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Risk and safety assessment on the consumption of Licorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin

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Abstract

Licorice (or 'liquorice') is a plant of ancient origin and steeped in history. Licorice extracts and its principle component, glycyrrhizin, have extensive use in foods, tobacco and in both traditional and herbal medicine. As a result, there is a high level of use of licorice and glycyrrhizin in the US with an estimated consumption of 0.027–3.6 mg glycyrrhizin/kg/day. Both products have been approved for use in foods by most national and supranational regulatory agencies. Biochemical studies indicate that glycyrrhizinates inhibit 11β-hydroxysteroid dehydrogenase, the enzyme responsible for inactivating cortisol. As a result, the continuous, high level exposure to glycyrrhizin compounds can produce hypermineralocorticoid-like effects in both animals and humans. These effects are reversible upon withdrawal of licorice or glycyrrhizin. Other *in vivo* and clinical studies have reported beneficial effects of both licorice and glycyrrhizin consumption including anti-ulcer, anti-viral, and hepatoprotective responses. Various genotoxic studies have indicated that glycyrrhizin is neither teratogenic nor mutagenic, and may possess anti-genotoxic properties under certain conditions. The pharmacokinetics of glycyrrhizin have been described and show that its bioavailability is reduced when consumed as licorice; this has hampered attempts to establish clear dose-effect levels in animals and humans. Based on the *in vivo* and clinical evidence, we propose an acceptable daily intake of 0.015–0.229 mg glycyrrhizin/kg body weight/day.

Keywords: Licorice; Glycyrrhizin; Toxicity; GRAS; Additive; Burdock; Food; Flavor; FEMA; Generally recognized as safe; JECFA; Food additive; Sweetener; 11β-Hydroxysteroid dehydrogenase; Enzyme; Cortisol; Hypermineralocorticoid; Teratogen; Mutagen; Cytotoxicity; Carcinogenicity; Safetyl

1. Introduction

The licorice (liquorice) plant has a long and storied history of use in both Eastern and Western cultures pre-dating the Babylonian and Egyptian empires (Fenwick et al., 1990; Olukoga and Donaldson, 1998). The genus name *Glycyrrhiza* is derived from the ancient Greek word for 'sweet root' (Gr. *glykos* (sweet) + *rhiza* (root)), which was later Latinized to *liquiritia* and eventually to licorice (Schulz et al., 1998). The two principal forms in commerce are licorice root (*Liquiriti radix*) and the extract (*Glycyrrhizae extractum crudum* or *Succus liquiritiae*).

The ancient Greeks and Romans are known to have cultivated the plants in the third century. Licorice was a prescriptive agent of Hippocrates in the treatment for asthma, dry cough, and other "pectoral diseases,"and was also thought to be effective in preventing thirst. In Chinese traditional medicine, licorice (*Gan Cao*) remains one of the oldest and most commonly prescribed herbs and has been used in the treatment of numerous ailments ranging from tuberculosis to peptic ulcers (Huang, 1993). Licorice has held claim for therapeutic use for fevers, liver ailments, dyspepsia, gastric ulcers, sore throats, asthma, bronchitis, Addison's disease and rheumatoid arthritis and has been used as a laxative, antitussive and expectorant (Anon, 2005; Schulz et al., 1998; Wang et al., 2000). Among its most consistent uses are as a demulcent for the digestive system, to

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treat coughs, to soothe sore throats, and as a flavoring agent. According to Duke (1985), the tobacco industry is the primary user of licorice derivatives in the United States, with the remainder equally divided among the food and pharmaceutical industries.

In light of the historical use of licorice as a medicinal herb, its use as a flavorant, its increasing presence as a self-prescribed herbal remedy in Western societies, and the growing body of scientific publications describing its biological effects, it is prudent to review the safety of licorice products on a frequent basis. This article summarizes the current pharmacological and toxicological effects of licorice root products with an emphasis on glycyrrhizin, its principal active ingredient. Through the establishment of no observed effect levels (NOEL) and the implementation of relevant safety factors, it is possible to determine acceptable daily intake[1] (ADI) levels. The ADI is discussed in relation to current estimated levels of consumption as an ingredient added to food.

1.1. Description, occurrence, sources, and economic uses

Native to Asia and the Mediterranean region, licorice (*Glycyrrhiza glabra*) is a tall shrub of the Leguminosae family (Fenwick et al., 1990; Olukoga and Donaldson, 1998). There are about 14 species known, although most commercial licorice is extracted from varieties of *G. glabra* grown in southern and central Europe (var. *typica*), in central and southern Russia (var. *glandulifera*), and in Iran and Iraq (var. *violacea*). Licorice also grows in the United States (var. *lepidota*) and England (var. *typica*) but neither represents a significant contribution to world production. Commercially important sources are Spain, Iraq, Iran, Turkey, Russia and China, and although there are no known prohibitions against use of any species, variety or country of origin, some types are not sweet enough to have commercial value. Chinese licorice (*G. uralensis* and *G. pallidiflora*) are somewhat smaller, related plants, regarded as separate species of *Glycyrrhiza*.

Commercial licorice products are derived from extracts of the root system. As noted, the genus name, *Glycyrrhiza*, well describes the main feature of the plant as it derives from the Greek words "glykos," meaning sweet and "rhiza," meaning root. The sweet taste of the root comes from the substance glycyrrhizin, reputed to be 50 times sweeter than refined sugar. The harvesting of licorice root occurs in the autumn of its third or fourth year of growth (Olukoga and Donaldson, 1998). The roots are dug up, washed and transported to warehouses for bailing, sorting and drying. The dried roots are crushed by millstones and the pulp is boiled to make the extract. After removal of the solids, the extract is vacuum dried to a dark paste, which is cast into blocks or short sticks, or may be dried to a powder. Licorice paste is the preferred form for flavoring tobacco (Carmines et al., 2005) whereas licorice powder is preferred for confectionery and pharmaceuticals.

As with most plant extracts, the number of chemical constituents is potentially vast and greatly influenced by a constellation of genetic, environmental, and processing factors; licorice root extract is no exception. A detailed examination of the components identified in licorice root extract is beyond the scope of this assessment, but has been reviewed by other authors (Duke, 2000; Fenwick et al., 1990; Wang et al., 2000). The fresh root contains about 20% of water-soluble extractives, and much of this—typically 3–5% of the root—is composed of glycyrrhizin, present as a mixture of potassium and calcium salts. The bright yellow color of licorice root is provided by flavonoids, particularly liquiritin, isoliquiritin and their corresponding aglycones, which typically comprise 1–1.5% of the water soluble extract. Licorice extract also contains reducing and non-reducing sugars, starch, plant gums, resins, essential oils, inorganic salts and low levels of nitrogenous constituents such as proteins, individual amino acids, and nucleic acids.

Glycyrrhizin (glycyrrhizic acid; glycyrrhizinate) constitutes 10–25% of licorice root extract and is considered the primary active ingredient. Minor constituents which may also confer some pharmacological activities, include liquiritigenin, isoliquiritigenin, and their corresponding aglycones (Leung and Foster, 1996). Glycyrrhizin (Fig. 1) is a saponin compound comprised of a triterpenoid aglycone, glycyrrhetic acid (glycyrrhetinic acid; enoxolone) conjugated to a disaccharide of glucuronic acid. Both glycyrrhizin and glycyrrhetic acid can exist in the 18α- and 18β-stereoisomers (Wang et al., 2000). As a tribasic acid, glycyrrhizin can form a variety of salts and occurs naturally in licorice root as the calcium and potassium salts. The ammoniated salt of glycyrrhizin, which is manufactured from licorice extracts, is used as a food flavoring agent and specifications for this salt form have been established in the Food Chemicals Codex (FCC, 2003). Carbenoxolone (18β-glycyrrhetinic acid hydrogen succinate), an analog of glycyrrhetic acid, is used in the treatment of some alimentary tract ulcerative conditions, such as peptic ulcers.

Although glycyrrhizin is considered much sweeter than sucrose, the associated licorice flavor makes direct comparison difficult and affords it little commercial value as a sweetener. Because glycyrrhizin also imparts an undesirable brownish color to foods and the sweetness is lost in acidic solutions, as occurs in most beverages, glycyrrhizin remains of little value to the food and beverage industries. The primary use for licorice products and glycyrrhizin is limited

[1] *Abbreviations used:* ACTH, adrenocorticotropic hormone; ADI, acceptable daily intake; ALT, alanine transaminase; AST, aspartate transaminase; CFR, Code of Federal Regulations; EMS, ethyl methanesulfonate; FCC, Food Chemicals Codex; FDA, U.S. Food and Drug Administration; FEMA, Flavor and Extract Manufacturers' Association; GRAS, Generally Recognized as Safe; 11βHSD, 11β-hydroxysteroid dehydrogenase; IC_{50}, inhibitory concentration causing 50% of response; *i.m.*, intramuscular; *i.p.*, intraperitoneal; *i.v.*, intravenous; JECFA, Joint FAO/WHO Expert Committee on Food Additives; 3MGA, 3β-(Monoglucuronyl)-18β-glycyrrhetinic acid; NACGM, National Association of Chewing Gum Manufacturers; NOEL, no-observed effect level; PADI, Possible Average Daily Intake; *p.o.*, *per os* (oral); SNMC, Stronger Neo Minophagen C; NK cells; LDH; CCl_4; *s.c.*; LD_{50}.

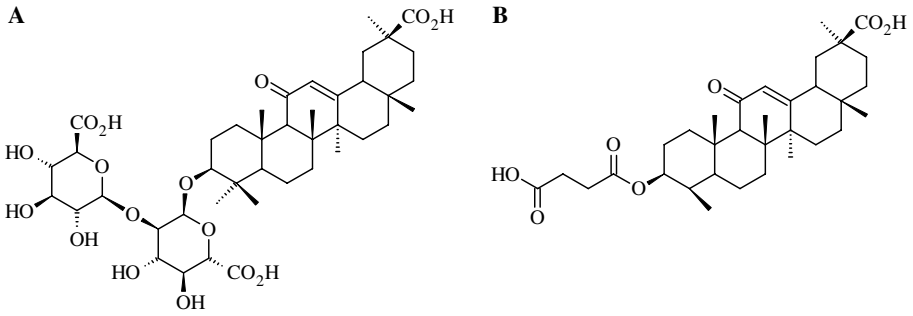

Fig. 1. Chemical structure of glycyrrhizin (A) and carbenoxolone (B).

to flavoring tobacco and candy (Fenwick et al., 1990; Reineccius, 1999).

Some minor consumer use for licorice root extract and glycyrrhizin has been in beer and ales, where they provide good surfactant (foaming) properties and take the edge off of these potentially bitter-tasting beverages. Similarly, licorice root extract and glycyrrhizin may be used to alleviate the bitter after taste in some saccharinated products and pharmaceutical preparations. According to Duke (1985), glycyrrhizin may be used as a flavor enhancer for cocoa, replacing as much as 25% of the cocoa in manufactured products. Industrial uses for glycyrrhizin include an adhesive agent in insecticides and a wetting agent (surfactant) for various industrial processes. Spent licorice root solids serve in fire-extinguishing agents, to insulate fiberboard, compost for growing mushrooms and as feed for cattle, horses and chickens (Duke, 1985; Fenwick et al., 1990).

1.2. Regulatory assessments

Licorice and licorice derivatives, including ammoniated glycyrrhizin, are affirmed as Generally Recognized as Safe (GRAS) for use in foods by the U.S. FDA (21 CFR 184.1408). This chapter of the regulations includes descriptions, specifications, and maximum use levels (Table 1) for licorice and licorice derivatives. FDA assumes that glycyrrhizin levels in foods do not pose a health hazard, provided that these foods are not consumed in excess or by individuals who are sensitive to low levels of glycyrrhizin (Anon, 1983). Licorice extract and its derivatives are also approved for use in some over-the-counter drugs (21 CFR 310.528; 310.544; 310.545), and licorice is included as a GRAS ingredient in animal feeds (21 CFR 582.10; 582.20).

Table 1
US Food and Drug Administration limitations for the use of licorice and its derivatives in foods (21 CFR 184.1408c)

Food category	Maximum allowable levels in foods as % glycyrrhizin content	Functional use[a]
Baked goods	0.05	1, 2
Alcoholic beverages	0.1	1, 2, 3
Non-alcoholic beverages	0.15	1, 2, 3
Chewing gum	1.1	1, 2
Hard candy	16.0	1, 2
Soft candy	3.1	1, 2
Herbs and seasonings	0.15	1, 2
Plant protein products	0.15	1, 2
Vitamin or mineral dietary supplements	0.5	1, 2
All other foods, except sugar substitutes	0.1	1, 2

[a] 1, flavor enhancer; 2, flavoring agent; 3, surface-active agent.

Licorice root, licorice extract, licorice extract powder and glycyrrhizin were included in the Flavor and Extract Manufacturers' Association (FEMA) list of GRAS substances at use levels indicated in Table 2 (Hall and Oser, 1965). At the 1977 meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), a decision on an acceptable daily intake (ADI) for licorice was held in abeyance. Glycyrrhizinic acid was evaluated during a more recent JECFA meeting (WHO, 2005). Although a formal ADI was not established, the committee indicated that consumption of 100 mg/day would be unlikely to cause adverse effects in the majority of adults and recognized that a subset of the population may be more susceptible to its physiological effects even at lower doses.

Both the Council of Europe and the UK Food Additive and Contaminants Committee consider licorice as a natural plant product intended for use in small quantities as a food additive with the intention that its consumption be limited by the glycyrrhizin levels and not to exceed those occurring naturally in foods (Fenwick et al., 1990). A limit of less than 50 ppm glycyrrhizin was established by these organizations. The Dutch Nutrition Information Bureau advised against a daily glycyrrhizin consumption in excess of 200 mg, assumed to correspond to 150 g of licorice confectionery (Fenwick et al., 1990); although it should be cautioned that the glycyrrhizin content of confectionery products can vary by as much as 30-fold (Spinks and Fenwick, 1990). As with JECFA, the European Community's Scientific Committee on Food recently re-evaluated the use of glycyrrhizinic acid in foods and was unable to establish an ADI, but considered 100 mg/day to be a reasonable upper limit of consumption for the majority of the population (SCF, 2003).

