pre-eclampsia in a 18-aged girl at less than 20 weeks gestation possibly aggravated by the consumption of liquorice has been published (Hauksdottir *et al.*, 2015).

Finally, there is a number of case-reports dealing with contact dermatitis developed with topical products containing liquorice.

***EudraVigilance***

A search carried out on Eudravigilance database (Eudravigilance Post Marketing Module – EVPM) on 3rd of December 2024 returned 1370 reports of suspected adverse drug reactions (ADR) associated with liquorice as suspected/interacting active substance. The search terms were "liquorice", "liquiritiae" and "Glycyrrhiza glabra". For the analysis, all reports where liquorice has been used as a single substance or in combination to other compounds were considered. A number of 253 reports (18.5%) referred specifically to liquorice extracts, including herbal teas. Among the ADRs, the preferred term hypokalaemia was the most reported ADR (5.93%), followed by diarrhoea (2.70%), rhabdomyolysis (2.70%), gastro-intestinal disturbances (2.56%) and nausea (1.89%). Twelve cases were fatal, but in none of them the medicinal product containing liquorice has been judged as correlated to the fatal outcome. Only 14 reports of severe ADRs reported medicinal product containing liquorice as the only suspected drug and the most observed ADRs were again hypersensitivity reactions, diarrhoea and vomiting; however, in only one report (severe ADR: hypertension) liquorice as a herbal tea was the only substance taken by the patient. In total, only 9 reports refer to liquorice as the only active ingredient taken by the subjects:

• 1 serious case report related to hypertension;

• 8 non-serious case reports related to hypertension and gastrointestinal disorders (e.g. vomiting, diarrhoea, and nausea).

Hypertension and mineralcorticoid effects are well known effects of liquorice, already included in the monograph. As for the gastrointestinal ADRs a clear causal relationship cannot be established due to the the lack of enough information in the report which do not allow for a thorough assessement.

*Assessor's comment: Hypertension and mineralcorticoid effects are usually observed with higher doses and with longer duration of treatment compared to those reported in the monograph. Liquorice preparations are not recommended to be used in patients affected by hypertension, kidney diseases, liver or cardiovascular disorders or hypokalemia, as they are more sensitive to the adverse effects of liquorice (see section 4.4 of the monograph). Other adverse events, such as those reported in clinical trials (e.g. diarrhoea, nausea, gastric complaints, and increase in blood sugar) were observed with preparations different from those reported in the monograph or they were associated with long duration of treatment. The search on EudraVigilance database returned a total of 8 reports of gastro-intestinal complaints (e.g. vomiting, diarrhoea, and nausea) where liquorice preparations were the only products taken by the subjects. However, information reported for these few cases did not allow for the identification of a clear causal relationship, therefore section 4.8 of the monograph does not mention any specific adverse event.*

## *5.4. Laboratory findings*

Liquorice assumption, particularly at high dosages and for longer periods, induces a mineralcoid effect which results in an increase of blood pressure, hypokalemia, lowered levels of blood PRA and aldosterone, increased cortisol/cortisone ratio in urine. In addition, in several case-reports ECG alterations, including sinus bradycardia, prominent U-wave, wide T-wave and prolongation of QT interval were reported as a consequence of severe hypokalemia (van Beers *et al.*, 2011; Russo *et al.*, 2000; Kormann *et al*., 2012; Taylor *et al.*, 2012; Desmet *et al*., 2013; Öztürk *et al*., 2013; Flores Robles *et al.*, 2013; Yamada S et al., 2013; Panduranga & Al-Rawahi, 2013; Piva *et al*., 2014; Bisogni *et al.*, 2014; Smith *et al.*, 2016; He *et al*., 2018; Smedegaard & Svart 2019; Angus & Stranks, 2020; Petersen *et al*., 2020; Edelman *et al.*, 2020; Yoshida *et al*., 2022).

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

No data available.

### 5.5.1. Use in children and adolescents

There is insufficient data to support the safety of liquorice root in children and adolescents under 18 years.

### 5.5.2. Contraindications

Contraindicated in case of hypersensitivity to the active substance(s).

