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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}.