Table 2
FEMA usual use levels[a] (in ppm)

Food classification:	Licorice	Licorice extract	Licorice extract powder	Glycyrrhizin, ammoniated
FEMA No.:	2630	2628	2629	2528
Baked foods	113.8	570.3	1666	61.07
Frozen dairy	518.3	468.2	200	50.14
Meat products	2100	600	2200	—
Sweet sauce	—	10.9	—	—
Gelatin, pudding	—	192.9	—	52
Non-alcoholic beverages	176.6	169.3	24.6	24.5
Alcoholic beverages	—	1393	1800	27.8
Confection, frosting	—	—	—	625
Soft candy	1863	—	—	762
Hard candy	10.07	—	4010	27.8
Hard candy[b]	30,099	3195	41714.7	479.9
Chewing gum	—	—	5825	400
Chewing gum[b]	—	—	200	—

[a] Average usual use levels are considered Generally Recognized As Safe (GRAS) by the FEMA panel of experts.
[b] Use levels reported by the National Association of Chewing Gum Manufacturers (1977 survey).

1.3. Consumption

Estimates for the amount of licorice root and its derivatives consumed by the public are determined via the use of per capita estimates of intake, based on "disappearance data." This calculation is facilitated by the periodic surveys conducted by the National Academy of Sciences survey of ingredients added to food (NAS, 1989). The last survey conducted was in 1987 and based on voluntary reporting by manufacturers of the volume of ingredients produced during the survey year. The assumption is that there is a finite amount of a substance available and it is ingested regardless of source at the retail level, whether through candy, chewing gum, or other foods. The method is easy to use because it divides the total yearly poundage by the population in the survey year and the number of days per year. The resulting quotient is daily exposure. Some caveats are necessary in the use of the survey data because (1) not all producers participate so it is generally held that the amount reported is a fraction of the actual volume and (2) not all persons eat all foods each day in each category in which the substance may be found and, conversely, some consumers may seek out the substance (such as sweet substances, or in this case, anise-type flavors); therefore, the distribution of consumption may be uneven. In order to compensate for these variables, the FDA assumes that only 60% of the actual value was reported and only 10% of the US population (243.9 million in 1987) consumes 100% of the calculated amount. Because the primary active ingredient is glycyrrhizin and a comparison of consumption is made on this basis, the total poundage reported for use as a flavor yields an estimated daily per capita consumption of 1.6 mg/day, or 0.027 mg/kg body weight (Table 3).

An alternate method for estimating consumption of glycyrrhizin is to determine the Possible Average Daily Intake (PADI) from published FEMA data and values generated in a survey of National Association of Chewing Gum Manufacturers (NACGM) participating companies. Combining these PADIs and calculating the amount of glycyrrhizin present in the materials gives an estimate of the high end of consumption of these materials. By this method, an estimated 215 mg/day, or 3.6 mg/kg body weight, is consumed (Table 4). This is similar to the amount estimated by the FDA (246 mg/day) for US consumption (Anon, 1983) and may only be exceeded by estimated consumption for the Netherlands at 4–5 g/day (Fenwick et al., 1990). It is possible that individuals with a pica for this particular flavor could consume even more glycyrrhizin, but it is unlikely as licorice flavor is often produced with similar tasting substances such as anise or anethole.

According to Fenwick et al. (1990), approximately 90% of the US licorice supply is used by the tobacco industry,

Table 3
Per capita glycyrrhizin consumption

Substance	Amount consumed (US Pop.) (lbs)	Per capita consumption (mg/day)	Glycyrrhizin content (%)	Adjusted per capita consumption (mg/day)
Licorice root	58,333	2.9658	15[a]	0.4448
Licorice extract powder	47,166	2.3982	25[a]	0.5995
Licorice extract	37,666	1.9152	20[a]	0.3830
Ammoniated glycyrrhizin	5450	0.2766	73[b]	0.2019
Total per capita consumption				1.6292

[a] Fenwick et al. (1990).
[b] FCC provides for a value of 22–32% ammonium (FCC, 2003).

Table 4
Glycyrrhizin consumption via use in food

Substance	FEMA PADI[a] (mg/day)	NACGM[b] (mg/day)	Total EDI (mg/day)	Glycyrrhizin content (%)	Adjusted consumption (mg/day)
Licorice root	223.021	18.059	241.08	15[c]	36.162
Licorice extract powder	470.084	25.068	495.152	25[c]	123.788
Licorice extract	204.160	1.917	206.077	20[c]	41.215
Ammoniated glycyrrhizin	19	0.287	19.287	73[d]	14.079
Total per capita consumption					215.244

[a] PADI (Possible Average Daily Intake), calculated on Market Research Corporation of America values and highest use levels approved by FEMA.
[b] Daily intake values using NACGM use levels and MRCA consumption values (600 mg hard candy per day and 200 mg chewing gum per day). (More recent values (USDA, 2000) indicate roughly 1% of the persons completing day one of the food intake survey (147 of 15,016 people between the ages of one and 70 years) reported consuming, on average, 41 g of licorice candy (the standard deviation is 54 g). A survey conducted by the National Association of Chewing Gum Manufacturers' (NACGM, 1977) reported an average amount of 4,097 ppm of licorice extract as being added to hard candy. Using these values, approximately 168 mg licorice extract (33.6 mg glychyrrhizin) was consumed per day. The average amount plus one standard deviation would be 33.6 + 44.2 mg glychrrhizin or a total of 77.85 mg/day.)
[c] Fenwick et al. (1990).
[d] FCC provides for a value of 22–32% ammonium (FCC, 2003).

with the remainder split evenly between the food and pharmaceutical industries. However, consumption of glycyrrhizin as a dietary supplement may also be significant. For example, Schulz et al. (1998) suggested an average daily dose not to exceed 5–15 g of dried root (equivalent to 200–600 mg glycyrrhizin) for the treatment of gastrointestinal ailments, with a recommended course of treatment not exceeding 4–6 weeks.

Therefore, there is a range of daily intake of licorice (as glycyrrhizin) from 1.6 to 215 mg/day (0.027–3.6 mg/kg), with a likely average intake of less than 2 mg/day. It is quite possible that some acute exposures may well exceed the average amount and might, in fact, exceed the high end of consumption as the result of an indulgence in licorice-flavored candy, although this occurrence may be ameliorated by the fact that many licorice flavors often contain anise or anethole as a flavoring agent, thereby reducing the actual amount of glycyrrhizin consumed. A high consumption of nearly equal probability, but not quantitatively as great in dose, may occur among those with a chronic high intake of tobacco flavored with licorice and/or glycyrrhizin, or among those individuals who consume licorice capsules as a health product.

1.4. Absorption, distribution, metabolism and elimination

Glycyrrhizin has a poor oral bioavailability in both rats and humans. In rats, glycyrrhizin was detectable in plasma samples only after the administration of oral doses exceeding 50 mg/kg (Cantelli-Forti et al., 1994; Wang et al., 1994; Yamamura et al., 1995). Similarly, in humans, glycyrrhizin was detected at very low levels after a single oral dose in the range of 100–1600 mg/kg (Gunnarsdottir and Johannesson, 1997; Raggi et al., 1994; Yamamura et al., 1992). However, these studies showed that glycyrrhetic acid, the aglycone of glycyrrhizin, is readily detected in plasma following the ingestion of glycyrrhizin or licorice extract by rats and humans.

In most species, the peak plasma glycyrrhetic acid levels are lower and occur later when glycyrrhizin is administered in licorice extract than when provided at an equivalent dose as a pure compound; however, the contrary seems to occur in rabbits (Hou et al., 2005). In rats fed glycyrrhizin (160 or 200 mg/kg) or licorice extract with the same glycyrrhizin load, the area under the plasma curve (AUC) for glycyrrhetic acid was approximately 2.5 times greater for the pure compound (Cantelli-Forti et al., 1994; Wang et al., 1995). Licorice extract was also found to increase bile flow in rats (Cantelli-Forti et al., 1997), which could partially explain the altered pharmacokinetics. The time to maximum glycyrrhetic acid plasma concentration was 10 h for glycyrrhizin and 2 h longer with licorice extract. Similar results were found in a study involving eight healthy adult volunteers fed 800 or 1600 mg glycyrrhizin as its ammoniated salt or in licorice extract (Cantelli-Forti et al., 1994). The lipophilic components of licorice extract were found to reduce the gastric emptying rate and absorption of glycyrrhizin (Wang et al., 1995), but the compounds involved in this activity have not been identified.

Interestingly, neither glycyrrhizin nor glycyrrhetic acid are detected in the plasma of germ-free rats fed glycyrrhizin (Akao et al., 1994; Takeda et al., 1996). These data, along with the relatively long lag time to maximum plasma glycyrrhetic acid concentrations, suggest a presystemic metabolic process involving the de-glucuronidation of glycyrrhizin by intestinal flora prior to the absorption of glycyrrhetic acid (Ploeger et al., 2001) (Fig. 2). Indeed, researchers have identified various intestinal bacterial strains, including *Streptococcus* and *Eubacterium* sp., with specialized β-glucuronidase activity capable of glycyrrhizin hydrolysis (Akao et al., 1994; Kim et al., 1999, 2000). Oral bioavailability of glycyrrhetic acid following the administration of glycyrrhizin to rats appears to be dose limiting and may be due to limitations in either the uptake of glycyrrhetic acid or the hydrolysis process (Ploeger et al., 2001).

Kato et al. (1995) examined the serum metabolites of 21 patients administered 80–375 mg/day of glycyrrhizin-containing agents. Ten of these patients showed signs of pseudoaldosteronism and had detectable plasma levels of

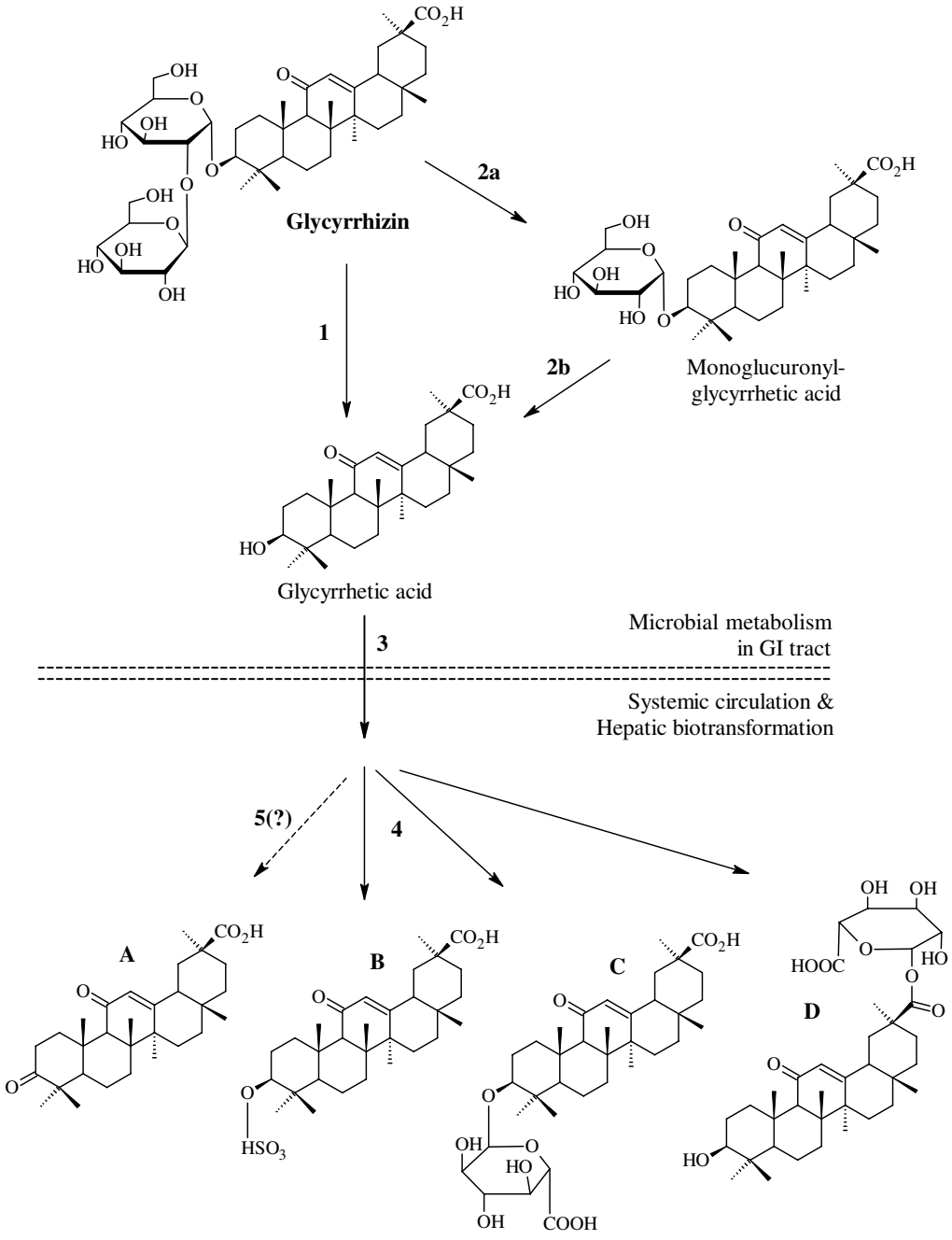

Fig. 2. Glycyrrhizin metabolism. Following ingestion glycyrrhizin is deglucuronidated by intestinal microbes either completely (1) or through a two-step intermediary process (2a, 2b). Glycyrrhetic acid is absorbed (3) and undergoes hepatic biotransformation (4) to produce 18β-glycyrrhetyl-3-*O*-hydrogen sulfate (B), 18β-glycyrrhetyl-3-*O*-monoglucuronide (C), or 18β-glycyrrhetyl-30-monoglucuronide (D). Glycyrrhetic acid may also undergo hepatic dehydrogenation (5) to form 3-ketoglycyrrhetic acid (A), although this step is undocumented in humans.

