Final Report of the Cosmetic Ingredient Review Expert Panel

Safety Assessment of Glycyrrhiza Glabra (Licorice) Rhizome/root, Glycyrrhiza Glabra (Licorice) Leaf Extract, Glycyrrhiza Glabra (Licorice) Root, Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) Root Juice, Glycyrrhiza Glabra (Licorice) Root Powder, Glycyrrhiza Glabra (Licorice) Root Water, Glycyrrhiza Inflata Root Extract, and Glycyrrhiza Uralensis (Licorice) Root Extract

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Cosmetic Ingredient Review

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Final Report on the Safety Assessment of
Glycyrrhiza Glabra (Licorice) Rhizome/root, Glycyrrhiza Glabra (Licorice) Leaf Extract, Glycyrrhiza Glabra (Licorice) Root, Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) Root Juice, Glycyrrhiza Glabra (Licorice) Root Powder, Glycyrrhiza Glabra (Licorice) Root Water, Glycyrrhiza Inflata Root Extract, and Glycyrrhiza Uralensis (Licorice) Root Extract

ABSTRACT: Glycyrrhiza Glabra (Licorice) Rhizome/Root, Glycyrrhiza Glabra (Licorice) Leaf Extract, Glycyrrhiza Glabra (Licorice) Root, Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) Root Juice, Glycyrrhiza Glabra (Licorice) Root Powder, Glycyrrhiza Glabra (Licorice) Root Water, Glycyrrhiza Inflata Root Extract, and Glycyrrhiza Uralensis (Licorice) Root Extract are cosmetic ingredients derived from *Glycyrrhiza glabra*, *G. inflata*, or *G. uralensis* plants. These ingredients perform a wide range of functions in cosmetics, including flavoring and fragrance, skin conditioning, antioxidant, and astringent, and are used at low concentrations, generally in the range of 0.0001% to 0.5%. Not all of these licorice-derived ingredients are currently used in cosmetics. Any future use of currently unused ingredients should be at levels comparable to current use. Extracts are prepared in a single step using water or in multiple steps using water, ammonia, ethanol, ethyl acetate, and/or acetone. The composition varies as a function of extraction method and other factors, including growth conditions of the plant, but little information is available regarding specific composition of any given preparation. The available data on the general composition, however, demonstrated that the range of different compounds found in licorice was remarkably similar across species. Licorice extracts are used as flavoring agents and are generally recognized as safe by the U.S. Food and Drug Administration. Impurities in licorice extracts may include heavy metals and pesticide residues. Limited data were available on the dermal penetration of these ingredients or their constituents. Licochalcone A penetrated the horny layer, epidermis and dermis of pig skin but none was detected in the receptor fluid, suggesting that it remained bound to the skin. These ingredients were not significantly toxic in acute, subchronic, and chronic animal toxicity studies. For example, an acute intraperitoneal LD_{50} of > 5 g/kg was reported in rats, and ingestion of licorice extracts produced both memory enhancement and cognitive deficits in mice. These ingredients were not ocular or dermal irritants, or dermal sensitizers or photosensitizers in animals studies. Glycyrrhiza Glabra (Licorice) Root Extract was not a reproductive toxicant at oral doses up to 2 g/kg/d in Sprague-Dawley rats. Licorice extracts did not have estrogenic properties. In Ames testing, the water and methanol extracts of *G. uralensis* were negative and other genotoxicity tests demonstrated that licorice extracts can reduce the mutagenic effect of other agents. In vitro studies suggested possible cancer chemoprotective effects of licorice extracts, but rats fed Licorice Extract up to 3.0% of the diet had no such effects. In multiple clinical assessments of safety, oral administration of Licorice Extract resulted in various levels of hypokalemia; sodium retention; edema of the face, hands and ankles; headaches; lethargy; electrolyte imbalance; increased urinary unconjugated cortisol; reduced plasma renin activity; increased plasma atrial natriuretic peptide concentration; and hypertension. All symptoms resolved after termination of exposure to licorice extract. These licorice-derived ingredients were not dermal irritants, sensitizers, or photosensitizers in clinical tests. There are many specific chemicals that have been identified as constituents of licorice plants, principal among which are Glycyrrhizic Acid and Glycyrrhetinic Acid and their derivatives. In a separate safety assessment, Glycyrrhizic Acid and Glycyrrhetinic Acid and their derivatives were found to be safe for use in cosmetics. An appendix includes many other chemicals that have been isolated from licorice plants and studied primarily for their medicinal properties. These individual chemical constituents vary in their dermal penetration and intestinal absorption, but once absorbed are metabolized and widely distributed. For example, while licochalcone A was not dermally absorbed; liquiritigenin, davidigenin, liquiritin, and liquiritin apioside were dermally absorbed. Most of the licochalcone A injected into rats metabolized into 4'-phenolic glucuronides in the plasma and urine. Oral absorption and oral bioavialability were low and dose-dependent for glabridin. Glabridin, licochalcone A, and licorisoflavan A decreased the effects of induced nephritis in mice. Hepatic protection has been reported for Glycyrrhizic Acid, glabridin, isoliquiritigenin, licopyranocoumarin, liquiritin, liquritin and glycyrrhizin. Deglycyrrhizinized licorice had gastrointestinal protective effects from ulcers in rats and dogs as did isoliquiritigenin in rats. Muscular contraction was reported for glycycoumarin, while inhibition of contraction was reported for isoliquiritigenin and davidigenin, and liquiritin, liquirtitin apioside, and isoliquirtin were inactive. Isoliquiritigenin reduced platelet aggregation in human and rat blood. Several flavonoids, including glabridin, were inhibitors of cAMP. Licochalcone A and E were not cytotoxic to human lung, ovarian melanoma, and colon cell lines. Isoliquiritigenin inhibited histamine mediated signals and is a selective antagonist in U937 cells and inhibited monocyte adhesion in a dose-dependent manner. Licochalcone A reduced cytokine production in human skin cells in a dose-dependent manner. Anti-inflammatory effects were found with glabridin, Glycyrrhizic Acid, licochalcone A and B, and various other licorice constituents. Antioxidant effects were found for licochalcone A and B, glabridin, hispaglabridin A and B, formononetin, hemileiocarpin, glycycoumarin paratocarpin B, and 4'-*O*-methylglabridin, but not for licocoumarone. Anti-parasitic and anti-microbial activity varied as a function of the compound tested, with some effective and some not. Glabridin was not phototoxic to guinea pigs with UVB exposure. Gancaonis R, glabrene, glycyrol, gismoidin B and liquiritigenin, prunetin, daidzein, formononetin, datiscetin, fisetin, and isoliquiritigenin had weak estrogen receptor binding affinties. Glabridin stimulated the growth of human breast cancer cells at concentrations up to 10 µM, but inhibited cell growth at concentrations

>15 µM. Uterine weights were increased after rats were injected with glabridin, but not as much as estradiol. Glabradin and glabrene demonstrated estrogen activity when incubated with human female bone cells from pre- and post menopausal women. In an Ames tests, glabrene was antimutagenic against spontaneous and ethylmethanesulfonate-induced mutations up to 1.0 µg/plate; it was toxic at 10 µg/plate. Glabrol, fromononet, glabridin, and Hispaglabridin A were not antimutagenic. The flavones, gancaonin O and P and glyasperin A, were more cytotoxic to oral tumor cells than normal cells. Isoliquiritigenin has tumorigenesis inhibiting properties in in vitro and in vivo assays for several types of cancer cells. Licochalcone A was not cytotoxic to PC-3 prostate cancer cells but caused growth inhibition and decreased both the number and size of DMBA-induced tumors in mice. The CIR Expert Panel recognized the available data do not address all licorice-derived ingredients. The available data on the constituents of licorice, however, were consistent across species, demonstrating that the composition of the plants were similar enough that toxicity data on *G. glabra* extracts could be extrapolated to the others, and vice versa. The Panel also recognized different constituents could be present in a licorice extract depending on the method of extraction. In this regard, the Panel considered the available data on a wide range of individual chemical constituents to consistently demonstrate an absence of significant toxicity, with many studies reporting protective effects. These data on chemical constituents also are consistent with the safety test data for the individual extracts in the main body of the report. The Panel concluded the variation in constituents with different extraction solvents did not present different toxicity issues. The Panel recognized that glabridin can have depigmentation properties, but would be present in cosmetic formulations at such a low concentration that it should not have any dipigmentation activity. Cosmetic formulators should only use licorice extracts in products in a manner that does not cause depigmentation. The Expert Panel expressed concern regarding pesticide residues and heavy metals that may be present in botanical ingredients and stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulation. Likewise, the cosmetic industry should limit mycotoxins before blending into cosmetic ingredients. The Panel believes that licorice-derived ingredients should be methanol and aflatoxin free; with ≤ 20 ppb corresponding to a "negative" aflatoxin content. The CIR Expert Panel noted the weak estrogen receptor activity of some low-level constituents. Because licorice-derived ingredients do not significantly penetrate the skin, have low levels of use, the constituents in question exist only at low levels in the ingredient, significant estrogenic activity is precluded. In the absence of inhalation toxicity data, the Panel determined that these ingredients can be used safely in hair sprays, because the ingredients particle size is not respirable. Overall, the available data supported the safety of these ingredients. Accordingly, these licorice-derived ingredients were considered safe as cosmetic ingredients in the practices of use and concentration described.

INTRODUCTION

This report reviews the available data relevant to the safety of Glycyrrhiza Glabra (Licorice) Leaf Extract, Glycyrrhiza Glabra (Licorice) Rhizome/Root, Glycyrrhiza Glabra (Licorice) Root, Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) Root Juice, Glycyrrhiza Glabra (Licorice) Root Powder, Glycyrrhiza Glabra (Licorice) Root Water, Glycyrrhiza Inflata Root Extract, and Glycyrrhiza Uralensis (Licorice) Root Extract as used in cosmetic products. These are the International Nomenclature Cosmetic Ingredient (INCI) names for licorice-derived cosmetic ingredients as given in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008).

Part I of this safety assessment presents data on the ingredients listed above.

Since the ingredients included in this safety assessment are derived from plant material, they may contain many different constituents depending on the method of preparation.

Part II of this safety assessment includes information on specific constituents (e.g., glabridin, lichochalcone A, etc.). There are large amounts of data on some constituents while very little or none on others.

The Cosmetic Ingredient Review (CIR) previously reviewed the safety of Glycyrrhizic Acid and Glycyrrhetinic Acid and their derivatives, which are constituents of licorice-derived ingredients as well as cosmetic ingredients in their own right (CIR 2005). It should be expected that these chemicals are present at some level in any licorice-derived substance, except for deglycyrrhizinated licorice extract. The relevant information from the safety assessment on Glycyrrhizic Acid and its metabolite Glycyrrhetinic Acid are summarized in Part II under the relevant headings. Overall, the CIR Expert Panel concluded that Glycyrrhizic Acid and its derivatives "...are safe for use in cosmetic formulations in the practices of use and concentration as described in this safety assessment" (CIR 2005).

Unpublished data provided through the Personal Care Products Counsel (Council) formerly the Cosmetic Toiletry, and Fragrance Association (CTFA) are cited according to the name of the organization when submitted.

A list of acronyms and abbreviations used in this report is located at the end of the document for readers' convenience.

PART I - Licorice-derived Ingredients

CHEMISTRY

Definition and Origin

The term "licorice" generally refers to certain perennial herbs or subshrubs with horizontal underground stems. The plants stand 1 to 2 meters high, with *Glycyrrhiza uralensis* being the smallest among those reviewed here (Leung and Foster 1996). The

licorice plants are native to the Mediterranean region and parts of Asia and are cultivated worldwide (Wren et al. 1988). Yamazaki et al. (1994) found that *G. glabra* and *G. uralensis* were found to have a genetic similarity coefficient (GS) of 0.61, making them more closely related to each other than they were to *G. pallidiflora* (GS = 0.42 and 0.35, respectively) and *G. echinata* (GS = 0.41 and 0.45, respectively). The plants of the species *Glycyrrhiza glabra* are also known as Spanish licorice, Persian licorice, and Russian licorice (Leung and Foster 1996).

As given in the International Cosmetic Ingredient Dictionary and Handbook (Gottschalck and Bailey 2008), Glycyrrhiza Glabra (Licorice) Rhizome/Root (CAS No. 68916-91-6), formerly known as Glycyrrhiza Glabra (Licorice), is a plant material derived from the dried rhizomes and roots of *G. glabra*. Its chemical class is biological products. Other names for Glycyrrhiza Glabra (Licorice) Rhizome/Root include Glycyrrhiza, Kanzou (Japan), Licorice, Licorice Extract (Glycyrrhiza spp.), Licorice Extract Powder (Glycyrrhiza glabra L.), Licorice Root, Licorice Root Extract (Glycyrrhiza glabra L.), Licorice Root (Glycyrrhiza glabra L.) and Powdered Glycyrrhiza.

Herbasol® Extract PG is listed as a trade name by Cosmetochem (2004a,b).

Glycyrrhiza Glabra (Licorice) Leaf Extract (no CAS No.) is defined as an extract of the leaves of *G. glabra*. Its chemical class is biological products. It is also called Licorice Leaf Extract and supplied under the trade name ABS Licorice Extract Powder (Gottschalck and Bailey 2008).

Glycyrrhiza Glabra (Licorice) Root (no CAS No.) is defined as the root of *G. glabra*. Its chemical class is biological products. AEC Liquorice Root Whole and Licorice GLS are listed as a trade names (Gottschalck and Bailey 2008).

Glycyrrhiza Glabra (Licorice) Root Extract (CAS No. 84775-66-6) is the extract of the root *G. glabra*. Its chemical class is biological products. Synonyms for Glycyrrhiza Glabra (Licorice) Root Extract include Glycyrrhiza Extract, Glycyrrhiza Extracted Powder, Glycyrrhiza Flavonoids, Glycyrrhiza Glabra Extract, Kanzou Ekisu (Japan), and Kanzou Furabonoido (Japan). It has many trade names and trade name mixtures (Gottschalck and Bailey 2008).

Glycyrrhiza Glabra (Licorice) Root Juice (no CAS No.) is defined as the juice expressed from the roots of *G. glabra*. Its chemical class is biological products (Gottschalck and Bailey 2008).

Glycyrrhiza Glabra (Licorice) Root Powder (no CAS No.) is defined as the powder obtained from the dried, ground roots of *G. glabra*. Its chemical class is biological product. AEC Liquorice Root Powdered and Mukti Yastimadhu listed as a trade name (Gottschalck and Bailey 2008).

Glycyrrhiza Glabra (Licorice) Root Water (no CAS No.) is defined as an aqueous solution of the steam distillate obtained from the roots of *G. glabra*. Its chemical class is essential oils and waters. Glycyrrhiza Glabra Extract is listed as a trade name (Gottschalck and Bailey 2008).

Glycyrrhiza Inflata Root Extract (no CAS No.) is an extract of the roots of *G. inflata*. Its chemical class is biological product. Polyol Soluble Licorice Extrace P-U is listed as a trade name (Gottschalck and Bailey 2008).

Glycyrrhiza Uralensis (Licorice) Root Extract (no CAS No.) is defined as an extract of the roots of *G. uralensis*. Its chemical class is biological product. Its technical name is listed as Licorice Root Extract (Gottschalck and Bailey 2008).

The licorice root of *G. glabra* is also referred to as *Glycyyrrhizae radix* (Chin et al. 2007).

Physical and Chemical Properties

Cosmetochem (2004a,b) provided data sheets on Glycyrrhiza Glabra (Licorice) Root Extract under the trade name mixture Herbasol® Extract PG. The product also contains water, propylene glycol, sorbitol, phenoxyethanol, methylparaben, butylparaben, ethylparaben, propylparaben and isobutylparaben. The chemical and physical properties of this product are given in **Table 1.**

Table 1. Chemical and physical properties of Glycyrrhiza Glabra (Licorice) Root Extract (trade name mixture Herbasol® Extract PG) (Cosmetochem 2004a,b).

Property	Description/Value
Appearance	dark brown liquid
Odor	characteristic
Density 20°C	1.045 - 1.058 g/cm^3
Index of refraction 20°C (Na 589.3 nm)	1.376 - 1.386 n
pH - value 25°C	5.0 -6.3
Dry matter (IR; Halogen)	5.5 - 7.5
Identification test amino acids	positive
Melting point/melting range	not determined
Boiling point/boiling range	95 - 185°C
Flash point	> 101°C
Self-inflammability	product not self-igniting
Danger of explosion	not explosive
Solubility (10%) in water	clear; fully miscible
Solubility (10%) IPA	sedimentation
Total plate count (media Eugon LT 100; Agar B)	max. 100 cfu/g
Yeasts (media Eugon LT 100; Agar C)	max. 20 cfu/g
Mold (media Eugon LT 100; Agar C)	max. 20 cfu/g
Gram - germs (media Eugon LT 100; Agar H)	negative

The ultraviolet (UV) absorbances of Glycyrrhiza glabra (Licorice) Root Extract and Glycyrrhiza Inflata Root Extract are shown in **Table 2** (CTFA 2004e,f).

Table 2. UV absorbance maxima of Glycyrrhiza Glabra (Licorice) Root Extracts and a Glycyrrhiza Inflata Root Extract in ethanol by trade name (CTFA 2004e,f).

Trade Name (ingredient name or source)	Max (nm)	E_{1cm} 1%	Marker compound (%)
Oil soluble licorice extract P-T(40) (Glycyrrhiza Glabra (Licorice) Root Extract)	240.5	117.2	Glabridin (40%)
	282.0	364.7	
	227.5	775.5	
Oil soluble licorice extract P-Th (Glycyrrhiza Glabra (Licorice) Root Extract)	282.0	371.0	Glabridin (20%)
Oil soluble licorice extract P-T (Glycyrrhiza Glabra (Licorice) Root Extract)	282.0	331.5	Glabridin (20%)
Polyol soluble licorice extract P-U (Glycyrrhiza Inflata Root Extract)	347.0	235.2	Licochalcone A (0.6% - 1.6%)
	289.5	291.3	
Polyol soluble licorice extract BG100 (Glycyrrhiza Glabra (Licorice) Root Extract mixture)	282.0	3.5	Glabridin (0.4%)
	227.0	7.5	

Wojcikowski et al. (2007) sequentially extracted *G. glabra* root with ethyl acetate, methanol, and aqueous methanol (1:1; v:v). The authors report that the oxygen radical absorbance capacities were 196.44 ± 7.48, 416.93 ± 41.72, and 416.08 ± 41.99 µmol TE/g dw, respectively

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Method of Manufacture

According to the Food and Drug Administration (FDA 1983a), in the process of manufacturing licorice for foods, the extract is that portion of the licorice root that is, after maceration, extracted by boiling water. The extract can be further purified by filtration and by treatment with acids and ethyl alcohol. Licorice extract is sold as a liquid, paste (''block''), or spray-dried powder.

According to Leung and Foster (1996), licorice extracts are commonly prepared by hot water extraction followed by evaporating off the water and drying the extracts to form sticks or blocks.

Data sheets on Glycyrrhiza Inflata Root Extract state that production is by extraction of *G. inflata* root with ethanol, concentration of the filtrate, extraction of residue with ethyl acetate, and then drying of filtrate to yield the commercial product (CTFA 2004a,b).

The Committee of Experts on Cosmetic Products (2002) summarizes the manufacturing process for Licorice Extract as follows: 1) digestion with hot water (90°C) and concentration under vacuum to dryness; 2) precipitation of aqueous phase or extract with solution of mineral acids and separation of the solid phase obtained; the solid is dissolved in diluted ammonia solution, filtered, and the filtered solution is concentrated under vacuum to dryness; 3) purification of ammoniated glycyrrhizin; and 4) acid hydrolysis of ammoniated glycyrrhizin or glycyrrhizic acid and purification.

Mae et al. (2003) conducted several extractions of *G. uralensis* root using ethanol, ethyl acetate, acetone, and water. The yields of these extracts were 14.8, 8.2, 11.0, and 21.0 g/100g G. uralensis root, respectively.

Majima et al. (2004) reported that licorice processing may include roasting.

Composition

While this section describes the chemical constituents that may be found in different licorice-derived ingredients, additional discussion of chemical constituents and safety test data may be found in **Part II.**

Molhuysen et al. (1950) described a "watery extract" of the roots of *G. glabra* as containing 15% protein substance, 15% Glycyrrhizic Acid, 40% "extractable" substances, 5% sugar, and 5% insoluble substance.

Duke (1985) stated that an analysis of licorice root showed 20% moisture, 12% to 16% glycyrrhizin, 8% reducing sugars, 8% non-reducing sugars, 30% starch and gums, 5% ash, and 12 to 17% undetermined.

Zava et al. (1997) found that a 2 g capsule of licorice contained the equivalent of 4 µg estradiol.

Because they are plant-derived materials, these ingredients are a mixture of many constituents. Informatics Inc. (1972) and Leung and Foster (1996) reported findings of Glycyrrhizic Acid (1% to 24 % in the root); glucuronic acid; sapogenin; mono-, di-, and polysaccharides; fat; L-asparagine; and "bitter compounds" with minute amounts of essential oil, tannin, and methylsalicylate.

Han and Chung (1990) reported that alkaloids also are found in licorice plant extracts and Han et al. (1990) isolated 5,6,7,8-tetrahydro-2,4-dimethylquinoline and 5,6,7,8-tetrahydro-4-methylquinoline from *G. uralensis*.

Shimizu et al. (1991) isolated glycyrrhizan GA (l-arabinose, d-galactose, l-rhamnose, d-galacturonic acid, d-glucuronic acid in the ratio of 22:10:1:2:1) from the hot water extract of the stolon of *G. glabra* L. var. glandulifera through a multistep fractionation procedure.

Zhang and Wang (1997) stated that naringenin is a licorice constituent.

Nomura and Fukai (1998) stated that the main phenols of licorice are glycosides of liquiritigenin (4',7-dihydroxyflavanone) and isoliquiritigenin (2',4,4'-trihydroxychalcone) such as liquiritin, isoliquiritin, liquiritin apioside, and licuraside.

Shibata (2000) stated that sweet-tasting saponin is, on average, 4 to 5% of the dried root and the total content of flavonoid in the root is 1 to 2%. There are about 50 aglycones of saponins found in *G. glabra, G. uralensis* and *G. inflata* roots. *G. glabra* root contains about 70 phenolic compounds, *G. uralensis* about 60 and *G. inflata* about 60. The dried roots of licorice plants yield 1 to 2% phenolic compounds; the content of licochalcone A in the root of *G. inflata* is approximately 0.8%.

Piersen (2003) stated that the triterpenoids in licorice are sometimes classified as phytoestrogens but meet neither a strict structural nor a rigorous functional definition.

Agricultural Research Service (ARS 2004, 2007) listed many constituents and their concentrations found in *G. glabra* in **Table 4** in alphabetical order.

Table 4. Alphabetical list of substances found in *G. glabra* for which the concentration was measured (ARS 2004).

Chemical	Plant part	Concentration (ppm)	Chemical	Plant part	Concentration (ppm)
7-acetoxy-2-methylisoflavone	root	4 &17	formononetin	root	33 - 66
	plant	25	furfural	root	2
acetic-acid	root	2	gamma-nonalactone	root	6
alpha-terpineol	rhizome essent. oil	75,000	glabranin	shoot	3,100
alpha-terpinolene	root	0.1	glabrene	rhizome	129
aluminum	root	182		root	80 - 800
anethole	root	1	glabridin	rhizome	1,433
apioglycyrrhizin	root	157		root	400 - 4,000
araboglycyrrhizin	root	75	glabrol	root	129 - 440
ascorbic-acid	root	626		root	12
ash	root	100,000 - 107,000	glucose	root	30,000 - 42,300
asparagine	root	20,000 - 40,000	glycocoumarin	root	110 - 710
benzaldehyde	rhizome essent. oil	75,000	glycyrin	root	100
beta-carotene	seed	5	18-alpha-glycyrrhetinic-acid	root	1,300 - 5,200
	root	trace	18-beta-glycyrrhizinic acid	root	16,800 - 95,200
beta-sitosterol	root	500	glycyrrhetinic acid	root	6,000 - 22,400
2-butyl-2-octenal	root	2	glycyrrhetinic acid monoglucuronide	root	6,000 - 22,400
butanoic-acid	root	2	glycyrrhizic acid	plant	100,000 - 240,000
calcium	root	8,780	glycyrrhizin	root	16,000 - 152,000
carbohydrates	root	773,000	heneicosane	root	0.1 - 7
cellulose	plant	300,000		root	4 - 38
chromium	root	17	heptadecane	root	7
cobalt	root	101	heptanoic acid	root	12
cumic-alcohol	root	4	hexacosane	root	5 - 40
decanoic-acid	root	1		root	0.8
1,7-demethyl-7(4-methyl-3-pentenyl)-tricycloheptane	root	0.1 - 6	Hexadecanoic acid	root	16
dihydro-5,5-dimethyl-2(3H)-furanone	root	2	hexadecyl acetate	root	trace
2,3-dihydro-4-methyl-furan	root	2	hexanoic acid	root	148
docosane	root	0.1 - 25	2-hexanal	root	2
eicosane	root	19	hexanol	root	8
essential oils	root	400 - 590	hexyl formate	root	4
estragole	root	1	hispaglabridin A	root	126
2-ethyl-1,4-dimethyl-benzene	root	1	hispaglabridin B	root	119
eugenol	root	1	hispaglabrin A	rhizome	127
fat	root	8,000 - 10,000	hispaglabrin B	rhizome	119
fiber	root	84,000	glabrene	rhizome	129
fluoride	root	4.2		root	80 - 800
			glabridin	rhizome	1,433
				root	400 - 4,000

Table 4 (continued). Alphabetical list of substances found in *G. glabra* for which the concentration was measured (ARS 2004).

Chemical	Plant part	Concentration (ppm)	Chemical	Plant part	Concentration (ppm)
glabrol	root	129 - 440	magnesium	root	9,650
	root	12	manganese	root	47
glucose	root	30,000 - 42,300	2-methalyl-phenol	root	1
glycocoumarin	root	110 - 710	3'-methoxyglabridin	root	117
glycyrin	root	100	1-methoxy-4-isopropyl-cyclohexane	root	2
18-alpha-glycyrrhetinic-acid	root	1,300 - 5,200	6-methyl-3,5-heptadien-2-one	root	2
18-beta-glycyrrhizinic acid	root	16,800 - 95,200	methyl hexadecanoate	root	1
glycyrrhetinic acid	root	6,000 - 22,400	methyl hexanoate	root	trace
glycyrrhetinic acid monoglucuronide	root	6,000 - 22,400	4-methyl-isopropyl-3-cyclohexen-1-ol	root	6
glycyrrhizic acid	plant	100,000 - 240,000	2-methyl-6-methylene-7-octen-2-ol	root	1
glycyrrhizin	root	16,000 - 152,000	3-methyl-2-methyl-propane	root	2
heneicosane	root	0.1 - 7	5-methyl-2-undecene	root	2
	root	4 - 38	9-methyl-3-undecene	root	2
heptadecane	root	7	myrtenal	root	trace
heptanoic acid	root	12	N-methyl-2-pyrrolidone	root	5
hexacosane	root	5 - 40	N-tetradecane	root	trace
	root	0.8	nicotinic acid	leaf	100 - 1,000
Hexadecanoic acid	root	16	nonadecane	root	0.1 - 2
hexadecyl acetate	root	trace	O-acetyl-salicylic aced	root	1,500 -1,636
hexanoic acid	root	148	O-cresol	root	trace
2-hexanal	root	2	4'-O-methylglabridin	root	169
hexanol	root	8	O-tolunitrile	root	1
hexyl formate	root	4	octacosane	root	4 - 34
hispaglabridin A	root	126	octadecanal	root	15
hispaglabridin B	root	119	octadecane	root	trace
hispaglabrin A	rhizome	127	octanoic acid	rhizome essent. oil	114,000
hispaglabrin B	rhizome	119		root	7
3-hydroxy-glabrol	rhizome	23.3	ononin	root	320
2-hydroxy-4-methyl-benzaldehyde	root	1	paeonol	rhizome essent. oil	89,000
indole	root	2	pectin	shoot	58,000
iron	root	880	pentacosane	root	0.1 - 41
isobutyl adipate	root	1	pentadecane	root	trace
isoglycycoumarin	root	50	pentadecanoic acid	root	trace
isoglycyrol	rhizome	80	1-pentadecanol	root	1
isoliquiritigenin	rhizome	160	pentanoic acid	root	4
	root	9,610	2-pentyl-furan	root	2
isoliquiritin	rhizome	470 - 920	5-pentylpyran-2-one	root	1
	root	470 - 920	phaseollinisoflanan	root	27
licoflavanone	leaf	4,900	phenethyl alcohol	root	1
lignin	root	100,000	phenyl acetaldehyde	root	trace
linalol	root	2	phosphorus	root	790
liqcoumarin	root	23	pinocembrin	shoot	9,500 - 9,700
liquiritigenin	root	140	potassium	plant	11,4000

Table 4 (continued). Alphabetical list of substances found in *G. glabra* for which the concentration was measured (ARS 2004).

Chemical	Plant part	Concentration (ppm)	Chemical	Plant part	Concentration (ppm)
protein	root	110,000	tetradecanoic acid	root	trace
pseudoionone	root	1	thiamin	root	2.1
salicylic acid	root	567	tin	root	24
silicon	root	158	tricosane	root	0.1 - 34
sodium	root	8,180	tridecane	root	trace
starch	root	50,000 - 200,000	tridecanoic acid	root	trace
	shoot	15,000	undecane	root	trace
sucrose	root	24,000 - 91,700	undecanoic acid	root	trace
sugar	root	30,000 - 140,000	uralenic acid	shoot	15,000
terpinen-4-ol	rhizome essent. oil	72,000	uralozide acid	shoot	15,000
tetracosane	root	0.1 - 39	zinc	root	3

Table 5 presents these same substances found in *G. glabra* for which the concentration was measured (ARS 2004), but as a function of plant part.

Dibenzoylmethane (DBM) is a minor component of licorice (Frazier et al. 2004).

Di Mambro and Fonseca (2005) reported that *G. glabra* contains 115.7 ± 2.4 µg protein/mg plant extract, 7.42 ± 0.480 µg polyphenol/mg plant extract, and 0.88 ± 0.015 µg flavonoid/mg plant extract.

Table 5. Substances found in *G. glabra* for which the concentration was measured sorted by plant part (ARS 2004).

Plant Part/Chemical	Concentration (ppm)	Plant Part/Chemical	Concentration (ppm)	Plant Part/Chemical	Concentration (ppm)
Leaf		**Seed**		**Root (continued)**	
licoflavanone	4,900	beta-carotene	5	1,7-demethyl-7(4-methyl-3-pentenyl)-tricycloheptane	0.1 - 6
nicotinic acid	100 - 1,000	**Shoot**		18-alpha-glycyrrhetinic-acid	1,300 - 5,200
Rhizome Essential Oil		glabranin	3,100	18-beta-glycyrrhizinic acid	16,800 - 95,2000
alpha-terpineol	75,000	pectin	58,000	2-butyl-2-octenal	2
benzaldehyde	75,000	pinocembrin	9,500 - 9,700	2-ethyl-1,4-dimethyl-benzene	1
octanoic acid	114,000	starch	15,000	2-hexanal	2
paeonol	89,000	uralenic acid	15,000	2-hydroxy-4-methyl-benzaldehyde	1
terpinen-4-ol	72,000	uralozide acid	15,000	2-methalyl-phenol	1
Rhizome		**Plant**		2-methyl-6-methylene-7-octen-2-ol	1
3-hydroxy-glabrol	23.3	7-acetoxy-2-methylisoflavone	25	2-pentyl-furan	2
glabrene	129	cellulose	300,000	2,3-dihydro-4-methyl-furan	2
glabridin	1,433	glycyrrhizic acid	100,000 - 240,000	3'-methoxyglabridin	117
hispaglabrin A	127	potassium	11,4000	3-methyl-2-methyl-propane	2
hispaglabrin B	119	**Root**		4'-O-methylglabridin	169
isoglycyrol	80	1-methoxy-4-isopropyl-cyclohexane	2	4-methyl-isopropyl-3-cyclohexen-1-ol	6
isoliquiritigenin	160	1-pentadecanol	1	5-methyl-2-undecene	2
isoliquiritin	470 - 920			5-pentylpyran-2-one	1

Table 5 (continued). Substances found in *G. glabra* for which the concentration was measured sorted by plant part (ARS 2004).

Plant Part/Chemical	Concentration (ppm)	Plant Part/Chemical	Concentration (ppm)	Plant Part/Chemical	Concentration (ppm)
Root (continued)		**Root (continued)**		**Root (continued)**	
6-methyl-3,5-heptadien-2-one	2	heneicosane	0.1 - 7	pentanoic acid	4
7-acetoxy-2-methylisoflavone	4 &17	heptacosane	4 - 38	phaseollinisoflanan	27
9-methyl-3-undecene	2	heptadecane	7	phenethyl alcohol	1
acetic-acid	2	heptanoic acid	12	phenyl acetaldehyde	trace
alpha-terpinolene	0.1	hexacosane	5 - 40	phosphorus	790
aluminum	182	hexadecane	0.8	protein	110,000
anethole	1	Hexadecanoic acid	16	pseudoionone	1
apioglycyrrhizin	157	hexadecyl acetate	trace	salicylic acid	567
araboglycyrrhizin	75	hexanoic acid	148	silicon	158
ascorbic-acid	626	hexanol	8	sodium	8,180
ash	100,000 - 107,000	hexyl formate	4	starch	50,000 - 200,000
asparagine	20,000 - 40,000	hispaglabridin A	126	sucrose	24,000 - 91,700
beta-carotene	trace	hispaglabridin B	119	sugar	30,000 - 140,000
beta-sitosterol	500	indole	2	tetracosane	0.1 - 39
butanoic-acid	2	iron	880	tetradecanoic acid	trace
calcium	8,780	isobutyl adipate	1	thiamin	2.1
carbohydrates	773,000	isoglycycoumarin	50	tin	24
cobalt	101	isoliquiritigenin	9,610	tricosane	0.1 - 34
cumic-alcohol	4	isoliquiritin	470 - 920	tridecane	trace
decanoic-acid	1	lignin	100,000	tridecanoic acid	trace
dihydro-5,5-dimethyl-2(3H)-furanone	2	linalol	2	undecane	trace
docosane	0.1 - 25	liqcoumarin	23	undecanoic acid	trace
eicosane	19	liquiritigenin	140	zinc	3
essential oils	400 - 590	magnese	47		
estragole	1	magnesium	9,650		
eugenol	1	methyl hexanoate	trace		
fat	8,000 - 10,000	methyl hexadecanoate	1		
fiber	84,000	myrtenal	trace		
fluoride	4.2	N-methyl-2-pyrrolidone	5		
formononetin	33 - 66	N-tetradecane	trace		
furfural	2	nonadecane	0.1 - 2		
gamma-nonalactone	6	O-acetyl-salicylic aced	1,500 -1,636		
glabrene	80 - 800	O-cresol	trace		
glabridin	400 - 4,000	O-tolunitrile	1		
glabrol	129 - 440	octacosane	4 - 34		
glabrone	12	octadecanal	15		
glucose	30,000 - 42,300	octadecane	trace		
glycocoumarin	110 - 710	octanoic acid	7		
glycyrin	100	ononin	320		
glycyrrhetinic acid monoglucuronide	6,000 - 22,400	pentacosane	0.1 - 41		
glycyrrhetinic acid	6,000 - 22,400	pentadecane	trace		
glycyrrhizin	16,000 - 152,000	pentadecanoic acid	trace		

G. uralensis constituents are given in **Table 6** in alphabetical order and in **Table 7** as a function of plant part. Constituents for which the concentrations were not given were not included in these tables.