### 5.5.3. Special warnings and precautions for use

The ability of liquorice to increase blood pressure is well established and its well known mineralcorticoid effects include hypokaliemia, which is associated with sodium retention, peripheral edema and potassium loss. The sodium retention is associated with suppression of plasma levels of

renin and aldosterone. In addition, as a result of pseudoaldosteronism-associated hypokalemia, muscle injury (such as myopathy or rhabdomyolysis), heart injury (such as heart failure or arrhythmia), or glucose intolerance could develop (Shimada *et al.*, 2019).

Liquorice medication is not recommended to be used in patients affected by hypertension, kidney diseases, liver or cardiovascular disorders or hypokalemia, as they are more sensitive to the adverse effects of liquorice.

Patients taking liquorice medication should not take other liquorice containing products as serious adverse events may occur such as water retention, hypokalemia, hypertension, cardiac rhythm disorders.

Concomitant use with diuretics, cardiac glycosides, corticosteroids, stimulant laxatives or other medications which may aggravate electrolyte imbalance is not recommended (see section 4.5).

If dyspnoea, fever or purulent sputum occurs when liquorice is used as an expectorant in cough associated with cold, a doctor or a qualified health care practitioner should be consulted.

## 5.5.4. Drug interactions and other forms of interaction

As a consequence of its mineralcorticoid effects, particularly due to marked hypokaliemia, liquorice should not be used concomitantly with diuretics, cardiac glycosides, corticosteroids, stimulant laxatives, or other medications which may aggravate electrolyte imbalance.

There is clinical evidence that liquorice increases blood pressure, therefore it may counteract antihypertensive action of prescribed medications.

An *in vivo* study found that glycyrrhetic acid induced P-glycoprotein and CYP3A4, resulting in reduced oral bioavailability of cyclosporin (Hou *et al.*, 2012). In humans, glycyrrhizin has been shown to cause a moderate, clinically relevant, induction of CYP3A4 (Tu et al 2010a). Clinical studies have shown that the oral assumption of 300 mg/day of glycyrrhizin for 14 days can significantly decrease the systemic exposure to midazolam and omeprazole, through induction of CYP3A4-mediated metabolism. Since, based on literature data, there is a high variability of glycyrrhizin intake with different liquorice preparations, a definite daily intake of glycyrrhizin to rule out potential clinically meaningful interactions cannot be predicted according to the posology reported in the monograph; therefore, caution is adviced when the herbal preparations described in the monograph are taken concomitanly with drugs known to be substrate of CYP3A4 (e.g. midazolam, ciclosporin A, omeprazole).

It has been hypothised that liquorice could increase the risk of bleeding or potentiate the effects of warfarin therapy due to the presence of coumarines (Heck et al., 2000; Singh et al., 2012), although clinical evidence is limited to a case-report of a 80-year-old woman with atrial fibrillation, anti-coagulated with warfarin, who on two separate occasions developed black tarry stools and an elevated international normalized ratio (INR) after eating a pound of Black Liquorice (Liu et al., 2010).

Interestingly, a more recent case report showed that the consumption of 1.5 kg of hard-boiled candy liquorice significantly reduced INR values in a 92-year-old female patient, who was diagnosed with atrial fibrillation and treated with phenprocoumon to prevent strokes. The authors hypothesed that liquorice may have stimulated the function of CYP3A4 leading to reduced bioavailability of phenprocoumon; in addition, the influence of liquorice on peroxisome proliferator-activated receptor alpha transactivation may have also played a role (Roemer *et al.*, 2021).

The clinical evidence of interactions between liquorice and anticoagulants is based only on few case-reports, and theoretical rationale is not enough robust to make any specific recommendations

regarding concurrent use; indeed, the coumarin constituents of liquorice are not known to be anticoagulants, and there is no evidence of liquorice acting as an anticoagulant (Williamson *et al.*, 2009).

The same conclusion applies for possible interactions of liquorice with oral contraceptives. Indeed, although interaction with oral contraceptives, with a similar clinical pattern (hypokalaemia and water retention), has also been suggested (Di Lorenzo et al., 2014), clinical evidence is limited to only one case-report of a 39-year-old woman taking no drugs except for oral contraceptives who experienced headache, generalized weakness and upper-limb edema, hypokalaemia, low plasma levels of renin and aldosterone without hypertension (Francini-Presenti et al., 2008).