3β-monoglucuronyl-18β-glycyrrhetic acid (3MGA), a monoglucuronyl metabolite of glycyrrhizin, whereas those not exhibiting signs of adverse effects did not have detectable serum levels of 3MGA. Because orally administered glycyrrhizin is poorly absorbed, and is hydrolyzed to glycyrrhetic acid by intestinal bacteria, the authors suggested a two-step microbial hydrolysis of glycyrrhizin first to 3MGA and then to glycyrrhetic acid prior to absorption. This was later confirmed when microbial glucuronidases were isolated from various intestinal bacteria (Kim et al., 1999, 2000). Some microbial glucuronidases, such as those isolated from *Eubacterium* and *Bacteroides* sp. completely de-glucuronidate glycyrrhizin, whereas that isolated from *Streptococcus* sp. only removed one glucuronide moiety (Fig. 2). Interindividual differences in glycyrrhizin response, metabolism and kinetics are therefore influenced, at least in part, by the intestinal microflora profile.

Neither glycyrrhizin nor glycyrrhetic acid accumulate in tissues. However, both compounds adhere extensively to human and rat serum albumin, and although binding sites

may be specific and non-specific, it is a saturable process (Ishida et al., 1989, 1992; Ploeger et al., 2001). A recent study showed that glycyrrhetic acid is somewhat able to cross the placental barrier and can be detected in the rat fetuses (Hundertmark et al., 2002). In this study, dams were fed 100 mg glycyrrhetic acid/kg/day commencing on the 13th day of gestation. On the 17th, 19th and 21st days of gestation the maternal plasma glycyrrhetic acid concentrations were approximately 100 μg/ml whereas the fetal concentrations were 5, 18 and 32 μg/ml, respectively.

The enterohepatic circulation of glycyrrhetic acid was nicely demonstrated by Iveson et al. (1971) where the bile was collected from one rat administered [^{3}H]glycyrrhetic acid and introduced into the duodenum of a second rat. Approximately 14% of the radioactivity was excreted in the bile of the second animal within 6 h with totals of 29% and 31% excreted in the bile after 24 and 48 h, respectively. Analysis of the collected bile revealed the presence of three products of glycyrrhetic acid: 18β-glycyrrhetyl-30-glucuronide, 18β-glycyrrhetyl-3-*O*-glucuronide, and 18β-glycyrrhetyl-3-*O*-sulfate, indicating the hepatic conjugation of glycyrrhetic acid prior to biliary excretion (Fig. 2). Less than 2% of the initial dose was excreted in the urine illustrating that this is not considered to be a major elimination pathway. Fecal extracts of rats dosed orally with [^{3}H]glycyrrhetic acid revealed the presence of only the parent compound as an unmodified substance indicating the complete hydrolysis of glucuronyl and sulfate conjugates by intestinal microflora prior to excretion in the feces. The enterohepatic circulation of glycyrrhetic acid has not been studied in humans, but can be expected because glycyrrhetic acid metabolites can be hydrolyzed by human gastrointestinal bacteria (Ploeger et al., 2001). Time curve data for glycyrrhetic acid also provide evidence of enterohepatic circulation in humans (Ploeger et al., 2000a).

Glycyrrhetic acid was also found to be metabolized by rat liver homogenates into 3-ketoglycyrrhetic acid by an enzyme described as glycyrrhetinate dehydrogenase (Akao et al., 1990). This metabolic step was reversible and the *in vitro* 3-ketoglycyrrhetic acid-reducing rate was three times faster than the oxidative rate of glycyrrhetic acid. In a subsequent study, the activity of glycyrrhetinate dehydrogenase was found to occur only in male rats commencing at approximately the sixth week of age (Akao et al., 1991). Maximal enzyme activity was achieved by the twelfth week of age. Administration of estradiol, or surgical hypophysectomy to adult male rats reduced the activity of glycyrrhetinate dehydrogenase. In contrast, hypophysectomy or testosterone administration induced some glycyrrhetinate dehydrogenase activity in female rats. These results suggest that the enzyme is regulated through the hypothalamus–pituitary system. Although this metabolic pathway likely plays a minor part in glycyrrhetic acid metabolism in rats, it is still unknown if this pathway exists in humans.

The pharmacokinetics of glycyrrhizin and glycyrrhetic acid have been reviewed extensively by Ploeger et al. (2000a, 2001, 2000b). The plasma clearance of glycyrrhizin and glycyrrhetic acid is dose dependent when administered to rats or humans at levels which exceed the saturation of serum protein binding. Yamamura et al. (1992) reported that the plasma clearance of *i.v.* glycyrrhizin was not dose dependent at doses below 120 mg in healthy volunteers. The plasma clearance was in the range of 38–64 ml/h/kg and the volume of distribution at steady state (38–64 ml/kg) was close to the mean serum volume for humans, 43 ml/kg. Plasma clearance was significantly decreased in patients with chronic hepatitis C and liver cirrhosis (Ploeger et al., 2001; van Rossum et al., 1999b). Together, these data suggest a hepatic related capacity-limited process in metabolism or excretion in the bile. Because of the enterohepatic recycling of glycyrrhetic acid and the biliary storage process of glycyrrhetic acid metabolites in the gallbladder, plasma glycyrrhetic acid concentrations show several peaks following the oral administration of either glycyrrhizin glycyrrhetic acid or licorice. For example, after 16 healthy adults consumed licorice containing 225 mg glycyrrhizin, a peak plasma glycyrrhetic acid concentration of 1 μg/ml was reached after 10 h with second and third peaks of approximately 0.2 and 0.1 μg/ml appearing at 30 and 50 h, respectively (Ploeger et al., 2000a). It is believed that these latter peaks reflect a bolus re-administration of glycyrrhetic acid metabolites into the intestines through an emptying of the gallbladder following a fat-containing meal. Therefore, the complete elimination of glycyrrhetic acid from the body takes several days following a single oral dose of glycyrrhizin and the potential for systemic accumulation of glycyrrhetic acid becomes more apparent when administration occurs on a daily basis.

In summary, the metabolic fate of glycyrrhizin is complex and involves several inter-dependent steps. Following an oral administration, there is a primary metabolic step involving the enteric microbial metabolism of glycyrrhizin to a monoglycone and/or aglycone compound. The resulting 3MGA and glycyrrhetic acid are absorbed through the intestines with only a minimal absorption of glycyrrhizin. In humans, the hepatic metabolism and processing of 3MGA and glycyrrhetic acid are not yet clearly defined, but it is apparent that each can undergo further conjugation or reduction followed by biliary excretion. Glucuronide compounds and metabolites excreted in the bile are likely re-metabolized by the intestinal flora and thereby subject to enterohepatic recycling. In general, the clearance capacity for glycyrrhetic acid would be reduced in patients with compromised hepatic function.

1.5. Biochemical aspects

Most research into the biochemistry and pharmacology of glycyrrhizate compounds has focused on the possible mechanism by which they induce "pseudohyperaldosteronism", or hypermineralocorticosteroid effects. Based on the similarities in the corticosteroid hormones and the glycyrrhizate structure, initial theories assumed a direct binding of glycyrrhetic acid to the mineralocorticoid and glucocor-

ticoid receptors in various tissues. However, competitive binding assays revealed that the affinities of glycyrrhizin and glycyrrhetic acid for the corticoid receptors were 3000–10,000 times less than that for endogenous adrenocortical hormones (Armanini et al., 1983, 1989; Tamaya et al., 1986a,b).

More recently, the theory of direct corticosteroid receptor agonist activity by glycyrrhizate compounds has come into question, given the low relative affinity for the receptors as compared to aldosterone. Serum levels of glycyrrhizin or glycyrrhetic acid, following consumption of licorice extract, do not approach the concentrations necessary to affect aldosterone or cortisol binding to mineralocorticoid and glucocorticoid receptors in humans (Stormer et al., 1993). Also, hypermineralocorticosteroid effects do not seem to occur in patients or animals with severe adrenal insufficiency. Studies in normal subjects fed licorice showed that the corticosteroid-like effects were associated with a change in cortisol metabolism, which suggested an inhibition of the 11β-hydroxysteroid dehydrogenase (11βHSD) enzyme (Stewart et al., 1987). 11βHSD catalyzes the oxidation of the active mineralocorticoid, cortisol, to the inactive cortisone and is also responsible for the reverse, a reduction reaction. A congenital deficiency of this enzyme produces similar effects of apparent mineralocorticoid excess. The oral administration of glycyrrhizin to rats inhibited renal isoform of 11βHSD activity in a dose-dependent manner (Monder et al., 1989; Tanahashi et al., 2002). Similarly, carbenoxolone, an analog of glycyrrhetic acid used in the treatment of ulcers, was reported to inhibit the metabolism of ^{3}H-cortisol in six healthy adults (Stewart et al., 1990). *In vitro* analysis using rat kidney homogenates found a strong inhibitory effect of glycyrrhetic acid for the 11β-dehydrogenase, but not the 11-oxoreductase activity (Monder et al., 1989). The K_i of glycyrrhetic acid was approximately 1–10 nM in the homogenates, but only 1–10 μM in intact renal tubules. 3-Ketoglycyrrhetic acid and 3MGA, both metabolites of glycyrrhizin, were also found to inhibit 11βHSD activity (Akao et al., 1992; Kato et al., 1995). In addition to the competitive inhibition of 11βHSD activity, the oral administration of 75–120 mg glycyrrhizin/kg/day to rats was also found to reduce 11βHSD protein and messenger RNA levels in kidney, colon, liver, and pituitary tissues (Tanahashi et al., 2002; Whorwood et al., 1993). Another study reported the inhibitory effect of glycyrrhetic acid on Δ^4-5β-steroid-reductase activity—an enzyme which inactivates both glucocorticoids and mineralocorticoids by reduction of the steroid A ring (Latif et al., 1990; Morris et al., 1990). Collectively, these studies indicate that the metabolites of glycyrrhizin promote the development of a pseudoaldosteronism by inhibiting cortisol metabolising processes at several levels.

Glycyrrhetic acid has been reported to alter several other enzymatic processes in animals and cell culture systems. Inhibition of mitochondrial oxidative phosphorylation (Whitehouse et al., 1967), cytochrome P450 monoxygenase systems (Paolini et al., 1998, 1999), and *N*-acetyltransferase activity (Chung et al., 2000) have all been described in isolated enzyme systems or animal models. Others have reported the induction of a cytochrome P450 isozyme (Jeong et al., 2002). Although the potential physiological effects of these finding are important, clinical studies and case reports have not yet reflected their impact on the human condition.

1.6. Pharmacological studies

Licorice root has a long history of use in both Eastern and Western cultures as a remedy in the treatment for a wide range of ailments. Although some of these uses have fallen out of favor, others have persisted and appear to have some scientific merit.

1.6.1. Anti-ulcer effects

Perhaps the most predominant and consistent medicinal use for licorice has been as a demulcent for the digestive system. Indeed, carbenoxolone, a widely used pharmaceutical treatment for gastric ulcers, is a synthetic derivative of glycyrrhetic acid (Fig. 1). Despite its historical use, studies in animals and humans have provided inconsistent results in the efficacy of licorice, glycyrrhizinate compounds, and carbenoxolone.

Revers (1956) was one of the first authors to systematically study anti-ulcer properties of licorice extract. In an un-blinded and un-controlled study 45 patients with confirmed gastric ulcers were administered 10 g/day of powdered licorice extract (duration unknown). The ulcers were found to disappear in 17 of the cases, were diminished in 22 cases, and were unchanged in six of the cases. Patients with duodenal ulcers did not react as favorably. Approximately 20% of the patients were noted to develop edema, some with complications, including violent headache, dizziness, upper right quadrant pain, compression in the chest, and hypertension. A reduction of the dosage to 3 g/day reduced the occurrence of edema, although not in all cases. Crude fractionation of the licorice extract revealed that glycyrrhizin was the probable agent responsible for the edematous effect and an unknown component was therefore considered to be the active anti-ulcerogenic agent.

Uncertainty still remains surrounding the involvement of glycyrrhizate compounds in the anti-ulcerogenic efficacy of licorice. Carbenoxolone was developed as glycyrrhizate analog and has shown success in clinical trials for gastric (Bianchi et al., 1985; Cocking and MacCaig, 1969; Ganguli and Mohamed, 1980; Hadzic et al., 1974; Schwamberger and Reissigl, 1980) and duodenal (Cook et al., 1980; Hadzic et al., 1974; Nagy, 1978; Young et al., 1979) ulcers. Hypermineralocorticoid-like side effects were noted in all of these studies although most authors considered these effects to be tolerable. The mechanism of action of carbenoxolone remains unknown. Peskar (1980) reported the inhibition of prostaglandin metabolising enzymes by carbenoxolone in human gastric tissue; however, Bennett et al. (1985) found that neither carbenoxolone (50 mg/kg), nor deglycyrrhizi-

nated licorice (1 g/kg) affected the amounts of prostaglandin E, 6-keto-prostaglandin F_{1a}, or thromboxane B_2 produced by the gastric mucosa in rats.

The anti-ulcer activity of deglycyrrhizinated licorice was demonstrated by Bennett et al. (1980) using a rat model of aspirin-induced gastric mucosal damage. Male Wistar rats were administered 60 mg of aspirin by gastric tube and divided into four different treatment groups of saline (control), 50 mg deglycyrrhizinated licorice, 5 mg/kg cimetidine, or both deglycyrrhizinated licorice and cimetidine. Four hours after treatment the stomachs were removed and gastric mucosal damage was scored. Protection against gastric damage by aspirin was greatest in the group receiving both the licorice and cimetidine (80% reduction in lesion score), whereas those animals receiving only the licorice or cimetidine had reductions of 50% and 25%, respectively.

In order to further elucidate the possible anti-ulcer activity of a methanolic extract of licorice root (F_m 100), Ishii and Fujii (1982) examined its effects on gastrin production in male rats and female mongrel dogs. In fasting rats, serum gastrin concentrations were slightly, but significantly, elevated as compared to control animals following the administration of 800 mg F_m 100/kg but not in those animals receiving a dose of 400 mg/kg. When entry of gastric juice from the fundus into the antrum was physically blocked in rats prior to the intraduodenal administration of 800 mg F_m 100/kg there was a significant decrease in the serum concentration of gastrin. The serum gastrin concentration of dogs that were fasted overnight was significantly increased within minutes after feeding, but this increase was not affected by pretreatment of the animals with 400 mg F_m 100/kg, *p.o.*. Serum gastrin concentrations were significantly increased following the intra-antral administration of peptone solution to dogs with a fundus–antrum block. This increase in serum gastrin was nearly completely abolished following the intraduodenal administration of 200 mg F_m 100/kg. Together, these results suggest that the anti-ulcer effects of licorice extract may be due to reduced gastric secretions caused by an inhibition of gastrin release.