Impurities

Liquiritin extracted from licorice root had purity approaching 99% as the recrystallized product (Cong and Lin 2007).

Yu et al. (2008) reported that glabridin obtained in China was > 99.0% pure.

Variation of Composition

A 10-fold difference in Glycyrrhizic Acid values due solely to different assay methods of the same samples have been reported. The number of constituents and their relative concentrations in plant-derived ingredients vary with the source and growth conditions (Leung and Foster 1996).

Table 6. Alphabetical list of substances found in *G. uralensis* for which the concentration was measured (ARS 2007).

Chemical	Plant part	Conc. (ppm)	Chemical	Plant part	Conc. (ppm)
4'-O-(beta-D-Apio-D-furanosyl-(1,2)-beta-D-glucopyranosyl)-liquiritigenin	root	120,000	Glycyrrhizin	root	11,200 - 84,000
Apioglycyrrhizin	root	100	Glycyrrhizin	rhizome	2,000 - 81,670
Araboglycyrrhizin	root	600	p-Hydroxy-benzoic-acid	sprout seedling	0.5
Arsenic	root	0.3	Iron	root	180 - 280
Astragalin	leaf	16	Isobavachalcone	sprout seedling	0.8
Calcium	root	6,850 - 23,500	Isoglycycoumarin	rhizome	180
Clycosin	sprout seedling	0.5	Isoglycyrol	root	200
Copper	root	13 - 14	Isoglycyrol	rhizome	270
4',7-Dihydroxyflavone	root	240	Isolicoflavonol	rhizome	10
EO	root	50	Isoliliquiritigenin	rhizome	60 - 20,000
Formononetin	sprout seedling	25	Isoliquiritigenin	root	100 - 1,050
Formononetin	shoot	3	Isoliquiritin	rhizome	800 - 23,280
Gancaonin-A	shoot	3	Isoliquiritin	root	120 - 4,000
Gancaonin-B	shoot	20	Isoliquiritin-apioside	root	20 - 1,650
Gancaonin-C	shoot	3	isoquercitrin	leaf	21
Gancaonin-D	shoot	0.3	Kanzonol-F	root	2
Gancaonin-E	shoot	8	Kanzonol-G	root	4
Gancaonin-L	shoot	7	Kanzonol-H	root	1
Gancaonin-M	shoot	6	Kanzonol-I	root	4
Gancaonin-N	shoot	1	Kanzonol-J	root	2
Gancaonin-O	shoot	5	Kanzonol-K	rhizome	0.04
Gancaonin-P	shoot	6	Kanzonol-L	rhizome	0.1
Gancaonin-P3'-methyl-ether	root	1	Kanzonol-M	root	0.6
Gancaonin-Q	shoot	1.5	Kanzonol-N	root	0.4
Gancaonin-R	shoot	14	Kanzonol-O	root	0.4
Gancaonin-S	shoot	3	Kanzonol-P	root	0.8
Gancaonin-T	shoot	1	Licocoumarone	rhizome	19 - 400
Gancaonin-U	shoot	12	Licocoumarone	root	900
Glyasperin-D	root	0.4	Liconeolignan	root	15
Glycycoumarin	rhizome	10 - 1,380	Licopyranocoumarin	root	500
Glycycoumarin	root	1,600 - 1,750	Licorice-saponin-A-3	rhizome	290
18-Alpha-Glycyrrhizin	root	200	Licorice-saponin-A-3	root	5.8 - 1,000
Glycyrin	root	400	Licorice-saponin-B-2	rhizome	40
Glycyrol	root	800	Licorice-saponin-B-2	root	0.8
Glycyrol	rhizome	440	Licorice-saponin-C-2	rhizome	50

Table 6 (continued). Alphabetical list of substances found in *G. uralensis* for which the concentration was measured (ARS 2007).

Chemical	Plant part	Conc. (ppm)	Chemical	Plant part	Conc. (ppm)
Licorice-saponin-C-2	root	1	Magnesium	root	3,690 - 5,070
Licorice-saponin-D-3	rhizome	70	Manganese	root	13 - 26
Licorice-saponin-D-3	root	1	1-Methoxy-Ficifolinol	root	3.4
Licorice-saponin-E-2	rhizome	120	Narcissin	leaf	16
Licorice-saponin-E-2	root	2.4 - 700	Neoisoliquiritin	root	200
Licorice-saponin-F-3	root	0.4	nicotiflorin	leaf	32
Licorice-saponin-G-2	root	0.6 - 1,000	Ononin	root	200 - 6,000
Licorice-saponin-H-2	root	1.4 - 2,100	Potassium	root	2,500 - 3,140
Licorice-saponin-J-2	root	0.4	8-C-Prenyl-eriodictyol	root	13
Licorice-saponin-K-2	root	0.4	Quercetin	leaf	7
Licorice-saponin-L-3	root	300	Quercetin-3,3'-dimethylether	leaf	48
Licoricidin	root	11	Rutin	leaf	53
Licorisoflavan-A	root	16	Saponins	root	60,000 - 140,000
Licraside	root	600	Scoponetin	shoot	2
Liquiritigenin	rhizome	30 - 1,210	Signoidin-B	shoot	7
Liquiritigenin	root	70,000	Sodium	root	323 - 1,340
Liquiritigenin-4',7-diglucoside	root	20	Uralene	leaf	26
Liquiritin	rhizome	7,900 - 36,490	Zinc	root	11 - 13
Liquiritin	root	120 - 300,000			
Liquiritin-apioside	root	120 - 9,000			
Lupiwighteone	shoot	6			

Origin Variation

G. glabra is native to the Mediterranean region and central and southwest Asia, and it is extensively cultivated in Russia, southern Europe, the Middle East, India, and in the United States (D'Amelio 1999; Kowalchik and Hylton 1998). Kimura et al. (1999) found that the aqueous extract of licorice root collected in autumn in China had a Ca+ content of 0.39% and a Mg2+ content of 0.69%.

G. glabra L. var. typica is native to Spain and Italy. G. glabra L. var. violacea is native to Turkey and Iran. G. glabra L. var. gladulifera is native to China, Russia and central Asia. G. uralensis is native to northeastern China and far east Russia. G. inflata is native to the Xinjiang province of China (Shibata 2000).

Nomura et al. (2002) reported that *G. glabra* has about 90 kinds of phenolic compounds isolated from the plants; ~ 50 are substituted with isoprenoid groups(s) (i.e., 3-methyl-2-butenyl (prenyl) group; 2,2-dimethylpyran ring). In Spanish and Russian *G. glabra*, the main isoprenoid-substituted flavonoid is the pyranoisoflavan, glabridin; and other flavonoids that are unsubstituted in the 5-position (i.e., glabrene, glabrol, 3-hydroxyglavrol).

In Chinese *G. glabra*, both 5-unsubstituted flavonoids and 5-oxygenated flavonoids (i.e., 3',8-diprenylated dalbergioidin) have been isolated. *G. uralensis* has had about 100 kinds of isolated phenols in which ~70 compounds are isoprenoid-substituted phenols. The main isoprenoid-substituted flavonoids are isoflavans with 2 prenyl groups, licoricidin and licorisoflavan A.

G. inflata has had about 40 flavonoids isolated including 20 isoprenoid-substituted flavonoids, the main one being licochalcone A (Nomura et al. 2002).

Hayashi et al. (2003a) reported that the major leaf flavonol glycoside of *G. glabra* collected in Turkey, Italy and Spain was isoquercitrin. However, it was was rutin in Kazakhstan. Glabridin is species specific for *G. glabra* L., glycycoumarin for *G. uralensis* Fish. and lichochalcone A for *G. inflata* Bat.

Hayashi et al. (2003b) reported in a review article that the glycyrrhizin content varied from 4.76% to 6.13% of dry weight in the roots and from 3.33% to 5.98% of dry weight in the stolons of *G. glabra* collected in Uzbekistan. The authors stated that these values are higher than that collected in Spain (0.7% to 4.4%) and Italy (0.07% to 0.27%). The glabridin content of *G. glabra* in Uzbekistan varied from 0.08% to 0.35% and was similar to the content of plants collected in Spain (0.21% to 0.80%) and Italy (0.07% to 0.27%).

Table 7. Substances found in *G. uralensis* for which the concentration was measured sorted by plant part (ARS 2007).

Plant Part/Chemical	Concentration (ppm)	Plant Part/Chemical	Concentration (ppm)	Plant Part/Chemical	Concentration (ppm)
Leaf		**Shoot - continued**		**Root - continued**	
Astragalin	16	Lupiwighteone	6	Licorice-saponin-A-3	5.8 - 1,000
isoquercitrin	21	Scoponetin	2	Licorice-saponin-B-2	0.8
Narcissin	16	Signoidin-B	7	Licopyranocoumarin	500
nicotiflorin	32	**Sprout Seedling**		Licorice-saponin-C-2	1
Quercetin	7	Clycosin	0.5	Licorice-saponin-D-3	1
Quercetin-3,3'-dimethylether	48	Formononetin	25	Licorice-saponin-E-2	2.4 - 700
Rutin	53	Isobavachalcone	0.8	Licorice-saponin-F-3	0.4
Uralene	26	p-Hydroxy-benzoic-acid	0.5	Licorice-saponin-G-2	0.6 - 1,000
Rhizome		**Root**		Licorice-saponin-H-2	1.4 - 2,100
Glycycoumarin	10 - 1,380	1-Methoxy-Ficifolinol	3.4	Licorice-saponin-J-2	0.4
Glycyrol	440	18-Alpha-Glycyrrhizin	200	Licorice-saponin-K-2	0.4
Glycyrrhizin	2,000 - 81,670	4'-O-(beta-D-Apio-D-furanosyl-(1,2)-beta-D-glucopyranosyl)-liquiritigenin	120,000	Licorice-saponin-L-3	300
Isoglycycoumarin	180	4',7-Dihydroxyflavone	240	Licoricidin	11
Isoglycyrol	270	8-C-Prenyl-eriodictyol	13	Licorisoflavan-A	16
Isolicoflavonol	10	Apioglycyrrhizin	100	Licraside	600
Isoliliquiritigenin	60 - 20,000	Araboglycyrrhizin	600	Liquiritigenin	70,000
Isoliquiritin	800 - 23,280	Arsenic	0.3	Liquiritigenin-4',7-diglucoside	20
Kanzonol-K	0.04	Calcium	6,850 - 23,500	Liquiritin	120 - 300,000
Kanzonol-L	0.1	Copper	13 - 14	Liquiritin-apioside	120 - 9,000
Licocoumarone	19 - 400	EO	50	Magnesium	3,690 - 5,070
Licorice-saponin-A-3	290	Gancaonin-P3'-methyl-ether	1	Manganese	13 - 26
Licorice-saponin-B-2	40	Glyasperin-D	0.4	Neoisoliquiritin	200
Licorice-saponin-C-2	50	Glycycoumarin	1,600 - 1,750	Ononin	200 - 6,000
Licorice-saponin-D-3	70	Glycyrin	400	Potassium	2,500 - 3,140
Licorice-saponin-E-2	120	Glycyrol	800	Saponins	60,000 - 140,000
Liquiritigenin	30 - 1,210	Glycyrrhizin	11,200 - 84,000	Sodium	323 - 1,340
Liquiritin	7,900 - 36,490	Iron	180 - 280	Zinc	11 - 13
Shoot		Isoglycyrol	200		
Formononetin	3	Isoliquiritigenin	100 - 1,050		
Gancaonin-A	3	Isoliquiritin	120 - 4,000		
Gancaonin-B	20	Isoliquiritin-apioside	20 - 1,650		
Gancaonin-C	3	Kanzonol-F	2		
Gancaonin-D	0.3	Kanzonol-G	4		
Gancaonin-E	8	Kanzonol-H	1		
Gancaonin-L	7	Kanzonol-I	4		
Gancaonin-M	6	Kanzonol-J	2		
Gancaonin-N	1	Kanzonol-M	0.6		
Gancaonin-O	5	Kanzonol-N	0.4		
Gancaonin-P	6	Kanzonol-O	0.4		
Gancaonin-Q	1.5	Kanzonol-P	0.8		
Gancaonin-R	14	Licocoumarone	900		
Gancaonin-S	3	Liconeolignan	15		

Statti et al. (2004) evaluated 9 specimens of *G. glabra* collected in different parts of Calabria, Italy. After preparing methanol extracts from each specimen, it was found that the yields of extract ranged from 9.79% to 18.81%. The Glycyrrhizic Acid content ranged from 0.425 ± 0.001% to 2.080 ± 0.003% and the isoliquiritigenin content ranged from 0.138 ± 0.003% to 0.316 ± 0.005%.

Rauchensteiner et al. (2005) reported that Chinese commercial licorice preparations were, on average, higher in Glycyrrhizic Acid than European commercial licorice and licorice extraction performed in the laboratory.

Nagai et al. (2006) reported that the amount of glycycoumarin (~0.10%) was similar in 4- and 5-year-old cultivated G. uralensis roots and wild plants of the same species from Inner Mongolia.

Species Variation

Kondo et al. (2007) compared the constituents of licorice root extracts (80% methanol) from *G. glabra* (n = 17), *G. uralensis*, (n = 87) and *G. inflata* (n = 8) as well as hybrids between *G. uralensis* and *G. glabra* or *G. inflata* (n = 5). There were no differences among the 3 species for the contents of glycyrrhizin, liquiritin apioside, and isoliquiritin apioside. The content of the 6 main constituents of *G. glabra* and *G. inflata* (glycyrrhizin, liquiritin apioside, isoliquiritin apioside, liquiritin, isoliquiritin, and apioside) were similiar. In *G. glabra*, 15 samples had glabridin, 1 had no species-specific constituents. In *G. inflata*, 7 had licochalcone A and 1 did not, and in *G. uralensis*, 83 samples had glycycoumarin and 4 had no species-specific constituent.

Table 8 presents the concentrations of each constituent. In the hybrids between *G. uralensis* and *G. glabra*, 40% had glycyroumarin and 60% had licochalcone A.

Structures of constituents of licorice are included in the Part II.

Processing/manufacturing Variation of Composition

Sovak et al. (2002) tested an herbal mixture, PC-SPES (used for the management of hormone-responsive and hormone-refractory prostate cancer), for consistency of composition.

PC-SPES has *G. glabra* listed as one of its ingredients, which contains licochalcone A, an antitumor phytoestrogen with Bcl-2 protein-modulating properties. The authors examined 8 lots of this substance produced from 1996 to 2001 using HPLC, 1H and 13C{1H} NMR and GC/MS. The average licochalcone A content varied with a low of 3.8 µg/g (2.8 to 4.8 µg/g) in 1999 and the high of 289.2 µg/g (276.6 to 301.4 µg/g) in 2001 (Sovak et al. 2002) .

Majima et al. (2004) found that the content of glycyrrhizin, isoliquiritin, and isoliquiritin apioside were similar in extracts of *G. uralensis* whether roasted or unroasted.

Sung and Li (2004) used capillary electrophoresis to explore the changing composition of dried *G. glabra* root as it is roasted, as it is sometimes processed in herbal medicine. The dried root powder was roasted at 150°C for 30 and 60 min; 200°C for 15 and 30 min; and 250°C for 30 min. The root powder (1 g) was also mixed with 1 ml honey and roasted for 30 min at 200°C and 250°C. In raw licorice powder, glycyrrhizin is clear on the electropherograms while isoliquirtigenin is not very clear except with "spiking", and 18-β glycyrrhetinic acid was not evident.

When dry roasted at 200°C for 30 min, there is an 18-β glycyrrhetinic acid peak; it is more prominent when honey roasted. This is accompanied by a decrease in glycyrrhizin. Increasing the temperature to 250°C resulted in a further increase in 18-β glycyrrhetinic acid and a still higher amount when honey roasted. The amount of glycyrrhizin again decreases; at 250°C, none is detected (Sung and Li 2004).

In a data sheet, lichocalcone A was used as the assay marker for the presence of Glycyrrhiza Inflata Root Extract (CTFA 2004a). The specific minimum was 15%; it was noted that the specific minimum was typically ~25%. In another safety data sheet, licorice extract containing 5% by weight Glycyrrhiza Inflata Root Extract, had a specific minimum of 0.6%; it is typically ~1.1% (CTFA 2004b). A compound containing 1% Glycyrrhiza Inflata Root Extract was 0.012% lichochalcone A (CTFA 2004c); and another compound containing 0.05% Glycyrrhiza Inflata Extract contained 0.019% lichochalcone A (CTFA 2004d).

Table 8. The concentration (%) of the main constituents of *G. uralensis*, *G. glabra*, *G. inflata* (Kondo et al. 2007).

Constituent	*G. uralensis*	*G. glabra*	*G. inflata*
Glycyrrhizin	3.37 ± 1.57	3.54 ± 1.57	3.27 ± 1.53
Liquiritin apioside	1.27 ± 0.73	1.60 ± 0.67	1.47 ± 0.65
Isoliquiritinin apioside	0.40 ± 0.26	0.53 ± 0.24	0.51 ± 0.25
Liquiritigenin	0.11 ± 0.12	0.02 ± 0.02[a]	0.02 ± 0.03
Liquiritin	1.68 ± 1.06	0.17 ± 0.15[a]	0.27 ± 0.12[a]
Isoliquiritin	0.32 ± 0.22	0.02 ± 0.02[a]	0.04 ± 0.02[a]
Glycycoumarin	0.09 ± 0.05	nd[b]	nd
Glabridin	nd	0.14 ± 0.12	nd
Licochalcone A	nd	nd	0.74 ± 0.53*

[a] $p < .001$ vs *G.uralensis* [b] nd = none detected

Seasonal Variation

Hayashi et al. (1998) collected *G. glabra* seed in Turkey, raised them in pots in Japan starting in April, and analyzed the roots for glycyrrhizin and isoliquiritigenin glycoside content when the plants were young (first year) and mature (third year). In the first year, the glycyrrhizin content in the main roots increased from < 0.1% of dry weight in August to ~0.5% in November and ~0.4% in December. Isoliquiritigenin glycoside content was ~0.1% of dry weight in August and increased to ~0.3% for October through December. In year 3, the glycyrrhizin content increased from ~0.5% of dry weight of the main roots in January to >1.5% in September through December. Isoliquiritigenin glycoside content increased from <0.25% in January to ~0.5% in August through December. Glycyrrhizin content increased during senescence of the aerial parts as well as during the early stage of shoot elongation. Isoliquiritigenin glycosides increased from June to July in the mature plants where as the glycyrrhizin did not. The authors stated that this indicates that the biosynthesis of these 2 compounds are differently regulated in the plant.

Growth Condition Variation

Afreen et al. (2006) reported that the melantonin content in *G. uralensis* is higher in the root (~34.0 µg/g) than the seed (not detectable), leaf (~0.28 µg/g) or stem (not detectable) tissues. The melantonin content increased with increased exposure to red light to ~ 85 µg/g. The concentration of melantonin was highest in the root when the plant was exposed toUV-B radiation for 3 d followed by low intensity UV-B.

Marker Chemicals

Access Business Group (2007) analyzed 9 samples of licorice root extract, used as cosmetic ingredients, from different commercial sources, for chemicals that are characteristic of a species or type of extract. Glabridin (0.02 mg/ml detection limit) was detected in 4 oil extracted samples at levels that ranged from 31.9% to 51.9%. Glycyrrhizin (0.1 mg/ml detection limit) was detected in only 1 sample at 5.4%. Lichochalcone (0.04 mg/ml detection limit) was detected in 2 water extracted samples at 16.0% and 19.6%. None of the marker chemicals were detected in 2 of the samples. The authors note that the chromatographs for glabridin and lichochalcone were clean therefore free of excipients and other phytochemicals of related molecular structure. The chromatograms for Glycyrrhizic Acid showed a variety of peaks demonstrating various excipients and other phytochemicals of related molecular structure.

Impurities

Uematsu et al. (2002) reported that methanol in commercial licorice extract (to be used as a food additive) exceeded 50 µg/g in 6 of 9 samples collected in 1999 from a Japanese market using large volume head-space GC. The highest concentration was 12,000 µg/g. Methanol exceeding 50 µg/g was found in 2 of 9 samples collected in 2000; the highest concentration was 270 µg/g. In these same samples, ethanol exceeding 50 µg/g was found in all 9 samples from 1999; the highest concentration was 27,000 µg/g. Seven of the 9 samples from 2000 exceeded 50 µg/g; the highest concentration was 17,000 µg/g. One sample of licorice powder from 1999 and 2000 was found to have ≤50 µg/g of both methanol and ethanol.

Carbonell-Barrachina et al. (2003), in a study of 22 licorice-containing hard and soft candy and throat pearls, found that the higher the Glycyrrhizic Acid concentration in the candy the higher the arsenic content (R2 = 0.9357). The amount of arsenic ranged from trace levels (0.01 to 0.02 µg/g) in some soft candies that did not contain licorice (according to the labels) to 1.13 ± 0.06 µg/g in licorice throat pearls with menthol flavor. The mean level of arsenic was 0.20 µg/g and the median was 0.05 µg/g. The maximum allowable level of arsenic in Spain is 0.1 µg/g; 5 of the candies had levels in the range of 0.1 to 1.0 µg/l and 2 candies had concentrations of > 1.0 µg/l. The Glycyrrhizic Acid levels ranged from < 0.1 to 65.5 ± 1.3 mg/g.

In an analysis of Glycyrrhiza Glabra (Licorice) Root Extract, containing 35% to 40% glabridin, it was found to be positive for flavones, have a specific value of <100 cfu/g and an analytical value of <10 cfu/g for total bacteria, a specific value of <20 ppm and an analytical value of <4.0 ppm for lead, and a specific value of <2 and an analytical value of <1.0 for arsenic (Shangai OLI Enterprises Co., LTD. 2006).

Ariño et al. (2007) tested several licorice products, purchased from public sources, for ochratoxin A, a secondary metabolite of several species of fungus. It is a mycotoxin with carcinogenic, nephrotoxic, teratogenic, and immunotoxic properties. Dry licorice root (n = 15) was found to have a mean of 63.6 ± 20.8 ng/g (range 1.4 to 252.8 ng/g), fresh licorice root (n = 8) 9.2 ± 1.3 ng/g (3.3 to 14.7 ng/g), licorice sweets (n = 4) 3.8 ± 1.9 ng/g (0.5 to 8.2 ng/g), licorice extract (liquid) (n = 2) 16.0 ± 1.4 ng/g (14.6 to 17.3), and licorice block (solid) (n = 1) 39.5 ng/g.

The authors reported that ochratoxin A content in *G. glabra* root extract was stable to heat treatment at 150°C for 60 min. The ochratoxin A concentration was unaffected by sorting or washing but was reduced by peeling (53.2%) compared to dry root powder. Levels were reduced by the production of licorice extract (78.6%) and block licorice (91.8%).

USE

Cosmetics

Current uses of cosmetic ingredients as a function of product category are provided by industry to the U.S. Food and Drug Administration (FDA) under the Voluntary Cosmetic Ingredient Registration Program (VCRP). An industry survey conducted by the Personal Care Products Council (Council) provided data on current use concentrations. These data for the licorice extracts currently in use are presented in **Table 9**.

Glycyrrhiza Glabra (Licorice) Rhizome/Root, as given in the *International Cosmetic Ingredient Dictionary and Handbook*, functions as a flavoring agent and fragrance ingredient in cosmetic products (Gottschalck and Bailey 2008).

According to information supplied to the FDA by industry as part of the VCRP, Glycyrrhiza Glabra (Licorice) Rhizome/Root (listed as Glycyrriza Glabra (Licorice) Root Extract and Glycyrrhiza Glabra (Licorice) Extract) is used in 141 cosmetic products (FDA

2007). In a survey of use concentrations in the industry, the Personal Care Products Council (Council 2008) reported that Glycyrrhiza Glabra (Licorice) Rhizome/Root is used at concentrations ranging from 0.0001% to 0.4% in cosmetic products.

Glycyrrhiza Glabra (Licorice) Leaf Extract functions in cosmetics as a skin-conditioning agent - miscellaneous (Gottschalck and Bailey 2008). FDA (2007) reports that this ingredient is used in 5 products. There are no reported concentrations of this ingredient by the Council (2008).

Glycyrrhiza Glabra (Licorice) Root functions in cosmetics as a skin-conditioning agent - miscellaneous (Gottschalck and Bailey 2008). There are no reported uses of this ingredient in the VCRP (FDA 2007) and concentrations of use data were not reported to the Council (2008).

Glycyrrhiza Glabra (Licorice) Root Extract functions in cosmetics as an antioxidant, skin conditioning agent-miscellaneous, and skin conditioning agent-humectant (Gottschalck and Bailey 2008). There are no reported uses of this ingredient in the VCRP (FDA 2007) and concentrations of use data were not reported to the Council (2008).

Glycyrrhiza Glabra (Licorice) Root Juice functions in cosmetics as a skin-conditioning agent - miscellaneous (Gottschalck and Bailey 2008). There are no reported uses of this ingredient in the VCRP (FDA 2007) and concentration of use data were not reported to the Council (2008).

Glycyrrhiza Glabra (Licorice) Root Powder functions in cosmetics as a skin-conditioning agent - miscellaneous (Gottschalck and Bailey 2008). There are no reported uses of Glycyrrhiza Glabra (Licorice) Root Powder in the VCRP (FDA 2007) and concentration of use data were not reported to the Council (2008).

Glycyrrhiza Glabra (Licorice) Root Water functions in cosmetics as an antioxidant, cosmetic astringent, and a skin-conditioning agent - emollient (Gottschalck and Bailey 2008). There are no reported uses of this ingredient in the VCRP (FDA 2007) and concentration of use data were not reported to the Council (2008).

The Council (2008) reported use concentrations as a function of product type for a polyol-soluble Glycyrrhiza Glabra (Licorice) Root Extract, which contains a higher concentration of glabridin, and is used at concentrations of 0.0001% to 0.06%. These data are shown in **Table 10.**

Glycyrrhiza Inflata Root Extract functions in cosmetics as a skin-conditioning agent - miscellaneous (Gottschalck and Bailey 2008). There are no reported uses (FDA 2007) or concentrations of use (Council 2008) for this ingredient.

The Council (2008) reported the use of a polyol-soluble extract of Glycyrrhiza Inflata Root Extract, which contains high concentrations of licochalcone A) in the range of 0.0001% to 0.05%, as shown in **Table 11.**

Glycyrrhiza Uralensis (Licorice) Root Extract functions in cosmetics as a skin-conditioning agent-miscellaneous (Gottschalck and Bailey 2008). There are no reported uses of this ingredient in the VCRP (FDA 2007) and concentration of use data were not reported to the Personal Care Council (2008).

Table 9. Frequency of use and concentration of Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) and Glycyrrhiza Glabra (Licorice) Leaf Extract.

Product Category (Total number of products in each category (FDA 2008))	Frequency of use (FDA 2007)	Concentration of use (%) (Council 2008)
Glycyrrhiza Glabra (Licorice) Rhizome/Root[a]		
Bath products		
Soaps and detergents (594)	-	0.002
Eye makeup		
Eye lotions (177)	3	0.001-0.05
Mascara (463)	-	0.1
Other (288)	4	-
Fragrance products		
Colognes and toilet waters (1288)	-	0.06
Other (399)	4	-
Noncoloring hair care products		
Conditioners (1249)	2	-
Sprays/aerosol fixatives (371)	1	-
Straighteners (144)	2	0.004
Permanent waves (141)	-	0.0001
Shampoos (1403)	3	-
Tonics, dressings, etc. (1097)	3	0.0005
Other (716)	3	-

Table 9 (continued). Frequency of use and concentration of Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) and Glycyrrhiza Glabra (Licorice) Leaf Extract.

Product Category (Total number of products in each category (FDA 2008))	Frequency of use (FDA 2007)	Concentration of use (%) (Council 2008)
Glycyrrhiza Glabra (Licorice) Rhizome/Root[a] *(continued)*		
Hair coloring products		
Dyes and colors (2481)	-	0.01
Shampoos (48)	1	-
Makeup		
Face powders (613)	-	0.002
Foundations (635)	4	0.001-0.01
Lipsticks (1912)	2	-
Other (406)	-	0.01[B]
Nail care products		
Other (124)	2	-
Oral hygiene products		
Mouthwashes and breath fresheners (85)	-	0.01-0.07
Other (48)	2	-
Personal hygiene products		
Other (514)	2	-
Shaving products		
Aftershave lotions (395)	4	-
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (1368)	9	0.002-0.04
Face and neck creams, lotions, powder and sprays (1195)	29	0.05-0.4[C]
Body and hand creams, lotions, powder and sprays (1513)	9	0.0005-0.2
Foot powders and sprays (48)	1	-
Moisturizers (2039)	20	0.008-0.4
Night creams, lotions, powder and sprays (343)	3	0.05-0.1
Paste masks/mud packs (418)	6	0.001
Skin fresheners (285)	3	0.02
Other (1244)	16	0.003-0.008
Suntan products		
Suntan gels, creams, liquids and sprays (156)	3	-
Total uses/ranges for Glycyrrhiza Glabra (Licorice) Root Extract	**141**	**0.0001-0.4**
Glycyrrhiza Glabra (Licorice) Leaf Extract		
Makeup		
Lipsticks (1912)	1	-
Shaving products		
Aftershave lotions (395)	1	-
Skin care products		
Moisturizers (2039)	2	-
Night creams, lotions, powder and sprays (343)	1	-
Total **uses/ranges for Glycyrrhiza Glabra (Licorice) Leaf Extract**	5	-

Table 9 (continued). Frequency of use and concentration of Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) and Glycyrrhiza Glabra (Licorice) Leaf Extract.

Product Category (Total number of products in each category (FDA 2008))	Frequency of use (FDA 2007)	Concentration of use (%) (Council 2008)
Glycyrrhiza Glabra (Licorice)[D]		
Oral hygene products		
Dentifrices (59)	1	-
Skin care products		
Skin fresheners (285)	1	-
Other (1244)	1	-
Total **uses/ranges for Glycyrrhiza Glabra (Licorice) Extract**	3	-

[a] Listed as Glycyrrhiza Glabra (Licorice) Root Extract and Glycyrrhiza Glabra (Licorice) Extract by FDA (2007).
[b] 0.1% in a spray concealer.
[c] 0.01% in face and neck sprays.
[d] Listed in the FDA database as Glycyrrhiza Glabra (Licorice). There was no matching name in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and Bailey 2008).

Table 10. Concentration of use of a polyol-soluble Glycyrrhiza Glabra (Licorice) Root Extract containing higher concentrations of glabridin.

Product Category	Concentration of use (%) (Council 2008)	Concentration of glabridin in extract (%) (Council 2008)
Bath products		
Soaps and detergents	0.0001	40
Eye makeup		
Eye lotions	0.0003-0.004	36-42
Makeup		
Foundations	0.0005-0.004	17-27, 36-42
Makeup bases	0.001	40
Other	0.0003	40
Skin care products		
Skin cleansing creams, lotions, liquids, and pads	0.0003-0.001	20, 35
Face and neck creams, lotions, powder and sprays	0.001-0.05	24, 35-42
Body and hand creams, lotions, powder and sprays	0.001-0.05	35-40
Moisturizers	0.001-0.05	35-40
Night creams, lotions, powder and sprays	0.001-0.004	36-42
Paste masks/mud packs	0.001	40
Other	0.001-0.02[a]	17-27, 40
Suntan products		
Suntan gels, creams, liquids and sprays	0.06	35
Concentration ranges for Polyol-Soluble Glycyrrhiza Glabra (Licorice) Root Extract and concentration of glabradin in the extract	**0.0001-0.06**	**17-42**

[a]0.02% (17%-27% glabridin) in a skin brightener.

Cosmetic Aerosol Use

Glycyrrhiza Glabra (Licorice) Root/Rhyzome is used in a spray/aerosol fixative.

Jensen and O'Brien (1993) reviewed the potential adverse effects of inhaled aerosols, which depend on the specific chemical species, the concentration, the duration of the exposure, and the site of deposition within the respiratory system.

The aerosol properties associated with the location of deposition in the respiratory system are particle size and density. The parameter most closely associated with this regional deposition is the aerodynamic diameter, da, defined as the diameter of a sphere of unit density possessing the same terminal setting velocity as the particle in question. These authors reported a mean aerodynamic diameter of 4.25 ± 1.5 μm for respirable particles that could result in lung exposure (Jensen and O'Brien 1993).

Table 11. Concentration of use of a polyol-soluble Glycyrrhiza Inflata Root Extract containing higher concentrations of licochalcone A.

Product Category	Concentration of use (%) (Council 2008)	Concentration of licochalcone A in extract (%) (Council 2008)
Noncoloring hair care products		
Conditioners	0.0001	15
Shampoos	0.0001	15
Other	0.0001[a]	15
Skin care products		
Skin cleansing creams, lotions, liquids, and pads	0.03	0.14
Face and neck creams, lotions, powder and sprays	0.05	0.008
Total uses/ranges for Glycyrrhiza Inflata (Licorice) Root Extract	**0.0001-0.05**	**0.008-15**

[a]0.0001% in a hair/scalp treatment, leave-in conditioner and hair gel.