## 5.5.5. Fertility, pregnancy and lactation

*Pregnancy*

The exposure to liquorice is particularly critical during pregnancy. The fetus is protected from the 5-10-fold increased maternal cortisol levels during pregnancy by highly expressed placental 11β-HSD2. The placental glucocorticoid metabolism was examined in dually perfused freshly isolated intact human placentas, obtained from randomly selected normal term deliveries. The maternal circuit was perfused with physiological concentration of cortisol, the fetal effluent collected, and steroid metabolites separated and quantified using silica columns and HPLC. Most of the maternal administered cortisol was metabolized o cortisone, and no conversion of cortisone to cortisol was detected. Cortisone was the only product of cortisol metabolism. Ihibition of 11β-HSD with glycyrrhetinic acid allowed cortisol to gain direct access to fetal circulation (Benediktsson *et al.*, 1997).

Observational studies implicated shorter gestation times, poorer cognitive function and behavioral disturbances associated with an increased activity of the hypothalamic-pituitary-adrenal axis after maternal consumption of large amounts of liquorice (Beck *et al.*, 2020).

A sample of 1049 Finnish women and their healthy singleton infants was studied in 1998. 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% CI: 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 *et al.* 2001).

In another study, the same group of researchers tested whether this association also applied to preterm births collecting other data through the same methodology in the years 2000-2001. A sample of 95 Finnish women who delivered preterm singletons was compared with controls (*n* = 107) who delivered babies of normal gestational age. Heavy consumption versus a lower level of consumption was associated with a more than two-fold increased risk of preterm (<37 weeks) delivery. The association was stronger when only the 40 births classified as early preterm delivery (<34 weeks) were included (odds ratio = 3.07, 95% CI: 1.17, 8.05 for the fully adjusted model). The authors concluded that heavy glycyrrhizin exposure was associated with preterm delivery (Strandberg *et al.*, 2002).

An Italian study collected data over a 10-month period through a face-to-face interview of 392 pregnant women to explore the use of herbal products and the possible influence of herbal consumption on pregnancy outcome. Fifteen women (13.8%) regularly took oral liquorice during pregnancy for reasons such as hypotension, digestive problems, strengthen immune system. Among the regular users of liquorice, a higher frequency of threatening miscarriages (35.7%) and preterm labours of low-birth-weight infants (16.7%) compared to non-users were observed (Cuzzolin *et al.*, 2010).

The fetal and neonatal outcomes of 185 singleton pregnant Korean women reporting exposure to liquorice as a constituent of over the counter (OTC) medications and other pharmaceutical preparations was evaluated in a prospective cohort study age-matching with 370 singleton pregnant controls that were not exposed to any potential teratogen. The indication in 56.8% of the women taking liquorice was for cough and cold control, followed by treatment of functional gastrointestinal disorders in 22.2%, with the maximum dose of 2104 mg/day and exposure occurring between the 4$^{th}$ day and 25$^{th}$ week of gestation. The rate of stillbirths was marginally higher among women who took liquorice than those who did not (OR = 7.9; 95% CI 0.9–71.5; p = 0.048), and significantly higher when compared to the general population in the Republic of Korea (OR = 13.3; 95% CI 4.9–35.8; p < 0.001). Among women who delivered, gestational age at birth, birth weight and length, head circumference at birth, and 1-min and 5-min Apgar scores were similar between the two study groups. There were two babies born with major malformations in the liquorice group and one in the control group (OR = 3.9; 95% CI 0.4–43.5; p = 0.27). The authors concluded that liquorice as a constituent of OTC and naturopathic formulations is not a major human teratogen and is not associated with adverse fetal and neonatal outcomes. However, the study demonstrates preliminary evidence that liquorice may increase the risk of stillbirth. These findings require further confirmation in studies with a larger sample size (Choi *et al.*, 2013).