The results from clinical studies evaluating the efficacy of deglycyrrhizinated licorice suggest that several components exist in the extract which promote gastric healing, although inconsistencies are apparent between these studies. For example, Cooke and Baron (1971) studied the effects of deglycyrrhizinated licorice extract in two female patients with gastric ulcer. The patients were fed 2.28 g deglycyrrhizinated licorice/day for 21 days. In neither patient were there any changes in weight, blood pressure, serum levels or urinary excretion of sodium, potassium or chloride. Final barium meal examinations showed no evidence of gastric ulceration, and the authors suggested from these data that a non-glycyrrhizin component of licorice is responsible for its gastric healing properties.

A more formal, double-blind clinical trial with a crossover design was later conducted to evaluate the efficacy of deglycyrrhizinated licorice in the treatment of gastric ulcers (Engqvist et al., 1973). Thirty-eight patients with confirmed chronic gastric ulcers were initially recruited into the trial and 30 patients completed both sections. Patients were administered 760 mg deglycyrrhizinated licorice, or placebo, three times daily for four weeks, and then switched treatments for the following four weeks. No side effects of the treatments were observed and, contradictory to the findings of Cooke and Baron (1971), there was no demonstrable healing effect of the deglycyrrhizinated licorice extract on gastric ulcers.

In contrast, a subsequent retrospective trial on the efficacy of deglycyrrhizinated licorice, studied the effect of 760 mg deglycyrrhizinated licorice extract administered five times daily for 24 weeks to 36 patients with histories of chronic gastric ulcers (Larkworthy and Holgate, 1975). Healing of the ulceration was observed in all patients and in the majority the mucosa appeared normal. There were no side effects of the treatment reported by the authors.

1.6.2. Anti-viral and immunostimulatory effects

Licorice and glycyrrhizate compounds have long been used in the treatment of chronic viral hepatitis in China and Japan (Wang et al., 2000), but the possible mechanism of anti-viral activity remains unknown. *In vitro* studies have demonstrated that glycyrrhizin is effective at inhibiting the growth of a host of viruses under culture conditions including pathogenic flaviviruses (Crance et al., 2003), alphaviruses (Briolant et al., 2004), herpes simplex virus (Ikeda et al., 2005), vaccinia and vesicular stomatitis viruses (Pompei, 1979) and human immunodeficiency virus (Sasaki et al., 2002a). Studies such as these suggest a direct effect of glycyrrhizin on viral growth, possibly through an inhibition of viral particle to cell membrane binding, replication mechanisms, or through cellular signal transduction mechanisms. *In vivo* and human studies tend to agree with the anti-viral efficacy of glycyrrhizin, but the mechanism of action may be more complex and promote an immune response.

Abe et al. (1982) examined whether glycyrrhizin or glycyrrhetic acids induce the production of interferon, an endogenous lymphokine known to inhibit viral replication. Groups of three male DDI mice were administered either glycyrrhizin (20 mg/kg *i.v.* or 50 mg/kg *i.p.*) or glycyrrhetic acid (5 mg/kg *i.v.*). Serum was then collected over a 30-h period and tested for *in vitro* anti-viral activity in L-929 cells infected with vesicular stomatitis virus. The intravenous dose of glycyrrhizin induced two peaks of activity, resulting in 220 U/ml after 2 h and 110 U/ml after 18 h. Intraperitoneal administration of glycyrrhizin produced one peak of anti-viral activity equivalent to 220 U/ml at 20 h. The intravenous administration of glycyrrhetic acid produced a titer of 80 U/ml 2 h after dosing. The anti-viral substance in the serum was determined to be interferon as it was inactive in human WISH or rabbit RK-13 cells. Similar anti-viral activity was observed when C3H, ddY, CDF-1 and C57BL mice were used, but a greatly reduced activity was obtained with athymic (nu/nu) BALB/c mice. This suggests that interferon induction by glycyrrhizin is dependent

on T-cell function. Inhibition of a glycyrrhizin-induced anti-viral titer by the previous treatment of DDI mice with hydrocortisone, trypan blue, or X-rays may imply that lymphocytes and macrophages are also required for the induction of interferon by glycyrrhizin.

Natural killer (NK) cell activity is known to play an important role in the elimination of virus-infected cells during chronic hepatitis. NK cell activity is also elevated in response to γ-interferon and interleukin-2. Due to the prior reports on elevation of γ-interferon by glycyrrhizin (Abe et al., 1982), Wada et al. (1987) investigated NK cell activity among control subjects and patients with chronic liver diseases following the intravenous (*i.v.*) administration of 80–200 mg glycyrrhizin/day (duration unknown). Glycyrrhizin administration was found to have no effect on NK cell activity and no further data was reported.

Clinical studies on the efficacy of glycyrrhizin in the treatment of hepatitis B have generally shown favorable outcomes with the treatment normalizing elevated serum transaminases and improving liver function (Sumiyama et al., 1991; Zhang and Wang, 2002). In Japan, Stronger Neo Minophagen C (SNMC) is a commonly prescribed glycyrrhizin solution administered intravenously for the treatment of chronic hepatitis. The European experience with SNMC in the treatment of hepatitis has also shown good results, equivalent to those produced with interferon treatment (van Rossum et al., 1998). However, in a double-blind, randomized, placebo-controlled trial involving 57 patients with hepatitis C, the *i.v.* administration of 80–240 mg glycyrrhizin/day, three days per week for four weeks improved serum alanine aminotransferase, but had no effect on hepatitis C RNA levels (van Rossum et al., 1999a, 2001).

The immunostimulatory properties of glycyrrhizin were also studied by Utsunomiya et al. (1997). BALB/c mice infected with influenza virus A_2 (H_2N_2) were unable to survive 10 times the mean lethal dose (LD_{50}) of virus. However, a complete survival was observed when these animals were treated with 10 mg glycyrrhizin/kg, *i.p.*, on the day prior to, the day after, and on the fourth day after infection. This same dosing regimen conferred 70% survival in mice infected with 50 times the viral LD_{50}. This response was demonstrated to be dose-dependent with improved survival in animals administered greater than 2.5 mg glycyrrhizin/kg. Although the lung viral titers were decreased in glycyrrhizin-treated animals, *in vitro* assays demonstrated that this compound did not inhibit viral replication in cell cultures. This suggested that the anti-viral mechanism of glycyrrhizin was indirect, and possibly stimulated endogenous viral defense mechanisms. In examining this theory, the authors reported that the transplanting of splenic T-cells from glycyrrhizin-treated mice conferred resistance to infected mice that had not been treated with glycyrrhizin. The transplanting of other splenic cell subsets did not improve the survival of infected mice, indicating that glycyrrhizin was a specific inhibitor of the cell-mediated immunological response. The administration of γ-interferon monoclonal antibody to infected mice blocked the anti-viral activity of glycyrrhizin treatment (Utsunomiya et al., 1997). These results confirm that the anti-viral activity of glycyrrhizin is due to its stimulating of γ-interferon production by T-cells.

Glycyrrhizin has also been demonstrated as effective in the treatment of herpes simplex-induced encephalitis in mice (Sekizawa et al., 2001). Animals were administered 50 μg glycyrrhizin/g *i.p.*, on the third, fourth and fifth days following viral inoculation. The survival rate of among treated mice was 82% and only 38% in untreated animals. The mean virus yield per brain hemisphere in glycyrrhizin treated animals was approximately half that of control.

The *in vivo* effects of licorice extract on the immune response were tested in female CD-1 mice challenged with either *Listeria monocytogenes*, to measure cell-mediated immunity, or antibody formation to sheep red blood cells, to measure the humoral response (Gaworski et al., 1994). Animals were dosed with 1250, 2500 or 5000 mg licorice extract/kg/day for five days by oral gavage. On the third day, some of the animals were inoculated *i.v.* with live *L. monocytogenes* cultures and monitored for ten days post-challenge. The remaining mice were injected with sheep red blood cells at the end of the five day treatment period and the spleens were removed four days later. Splenic cell suspensions were evaluated for their plaque-forming ability in the presence of sheep red blood cells and complement. Licorice extract did not affect the mortality or average survival times of infected mice at any dose tested. There were no effects of licorice extract in the anti-sheep red blood cell plaque forming assay as compared to vehicle-control animals. These results indicate that short term administration of licorice extract at concentrations up to 5000 mg/kg have no effect on either the cell-mediated or humoral response functions in mice.

1.6.3. Hepatoprotective effects

In two brief abstracts, Watari (1976) and Watari and Hotta (1980) demonstrated the hepatoprotective properties of glycyrrhizin in hepatotoxin treated ddy mice. In the first study, animals were administered the hepatic carcinogen 3′-methyl-4-dimethyl-aminoazobenzene in the diet and injected 1 mg glycyrrhizin twice per week for a period of three months. Animals treated with glycyrrhizin had a reduced incidence of hepatic cells showing morphological evidence of injury, including degenerated mitochondria, increased number of lysosomes, atrophied Golgi apparatus, pseudonuclear inclusions and increased mitotic cells (Watari, 1976). The second study reported that glycyrrhizin (1 mg injection, *i.m.*, twice per week) protected mice from the hepatocellular injuries caused by the 1–9 month administration of 10% ethanol in drinking water (Watari and Hotta, 1980). Liver sections from mice receiving the concurrent administration of glycyrrhizin and 10% ethanol had no microscopic signs of alterations or injuries whereas those receiving ethanol alone had greatly changed morphological features typical of chronic ethanol exposure.

Carbon tetrachloride (CCl_4), a known inducer of hepatic fibrosis in rats, was found to cause the leakage of aspartate transaminase (AST) and lactate dehydrogenase (LDH) from cultured primary hepatocytes (Nakamura et al., 1985). Glycyrrhizin, at concentrations of 25–200 μg/ml, was found to significantly inhibit the CCl_4-induced release of AST and LDH. The authors speculated that this function was due to an alteration of membrane fluidity by the glycyrrhizin, or perhaps an inhibition of CCl_4-induced membrane lipid peroxidation.

Kiso et al. (1984) examined the effects of glycyrrhizin and its metabolites on the CCl_4-induced free radical generation and lipid peroxidation in cultured rat hepatocytes and microsomes. In agreement with the findings of Nakamura et al. (1985), Kiso et al. (1984) found that 1 mg/ml of glycyrrhizin, 18α-glycyrrhetinic acid, 18β-glycyrrhetinic acid, 3-dehydro-18α-glycyrrhetinic acid, 3-dehydro-18β-glycyrrhetinic acid, and 3-epiglycyrrhetinic acid significantly reduced the release of alanine transaminase (ALT) from primary cultured rat hepatocytes exposed to CCl_4. The strongest congener was 18β-glycyrrhetic acid. Glycyrrhizin exhibited no significant suppressive activity of free radical generation, whereas 18β-glycyrrhetic acid inhibited this activity at 1 mg/ml. Similar results were noted for the effect of these two compounds on aniline hydroxylase activity in rat liver microsomal preparations. Interestingly, glycyrrhizin did not inhibit lipid peroxidation, whereas 18β-glycyrrhetic acid elicited a significant inhibitory action stronger than vitamin E. The authors concluded from these results that the *in vivo* hepatoprotective mechanism of glycyrrhizic acid is due to its aglycone, 18β-glycyrrhetic acid, which inhibits both free radical generation as well as lipid peroxidation (Kiso et al., 1984).

The *in vivo* protection of glycyrrhizin against CCl_4-induced hepatotoxicity was more recently illustrated by Jeong et al. (2002). Pretreatment of mice with 10–100 mg/kg, *s.c.*, significantly reduced the elevated serum ALT and AST as well as the liver lipid peroxidation caused by CCl_4. The depletion of hepatic glutathione was also reduced in a dose-dependent manner by glycyrrhizin treatment. Examination of hepatic cytochrome P450 2E1, which is involved in the bioactivation of CCl_4, showed a significant reduction in protein expression. These data suggest that the protection conferred by glycyrrhizin is, at least in part, due to the reduced *in vivo* activation of CCl_4.

1.6.4. Anti-carcinogenic effects

Studies evaluating the potential anti-carcinogenic effects of licorice extract and glycyrrhizate compounds have been recently reviewed (Wang and Nixon, 2001; Wang et al., 2000). The *in vitro* anti-mutagenic properties of triterpene compounds, such as glycyrrhizin, have been well documented, although the mechanism of this action is still poorly understood. An early report on the anti-mutagenic effects of glycyrrhizin and glycyrrhetic acid demonstrated, using a modified Ames test, that both of these compounds inhibited the mutagenicities of 3-amino-1-methyl-5*H*-pyrido[2,3-*b*]indol (Trp-p-2), 2-acetyl aminofluorene, and benzo(α)pyrene, in the presence S9 fraction hepatic enzymes (Yamaguchi and Watanabe, 1984). When the assay was repeated using mutagens not requiring metabolic activation, such as methyl glyoxal, glyceraldehyde and glucose pyrolysate, glycyrrhizin inhibited the number of induced *Salmonella typhimurium* TA98 revertants, whereas glycyrrhetic acid promoted the number of revertants per plate. These results prompted the authors to speculate that glycyrrhetic acid may act by inhibiting the metabolic activation of some mutagens.

The diversity of the antimutagenic effects of glycyrrhizin is further illustrated by Tanaka et al. (1987) and Ikken et al. (1999). Both licorice extract and glycyrrhizin inhibited the mutagenic effects of Trp-p-1 and Trp-p-2 in *S. typhimurium* TA98 (Tanaka et al., 1987) whereas licorice extract exerted a moderate to strong antimutagenic effect against several *N*-nitrosamine mutagens (Ikken et al., 1999). Licorice extract and glycyrrhizin were also effective at inhibiting the mutagenic effects of metabolically pre-activated Trp-p-1, suggesting that the antimutagenic effects are not due solely to the inhibition of the activating enzymes (Tanaka et al., 1987).