Bower (1999), reported diameters of anhydrous hair spray particles of 60 - 80 μm and pump hair sprays with particle diameters of ≥80 μm. Johnsen (2004) reported that the mean particle diameter is around 38 μm in a typical aerosol spray. In practice, he stated that aerosols should have at least 99% of particle diameters in the 10 - 110 μm range.

The Ministry of Health, Labor and Welfare (MHLW 2007) of Japan listed Licorice Extract and Glycyrrhetinic Acid as acceptable medicinal ingredients to be used in cosmetics.

Non-cosmetic Use

Medicinal Uses

Licorice Extract was used as a treatment for gastric ulcers during World War II (Molhuysen et al. 1950). According to Gibson (1978), licorice has been used as a medicine as early as 2100 B.C. Throughout history, there are recorded uses of Glycyrrhiza herbal remedies for stomach and mouth ulcers, thirst, hepatitis, scabies, renal dysfunction, wounds, cough, inflammation, and constipation, among others.

Oil soluble Licorice (Glycyrrhiza) Extract (0.05%) is on the list of active ingredients that help lighten the skin that are exempted (KFDA no date b) from the required safety testing of cosmeceuticals in Korea (KFDA 2007a,b).

Licorice also has been studied for its anticaries effect (Edgar 1978), antimicrobial effect (Fukai et al. 1988), xanthine oxidase effect, monoamine oxidase inhibitor effect (Hatano et al. 1989), antimutgenic activity (Zani et al. 1993), antitumor promoting activity (Kelloff et al. 1994; Wang and Nixon 2001), anti-angiogenic effects (Kobayashi et al. 1995), antioxidant effects (Konovalova et al. 2000), and anti-inflammatory activities (Shibata 2000).G. glabra makes up 5.9% of a popular anti-asthmatic Chinese herbal medicine consisting of 10 total herbs (Homma et al. 1994).

The German Federal Institute for Drugs and Medical Devices (1998) stated that G. glabra is used as a medicinal plant. The World Health Organization (1999) indicated that both G. glabra and G. uralensis are used as medicinal plants.

Kumar et al. (2002) reported that G. glabra is used as a complementary/integrative nutritional therapy by cancer patients. Isoliquiritigenin from G. uralensis has strong inhibitory effects on tyrosinase activity (Nerya et al. 2003), which is known to be a key enzyme in melanin biosynthesis. Licorice is reported to be used as a depigmentation agent (Halder and Richards 2004).

Non-medicinal Uses

The FDA (1983a) determined that licorice, as G. glabra and other species of Glycyrrhiza, is generally recognized as safe (GRAS) as a spice and other natural seasoning and flavoring. Additionally, the FDA (1983b) included G. glabra and other species of Glycyrrhiza in a list of essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are GRAS. Licorice is GRAS as an ingredient in animal feed.

While licorice and licorice derivatives are GRAS, the FDA (1983a) has established the following maximum restrictions on the levels of Glycyrrhizic Acid in certain types of foods: 0.05% in baked goods; 0.1% in alcoholic beverages; 0.15% in nonalcoholic beverages; 1.1% in chewing gum; 16.0% in hard candy; 0.15% in herbs and seasonings and in plant protein products; 3.1% in soft candy; 0.5% in vitamins and dietary supplements; and 0.1% in all other food except sugar substitutes. Glycyrrhizic Acid may not be used as a non-nutritive sweetener in sugar substitutes.

Olukoga and Donaldson (2000) summarized some medical and non-medical products containing licorice in **Table 12.**

Licorice extracts are used to flavor tobacco, chewing gums and candies (Nerya et al. 2003). Licorice extracts also are used as sweetening agents (Nerya et al. 2003; Choi 2005).

Licorice Root Extract has been used in beers and ales to provide surfactant properties and to take the edge off these bitter-tasting beverages as well as to alleviate bitter after tastes in some saccharine-containing products and pharmaceutical preparations (Isbrucker and Burdock 2006).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Glycyrrhiza Uralensis (Licorice) Root Extract

Yamamoto et al. (2003) fasted rats (strain not specified; n = 5) overnight then orally administered Glycyrrhiza Uralensis

Table 12. Preparations/products containing licorice (Olukoga and Donaldson 2000).

Health products
Herbal cough mixtures
Licorice flavored cough mixtures
Laxatives (including Cascara, Compound licorice powder)
Licorice tea
Throat pearls
Liquirizia naturale
Antibron tablets
Licorice flavored diet gum
Confectionery
Licorice sticks, toffee, bars, cakes, bricks, tubes, balls, Catherine wheels, pastilles, pipes, allsorts
Pomfret (Pontefract) cakes
Blackcurrant
Torpedos
Sorbits chewing gum
Stimorol chewing gum
Servez vous
Alcoholic drinks
Belgian beers
Pastis brands
Anisettes - raki, ouzo, Pernod
Chewing Tobacco

(Licorice) Root Extract, either from a cultivated source (756 mg/10 ml/kg) or a wild source from China (452 mg/10 ml/kg).

Each preparation was determined to have 45 mg/kg Glycyrrhetic Acid.

Blood samples were collected from the tail vein at 0, 1, 2, 4, 6, 9, and 12 h then analyzed for Glycyrrhetic Acid by HPLC. The detected amounts of Glycyrrhetic Acid in the blood samples were similar between the 2 sources.

The peak concentration was at 9 h (1.90 ± 0.40 µg/ml for the cultivated source and 1.51 ± 0.91 µg/ml for thewild source). There was no Glycyrrhetic Acid detected at 24 h (Yamamoto et al. 2003).

Majima et al. (2004) orally administered G. uralensis extract (roasted and unroasted; containing 45 mg/kg glycyrrhizin) to male Wistar rats after overnight fasting.

Blood samples (0.3 ml) were collected from the tail vein periodically for 24 h. The plasma Glycyrrhetic Acid levels and the area under the mean concentration vs time curve for 24 h (AUC0-24 h) was determined. Glycyrrhetic Acid (but not glycyrrhizin) was detected in plasma and peaked at 9 h after oral administration of both extracts. There was no difference in Glycyrrhetic Acid levels in the plasma between the roasted and unroasted licorice extracts. The AUCs0-24 h were similar for the roasted and unroasted licorice (14.2 ± 9.0 µgh/ml and 12.5 ± 4.9 µgh/ml, respectively) as were the maximum concentrations of 1.48 ± 0.86 µg/ml, 1.47 ± 0.63 µg/ml, respectively(Majima et al. 2004).

Systemic Hormone Effects

Licorice Extract

Al-Qarawi et al. (2002) tested the effect of a water extract of G. glabra on the pituitary-adrenal-renal axis of adult male Wistar rats (120 to 150 g). The rats were allowed food and water ad libitum and divided into 4 groups (n = 7). The licorice extract was administered by oral gavage in distilled water at 0, 100, 250, or 500 mg/kg for 15 consecutive days. The rats were weighed at the beginning and end of treatment. The rats were killed 24 h after the last dose and blood collected, centrifuged, and analyzed. Plasma concentrations of cortisol, adrenocorticotrophic hormone (ACTH), renin, and aldosterone were measured by radioimmunoassay and sodium and potassium concentrations were measured by flame photometer. As shown in **Table 13**, there was a dose-dependent decrease in the concentration of cortisol, ACTH, aldosterone and postassium; there was a dose-dependent increase in the concentration of renin and sodium.

Table 13. The effect of the water extract of *G. glabra* on the concentration of ACTH, cortisol, aldosterone, renin, sodium, and potassium in rat plasma (Al-Qarawi et al. 2002).

Treatment	ACTH (pg/ml)	Cortisol (pg/ml)	Aldesterone (pg/ml)	Renin (ng/ml/h)	Sodium (mEq/l)	Potassium (mEq/l)
Control	297.3 ± 12.3[a]	1.4 ± 0.001[a]	470 ± 19.8[a]	6.0 ± 1.1[a]	131.4 ± 2.4[a]	4.36 ± 0.19[a]
100 mg/kg	215.8 ± 15.7[b]	1.3 ± 0.001[a]	425.9 ± 20.1[b]	6.2 ± 0.09[a]	149.5 ± 4.2[b]	3.52 ± 0.01[b]
250 mg/kg	194.3 ± 13.6[c]	0.5 ± 0.001[b]	415.3 ± 20.2[b]	6.7 ± 0.10[b]	160.1 ± 3.0[c]	2.90 ± 0.26[c]
500 mg/kg	98.1 ± 8.5[d]	0.2 ± 0.00[d]	380.1 ± 17.9[c]	7.3 ± 0.08[c]	171.2 ± 1.5[d]	2.24 ± 0.26[d]

[a] For each variable values with different superscripts are significantly different from each other; [b] $p < .05$; [c] $p < .001$; [d] $p < .005$ compared to control

Hepatic Effects

Licorice Extract

Mirsalis et al. (1993) administered feed containing 0%, 0.8%, 2.5%, 8.0%, or 25% licorice root extract to B6C3F1 mice for 30 or 90 d. At the end of the respective dosing periods, the mice were killed, and liver enzyme activities were evaluated. Licorice root extract at the 8 and 25% levels induced 7-ethoxycoumarin O-deethylase activity after both 30 and 90 d. Licorice Root Extract dose-dependently induced uridine diphosphate (UDP)-glucuronyl transferase activity and inhibited glutathione-S-transferase (GST) activity, but the degree of these effects were not statistically significant. Benzo[a]pyrene (B[a]P) hydroxylase and superoxide dismutase (SOD) were not affected by treatment. Other results of this study are described in the Animal Toxicology section of this report.

Paolini et al. (1998) studied the effects of licorice extract and glycyrrhizate on the cytochrome P450 (CYP) monooxygenase activities in Swiss albino CD1 mice. The mice were given daily oral doses of 3138 or 6276 mg/kg licorice extract or 240 or 480 mg/kg Glycyrrhizic Acid for 1, 4, or 10 d. Controls received vehicle (saline) only. Animals were fasted for 16 h after the last dose and then killed. The livers were rapidly removed and prepared for enzymatic assays, electrophoresis, Western immunoblot, RNA isolation, and Northern hybridization.

The single dose of licorice extract or Glycyrrhizic Acid did not induce any enzymes. However, hepatic CYP3A-, 2B1-, and 1A2-dependent microsomal monooxygenase were induced by multiple doses of licorice extract and/or Glycyrrhizic Acid. The following testosterone hydroxylase (TH) enzymes in the liver were also induced by multiple doses of the 2 test materials: 6β-TH, 2α-TH, 6α-TH, 7α-TH, and 16β-TH. The authors suggested that these results indicate that the induction of CYP-dependent activities by the prolonged intake of licorice extract or Glycyrrhizic Acid at high doses may cause accelerated metabolism of co-administered drugs, and that the adverse effects associated with CYP changes may also have clinical consequences (Paolini et al. 1998).

Paolini et al. (1999) tested the effects of aqueous *G. glabra* root extract on rat livers. Male and female Sprague-Dawley rats (150 to 170 g) were fed rodent chow and allowed water ad libitum. The rats were administered a single dose of licorice extract in saline (3138 or 6276 mg/kg) orally or the same dose for 4 consecutive days (n = 6). The rats were killed 24 h after the last treatment after 16 h of fasting. The livers were quickly removed and S9 prepared. The post-mitochondrial supernatant was centrifuged, resuspended, washed, and centrifuged again. The pellet was homogenized and fractions frozen in liquid nitrogen. Enzymatic analyses were performed within a week.

Using male rats, the lower dose administered over 4 d induced CYP content ($p < .01$) and the *N*-demethylation of aminopyrine (~2-fold increase). The CYP1A1-associated *O*-deethylation of ethoxyresorufin was induced by licorice extract (up to 2-fold, higher dose). A 3.7-fold increase in the *O*-dealkylation of pentoxyresorufin (supported by CYP2B1) was achieved at the higher dose. The authors stated that male rats were highly responsive to licorice extract as shown by the types and activity of the various hydroxylases as shown in **Table 14.**

Results using female rats were similar in the susceptibility to CYP3A1/2 induction; aminopyrine *N*-demethylase activity was enhanced at the lower dose ($p < .01$). A marked induction in the methoxyresorufin *O*-demethylase activity activity (CYP1A2) was obtained by licorice extract (2.3- and 2.7-fold increase for the lower and higher dose, respectively). An increase in the "mixed" ethoxycoumarin *N*-deethylase activity ($p < .01$) was recorded.

Results using female rats were similar in the susceptibility to CYP3A1/2 induction; aminopyrine N-demethylase activity was enhanced at the lower dose ($p < .01$). A marked induction in the methoxyresorufin O-demethylase activity activity (CYP1A2) was obtained by licorice extract (2.3- and 2.7-fold increase for the lower and higher dose, respectively). An increase in the ethoxycoumarin N-deethylase activity ($p < .01$) was recorded only in the lower dose.

There was no difference between control and single dose rats with regards to the CYP-linked mono-oxygenases in either absolute or relative liver weight (Paolini et al 1999).

Tsukamoto et al. (2005) reported that the methyl alcohol extract of *G. uralensis* had CYP3A4 inhibitory activity with an IC_{50} of 0.022 mg/ml.

Glycyrrhizia Uralensis (Licorice) Root Extract

Leung et al. (2003) tested the effects of aqueous licorice root extract (G. uralensis Fisch) on the gene expression in rat liver cells. Rat liver cells (Clone 9) were exposed to aqueous licorice extract (3 mg/ml) for 24 h then 50 µg total RNA was isolated using Trizol reagent. A control was treated with medium only. The mRNA of both samples was purified and used to synthesize [α-^{32}P]dATP-labeled cDNA. The hybridized signals printed on the X-ray film were measured by densitometer. The threshold value was based on background signal; the levels of gene up-regulation (cut-off at 2-fold) and down-regulation (cut-off at > 50%) were calculated by the ratio of signal of that gene in the control and the licorice extract treated gene.

In the control, 84 genes were detected and 89 were detected in the treatment group. Five genes were differentially expressed. Six genes were up-regulated and 4 genes were down-regulated (-) as shown in **Table 15.**

The authors then seeded the cells (3×10^6) in 100 mm culture dishes, allowed them to attach for 48 h, and treated the cells with licorice extract in phosphate-buffered saline (PBS) diluted to various concentrations (0.25, 0.5, 1, 2, and 3 mg/ml) with Dulbecco Modified Eagle Medium (DMEM). The cells were harvested at 8, 16, 24, and 48 h. Total RNA was extracted with Trizol reagent. A parallel experiment was run with medium only for the control. From the total RNA, the 5 differentially expressed genes identified in the first part of the experiment (GST-pi, uPAR, PAI-1, fosl-1, and DT-diaphorase) were subjected to Northern blot analysis.

Table 14. Drug metabolizing enzyme activities and testosterone hydroxylase in liver microsomes from licorice extract treated rats (Paolini et al. 1999).

	Control		Licorice Extract (3138 mg/kg)		Licorice Extract (6276 mg/kg)	
Induced Enzyme Activity (units)	M	F	M	F	M	F
CYP (nmol/mg)	0.45 ± 0.07	0.43 ± 0.06	0.68 ± 0.05[a]	0.67 ± 0.04[a]	0.38 ± 0.04	0.36 ± 0.02
Aminopyrine *N*-demethylase (nmol/mg/min)	5.06 ± 0.52	4.62 ± 0.23	9.84 ± 0.51[a]	9.59 ± 0.43[a]	6.38 ± 0.15	6.42 ± 0.23
p-Nitrophenol hydroxylase (nmol/mg/min)	1.18 ± 0.11	0.81 ± 0.05	1.05 ± 0.04	0.73 ± 0.04	0.97 ± 0.06	0.75 ± 0.06
Ethoxyresorufin *O*-deethylase (pmol/mg/min)	38.48 ± 2.58	29.11 2.75	59.69 ± 5.45[a]	45.41 ± 3.39[a]	74.58 ± 2.47[a]	56.58 ± 2.71[a]
Pentoxyresorufin *O*-dealkylase (pmol/mg/min)	26.51 ± 2.45	31.53 ± 4.76	25.47 ± 2.39	31.34 ± 4.01	26.22 ± 3.59	36.32 ± 3.04
Metoxyresorufin *O*-demethylase (pmol/mg/min)	39.65 ± 2.86	38.96 ± 1.24	37.97 ± 2.13	103.48 ± 6.87[a]	36.67 ± 2.21	90.94 ± 3.81[a]
Ethoxydoumarin *O*-deethylase (nmol/mg/min)	0.41 ± 0.02	0.32 ± 0.01	0.68 ± 0.03[a]	0.61 ± 0.02[a]	0.47 ± 0.02	0.44 ± 0.04
Testosterone hydroxylase activity						
6α-Hydroxy-testosterone (pmol/mg/min)	24.02 ± 2.62	35.18 ± 1.48	23.13 ± 1.08	33.69 ± 1.58	25.98 ± 2.23	34.66 ± 2.21
7α-Hydroxy-testoterone (pmol/mg/min)	52.53 ± 1.86	51.33 ± 4.72	149.00 ± 4.87[a]	109.77 ± 6.34[a]	73.42 ± 3.57[a]	85.03 ± 3.74[a]
6β-Hydroxy-testoterone (nmol/mg/min)	76.22 ± 9.56	83.25 ± 7.68	890.92 ± 29.14[a]	373.85 ± 15.10[a]	725.65 ± 22.65[a]	270.16 ± 22.71[a]
16α-Hydroxy-testosterone (pmol/mg/min)	73.22 ± 7.51	45.27 ± 4.91	428.25 ± 37.49[a]	47.57 ± 2.03	155.33 ± 8.80[a]	21.99 ± 2.18[a]
16β-Hydroxy-testosterone (pmol/mg/min)	22.90 ± 4.11	27.12 ± 2.21	24.87 ± 1.08	39.75 ± 1.26[a]	27.29 ± 1.39[b]	35.94 ± 3.85[a]
2α-Hydroxy-testosterone (pmol/mg/min)	15.27 ± 8.01	13.56 ± 1.13	256.68 ± 31.73[a]	17.35 ± 0.54[b]	173.21 ± 18.21[b]	12.04 ± 1.60
2β-Hydroxy-testosterone (pmol/mg/min)	33.33 ± 2.28	25.30 ± 2.80	57.27 ± 2.89[a]	18.66 ± 2.10[a]	63.98 ± 4.89[a]	21.55 ± 1.14[b]
Androst-4-ene-3,17-dione (nmol/mg/min)	420.35 ± 9.09	395.02 ± 17.95	874.67 ± 25.90[a]	521.06 ± 24.25[a]	583.79 ± 18.66[a]	476.39 ± 25.45[a]

[a] $p < .01$; [b] $p < .05$

The expression of GST-pi was both concentration and time dependent with regard to its exposure to licorice root extract; maximum level was reached after 16 h of treatment with 3 mg/ml licorice extract. The expression of uPAR was increased 5-fold after 24 h in 3 mg/ml licorice root extract. Prolonged treatment (48 h) resulted in diminished effectiveness.

Licorice extract exposure could not prevent degradation but only induce uPAR expression. PAI-1 increased expression up to 8 h; prolonged exposure could not sustain the high level of expression. Fosl-1 was almost undetectable in the control; expression was induced at 8 h of incubation at 2 mg/ml licorice extract.

Treatment with 3 mg/ml licorice extract exponentially promoted expression of fosl-1; prolonged exposure resulted in diminished expression.

DT-diaphorase was the most abundant gene to increase expression after exposure to licorice root extract at >6-fold. The Northern blot at 500 µg/ml and a short incubation time of 8 h activated DT-diaphorase transcription. The authors suggest that licorice root extract is an effective inducer in the treatment of Clone 9 cells; licorice root extract was able to up-regulate the transcription of genes which are associated with phase II enzymes and cell mobility thus this ingredient has hepatoprotective properties (Leung et al. 2003).

Anti-hepatotoxic Activity

Glycyrrhiza Uralensis (Licorice) Root Extract

Shen et al. (2006) tested aqueous *G. uralensis* extract for protection properties against hepatotoxicity by isoniazid. Hepatocytes from female Sprague-Dawley rats were placed in a monolayer culture (2 x 105 cells/well) for 4 h. Isoniazid (1.1 mM) was added with or without licorice extract (5 g/l) or Glycyrrhizic Acid (0.05 g/l). The medium was changed every 48 h. Viability, intracellular glutathione levels, and albumin secretion were measured.

Table 15. Summary of rat cDNA expression after exposure to Glycyrrhiza Uralensis (Licorice) Root Extract (Leung et al. 2003).

Genes	Ratio[a]
c-Met oncogene; hepatocyte growth factor receptor	-2.41
Insulin-like growth factor binding protein 2 (IGF-binding protein 2; IGFBP2; IBP2); BRL-BP	-4.13
RL.IF-1	3.46
Extracellular signal-regulated kinase 3 (ERK3); mitogen-activated protein kinase 3 (MAP kinase 3; MAPK3); p55-MARK	2.10
Gluathione S-transferase P subunit 7 pi (GST7-7)	4.99
Uronkinase receptor; urokinase plasminogen activator surface receptor (GPI-anchored from; uPAR); CD87	4.91
Na, K-ATPase beta 3 subunit	2.32
Tissue inhibitor of metalloproteinase 3 (TIMP-3)	-4.08
Type I procollagen C proteinase enhancer protein	-2.81
Plasminogen activator inhibitor-1 (PAI-1)	3.77

[a] the ratio of signal of that gene in the control and the licorice extract treated gene

The addition of licorice extract and Glycyrrhizic Acid increased the viability of the cells for up to 72 h ($p < .01$) when added to isoniazid. Intracellular glutathione levels were also greater in both cases ($p < .01$), but not as high as the untreated control ($p < .01$); albumin secretion was also increased compared to isoniazid alone ($p < .05$) but lower than the untreated control ($p < .01$) (Shen et al. 2006).

Licorice Root Powder

Rajesh and Latha (2004) tested for the preventive properties of *G. glabra* root powder against the effects of carbon tetrachloride (CCl_4)-induced oxidative damage in rats. Three groups of albino Sprague-Dawley rats (n = 6) were fed either a 1) standard pellet diet (control), 2) a standard pellet diet with a daily dose of 0.3 ml CCl_4 in liquid paraffin, or 3) a standard diet, a daily dose of 0.3 ml CCl_4 in liquid paraffin, and 1000 mg/kg licorice root powder for 2 months. At the end of the experimental period, the rats were killed and the liver and kidney removed. Liver and kidney homogenate was prepared using tris-HCl buffer. Lipid peroxidation was assessed in terms of thiobarbituric acid reactive substances (TBARS; an index of lipid peroxidation and oxidative stress) and conjugated dienes (CD). Changes in the antioxidant status were determined by estimating the activities of catalase, SOD, GST, glutathione (GSH), and glutathione peroxidase (GSH-Px) in the liver and kidney.

There was an increase in the concentrations of TBARS and CD during CCI4 treatment compared to control. Administration of G. glabra root powder resulted in a decrease of TBARS and CD in liver and kidney compared to group 2 ($p < .05$). The activities of SOD, catalase, GST, and GSH-Px in the tissues were decreased in the CCl_4-treated group compared with controls ($p < .05$). There was a decrease in the content of GSH in group 2 ($p < .05$). Administration of G. glabra with CCI4 restored the activities of these parameters to near normal levels.

Histopathological studies demonstrated fatty change and ballooning degeneration of hepatocytes induced by CCI4. The liver also had distorted architecture with nodule formation, a distorted central vein, and fibrous expansion in the portal triad with moderate fibrosis and moderate inflammation. Administration of G. glabra root powder exhibited protective activity to livers and kidneys or rats exposed to CCl_4 (Rajesh and Latha 2004).

Gastrointestinal Protective Effects

Licorice Extract

Dehpour et al. (1994) tested how well licorice extract from the roots and underground stem of *G. glabra* and simple derivatives thereof protect the stomach from ulcer formation by aspirin. Aspirin powder was coated with licorice, Glycyrrhizinic Acid, deglycyrrhized licorice, high glycyrrhized licorice (15% Glycyrrhizinic Acid added to licorice extract), carbenoxolone or enoxolone by a wet granulation. The granules were suspended in 0.9% saline (equivalent to 26.6 mg/ml aspirin) and administered by oral gavage (266 mg/kg). The rats were killed 4 h later and their stomachs excised immediately. Mucosal damage was assessed macroscopically. Licorice and its derivatives reduced the size and number of ulcers in gastric mucosal. Aspirin absorption was not affected by licorice or its derivatives.

Khayyal et al. (2001) used male Wistar rats to test the anti-ulcerogenic activity of *G. glabra* root alcohol extract. The rats, weighing 120 to 150 g, were kept individually in starvation cages for 36 h with no feed but access to water. Water was withdrawn and the rats were dosed with 2.5, 5, or 10 ml/kg in water (n = 10). One h later, the rats were orally dosed with 10 mg/kg indomethacin to induce stomach ulcers. Five h later, the rats were killed and the stomachs removed. The stomachs were examined for ulcers and evaluated using a scale of 0 to 4 for the extent of the ulceration: 0 indicating a normal mucosa with no ulcers and 1 to 4 indicating the presence of lesions varying in severity from minor to severe with bloody erosion of the gastric mucosa. The effects were assessed by adding the percent incidence of animals with ulcers, mean severity (scale 0 to 4), and mean number of ulcers per stomach. The protective anti-ulcerogenic effect of G. glabra extract was defined as the percentage change in the ulcer index as compared to the indomethacin/alcohol only treated group.

The reduction in the ulcer index of the *G. glabra* root extract-treated groups was found to be >40%, >60%, and >90% for 2.5, 5, and 10 ml/kg, respectively. The rats tolerated the treatment well and had no evidence of sedation or hyperactivity that could be associated with the *G. glabra* extract.

In an additional experiment, male Wistar rats were individually placed into starvation cages for 48 h. The G. glabra extract was orally administered at the same doses as above (n = 11). One more group was administered water. One h later, the rats were lightly anesthetized with ether and a pyloric ligation performed. After cleaning and closing the incision, indomethacin was injected intraperitoneally (10 mg/kg) to all animals except for one group that served as the negative control. The positive control group

was dosed with indomethacin with no previous treatment. The rats were allowed to recover for 4 h in their cages with no food or water. The rats were anesthetized again and a ligature placed at the oesophageal-cardiac junction. The stomach was removed and the animals killed. Stomachs from 3 rats in each group were washed in saline and placed in 10% formalin for histological examination. The stomachs from the other 8 rats were opened along the greater curvature and the gastric contents drained and centrifuged and free acidity, mucin concentration, pepsin concentration, and prostaglandin and leukotriene were determined. The stomachs were laid open and the mucosa scrubbed off and weighed.

Indomethacin increased the free acidity from < 88 mEq/l in the control rats to 200 mEq/l and increased the acid output from 16.2 to 71.2 mEq/h. G. glabra root extract reduced the effect of indomethacin on free acidity to < 130 mEq/l ($p < .05$) and acid output to < 30 mEq/h ($p < .05$). Mucin content was reduced by indomethacin from ~2 mg hexose/ml to ~ 1 mg hexose/ml. G. glabra root extract increased the mucin content to > 4 mg hexose/ml ($p < .05$) in indomethacin-treated rats. Pepsin content was not affected by indomethacin or G. glabra root extract. Indomethacin reduced gastric mucosal prostaglandin content from 696 ng/g to 47.8 ng/g. G. glabra root extract inhibited the effect of indomethacin by increasing prostaglandin content to >200 ng/g ($p > .05$). Indomethacin increased leukotrienes content from ~200 ng/g to ~1800 ng/g. G. glabra root extract decreased the effect to ~ 400 ng/g. The authors concluded that the alcohol extract of G. glabra root has anti-ulcerogenic acitivty (Khayyal et al. 2001).

Aly et al. (2004) tested the anti-ulcer properties of aqueous licorice extract (species not provided). Albino rats (n = 6) were fasted then intragastrically administered famotidine (1.14 mg/kg), 2) licorice extract (100 mg/kg), 3) licorice (100 mg/kg) and famotidine (0.57 mg/kg), or 4) saline as the control. After 30 min, indomethacin (20 mg/kg) was administered intragastrically. The rats were killed 4 h later and the stomach removed and the lesion area quantified. The lesion area for the rats administered licorice extract (4.9 ± 0.26 mm2) and licorice extract with famotidine (2.0 ± 0.06 mm2) was reduced compared to control (24.9 ± 0.37 mm2; $p < .05$).

Deglycyrrhizinated Licorice Extract

Aarsen (1973) tested the effects of deglycyrrhizinized licorice (DGL; licorice processed to remove glycyrrhizin) extract on naturally occurring ulcers of male Cpb Wistar rats. The rats (150 to 180 g) were fasted for ~48 h with free access to water. Under anesthesia, the pylorus was ligated. Before closure, DGL (12.5, 25, 50, or 100 mg) in 2 ml distilled water was injected into the abdominal cavity. The control group was administered 2 ml saline. About 17 h later, the animals were killed. The stomachs with the ligation intact were removed and weighed. The stomachs were opened and the contents were titrated with 0.5 N NaOH to pH 7. The stomachs were stretched out and ulcers counted under microscopy.

The control rats had an average of 33.8 ulcers/stomach; 5 of 10 had perforated stomachs. The number of animals with no ulcers/group increased as the dose of DGL increased. The authors noted this "all or none" response and repeated the experiment with doses of 37.5, 62.5, and 87.5 mg. The authors determined that the number of rats without ulcers increased with increasing dose and concluded that DGL has anti-ulcer properties (Aarsen 1973).

Håkanson et al. (1973) tested the effects of DGL on gastric acid secretion using Wistar rats (150 to 250 g). Eight rats that had chronic gastric fistulas were fasted for 24 h and then restrained. The fistulas were opened and the stomachs rinsed with 0.9% saline until clear. Saline (10 ml) was administered subcutaneously (s.c.) to replace fluid loss. After the fistula had been draining freely for 1 h, basal acid secretion was collected for 2 1-h periods. DGL, (200 mg/kg) suspended in 0.9% saline (40 mg/ml), was administered interperitoneal (i.p.) and acid collected. Four further 1-h portions were collected. Acid output was determined by titration with 0.02 N NaOH. The procedure was repeated with the same animals except with the same volume of saline for the control.

DGL reduced the acid output in gastric fistula rats. The inhibition was apparent during the first hour after the injection ($p < .05$) and lasted for at least 4 h (hour 2 and 3, $.01 < p < .001$; hour 4, $p < .001$).

To determine histidine decarboxylase activity (HDA) and gastrin in serum, 12 normal and 8 anterectomized rats were fasted for 48 h. The rats were then administered 200 mg/kg DGL i.p.; they were dosed again 3 h later. Seven more fasted rats were administered the same amount of saline. All rats were lightly anesthetized with ether 3 h later, their abdomens opened, and blood drawn from the caval vein. The blood was allowed to clot and the serum was freeze-dried. The concentration of immunoreactive gastrin was determined. The rats were exsanguinated and the stomach was removed, cut open along the greater curvature, and rinsed in cold saline. The mucosa of the oxyntic gland area was scraped off and homogenized in phosphate buffer (pH 6.9) to a final concentration of 100 mg wet weight/ml. The mucosa was then centrifuged. HDA in the supernatant was determined by incubation with 14C-carboxyl-labeled histidine.

The HDA and serum gastrin level were increased in normal, but not in anterectomized rats (HDA: control, 5.2 ± 2.3; normal DGL, 18.4 ± 3.8; anterectomy DGL, 4.6 ± 1.2 pmoles CO2/mg/h; gastrin: control, 37 ±3; normal DGL, 122 ± 30; anterectomy, 27 ± 6 pg E synthetic human gastrin I/ml) (Håkanson et al. 1973).

Morris et al. (1974) instilled Heidenhain pouches in 4 dogs to test the effectiveness of DGL protection of gastric mucosa against the damaging effect of bile. There were 4 parts to each run of the experiment: 1) the pouch was perfused for 1 h with DGL preparation (312 mg DGL ; < 3% glycyrrhizinic acid) in 25 ml acid solution; 2) the pouch was perfused with 30 ml of the acid solution containing known amounts of H+, Na+, and Li+; 3) a 10 mM solution of sodium taurocholate solution (10 mM sodium taurocholate, 15mM sodium chloride and mannitol; osmolarity 314 mOsm/l; HCl added to pH 2 as a bile mimic); and 4) a repeat of part 2.

The volume and ionic composition of the standard acid solution were determined at the beginning and end of parts 2 and 4; differences between net flux of H+ and Na+ across the mucosa during parts 2 and 4 were a measure of the effect of taurocholate on the gastric mucosal barrier. An increased loss of H+ or gain of Na+ in the acid solution in part 4 compared to part 2 indicated an increase in permeability of the mucosal barrier of the pouch. The control was the acid solution in part 1 and NaCl with mannitol (300 mOsm/l) in part 3.

The dogs were fasted for 24 h before each experiment and administered an s.c. injection of atropine (0.015 mg/kg) 1 h before experiments to suppress acid secretion. Each experiment was repeated 3 times on each dog over 2 months.

In the controls, taurocholate increased the permeability of gastric mucosa to both hydrogen and sodium ions. Licorice reduced the effect of bile acids on the mucosal permeability to hydrogen ions (Morris et al. 1974).

Rees et al. (1979) tested DGL for protection from gastric mucosal damage by aspirin on male Wistar rats. The rats were fasted for 24 h with free access to water and administered 60 mg aspirin in 1% tragacanth either alone or with 100, 300, or 500 mg DGL. After 4 h the rats were killed and the stomachs removed, cut open, rinsed, and examined blind for mucosal damage (only the corpus was affected). There was less mucosal damage from aspirin when administered with DGL ($p < .02$ to .01).

Bennett et al. (1980) administered aspirin (60 mg/rat; in 2 ml tragacanth at 1%) by stomach tube to male Wistar rats after fasting. The rats were then administered sodium chloride (0.5 ml; 0.15 M) i.p. (n = 28); cimetidine (5 mg/kg; in 0.15 M NaCl) i.p. (n = 28); deglycyrrhizinated licorice (50 mg) mixed with the aspirin and NaCl (0.15 M) i.p. (n = 20); or deglycyrrhizinated licorice (50 mg) by stomach tube and cimetidine i.p. (n = 27). The rats were killed 4 h later and gastric mucosal damage assessed. Protection against damage was greater with the combination of deglycyrrhizinated licorice and cimetidine ($p = .00014$) than with either cimetidine ($p = .049$) or deglycyrrhizinated licorice extract ($p = .0253$) alone. The authors suggest that low doses of cimetidine and deglycyrrhizinated licorice extract together may reduce problems of drug toxicity.