The conclusions of the studies by Strandberg *et al*. (2002) and Choi *et al*. (2013) were confuted in a re-analysis conducted by MacLennan & Koog (2014). The authors considered that the conclusion drawn from the study by Strandberg *et al*. (2002) should be viewed with caution. First, because data on liquorice consumption was collected through the use of a retrospective postnatal questionnaire, the actual amount of liquorice consumed by a subject may be different to that reported. Second, because the odds ratio, defined as the ratio of odds in the case group (i.e. the proportion of people given exposure divided by that of people not given exposure) to the corresponding odds in the control group, was 2.18 (95% CI: 0.98–4.86, p = 0.056), thus termed as insignificant. The authors identified a number of critical flaws also in the study by Choi et al. (2013), i.e. the data collection method used in this trial was reliant on the trial subjects' memory ("memory bias"), the presence of an acute illness in early gestation in more than half of the pregnant women which could have influenced the number of stillbirths, unclearness if all the women in the experiment group actually received medication containing liquorice since it was reported in the study that women in the experiment group took a commercially available substance or a prescribed herbal medicine. Therefore, the authors highlighted the need for a systematic and unbiased evaluation of the evidence relating to the use of liquorice during pregnancy (MacLennan & Koog 2014).

The intake of high amounts of liquorice-containing confectionery between the 3$^{rd}$ day and the 25$^{th}$ week of gestation by Finnish pregnant women was associated with pubertal maturation (height, weight, BMI for age, difference between current and expected adult height, Tanner staging, score on the Pubertal Development Scale), neuroendocrine function (diurnal salivary cortisol, dexamethasone suppression), cognition (neuropsychological tests), and psychiatric problems (as measured by the Child Behavior Checklist) in their offspring. These cognitive, behavioral, and neuroendocrine changes persisted during the adolescence. Girls exposed to high maternal glycyrrhizin consumption (≥500

mg/week) were more than 3 cm taller (mean difference (MD) = 0.4 SD, 95% CI: 0.1, 0.8), were 8 kg heavier (MD = 0.6 SD, 95% CI: 0.2, 1.9), and had 2.2 higher body mass index for age (MD = 0.6 SD, 95% CI: 0.2, 0.9) than girls whose mothers consumed none or low amounts. They were also 0.5 standard deviations (95% CI: 0.2, 0.8) closer to adult height and reported more advanced pubertal development ($P < 0.04$). Girls and boys exposed to high maternal glycyrrhizin consumption scored 7 (95% CI: 3.1, 11.2) points lower on tests of intelligence quotient, had poorer memory ($P < 0.04$), and had 3.3-fold (95% CI: 1.4, 7.7) higher odds of attention deficit/hyperactivity disorder problems when compared to children whose mothers consumed little to no glycyrrhizin at all (≤249 mg/week). The authors admitted some limitation of the design of the study. Indeed, average weekly consumption levels of glycyrrhizin during pregnancy and consumption of liquorice confectionery only was measured, since the authors were unable to determine consumption of other glycyrrhizin-containing products. In addition, the authors could not determine pubertal timing, and the stage of puberty was self-reported (Räikkönen *et al.*, 2017).

A total of 225 Danish pregnant women were included in a prospective cohort study to assess the prevalence and characteristics of alternative medicine, ginger and liquorice use. No less than 87.1% (n = 196) reported consuming liquorice, 38.2% (n = 86) reported liquorice consumption at least "a couple of times a week," and 7.1% (n = 16) reported daily use. A minor increase in maternal SBP ($p = 0.04$) in women reporting a mean daily intake of liquorice compared to those reporting rare or no intake of liquorice. Moreover, the frequency of liquorice intake was also associated, albeit not significantly, with reduced birthweight (Volqvartz *et al.*, 2019).

*Lactation*

Glycyrrhizin is detectable in the breastmilk of some women taking liquorice, but studies measuring glycyrrhetinic acid have not been performed. Ten lactating women with breast engorgement were treated with granules containing a mixture of various herb extracts, including liquorice. The dose was 2.5 grams 3 times daily (about 400 mg daily of liquorice extract). Glycyrrhizin was measured in the breastmilk at unspecified times. Glycyrrhizin was detected in 2 women at 1.14 mg/L and 0.15 mg/L, and in 3 women at <0.1 mg/L. It was undetectable in the remaining 5 women. (Drugs and Lactation Database – Liquorice, 2021).

A woman with a history of excessive liquorice intake had amenorrhea, severe headaches, hypertension, and hypokaliemia. She had elevated serum prolactin levels that remained abnormal for one month after liquorice discontinuation and normalized by 6 months after discontinuation (Werner *et al.*, 1979).

In a study of 25 men and 25 women, the baseline and thyrotropin-stimulated serum prolactin levels were measured to determine normal serum prolactin values. Subjects who regularly ingested liquorice had lower basal and lower stimulated serum prolactin concentrations (Le Moli *et al.*, 1999).