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

Mitscher et al. (1986) had also reported that licorice extracts were highly effective against the mutagenic effects of EMS in the Ames test. The antimutagenic activity of licorice extract was confirmed in the *rec*-assay in *Bacillus subtilis* strain M45, which is deficient in the genetic recombination function. However, licorice extract was not antimutagenic to the activities of the frameshift mutagens 9-aminoacridine or acriflavine, suggesting a specificity in its mechanism of action. These results led to the possibility 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, indicated that licorice extract improved the survival of the bacteria when applied prior to exposure of the cells

to EMS. This result led the authors to conclude that licorice extract exerts an antimutagenic effect by inducing the adaptive response in bacterial cells.

In a study examining effects of glycyrrhizin and glycyrrhetic acid on B16 melanoma cell growth and melanogenesis, Abe et al. (1987) reported that both compounds were inhibitory to these cells, but that glycyrrhetic acid was much more potent. Glycyrrhetic acid inhibited the growth of B16 cells in a concentration-dependent manner and caused the complete inhibition at concentrations over 10 μg/ml (approx. 21 μM), while 200 μg glycyrrhizin/ml (243 μM) inhibited growth by 40%. Flow cytometric analysis of the culture after four days exposure to 12.5 μg/ml glycyrrhetic acid revealed an accumulation of cells in the G_1 phase of the cell cycle and a reduction in the population of S and G_2/M phase cells. This result confirms that the growth inhibiting properties of glycyrrhetic acid is not due to a cytotoxic effect. Glycyrrhetic acid (10 μg/ml) also promoted the accumulation of melanin within the B16 cells after seven days exposure whereas glycyrrhizin had only nominal melanogenic properties at 200 μg/ml. These results did not allow the authors to speculate on the possible mechanisms of action of the glycyrrhizate compounds.

In an early *in vivo* study Nozawa (1967) briefly reported the potential anti-carcinogenic effects of glycyrrhizin in a methylcholanthrene-induced mouse model of skin carcinogenesis. Twice weekly dermal applications of 1.2 mg glycyrrhizin significantly reduced the average number of nodules and the mortality rate as compared to untreated controls. Oral and topical glycyrrhizin or glycyrrhetic acid were also reported to have anti-carcinogenic activity in the two-stage mouse skin tumorigenesis model induced with 7,12-dimethylbenz(α)anthracene and 12-*O*-tetradecanoylphorbol-13-acetate (Wang and Nixon, 2001).

Glycyrrhizin has also been shown to reduce hepatocellular carcinomas in mice induced with diethylnitrosamine (Shiota et al., 1999). The intramuscular (*i.m.*) administration of 2 mg glycyrrhizin/mouse, 3 days per week, reduced the incidence and total number of tumors or hepatocellular carcinomas per liver at week 32 of treatment. Similarly, glycyrrhizin (10 mg/kg, *i.p.*, on days 1, 3, 5, and 7) significantly reduced the incidence of B16F10 melanoma metastases in the lungs of mice (Kobayashi et al., 2002). Pulmonary metastasis by this cell line is strongly influenced by interleukin-4 (Il-4) produced by tumor-associated Th2 cells. The authors found that isolated $CD4^+$ T cells from treated mice produced significantly less IL-4 than those isolated from control animals, suggesting that this may be a mechanism by which glycyrrhizin reduces this specific form of tumorigenesis.

The isoflavanoids liquiritin and isoliquiritin (the glycone forms of which are liquirtigenin and isoliquiritigenin, respectively) found in licorice extracts, have also been the subject of investigation. Liquirtin was found to decrease the number of some biomarkers in the development of colonic aberrant crypt foci induced by azoxymethane in male F344 rats (Kawamori et al., 1995). The potency of isoliquiritin was measured against those of glycyrrhizin and licorice extract in an adjuvant-induced pouch granuloma model of angiogenesis in mice (Kobayashi et al., 1995). Isoliquiritin (0.31–1.3 mg/kg, *i.p.*) inhibited angiogenesis in a dose-dependent manner with an estimated IC_{50} of 1.46 mg/kg. The potency of isoliquiritin was approximately 50 times greater than that of licorice extract and glycyrrhizin. *In vitro* studies with vascular endothelial cells also found a concentration-dependent inhibition of tube structure formations.

1.6.5. Anti-cariogenic studies

Several studies have been conducted on the effects of licorice and glycyrrhizin on the growth and acid production of oral bacteria associated with the development of dental caries. Berry and Henry (1982, 1984) reported in two brief abstracts that glycyrrhizin could significantly reduce the growth and acid production of *Streptococcus*, *Actinomyces*, and *Bacterionema* species. Licorice powder, ammoniated glycyrrhizin, and monoammonium glycyrrhetic acid competitively reduced the metabolism of sucrose, glucose, and fructose, but were themselves minimally fermentable.

In contrast to these results, Segal et al. (1985) reported that neither licorice "juice," nor glycyrrhizin inhibited the growth of seven *Streptococcus mutans* strains. In the presence of sucrose, 0.5–1% glycyrrhizin had no effect on growth, but significantly inhibited bacterial adherence to glass by nearly 100% at the highest concentration tested. Licorice juice had similar anti-adherent properties with concentrations of 5 and 10% providing almost 100% activity. The buffering capacity of glycyrrhizin was not sufficient to affect the fall in pH caused by bacterial sucrose degradation.

In an additional study evaluating the mechanism of the anti-adherent property of glycyrrhizin, Sela et al. (1987) examined its effect on bacterial glucosyltransferase activity—an enzyme required in the formation of insoluble glucans required in plaque development. A crude preparation of *S. mutans* glucosyltransferase was significantly inhibited by glycyrrhizin in a concentration-dependent manner. At 12 mM glycyrrhizin there was a 50% inhibition of total glucan formation and a 90% inhibition of adhered glucans formation. Although glycyrrhizin was able to inhibit the activity of the soluble glucan-forming glucosyltransferase, the IC_{50} was 36 mM. The authors concluded that inhibition of bacterial glucosyltransferase activity may be a mechanism by which glycyrrhizin inhibits oral bacterial adherence, but that additional enzyme systems may also be affected.

1.6.6. Miscellaneous studies

Glycyrrhizin is classified as a saponin compound, and this property has been tested to determine its interaction with cellular membranes of erythrocytes and hepatic lysosomal preparations. Glycyrrhizin was found to protect erythrocytes against the hemolysis induced by other saponin compounds including digitonin, excin, tomatin, and

saponin A (Segal et al., 1977). The effect of glycyrrhizin was concentration-dependent but it was only effective at preventing hemolysis at concentrations approximately 400 times greater than the hemolysin. Glycyrrhizin was found to be as efficacious against the sapogenins digitogenin, tomatidine, and sapogenin A, indicating that its mechanism of action is not the result of the inhibition of membrane glycosidases of erythrocytes. The possibility remains that glycyrrhizin prevents access of hemolysin to its receptor, or alters membrane fluidic dynamics at these high concentrations. To test this possibility, Nakagawa and Asami (1981) investigated the effects of glycyrrhizin on the release and activity of acid phosphatases from hepatic lysosomal preparations. Both glycyrrhizin and 18β-glycyrrhetic acid attenuated acid phosphatase activity, but did not affect β-*N*-acetylglucosaminidase activity. The reduction of lysosomal acid phosphatase activity was due to its release from the lysosomes rather than a direct inhibition of the enzyme suggesting an alteration in membrane fluidity.

The dietary administration of glycyrrhizin to non-insulin dependent diabetic model mice was reported to significantly reduce their diabetic symptoms (Takii et al., 2001). KK-A^y mice were fed basal diets supplemented with 0, 2.7, or 4.1 g/kg of glycyrrhizin for nine weeks. At the end of the study, the highest dosed animals showed a significantly reduced weekly water intake and fasting blood glucose level as compared to the control and low-dosed groups. Body weight and weekly food intake were similar among all 3 groups. Blood glucose levels were also significantly lower in the highest dosed group 60 min after a glucose challenge. Blood chemistry analysis showed that this group also had significantly reduced insulin levels but free fatty acid, total cholesterol and triacylglycerol were unchanged.

Mendes-Silva et al. (2003) reported that glycyrrhizin reduced thrombosis formation in rats in a dose-dependent manner. The IC_{50} for intravenous glycyrrhizin was 75 mg/kg in a stasis-induced model, but only 230 mg/kg in an arteriovenous shunt model. Interestingly, the antithrombic activity was very time-dependent with an effective half life of less than 60 min in both models. The mechanism of action did not appear to be similar to that of heparin.

2. Toxicological studies

2.1. Acute toxicity studies

The acute toxicities of various glycyrrhizin salts have been tested in mice with oral LD_{50} values ranging from 1220 to 12,700 mg/kg, depending on the salt type tested (SCOGS, 1974); these data are summarized in Table 5. The pathological sequelae induced by acute lethal doses of glycyrrhizin have not been reported. Komiyama et al. (1977) reported similar acute toxicity doses in mice and rats for an extract of *Glycyrrhiza* containing approximately 53% glycyrrhizin (Table 6).

The *i.p.* administration of 2 g/kg of 18α-glycyrrhetic acid was lethal to adult female Sprague–Dawley rats (Rossi et al., 1999). This dose led to the progressive impairment of cardiac function within 60 min of dosing with subsequent atrio-ventricular block occurring within 140 min. Histopathology of these rats revealed brain, cerebellum and lung edema with renal hematic stasis. Focal changes of the papillary muscles as well as swollen cardiomyocytes were noted. These toxic responses were not found in rats treated with equivalent doses of 18β-glycyrrhetic acid or glycyrrhizin.

Segal et al. (1977) reported acute toxic effects of convulsions and slight hemolysis in mice administered 70 mg/kg glycyrrhizin intravenously in a solvent system consisting of propylene glycol, dimethyl sulfoxide and water. There were no toxic effects seen at lower doses of glycyrrhizin.

Table 5
Acute toxicities of glycyrrhizin salts in mice

Route	Glycyrrhizin form	LD_{50} (mg/kg)
p.o.	Ammonium, crude	12,700
p.o.	Diammonium	9600
p.o.	Potassium, crude	12,400
p.o.	Monopotassium	1220
p.o.	Dipotassium	8100
i.v.	Monopotassium	412
i.p.	Ammonium, crude	1050
i.p.	Monoammonium	1070
i.p.	Diammonium	1250
i.p.	Potassium, crude	1260
i.p.	Dipotassium	1400
i.m.	Monopotassium	695
s.c.	Monopotassium	697

Data from SCOGS (1974).

Table 6
Acute toxicity of *Glycyrrhiza* extract, containing 53% glycyrrhizic acid, in mice and rats by various routes of administration

Route	Species	Sex	*Glycyrrhiza* extract LD_{50} (g/kg)	Glycyrrhizic acid LD_{50} (g/kg)
p.o.	Rat	Male	18.0	9.54
		Female	14.2	7.53
	Mouse	Male	>7.5	>3.98
		Female	>7.5	>3.98
s.c.	Rat	Male	4.4	2.33
		Female	4.2	2.23
	Mouse	Male	4.0	2.12
		Female	4.2	2.23
i.p.	Rat	Male	1.58	0.84
		Female	1.42	0.75
	Mouse	Male	1.70	0.90
		Female	1.50	0.80

Data modified from Komiyama et al. (1977).

2.2. Short-term studies

Early reports on the "deoxycortone-like" effects of licorice and licorice extract in humans led to an examination of their effects in rats and guinea pigs (Card et al., 1953). Few details were given on the methodologies used by the authors. Guinea pigs and rats were administered 3 g crude

licorice/kg body weight/day (unknown number of days) by stomach-tube and provided physiological saline to drink. This treatment did not result in weight gain by either group of animals. Administration of glycyrrhetic acid (unknown dose or protocol) also failed to affect the weights of test animals compared to controls, whether given by stomach tube or subcutaneous (*s.c.*) injection. The same doses of glycyrrhetic acid did not prolong the survival time of adrenalectomized rats or guinea pigs.

Girerd et al. (1958) compared the effects of licorice extract, ammoniated glycyrrhizin and deoxycorticosterone acetate (DCA) on blood pressure and subsequent tissue lesions. Male Sprague–Dawley rats were divided into four treatment groups, all maintained on chow and 0.87% sodium chloride in water, unilaterally nephrectomized on the 25th day of the experiment, and monitored up to the 50th day. The results are summarized in Table 7. The authors concluded that rats treated with DCA, licorice extract, or glycyrrhizin showed identical trends in hypertension, but that the glycyrrhizin treatment was not polydipsic, and caused little change in body weight gain as compared to the control group. Since the licorice extract provided approximately equivalent doses of glycyrrhizin as was administered to the ammoniated glycyrrhizin group, these results suggest that other constituents of licorice, such as estrogenic compounds, may affect the normal physiological metabolism of water and salts.

Komiyama et al. (1977) examined the toxic effects of short-term licorice extract administration to Wistar rats (summarized in Table 8). Rats were orally administered

Table 7
Hypertensive and physiological effects of deoxycorticosterone (DCA), licorice extract and glycyrrhizin on unilaterally nephrectomized male rats after 50 days of treatment

Treatment group	Max. blood pressure (mm Hg)	Survival	Pathology
Control	128	10/10	No noted organ lesions
DCA (75 mg pellet implants)	188	12/19 (63%)	Elevated heart, kidney and adrenal weights, reduced hypophyses and testes weights. Lesions found in kidney, heart, brain. Arteriolar necrosis and hyalinization
Licorice extract (10 g/kg/day, *p.o.*)	186	5/14 (36%)	Elevated heart, kidney and adrenal weights, reduced hypophyses and testes weights. Lesions found in kidney and brain. Arteriolar necrosis and hyalinization
Ammoniated glycyrrhizin (1 g/kg/day, *p.o.*)	194	10/13 (77%)	Elevated heart, kidney and adrenal weights, reduced hypophyses and testes weights. Lesions found in kidney and brain, but less severe than in licorice and DCA groups. Arteriolar necrosis and hyalinization

All groups were maintained on drinking water containing 0.87% sodium chloride. Data from Girerd et al. (1958).