Van Marle et al. (1981) tested the effects of DGL on the renewal of male Cpb:WU(WI) rat stomachs. Twelve rats were fasted with access to water for 48 h and then the pylorus was ligated to induce peptic ulcers. DGL (0.85 mg/g) was injected ip in 6 of these rats immediately after ligation. All 12 of the rats were administered 1 µCi/g [3H]thymidine. Another 12 fasted rats were sham operated. Six of these also received 0.85 mg/g DGL; all 12 received 1 µCi/g [3H]thymidine. All 24 rats were denied food and water after the operation. Nine rats with full access to food and water were sham operated and administered 1 µCi/g [3H]thymidine. At 1, 24, and 36 h, 2 or 3 rats were killed and the stomach excised, opened along the major curvature, stretched and fixed for 48 h in alcohol:100% formal (2:1). The stomachs were embedded in paraffin or methacrylate and sectioned for autoradiography. Cell proliferation in the forestomach and in the glandular part of the stomach was assessed in microautogradiographs.

None of the pylorus-ligated rats survived for more than 40 h, so 36 h was used as the longest time period. There were no differences observed macroscopically in the stomachs at different times of necropsy. There were small black dots in the forestomach in the pylorus-ligated rats necropsied at 24 h; these dots were smaller in the animals that received DGL. The 2 rats not receiving DGL had died at 26 h; the rats receiving DGL survived to 36 h.

Ulcers were observed in the forestomach and in the glandular part of the stomach in all groups. The labeled cells in the fasted rats had less proliferation in the forestomach than the non-fasted rats.

When DGL was administered to fasted rats, proliferation became equal to that in non-fasted rats at 24 h. Compared to the 2 fasted rats not administered DGL, the proliferation of the epithelium of the forestomach was accelerated with DGL. Pylorus ligation reduced proliferation to almost none, observed as early as the 1 h mark. DGL administration to pylorus-ligated rats resulted in a proliferation comparable to the proliferation in rats fasted without DGL treatment; at 1, 24, and 36 h, comparable amounts of labeled cells were present in the 3 compartments of both groups.

In the glandular part of the stomachs, cellular proliferation was similar in fasted and non-fasted normal rats not treated with DGL at 1, 24, and 36 h. Pylorus ligation had no effect on proliferation compared to fasted rats at 1 h, but at 24 h, no further proliferation had taken place.

After staining with alcian blue-PAS-haematoxylin no differences could be observed between mucus cells from the surface and the pits of the fundus glands in the various goups at 1, 24, and 36 h. When combined with autoradiography, labeled fully differentiated mucus cells occurred in the pit, isthmus, and neck of the fundus glands as soon as 1 h. The authors concluded that DGL increased mucosal secretion giving protection against peptic ulcers (Van Marle et al. 1981).

Morgan et al. (1983) administered DGL before, during, or after the administration of stomach damaging dose of aspirin (acetylsalicylic acid) in fasted male Sprague-Dawley rats. Four groups of rats were administered 1) water at time 0 and aspirin (128 mg/kg) at 30 min (n = 24); 2) DGL (2000 mg/kg in water) at time 0 and aspirin at 30 min (n = 24); 3) nothing at time 0 and aspirin at 30 min (n = 23); or 4) nothing at time 0 and aspirin and DGL at 30 min (n = 24). Four h later, the rats were killed and their stomachs removed, opened, examined, and scored for hemorrhagic lesions. Previous administration of DGL was not effective in preventing lesions; co-administration of DGL and aspirin (group 4) decreased the number of lesions compared to control (group 3; $p < .0005$).

In a second experiment, the authors again used 4 groups of rats to test for the effects of DGL on the administration of aspirin plus bile acid (taurodeoxycholic acid [TDC]). The rats were administered 1) aspirin (64 mg/kg; n = 22); 2) aspirin and TDC (5mmol/l; n = 22); 3) aspirin and DGL (2000 mg/kg; n = 21) or

4) aspirin, TDC, and DGL (n = 22). The rats were killed and the stomachs processed as above. TDC and aspirin caused an increase in the number of lesions compared to controls ($p < .05$); this was not observed with the co-administration of DGL. DGL decreased the number of lesions caused by the combination of aspirin and TDC ($P < .0002$) and by aspirin alone ($p < .0005$).

In a third experiment, aspirin (128 mg/kg) was administered by intubation with or without DGL (2000 mg/kg; n = 7). The rats were then anesthetized and blood samples were taken at 20, 40, 60, and 80 min. Total serum salicylates were measured. None of the rats survived after 80 min. Absorption of aspirin was delayed by the DGL at the 20 min sample (0.9 vs 1.2 mmol/l; $p < .05$). There were no other differences found at the other measurements. The authors concluded that combining aspirin and DGL might lessen gastric mucosal injury without compromise by biliary reflux and impairment of aspirin absorption (Morgan et al. 1983).

Russell et al. (1984) tested the protective effects of DGL against aspirin- and TDC (5 mmol/l) induced gastric mucosal damage. In the first experiment, 4 groups of male Sprague-Dawley rats (150 to 200g; n = 22) were fasted for 16 h with free access to water. The rats were intubated and administered at 0 and 30 min: 1) water then aspirin (64 mg/kg); 2) DGL in water (2000 mg/kg) then aspirin; 3) nothing then aspirin; and 4) nothing then aspirin and DGL. The rats were killed 4 h later and the stomachs removed. The stomachs were examined for bleeding lesions. Hemorrhagic lesions measuring <1, 1 to 2, 2 to 3, or >3 mm were scored 1, 2, 3, or 4 points, respectively. The median scores were calculated.

Exposure to DGL before aspirin by the gastric mucosa did not affect the degree of aspirin-induced damage. The combined administration of DGL and aspirin reduced the number of lesions compared to only aspirin ($p < .0005$). Lesion scores are given in **Table 16.**

In a second experiment, the rats (n = 21 or 22) were exposed once to 1) aspirin (64 mg/kg, the amount that caused hemorrhagic lesions in 50% of the rats in the first experiment); 2) aspirin and TDC (5 mmol/l); 3) aspirin and DGL (2000 mg/kg) or 4) aspirin, DGL, and TDC. The addition of TDC increased the lesion score ($p < .05$) over that of aspirin alone. DGL decreased the lesion score compared to aspirin ($p < .0005$) and aspirin plus TDC ($p < .0002$).

The effect of i.p. DGL (500 mg/kg) on gastric mucosal damage induced by aspirin (125 mg/kg) was also studied. Intraperitoneal DGL given together with aspirin reduced lesion scores from 14 to 7 ($p < .03$).

To study the effects of DGL on the gastric absorption of aspirin, 2 groups of rats (n = 7) were administered aspirin (128 mg/kg) or aspirin and DGL (2000 mg/kg).

The rats were immediately anesthetized and tail blood samples were taken at 20, 40, 60, and 80 min. Total serum salicylate levels were measured. DGL delayed absorption of aspirin at 20 min (0.9 mmol/l vs 1.2 mmol/l; $p < .05$). No effects were found at the other times.

Table 16. Effect of DGL on hemorrhagic lesions induced by aspirin (Russell et al. 1984).

	Treatment group			
Time	**1**	**2[a]**	**3**	**4[b]**
0	Water	DGL	--	--
30	Aspirin	Aspirin	Aspirin	Aspirin and DGL
Median lesion score	11	15	17	8

[a] 1 vs 2 NS
[b] 3 vs 4 significant at $p < .0005$

The authors concluded that a combination of aspirin and DGL could have a therapeutic role in diminishing injury due to aspirin and the increased damage occurring when aspirin and bile are present together without affecting aspirin absorption (Russell et al. 1984).

Bennett et al. (1985) administered deglycyrrhized licorice (1 g/kg in aqueous suspension in 1% gum arabic) to rats (n = 28) by gastric intubation. Controls received vehicle alone (n = 14). The dosing was repeated 22 h later. The rats were killed 2 h after that and the stomachs removed and analyzed for prostanoids. There was little or no effect by the deglycyrrhized licorice on the prostanoids in the stomachs of rats. The authors concluded that deglcyrrhized licorice may exert its anti-ulcer effect by mechanisms other than prostaglandin.

Cerebral and Memory Effects

Licorice Extract

Parle et al. (2004) tested the effects of G. glabra aqueous extract on the memories and step-down (transfer latency [TL] parameters) of male Swiss albino mice (3 mo old; 25 g) using an elevated plus-maze with 2 open arms and 2 closed arms. The mice had free access to feed consisting of wheat flour and water with a small amount of vegetable oil and salt. The volume of all doses of licorice extract (oral and intraperitoneal [i.p.]) were 1 ml/100 g.

Group I (n = 6) was the control and was administered 1 ml/100 g distilled water for 7 d. Groups II and III (n = 5) were administered 150 or 300 mg licorice extract/kg, respectively, orally for 3 d. Groups IV, V, and VI (n = 5) were administered 75, 150, or 300 mg licorice extract/kg, respectively, orally for 7 d. TL was recorded at 90 min and 24 h after last administration for groups I through VI. Groups VII and VIII (n = 5) were administered scopolamine hydrobromide (0.4 mg/kg ip) or diazepam (1 mg/kg ip), respectively, before training. TL was recorded 45 min and 24 h after injection. Group IX (n = 5) was administered scopolamine hydrobromide (0.4 mg/kg ip) after training on day 2. TL was recorded 45 min after injection.

Groups X and XI (n = 5) were administered 150 mg/kg per os (p.o.) licorice extract for 7 d. Scopolamine hydrobromide (0.4 mg/kg) or diazepam (1 mg/kg ip), respectively, were injected 90 min after the final licorice injection. TL was recorded 45 min and 24 h after last injection. Group XII (n = 5) was administered 150

mg licorice extract/kg for 7 d. TL was recorded at 90 min after last administration. Scopolamine hydrobromide (0.4 mg/kg ip) was administered 45 min prior to recording TL on day 8. Group XIII (n = 5) was administered ethanol (1 g/kg ip) before training. TL was recorded at 30 min and 24 h.

Group XIV (n = 5) was administered ethanol (1 g/kg ip) after training on day 2. TL was recorded at 30 min. Group XV (n = 5) was administered 150 mg licorice extract/kg po for 7 d. Ethanol (1 g/kg ip) was administered at 90 min after last licorice administration. TL was recorded at 30 min and 24 h. Group XVI (n = 5) was administered 150 mg licorice extract/kg po for 7 d. TL was recorded at 90 min after last administration. Ethanol (1 g/kg ip) was administered 30 min prior to recording TL on day 8.

Administration of 150 mg licorice extract/kg for 7 d decreased TL on the first day and the second day ($p < .05$). Administration of 300 mg licorice extract/kg for 7 d increased TL in training, indicating impairment in learning. Scopolamine hydrobromide, diazepam, or ethanol injected before training impaired learning (increased TL, $p < .05$), whereas, scopolamine or ethanol injected after training impaired memory ($p < .05$). Licorice extract (150 mg/kg) administered orally for 7 d protected the mice from learning impairment and memory loss by scopolamine, diazepam and ethanol ($p < .05$).

The authors performed a passive avoidance paradigm experiment to test long-term memory effects of G. glabra aqueous extract. A box (27 x 27 x 27 cm) with 3 wooden walls, one plexiglass wall and a floor of stainless steel rods with a wooden platform in the middle (10 x 7 cm) was constructed. The mice were placed on the wooden platform and timed for how long to place all 4 feet on the steel rods. The mice were shocked with 20 V AC for 15 sec when all 4 feet touched the rods. Step-down latency (SDL), the time taken by the mouse to step down from the wood platform with all paws on the grid, was recorded. Animals with an SDL of 2 to 15 sec were used for the experiment. They were retested 90 min later and removed if they did not step down after 60 sec. The mice were tested again at 24 h with no shock with a maximum time of 300 sec.

Group XVII (n = 6) was the control and administered 1 ml/100 g distilled water orally for 7 d. At 90 min after last administration, SDL was recorded during both sessions of training. Retention was examined at 24 h. Group XVIII, XIX, and XX (n = 5) were administered 75, 150, and 300 mg licorice extract/kg orally for 7 d. SDL was recorded at 90 min and 24 h after last administration. Group XXI (n = 5) was administered scopolamine hydrobromide (0.4 mg/kg ip) after training on day 2; SDL was recorded at 45 min. Group XXII (n = 5) was administered licorice extract (150 mg) for 7 d. SDL was recorded at 90 min after last administration. Scopolamine hydrobromide (0.4 mg/kg i.p.) was administered 45 min prior to recording SDL on day 8. Group XXIII (n = 5) was administered ethanol (1 g/kg ip) after training on day 2; SDL was recorded at 30 min. Group XXIV (n = 5) was administered 150 mg licorice extract/kg for 7 d. SDL was recorded at 90 min after last administration. Ethanol (1 g/kg ip) was administered 30 min prior to recording SDL on day 8. Groups XXV and XXVI (n = 5) were administered 150 mg licorice extract/kg po for 7 d and locomotor function was studied using a photoactometer.

G. glabra aqueous extract (75 and 300 mg/kg) did not have an effect on SDL compared to negative controls. G. glabra aqueous extract (150 mg/kg) for 7 d increased SDL compared to control group on the second day ($p < .05$), indicating improved memory. Scopolamine hydrobromide and ethanol decreased SDL on the second day after training ($p < .05$), indicating memory impairment. G. glabra aqueous extract (150 mg/kg) administered orally for 7 d reversed amnesia induced by scopolamine hydrobromide or ethanol ($p < .05$).

The authors stated that *G. glabra* aqueous extract had memory enhancing capabilities, but did not affect locomotor function or have any other adverse effects on the mice up to 300 mg/d (Parle et al. 2004).

Ahn et al. (2006) tested the inhibition effects of the aqueous extract of *G. uralensis* on the cognitive deficits caused by β-amyloid peptide 25-35 (thought to cause oxidative stress in the brain of Alzheimer's patients) in mice. The male ICR mice (n = 10) were divided into 4 groups and allowed free access to water and fed a standard diet for 6 wk; 2 of these groups had diets augmented with the licorice extract (0.5% or 1%). At the beginning of weeks 5 and 6, the mice in the augmented feed groups and one of the un-augmented feed groups were administered β-amyloid peptide 25-35 (10 µg/10 µl/mouse in phosphate buffer solution [PBS]) by intracerebroventricular injection; the fourth group was administered PBS. The day after the first injection, the mice were trained on a one-trial passive avoidance box (half light and open; half closed, dark, with electric shock). The next day the mice were placed back in the light side of the box and the time it took them to move to the dark side measure up to 240 s. On the day of the second injection (week 6) the mice were placed in a water-maze performance test.

In the passive avoidance test, treatment with licorice extract inhibited the effects of β-amyloid peptide 25-35 in a dose-dependent manner. The mice in the groups fed 0.5% and 1% licorice extract went into the other side of the test box sooner than the control group fed no licorice extract and not administered β-amyloid peptide 25-35 ($p < .05$ compared to control). The group of mice fed 1% licorice extract waited 81.3% as long as the control group to enter the other side of the box ($p < .05$ compared to groups fed 0 and 0.5% licorice). In the water-maze performance test, treatment with licorice extract inhibited the effects of β-amyloid peptide 25-35 in a dose-dependent manner. The mice fed no licorice extract took twice as long as the control group to find the platform ($p < .05$) and the mice fed 1% licorice extract took the same amount of time to find the platform as the control group.

After the behavioral tests, the mice were killed and the antioxidant activity was measured in the serum and brain as was the acetylcholinesterase activity in the brain. Licorice extract reduced the increase in lipid peroxidation in the brain in a dose-dependent manner; there was no effect with regard to the serum. There was no effect of licorice extract on SOD and GSH-Px in the mouse

brain. The catalase activity was decreased in the 1% licorice extract group compared to controls ($p < .05$). There was no difference in acetylcholinesterase activity in the serum and brain among the control, 0, and 0.5% licorice groups. The mice in the 1% licorice extract group had decreased acetylcholinesterase activity in both the serum ($p < .05$) and the brain ($p < .01$). The authors conclude that licorice extract in the feed of mice improves cognitive deficits induced by β-amyloid peptide 25-35 by decreasing oxidative stress; licorice extract may also be scavenging reactive oxygen species (Ahn et al. 2006).

Dhingra et al. (2006) compared the effect of aqueous *G. glabra* extract on cholinesterase-inhibiting activity with metrifonate, a standard acetylcholinesterase-inhibiting drug. Male Swiss albino mice (3 months old; n = 5) were adminisitered metrifonate (50 mg/kg) by a single i.p. injection, normal saline (10 ml) by i.p. injection for 3 consecutive days, G. glabra root extract (150 mg/kg) orally for 7 consecutive days, or distilled water (10 ml) orally for 7 consecutive days. The mice were killed, their brains extracted, homogenized, and analyzed for acetylcholinesterase activity. Metrifonate inhibited cholinesterase activity by 20% compared to its control and G. glabra aqueous extract by 25.4% compared to its control.

Other Effects - Absorption of Iron

Licorice Extract

El-Shobaki et al. (1990) tested the effect of licorice beverage extracts on intestinal iron absorption in male and female Sprague-Dawley rats (95 to 140 g). The beverage was prepared using 5 g licorice drink powder/100 ml distilled water infused for 3 h. Twenty cm segments of intestine, comprising both the duodenum and the front part of the jejunum, were tied off. After an overnight fast, the iron/licorice dose, consisting of 1 ml licorice beverage and 1 ml iron solution containing 500 nmol Fe labelled with active iron (^{59}Fe), was injected into the tied off segment under anesthesia. One h later, blood was drawn, the intestinal section was removed and washed, the liver was removed, and the 59Fe content was determined. The absorption index was calculated as: ^{59}Fe in blood + liver + body/ ^{59}Fe injected x 100.

Licorice drink extract injected directly into the intestine was found to enhance the absorption of iron in the intestine; the absorption index was 20.3 compared to 14.1 for iron alone (El-Shobaki et al. 1990).

Effects on Tyrosinase

Glycyrrhiza Glabra (Licorice) Root Extract

Lee et al. (1997) assayed tyrosinase inhibition by methanol Glycyrrhiza Glabra (Licorice) Root Extract (0, 3.3 and 333 µg/ml) using mushroom tyrosinase. The inhibition was 25% and 68% for 3.3 and 333 µg/ml, respectively. The authors stated that G.glabra has high inhibitory activity compared to the other 99 plant extracts tested.

Khanom et al. (2000) tested tyrosinase inhibitory activity of methanol, acetone, and aqueous extracts of G. glabra (using the "trunk" part of the plant). Tyrosinase catalyzes 2 distinct reactions involving molecular oxygen: the hydroxylation of monophenols to o-diphenols and the oxidation of the o-diphenols to o-quinones. Quinones are highly reactive compounds and can polymerize spontaneously to form high-molecular-weight compounds or brown pigments (melanins) or react with amino acids and proteins that enhance the brown color produced. McIlvaine buffer (1.0 ml) solution, 1.0 ml distilled water containing 0.3 mg/ml l-tyrosine, and 0.9 ml licorice extract were combined in a test tube. After mixing and preincubation for 10 min, 0.1 ml of 480 units/ml of tyrosinase (from Agricus biporus) was added and incubated for another 10 min. Sodium azide (0.1 ml of 1 M) was added to stop the reaction. Absorbance was measured at 475 nm and the results compared to control. Methanol extract of G. glabra (50%) inhibited tyrosinase by 92.1%, acetone extract by 94.7%, and water extract by 48.6%.

Neuromuscular Effects

Licorice Extract

Ambawade et al. (2002) tested the effect of an ethanolic extract of *G. glabra* roots and stolons on several convulsive endpoints using male albino Swiss mice (22 to 25 g) and male albino Wistar rats (125 to 150 g). The animals had free access to food and water until 8 h before and during the experiments when food was withdrawn. The mice (n = 6) were injected i.p. with the licorice extract in water at 10 to 500 mg/kg 30 min prior to application of electric shock (42 mA, 0.2 sec) using corneal electrodes. The controls were injected with the vehicle. The duration of tonic hind leg extension was measured. Doses of 10, 30, 100, and 500 mg/kg did not reduce the time of the hind leg extension.

The authors injected i.p. the licorice extract at varying doses (10 to 100 mg/kg) to the mice (n = 6) 30 min prior to the subcutaneous (s.c.) injection of pentylenetetrazol (PTZ) (80 mg/kg). The controls were injected with just the vehicle and another group was injected with diazepam (2.0 mg/kg). The mice were observed for onset of myoclonic spasm and clonic convulsions for up to 30 min.

In the mice treated with just the vehicle, clonic convulsions began 140.3 ± 13.8 sec after PTZ injection and all mice died after the seizures. The licorice extract dose-dependently inhibited the onset and occurrence of seizures as shown in **Table 17.**

At 10 mg/kg, only 66.6% of the mice had seizures and all the mice that had seizures died. No mortality occurred in the 30 and 100 mg/kg groups for 24 h. No mice in the diazepam group had seizures.

Five groups of rats (n = 6) were injected i.p. with lithium sulfate (3 mEq/kg). After 23.5 h, they were injected i.p. with G. glabra extract (10, 30, or 100 mg/kg), diazepam (1.0 mg/kg) or just the vehicle. Twenty-four h after the lithium sulfate injection, the rats were injected i.p. with pilocarpine (30 mg/kg). The severity of status epilepticus was observed every 15 min for 90 min then every 30 min for another 90 min. The convulsions were scored: 0 - no response to the drugs; 1 - fictive scratching; 2 - tremors; 3 - head nodding; 4 - forelimb clonus; and 5 - rearing and falling back.

Table 17. Effect of ethanolic extract of *G. glabra* on PTZ-induced seizures in mice (Ambawade et al. 2002).

Treatment	Onset of clonic convulsions (sec)	Incidence of convulsions (%)
Vehicle	140.0 ± 13.8	100
G. glabra extract (10 mg/kg)	664.5 ± 93.0[a]	66.6
G. glabra extract (30 mg/kg)	780.0	16.6
G. glabra extract (100 mg/kg)	No convulsions	0
Diazepam	No convulsions	0

[a] $p < .001$

All the control rats had stage 4 seizures by 75 min after pilocarpine injections. The *G. glabra* extract diminished the intensity of seizures and none of the animals exhibited stage 4 seizures; all exhibited normal behavior by 180 min. The authors stated that the ethanolic extract of *G. glabra* inhibits convulsions caused by PTZ and lithium-pilocarpine but not maximum electroshock seizures (Ambawade et al. 2002).

Enzyme Effects

Glycyrrhiza Glabra (Licorice) Root

Oganesyan (2002) tested the antioxidant effects of *G. glabra* root on stressed chinchilla rabbits in a vibration stress test. Fifteen rabbits (2.5 to 3 kg) were divided into 4 groups. The animals had free access to feed and water; licorice root was provided in the feed (150 mg/100 g). Blood was collected from the marginal ear vein before the experiment and every fifth day of treatment. Group 1 was exposed to vibration for 30 d; group 2 was exposed to vibration for 30 d and received licorice root; group 3 received licorice root and no vibration; and group 4 was exposed to vibration for 30 d after 30 d pretreatment with licorice root. Catalase activity was measured using hydrogen peroxide (H_2O_2) degradation.

In group 1, vibration increased catalase activity; group 2 (vibration and licorice root) had decreased enzyme activity to below normal at all times ($p < .001$). Group 3 had decreased catalase activity to below normal on days 5, 10, and 15 ($p < .001$); enzyme activity increased from these levels on days 15 through 30, but stayed below normal ($p < .001$). Group 4 animals had catalase activity lower than groups 1 and 2 throughout the experiment . The authors concluded that G. glabra root decreased catalase activity in the peripheral blood and increased resistance to vibration stress (Oganesyan 2002).

Clot Lysing Effects

Glycyrrhiza Glabra (Licorice) Root Extract

Prasad et al. (2007) tested the ability of Glycyrrhiza Glabra (Licorice) Root Extract (multiple solvent extraction of methanol: isopropyl alcohol: acetone) to break up blood clots. Whole human blood (4 ml) from 20 volunteers was distributed into 8 preweighed sterile microcentrifuge tubes and incubated for 45 min to form a clot. Serum was removed and the tubes weighed. The extract (100 µl) was added to the tubes (n = 20) and incubated for 90 min. Released fluid was removed and the tubes weighed again. Water served as the negative control and streptokinase served as the positive control. Glycyrrhiza Glaba (Licorice) Root Extract caused negligible clot lysis (17.8%).

Cholesterol Effects

Glycyrrhiza Glabra (Licorice) Root Powder

Sitohy et al. (1991) fed 60 albino rats (60 d old, 90 to 110 g) either a basal diet (n = 15) or basal diet supplemented with 2% cholesterol and 0.5% cholic acid (replacing starch) to induce hypercholesterolemia for 4 wk. Rats receiving the supplemented diet were either continued on the same diet (n = 15) or continued on the same diet supplemented further with 5% or 10% licorice powder at the expense of starch (n = 15 each). At 4, 7, and 10 wk from the start of treatment, 5 rats were killed, blood samples collected, and the organs separated and chilled. Total lipids, total cholesterol, triglycerides, and phospholipids were determined and serum glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities assayed.

There was no difference between the 2 doses of licorice powder with regards to the cholesterol and total lipid or triglyceride contents in the kidneys. After 10 wk, both treated groups had total lipids, cholesterol, and triglycerides that were lower than the positive control ($p < .01$) and phospholipids that were higher than the positive control ($p < .01$) as shown in **Table 18.**

In the liver, total lipids, cholesterol, phospholipids, and triglycerides were reduced compared to controls by both licorice extract treatments. No difference between the 2 treatment levels were observed.

The untreated hypercholesterlemic rat group had the highest serum content of total lipids, triglycerides, and cholesterol and the lowest phospholipid content compared to rats with the same diet plus 5% or 10% licorice powder as shown in **Table 19**. Total lipids, tryglyceride, and cholesterol content in the serum of this group increased gradually with time and the phospholipids decreased with time. The level of serum cholesterol and total lipids or triglycerides was directly proportional; the proportion between cholesterol and phospholipids was inversely proportional.

The hypercholesterolemic rats receiving licorice at either dose gradually responded; all enzyme activities diminished with time and returned to normal values after 10 wk of daily licorice as shown in **Table 20.**

The GOT/GPT ratio was reduced by licorice ingestion compared to hypercholesterolemic level, thus, the level of GOT was more reduced than the level of GPT by licorice treatment. The authors state that this is a sign of improved heart function since the heart is the organ most affected by cholesterol precipitation.

Table 18. Accumulation of lipid fraction in the kidneys of hypercholesterolemic rats fed 2 levels of dietary *G. glabra* powder (Sitohy et al. 1991).

	Total lipids (g/100 g)			Cholesterol (mg/100 g)			Phospholipids (mg/100 g)			Triglyceride (mg/100 g)		
						Time (weeks)						
Treatment	**4**	**7**	**10**	**4**	**7**	**10**	**4**	**7**	**10**	**4**	**7**	**10**
Negative control	7.85 ± 0.45	8.02 ± 0.56[a]	7.64 ± 0.56	450.2 ± 41.5[a]	462.5 ± 40.6[a]	496. ± 38.6[a]	1108 ± 95.4	1098 ± 86.4	1162 ± 96.1	5.08 ± 0.49	5.10 ± 0.50[a]	5.10 ± 0.49*
Positive control	9.40 ± 0.91	9.90 ± 0.92	10.50 ± 0.89	930 ± 86.4	942.0 ± 64.3	1077.5 ± 91.6	1108 ± 99.4	956.4 ± 86.4	840.2 ± 51.6	6.42 ± 0.62	6.65 ± 0.63	7.15 ± 0.58
Low level 5%	8.92 ± 0.98	8.50 ± 0.74	7.86 ± 0.64[a]	815.6 ± 71.5	682 ± 62.4[a]	595 ± 48.2[a]	1080 ± 74.5	1102 ± 82.6	1150 ± 95.6[a]	6.15 ± 0.60[a]	5.60 ± 0.36	5.37 ± 0.43*
High level 10%	8.84 ± 0.68	8.40 ± 0.56	7.95 ± 0.46[a]	818.4 ± 80.6	661.4 ± 35.6[a]	572.0 ± 39.4[a]	1074 ± 76.4	1112.5 ± 86.4	1138 ± 90.4[a]	610 ± 0.41	5.52 ± 0.36[a]	5.20 ± 0.50*

[a] $p < .01$ when compared to positive control

Table 19. Serum lipid fractions of hypercholesterolemic rats as fed 2 levels of dietary *G. glabra* powder (Sitohy et al. 1991).

	Total lipids (mg/dl)			Cholesterol (mg/dl)			Phospholipids (mg/dl)			Triglyceride (mg/dl)		
						Time (weeks)						
Treatment	**4**	**7**	**10**	**4**	**7**	**10**	**4**	**7**	**10**	**4**	**7**	**10**
Negative control	421.6 ± 30.6[a]	456..2 ± 25.8[a]	542 ± 43.5[a]	136.2 ± 8.9[a]	157.4 ± 12.6[a]	164.5 ± 9.4[a]	132.6 ± 10.4[a]	130.5 ± 11.4[a]	139.5 ± 10.6[a]	62.4 ± 3.2[a]	70.4 ± 6.2[a]	78.4 ± 5.6a
Positive control	784.6 ± 61.5	896.4 ± 73.5	1042.5 ± 52.4	297.5 ± 18.5	320.6 ± 30.4	362.4 ± 28.4	102.6 ± 9.4	76.5 ± 6.8	38.6 ± 2.9	182.6 ± 16.4	242.6 ± 21.2	285.4 ± 22.4
Low level 5%	764.6 ± 46.4	721.4 ± 61.5[a]	624.6 ± 51.8[a]	284.6 ± 21.6	250.4 ± 20.5[a]	182.4 ± 14.5	104.6 ± 8.6	106.2 ± 6.4	110.5 ± 7.4[a]	176.2 ± 15.6	138.4 ± 10.6[a]	104.2 ± 8.4[a]
High level 10%	776.6 ± 43.2	692.4 ± 36.2[a]	598.5 ± 41.9[a]	288.6 ± 25.4	236.4 ± 20.2[a]	180.6 ± 16.3[a]	106.5 ± 9.2	108.6 ± 8.1	114.5 ± 6.3[a]	184.6 ± 10.4	130.6 ± 11.5[a]	92.6 ± 9.2[a]

[a] $p < .01$ when compared to positive control

Table 20. Phosphatase and transaminase activities in the serum of hypercholesterolemic rats as fed 2 levels of dietary *G. glabra* powder (Sitohy et al. 1991).

Treatment	**Acid phosphatase (IU/l)**			**Alkaline phosphatase (IU/l)**			**GOT (U/ml)**			**GPT (U/ml)**			**GOT/GPT**		
							Time (weeks)								
	4	**7**	**10**	**4**	**7**	**10**	**4**	**7**	**10**	**4**	**7**	**10**	**4**	**7**	**10**
Negative control	6.24 ± 0.54[a]	6.56 ± 0.62[a]	6.38 ± 0.36[a]	32.4 ± 28.5[a]	31.8 ± 26.4[a]	32.6 ± 3.5[a]	48.51 ± 3.68[a]	48.56 ± 4.18[a]	49.62 ± 4.36[a]	19.82 ± 1.85[a]	20.6 ± 2.0[a]	20.8 ± 1.64[a]	2.45 ± 0.18	2.36 ± 0.18	2.38 ± 0.21
Positive control	10.25 ± 0.64	10.42 ± 0.84	11.64 ± 1.04	40.5 ± 2.92	43.6 ± 2.84	46.6 ± 3.15	71.6 ± 4.26	76.4 ± 6.52	81.62 ± 7.45	27.2 ± 2.21	31.6 ± 2.91	34.5 ± 2.84	2.63 ± 0.15	2.42 ± 0.21	2.37 ± 0.21
Low dose 5%	10.24 ± 0.92	8.86 ± 0.74[a]	7.24 ± 0.61[a]	40.2 ± 3.2	40.1 ± 2.84	36.4 ± 2.68[a]	68.5 ± 7.45	56.7 ± 5.24	51.6 ± 3.62[a]	28.4 ± 1.64	27.4 ± 2.12	24.6 ± 1.92[a]	2.41 ± 0.20	2.07 ± 0.20	2.10 ± 0.20
High dose 10%	10.20 ± 0.92	8.42 ± 0.46[a]	7.10 ± 0.38[a]	40.6 ± 3.62	38.5 ± 3.72	35.4 ± 2.42	69.5 ± 4.56	58.5 ± 4.24	50.2 ± 5.00[a]	27.24 ± 1.24[a]	26.5 ± 2.41	25.5 ± 2.35[a]	2.62 ± 0.16	2.21 ± 0.21	1.96 ± 0.16

[a] $p < .01$ when compared to positive control

Hypercholesterolemic rats receiving either dose of licorice root powder had lower serum glucose levels compared to untreated rats but higher levels compared to the negative control. The authors state that this is evidence of improved liver function which involves blood glucose regulation. The hypercholesterolemic rats contained higher levels of serum creatinine compared to the negative control.

The authors concluded that cholesterol precipitation in the kidneys of hypercholesterolemic rats might have an impairing effect on renal function because the rat administered licorice root powder showed diminished values, especially in the third stage. The authors also suggested that licorice root could improve renal function due to reduction of blood and kidney cholesterol levels (Sitohy et al. 1991).

Visavadiya and Narasimhacharya (2006) tested the effect of Glycyrrhiza Glabra (Licorice) Root Powder on male albino rats with and without hypercholesterolemia induced by diet. The root powder was extracted in petroleum ether to remove fat. The normal rats (n = 8; 3.5 month old) were fed a basal diet, a basal diet incorporated with root powder (5 g%), or a basal diet incorporated with root powder (10 g%) for 4 wk. The hypercholestrolemic rats (n = 8) were fed the same as the normal rats. At the end of the experiment, blood and feces were analyzed. The rats were killed and the liver examined.

There were no differences among the groups with regards to body weight and feed intake. The body weights of the normal rats fed both doses of root powder were lower than the control ($p < .01$); all the hypercholestolemic rats had similar body weights to the controls. All groups had similar liver weights except for an increase in the hypercholestolemic group fed the basal diet ($p < .0001$).