*Assessor's comment: experimental studies on human placentas have suggested that glycyrrhetinic acid inhibits 11ß-HSD2 function allowing a direct placental by-pass for maternal circulating cortisol to the fetus. In the literature, concerns have been raised from high liquorice consumption with intakes of glycyrrhizin > 500mg/week during pregnancy.*

*In conclusion, there are limited data from use in pregnant women; in the absence of sufficient data, the use during pregnancy and in women of childbearing potential not using contraception is not recommended.*

*Glycyrrhizin is detectable in the breastmilk of some women taking liquorice, but studies measuring glycyrrhetinic acid have not been performed. The effect of liquorice root or its constituents on newborns/infants is unknown. The use during breast-feeding is not recommended.*

*Fertility*

The effect of liquorice on gonadal function was assessed in seven normal men, 22 to 24 years of age. The men were given 7 g daily of a commercial preparation of liquorice in the form of tablets containing 0.5 g of glycyrrhizic acid for seven days and four days after it was discontinued. During the period of liquorice administration, the men's serum testosterone concentrations decreased and their serum 17-hydroxyprogesterone concentrations increased; however, levels reverted to pre-treatment values 4 days after discontinuation of liquorice administration (Armanini *et al.*, 1999).

Later investigations by the same study group seemingly replicated these findings, confirming that the oral administration of 7 g liquorice per day (0.5 g of glycyrrhizic acid) in 17 healthy men resulted in a significant decrease (—25%) in total serum testosterone levels, as well as a significant increase in serum 17-hydroxyprogesterone (+39%) and LH levels (+24%) after 8 d of treatment, but no significant changes in serum androstenedione levels were detected (Armanini *et al.*, 2003b).

Finally, the same group of authors investigated the effect of liquorice on androgen metabolism in 9 healthy women 22-26 years old, in the luteal phase of the cycle. They were given 3.5 g of a commercial preparation of liquorice (containing 7.6% w/w of glycyrrhizic acid) daily for two cycles. Total serum testosterone decreased from 27.8+/-8.2 to 19.0+/-9.4 in the first month and to 17.5+/-6.4 ng/dL in the second month of therapy ($p < 0.05$). It returned to pre-treatment levels after discontinuation. Androstenedione, 17-hydroxy-progesterone, and LH levels did not change significantly during treatment. Plasma renin activity and aldosterone were depressed during therapy, while blood pressure and cortisol remained unchanged (Armanini *et al.*, 2004).

On the other hand, administration of 5.6 g/day of liquorice (containing 0.5 g/day of glycyrrhizic acid) in 20 healthy men over 4 consecutive days did not significantly decreased salivary testosterone levels (—9.5%) measured by RIA, even after repeating the trial using liquorice from different sources and after recruiting a new group of participants (11 men; 10 women) (Josephs *et al.*, 2001).

The effect of glycyrrhizin on serum testosterone concentrations was also investigated in a study including 18 male patients with type 2 diabetes and chronic hepatitis who were given weekly glycyrrhizin, which contained 240–525 mg glycyrrhizic acid, for >1 year and 21 male patients not given glycyrrhizin. Serum concentrations of total and free testosterone were significantly lower in patients given glycyrrhizin than those in patients not given glycyrrhizin (4.3 ± 2.2 vs. 5.9 ± 1.7 ng/ml, P = 0.0113; 6.7 ± 3.8 vs. 11.1 ± 3.8 pg/ml, P = 0.0009, respectively). Mean intima-media thickness and plaque score by carotid ultrasonography were significantly greater in patients given glycyrrhizin than in patients not given glycyrrhizin (1.12 ± 0.29 vs. 0.89 ± 0.23 mm, P = 0.0385; 6.8 ± 3.1 vs. 3.7 ± 3.3, P = 0.0326, respectively). Glycyrrhizin treatment was an independent risk factor (β = 0.464, P = 0.0433) for atherosclerosis (plaque score) after adjustment for age, hypertension, hyperlipidemia, smoking history, and glycemic control (HbA1c) (Fukui *et al.*, 2003).