Table 8
Summary of *in vivo* studies involving repeated dose administration of glycyrrhizin

Species—route	Dose (as glycyrrhizin)	Duration	Results	Reference
Rat—drinking water	1600 mg/kg	5 days	Inhibition of urine production ↑Na^+ & K^+	Mori et al. (1987)
Rat—drinking water	75 mg/kg	5 days	↓ in relative activity of 11βHSD, ↓ 11βHSD mRNA	Whorwood et al. (1993)
Rat—*i.p.*	50 mg/kg twice daily	7 days	Hypertenion, ↓ nitric oxide response, ↑ endothelin response	Ruschitzka et al. (2001)
Rat—diet	2000 mg/kg	14 days	↑BW over controls, fecal excretion of iron, ↓liver iron content	West et al. (1979)
Rat—gavage	0, 20, 50 or 100 mg/kg	15 days	↑ renin and Na. ↓ cortisol, ACTH, aldosterone, K	Al Qarawi et al. (2002)
Rat—gavage	15 mg glycyrrhizin/kg, 30 mg glycyrrhetic acid/kg	30 days	↑BP and other changes. Return to control values upon withdrawal	Rossi et al. (1994, 1999)
Rat—gavage	15 mg 18α-glycyrrhetic acid/kg	30 days	Mild papillary myolysis, renal calculi, bronchial lymphoid tissue expansion	Rossi et al. (1999)
Mouse—drinking water	200, 375, 750, 1500, 3125 mg/kg	10 weeks	No survivors at two highest doses. MTD 375 mg/kg for males and 750 mg/kg for females	Kobuke et al. (1985)
Rat—drinking water	10, 100 mg/kg	12 weeks	↑BP, histologic changes in lung arteries	Ruszymah et al. (1995)
Rat—gavage	164, 334, 663, 1325 mg/kg	90 days	↓ BW, formed elements of blood, serum cholesterol; ↑ liver and kidney weights at two highest doses NOEL = 334 mg/kg	Komiyama et al. (1977)
Rat—diet	1200, 1900, 2600 mg NH_4 glycyrrhizin	4–6 months	Hypertension, ↑ kidney and heart weight, polydipsia, bracycardia, behavioral changes	Sobotka et al. (1981)
Mouse—drinking water	Males: 71, 166, 229; females: 117, 217, 407	96 weeks	Reduction in amount of water consumed. No effect on BW; mortality rate; time to death; incidence of tumors, type or distribution. No evidence of chronic toxicity or carcinogenicity	Kobuke et al. (1985)

Abbreviations: ACTH, adrenocorticotropic hormone; BP, blood pressure; BW, body weight; MTD, maximum tolerated dose; NOEL, no-observed effect level.

0.31, 0.63, 1.25, or 2.5 g licorice extract/kg/day for 90 days with licorice extract estimated to contain 53% glycyrrhizin. Body weight gain was slightly inhibited in animals that received 2.5 g/kg/day. Hematological evaluation revealed a significant decrease in the red blood cell counts with an accompanying decrease in hematocrit of the male, but not female, rats receiving the two highest doses of licorice extract. Male rats had a slightly, but significantly, elevated neutrophil and decreased lymphocyte count at the highest dose. Female rats also had some significant changes in the differential white cell count, but these were not dose-related. There were no abnormal findings in the urinalysis of male or female rats at any dose level, although electrolytes were not determined. Serum biochemistry revealed an interesting finding of significantly elevated total protein, albumin, AST and ALT in the male rats receiving the highest doses, whereas these values were significantly depressed 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 licorice 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 licorice extract. The authors considered the NOEL to be 0.31–0.63 g extract/kg (approximately 165–334 mg glycyrrhizin/kg) for 90 days of treatment.

In a study on the immediate effects of glycyrrhizin on urine volume and electrolyte metabolism in rats, Mori et al. (1987) found that this compound could alter renal functions by the third day of treatment, but that the effects were terminated upon withdrawal for four days. Male Slc:Wistar/K4 rats were administered 4 ml/day by oral gavage of a 5% glycyrrhizin solution (~1600 mg/kg)[2] for five days. Glycyrrhizin administration significantly inhibited urine production, as well as urine sodium and potassium excretion, during the five day treatment. These effects were reversible as all measured parameters returned to control levels following four days removal from the glycyrrhizin.

In a range-finding study preliminary to a chronic, two-year, toxicity study Kobuke et al. (1985) administered male and female B6C3F$_1$ mice 0, 0.08, 0.15, 0.3, 0.6, or 1.25% disodium glycyrrhizin in drinking water (~200, 375, 750, 1500 or 3125 mg/kg) for 10 weeks. None of the animals receiving the two highest doses of glycyrrhizin survived, with animals showing histological signs of marked starvation atrophy. From this study, the authors determined that the maximum tolerated dose of disodium glycyrrhizin was 0.15% for male and 0.3% for female mice.

In a brief report summarizing the effects of glycyrrhizin administration on the administration of other physiological minerals, West et al. (1979) measured the effects on fecal, tibia, and liver concentrations of iron, magnesium, and zinc. Male Sprague–Dawley rats were fed 2% glycyrrhizin mixed in rat chow (~2000 mg/kg) for 14 days. Glycyrrhizin treatment was found to significantly increase body weight as well as average fecal weight. Neither the liver nor tibia weights were affected in the treatment group compared to controls. There was a significant increase in the fecal excretion of iron and a concurrent decrease in liver iron content. Tibia iron levels were not altered, nor were the magnesium or zinc levels in the tissues or feces of the treated rats.

Ruszymah et al. (1995) examined the effects of subchronic glycyrrhizin administration on blood pressure, atrial pressure and the pulmonary vasculature in male Sprague–Dawley rats. Animals were provided drinking water containing either 0, 0.1 or 1.0 mg glycyrrhizin/ml (~10 and 100 mg/kg) for 12 weeks. There was a significant increase in tail blood pressure at 3, 6, 9 and 12 weeks in both glycyrrhizin-treated groups, which was associated with an increase in serum sodium and decrease in serum potassium levels. After 12 weeks of treatment, the mean right atrial pressure increased significantly from 2.69 mmHg in control animals to 4.47 and 6.86 mmHg in the 0.1 and 1.0 mg/ml treatment groups, respectively. Histological examination of lung tissue revealed a significant medial thickening of the arterial vessels in the treated groups, but there was no effect of glycyrrhizin treatment on the thickness of the left ventricular wall.

A recent study by Al Qarawi et al. (2002) further illustrates the effect of licorice extract consumption on the renin–aldosterone–angiotensin system. Male Wistar rats were administered daily by gavage a solution of 0, 100, 250 or 500 mg/kg of licorice extract for 15 days (approximately 0, 20, 50 or 100 mg/kg glycyrrhizin[3]). Treatment with licorice extract resulted in dose-dependent increases in plasma renin and sodium with concomitant decreases in plasma cortisol, adrenocorticotropic hormone (ACTH), aldosterone and potassium levels.

In another study on the hyperaldosterone effects of glycyrrhizin, Rossi et al. (1994, 1999) examined the role of 18α- and 18β-glycyrrhetic acid on blood pressure, urine volume, and urinary excretion of electrolytes as well as major organ histology. Male Sprague–Dawley rats were orally administered either 30 mg glycyrrhizin/kg/day, or 15 mg 18α- or 18β-glycyrrhetic acid/kg/day, for 30 days. Blood pressure was measured on the 7th, 15th and 30th day of the study and urine was collected by housing rats individually in metabolic cages. Rats treated with both isoforms of glycyrrhetic acid showed a significant increase in blood pressure, and decrease in urine volume on days 15 and 30, whereas those animals receiving glycyrrhizin exhibited hypertension only on day 15 and their urinary output was not affected. There was a significant increase in the urinary excretion of calcium ions in the 18β-

[2] Assumes 200 mg glycyrrhizin administered to a 125 g rat.

[3] Assumes a 20% glycyrrhizin content in the licorice extract.

glycyrrhetic acid-treated animals, whereas those treated with the 18α-isoform excreted higher levels of sodium and potassium. Removal of animals from the 18β-glycyrrhetic acid and glycyrrhizin treatments returned their blood pressure and urinary parameters to control levels within 30 days. However, those animals receiving 18α-glycyrrhetic acid showed a significant continued elevation in urine potassium and calcium output as compared to control animals. Histological examination revealed a mild papillary myolysis, some renal calculi and an expansion of bronchial-associated lymphoid tissue of animals treated with 18α-glycyrrhetic acid only. These data suggest that the 18α-isoform of glycyrrhetic acid is more toxic than either ammoniated glycyrrhizin or the 18β-isoform.

The mechanism of glycyrrhizin-induced hypertension was further examined by Ruschitzka et al. (2001). The effects of glycyrrhizin (50 mg/kg, *i.p.*, twice a day for seven days) on nitric oxide production and vascular endothelin response were monitored in male Wistar rats. The aortic endothelial nitric oxide response was significantly inhibited by glycyrrhizin treatment, as indicated by reduced aortic tissue nitrate concentrations and decreased endothelial nitric oxide synthase protein levels. In contrast, the endothelin response was enhanced by glycyrrhizin. Prepro-endothelin-1 gene expression and protein levels as well as the vascular response to endothelin-1 were all significantly increased as compared to untreated animals. Chemical inhibition of the endothelin-A receptor prevented the hypertensive effects of glycyrrhizin, suggesting a plausible mechanism for this physiological response to increased cortisol levels.

In an early study on the effects of glycyrrhizin on the pituitary–adrenal axis, Kraus (1958) measured the effects of treatment on the cold-stress response and the ability of fasting mice to mobilize glucose stores. Male mice were administered 0.4% ammoniated glycyrrhizin in drinking water (~1000 mg/kg) for 4–7 days. Some of the animals were housed at 5 °C and their survival time recorded. Glycyrrhizin-treated animals died more rapidly than did control animals. In mice fasted for 48 h, a one week pretreatment with ammoniated glycyrrhizin resulted in significantly lower blood glucose levels indicating a decreased ability of the animals to mobilize glucose stores. The author suggested these results indicated a decrease in the output of adrenal corticotrophic hormone following a short-term exposure to glycyrrhizin (Kraus, 1958).

The estrogenic-like effects of licorice extract were examined in female Wistar rats fed 25 μg extract/day for 2 weeks (Tamir et al., 2001). Treated animals showed a significant increase in creatine kinase activity in the pituitary, uterus, diaphysal and epiphysal bone, left ventricle and aortic tissues as compared to controls. The increase in creatine kinase was comparable to other animals administered 0.5 μg/day of 17β-estradiol for the same period. *In vitro* experiments suggested the active components in licorice root extract were glabrene and isoliquiritigenin.

Evidence that the pharmacological effects of glycyrrhizin involve the pituitary–adrenal axis led to the investigation of its potential neurobehavioral toxicity (Sobotka et al., 1981). Male Sprague–Dawley rats were fed 0, 2, 3, or 4% ammoniated glycyrrhizin in chow, providing approximately 0, 1.2, 1.9, or 2.6 g ammoniated glycyrrhizin/kg/day for 4–6 months. Expected changes in the basic physiological measurements were noted, including hypertension, increased kidney and heart weight, polydipsia, and bradycardia. Motor coordination and balance were unaffected by the glycyrrhizin treatment. Behavioral studies demonstrated that there was no effect on the passive avoidance or fixed interval responses, indicating that glycyrrhizin has no obvious effect on response inhibition learning, retention, or shock sensitivity. The conditioned avoidance response was found to be facilitated at the 4% glycyrrhizin dose, unaffected by the 3% dose, and depressed in those animals administered the 2% dose. Although these data do not provide information on the neuropharmacological mechanism of glycyrrhizin, the authors do note that its actions are specific, rather than general, and that they are similar to those associated with other neuropeptides such as ACTH (Sobotka et al., 1981).

2.3. Chronic studies

Kobuke et al. (1985) studied the chronic effects of disodium glycyrrhizin consumption in male and female $B6C3F_1$ 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. The significance of these differences in the maximum tolerated doses between the sexes was not discussed by the authors. Glycyrrhizin was administered in drinking water for 96 weeks at concentrations of 0, 0.04, 0.08, 0.15, or 0.3%, delivering an approximate daily dose of 0, 71, 166, or 229 mg/kg to the male mice and 0, 117, 217, or 407 mg/kg to the female animals. Following the 96 weeks exposure to glycyrrhizin, the animals were maintained on basal diets absent of glycyrrhizin for an additional 14 weeks prior to terminating the study. Although there was a dose-related reduction in the amount of drinking water consumed by treated mice the total intake of glycyrrhizin by male or female mice in the highest dose group was 3.2 or 3.5 times greater than those in the lowest dose groups, respectively. Glycyrrhizin treatment did not significantly affect average body weights, cumulative mortality rates, mean time to death, incidence of tumors, or types or distribution of tumors. The authors concluded that the long-term daily administration of glycyrrhizin to these mice did not provide any evidence of chronic toxicity or tumorigenicity.

2.4. Teratology and reproductive studies

Several studies have examined the potential teratogenic effects of glycyrrhizinate compounds. These are summarized in Table 9. In 1971, the Food and Drug Research

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

Itami et al. (1985) examined the potential teratogenic effects of disodium glycyrrhizin in pregnant Wistar rats. Rats were administered 0, 0.08, 0.4, or 2% disodium glycyrrhizin in their diet (80, 400 or 2000 mg/kg) during days 0–20 of gestation. Rats were either sacrificed and the fetuses 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 fetuses, sex ratios, fetal body weights, placental weights, degrees of ossification, live birth index, or body weight gain after birth. One fetus in the 0.08% treatment group was found with dilatation of the renal pelvis, but no other malformations or anomalies were noted in the treatment groups. There was a significant reduction in the maternal weight gain following delivery in the 0.4 and 2% dose groups. The authors concluded that disodium glycyrrhizin is not teratogenic in rats under the conditions of this study.