Plasma analysis of the normal rats revealed a dose-dependent decrease in cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and atherogenic index ($p < .05$) and a rise in high density lipoprotein (HDL; $p < .05$). The hypercholestolemic group had dose-dependent responses in the decrease in total lipids, total cholesterol, triglycerides, LDL, VLDL, and atherogenic index ($p < .05$) and an increase in HDL ($p < .05$). Both normal rat groups had no variation in hepatic lipid profiles compared to the controls; both the hypercholestolemic rat groups had declines in hepatic total lipids, total cholesterol, and triglycerides ($p < .05$). The hepatic 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase was not affected in the normal rats but rose in the hypercholestolemic rat groups ($p < .05$).

Fecal cholesterol, neutral sterol, and bile acid content were similar between the normal rats and the controls; all levels were increased in the hypercholestolemic rat groups ($p < .05$). The normal rats had decreased hepatic lipid peroxidation ($p < .05$) and an increase in ascorbic acid ($p < .05$) compared to controls. The hypercholestolemic rats groups had decreased lipid peroxidation ($p < .05$) and an increase in catalase ($p < .05$), SOD ($p < .05$) and ascorbic acid ($p < .05$) compared to controls.

The authors suggested that the hypolipidemic and hypocholesterolemic effect of Glycyrrhiza Glabra (Licorice) Root Powder is mediated through an increased cholesterol turnover via higher fecal sterol excretion and that the increased hepatic antioxidant activities indicated a decreased oxidative susceptibility in rats (Visavadiya and Narasimhacharya 2006).

Cytotoxicity and Cellular Effects

Licorice Extract

Budzinski et al. (2000) tested serial dilutions of the ethanol extract of *G. glabra* for in vitro inhibitory effect on CYP 3A4 using a fluorometric microtitre plate assay. The ethanol concentration for the stock solution of the extract was 55% and was serially diluted with ethanol (100% of the stock solution to 1.56%). The control was 55% ethanol and buffer solution. Test wells (n = 3) were extract and nicotinamide adenosine dinucleotide phosphate (NADPH) solution. The test blank was extract and buffer solution. Enzyme solution was added to all wells. Incubation was for 1 h at 37°C. Florescence was measured at 530 nm (35 nm bandwidth). The median inhibition concentration (IC_{50}) of G. glabra ethanol extract was found to be 1.83% of the original extract.

Anti-inflammation and Immunity Effects

Licorice Extract

Matsushima and Baba (1992) tested the antigranulomatous effects of aqueous extract of licorice root. Normal human fibroblasts were incubated in culture well plates with DMEM-FBS. After washing the culture, medium was replaced with 0, 0.2%, 1%, or 5% licorice extract for 24 h. The fibroblasts were washed and the medium replaced, without licorice extract, and incubated for 16 h. The supernatant was extracted. Normal human monocytes were incubated for 6 h on a culture slide with 200 µl of one of the supernatant from the fibroblasts and 50 µl of the activating factor of sarcoidosis serum (n = 5) and the spread of the monocytes was measured by counting the monocytes after staining.

The control monocytes spread increased by ~65%. The spread of the monocytes exposed to 0.2%, 1%, and 5% licorice extract increased by ~42%, ~28%, and ~25%, respectively.

The authors then tested licorice extract on fibroblasts in the suppression of granuloma formation. Pulmonary granulomas were induced in female Hartley albino guinea pigs by the 0.1 ml intratracheal injection of Sephadex beads suspended in Delbecco's PBS at 1.5 x 105/ml. Licorice extract (2 ml) was intraperitoneally injected daily for 3 d. Control animals were injected with saline. The guinea pigs were killed and at least 5 histological sections were made from each excised lung and stained with hematoxylin and eosin.

The control animals had large granuloma formation; the inflammatory infiltrate surrounding the bead was mainly composed of epithelioid macrophages and multinucleated giant cells with a mixture of lymphocytes and scattered neutrophils. There was no marked granuloma formation in any of the treated guinea pigs (Matsushima and Baba 1992).

Kobayashi et al. (1995) investigated the effects of Glycyrrhiza Uralensis [Licorice] Root Extract, glycyrrhizin, Glycyrrhitinic Acid and the flavonoids isoliquiritin, isoliquiritin apioside, isoliquiritigenin, and liquiritigenin on granuloma angiogenesis by measuring carmine content in newly formed blood vessels. Male ddY mice (6 to 7 wk old) were injected with 3 ml air s.c. into the dorsum under anesthesia to produce a regular ellipsoid air sac. Freund's complete adjuvant (FCA) emulsion (with 0.1% croton oil and 2 mg heat-killed Mycobacterium tuberculosis; 0.5 ml) was injected into the air pouch under anesthesia 24 h later. Two h later, isoliquiritin (n = 22), glycyrrhizin (n = 23) or licorice extract (n = 21) was injected i.p. daily for 4 d.

The mice were injected with 10% carmine solution containing 5% gelatin into the tail vein on day 5 then killed. The weights of granuloma tissues and pouch fluid isolated from the pouch were measured. Optical density was used to determine the carmine content, an index of newly formed blood vessels in the pouch granuloma.

Glycyrrhiza Uralensis (Licorice) Root Extract (12.5 to 100 mg/kg) inhibited carmine content in a dose-dependent manner; it also reduced carmine content to up to ~45% of controls. The extract inhibited granuloma weight by <50% of controls and inhibited the weight of pouch fluid by up to ~20%. The control values for carmine content, granuloma weight, and pouch fluid weight were 0.256 ± 0.017 mg, 353 ± 15 mg, and 144 ± 26 mg, respectively.

To test the effect of licorice constituents on angiogenisis by measuring tube formation, the authors also cloned EC from the thoracic aorta of male Wistar rats in DMEM supplemented with 10% heat-inactivated FBS, benzyl penicillin potassium and streptomycin sulfate. Two to 11 wk after confluence (5th to 14th passage) the cells were washed and replated (2.6 x 104 cells/well). The DMEM was supplemented with various concentrations of Glycyrrhiza Uralensis (Licorice) Root Extract except for controls. The cells were cultured for 4 d with the medium changed every other day. Tube formation was measured and total tube length was quantified. The experiment was repeated 3 times.

The extract (0.01 to 1 mg/ml) inhibited tube formation in a concentration-dependent manner; the IC_{50} was 0.518 mg/ml (0.352 to 0.764). The results for isoliquiritin and the other constituents are presented in the ANTI-INFLAMMATION EFFECTS section of the Appendix (Kobayashi et al. 1995).

Tokiwa et al. (2004) found that the aqueous extract from G. glabra slightly inhibited the growth (~20%) of rheumatoid arthritis cells with an MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay.

Aly et al. (2005) tested the anti-inflammatory effects of aqueous licorice extract (species unknown). Albino rats (n = 6) were injected i.m. with 1) sesame oil (0.2 ml; negative control), 2) diclofenac sodium (10 mg/kg; positive control), 3) licorice extract (250 mg/kg), or 4) licorice extract (250 mg/kg) and diclofenac sodium (10 mg/kg). After 30 min, carrageenan (0.1 ml; 1% in saline) was injected into the plantar surface of the right hind paw. The left hind paw was administered saline. The paws were removed after 5 h and weighed. Aqueous licorice extract decreased edema by 66.8% compared to the control ($p < .05$). The extract in combination with diclofenac sodium decreased edema 76.1% compared to control ($p < .05$).

Kim et al. (2006) tested the anti-inflammatory effects of aqueous and ethanol extracts of raw and roasted *G. inflata* root using RAW264.7 murine macrophages. To test viability, the cells were plated (2 x 104/well) and incubated with each licorice extract (0, 10, 30, or 100 µg/ml) for 24 h. The viable cells were counted by assay kit. There were no signs of cytotoxicity up to 30 µg/ml. Cell viability was decreased with both ethanol extracts, but not the water extracts, at 100 µg/ml ($p < .001$) compared to controls.

The amount of nitrite and prostaglandins (PGE2) produced by RAW264.7 cells exposed to each type of licorice extract was measured. The cells were plated (5 x 105 cells/well) for 12 h then treated with each licorice extract (10 µg/ml) in lipopolysaccharides (LPS; 1 µg/ml) for 18 h. The culture supernatant was analyzed by enzyme linked immunosorent assay (ELISA) for PGE2 production and by the Griess reagent system for nitrite production. Both roasted and raw ethanol extracts inhibited nitrite production (9.51 ± 0.1 and 19.8 ± 1.4 µM, respectively) compared to controls (~32 µM; $p < .001$). PGE2 production was inhibited by 76% and 69%, respectively ($p < .001$). The authors stated that these results suggest ethanol extracts of roasted and raw licorice root exerted anti-inflammatory effects.

Immunoblotting analysis was performed to measure the ability of these extracts to induce nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) activity in RAW264.7 cells. The cells were plated and incubated in each extract (30 µg/ml) in LPS (1 µg/ml) for 18 h. iNOS and COX-2 levels were reduced in the ethanol extract of roasted and slightly reduced in the ethanol extract of raw licorice. The water extracts did not have any inhibitory effects.

Cytokine assays (ELISA) were performed to measure the amount of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6; pro-inflammatory cytokines) in the supernatant of RAW264.7 cells (5 x 105 cells/well) incubated in both ethanol extracts (10 µg/ml) in LPS (1 µg/ml) for 24 h. Ethanol extract of roasted licorice inhibited TNF-α production ($p < .0001$) and IL-6 production ($p < .0001$). Ethanol extract of raw licorice only inhibited IL-6 production ($p < .001$).

A fluorescence-activated cell sorting analysis was performed to detect accessory molecules on RAW264.7 cells (5 x 105 cells/well) exposed to the ethanol extracts of roasted and raw licorice root (0, 10 or 30 µg/ml) with LPS (1 µg/ml). CD14 and CD86 cell surface molecules were expressed in large numbers on the LPS-simulated RAW264.7 cells; TLR4 expression was down-regulated on LPS-stimulated cells. The ethanol extract of roasted licorice decreased the expression of CD14 by ~20% ($p < .01$) and ~35% ($p < .001$) at 10 and 30 µg/ml; ethanol extract of raw licorice decreased expression by ~20% ($p < .01$). Neither extract affected the expression of CD86 or TLR4.

The effect of exposure of ethanol extract of roasted licorice root (30 µg/ml) on transcription nuclear factor-κB (NF-κB; a major

transcription factor involved in the release of proteins that mediate the inflammatory response) activation in RAW264.7 cells exposed to LPS (1 µg/ml) was observed by immunoblotting over 120 min. LPS-induced DNA binding of NF-κB was inhibited in LPS-stimulated RAW264.7 cells exposed to ethanol extract of roasted licorice root ($p < .001$).

In an in vivo study, female ICR mice (8 wk old; n = 10) were orally administered ethanol extract of either roasted or raw licorice root (10 mg/kg) 24 and 2 h before an i.p. injection of LPS (15 mg/kg). The mice were observed for 5 d for survival. The 3-day survival rate for LPS-exposed mice without any licorice treatment was 30%, 90% with treatment of ethanol extract of roasted licorice, and ~50% with ethanol extract of raw licorice. The survival rates were maintained until day 5.

An ethanol extract of roasted or raw licorice root (10 mg/kg) was orally administered to another set of mice (n = 5, 3 sets of experiments) 24 and 2 h before an i.p. injection of LPS (200 µg/kg). Blood samples were collected 6 h later and TNF-α, IL-6, and IL-10 (which has a strong anti-inflammatory effect) were measured. The ethanol extract of roasted licorice root decreased the TNF-α level ($p < .0001$) and the IL-6 level ($p < .0001$) and increased the IL-10 level ($p < .001$) compared to controls. The ethanol extract of raw licorice root decreased the TNF-α level ($p < .001$) and IL-6 level ($p < .001$) and increased the IL-10 level ($p < .0001$). The authors stated that ethanol extract of roasted licorice root exerts anti-inflammatory effects on both in vitro cell cultures and animal models (Kim et al. 2006).

Liao et al. (2007) tested the antioxidative activity of aqueous Glycyrrhiza Uralensis (Licorice) Root Extract using the oxygen radical absorbance capacity (ORAC) assay. The extract (12.5, 25, 50, or 100 mg/l; 20 µl;) was compared to Trolox (6-hydroxy-2,5,7,8-tetramethyl-2-caroxylic acid; a water-soluble analog of vitamin E). The extract had a similar antioxidant activity (AUC; 1184 ± 87 µmol Trolox equivalence/g) than vitamin E (1162 µmol Trolox equivalence/g). Inhibition of iNOS activity by the extract was determined in a nitric oxide assay using RAW 264.7 macrophages and compared to unstimulated and LPS-stimulated assays. Unstimulated macrophages produced 0.5 ± 0.1 µM nitrite. LPS-stimulated macrophages produced 38.9 ± 2.3 µM nitrite. Glycyrrhiza Uralensis (Licorice) Root Extract at 50 and 500 mg/l stimulated macrophages to produce 28.8 ± 8.3 (26.0% inhibition effect) and 31.0 ± 7.4 µM (20.3 inhibition effect) nitrite, respectively.

Račková et al. (2007) investigated the mechanism of the anti-inflammatory effects of Glycyrrhiza Glabra (Licorice) Root Extract. The extract (0.001 to 0.06 mg/ml) was tested using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay. The extract had high antiradical activity (EC_{50} = 0.130 ± 0.006 mg/ml) compared to Trolox, a standard antioxidant (concentration producing 50% contraction relaxation (EC_{50}) = 0.0028 ± 0.00005 mg/ml). A test using unilamellar 1-alpha-dioleylphosphatidyl choline (DOPC) liposomes (0.8 mM) was used to evaluate antioxidant activity of Glycyrrhiza Glabra (Licorice) Root Extract. The inhibition of lipid peroxidation was potent with the extract (IC_{50} = 0.009 ± 0.0025 mg/ml) but lower than for Trolox (IC_{50} = 0.0182 ± 0.0049 mg/ml). In a chemiluminescence assay using fresh human blood, the extract showed a high inhibitory activity with both phorbol-12-myristate-13-acetate (receptor-bypassing stimuli; IC_{50} = 0.0049 ± 0.0017%) and opsonized zymosan (receptor operating stimuli; IC_{50} = 0.058 ± 0.019%) stimulation. The authors concluded that Glycyrrhiza Glabra (Licorice) Root Extract has beneficial effects on neutrophil functions.

Anti-diabetic Effects

Licorice Extract

Yun-ping and Jia-qing (1989) examined the effect of licorice extract on diabetes. Male Sprague-Dawley rats were made diabetic by a single injection of streptozotocin (STZ; 70 mg/kg, i.p.) following an overnight fast. The animals were found to be hyperglycemic at 48 h. The rats were kept on routine feed and water without treatment for 2 wk. At 2 wk, 7.5 ml/kg/d of licorice was given orally by gavage for 1 week. Blood samples were taken before treatment, on the last day of treatment and 1 week after treatment. There was no change in blood glucose levels at the end of treatment. However, 1 week after treatment blood glucose levels rose ($p < .01$). Since this also occurred in the group without any treatment, it was attributed to the gradual deterioration of the function of the pancreatic islets. The sorbitol levels in the red blood cells (RBC) of the treated rats before treatment were greater than the control rats (120.3 ± 26.1 nmol/g hemoglobin vs. 61.9 ± 15.4 nmol/g hemoglobin). After treatment with licorice extract, the sorbitol levels decreased ($p < .05$). The sorbitol levels were restored to their untreated levels after 1 week without treatment. The authors concluded that due to the reduction of RBCs sorbitol levels, possibly through inhibition of aldose reductase, licorice extract may be useful in the control of clinical complications of diabetes.

Swanston-Flatt et al. (1990) tested the use of aqueous licorice root extract (from *G. glabra*) as a preventive measure in the development of diabetes in adult male mice. Licorice root was incorporated into the feed pellets of the mice (n = 5 to 7) at 6.25% by weight supplied ad libitum. Licorice root extract was also supplied to another group of mice in drinking water supplied ad libitum. The treatment water was prepared by adding 1 g licorice root extract to 400 ml cold water which was boiled for 5 min and infused for 15 min. On day 12 of treatment, the mice were injected with 200 mg/kg i.p. STZ in 0.5 mol/l citrate buffer. Blood samples were obtained on days -4, 3, 10, 17, 24, 30, and 40. Blood glucose was assayed and plasma insulin was determined.

Licorice root extract did not alter any of the parameters measured (body weight, food and fluid intake, basal plasma glucose, insulin) during the 12 d before induction of diabetes. Licorice root extract did not affect basal plasma glucose and insulin concentrations in STZ diabetic mice. Licorice root extract decreased the hyperphagia (~20%) and polydipsia (~45%) compared with controls but did not alter the hyperglycemia or hypoinsulinemia. The authors suggested that licorice root extract would not retard the development of STZ diabetes in mice (Swanston-Flatt et al. 1990).

Kuroda et al. (2003) tested the ethyl acetate extract of *G. uralensis* root for peroxisome proliferator-activated receptor (PPAR)-γ ligand-binding activity. PPAR-γ agonistic activity is useful for the prevention and improvement of type-2 (non-insulin dependent) diabetes.

PPAR-γ ligand binding activity was accomplished with a GAL-4-PPAR-γ chimera assay system using CV-1 monkey kidney cells. The cells were incubated for 24 h, washed, and transfected with pM-hPPAR-γ and p4xUASg-tk-luc. A mock control used pM-hPPAR-γ and p4xUASg-tk-luc tranfected into CV-1 cells. After 24 h of transfection, the medium was changed to DMEM and various concentrations of the extract and the cells were incubated for an additional 24 h. The cells were washed with Ca2+ and Mg2+ containing PBS with a luminescent signal. Intensity of emitted luminescence, measuring ligand-binding activity, was determined.

The relative luminescence intensity was 2.8 at a sample concentration of 30 µg/ml, which was approximately equivalent to 1.0 µM (0.44 µg/ml) troglitazone, a potent synthetic PPAR-γ agonist. The ethyl acetate extract of G. uralensis had stronger PPAR-γ ligand-binding activity than 70 other spice and herb extracts tested. The extract was fractioned using silica gel column chromatography and the fractions were tested. Six compounds were found to have PPAR-γ agonist activity: an unnamed pale-yellow gum with a molecular formula of $C_{22}H_{24}O_5$, glyasperin D, glyasperin B, glycyrin, dehydroglyasperin C, and glycycoumarin, in descending order. The aqueous extract was also tested and exhibited no activity compared to control (Kuroda et al 2003).

Mae et al. (2003) tested the effects of licorice extracts (ethanol, ethyl acetate, acetone, and water) on diabetes in a series of experiments. In the first experiment, PPAR-g ligand-binding activity was assayed in a GAL4-PPAR-g chimera assay system. CV-1 monkey kidney cells were grown in supplemented DMEM and 2 plasmids, pM-hPPAR-g and p4xUASg-tk-luc, were transfected into the cells. Controls were transfected with pM and p4xUASg-tk-luc. After 24 h of transfection, the medium was changed to DMEM supplemented with 100 ml/l charcoal-treated FBS and the licorice extract at 10 and 30 mg/l in dimethyl sulfoxide (DMSO). The cells were washed with PBS containing Ca2+ and Mg2+ and luciferase activities were measured in a TopCount plate-reader. Relative luciferase activity was calculated as a ratio of mean luciferase activity (n = 4) with that of the controls.

Of 70 edible plants such as spices and herbs tested, the licorice extracts had the highest PPAR-g ligand-binding activity, except for the aqueous extract. The aqueous extract was not different from controls whereas the other extracts increased activity 2- to 4-fold ($p < .05$).

In a second experiment, the prevention and amelioration of diabetes by the ethanol licorice root extract was tested using KK-Ag mice. Three groups of 6-week-old mice (n = 5) were fed purified powdered diet or diet plus 0.1 or 0.2 g/100 g licorice extract for 4 wk. Food and water were available ad libitum. Blood glucose levels were measured before and after the treatment period. After the treatment period, the mice were killed to collect blood and periuterine, perirenal, and mesenteric adipose tissues. The tissues were pooled to calculate relative weight to body weight.

Dose levels of the extract were calculated to be 152 and 285 mg/kg/d at intake levels of 0.1 and 0.2 g/100 g, respectively, corresponding to an intake of 25 and 48 mg/kg of the total of the 4 active compounds found to be in the extract (glycycoumarin, glycyrin, dehydroglyasperin C, and dehydroglyasperin D). The inclusion of ethanol extracted licorice did not affect body weight or food intake. The blood glucose level in the control was 8.2 ± 0.5 mmol/l before feeding and 28.0 ± 1.5 mmol/l after 4 wk of treatment, indicating the mice were becoming hyperglycemic. The blood glucose levels for both treatment groups were decreased ($p < .001$) indicating suppression of the increase in blood glucose levels. No ill effects were reported in the mice. Serum insulin levels in the treatment groups were lower than controls ($p < .05$) and the relative weight of intra-abdominal adipose tissue was lower in the 0.2 g/100 g licorice treatment group when compared to the control ($p < .05$).

A third experiment was similar to the second. Thirteen-week-old female KK-Ag mice were fed a powdered standard diet or that diet containing ethanol extracted licorice at 0.1 or 0.3 g/100 g diet ad libitum for 4 wk (n = 6). The same measurements as the second experiment were performed. Body weight gain and feed consumption did not differ between the groups. Licorice extract intake was 110 and 322 mg/kg/d for the 0.1 and 0.3 g/100 g diets, respectively, and the licorice extract intake corresponded to 18 and 54 mg/kg/d, respectively, of the total of the 4 active compounds (glycycoumarin, glycyrin, dehydroglyasperin C, and dehydroglyasperin D). The blood glucose levels in the treatment groups were lower than the control after treatment ($p < .05$). There was no difference in serum insulin or relative weight of intra-abdominal adipose tissue.

In a fourth experiment, the authors fed a high-fat diet to 8-week-old male C57BL mice for 8 wk to induce obesity. The mice then were divided into 3 groups (n = 7). The control group was fed powdered commercial diet and the other 2 groups were fed the same diet containing licorice ethanol extract at 0.1 and 0.2 g/100g ad libitum for 4 wk. The mice were killed and perirenal, periuterine, and mesenteric adipose tissues were collected and relative weights were calculated.

The total adipose tissue weights for mice fed a high fat diet for 8 wk were approximately twice that of mice on a normal diet (data not shown by authors). In this experiment, body weight gain and feed consumption did not differ between the 3 groups. Relative weighs of periuterine and perirenal adipose tissues of the mice fed licorice extract were lower than controls ($p < .05$) but relative weights of mesenteric adipose tissue were not different. Relative weight of intra-abdominal adipose tissue of the licorice extract fed mice was lower than controls ($p < .05$).

In a final experiment, the authors divided male SHR rats into 3 groups (n = 8) and orally administered ethanol extracted licorice

suspended in propylene glycol (300 mg/kg/d), propylene glycol only (3 ml/kg/d), or enalapril maleate in methyl cellulose (20 mg/kg/d; positive control) for 3 wk. Systolic blood pressure (BP) was measured weekly.

Body weight gain did not differ between the groups. The control group's BP increased over the 3 wk of treatment ($p < .001$). The BP of the positive control group was lower than the control group ($p < .01$). The treatment group was not different than the negative control for the first 2 wk but was lower at the end of the third week ($p < .01$). No ill effects to the rats were reported. The authors concluded that ethanolic licorice extract prevents and ameliorates diabetes and associated abdominal obesity and hypertension so it may be effective in preventing and/or ameliorating the metabolic syndrome (Mae et al. 2003).

Nakagawa et al. (2004) found that licorice flavonoid oil (prepared by further extracting licorice ethanolic extract with medium-chain triglycerides) placed in the food of female genetically type 2 diabetic KK-Ay/Ta mice at 2% resulted in lower weights of abdominal adipose tissues ($p < .05$). The licorice flavonoid oil suppressed the body weight gain and abdominal fat accumulation induced by a high-fat diet. No ill effects of the licorice extract were noted.

Ko et al. (2007) tested the anti-diabetic effect of (ethanol, 70%) Glycyrrhiza Uralensis (Licorice) Root Extract and roasted Glcycyrrhiza Uralensis (Licorice) Root Extract on male C57BL6J mice. The splenic portion of the pancreas was removed from each mouse (8 wk old) to induce diabetic signs. The mice ($n = 10$) were administered cellulose (orally; control); exendin-4 (150 pmol/kg s.c.; positive control); the extract (orally; 1 mg/kg in DMSO); or roasted extract (orally; 1 mg/kg in DMSO) daily for 8 wk. At 3-week intervals the mice were administered an oral glucose tolerance test.

There were no differences in body weights between the groups. The 2 extract groups had decreased fasting serum glucose levels, similar to that of the exendin-4 group and lower than the cellulose control group ($p < .05$). The same was true for post-prandial serum glucose ($p < .05$). There was no differences among the groups for fasting serum insulin. The control and the Glycyrrhiza Uralensis (Licorice) Root Extract had similar values for post-prandial serum insulin; both roasted extract and exendin-4 had similar values, higher than the other 2 groups ($p < .05$).

When 3T3-L1 fibroblast were pretreated with various concentrations of Glycyrrhiza Uralensis (Licorice) Root Extract (roasted and unroasted) for 8 h, followed by stimulation with insulin (0.2 nm) for 30 min, insulin-stimulated glucose uptake was increased by both extracts in a dose-dependent manner ($p < .05$). Triglcyceride accumulation of 3T3-L1 fibroblasts during differentiation to adipocytes when treated with the extracts was increased ($p < .05$). Isolated pancreatic islets from the mice were incubated in high (2 mm) or low (20 mm) glucose media then treated with Glcycrrhiza Uralensis (Licorice) Root Extract (roasted and unroasted). There was no effect in the low glucose treatment. In the high glucose treatment, both extracts increased glucose-stimulated insulin secretion, the roasted extract more than the unroasted. The authors suggest that both the roasted and unroasted Glycyrrhiza Uralensis (Licorice) Root Extracts improve impaired glucose tolerance, possibly by enhancing insulin sensitivity. Because of the greater reaction of the islets, the roasted extract has the better anti-diabetic action (Ko et al. 2007).

Antioxidant Effects

Licorice Extract

Shetty et al. (2002) exposed plasmid pBR322 DNA (20 to 25 µg/ml) and rat liver microsomal membranes (400 to 600 µg microsomal protein/ml) in 0.1 M sodium phosphate buffer (pH 7.0) to 60Co-gamma rays at 9.6 Gy/min in the presence (5 to 100 µg/ml) and absence of G. glabra root methanol extract. There was dose-dependent protection from gamma rays by the licorice extract; there was almost complete protection from up to 500 Gy at 100 µg/l. Lower concentrations of the root extract (10 µg/ml) offered complete protection against radiation damage to the membranes. Protection to DNA in vitro was dose-dependent, as well. There was a reduction of 40% of supercoiled form of plasmid DNA (band 2) by 500 Gy of gamma radiation and the presence of the root extract (20 µg/l) resulted in almost complete (99%) protection.

Naik et al. (2003) measured the potential of G. glabra aqueous extract as an antioxidant. Male albino Wistar rats (180 to 200 g) were killed and the livers removed, washed, and homogenized. Mitochondria were isolated by centrifugation, washed and centrifuged again. The mitochondria were washed and suspended in 10 mM phosphate buffer at pH 7.4 at 2 mg protein/ml in glass vials. All procedures were carried out at 0 to 4°C. Protein was estimated by the Lowry method. Lipid peroxidase assay (LPO) was carried out by γ-radiolysis of rat liver microsomes with or without licorice extract. The lipid peroxidation was estimated in terms of TBARS.

In the controls, LPO increased with increasing absorbed dose. In the presence of licorice extract there was a decrease in TBARS, which the authors interpreted as indicating the extract protected the microsomes from the radiation induced LPO (p-value not provided). The extract protected in a dose-dependent manner and the effect was greater at lower doses of radiation up to 60 µg/ml extract.

To examine licorice extract's effect on antioxidant SOD, the mitochondria were exposed to a total dose of 570 Gy with and without the licorice extract. The change in absorbance was monitored at 320 nm for 6 min against the blank. SOD units/mg protein was computed from the difference between the absorbance of standard and the sample.

With all samples, irradiation with γ-radiation initially lead to increased SOD generation to combat oxidative stress, but further irradiation caused a decrease in the enzyme due to its own damage by the radiation.

A 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay to evaluate the antioxidant activity of licorice extract was conducted using 500 µM DPPH in methanol mixed with equal volume of licorice extract and kept in the dark for 20 min or with just DPPH as a

control. The absorbance at 517 nm was monitored with different concentrations of the extract. The amount of extract at which the absorbance at 517 nm decreased to half its initial value was determined to be the IC_{50}. The IC_{50} was reported to be >70 µg/ml. (Naik et al. 2003).

Di Mambro and Fonseca (2005) evaluated the free radical scavenging activity of various concentrations of *G. glabra* extract using changes of chemiluminescence intensity of the H_2O_2-luminol-horseradish peroxidase (HRP) system. Hydrogen donor ability was evaluated by measuring absorbance at 517 nm using an ethanol solution of DPPH•. LPO was assayed as malondialdehyde (MDA) using mitochondria as the lipid source. No controls were presented.

It was found that *G. glabra* extract showed great antioxidant and free radical scavenging activities. The IC_{50} for the chemiluminescence assay was 0.09 ± 0.004 µl/ml and 0.32 ± 0.007 µl/ml for stable free radical scavenging activity. The DPPH• assay showed an ~20% inhibition of hydrogen donor activity. The IC_{50} for the lipid peroxidation assay was 0.07 ± 0.002 µl/ml (Di Mambro and Fonseca 2005).

Yadav and Bhatnagar (2007) tested the lipid peroxidation effects of water:ethanol (1:1) Glycyrrhiza Glabra (Licorice) Root Extract on Wistar rat liver homogenate. The supernatant of the liver homogenate was incubated with iron chloride (Fe3+; 50 µM); iron (50 µM) and ascorbic acid (50 µM); iron (50 µM), ascorbic acid (50 µM), and hydrogen peroxide (50 µM), or nothing with or without the extract at various concentrations for 1 h. LPO by all oxidants was inhibited Glycyrrhiza Glabra (Licorice) Root Extract compared to the control ($p < .05$ and .001).

Various concentrations of Glycyrrhiza Glabra (Licorice) Root Extract were incubated with the supernatant above and iron chloride. The IC_{50} for the inhibition of iron chloride-induced LPO was 28.0 ± 2.0 µg.

The effect of Glcyrrhiza Glabra (Licorice) Root Extract on the initiation and propagation phases of oxidation of lipids in liver homogenate was measured in the presence of iron chloride. LPO was measured at 1 to 6 h in the presence or absence of iron chloride (50 µM). The extract inhibited the initiation phase only, not the propagation phase.

The authors used ferric ions to measure the reducing power of the extract as an indicator of potential antioxidant activity. There was concentration-dependent reduction by the extract. The authors measured the superoxide anion scavenging activity of the extract using phenazine methoxulphate-nicotinamide adenine dinucleotide (NADH). Glycyrrhiza Glabra (Licorice) Root Extract had 30% inhibition of superoxide radical scavenging activity at 200 µg. The polyphenol content of the extract was found to be 0.46 ± 0.01 mg/ml (Yadav and Bhatnagar 2007).

Licorice Root Powder

Konovalova et al. (2000) compared the antioxidative effects of *G. glabra* root powder to other antioxidants. Male Wistar rats (180 ± 20 g) were administered a 0.5 ml water suspension of a vitamin preparation (2 mg β-carotene/100 g), licorice root powder (200 mg/100 g body weight), or the vitamin preparation and licorice root powder by gastric tube (n = 6 to 8). Control rats were administered 0.5 ml water (n = 8).

The rats were killed and the liver perfused and the heart washed with isotonic potassium chloride. The organs were cooled and homogenized in buffer (pH 5.9). The homogenates were incubated with 0.5 mM ascorbate with constant shaking and aerobic conditions. The medium was sampled every 1 to 5 min and content of secondary products of lipid peroxidation estimated by reaction with thiobarbituric acid. Optic density of the original medium and the samples were measured at 532 nM. Aliquots of liver homogenate (0.5 ml) were extracted with a chloroform-methanol mixture (2:1) and the chloroform evaporated in vacuum and dried under argon flow to constant weight. Lipid content was measured gravimetrically.

G. glabra root powder administered for 30 d inhibited ascorbate-dependent oxidation of endogenous polyenic lipids in rat liver and myocardium. The lag phase in rat liver increased from 321 ± 77 sec in controls to 878 ± 209 sec in those treated with licorice root powder ($p < .05$). This was similar to the effect of carinat (960 ± 161 sec, $p < .05$). There were no oxidation products in the myocardium medium of rats treated with licorice powder after 3 h. Livers from rats treated with licorice root powder had decreased lipid peroxide content ($p < .05$) by 25%. The antioxidant effects of licorice root powder were comparable to those of β-catotene (Konovalova et al. 2000).

Immune Effects

Licorice Extract

Nose et al. (1998) tested the effects of the water extracts of the shoots and roots of *G. glabra* and the hairy roots of *G. uralensis* on the nitrous oxide production of murine peritoneal macrophages. The macrophages were obtained from ICR mice that were lavaged with 8.0 ml cold RPMI-1640 4 d after injection of 2.0 ml 3.0% thioglycolate broth. The cells were plated in 96-well cluster plates at 2 x 106 cells/ml. After 2 h, non-adherent cells were washed away. The medium was replaced with new medium containing various concentrations of the extracts and the cells incubated for 20 h. The cell culture supernatant was extracted (100 µl) and combined with the same amount of Griess reagent and incubated at room temperature for 10 min in a 96-well microplate. Absorbance was read at 577 nm. Sodium nitrite was diluted in the medium and used as a standard. The nitrous oxide production increased in a dose-dependent manner from ~1 µM for the control to ~6 µM nitrous oxide for 200 µg extract/ml.

Antiviral Effects

Licorice Extract

Badam (1997) tested the anti-viral activities of licorice root on the Japanese encephalitis virus (JEV). Stable porcine kidney cells in culture were infected with JEV strains Nakayama, P-20778, or 821564 XY48. One hour after infection, the cells were exposed to various concentrations of licorice root for 96 hours. Infected control cells received no treatment. The cells were then stained with Amido black, rinsed, dried, and the plaques were counted.

The experiment was repeated 3 times. Licorice at 1000 µg/ml completely inhibited plaque formation in all strains. No signs of cytotoxicty were observed at these concentrations.