In a further study, the effects of 100 g/day liquorice confectionery administration (containing 3% of liquorice extract) on salivary steroids profiled by enzyme-linked immunosorbent assay in 20 healthy male (n = 10) and female (n = 10) volunteers, were compared to those found in healthy matched controls receiving 100 g/day of non-liquorice confectionery using a crossover design. After 1 wk of treatment, the authors observed a significant increase in deoxycorticosterone, dehydroepiandrosterone, and testosterone concentrations in volunteers receiving liquorice compared with controls (Al-Dujaili *et al.*, 2011).

An open-treatment trial compared sex steroid hormone levels in 21 male and 15 healthy female volunteers with essential hypertension receiving a daily intake of 100 g of sweet liquorice (containing 0.15 g of glycyrrhetinic acid). The study period consisted of three parts: 1 run-in week with definition of baseline values, one 4-week period with a daily intake of 100 g of sweet liquorice, and, finally, a washout period of 4 weeks. The women commenced the consumption of liquorice on days 1–4 of the menstrual cycle. After 4 wk of liquorice administration, the serum concentrations of androstenedione, progesterone, prolactin, SHBG, LH, follicle-stimulating hormone (FSH), and estradiol measured by RIA were not affected by liquorice consumption in neither the male nor female participants. Conversely, a sex difference was found with regard to the variation in dehydroepiandrosterone sulfate (DHEAS) and testosterone levels. In more detail, a moderate decrease in serum DHEAS levels was observed in men, but not women, whereas serum DHEAS and testosterone levels increased after a 4-wk washout period in women only; thus, suggesting a possible sex-dependent effect on androgens (Sigurjónsdóttir *et al.*, 2006).

*Assessor's comment: Several clinical studies have investigated the effect of liquorice on testosterone levels both in men and women, but results are conflicting. Some studies examined salivary testosterone, whereas others assessed serum androgen levels. The assessment of salivary testosterone might not be a reliable method to detect hypogonadism, defined by low serum total testosterone. These studies do not contribute to establish a clear link between reduced testosterone levels and symptoms related with hypogonadism or sexual dysfunction, therefore, in this prospect, further clinical invest*
*igation is needed.*

*In conclusion, the statement "No adequate fertility data available" is reported in the monograph.*

## 5.5.6. Overdose

Abnormal vision has been reported by WHO as a possible adverse ocular reaction associated with large doses of liquorice (Fraunfelder, 2004).

Excess liquorice intake may cause various cardiovascular complications due to its mineralcorticoid effect. A review including 36 published articles until year 2010 addressing the hazardous effects of liquorice on the cardiovascular system revealed that the most serious cardiovascular complication arising from liquorice intake relates to its arrhythmogenic potential. Chronic ingestion can lead to depletion of the body potassium stores. The extent of metabolic derangement can be severe enough to cause profound hypokalemia – a precursor of QT interval prolongation and ventricular arrhythmias. Cardiac arrest is a common associated feature, with a subsequent recovery in the majority of patients. Another complication frequently encountered after copious liquorice intake is systemic hypertension. There is a wide continuum of presentations ranging from mild to severe resistant hypertension requiring hospitalization. Several patients developed hypertensive encephalopathy. The $Na^+$-retaining effect of liquorice can lead to generalized edema or more seriously acute heart failure and pulmonary edema, which usually occur after a liquorice binge. Liquorice-induced hypertension and hypervolemia is reversible once intake is stopped. Cessation of liquorice intake should be immediately implemented in patients with toxicity. Aggressive potassium supplementation should be instituted in case of profound hypokalemia and arrhythmias. Cardiac monitoring is mandatory in patients with electrolyte derangements, especially in the presence of an underlying cardiac disease or arrhythmias. Serial electrocardiograms is crucial for monitoring patients with evidence of QTc interval prolongation on presentation. Aldosterone receptor antagonism (e.g. spironolactone) can be used as they counteract the action of liquorice. Occasionally, more potent antihypertensives are required for resistant cases and for hypertensive emergencies (Omar, 2013).

Prolonged use of large doses (> 50g/day) of the drug for extended periods (>6 weeks) may increase water accumulation, causing swelling of the hands and feet. Sodium excretion is reduced and potassium excretion is increased. Blood pressure may rise (WHO 2010).

On prolonged use and with higher doses, mineralcorticoid effects may occur in the form of sodium and water retention and potassium loss, accompanied by hypertension, edema, hypokalemia, and, in rare cases, myoglobinuria (Commission E monograph, 1991).