A similar study, evaluating the teratogenicity of ammoniated glycyrrhizin in pregnant Sprague–Dawley rats, was conducted by Mantovani et al. (1988). Commencing on the seventh day of gestation dams were provided with 0, 10, 100, or 250 mg ammoniated glycyrrhizin/100 ml drinking water (10, 100 or 250 mg/kg) and maintained up to the 20th day of pregnancy. All rats were sacrificed, blood was collected, adrenals removed and fixed, and the uterus was excised. The authors recorded implant numbers, malformations and histological markers of skeletal and soft tissues. 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 fetuses. Skeletal abnormalities were significantly elevated in the two highest treatment groups but the authors noted that similar anomalies were also found in high numbers of control litters. These abnormalities included misaligned, asymmetric and bipartic sternebrae and hemisternebrae. Soft-tissue abnormalities were mostly renal and were significantly elevated in the 100 and 250 mg/100 ml (100 and 250 mg/kg) dose test groups. External hemorrhages were also observed in some fetuses. From these data, the authors concluded that ammoniated glycyrrhizin exhibited some embryotoxicity to the developing rat fetus, but no toxicity to the mother, and that the fetal effects were minor.

A recent study examined more closely the effects of glycyrrhetic acid on rat fetal lung development (Hundertmark et al., 2002). Since 11βHSD is important in the regulation of pulmonary surfactant synthesis during development, the authors explored the fetal implications of maternal glycyrrhetic acid consumption. Pregnant Wistar rats were fed a daily diet delivering 0, 10, 100, or 1000 mg/kg glycyrrhetic acid commencing on the 13th day of gestation. Fetuses were examined on days 17, 19 and 21 of gestation as well as on the 1st post partum day. Fetal lung 11βHSD activity was moderately, but significantly, reduced in the highest dosed group as compared to controls. Fetal and maternal

Table 9
Summary of teratogenicity studies of oral administered glycyrrhizinates

Species	Dose	Dosing regimen	Comment	References
Mouse	0, 27, 90, 300, 1000 mg/kg/day[a]	Gestational days 6–15	No treatment related effects noted	FDRL (1971)
Rat	0, 27, 90, 300, 1000 mg/kg/day[a]	Gestational days 6 – 15	No treatment related effects noted	FDRL (1971)
Hamster	0, 27, 90, 300, 1000 mg/kg/day[a]	Gestational days 6–10	No treatment related effects noted	FDRL (1971)
Rabbit	0, 27, 90, 300, 1000 mg/kg/day[a]	Gestational days 6–18	No treatment related effects noted	FDRL (1971)
Rat	0, 0.08, 0.4, 2% in diet[b]	0–20 days of gestation or up to 8 weeks post partum	Reduced maternal weight gain at 0.4 and 2% doses No other treatment related effects noted	Itami et al. (1985)
Rat	0, 10, 100, 250 mg/100 ml drinking water[a]	Gestation days 7–20	Some embryotoxicities noted at 100 and 250 mg dose groups including skeletal and renal abnormalities	Mantovani et al. (1988)
Rat	0, 10, 100, 1000 mg/kg/day[c]	Gestation day 13 to 1 day post partum	Highest dose showed reduced fetal lung 11βHSD activity, reduced surfactant protein A mRNA, reduced lamellar body content and reduced surfactant clusters No other treatment related malformations, abnormal behaviour or fetal death rate	Hundertmark et al. (2002)

[a] Ammoniated glycyrrhizin.
[b] Disodium glycyrrhizin.
[c] Glycyrrhetic acid.

plasma corticosteroid, sodium and potassium levels were not affected by the treatment at any doses but there was a significant decrease in the fetal lung surfactant protein A mRNA levels in the 1000 mg/kg group. Histological examination of fetal 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 fetal death rate associated with glycyrrhetic acid exposure nor was any abnormal behavior observed in the neonatal rats.

Two studies have evaluated ammoniated glycyrrhizin in rodent dominant lethal tests to determine its *in vivo* mutagenic potential to rodent germ cells. In the first study, male Sprague–Dawley rats were provided with feed containing 0, 4000, 13,333, or 40,000 ppm glycyrrhizin for a period of 10 weeks prior to mating (SRI, 1977). These concentrations delivered approximately 0, 500, 1667 or 5000 mg/kg/day of glycyrrhizin to the animals. Dominant lethality was evaluated on the basis of dead implants per pregnant female and the number of dead implants per total implants. The highest dosed group showed a statistically significant increase in the number of dead implants as well as dead per total implants. No other effects of glycyrrhizin treatment were noted and the authors concluded that glycyrrhizin was mutagenic at 40,000 ppm.

Sheu et al. (1986) evaluated ammoniated glycyrrhizin in the rat and mouse dominant lethal tests as well as a mouse heritable translocation test. In the first assay, male Sprague–Dawley rats were fed 0.4, 1.3, or 4.0% glycyrrhizin in their diet (~400, 1300 or 4000 mg/kg) for a period of 10 weeks prior to mating with females. As with the Stanford Research Institute study (SRI, 1977), the highest dose of glycyrrhizin produced a weak, but statistically significant, increase in the number of dead implants per pregnant female, whereas the 1.3% dose produced a small decrease in the number of live implants per female. In the second assay, male $(101 \times C3H)F_1$ mice were fed 1.5 or 2.5% glycyrrhizin in their diet (~2250 or 3750 mg/kg) for eight weeks prior to mating with $(SEC \times C57BL)F_1$ and $(C3H \times C57BL)F_1$ females to test for a dominant lethal effect, and the male progeny were subjected to sequential fertility to test for the presence of heritable translocations. Glycyrrhizin did not induce a detectable level of a dominant lethal effect in male mice. In the heritable translocation test, those progeny carrying confirmed translocations due to maternal heritance were eliminated from the final calculations. In considering only the newly arisen translocations, the number of carrying male progeny detected in the glycyrrhizin-treated groups were comparable to those of the control group. From these data, the authors concluded that ammoniated glycyrrhizin did not have the capacity to induce heritable chromosomal defects and, in contrast to the conclusions of SRI (1977), the relevance of the weak embryotoxicity found in this study could be questioned (Sheu et al., 1986).

Although there are no known studies evaluating the reproductive effects of licorice extract, or glycyrrhizinate compounds the results from the dominant lethal testing studies suggest that the consumption of glycyrrhizin (4000–5000 mg/kg/day) for 10 weeks does not affect the ability of male rats to conceive (Sheu et al., 1986; SRI, 1977).

2.5. Genotoxicity studies

The majority of bacterial genotoxicity studies have reported an absence of genotoxic effects from licorice extracts or glycyrrhizinate compounds. Table 10 summarizes those assays performed using *S. typhimurium* strains. Genotoxicity studies using *Escherichia coli* WP2 (SRI, 1979) or *Saccharomyces cerevisiae* D-3 (Green, 1977) also showed an absence of mutagenic effects to glycyrrhizin. Only one researcher reported a positive genotoxic response (Martinez et al., 1999). Licorice extract was mutagenic in *S. typhimurium* TA100 at all concentrations tested, but not in TA98. However, this response was not clearly concentration-dependent suggesting either some toxicity or influence on the DNA repair mechanisms at the higher concentrations. Pre-incubation of the extract with rat liver S9 fraction did not change the responses.

Table 10
Reported genotoxicities of licorice extracts, glycyrrhizin and glycyrrhetic acid in *Salmonella typhimurium* strains

S. typhimurium strain	Compound	Concentration	Results	Reference
TA100	Licorice extract	25–100 μl/plate	Negative	Zani et al. (1993)
TA100	Glycyrrhizin	100–2000 μg/plate	Negative	Zani et al. (1993)
TA100	18α-glycyrrhetic acid	250–2000 μg/plate	Negative	Zani et al. (1993)
TA100	18β-glycyrrhetic acid	250–1500 μg/plate	Negative	Zani et al. (1993)
TA98, TA100, TA1535, TA1537, TA1538	Glycyrrhizin	33–10,000 μg/plate	Negative[a]	SRI (1979)
TA98	Licorice extract	1–10 mg/ml	Negative	Martinez et al. (1999)
TA100			Positive	
TA98, TA100	Glycyrrhizin	200 μg/plate	Negative	Yamaguchi and Watanabe (1984)
TA98, TA100	Glycyrrhetic acid	200 μg/plate	Negative	Yamaguchi and Watanabe (1984)
TA100	Licorice extract	3.9–250 μg/plate	Negative[a]	Mitscher et al. (1986)
TA100	Polar lipid fraction of licorice extract	0.001–100 μg/plate	Negative[a]	Mitscher et al. (1986)

[a] Extracts were toxic to cells at the highest concentration tested.

One study was found which examined the *in vivo* genotoxic effect of 39 food additives, including glycyrrhizin (Sasaki et al., 2002b). A single oral dose of 2000 mg glycyrrhizin/kg was administered to male ddY mice and DNA damage was measured in various organs three and 24 h later by the COMET assay. Glycyrrhizin did not increase DNA damage in any of the 8 organs examined.

2.6. Human studies

The pharmacologic and toxic effects of licorice extract, glycyrrhizinate compounds and carbenoxolone have been well studied and documented in humans over the past 30–40 years. Pharmacological studies, such as the anti-ulcer effects, are summarized above whereas the toxic responses, such as the pseudohyperaldosterone effects, are discussed here. Clinical case reports relating to the effects of licorice consumption are numerous in the medical literature and predominantly reflect the physiological responses to an imbalance of the renin–angiotensin–aldosterone system caused by excessive exposure to glycyrrhizinates. A simple search of the Medline literature database reveals almost 100 case reports published since 1980 involving licorice-induced hypertension, hypokalemia, decreased plasma renin and aldosterone levels, myopathies, edema and/or muscle weakness. Most patients recover after withdrawal of the licorice source although some deaths have been reported in more extreme cases. Unfortunately, the glycyrrhizin dose producing these effects is rarely available and patients are only able to provide rough estimates of their licorice consumption. Differences in licorice sources, manufacturers and processing methods makes the glycyrrhizin estimate even more difficult; as was highlighted by Spinks and Fenwick (1990), the glycyrrhizin content of confectionary products in the United Kingdom ranged from 0.26 to 7.9 mg/g while health products had an even broader range of 0.3–47.1 mg/g.

The earlier clinical studies on licorice consumption were aimed at determining its mechanism of causing edema as well as isolating the active component of licorice extract. Molhuysen et al. (1950) fed 20–45 g *succus liquiritiae* to seven patients with gastric ulcer and monitored serum and urinary electrolyte levels, blood pressure, urea excretion and creatinine excretion. The authors noted a quick reduction in urine volume as well as decreased chloride output, but that neither urea nor creatinine excretion were affected. Urinary potassium levels increased with continued administration of the licorice. In order to determine its site of action, comparisons were made to the effects of deoxycortone and ACTH. The authors found few differences in the effects of deoxycortone and *succus liquiritiae*. To compare licorice and ACTH, the authors examined the responses of a patient with rheumatoid arthritis to either substance. The patient was found to be unresponsive to 45 g daily doses *of succus liquiritiae*, but responded favorably to a 25 mg injection of ACTH within 6 h. From their data, the authors determined that the licorice compound had similar properties to deoxycortone, but that its mechanism remained unknown.

It was not until 1952 that glycyrrhizin was suspected as the active ingredient of licorice, although these findings were questioned when the result of studies involving patients with Addison's disease produced inconsistent results (Card et al., 1953). Louis and Conn (1956) were among the first scientists to clearly describe the effects of purified glycyrrhizin on the pituitary–adrenal axis in humans. Seven healthy control adult subjects and three patients with either adrenal hyperplasia or Cushing's syndrome were recruited for the investigation. Ammoniated glycyrrhizin (4–6 g/day) was administered to the subjects for 3–6 days and monitored for effects on organic metabolism, electrolyte metabolism, excretion of 17-ketosteroids, electrolyte composition of thermal sweat, and urinary excretion of melanocyte-stimulating hormone. Glycyrrhizin was found to have no effect on organic metabolism, but induced the retention of sodium, chloride, and water and increased urinary potassium. Sodium and chloride content of thermal-induced sweat were also decreased. There was a mild, but consistent, decrease in the excretion of 17-ketosteroids and glycyrrhizin was found to suppress the urinary excretion of melanocyte-stimulating hormone in one patient. From these results, the authors concluded that glycyrrhizin has properties similar to adrenal cortical steroid, but that its mechanism of action was unknown.

A study conducted by Hausmann and Tarnoky (1966) found similar, although less severe, effects of carbenoxolone administration to 15 patients with various gastro-intestinal disorders. Carbenoxolone disodium (200–300 mg/day) was administered to the patients for two weeks and they were monitored for weight changes, fluid balance, blood pressure, electrolyte status, and gastric, renal and liver functions. Most of the results remained within normal limits, although some patients developed symptomless hypertension. There was a tendency towards lowered serum potassium and increased bicarbonate levels in four patients, but none developed edema. The authors suggested that carbenoxolone has a limited similarity to primary aldosteronism, but that much of this effect differs between ambulatory and bed-ridden patients.

Epstein et al. (1977) studied the effects of daily confectionary licorice consumption on electrolyte status and the renin–angiotensin–aldosterone axis in 14 volunteers to further ascertain the effects of glycyrrhizin in healthy subjects. Volunteers ate 100–200 g licorice candy daily for 1–4 weeks and were monitored for blood pressure, plasma aldosterone, plasma renin activity, plasma angiotensin II, plasma electrolytes, as well as urinary electrolytes and aldosterone concentrations. Licorice was prematurely withdrawn for 6 of the 14 subjects due to hypokalemia or edema. Electrolyte imbalance was demonstrated in most subjects on both dose schedules. In 12 subjects, the plasma potassium fell by 1.5 mEq/l. There was a significant reduction in the levels of plasma renin activity, angiotensin II, and plasma and urinary aldosterone concentrations. These values returned to

control levels within 1–2 weeks after licorice withdrawal. Subjects with the most prolonged renin–angiotensin–aldosterone axis suppression had the most severely affected hypokalemia and edema that necessitated their early withdrawal from the study. A subsequent study by these authors, using similar licorice doses and schedules in healthy volunteers, reported the urinary unconjugated cortisol more than doubled in 10 of 13 subjects, but there was no effect on plasma ACTH or cortisol levels (Epstein et al., 1978). Despite the increased cortisol excretion, there was no effect of licorice consumption on urinary steroid metabolite excretion or on the normal diurnal variation of plasma cortisol levels.