Kapadia et al. (2002) tested the ethanol extract of *G. glabra* root on the inhibition of TPA-promoted Epstein-Barr virus early antigen (EBV-EA) activation. The Epstein-Barr virus activation was assayed using the EBV genome carrying lymphoblastoid Raji cells. The cells were incubated for 48 h in 1 ml of medium containing butyric acid, TPA, and various amounts of extract. The EBV-EA inhibitory activity of the ethanol extract of licorice was compared with a positive control experiment (100%). At 100 µg/ml, inhibition EBV-EA activation was 7.4 ± 0.6% with 70% viability; at 10 µg/ml, inhibition was 52.9 ± 1.3% with 100% viability; and at 1 µg/ml, inhibition was 94.0 ± 0.2% with 100% viability.

Anti-parasitic Effects

Licorice Extract

Rhee et al. (1982) tested licorice extract as a wormicide on *Clonorchis sinensis*, a Chinese river fluke that transmits clonorchiasis to humans, dogs, and cats. *G. uralensis* (75 g) was dried then boiled in 500 ml water for 1.5 to 2 h. The reduction was filtered and boiled again in ~200 ml water until reduced to 20 ml. One ml of the stock solution was diluted with 10 ml normal saline and placed in convex dishes. Twenty metacercaria, 20 cercaria, or 10 adults were placed in each dish and observed under a stereomicroscope. The metacercaria had persistent normal movement for 10 min, first death at 15 min, and all were dead at 20 min. The cercaria had tails separating from the body at 2 min and all were dead at 5 min. The adults had persistent normal movement for 25 min, first death at 45 min, and all were dead at 50 min.

Antimicrobial Effects

Licorice Extract

Motsei et al. (2003) tested the antifungal effects of water and solvent extracts of *G. glabra* on *Candida albicans* obtained from clinical isolates from a 5-month-old baby and an adult. A standard strain (ATCC 10231) also was used. The rhizomes of *G. glabra* (1 g) were extracted with 10 ml water, ethanol, ethyl acetate, or hexane for 1.5 h. Using water with the water extract and DMSO for the other extracts, the extracts were dissolved to the concentration of 100 mg/ml. The extracts were tested using a broth microdilution test with serial dilutions.

The minimum inhibitory concentration (MIC) for each extract are shown in **Table 21**.

To test the stability of the stored extracts, the test was repeated while the extracts were being stored at 4, 23, and 33°C over 7 d. All the extracts lost activity after only 1 d of storage at all temperatures and the MIC had increased to >25 mg/ml by day 3 (Motsei et al. 2003).

Nam et al. (2003) tested the effects of the extract of *G. glabra* on the anti-chemotactic effect on polymorphonuclear neutrophils (PMNs) by *Propionibacerium acnes*. *G. glabra* (part of the plant not specified) was minced and boiled twice in ethanol for 2 h. It was then filtered and evaporated. P. acnes (ATCC 6919) was anaerobically cultured on brain-heart infusion (BHI) agar for 5 d. A 2 ml aliquot of bacterial suspension in PBS was transferred to a culture flask containing 300 ml fresh BHI medium. The flask was anaerobically incubated for 72 h. The cells were cooled and centrifuged. The pellet was washed in PBS and resuspended in PBS to make a 1.0% *P. acnes* suspension. PMNs were isolated from rat arterial blood by centrifuge. Red blood cells were removed by washing with saline. The PMNs were washed with PBS. Neutrophil chemotactic activity was measured using the microchemotaxis assay using 30 µl of the licorice extract and 30 µl of the PMN suspension for 1 h. The methanol extract of G. glabra inhibited the chemotactic effect of *P. acnes* on PMNs ($p < .05$).

The antibacterial activity against P. acnes (strains ATCC 6919 and 11827) was tested by the medium dilution method. The bacteria were plated at 1 x 106 colony forming units/ml in BHI medium.

G. glabra extract had an MIC of 200 µg/ml for ATCC 6919 and 100 µg/ml for ATCC 11827. Neither strain of P. acnes developed resistance (Nam et al. 2003).

Hwang et al. (2004) reported that methanol extracts of *G. glabra* exhibited antibacterial activity against *Streptococcus mutans* ATCC 25175.

Table 21. MIC of extracts of G. glabra rhizomes against C. albicans clinical isolates from a 5-month-old baby and an adult, and a standard of the strain ATCC 10231 (Motsei et al. 2003).

	MIC (mg/ml)		
Rhizome extracted with:	**5-month old baby**	**adult**	**Atcc 10231**
Water	1.56	12.5	12.5
Ethanol	0.52	2.09	2.09
Ethyl acetate	2.09	1.03	2.09
Hexane	>8.35	>8.35	>8.35

Statti et al. (2004) collected *G. glabra* from various locations around Calabria, Italy and tested each specimen for its antimicrobial and antifungal properties. The licorice extract was obtained through methanol extraction process at room temperature by maceration. Antibacterial activity was determined by the MIC method on various bacteria. The effective concentrations ranged from 50 µg/ml to > 400 µg/ml. The antifungal activity was determined by the disc diffusion assay. The inhibitory effects ranged from 1 ± 0.01% to 6.7 ± 0.9% for *Trichophyton mentagrophytes* at 100 µg/ml and 15 ± 0.3% to 23.3 ±1.8% at 500 µg/ml. For the inhibition of growth of *Phytlum ultimum* at 100 µg/ml the range was 8.3 ± 1.2% to 34.5 ± 1.4% and 51 ± 2.0% to 66.2 ± 1.0%. Control was 0.3% DMSO. The authors did not clarify which samples came from which part of Calabria. The authors concluded that the samples differed remarkably in chemical composition and biological activity.

Gupta et al. (2008) incubated *Mycobacterium tuberculosis* H37Rv (Atcc 27294) and *M. tuberculosis* H37Ra (ATCC 25177) in the ethanol extract of *G. glabra* root using the BACTEC assay. The MICs were 500 ± 0.0 for both strains tested.

Drug Interactions

Licorice Extract

Moon and Kim (1996) tested the effects of the methanol extract of *G. glabra* roots on the metabolism of acetaminophen with regards to the uridine diphosphoglucuronosyltransferase (UGT) enzymatic activity. UGT1A accommodates flat, planar substrates such as acetaminophen and p-nitrophenol and is induced by 3-methylcholanthrene (3-MC). Male Sprague-Dawley rats (220 to 240 g) were provided food and water ad libitum. They were treated with the licorice root extract (1 g/kg p.o.) or glycyrrhizin (23 mg/kg p.o.) for 6 d. 3-MC (20 mg/kg in corn oil ip) for 4 d was the positive control.

Acetaminophen (150 mg/kg in saline containing 10% mannitol) was injected into the saphenous vein. The concentrations of acetaminophen and its metabolites in bile, urine, and blood samples, collected for 2 h, were determined by HPLC. Acetaminophen and its metabolites were eluted with water/methanol/acetic acid (900:80:8 v/v) and monitored by absorbance at 254 nm. The rats were then killed by decapitation without anesthetic and the liver excised, rinsed with saline, blotted, and frozen. The UGT1A activity was measured in hepatic microsomes that had been isolated by centrifugation with p-nitrophenol as a substrate. Total RNA was isolated from the rat liver by the acid guanidinium thiocyanate-phenol-chloroform extraction method then processed by Northern blot analysis with 3-MC inducible UGT1A1 cDNA probe.

Pretreatment with the methanol extract of G. glabra root increased the biliary excretion rate of acetaminophen (AAP)-glucuronide over 2 h (76.2 ± 5.9 vs 119.0 ± 10.9 µmol/kg; $p < .05$) while decreasing that of AAP-sulfate (7.3 ± 0.8 vs 4.6 ± 0.3 µmol/kg; $p < .05$) compared to controls. AAP-glutathione was not affected. The pretreatment increased the biliary (156%) and urinary (132%) excretion of AAP-glucuronide conjugate; the biliary excretion of AAP-sulfate was decreased by 63%. The pretreatment did not affect the blood concentrations of any of the AAP conjugates.

The methanol extract of G. glabra root and glycyrrhizin increased the specific activity of UGT1A by 111% ($p < .01$) and 96% ($p < .05$), respectively, over controls. 3-MC increased the activity by 416% over controls ($p < .01$). UDP-glucuronic acid concentrations increased by 257% and 484%, respectively.

A single band of ~2.3 kilobases was detected in all lanes of RNAs tested indicating that the UGT1A1 gene is transcribed in rat livers. The mRNAs for the enzyme were induced by 3-MC treatment; neither the methanol extract of G. glabra nor glycyrrhizin influenced the enzyme. The authors conclude that the activation of UGT1A by G. glabra root extract was not due to the induction of mRNAs for the enzyme (Moon and Kim 1996).

Miller (1998) stated that the immunostimulating effects of licorice may offset the immunosuppressive effects of cyclosporine as well as the aldosterone antagonist and diuretic effects of spironolactone. The author speculated that licorice may adversely interact with monoamine oxidase inhibitors, and since licorice contains phytoestrogens, it may function similar to estradiol and may lead to symptoms of excess estrogen in women undergoing estrogen therapy.

Fugh-Berman (2000) stated in a review article that licorice may potentiate the action of corticosteroids.

Heck et al. (2002) stated that licorice root can interact with warfarin increasing the potential risk of bleeding.

Kumar et al. (2002) stated that *G. glabra*, taken as an complementary/integrative nutritional therapy by cancer patients may impair the action of drugs that cause potassium loss and may enhance the action of corticosteroids. These authors also suggested that *G. Glabra* It may also counteract the effectiveness of drugs used to treat hypertension.

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Consumer Product Testing Co. (1994) found Glycyrrhiza Glabra (Licorice) Root Extract in corn oil not to be orally toxic to albino rats (n = 10) up to 5 g/kg. All rats survived up to 14 day after a single oral administration.

Licorice Root Extract is listed as having an oral LD_{50} of 14,200 mg/kg for rats (Lewis 1996).

Maruzen Parmaceuticals Co., LTD. (1999) orally administered a single dose of Glycyrrhiza Glabra (Licorice) Root Extract (40% glabridin) in olive oil to Crj:CD-1(ICR) mice at 2250 to 5250 mg/kg with no mortality after 7 d. No abnormalities were observed at necropsy.

In a safety data sheet submitted by CTFA (2004e), Glycyrrhiza Glabra (Licorice) Root Extract, under the trade name Oil Soluble Licorice Extact P-TH (Polyol Soluble Licorice Extract P-TH), is listed as having a LD_{50} of > 5000 mg/kg in rats (p.o.). The LD_{50} was > 2000 mg/kg in mice (p.o.) for the Glycyrrhiza Glabra (Licorice) Leaf Extract.

Acute Parenteral Toxicity

Informatics, Inc. (1972) gave the LD_{50} values of Licorice Extract in mice via i.v. and i.p. routes are 251 and 641 mg/kg, respectively. *Sax's Dangerous Properties of Industrial Materials* lists Licorice Root Extract as having a parenteral LD_{50} of 1420 mg/kg for rats and 1500 mg/kg for mice (Lewis 1996).

Ambawade et al. (2002) i.p. administered ethanolic extract of G. glabra root (600 to 1000 mg/kg) to groups of male albino Swiss mice (n = 10; 22 to 25 g) and observed them for 24 h. There was no mortality to the mice up to 1000 mg/kg i.p.

Acute Subcutaneous Toxicity

Sax's Dangerous Properties of Industrial Materials lists Licorice Root Extract as having a subcutaneous LD_{50} of 4200 mg/kg for rats and 4000 mg/kg for mice (Lewis 1996).

Short-term Oral Toxicity

Licorice Extract

Webb et al. (1992) fed male F344 rats (~ 50 d old) powdered diets containing 0.38%, 1.5% or 3% (wt/wt) licorice extract (from Chinese and Russian licorice) ad libitum for 1 month or 3 months (n = 24). The rats were killed. Blood was collected for hematological, clinical, and phytochemical analysis. During necropsy, the liver and intestine were removed and preserved in formalin for histopathology. Aliquots of liver and intestine were processed for biochemical analyses. A second aliquot of each organ was frozen and used to determine adensoine 3',5'-cyclic monophosphate (cAMP), guanosine 3',5'-cyclic monophosphate (cGMP), and protein kinase C. Specimens of all organs were fixed in formalin, embedded, sectioned, and mounted on slides for review.

Feed consumption in the 0.38% and 1.5% groups was similar to controls; it was reduced by 8% in the 3.0% group (14.9 ± 0.22 vs 16.2 ± 0.30 g/week). Weight gain of the treatment groups were 5% to 15% greater than controls. Water consumption of the treatment groups was similar to controls.

Phytochemical analysis by HPLC was performed on the rats fed 3% licorice extract in their food for 1 month. They had 5.84 ± 0.43 µg/l Glycyrrhetinic Acid in the serum.

No diet additive-related anatomic lesions were found at necropsy in any of the animals at 1 or 3 months. Liver, kidney, and adrenal weights in test animals were similar to controls except for the mean liver weight of rats in the 3% group (10.62 g vs 9.43 g; p value not provided). All hemotological measurements were within normal ranges. Liver GST activity increased from 4.0% to 42.5% at 1 month and from 0 to 22.8% at 3 months over basal values as the licorice extract was increased to 3% ($p < .001$). There was no effect on the level of GST in the intestinal mucosa. None of the treatment groups were different from the control at 1 or 3 months for liver catalase or protein kinase C.

For ornithine decarboxylase, the activity in the liver in the 0.38% group was decreased by 46% compared to control; the activity in the intestine in the 1.5% group was decreased by 53% to 55%. The results were not dose- or time-dependent.

There were no differences observed in protein kinase C of the liver, xanthine oxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, bilirubin, 4-nitrophenol UDP glucuronosyl transferases, SOD, and GSH-Px. No change was observed in cAMP and cGMP levels in the liver or the intestine. The authors stated that the effects of 3% licorice root extract on the activities of the 4 enzymes, without unfavorable changes in clinical parameters, may partially account for the chemopreventive effects (Webb et al 1992).

Mirsalis et al. (1993) administered B6C3F1 mice feed containing 0, 0.8, 2.5, 8.0, or 25% licorice root extract for 30 and 90 d (n = 5). Mice in all groups except the 0.8% group had poor weight gain that coincided with low food consumption. Clinical signs in the 8 and 25% groups included thinness, hunched posture, a lump near the prepuce, alopecia, swollen irritation and redness around the anus and tail, rough fur, lethargy/depression, and weakness. Three mice in the 8% group and 3 mice in the 25% group died during the treatment period. At the 30- and 90-day necropsies, all treated mice had decreased body weights. Absolute liver weights were not affected by Licorice Root Extract treatment, but the relative liver weights (ratio of liver weight to body weight) were significantly increased in the 8% and 25% groups at 30 d and in the 2.5% and 25% groups at 90 d.

At 30 and 90 d, mice of the 8% and 25% groups also had multiple lesions on the kidneys, small thymuses, enlarged spleens, and mottled livers. These observations were also noted in mice of the 2.5% group at 90 d. The daily consumption rates (i.e., mg/kg/d) of licorice root extract were not calculated. The no observed effect level for licorice root extract in this study was 0.8% (Mirsalis et al. 1993).

Gaworski et al. (1994) orally administered food grade licorice extract (species andextraction solvent unknown; 1250, 2500, or 5000 mg/kg in water; 10 mg/kg) to female CD-1 mice (n = 30) for 5 d. The control group was administered water. Ten of the mice in each group were used for a plaque-forming cell assay where 5 mice were injected ip with cyclophosphamide (80 mg/kg) 24 h prior to the assay, serving as a positive immunosuppression control group. The mice were observed daily for signs of toxicity and necropsied. The remaining 20 were used for a host resistance assay. Following the third day of dosing, the mice were injected with *Listeria monocytogenes* in saline. The mice were observed daily for signs of toxicity for 10 d.

The mice were then injected with sheep red blood cells (from a single sheep; 2 x 108) at the end of the 5-day exposure period. The positive control mice received an injection of cyclophosphamide (80 mg/kg) and the naive group received saline. The mice were killed and the spleens removed and weighed. Single spleen cell suspensions were prepared and viability assessed.

There was no difference in mortality between the treated groups and control in the host resistance assay. There was no difference in the viability or activity of spleen cells between the treated groups and the control. The authors conclude that licorice extract

did not aversely modulate the immune response (Gaworski et al. 1994).

Licorice Root Powder

Girerd et al. (1958) compared the effects of Glycyrrhiza Glabra (Licorice) Root Powder to ammoniated glycyrrhzin and dichloroacetate (DCA). Male Sprague-Dawley rats were implanted with 3 DCA pellets (25 mg) in the interscapular area (n = 19). The rest of the rats had sham implantations. Within these groups, the rats were administered daily doses of Licorice Root Powder (10 g/kg/d dissolved in boiling water; n = 14), ammoniated glycyrrhizin (1 g/kg/d in boiling water; n = 13), or a daily sham gastric catherization (n = 10) for 50 d. All rats had free access to saline (0.87% NaCl). Daily observations were made and BP and weights were measured periodically. The rats were killed on day 50 and necropsied.

Survival was 10/10 of the control group, 12/19 of the DCA group; 5/14 of the licorice root powder group, and 10/13 of the Glycyrrhizin group. The control rats had a moderate BP rise to 120 mm Hg by the end of treatment. The BP of the other groups rose to hypertensive levels ($\geq$178 mm Hg; $p < .001$). Saline intake was similar in the glycycrrhizin and the control groups. Saline intake of the DCA and the licorice root powder groups were similar and greater than the control group ($p < .001$) and the glycycrrhizin group ($p < .01$). The glycyrrhizin and control groups were similar with regards to growth. The DCA group and the licorice root powder group were similar during the first week. After that, the growth leveled off then the rats began to lose weight from day 20. At the end of the experiment, both treatment groups were lower in weight than controls ($p < .001$) and the glycyrrhizin group ($p < .001$).

Kidney weights in the licorice root powder group were similar to controls and greater in the DCA ($p < .001$) and the Glycyrrhizin group ($p < .05$). All 3 had greater relative kidney weights ($p < .001$). Heart weights were similar to controls in the Licorice Root Powder and Glycyrrhizin groups and higher in the DCA group ($p < .05$). All groups had greater relative heart weights ($p < .001$), greater absolute ($p < .05$ and .01), and relative adrenal weights ($p < .02$ and .001) compared to controls. All the group had lower absolute ($p < .01$ and .001) but not relative pituitary weights. The DCA and Licorice Root Powder groups had lower testes weights ($p < .001$ and .01) but only the licorice root powder group had greater relative testes weights compared to controls ($p < .001$). There were no differences between the Glycyrrhizin and control testes weights.

Microscopic examination revealed marked arteriolar necrosis and hyalinization, especially in the kidneys, heart and testes of all the treated animals. The kidneys were the most severely damaged organs with intense glomerular hyalinization, tubular dilations with atrophy of the tubular cells, hyaline casts, and proliferation of the interstitial tissue surrounding the glomeruli and tubules. All lesions were severe in the DCA and licorice groups. No lesions were found in controls. The authors suggested that the effects of the 3 tested substances are similar and had a mineralocorticoid-like effect (Girerd et al.1958).

Ocular Irritation

Licorice Extract

Consumer Product Testing Co. (1994) instilled Glycyrrhiza Glabra (Licorice) Root Extract (0.15% in corn oil; 0.1 ml) in one eye of New Zealand white rabbits (n = 6). The eyes remained unwashed for 24 h. The untreated eye was the control. Observations were made at 24, 48 and 72 h. The test article was found to be non-irritating to rabbits and not an ocular irritant.

Cosmepar Conseil & Expérimentation (1997) instilled Glycyrrhiza Glabra (Licorice) Root Extract (1% in glycyerine product; 0.1 ml) in the fornix of the left conjuctiva of albino New Zealand rabbits (n = 3) with no rinsing. The rabbits were observed for 7 d unless there were no effects on day 3. The right eye was the control. Slight lacrimation to lacrimation with moist eyelids and fur around the eyelids at 1 h was resolved by day 1. Slight chemosis and enanthema observed at 1 h was cleared by day 2. Slight ocular opacity in all animals was observed at 1 h was completely cleared by day 1. The authors stated that the test substance provokes slight irritant lesions of the ocular mucosa under these conditions and determined the substance to be a slight ocular irritant.

BioInnovation Laboratories, Inc. (2004) used a corneal model system consisting of normal, human-derived keratinocytes that have been cultured to form a stratified, squamous epithelium similar to a cornea to test Glycyrrhiza Glabra (Licorice) Root Extract (10%) for ocular irritation. The cells were exposed to the test material (100 µl) for various times. The extract was rated as a non-irritant, equivalent to a Draize score of 0.

In a safety data sheet issued by Cosmetochem (2004b), Liquorice Herbasol Extract PG was said to have no irritant effect on the eye. No further information was provided.

Dermal Irritation and Sensitization

Licorice Extract

Consumer Product Testing Co. (1994) applied Glycyrrhiza Glabra (Licorice) Root Extract (20% glabridin; 0.15% in corn oil; 0.5 ml) to the intact and abraded skin of New Zealand white rabbits (n = 6). The sites were occluded for 24 h and observed at 24 and 72 h after application. The primary irritation index was 1.60 and the test substance was not a primary dermal irritant.

In a local lymph node assay (LLNA), RCC (2002b) treated 3 groups of 4 female mice with licorice extract (sample ID 7272; 25% licochalcone A) at concentrations of 1%, 2.5% and 10% (w/v) in acetone:olive oil, 4:1 (v/v) by topical application to the dorsum of each ear lobe (left and right) on 3 consecutive d. A control group of 4 mice was treated with the vehicle (acetone:olive oil, 4:1(v/v)) only. Five d after the first topical application, the mice were injected i.v. into a tail vein with radio-labeled thymidine (3H-methyl thymidine). Approximately 5 h after intravenous injection, the mice were killed and the draining auricular lymph nodes were excised and pooled per group. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes which were washed subsequently and incubated with trichloroacetic acid overnight. The proliferative capacity of

pooled lymph node cells was determined by incorporation of 3H-methyl thymidine measured in a β-scintillation counter. A test item is regarded as a sensitizer in the LLNA if the exposure to at least one concentration of the test item resulted in an incorporation of 3H-methyl thymidine at least 3-fold or greater than that recorded in control mice, as indicated by the stimulation index (SI).

No signs of local toxicity were observed at the ears of the animals and no systemic findings were observed during the study period. All treated animals survived the scheduled study period. A dose-response relation was observed as shown in **Table 22**, but none of the SI's exceeded the criteria for being a sensitizer. These authors concluded that licorice extract was a non-sensitizer when tested up to 10% (RCC 2002b).

In a safety data sheet by Cosmetochem (2004b), Liquorice Herbasol Extract PG was said to have no irritant effect on the skin nor any known sensitization effect.

In a safety data sheet submitted by CTFA (2004e), Licorice, under the trade name oil soluble licorice extract P-U, Glycyrrhza Inflata (Licorice) Root Extract was not a primary irritant at 1% in rabbits and not a sensitizer at 1% in guinea pigs. Under the trade name Glycyrrhiza Glabra (Licorice) Leaf Extract, Licorice is not a primary irritant at 10% in rabbits (primary skin irritation test and repeated skin irritation test) and is not a skin sensitizer at 100% in guinea pigs. No further details were provided.

Shanghai Institute of Pharmaceutical Industry (2006a) conducted a guinea pig maximization test on Glycyrrhiza Glabra (Licorice) Root Extract, containing 35% to 40% glabridin. The test solution (0.2 ml; 2%) was applied to the shaved skin of white guinea pigs (n = 40) and covered with gauze and cellophane. The patch was removed 6 h later. This was repeated on days 7 and 14. On day 28 the test solution was applied to a shaved, naive site on the guinea pig and covered for 6 h. The challenge site was observed at 24, 48, and 72 h. Distilled water and dinitrochlorobenzene were the controls. There were no signs of sensitization at any observation period for Glycyrrhiza Glabra (Licorice) Root Extract.

Shanghai Institute of Pharmaceutical Industry (2006b) conducted an irritation test of Glycyrrhiza Glabra (Licorice) Root Extract, containing 35% to 40% glabridin, on guinea pigs (n = 4). The test substance (2%) was applied to the guinea pigs daily for 13 day. The guinea pigs were observed for signs of irritation on days 3, 7, and 14. There were no signs of irritation at any observation period.

Table 22. LLNA Stimulation index in mice as a function of licorice extract concentration (RCC 2002b).

Licorice Extract Concentration [a]	Stimulation Index
1	1.2
2.5	2.0
10	2.2

[a] concentration % (w/v)

Anti-allergenicity

Licorice Extract

Yamamoto et al. (2003) performed an anti-allergic study of the water extract of Glycyrrhiza Uralensis (Licorice) Root Extract on BALB/c mice. This experiment also compared the effectiveness of cultivated and wild collected (from Nei-meng-Gu, China) licorice extract. The mice (n = 5) were administered an i.v. injection of anti-dinitrophenol (DNP) monoclonal antibody immunoglobulin E (IgE) mAB-containing fluid 24 h before the dinitrofluorobenzene challenge. Two h before the challenge, the mice were orally administered Glycyrrhiza Uralensis (Licorice) Root Extract (50, 100, or 200 mg/kg) either from the cultivated or wild source. For the challenge, dinitrofluorobenzene (DNFB; 10 µl 0.1% in ethanol) was applied to each side of each ear of the sensitized mice. Ear swelling was measured at 1 h, 1 d, and 7 d. It is not made clear, but it appears that the control did not receive Glycyrrhiza Uralensis (Licorice) Root Extract before the challenge. The treatment with Glycyrrhiza Uralensis (Licorice) Root Extract reduced ear swelling in both the cultivated and wild collected groups at all dose levels at all 3 measurements ($p < .05$). There was no difference between the 2 licorice sources nor among the doses.

Majima et al. (2004) compared the inhibitory potency of roasted and unroasted *G. uralensis* root on IgE-mediated triphasic ear swelling in female BALB/c mice. The mice were injected (i.v.) with 1 ml anti-DNP monoclonal antibody (mAb) IgE mAb-containing fluid 24 h prior to DNFB challenge. DNFB (10 µl, 0.1%) in ethanol was applied to each side of each ear to elicit ear swelling. The licorice extracts (50, 100, or 200 mg/kg) in water (1 ml) were administered orally 2 h before and 2 to 6 d after the challenge. Prednisolone-21 (10 mg/kg) was the positive control administered 2 h before and 4 to 6 d after challenge. Ear thickness was measured with a dial thickness gauge at 1 h, 24 h, and 6 d after DNFB challenge.

Roasted licorice extract (100 and 200 mg/kg) inhibited IgE-mediated triphasic ear swelling in a dose-dependent manner for 1 h and 24 h after challenge ($p < .05$). Roasted licorice extract (50 and 200 mg/kg) also exhibited inhibitory activity 6 d after challenge ($p < .05$). Unroasted licorice extract (200 mg/kg) exhibited inhibitory activity 1 h and 1 d after challenge ($p < .05$). Unroasted licorice extract (50, 100, and 200 mg/kg) exhibited inhibitory activity 6 d after challenge in a dose-dependent manner ($p < .05$). The authors suggest that roasted licorice may increase the anti-allergic properties of licorice extract (Majima et al. 2004).

Phototoxicity and Photosensitization

Licorice Extract

CTFA (2001a) submitted data on a Licorice Extract dissolved in Earle's buffered salt solution (EBSS). The extract was tested with the 3T3 Neutral Red Uptake Phototoxicity Test up to a concentration of 1,000 mg/l. Balb/c 3T3 cells were cultured and seeded into 96-well-microtiter plates (1 x 104 cells/well, incubation at 37°C with 7.5% carbon dioxide and 10% newborn calf serum in DMEM). Twenty-four hours post seeding two 96-well plates per test chemical dissolved in EBSS were preincubated

with 8 different concentrations of licorice extract for 1 h. One plate was then exposed to a dose of 5 J/cm2 UV-A while the other plate was kept in the dark. The treatment medium was then replaced by culture medium and after 24 h the cell viability was determined by NRU during 3 h and compared to controls. The amount taken up was determined photometrically. This sample of Licorice Extract caused no cytotoxic effects (NR50 > 1,000 mg/l), but photocytotoxic effects (NR50 = 13.2 mg/l) were observed in this in vitro test. The calculated photo irritation factor (PIF) [NR50 (-UV)/NR50 (+UV)] was > 76. According to this report, a substance was considered to have probable phototoxic potential if the PIF ≥5.

CTFA (2001b) submitted data on a licorice extract for phototoxicity using the EpiDermTM Phototoxicity Test. Each of 4 EpiDermTM tissues were preincubated with 5 different concentrations of licorice extract (up to 1%) for 21 h. Two tissues per concentration were then exposed to a dose of 6 J/cm2 UV-A while the other tissues were kept in the dark. After 21 h the cell viability was determined by the MTT cytotoxicity test and compared to controls. This sample of Licorice Extract, dissolved in water, caused no cytotoxic or photocytotoxic effects in this in vitro test.

CTFA (2001c) submitted data on a licorice extract for phototoxicity using the red blood cell phototoxicity test (RBC PT). Bovine erythrocytes were seeded in 4 24-well microtiter plates together with different concentrations (up to 10,000 mg/l) of licorice extract dissolved in PBS. Two plates, one for the hemolysis test and one for hemoglobin oxidation, were exposed to a dose of 15 J/cm2 UVA and approximately 1 J/cm2 UVB while the other 2 plates were kept in the dark. After irradiation, a post incubation period of 30 min in the dark followed. For the hemolysis test, both plates, irradiated and non-irradiated, were centrifuged and the supernatants were transferred into cuvettes. The degree of hemolysis was determined by measuring the released hemoglobin spectrophotometrically at 525 nm. Absorbance data obtained for each test concentration was compared with that of 100% controls (lysed by 1% Triton X-100) and the percent hemolysis was calculated.

This sample of licorice extract caused no hemolysis whether or not it was irradiated. Total met-hemoglobin was determined by absorbance (630 nm) of the lysed cells after the dark incubation above and by subtracting the absorbance of the unirradiated sample from that of the irradiated sample. Met-hemoglobin formation was determined to be, at the highest test concentration of 10,000 mg/l, 0.075% (CTFA 2001c).

RCC (2002a) tested for photosensitization in albino guinea pigs using licorice extract (Sample ID 7272). There were 20 test and 10 control animals. For the induction, licorice extract at 10% in PEG 300 was applied epicutaneously to a nuchal skin area of 6 to 8 cm2 (marked previously with 4 intradermal injections of FCA/physiological saline [1:1]). The test sites were then exposed to 10 J/cm2 UV-A irradiation and 1.8 J/cm2 UV-B. This procedure was repeated 4 times within 2 wk of the induction phase. Control animals were intradermally treated with FCA/physiological saline only. Three weeks after the beginning of the induction, a challenge was performed by treating the animals epicutaneously on both flanks with the test item at the concentrations of 2.5%, 0.5%, 0.1% and 0.02% (dilutions in PEG 300). Treated sites were then either exposed to 10 J/cm2 UV-A irradiation (left flank) or remained unirradiated (right flank). Cutaneous reactions, i.e., erythema and edema formations, were evaluated at 24, 48 and 72 hours after the challenge exposure. There were no positive reactions to any of the exposures to licorice extract with or without irradiation.

A safety data sheet submitted by CTFA (2004e) stated that licorice (Glycyrrhiza Glabra (Licorice) Leaf Extract) was neither phototoxic to guinea pigs at 10% and 100%, nor a photosentizer at 20% in guinea pigs. No further details were provided.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Embryonic and Fetal Development

Licorice Extract

Shin et al. (2005) tested the effects of licorice extract on development of fetuses. Sprague-Dawley rats were orally administered Glycyrrhiza Glabra (Licorice) Root Extract (0, 500, 1,000, or 2,000 mg/kg/d in water; 10 ml) for 9 wk before mating, starting at 6 wk old, for males and 2 wk before mating, starting at 8 wk old, for females. The rats were paired for mating for 1 week (n = 30 pairs/group). The females continued treatment until day 19 of gestation. The females were killed, blood collected, and necropsies performed. Fetuses were delivered by Caesarian section and corpora lutea, implantations, live fetuses, resorptions, and deaths were counted. The fetuses were examined for external abnormalities. Half of the fetuses in each litter were examined for visceral abnormalities and the other half for skeletal abnormalities.

No clinical signs were observed during treatment. Weight gain and food and water consumption were similar among groups. Glycyrrhiza Glabra (Licorice) Root Extract did not have any effect on estrous cycles or copulation rates. There were no effects observed with regard to organ weights. There were no effects observed with regard to placenta weights; numbers of corpora lutea, implantations, or pups/litter; fetal mortality, live fetsuses, or male and female pup weights. There was an increased percentage of visceral abnormalities for the low- (9.8%), mid- (8.7%), and high-dose (1.6%) groups compared to controls (4.6%). Since dose-dependency was not shown, the authors did not consider the differences significant. With regard to skeletal abnormalities, malformation and variation were observed at 1.9% and 12.3% for the low-dose group, 0.7% and 8.6% for the mid-dose group, and 0.0% and 20.2% for the high-dose group, respectively. Dose-dependency or significance were not observed. The authors concluded that Glycyrrhiza Glabra (Licorice) Root Extract did not demonstrate reproductive or developmental toxicity (Shin et al. 2005).

Male Reproductive Effects

Glycyrrhiza Uralensis (Licorice) Root Extract

Shin et al. (2008) tested the effects of aqueous Glycyrrhiza Uralensis (Licorice) Root Extract on the male reproductive system

of rats. Sprague-Dawley rats (n = 15; 6 wk old) were orally administered the extract (500, 1000, or 2000 mg/kg/d) for 9 wk. The controls were administered the water vehicle. Body weights and feed intake were monitored. At the end of the treatment period, blood was sampled then the rats were killed and necropsied.

There were no clinical signs during the treatment period. There were no differences in body weights nor feed consumption. There were no differences in organ weights, including the reproductive system. The weight of the prostates in the high-dose group was slightly decreased, but not to significance. The number of testicular sperm was slightly reduced in the high-dose group, but not to significance. There were no effects to the number of or daily production of sperm. There was no dose-dependent change in epididymal sperm counts. There were no effects on motility or morphology of the sperm. Serum testosterone levels decreased over the 9 wk (28.6% in the high-dose group) but not to significance. There were no remarkable findings under histopathological examination. The authors concluded that the no observed effects level (NOEL) was > 2000 mg/kg/d for rats (Shin et al. 2008).