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

No data available.

### 5.5.8. Safety in other special situations

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 and muscular weakness, which can be aggravated to myoglobinuria and rhabdomyolisis.

# 6. Overall conclusions

*Well established use monograph*

There are no clinical data in the scientific literature to support a "well-established medicinal use". Thus, the requirements for well-established use according to Article 10a of Directive 2001/83/EC are considered not fulfilled.

*Traditional use monograph*

The requirements for traditional use according to Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC are considered fulfilled. It has been demonstrated that *Glycyrrhiza glabra* L. and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch., radix have been in traditional medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA, with an acceptable level of safety for:

1) Traditional herbal medicinal product used for the relief of digestive symptoms including burning sensation and dyspepsia.

2) Traditional herbal medicinal product used as an expectorant in cough associated with cold.

| Herbal substance/preparation | Indication | Therapeutic area for browse search | Posology and method of administration | Duration of use |
|---|---|---|---|---|
| Comminuted herbal substance | THMP for the relief of digestive symptoms including burning sensation and dyspepsia | Gastrointestinal disorders | Adults:<br>1.5 - 2 g of comminuted herbal substance in 150 ml of boiling water as a herbal infusion or decoction, 2 to 4 times daily. Take one cup after meals.<br>Daily dose: 3 – 8 g<br>Oral use | Not to be used for more than 4 weeks. |
| | THMP to be used as an expectorant in cough associated with cold | Cough and cold | Adults:<br>1.5 g – 4.5 g of comminuted herbal substance in 150 ml of boiling water as a herbal infusion or decoction, 2-3 times daily.<br>Daily dose: 3 – 13.5 g<br>Oral use | If the symptoms persist longer than 1 week, a doctor or a qualified health care practitioner should be consulted. |
| Soft extract (DER 1:0.4-0.5), extraction solvent water | THMP for the relief of digestive symptoms including burning sensation and dyspepsia | Gastrointestinal disorders | Adults:<br>32 mg 2-3 times daily for oral use.<br>Daily dose: 64 – 96 mg. Not more than 160 mg daily<br>Oral use | Not to be used for more than 4 weeks. |

| Herbal substance/preparation | Indication | Therapeutic area for browse search | Posology and method of administration | Duration of use |
|---|---|---|---|---|
| Soft extract (DER 3:1), extraction solvent water | THMP to be used as an expectorant in cough associated with cold | Cough and cold | Adults:<br>1.2-1.5 g 3-4 times daily.<br>Daily dose: 3.6 – 6 g | If the symptoms persist longer than 1 week, a doctor or a qualified health care practitioner should be consulted. |

*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.

The comminuted herbal substance as herbal tea, prepared by means of infusion or decoction, is traditionally used in Spain and Poland for both the indications since more than 30 years. It is currently authorised in the same way in Germany for the treatment of catarrh of the upper airway since 1986.

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 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. The recommended single and daily dose is in line with the Polish posology and with the minimum Spanish posology, considering to reduce the maximum one used in gastric ulcers.
- Use as an expectorant: 1.5 g – 4.5 g of comminuted herbal substance as a herbal infusion in 150 ml of boiling water or as a decoction 2-4 times daily; the daily dose sould not be higher than 13.5 g. This posology is based on the products marketed in Poland and Germany

The soft extract (DER 1:0.4-0.5, extraction solvent water) is traditionally used in Germany to support gastric function for more than 30 years and the recommended posology is taken from this use.

The soft extract (DER 3:1, extraction solvent water) is documented to be traditionally used in Denmark for more than 70 years as an expectorant with the following posology: 1.2-1.5 g 3-4 times daily for oral use.

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

In susceptible people prolonged daily intake even of low doses of liquorice, corresponding to 80-100 mg of glycyrryzic acid, may provoke severe hypertension.

There is insufficient data to support the safety of Liquorice root during pregnancy and lactation in children and adolescents under 18 years. Therefore, the use is not recommended for these patient groups.

No constituent with known therapeutic activity or active marker can be recognised by the HMPC.

Adequate tests on reproductive toxicity, genotoxicity and carcinogenicity have not been performed. Therefore, a European Union list entry cannot be supported due to lack of adequate data.

# Annex

## *List of references*