A similar study conducted by Forslund et al. (1989) in 12 healthy volunteers, further confirmed the effects of licorice consumption (100 g/day) on plasma renin activity, plasma aldosterone levels, as well as urinary excretion of aldosterone and unconjugated cortisol. Subjects in this study consumed the licorice confectionary for a period of eight weeks, in which time nine developed mild-to-severe cases of edema, but blood pressure did not increase above normal levels in any of the volunteers. All symptoms disappeared within two weeks following discontinuation of licorice ingestion. In addition to these findings, the authors reported that plasma concentrations of atrial natriuretic peptide were significantly increased, whereas the plasma antidiuretic hormone levels were significantly decreased during the period of chronic licorice ingestion. The authors suggest that the increased plasma atrial natriuretic peptide is a reflection of sodium and water retention, and is secondary to licorice consumption.

In order to provide direct clinical evidence for an inhibition of the conversion of cortisol to cortisone, MacKenzie et al. (1990) studied the effects of pure glycyrrhetic acid on plasma cortisol and cortisone in ten healthy volunteers. Glycyrrhetic acid (500 mg/day) was administered orally for seven days and blood samples were collected daily for measurements of plasma cortisol and cortisone. Glycyrrhetic acid activity was confirmed to be similar to that of glycyrrhizin as there were similar changes in plasma renin activity, aldosterone, atrial natriuretic peptide, and electrolytes as reported in previous studies. Glycyrrhetic acid consumption did not affect plasma cortisol levels, but it significantly decreased plasma cortisone concentrations and increased the urinary excretion of unconjugated cortisol. The authors concluded that the results are consistent with the hypothesis that glycyrrhetic acid exerts mineralocorticoid action by a direct inhibition of the conversion of cortisol to cortisone.

Bernardi et al. (1994) evaluated the effects of graded doses of pure licorice root extract in order to identify the dosages leading to mineralocorticoid-like side effects. Four groups of six volunteers were fed licorice root extract containing 108, 217, 380 or 814 mg glycyrrhizin daily for 4 weeks. No significant effects occurred in the groups receiving the two lowest doses. One subject from the 380 mg/day group and two subjects from the 814 mg/day group were forced to withdraw from the study due to headaches, hypertension, hypokalemia and/or edema. A significant depression of plasma renin activity was found only in those subjects of the two highest dose groups. The presence of subclinical disease or the use of oral contraceptives seemed to favor the development of side effects. The authors concluded that, in healthy subjects, the untoward effects of pure licorice root extract were dose related and were less common than after the intake of comparable amounts of pure glycyrrhizin.

Sigurjonsdottir et al. (1995) monitored the effects of moderate consumption of licorice on blood pressure, plasma and urinary electrolytes, and urinary cortisol. Thirty healthy volunteers were fed 100 g licorice daily (containing 270 mg glycyrrhizin) for four weeks. There was a significant increase in the mean systolic blood pressure during the period of licorice consumption and a significant decrease in plasma potassium levels, which returned to basal levels 2–4 weeks after withdrawal. Nineteen subjects complained of edema, headache or gastrointestinal symptoms during the test period. There was a significant rise in the urinary cortisol:cortisone metabolite ratio at the end of the four week period, indicating the pharmacological action of glycyrrhizin. The authors concluded that moderate licorice consumption can induce hypertensive effects, but the extent of these effects varies greatly between individuals.

In a study examining the possible causes for the biphasic distribution of licorice-induced pseudoaldosteronism effects, Kato et al. (1995) examined the blood concentrations of two glycyrrhizin metabolites in 21 patients: ten who developed licorice-induced pseudoaldosteronism, and eleven who showed no adverse response to glycyrrhizin consumption. All subjects showing signs of pseudoaldosteronism had been receiving glycyrrhizin agents of different doses (80–375 mg/day) and durations (3 weeks–8 years), whereas the non-affected group was administered 150–200 mg/day of glycyrrhizin over a period of 4–6 weeks. There was no significant difference in the mean serum concentration of glycyrrhetic acid between the two groups of patients. However, 3β-(monoglucuronyl)-18b-glycyrrhetic acid (3MGA) was detected in the serum of all affected patients (mean serum concentration = 1.23 μM) whereas none of the unafflicted patients had detectable levels of 3MGA. This finding suggested that 3MGA may be more potent than glycyrrhetic acid at inducing pseudoaldosteronism.

More recently, efforts have been made to clarify the dose–response effects of licorice in order to ascertain levels of safe use, or No-Observed Effect Levels (NOEL) in humans. One randomized double-blind study evaluated the responses of 39 healthy female volunteers to glycyrrhizin consumption over an 8-week period (van Gelderen et al., 2000). Women were chosen for this study as a previous pilot project had found them to be more sensitive to the effects of glycyrrhizin than men. The women consumed either 0, 1, 2, or 4 mg glycyrrhizin/kg/day. Only the highest dosed group exhibited measurable adverse effects including decreased plasma aldosterone, renin and potassium levels.

From this study the authors proposed a NOEL of 2 mg/kg to which they applied a safety factor of 10 to derive an acceptable daily intake (ADI) of 0.2 mg/kg.

A second dose response study involved healthy men and women volunteers who consumed licorice for 4 weeks (Sigurjonsdottir et al., 2001). Subjects ate 50, 100, or 200 g of licorice candy per day, providing approximately 75, 170 or 540 mg/day of glycyrrhetic acid. Within 2 weeks there was a significant rise in the systolic blood pressure (bp) of all dose groups, although this increase was only by an average of 3.1 mm Hg in the lowest dose. The systolic bp responses to the licorice consumption were varied in all groups and followed a normal distribution, resulting in some individuals experiencing a reduction in systolic bp at the end of the study. Interestingly, the systolic bp in the lowest dosed group, which was significantly elevated at the second week of the study, was no longer elevated by the 4th week suggesting a compensatory or adaptive response.

Two recent studies reported a significant decrease in serum testosterone levels among young healthy men who consumed licorice providing 0.5 g glycyrrhizin/day (Armanini et al., 1999, 2003). The original study involved seven men and reported an average decrease in serum testosterone levels of 35% following seven days of licorice consumption (Armanini et al., 1999). A subsequent study by another group and involving 31 young healthy men was unable to replicate these findings and reported a non-significant decrease in saliva testosterone concentration of only 9.5% (Josephs et al., 2001). Therefore, the original study was repeated in 17 young men and found a significant decrease in serum testosterone of 25% (Armanini et al., 2003). The discrepancy between these studies is believed to lie in the methods used. Although saliva testosterone accurately reflects serum testosterone levels, it only comprises the free hormone. Armanini et al. (1999, 2003) measured total (free and bound) testosterone in the serum. From these studies it therefore appears that testosterone levels are affected by licorice consumption in young men.

Two Finnish epidemiological studies reported a significant correlation between licorice consumption among pregnant women and preterm delivery. The initial report was of a cross-sectional study involving 1049 women who delivered healthy infants (Strandberg et al., 2001). Glycyrrhizin intake was evaluated by a lifestyle questionnaire determining licorice consumption and was divided into low (<250 mg/week), moderate (250–499 mg/week) and heavy ($\geqslant$500 mg/week) levels. Although the study was not designed to detect an association with preterm births, there was an apparent and significantly increased risk of delivery prior to 38 weeks gestation. A second case-controlled retrospective study compared 95 women who delivered healthy preterm babies to 107 women delivering at a full gestational age (Strandberg et al., 2002). Again, a heavy consumption of licorice was strongly associated with an increased risk of preterm birth (odds ratio = 2.18). The mechanism by which licorice promotes preterm delivery is unknown, but may be related to the induction of pseudoaldosteronism effects in the mother or fetus.

3. Discussion and conclusions

Licorice (*Glycyrrhiza glabra*) root and its extract have a long history of use in traditional medicines, folk remedies, and as a sweetening and flavoring agent. Indeed, the history of licorice use pre-dates the Greek and Roman empires. Early Western and Chinese traditional medicine practitioners valued licorice for its effectiveness in relieving numerous maladies including gastrointestinal ailments, cough, bronchitis, and arthritis. Many of the historical uses for licorice are still practiced today among consumers preferring alternative or complementary medical treatment. Current, non-medicinal use for licorice is primarily as a flavoring agent for tobacco and oral pharmaceutical preparations. Despite the sweetening capacity of glycyrrhizin (the principal active component of licorice), its strong and distinctive flavor limits its use in foods.

Both licorice extract and glycyrrhizin have been approved for use in foods by the U.S. Food and Drug Administration (FDA), the Council of Europe, and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). It has also been included in the list of substances Generally Recognized as Safe (GRAS) by the Flavor and Extract Manufacturers' Association (FEMA). Current estimates for intake of licorice extract and glycyrrhizin in the United States are in the range of 1.6–215 mg/day (as glycyrrhizin) (0.027–3.6 mg/kg/day), with a probable average intake of less than 2 mg/day; although, it is possible that some acute exposures may exceed the average amount as the result of eating large amounts of licorice-flavored candy.

Studies in rodents and humans show that glycyrrhizin is poorly absorbed from the gastrointestinal tract, but is metabolized by intestinal microflora to glycyrrhetic acid and monoglucuronyl glycyrrhetic acid, both of which are more readily absorbed. Glycyrrhetic acid undergoes phase II metabolic processes and is primarily excreted into the bile as conjugates of glucuronate and sulfate. This allows for an enterohepatic circulation of glycyrrhetic acid, thus requiring several days for complete elimination from the body for even a single dose. Interestingly, the bioavailability of glycyrrhizin is much less when administered in licorice than as a purified compound. The combination of a long persistence and enterohepatic circulation along with the unpredictable bioavailability has led to a persistent difficulty among researchers in establishing a clear dose–response relationship for glycyrrhizinate compounds.

Biochemical studies have shown that glycyrrhizin is the principal active ingredient in licorice extract and is an inhibitor of the oxidation function of the 11βHSD enzyme, which catalyzes the inactivation cortisol.

Pharmacological studies have evaluated several of the traditional health claims behind licorice use although many of these reports have produced inconsistent results.

Carbenoxolone, an analog of glycyrrhetic acid, has shown success in clinical trials for gastric and duodenal ulcers, but the potential development of pseudoaldosteronism has limited its use. Deglycyrrhizinated licorice has also shown some effect in the treatment of gastrointestinal ulcers, suggesting the presence of active ingredients other than glycyrrhizin, although other studies have shown it has no beneficial effects. Licorice and glycyrrhizinate compounds have also been used in the treatment of some viral infections such as hepatitis. Although these studies indicated favorable changes in hepatic function, they do not all demonstrate a reduction in viral load. The potential mechanisms of anti-ulcer and anti-viral action of glycyrrhizin are unknown; however, modulation of the immune response seems to be indicated.

The acute toxicities of licorice extract and glycyrrhizin salts are low with oral LD_{50}s generally greater than 4 g glycyrrhizinate/kg b.w. in mice and rats. The majority of short-term toxicity studies have examined the effects of licorice and glycyrrhizin on the pituitary–adrenal axis. The induction of pseudoaldosteronism by glycyrrhizin is dose and time dependent, but the establishment of a clear NOEL is difficult due to the differences in agents tested, animals used and end-points studied. In rats, the 90-day NOEL for licorice extract is in the range of 0.31–0.63 g extract/kg, which delivers approximately 165–334 mg glycyrrhizin/kg (Komiyama et al., 1977). However, it should be noted that the licorice extract used in this study had an unusually high concentration of glycyrrhizin (53%). For purified glycyrrhizin compounds, the 30-day NOEL in rats is below 15 mg/kg (Rossi et al., 1994, 1999). In contrast, a 2-year disodium glycyrrhizinate feeding study in mice showed no significant effects on average body weights or mortality, nor any signs of tumorgenicity among male animals administered up to 229 mg/kg/day or females administered up to 407 mg/kg/day (Kobuke et al., 1985).

Other *in vivo* studies reported no teratogenic effects when glycyrrhizin salts were administered maternally to mice, rats, hamsters, or rabbits during gestation at doses as high as 1000 mg/kg/day (FDRL, 1971; Itami et al., 1985; Mantovani et al., 1988). However, dominant lethal testing in male rats suggest that an intake of 4000–5000 mg glycyrrhizin/kg/day could lead to mutagenic effects in offspring (Sheu et al., 1986; SRI, 1977). Microbial tests found that licorice extract and glycyrrhizinates were non-genotoxic and had some anti-genotoxic properties. Some *in vivo* studies have reflected the anti-carcinogenic potential of glycyrrhizin, although this mechanism is likely due, at least in part, to the induction of carcinogen metabolizing enzymes. Antioxidants present in licorice extract could also promote any anti-carcinogenic potential.

There is abundant evidence from both case reports and clinical studies that the habitual consumption of glycyrrhizin results in adverse effects marked by the development of pseudohypercorticosteroidism. As with animal studies, the establishment of a clear NOEL from human studies is difficult due to differences in the administered substance tested (licorice extract *vs* purified glycyrrhizinates), length of the administration, end-points measured, and inter-individual variation in response. However, from these studies it appears that there is a biphasic distribution in the degree of response to glycyrrhizin exposure and that an adaptive mechanism may re-balance the renin–angiotensin–aldosterone system with continued consumption in some people. One 8-week clinical study established a NOEL for purified glycyrrhizin consumption of 2 mg/kg/day (van Gelderen et al., 2000).

Based on the feeding studies in rats and mice, it is safe to assume a NOEL for purified glycyrrhizin is in the range of 15–229 mg/kg/day. Using this experimental data and applying uncertainty factors of 10 for intraspecies differences and 10 for interspecies differences, an ADI for glycyrrhizin of 0.015–0.229 mg/kg/day is posited. This range is in agreement with the ADI for glycyrrhizin proposed by van Gelderen et al. (2000) of 0.2 mg/kg/day, but is less than estimated U.S. consumption of 0.027–3.6 mg/kg/day. It is important to note that consumption estimates are derived from sources, which include licorice extract, as well as purified glycyrrhizinates, but that the bioavailability of glycyrrhizin from the extract is much lower than that of the pure compound. From these data it can be assumed that current levels of consumption of licorice extract products and glycyrrhizinates presents no concern for safe use.

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