Hormonal Effects

Licorice Extract

Zava et al. (1998) tested the estrogen and progestin bioactivity of a 50% ethanol/distilled water licorice (*G. glabra*) extract. The extract (2 µL) was combined with 1 ml 5% charcoal-treated FBS in growth medium. Estrogen receptor (ER)/progesterone receptor (PR) positive (MCF7 and T47D) and ER/PR negative (MDA-468) breast carcinoma cell lines were cultured then seeded in 96-well tissue culture plates (2,000 to 5,000 cells in 250 µl). One to 2 d later, the media was replaced with 250 µl/well 5% charcoal-treated FBS in growth media with insulin with or without the licorice extract. Media was changed on days 3, 5, 7, and 9. Growth was quantified by total DNA content by propidium iodide staining and/or total protein by sulforhodamine B staining. In the ER(+) T47D cells, the licorice extract had ~ 3 times the amount of protein than the control ($p < .05$). The licorice extract had similar effects on ER-binding of ER(-) breast cancer cells as the control.

To determine if licorice extract has progestin agonist properties, T47D cells were incubated in the extract (1/500 dilution) and alkaline phosphatase activity was monitored. Progesterone, over a physiological concentration, with and without 100-fold molar excess of RU486 (a down regulator inhibitor of progestin regulated ERs) were the controls. There was no increase in alkaline phosphatase activity, so licorice extract is categorized as either neutral or an antagonist. The effects on progesterone induction of alkaline phosphatase was measured. Licorice extract demonstrated progestin antagonist properties by partially or totally blocking enzyme induction by progesterone (Zava et al. 1998).

Liu et al. (2001b) found that licorice extract (*G. glabra* L.) was not active when testing for ERα binding, ERβ binding, or alkaline phosphatase induction with Ishikawa cells (up to 20 µl/ml). PR expression with Ishikawa cells exposed to licorice root extract had a ratio of intensity (net intensity of PR band/net intensity of β-actin band) of 0.04; median effective dose (ED_{50}) to Ishikawa cells was >20 µg/ml. pS2 expression with S-30 breast cancer cells had a ratio of intensity (net intensity of pS2 band/net intensity of β-actin band) of 0.28; toxicity to S-30 cells was >20 µg/ml.

Amato et al. (2002) used a transient gene transfection system to assess the estrogenic activity of the alcohol extract of licorice. Human ERα and ERβ expression plasmids were transfected into HeLa cells with 750 ng ERE-E1b-luciferase plasmid, 50 ng of the CMV-β-galactosidase internal control plasmid and 50 ng of either the hERα or hERβ plasmid. After 6 h of incubation the media was changed and the cells were incubated with 17β-estradiol (10-9 M) or licorice extract (1:500 dilution). The control (n = 3) was cells incubated in charcoal-stripped, phenol red-free DMEM with 0.001% ethanol. After 24 h, the cells were harvested and luciferase activity measured. β-Galactosidase activity was measured to correct for variations in transfection efficiencies. There was slight activation by both hERα and hERβ with the alcohol extract of licorice but it failed to reach a level of statistical significance.

To further explore estrogenic activity of the alcohol extract of licorice, an uterotrophic assay was used. The authors administered 500 µl/d alcohol extract of licorice by gavage for 4 d to ovariectomized CD-1 mice (18 to 20 g) starting 3 wk after ovariectomy (n = 4). Untreated mice and mice administered 17β-estradiol (100 µg/kg s.c. for 4 d) served as controls. On day 5 the mice were killed and weighed. The uteri were removed and weighed. There was no difference between the treatment group and the negative control group (Amato et al. 2002).

In a review article, Piersen (2003) summarized estrogenic activities of licorice as shown in **Table 23.**

Table 23. Estrogenic activities of licorice (Piersen 2003).

Estrogenic Activity	**Present (+) or absent (-) in licorice**
Competitive estrogen receptor (ER) binding	
ER-α	+
ER-β	+
Proliferation of ER + breast cancer cells	
Stimulation in absence of estrogen	+/–
Inhibition in presence of estrogen	+/–
Induction of estrogen-dependent proteins	
Alkaline phosphatase	–
Up-regulation of estrogen-dependent genes	
Presenelin 2 (pS2)	+
Progesterone receptor (PR)	+
Uterotrophic effects (animal)	+/–
Changes in human testosterone in males	+/–

Klein et al. (2006) reported that the ether extract of *G. glabra* root had the estradiol equivalent of 73.4 ± 11.0 (pmol/l)/µg licorice root by a recombinant cell bioassay; the potency compared with estradiol was 1/1650.

Dong et al. (2007) examined the effects of the water extract of Glycyrrhiza Glabra (Licorice) Root Extract on MCF-7 human breast cancer cells. In a sulforhodamine B assay, MCF7 cells were exposed to 10 nM of E2 then extract (0.1 to 100 µg/ml) or glycyrrhizin (0.1 to 100 µM) for 3 d. Glycyrrhiza Glabra (Licorice) Root Extract stimulated cell growth at a range of 0.1 to 10 µg/ml ($p < .01$ compared to control), the upper limit equal to that of 10 nM E2. Glycyrrhizin did not show any significant growth promoting activity.

The levels of the phosphorylated forms and total proteins were determined after the treatment of MCF7 cells with 10 µg/ml of the extract and then compared with the treatment with 10 nM E2 or 10 µM/ml glycyrrhizin. Phosphorylation of Erk1/s and Akt was detected in response to all materials. However, phosphorylation of Erk1.2 and Adt induced by the extract were inhibited by the pretreatment with estrogen antagonists (ICI182,780 or 4-hydroxytamoxifen); phosphorylation of Erk1/2 and Akt activation by glycyrrhizin was not inhibited by the antagonists. The authors suggest that the signaling pathways were very similar between E2 and the extract but glycyrrhizin contributed to a distinctly different pathway.

PC-12 cells were transfected with an ERα-expression vector or an empty vector then treated with the extract in the presence of nerve growth factor. A larger portion of the ERα-transfected cells had newly sprouting neurites after the treatment with the extract and E2 compared to those treated with glycyrrhizin. Neither the vector-transfected cells treated with the extract nor ERα-transfected cells without treatment exhibited enhanced neurite outgrowth. The authors suggested that the effect of the extract on neurite outgrowth was similar to that of E2.

A set of 120 genes with greater statistical stability were used for expression profiles when treated with E2, the extract, or glycyrrhizin. Similar expression profiles were obtained between the cells treated with E2 and the extract but not glycyrrhizin. A correlation was observed between the extract and E2 for 120 genes ($R = .47$, $P < .0001$); the authors suggest this indicates estrogenic activity in the extract. There was also a correlation between the extract and glycyrrhizin ($R = .27$, $P < .01$) but no correlation between E2 and glycyrrhizin. The authors suggest that glycyrrhizin modulated the expression of these genes and contributed to the correlation between the extract and glycyrrhizin, but different from that of E2.

When the genes were categorized (enzymes, signaling, proliferation, transcription, transport, and others), correlations were observed between the extract and E2 for signaling and proliferation. There was no correlation between E2 and glycyrrhizin. A correlation was observed between the extract and glycyrrhizin for signaling genes. The authors suggest that glycyrrhizin and the licorice extract share the common signaling pathways (Dong et al. 2007).

Lee et al. (2007b) examined the effects of the methanol extract of *G. glabra* or *G. uralenis* roots (1 mg/ml) and its n-hexane, ethyl acetate, butanol, and aqueous fractions (20 µg/ml) on the release of growth hormone using pituitary cells isolated from Sprague-Dawley (SD) rats (4 to 5 wk old). The cells (n = 9) were incubated with rat growth hormone releasing hormone (0, 0.1, 0.3, 0.5, and 1.0 µM).

Rat growth hormone was released after the addition of rat growth hormone releasing hormone (0.1, 0.3, 0.5, and 1.0 µM) at 1.12, 1.55, 2.97 and 7.41 times the control ($p < .01$), respectively. The addition of the methanol extract, n-hexane fraction, and ethyl acetate fraction increased growth hormone release by ~1.89, ~4.60, and 1.86 times ($p < .01$), respectively. The butanol and aqueous fractions had no effect. ACTH release was increased by ~5.13, 11.66, 8.53, 11.48, and 11.35 times as high as the control (0.14 ± 0.03 nM; n = 12) by the addition of the methanol extract, and the n-hexane, ethyl acetate, butanol, and water fractions ($p < .01$). The extract and the fractions had no effect on leutinizing hormone release.

In an in vivo experiment, male SD rats (n = 7) were anesthetized and injected i.v. with rat growth hormone releasing hormone (10 µg/kg) and the methanol extract (corresponding to 1 mg dried herb/kg) or the n-hexane or ethyl acetate fractions (20 µg/kg). Blood samples were taken at 0, 12, 20, 30, 45, 60, 90, and 120 min and analyzed for rat growth hormone. The maximum rat growth hormone concentration of 4.02 ± 0.52 nM was at 10 min and returned to basal levels at 90 min for the control (n = 7). The maximum rat growth hormone concentration of 0.75 ± 0.02 nM was at 30 min for rats (n = 11) injected with the methanol extract. The maximum rat growth hormone concentration of 1.42 ± 0.53 nM was at 10 min, 7.17 times as high as the control ($p < .01$). There was no effect observed from the ethyl acetate group. The authors concluded that the n-hexane fraction is the most active regarding rat growth hormone release, but that the mechanism for the increase in growth hormone and ACTH by the methanol extract, the n-hexane fraction and glycyrhizin was unknown (Lee et al. 2007b).

GENOTOXICITY

Licorice Extract

Morimoto et al. (1982) performed an Ames test using *Salmonella typhimurium* TA98 and TA100 on the water and methanol extracts of *G. uralensis* (5, 10, 20, 50, and 100 mg/ml) with and without S9 and the rec-assay with spores of *Bacillus subtilis* (100 mg/ml). The Ames test was negative for all conditions for the water extract. The Ames test was negative for all conditions for the methanol extract; however, TA98 without S9 and TA100, with and without S9, bacteria were killed. The rec-assay was positive for both extracts.

Mitscher et al. (1986) tested the mutagenicity of ethanol (95%) Glycyrrhiza Glabra (Licorice) Root Extract. In a rec-assay test using ethyl methanesulfonate (EMS; 4 µl/plate) as the mutagen, the extract (100 µg/plate) decreased the zones of inhibition from 15 to 17 mm to 3 to 5 mm when co-treated with the mutagen and 3 to 4 mm when pretreated in *B. subtilis* M45 Rec- (deficient in

genetic recombination), but it had no effect on the H17 Rec+ strain.

The authors repeated the experiment using 9-aminoacridine and acriflavine HCl (frameshift mutagens) and *S. typhimurium* TA100 and TA1535. There was >2-fold decrease in reversion frequency.

The authors also pre-incubated *Escherichia coli* K-12 AB1157 and GW5352 in the extract and added EMS. The survivial of AB1157 was enhanced but not of GW5352.

In the modified Ames Salmonella test, the extract reduced TA100 revertants by a half to a third at concentrations of 3.9 to 125 µg/plate. At 250 µg/plate, the extract was toxic (Mitscher et al. 1986).

Maruzen Pharmaceuticals Co., LTD. (2003) conducted a reverse mutation test on Glycyrrhiza Glabra (Licorice) Root Extract on *S. typhimurium* (TA98, TA100, TA1535, and TA1537) and E. coli (WP2uvrA) with and without activation. 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide, 2-aminoanthracene, and 9-aminoacridine were used as positive controls. The test substance was found to be non-mutagenic up to 250 µg/plate with and without activation.

In a safety data sheet submitted by CTFA (2004e), licorice, under the trade name oil soluble licorice extract P-T(40), was not a mutagen using the Umu test method. Under the trade names oil soluble licorice extract P-TH and oil soluble licorice extract P-U, licorice was not a mutagen per the Ames test. Further details were not provided.

Glycyrrhiza Glabra (Licorice) Leaf Extract was not a mutagen using the Ames test or in cultured hamster fibroblasts. No further information was provided on these tests (CTFA 2004e).

Anti-mutagenicity

Tanaka et al. (1987a) used a modified Ames test to test for inhibition of mutagenicity of Glycyrrhiza extract. Glycyrrhiza extract (200 µl) was applied at various concentrations to *S. typhimurium* TA98 and TA100 while being exposed to various mutagens. Glycyrrhiza extract inhibited the mutagenicity of each mutagen. Glycyrrhiza extract at 75 mg/plate decreased the frequency of His+ revertants induced by 3-amino-1,4-dimethyl-5 H-pyrido[4,3-b]indole (Trp-P-1) and 3-amino-1-methyl-5 H-pyrido-[4,3-b]indole (Trp-p-2) to levels equivalent to the frequency of spontaneous His+ revertants. A decrease in the surviving colonies was not observed at each dose. The authors conclude that the effects of Glycyrrhiza extract were due to a decrease in the generation of His+ revertant colonies.

Zani et al. (1993) used a modified version of the Ames test to test for desmutagenic activity by ethanolic extract of *G. glabra* on *S. typhimurium* TA100 . EMS (3 µl/plate), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG; 1 µg/plate), and ribose-lysine (RL; 25 µl/plate) were used as mutagens (n = 3). DMSO was ≤11 µl/plate.

There was no desmutagenic activity for EMS by licorice extract; licorice extract at 25 and 50 µl/plate was antimutagenic, however, at 50 µl/plate this was in part due to toxicity. There was no desmutagenic or antimutagenic activity against MNNG. There was desmutagenic activity against ribose-lysine induced mutations in a dose-dependent manner. Ethanolic licorice extract showed antimutagenic activity against ribose-lysine. The authors suggest that compounds in *G. glabra* extract may be effective chemopreventive agents (Zani et al. 1993).

Ikken et al. (1999) tested the antimutagenic effects of the ethanol extract of *G. glabra* against N-nitrosodimethylamine, N-nitrosopyrrolidine, N-nitrosodibutylamine, and N-nitrosopiperidine using the Ames test. The *G. glabra* extract showed an inhibitory effect against mutagenicity of all N-nitrosamines tested. The mutagenicity of N-nitrosodimethylamine was inhibited 45% by a concentration of ≥ 50 µg/plate, of N-nitrosopyrrolidine 29% to 39% at 500 to 1000 µg/plate, of N-nitrosodibutylamine 25% to 46% at concentrations ≥ 500 µg/plate, and of N-nitrosopiperidine 56% to 72% by concentrations of ≥ 50 µg/plate.

CARCINOGENICITY

Cell Proliferation

Licorice Extract

Amato et al. (2002) tested the effect of the alcohol extract of licorice root on the proliferation of MCF-7 cells (human breast cancer cell line). The cells were plated in DMEM supplemented with 10% FBS and antibiotics in 96-well plates at 5 x 103 for 24 h. The cells were then washed in PBS, the media changed to 10% charcoal-stripped serum in phenol red-free DMEM, and incubated for another 12 h. The cells were treated with licorice extract in ethanol and medium (1:500 to 1:5,000 dilution) or 17β-estradiol (10-9 M) and incubated for 48 h. An untreated group was the control. Cell growth was quantified by ELISA. Licorice root extract did not promote the growth of MCF-7 cells.

Jo et al. (2004) tested licorice extracts for their effects on MCF-7 human breast cancer cells. Fresh roots of *G. uralensis* were extracted with chloroform, ethyl acetate, hexane, and methanol:water (70:30). MCF-7 cells were cultured in D-media supplemented with 10% FBS and antibiotics and maintained at exponential growth in 5% CO2 at 37°C. Stock solutions of licorice root (100 mg/ml) were dissolved in ethanol and experimental concentrations were prepared in the basal medium with a final ethanol concentration of 1%. To determine the effects on cell proliferation, the cells were plated in a 6 well culture plates (2 ml/well) in triplicate and allowed to attach for 24 h then washed. The medium was removed and replaced by test medium and incubated for 3 d with the media being changed once. The cells were washed with PBS and lysed with 1 ml of 0.1 sodium hydroxide and centrifuged. The DNA content was determined (method not described). The optical density (OD260nm) value of the clear lysate was measured with a spectrophotometer. All measurements were performed in duplicate.

Trichloromethane and ethyl acetate extracts of licorice root inhibited the proliferation of MCF-7 cells in a dose- and time-dependent manner. After 72 h, 50 µg/ml of trichloromethane extract caused a 63% inhibition and 50 µg/ml caused a 83% inhibition of cell growth compared to controls. The 70%

methanol and hexane extracts inhibited cell growth also in a dose- and time-dependent manner. After 72 h, there was a 62% and almost 80% inhibition, respectively.

The apoptotic effect of the extracts was analyzed by nuclear DNA staining and DNA fragmentation assay using cells from the first experiment treated for 48 h. Cells were fixed in 4% paraformaldehyde in PBS for 20 min, washed, stained with Hoechst 33258 at 1 µg/ml in PBS for 15 min. Stained cells were washed and observed with a fluorescent microscope. All the test compounds induced chromatin condensation and nuclear fragmentation. The cells shrank, turned around, and had a smaller volume than controls.

MCF-7 cells in the exponential phase of growth were treated with the extracts (50 µg/ml) for 24 to 72 h. The cells were washed and fixed. Data from 10,000 cells/sample were collected and analyzed using FACS Calibur. The samples from all the extracts showed that cells accumulated in the sub G1 phase gradually from 24 to 72 h. The number of cells in G1 phase decreased in the same manner.

To test for effects on poly ADP ribose polymerase (PARP) and cleavage, MCF-7 cells were grown in a dish. When the cells reached 80% to 90% confluence, the cells were treated with the licorice extract (50 µg/ml) for various times. The cells were washed and lysed. The lysates were sonicated, aliquoted, and stored at -20°C. Protein concentrations were determined by the Bio-Rad DC protein assay. Antibody-bound proteins were detected by western blotting analysis. The methanol licorice extract induced PARP degradation fragments in MCF-7 cells. The authors concluded that the licorice root extracts induced apoptosis.

The extracts caused transcription factor Bcl-2 cleavage and an increase in the Bax protein level in the MCF-7 cells. The authors stated that this may be a cause of induced apoptosis. The authors concluded that the root of G. uralensis might be a good chemopreventive for human breast cancer (Jo et al. 2004).

Glycyrrhiza Glabra (Licorice) Powder

Hawthorne and Gallagher (2008) conducted a cell proliferation assay using the MTT method. Human prostate cancer cells (LNCaP, PC3, and DU145) were incubated in Glycyrrhiza Glabra (Licorice) Powder (0.1 µg/ml to 20 mg/ml; dissolved in 70% ethanol) for 48 h. LNCaP growth was inhibited (ED_{50} = 10.3 mg/ml). Glycyrrhiza Glabra (Licorice) Powder had no effect on the PC3 and CU145 cell lines.

A prostate-specific antigen ELISA kit was used to quantify the amount of the antigen secreted by LNCaP cells. Plated cells were incubated with Glycyrrhiza Glabra (Licorice) Powder (10 or 20 mg/ml) for 24 h. Normal excretion rate of prostate-specific antigen is ~27 ng/ml; the powder down-regulated the secretion of the antigen by 59.3% and 81.1%, respectively. The authors concluded that G. glabra may have a role in the modulation of androgen-dependent prostate cancer growth via inhibition of cell proliferation and down-regulation of prostrate-specific antigen production (Hawthorne and Gallagher 2008).

Cancer Inhibition and Tumor Suppression

Licorice Extracts

Rafi et al. (2002) tested the effects of water, ethyl acetate, DMSO, and ethanol extracts of *G. glabra* root on T47D (breast cancer), MCF-7 (breast cancer), and HL-60 (leukemia) cells. To determine the effect of the extracts on protein expression, the extracts were tested on Bcl-2 (a 26-kDa protein that blocks cell death by inhibiting cytochrome c release from the mitochondria) by Western block analysis. All the extracts induced phosphorylation of Bcl-2 as demonstrated by slower migrating bands in contrast to the control (ethanol) except the water extraction.

Cell cycle analysis was also performed on MCF-7 cells using quantitative flow cytometry with a minimum of 10,000 cells incubated in the extracts. The licorice extracts induced G2/M cell cycle arrest similar to paclitaxel (Rafi et al. 2002).

To test the effects licorice extracts have on tumor promotion due to angiogenesis, Sheela et al. (2006) collected *G. glabra* roots from the western ghats of India. Dried roots were made into a powder and extracted with 50% ethanol, solvents with increasing polarity (methanol, petroleum ether, hexane, benzene; activity-guided fractionation), or water. Ehrlich ascites tumor cells (5 x 106) were injected intraperitoneally into Swiss albino mice and growth was recorded from day 1 to 12. Approximately 500 µg of each solvent extract or 600 µg of aqueous extract was injected intraperitoneally from day 6 to 12. No injections were made for the control group. Growth was recorded from day 1 by weighing the mice.

At the end of day 12, the Ehrlich ascites tumor cells were counted by trypan blue dye exclusion method and the volume of ascites was obtained from controls and treated animals. The mice were dissected to observe effects of *G. glabra* root extract or its fractions on peritoneal angiogenesis and microvessel density. There was a nearly 45% increase in body weight of the Ehrlich ascites tumor cell injected mice in the control, petroleum ether, hexane and benzene on day 12. The total volume of ascites was 4.5 ml. The controls grew a total number of 7.92 x 108 Ehrlich ascites tumor cells in vivo.

Treatment with *G. glabra* crude root extract or with methanolic or water extract resulted in a 90% inhibition of growth of Ehrlich ascites tumor cells and formation of Ehrlich ascites tumor mass fluid. Petroleum ether, hexane, and benzene extract treatment resulted in inhibition to lesser extents. The dissection revealed that the control had 47 ± 2 microvessels present in the peritoneum of Ehrlich ascites tumor treated mice while the *G. glabra* crude root extract treated mice showed only 4 - 5 microvessels. The results of the other extracts were not provided.

To verify the in vivo effect of *G. glabra* root extract on proliferation of Ehrlich ascites tumor cells, the cells were cultured in vitro in NCTC 135 medium supplemented with 10% FBS and 1 mg/ml penicillin/streptomycin in 10% CO2 atmosphere at 37°C for 2 d. 3[H] Thymidine (1µCi/ml of medium) was added prior to the addition of curcumin (1 mM) or *G. glabra* protein (60 or 120 µg). After 2 d, the cells were processed for liquid scintillation

counting. The water extract, 60 or 120 µg, inhibited proliferation by 59% and 44%, respectively, when compared to 100% proliferation of Ehrlich ascites tumor cells in vitro in the absence of *G. glabra* proteins.

The authors performed a chorioallantoic membrane (CAM) assay for 12 d. There was inhibition of growth of new blood vessels by *G. glabra* root extract in chick chorioallantoic membranes.

The authors tested for the levels of vascular endothelial growth factor (VEGF) secreted by Ehrlich ascites tumor cells into peritoneal ascites by ELISA. In the control, the level was found to be 1750 ng and in the mice treated with crude *G. glabra* root extract the level was 200 ng.

The authors stated that root extracts from *G. glabra* may be a supplemental source for cancer therapy (Sheela et al. 2006).

Lee et al. (2007a) tested the effects of the ethanol extract of *G. inflata* as an anticancer and chemopreventive agent in combination with cispatin. Male BALB/c mice (n = 8) were orally administered PBS; CT-25 cell inoculate (s.c.); CT-26 cell inoculate with licorice extract (0.5, 1, or 2 mg/kg); CT-26 cell inoculate with cisplatin (5 mg/kg in PBS; i.p.); or CT-26 cell inoculate with licorice (0.5, 1, or 2 mg/kg) plus cisplatin (5 mg/kg). The CT-26 cells (2 x 106 cells in 0.1 ml PBS) were injected subcutaneously into the right flank of the mice. Twenty-four hours later, the mice were dosed with licorice extract in PBS by oral gavage. Two hours later cisplatin was injected i.p. The licorice extract and cisplatin were administered daily for 15 d. The control group received PBS. The tumor volume was measured biweekly. Sixteen h after final dosing, the mice were killed, weighed, blood sampled and the liver and kidneys removed and examined.

All the mice survived treatment. *G. inflata* ethanol extract (0.5, 1, and 2 mg/kg) inhibited tumor growth by 38%, 57%, and 71%, respectively. Cisplatin inhibited tumor growth 92%. Combined treatment resulted in decreased threapeutic efficacy of cisplatin at the lower doses, but treatment with 2.0 mg/kg extract was similar to cisplatin alone.

The protective effects of *G. inflata* ethanol extract against nephrotoxic effects was measured by kidney weight, blood urea nitrogen (BUN) levels, and serum creatinine. The licorice extract plus cisplatin reversed dose-dependently the cisplatin-induced decrease in kidney weight and increase in serum BUN and creatinine levels. The protective effects of *G. inflata* ethanol extract against hepatotoxic effects was measured by liver weight and the xenograft model. The licorice extract plus cisplatin reversed dose-dependently the cisplatin-induced decrease in kidney weight and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. The cisplatin-mediated increaes in the serum nitric oxide and tissue malondialdehyde (MDA) levels were prevented by the treatment with the G. inflata extract. The decrease in tissue GSH levels by cisplatin was almost to control levels. *G. inflata* extract administration blocked the cisplatin-induced reduction in the activity of GSH-Px ($p < .05$, .005), SOD ($p < .05$, .005), and catalse ($p < .05$, .005) in the kidney and liver tissues.

The authors suggested that even though oral administration of licorice extract inhibited growth in the tumors and had a potent protective activity against the cisplatin-induced toxicity, patients on cisplatin therapy should avoid licorice-containing food and supplements due to its interference of the therapeutic efficacy of cisplatin (Lee et al. 2007a).

CLINICAL ASSESSMENT OF SAFETY

Oral

Licorice Extract

Licorice Extract was found to be an effective treatment for gastric ulcers during World War II, but edema occurred in about 20% of the patients given this treatment. To investigate possible causes of this edema, Molhuysen et al. (1950) administered a Licorice Extract (succus liquiritiæ) containing 15% glycyrrhizin to 7 patients who had persistent gastric ulcers and to 3 patients who did not. Subjects were given 20 to 45 g of the Licorice Extract, administered in 8 equal parts (3-hour intervals) throughout the day and night (duration of treatment was not specified, but seemed to vary with each patient). Urine was collected at each dose interval. Subjects were on a strictly controlled diet during the experiment.

Urine output volume was decreased within the first 3 d of treatment, as was chloride (Cl)- excretion. Excretion of urea plus ammonia was not affected, nor was urea clearance. There was no albumin or abnormal urinary sediment detected in the urine. Blood hemoglobin level decreased with dosing. Venous blood pressure, pulse pressure, and systolic pressure all increased. Patients also gained several kilograms in body weight due to edema. The authors attributed this combination of effects to "stimulation of the renal tubules to an excessive reabsorption of water and chlorides, and probably also of sodium." Another patient was given an unspecified amount of pharmaceutical Ammonium Glycyrrhizate, and the effects were similar to those given the Licorice Extract (Molhuysen et al. 1950).

Epstein et al. (1977a) tested the effects of confectionery licorice on electrolyte status and the renin-angiotensin-aldosterone (RAA) axis of 14 healthy volunteers. The volunteers were in good health, normotensive, and had not taken any medication, oral contraceptives or licorice in the preceding month. They all continued to eat their usual diets. Five men and 4 women ate 100 g licorice/day (in the form of 2 confectionary twists) and 5 women ate 200 g/d for 1 to 4 wk. The subjects were examined before the study, each week of the study, and 1 and 2 wk after the study (n = 13 and 11, respectively). The examination included: lying and standing blood pressure; body weight; blood test for measurement of plasma aldosterone, plasma renin activity (PRA), plasma angiotensin II and plasma electrolytes; and a 24 h urine test for aldosterone, sodium and potassium concentrations.

Eight subjects completed the 4 wk. Licorice was withheld from 6 women because of either hypokalemia (4 subjects) or uncomfortable edema of the face, hands and ankles (2 subjects). Four other subjects (1 man, 3 women) developed mild, transient, generalized edema. Other side effects were headache in 3 subjects and lethargy in 4. Blood pressure did not rise in any subject. Mean weight gain was 1.5 ± 0.7 kg. Ten people gained

> 1 kg and the 2 with the most pronounced edema gained 2.3 kg in 1 week and 4 kg in 3 wk. Weights returned to normal 1 week after termination of licorice consumption.

Most of the subjects had electrolyte imbalance. Plasma potassium fell by as much as 1.5 mmol/l in 11 subjects; everyone returned to normal after the treatment period. One man who started with suppressed RAA axis values later showed normal values despite licorice consumption. In the remaining subjects, RAA axis suppression continued during licorice consumption although signs of recovery were observed in some subjects. PRA, plasma angiotensin II, and urinary aldosterone concentrations remained subnormal in 5, 2 and 7 subjects, respectively, 1 week after licorice was withdrawn. Urinary aldosterone concentrations were the slowest to recover and remained subnormal in 2 subjects 2 wk after treatment terminated.

The authors concluded that potentially serious metabolic effects may occur in some humans who consume small amounts of licorice in a short period of time (Epstein et al. 1977a).

Epstein et al. (1978) tested the effects of licorice ingestion (100 or 200 g/d) for 1 to 4 wk on normal, healthy volunteers. Volunteers were examined at weekly intervals starting before treatment and until 1 week after treatment. Twenty-four h urine collections were taken weekly. Plasma cortisol and ACTH were measured 4 times (0800, 1000, 1200, and 1600 h) the day before and on the last day of licorice ingestion.

Urinary unconjugated cortisol increased by more than 2-fold in 10 of 13 subjects, 8 of these in the first week of treatment (11 to 82 µg unconjugated cortisol/24 h increased to 120 to 185 µg unconjugated cortisol/24 h); mean levels for the entire group were elevated ($p < .01$) for the treatment period and the 1 week followup. Results were not dose-dependent. Even though there was no effect on plasma ACTH or plasma cortisol, there was an effect on steroid metabolite excretion observed (Epstein et al. 1978).

Stewart et al. (1987) explored the mineralocorticoid activity of licorice by feeding 7 healthy male volunteers 200 mg/day of confectionery licorice (containing 580 mg Glycyrrhizic Acid) for 10 d. The volunteers had a run-in period (5 to 6 d) where their caloric intake was fixed and their sodium and potassium intake were set at 130 mmol and 80 mmol, respectively. Twenty-four h urine collections were analyzed for sodium, potassium, creatinine, aldosterone, and urinary free cortisol. The day before treatment began and on days 4 and 10, the principal urinary cortisol and cortisone metabolites were measured by capillary column gas liquid chromatography. On the day before treatment and days 3, 5, 7 and 11, 3 BP measurements were taken after the volunteer laid down for 0.5 h. Blood was then sampled for the measurement of sodium, potassium, PRA, and cortisol. Plasma Glycyrrhetinic Acid was measured the day before treatment began and on day 11, 10 h after the ingestion of licorice.

Three volunteers were used to study the metabolism of tritiated 11α-cortisol the day before treatment and 7 d after treatment. They fasted over night then drank 500 ml water at 0830 h. Then 0.7 mg [11α-H3]-cortisol was given intravenously in 15 ml sterile water. Plasma was collected regularly for 120 min and urine at 0, 60, and 120 min.

The group of 7 volunteers had significant sodium retention by day 4 (urinary sodium 90.4 mmol/24 h vs 121.4 mmol/24 h on the day before treatment; $p < 0.05$). By days 8 to 10, 6 of the volunteers appeared to be in mineral corticoid escape. All subjects had a pronounced kaliuresis producing negative potassium balance. Plasma potassium fell in all subjects (mean from 4.11 ± 0.2 mmol/l to 3.69 ± 0.1 mmol/l on day 11; $p = 0.06$). There was suppression of the RAA system by day 6 of treatment. PRA when supine fell from 1.57 ng/ml/g on the day before treatment to 0.51 ng/ml/g on day 6 ($p < 0.05$). Urinary aldosterone fell from 40.1 ± 4.7 nmol/24 h to 25.9 ± 2.9 nmol/24 h ($p < 0.5$). There was no change in weight or BP during treatment.

Urinary free cortisol rose in all subject during treatment, achieving significance on days 2 ($p < 0.05$) and 8 ($p < 0.01$). Plasma cortisol was unchanged during the study. The ratio of allo-tetrahydrocortisol plus tetrahydrocortisol to tetrahydrocortisone increased in all subjects. The ratio of allo-tetrahydrocortisol to tetrahydrocortisol also increased during treatment but did not reach a significant difference. The plasma half-life of [11α-H3]-cortisol was doubled during treatment. The percentage of total tritium excreted in the urine as H3-H2O fell from 27.7% to 12.3 % after day 7.

The authors concluded that this study confirms the mineralocorticoid effect of licorice, sodium retention and kaliuresis with suppression of the RAA axis (Stewart et al. 1987).

Forslund et al. (1989) tested the effects of long-term licorice ingestion in healthy subjects on plasma atrial natriuretic pepetide (ANP) concentration, RAA, antidiuretic hormone (ADH), and blood pressure. Fifteen normotensive (BP ≤ 125/80 mm Hg) voluteers (13 females, 2 males) aged 21 to 46 years were used. The females entered the study during the second week of their menstrual cycle. No one was taking any medications or contraception. The week prior to treatment no licorice-containing food was allowed; there were no other dietary restrictions.

BP and heart rates were measured twice during the week before treatment and weekly during the next 10 wk. BP was measured after 15 min of lying down. Body weight was measured before treatment and at weeks 4 and 8. Clinical and subjective signs of fluid retention were recorded. For treatment, the subjects ingested 100 g licorice (corresponding to 0.7 g Glycyrrhizic Acid) daily. Blood was sampled (after 0.5 h lying down) the day before treatment began after weeks 4 and 8. Urine was collected between 20.00 and 0.80 hours before treatment and after weeks 4 and 8.

Three females dropped out of the study for reasons not related to the study. Mild to moderate edema occurred in 5 subjects, 4 severe cases during weeks 3 to 8. All symptoms disappeared within 2 wk of discontinuing licorice ingestion. Body weights increased from 0.5 kg to 7 kg (mean 1.6 kg). Weights returned to normal after 3 wk of discontinuing licorice ingestion. Plasma ANP concentration increased during treatment ($p < 0.01$). No correlation was observed between the increased plasma ANP

concentration and change in systolic or diastolic BP. PRA decreased during the first 4 wk and rebounded to pretreatment levels at week 8.

There was a negative correlation between the change in plasma atrial ANP concentration and PRA after 8 wk ($p < 0.001$). Plasma aldosterone concentration and urine aldosterone excretion decreased as did the plasma ADH concentration during treatment. The urine cortisol excretion increased, more so at week 4 than at week 8. No change in plasma cortisol concentration was observed. Mean BP measurements increased but remained within normal limits in all but 2 subjects. These 2 developed mild hypertension (140/95 mm Hg and 170/95 mm Hg) which returned to normal at week 10. There was a decrease in serum potassium concentration ($p < 0.005$ at week 4; $p < 0.01$ at week 8). Serum sodium concentration increased at week 4 ($p < 0.005$) and returned to normal at week 8. There was no change in serum calcium and phosphorus as well as excretion of sodium and potassium.

The authors suggested that the elevation of plasma ANP concentration during ingestion of licorice may be a physiological response to prevent fluid retention and hypertension (Forslund et al. 1989).

Pratesi et al. (1991) tested the effect of a "pure derivate of licorice" on male volunteers (n = 6) to explore the reasons for pseudohyperaldosteronism from licorice. The volunteers were administered 7.5 g licorice/d for 7 d. Before treatment began, before treatment on day 4 and day 7, and 5 d after treatment ended, blood samples were drawn in an upright position, and urine was collected for the measurement of free cortisol, aldosterone, electrolytes, tetrahydrocortisone (THE), tetrahydrocortisol (THF), and allotetrahydrocortisol (ATHF).

Body weight increased from day 0 to day 7 in all cases (75.8 ± 10.0 to 77.0 ± 10.3 kg; $p < .05$). There were decreases in serum potassium (4.2 ± 0.3 to 3.9 ± 0.3 mmol/l; $p < .05$) and aldosterone (405 ± 99.8 to 108 ± 41.6 pmol/l; $p > .05$) and PRA increased (4.8 ± 1.5 to 11 ± 1.1 ng/ml per 3 h; $p > .05$). On day 4, the ratios THE:THF and (ATHF + THF):THE were increased as shown in **Table 24.** On day 7 the ratio was increased in only 3 cases and returned to pretreatment values in the other 3 ($p < .05$). Values ranged widely after treatment ended. The authors concluded that the variation in response is probably due to individual variations in the metabolism and excretion of licorice (Pratesi et al. 1991).

Table 24. Urinary metabolites of cortisol and cortisone during licorice ingestion of 7.5 g/d for 7 d (Pratesi et al. 1991).

Metabolite	Day 0	Day 4	Day 7	4 d After treatment
THE (µmol/24 h)	9.2 ± 2.2	8.0 ± 2.7	7.2 ± 3.6	8.0 ± 3.3
THF (µmol/24 h)	6.9 ± 1.9	8.3 ± 1.9	7.2 ± 1.6	6.9 ± 3.6
THF:THE	0.7 ± 0.1	1.0 ± 0.1 [a]	1.1 ± 0.4	0.8 ± 0.3
(Allo-THF + THF):THE	0.9 ± 0.1	1.2 ± 0.2 [a]	1.2 ± 0.6	1.0 ± 0.4

[a] $p < .05$

Murakami and Uchikawa (1993) tested the use of licorice extract for treatment of aldosterone deficiency, in patients with diabetes mellitis (n = 8). Baseline serum measurements were taken before treatment, after 4 to 6 months of treatment with 15 g/day calcium polystyrene sulfonate (CPS; a potassium-binding resin), then every 2 wk for the 6 months the patients were taking 150 mg glycyrrhizin/day (one patient took 75 mg/day).

CPS was continued until it was confirmed that serum potassium concentrations were maintained in the normal range. All other therapies for diabetes mellitis were continued during the experiment.

The serum potassium concentrations measured during administration of licorice extract were lower than those taken prior to administration of CPS in 7/8 patients. Administration of licorice extract did not produce any change in the serum levels of sodium or chloride. The average of the mean potassium concentration of all subjects taking licorice extract was lower than the average of the mean potassium concentrations before administration of CPS. The average fasting plasma glucose and hemoglobin A1c was not different. The researchers concluded that low-dose licorice extract is effective in normalizing the elevated serum potassium in patients with hypoaldosteronism (Murakami and Uchikawa 1993).

Bernardi et al. (1994) tested for the effects of prolonged intake of "pure licorice". Four groups of 6 healthy volunteers (3 males, 3 females) were administered dried, aqueous extract of licorice root containing 108, 217, 380, and 814 mg glycyrrhizin for 4 wk in the form of pills. Before the experiment and at weeks 1, 3, and 4 the following parameters were measured: body weight, wrist and ankle circumference, triceps skin fold and midarm muscle circumference, heart rate, arterial pressure, glomerular filtration rate, daily diuresis, daily renal excretion of sodium and potassium, urine sodium/potassium ratio, sodium and potassium concentrations, PRA, and plasma aldosterone concentration. Fasting blood samples were taken and analyzed. Baseline results did not differ among groups.

During treatment, one woman in the 380 mg group experienced continuous headaches at the end of week 2. One woman in the 814 mg group complained of headache, increased body weight, and peripheral edema; she also had borderline arterial hypertension (144/91 mm Hg) and hypokalaemia (2.6 mmol/l). She was taking an estro-progestinic drug at the same time. A man in the 814 mg group developed arterial hypertension (170/107 mm Hg); he had a family history of high blood pressure. All side effects resolved within 24 to 48 h after suspension of the protocol.

There were no differences in measured parameters for the 108 or 217 mg groups for the length of the study. Body weight increased in week 2 for the 814 mg group then returned to baseline by week 4. Natriuresis (excretion of sodium in the urine) was higher in the 814 mg group at week 4 ($p = .045$). Both the 380 and 814 mg groups had a depression in PRA ($p = .025$; $p = .045$, respectively). Plasma aldosterone concentrations declined in the 814 mg group ($p = .04$). Kalemia (excess potassium in the blood) was lower at

week 1 than baseline for the 814 mg group, then returned to baseline levels. There were no other differences observed.

The authors concluded that the effects of licorice root extract were dose-dependent and more frequent in females which may be increased by disease or conditions favoring sodium retention such as premenstrual period or contraceptive use (Bernardi et al. 1994).

Sigurjonsdottir et al. (1995) studied the effect of licorice on BP by feeding licorice to 30 healthy, normotensive, volunteers (19 women, 11 men). After 2 wk of baseline measurements of BP, 24-h urinary sodium, and sodium intake, the volunteers were administered 100 g licorice (270 mg Glycyrrhizic Acid) daily for 4 wk. The volunteers were followed for an additional 4 wk after termination of licorice consumption. BP was measured 3x/week. Plasma potassium was measured twice at baseline, twice during licorice administration, and twice after withdrawal. Cortisol and cortisone metabolites were measured from 24-h urinary samples collected from 20 of the volunteers. The hormone metabolites THE, THF, and ATHF were measured by ion exchange separation and GC-MS. The metabolite ratio THF + ATHF/THE was calculated to determine if the degradation of cortisol to cortisone had changed after licorice consumption. A subgroup of 13 women repeated the study after a 2 week recovery with 50 g/d licorice consumption.

The mean systolic BP for the total group at 2 and 4 wk of licorice consumption increased by 3.5 mm Hg ($p < .001$) and 6.5 mm Hg ($p < .001$), respectively. The mean systolic BP increased at 2 and 4 wk for women, 3.7 mm Hg ($p < .001$) and 7.5 mm Hg ($p < .001$), respectively, and for men, 3.2 mm Hg ($p < .003$) and 4.8 mm Hg ($p < .001$), respectively. The increase in systolic BP was >10 mm Hg in 15 of 19 women and 6 of 11 men; the greatest increase was 19 mm Hg. As shown in **Table 25**, the difference between the increase in BP between men and women was not significant.

During licorice consumption, plasma potassium decreased by 0.24 mmol/l in the total group ($p < .001$) and 0.3 mmol/l in women ($p < .001$); plasma potassium decreased by >0.4 mmol/l in 13 of 19 women and 2 of 11 men. Systolic BP rose by 2.5 mm Hg for each 0.1 mmol/l decrease in plasma potassium.

Nineteen volunteers complained of edema, headache, and some gastrointestinal symptoms (mainly dyspepsia) during licorice consumption. Fourteen of 19 women gained an average of 0.59 kg ($p < .01$; one gained 6.8 kg) during licorice consumption.

Edema developed at the same time as BP rose. Systolic BP rose 0.35 mm Hg for each kg gain in body weight. The mean cortisol/cortisone metabolite ratio in urine increased (1.39 ± 0.45 vs 2.15 ± 0.9; $p < .001$).

The authors conducted a second experiment with 13 women consuming 50 g licorice/d for 4 wk. Baseline measurements were collected for 2 wk prior to treatment.

The mean increase in systolic BP was 5.6 mm Hg ($p < .001$) and 3.4 mm Hg in diastolic BP ($p = .002$). There was no weight gain in this group and there were no physiological complaints.

Table 25. Mean systolic BP before (period 1), during consumption of 100 g licorice/d (periods 2 and 3), and after licorice withdrawal (periods 4 and 5) (Sigurjonsdottir et al. 1995).

	Systolic BP (95% CI)		
Period	Total group (mm Hg)	Women (mm Hg)	Men (mm Hg)
1	115.0 (4.55)	111.6 (2.90)	120.9 (3.99)
2	118.5 (1.35) [a]	115.3 (1.74) [a]	124.1 (2.10) [b]
3	121.5 (1.78) [a]	119.2 (2.41) [a]	125.7 (2.17) [a]
4	119.0 (2.21) [a]	116.6 (3.18) [c]	123.2 (2.29)
5	117.3 (2.18) [d]	114.7 (2.97) [d]	121.8 (2.74) [e]

[a] $p < .001$; [b] $p < .003$; [c] $p < .002$; [d] $p < .04$; [e] $p < .05$

The authors suggested that licorice-induced hypertension may be more common than appreciated and should be considered in treatment (Sigurjonsdottir et al. 1995).

Armanini et al. (1996) tested for the effects of licorice consumption on 6 male volunteers (age 22 to 24 yr). The subjects consumed 7 g of an extra-pure commercial licorice over the course of each day for 7 d. HPLC showed that the licorice has a 7.6% Glycyrrhizic Acid content. The subjects were weighed, BP was taken, and blood samples were taken and processed prior to licorice administration and on days 4, 7, and 11.

Systolic BP increased in all cases on day 4 (124 ± 10 to 133 ± 6 mm Hg; $p < .05$); diastolic pressure did not change over the treatment period. Compared to baseline (4.2 ± 0.3 mEq/l), serum potassium levels in the treatment group measured on day 8 were decreased (3.9 ± 0.3 mEq/l; $p < .05$). Compared to baseline (75.8 ± 10.0 kg), body weight increased on day 8 (1.4 ± 0.4 kg; $p < .01$). Urinary sodium/potassium ratio decreased on days 4 and 8 ($p < .05$) and was beginning to recover on day 11. Compared to baseline (68 ± 25 nmol/d), urinary-tetrahydroaldosterone decreased on day 4 (29 ± 11 nmol/d; $p < .01$), day 8 (30 ± 19 nmol/d; $p < .01$) and day 11 (16 ± 9 nmol/d; $p < .01$). The ratio of the 2 hormones, THF and THE, did not change during the experiment. Compared to baseline (4.8 ± 1.5; ml/3h), renin activity was reduced on day 4 (2.1 ± 1.2; $p < .001$) and on day 8 (1.1 ± 1.1 ng ml/3h; $p < .001$). Renin activity values returned to normal by day 11 in all but one case.

The authors suggested that the pseudohyperaldosteronism from licorice consumption was initially related to activity reduction of 11β-hydroxysteroid dehydrogenase and then a direct effect of licorice derivatives on mineralocorticoid receptors may take place. In other cases, the effect on the enzyme was related to individual variation (Armanini et al. 1996).

Armanini et al. (1999) administered 7 g commercially prepared licorice tablets (0.5 g Glycyrrhizic Acid by GC-MS) to 7 men (22 to 24 years) for 7 d. Serum testosterone, androstenedione, and 17-hydroxyprogesterone were measured by radioimmunoassay before administration, after days 4 and 7, and 4 d after the last day of administration. Data are shown in **Table 26.**

Table 26. Serum hormone concentrations (ng/dl) in 7 men administered licorice for 7 d (Armanini et al. 1999).

Hormone	Day 0	Day 4	Day 7	4d After discontinuation
Testosterone	740 ± 216	414 ± 43[a]	484 ± 191[a]	704 ± 42
Androstenedione	159 ± 35	140 ± 29	177 ± 30	170 ± 20
17-Hydroxyprogestrone	189 ± 36	216 ± 31	229 ± 36	193 ± 55

[a] $p < .001$ compared to day 0

Testosterone concentrations decreased and the serum 17-hydroxyprogesterone concentration increase was not significant. The authors suggested that men with libido or other sexual complaints should be questioned about licorice consumption when examined.

Sigurjónsdóttir et al. (2001) demonstrated a linear dose-response relationship between Glycyrrhizic Acid and increases in BP. Thirty healthy Swedish and Icelandic volunteers aged 23 to 37 years ingested licorice amounting to 75, 270, or 540 mg/ day Glycyrrhizic Acid for 2 to 4 wk (n = 24, 30, or 10/dose group, respectively). After 2 wk of dosing, mean systolic BP rose 3.1, 5.2, and 14.4 mm Hg for the low, middle, and high dose groups, respectively, compared to BP measured prior to treatment ($p <$ 0.03 to 0.000). Increases in blood pressure were of similar magnitude after 4 wk of dosing. The authors concluded that a dose-response relationship was demonstrated, but not a time-response, and there did not seem to be a special demographic group that was especially sensitive to the hypertensive effect of licorice or Glycyrrhizic Acid.

Fuhrman et al. (2002b) tested the effect of the ethanol extract of G. glabra on aspects of atherosclerosis. The extract (amount not specified) was placed into gel capsules and administered blind to 12 hypercholestolemic patients (45 to 55 years old) with plasma cholesterol levels of 220 to 260 mg/dl and LDL cholesterol levels of 120 to 170 for 1 month followed by 1 month of placebo (control). The patients were instructed to continue with their regular eating habits. The patients were otherwise healthy and nonsmokers. Body mass index (BMI) was 25 ± 1.7 kg/m2 and did not change during the study. Blood samples after 12 h of fasting were drawn before, at 1 month, and at the end of the study.

The blood was tested for serum paraoxinase activity; plasma lipid peroxidation; LDL levels; LDL oxidation; LDL aggregation and LDL retention (chondroitin sulfate (CS) binding ability) after incubation with CS for 30 min by spectrophotometric assy after precipitation with phosphotungstic acid and magnesium chloride.

There were no changes in markers of liver, kidney, and heart functions as shown by blood chemistry analysis of serum BUN, creatinine, ALT, AST, bilirubin, and creatine phosphokinase after either the treatment month or the placebo month. Serum electrolytes (including potassium and sodium) and serum alkaline phosphatase did not change during treatment or placebo. Compared to baseline, there was a 7% reduction in serum glucose (90 ± 3.0 mg/dl to 83 ± 1.0 mg/dl; $p < .01$) and a 10% reduction in serum amylase concentrations (82 ± 8.0 U/l to 74 ± 8.0 U/l; $p < .01$) after licorice consumption; the levels returned to baseline after the placebo. Serum cholesterol levels were reduced by 5% (244 ± 8 mg/dl to 232 ± 9 mg/dl; $p < .01$) and LDL was reduced by 9% (156 ± 8 mg/dl to 142 ± 9 mg/dl;$p < .01$); these reductions were not sustained after 1 month of placebo. Serum triacylglycerol concentrations decreased by 13% (174 ± 23 mg/dl to 155 ± 18 mg/dl; $p < .01$) and VLDL levels reduced by 14% ($p < .01$) with licorice but returned to baseline after placebo. Systolic BP decreased by 10% ($p < .01$); there was no change in the diastolic BP.

Serum paraoxonase (PON1) was not affected by licorice consumption. After 1 month of licorice consumption, plasma showed a 19% decreased susceptibility to the AAPH-induced lipid peroxidation ($p < .01$) as measured in TBARS formation (37 ± 1 mM to 30 ± 1 mM TBARS/l plasma); this was not sustained during the placebo period. The susceptibility of LDL after consumption of licorice to copper ion-induced oxidation was reduced; the prolonged lag time of 55% was required for the initiation of LDL oxidation in comparison with the lag time of LDL isolated before licorice consumption ($p < .01$). This effect was partially sustained after placebo with an 18% increment in the lag time ($p < .01$). LDL aggregation was reduced by 28% ($p < .01$) after 1 month of licorice consumption and returned toward baseline after placebo. LDL CS binding ability decreased by 25% ($p < .01$) after licorice consumption and this effect was partially sustained after placebo ($p < .01$).

The authors concluded that licorice root consumption by hypercholesterolemic patients may be therapeutic against cardiovascular disease (Fuhrman et al. 2002b).

Armanini et al. (2003a) tested the effect of licorice extract on serum testosterone using 17 healthy male volunteers (22 to 24 years). The men were administered 7 g licorice extract (7.6% w/w Glycyrrhizic Acid) in the form of tablets daily for 7 d. On days 0, 4, and 8 serum or plasma hormonal parameters (leuteinizing hormone [LH], PRA, aldosterone, cortisol, total and free testosterone, androstenedione, 17-hydroxyprogesterone [17-OHP]) were measured as well as urine cortisol and cortisone. The same parameters were measured in 11 of the men 3 d after the last dose. In 6 other cases, the treatment was continued for 4 more d, and at the 1st and 3rd d, 2000 IU of β-human chronic gonadotrophin (βHCG) were injected, and total and free testosterone, androstenedione, and 17-OHP were measured following each injection.

There was a 25% reduction in serum testosterone during licorice consumption ($p < .05$) at both days 4 and 8 as shown in **Table 27.** There was a 39% increase in 17-OHP at day 8 ($p < .05$). PRA and aldosterone were suppressed by licorice ($p < .01$) and the cortisol/cortisone ratio in urine was increased (0.97 ± 0.42 to 1.44 ± 0.71).

Serum androstenedione was not changed by the treatment. Serum LH increased at day 8 ($p < .05$). In the 11 cases who were tested 3 d after treatment terminated, mean serum free and total testosterone returned to pretreatment levels (total testosterone: pretreatment, 21.2 ± 7.9; after withdrawal, 20.2 ± 6.1 nmol/l; free testosterone: pretreatment, 17.4 ± 6.0; after withdrawal, 16.5 ± 6.2). Plasma aldosterone remained reduced (0.36 ± 0.5 to 0.1± 0.07 nmol/l; $p < .001$).

In the men who received βHCG stimulation, serum total testosterone decreased on day 8 (mean decrease 25%; $0 < .005$). The increase of total free testosterone was ~25% ($p < .05$) in both licorice- and placebo-treated groups (total testosterone in licorice group: 90% after first and 107% after second injection of βHCG; control group: 82% and 105%; free testosterone in licorice treated group: 70% and 70%; control group: 102% and 109%, respectively). The values of 17-OPH after βHCG injection increased by 59% and 37% in the licorice group and by 96% and 44% in placebo group ($p < .05$). Serum androstenedione was not affected by βHCG. None of the volunteers had values of total and free testosterone lower than normal ranges during therapy and none complained of unwanted sexual effects.

BP was not affected. The authors concluded that licorice extract did not affect the response of testosterone and 17-OHP to stimulation with βHCG in men (Armanini et al. 2003a).

Armanini et al. (2003b) determined the effects of licorice consumption on the reduction of body fat mass (BFM). Healthy volunteers (7 male; 8 female; 22 to 26 yr old) with normal BMI (males, 24.3 ± 2.1; females, 20.2 ± 2.0) were administered 3.5 g licorice/d in the form of tablets (no sugar or other additives) made from the extract of licorice plant roots for 2 months.

The equivalent amount of Glycyrrhizic Acid was 0.25 g. The volunteers were requested to not change their eating habits; none were consuming any licorice products.

Before and after treatment, measurements of BP, PRA, plasma aldosterone, cortisol urinary cortisol, cortisone, water and fat distribution, and skin fold thickness were taken. Serum cholesterol, tryglycerides, and leptin were also measured in females.

The males had reduced BFM measured by measuring skin folds (12 ± 2.1% to 10.8 ± 2.9%; $p < .05$) and bioelectrical impedance analysis (BIA; 12 ± 2.3% to 11 ± 2.8%; $p < .02$). Extra cellular water (ECW), predicted by BIA, increased from 41.8 ± 2.0% to 47.0 ± 2.3% of total body weight in males ($p < .01$). The mean reduction of fat, calculated from the reduction in grams of % body weight, was ~ 800 g in 2 months. PRA and aldosterone were decreased; there was no change in serum potassium or in BP. The urine cortisol/cortisone ratio increased from 0.8 ± 0.2 to 1.25 ± 0.4 ($p < .01$). There was a relationship between male BFM measured by BIA and by skinfold thickness ($p < .01$).

BMI was unchanged in the female volunteers before and after licorice consumption and after 1 month recovery. The reduction of BFM was 24.9 ± 5.1% to 22.1 ± 5.4% body weight ($p < .01$). BFM was 24.7 ± 4.9% after 1 month recovery. ECW increased from 48.2 ± 1.5% to 49.3 ± 2.1% body water ($p < .01$) and was 47.5 ± 4.4% after 1 month recovery. The mean net loss of fat was 1.5 kg. Serum cholesterol, HDL cholesterol, tryglycerides, and leptin did not change due to treatment. The volunteers reported an increased number of evacuations and reduced hunger during treatment. The authors suggest that licorice can reduce fat by inhibiting 11β-hydroxysteroid dehydrogenase at the level of fat cells (Armanini et al. 2003b).

Sigurjonsdotir et al. (2003) tested the effects of licorice consumption on BP and compared its effects on women and men as well as normotensive (NT) and hypertensive (HT) subjects. The subjects were 25 volunteers, 13 men and 12 women (age 22 to 43 yr, mean 31.2 yr). The HT group was being treated for hypertension and had systolic BP below 140 mm Hg and diastolic BP below 90 mm Hg and had stable BPs for 3 months.

For the gender comparison, all volunteers of each gender were grouped. One NT woman was taking thyroxin for hypothyroidism; 9 of the HT group were taking β-receptor inhibitors and a Ca2+-antagonist. No one reported excessive alcohol use, tobacco use, or taking homones (including contraception).

Table 27. Serum homone concentration in 17 men before and during licorice consumption (7 g licorice/d) (Armanini et al. 2003a).

	Day 0	**Day 4**	**Day 8**
Total Testosterone (nmol/l)	21.0 ± 7.0	15.9 ± 5.11 [a]	15.8 ± 5.7 [a]
Free testosterone (pmol/l)	17.6 ± 6.1	16.4 ± 7.0	16.5 ± 6.8
Androstenedione (nmol/l)	5.0 ± 1.75	4.3 ± 1.1	4.9 ± 1.8
17OH-P (nmol/l)	2.8 ± 1.2	3.2 ± 2.0	3.9 ± 2.1 [b]
LH (IU/l)	3.1 ± 1.1	3.3 ± 1.3	4.0 ± 0.8 b
Plasma aldosterone (nmol/l)	0.38 ± 0.09	0.14 ± 0.04	0.09 ± 0.02 [a]
Urinary F/E	0.97 ± 0.42	-	1.44 ± 0.71 [b]

[a] $p < .01$ compared to day 0; [b] $p < .05$ compared to day 0

The experiment was 1 week of run-in when baseline measurements were taken, 4 wk of licorice consumption (100 g/day equal to 150 mg Glycyrrhetinic Acid), then a 4-week wash-out period. Women started licorice consumption on day 1 through 4 of their menstrual cycle. BP, heart rate and BMI were measured 3 times the week before licorice consumption began for baseline values. The 24-h ambulatory BP and heart rate were measured twice in this same time period. These measurements were repeated at 2 wk and 4 wk of licorice consumption then after 2 and 4 wk of the 4-week wash out period. Blood samples and 24-h urine samples were taken at baseline, after licorice consumption, and after the wash-out period.

Thirteen volunteers reported headaches and 9 reported edema. A few reported diarrhea, increased abdominal gas, slight dizziness, or joint pain in the fingers and wrists with no difference between the genders. Complaints were more pronounced in the women since more of the women (5 NT and 1 HT; no men) stopped the treatment ($p = .0008$) before 4 wk. One HT woman had an extreme rise in BP (+35.7 systolic and 22.2 mm Hg diastolic) after 14 d with no other symptoms. Two women stopped treatment for headache and edema after 9 and 14 d; 1 because of edema, shortness of breath, and tiredness after 14 d; 1 because of headache after 14 d; and 1 because of irregular menstrual cycle. These women still participated in physical and biochemical examinations throughout the study.

After 4 wk of licorice consumption, systolic BP rose by an average of 3.5 mm Hg ($p < .06$ compared to baseline) in the NT group and 1.53 mm Hg ($p < .003$ compared to baseline) in the HT group. The systolic BP and ($p < .02$) diastolic BP ($p < .04$) rose more in the HT group than in the NT group. There were no differences found between the genders at any time period.

After 4 wk of licorice consumption, the mean rise in 24-h systolic BP in the NT group was lower than the HT group ($p = .001$). After the wash-out period there was no difference between the baseline and current systolic BP in both groups.

In comparing genders, the mean rise in systolic BP for women was 6.2 mm Hg ($p = .003$ compared to baseline) and 8.7 mm Hg in men ($p = .0004$); there was no difference between the groups for systolic or diastolic BP and heart rate. BP was not affected by age.

BMI increase by 0.38 ± 0.42 for the group with the maximum increase at 2 wk of licorice consumption. There were no differences between the NT and HT or male and female groups. There was no correlation between BP and BMI. Weight increased in both groups ($p = .002$); men (+1.4 ± 1.2 kg) more than women (+1.0 ± 1.5 kg, $p = .01$). There was no difference between the NT and HT groups. There was a correlation between the change in systolic BP and weight increase ($p = .04$) after 4 wk of licorice consumption.

Serum sodium did not change with licorice consumption but serum potassium decreased for the entire group ($p < .0001$) which correlated inversely with the increase in systolic BP ($r = -.036$, $p = .03$). Serum creatinine decreased with licorice consumption ($p < .004$) and the change correlated inversely with the increase in diastolic BP ($r = -0.39$, $p + .02$).

The HT group had lower baseline PRA (0.46 ± 0.44 ng/ml/h) than the NT group (0.96 ± 0.78 ng/ml/h, $p = .03$). PRA decreased with licorice consumption ($p = .02$) which correlated inversely with the increase in systolic BP ($r = -0.44$; $p < .01$) and diastolic BP ($r = -0.42$; $p = .01$) for the entire group. There were no differences found between the NT and HT groups or between genders with regards to serum potassium, creatinine, or PRA.

Serum cortisol did not change in any group. The decrease in urinary cortisol metabolites ($p < .0001$) inversely correlated with the change in daytime systolic BP ($p = .02$) and daytime diastolic BP ($p = .01$). No differences were found between the NT and HT groups or the genders concerning change in urinary cortisol metabolites. The urinary free cortisol/free cortisone ratio increased in all groups ($p < .001$) without differences between the groups. This ratio correlated with the change in serum creatinine ($r = -0.588$, $p = .0002$), PRA ($r = -0.35$, $p < .05$), and 24-h systolic BP ($r = 0.40$, $p = .02$). The increase in systolic BP correlated with the cortisol ratio ($r = 0.50$, $p = .007$) in the NT group but not in the HT group.

The authors concluded that hypertensive people are more sensitive to 11β-hydroxysteroid dehydrogenase inhibition by licorice than normotensive people and this inhibition causes more clinical symptoms in women than men (Sigurjonsdottir et al. 2003).

Armanini et al. (2004) orally administered a commercial preparation of pure licorice (3.5 g/d; 7.6% w.w. Glycyrrhizic Acid) in the form of tablets to healthy women ($n = 9$; 22 to 26 years old) for 2 menstrual cycles. Blood samples were collected and BP measured at baseline and during the luteal phase of the cycles. There was no change in diastolic or systolic BP. Total serum testosterone decreased from 27.8 ± 8.2 ng/dl to 19.0 ± 9.4 ng/dl after the first cycle ($p < .05$) and to 17.5 ± 6.4 ng/dl after the second cycle ($p < .05$). Testosterone levels returned to baseline ranges after discontinuation of the licorice. Serum androstenedione, serum LH, and 17OH-P were unchanged. The RAA system was depressed; PRA decreased from 3.1 ± 1.2 ng/ml/g at baseline to 1.1 ± 0.7 ng/ml/h after 2 cycles; $p < .001$. Baseline plasma aldosterone decreased from 14.5 ± 6.3 ng/dl to 5.6 ± 3.5 ng/dl ($p < .001$) after 2 cycles. The authors suggested that licorice can reduce serum testosterone probably due to the block of 17-hydroxysteroid dehydrogenase and 17-20 lyase; licorice could be a therapy for hirsutism and polycystic ovary syndrome.

Armanini et al. (2007) tested the effectiveness of the addition of licorice extract to spironolactone (treatment of polycystic ovary syndrome) in alleviating side effects (gynecomastia, metrorrhagia and menstrual abnormalities). Two groups of hirsute women (21 to 28 years old; $n = 16$) with similar BMI, Ferriman-Gallwey score, and age were administered either spironolactone (100 mg/d) or sprionolactone (100 mg/d) plus 3.5 extrapure commercially prepared aqueous licorice extract (265 mg Glycyrrhetinic Acid). Serum potassium, total and free

testosterone, androstenediol glucuronide (a di-hydrotestosterone metabolite), PRA, aldosterone, cortisol, and sex hormone binding protein were measured before treatment and on days 4, 7, 30, and 60. Before and at the end of treatment, 24-h urine was collected from 7 members of each group to measure THF, ATHF, and THE (an indirect index of licorice effect on 11β-OHSD type 2). BP was measured before and during treatment.

With just spironolactone, systolic BP was reduced on days 30 and 60. There was no change in the combination treatment group. Diastolic BP and serum postassium did not change in either group. The group treated with spironolactone had metrorrhagia in 50% of the group; the combination treatment group had metrorrhagia in 12.5% of the group ($p < .05$). The spironolactone group had symptoms of fatigue, orthostatic symptoms, and polyuria more frequently in the first 2 wk of treatment which improved by day 60.

These side effects were not reported by the combination treatment group. BMI decreased in the spirolactone group at days 30 and 60 but not in the combination treatment group ($p < .05$). Plasma aldosterone and PRA increased over the treatment period in both treatment groups beginning on day 4 ($p < .0001$); the increases were reduced in the combination treatment group at all measurements ($p < .05, .01, .001$). Serum testosterone was increased on day 4 compared to baseline ($p < .005$) in the spironolactone group; there were no differences from baseline at any other measurement in either group. Plasma cortisol increased after days 30 and 60 in both groups ($p < .05$); no other measured parameters changed (Armanini et al. 2007).

Zwickey et al. (2007) tested the effects of the water extract of G. glabra dried root on CD25 expression. Subjects (n = 3) ingested 7.5 ml of the extract twice daily for 7 d. Peripheral blood was sampled at baseline, 24 h, and 7 d. After washing, the blood cells were resuspended in FBS with fluorescently labeled antibodies. G. glabra increased the mean CD25 expression at 24 h ($p < 0.4$) but not at 7 d.

Deglycyrrhizinated Licorice Extract

Larkworthy and Holgate (1975) administered deglycyrrhizinized licorice in the form of tablets (380 mg; 2 tablets, 5 times/day) to chronic duodental ulcer patients (n = 32 men) on an empty stomach. Progress of the ulcers were periodically evaluated by endoscopy. Healing of the ulceration was observed in all, varying from normal mucosa (n = 19) to mild duodenitis (n = 13) at 24 wk. There was residual scarring noted in 14 patients but no evidence of duodenal stenosis. One patient had a recurrence of symptoms after 5 months which subsided after resuming treatment. No adverse effects were noted.

Bardhan et al. (1978) tested the effectiveness of deglycyrrhizinized licorice for the treatment of gastric ulcers. In a double blind study, volunteers were administered 500 mg deglycyrrhizinized licorice (n = 48) or 200 mg sucrose (n = 48) daily for 4 wk. Before and at the end of the study, volunteers were subjected to blood testing, gastroscopy, and barium meal. There was no difference between the groups in the proportions of healed or improved ulcers. One volunteer in the treatment group became edematous and developed hypokalemia with a weight gain of 0.3 kg.

D'Imperio et al. (1978) tested the effectiveness of deglycyrrhizinized licorice for the treatment of duodenal and benign gastric ulcers. The volunteers (11 males; 4 females; age 31 to 73 years) were administered 5 tablets containing deglycyrrhizinized licorice (380 mg/tablet) per day (one after each meal and 2 at bedtime; n = 15) or cimetidine (200 mg; n = 15). At 2 wk, 43% of the cimetidine volunteers had healed ulcers compared to 31% receiving the licorice treatment; at 4 wk, 93% and 64%, respectively, were healed.

All volunteers were placed on cimetidine until healed. The groups were then put on maintenance doses of the medication, 400 mg cimetidine at bedtime or 3 tablets with deglycyrrhzinized licorice daily. At 2 and 4 months, 1 of 11 volunteers on cimetidine and 3 of 8 on licorice had a relapse.

The trial was repeated with 14 more volunteers. At 2 wk, 1 of 7 volunteers on cimetidine and 4 of 7 on deglycyrrhizinized licorice were healed. At 4 wk, 4 of 7 in both groups were healed. All unhealed volunteers were placed on cimetidine as above until healed then maintenance doses were administered: 400 mg cimetidine or 150 mg carbenoxolone (a glycyrrhitinic acid derivative) daily. After 2 months, 3 volunteers on cemetidine and 4 on carbenoxolone were still ulcer free. There were no reports of adverse effects for either treatment (D'Imperio et al. 1978).

Hollanders et al. (1978) tested deglycyrrhizinated licorice for use as a prophylactic drug for recurring ulcers. Volunteers were administered 5 capsules/d deglycyrrhizinated licorice (450 mg; n = 11) or placebo (n = 22) and were followed for at least 2 yr. The patients were examined monthly for recurrence of symptoms; gastroscopy and barium-meal examinations were performed at 6-month intervals or as symptoms presented. The relapse rates were 59% and 45%, respectively, for placebo and treatment groups, but the difference was not significant. No ill effects from the licorice were reported.

Rees et al. (1979) tested the protective effects of deglycyrrhizinated licorice on gastric mucosal damage by aspirin in 9 healthy male volunteers (24 to 64 yrs) in a double-blind cross over study. Aspirin tablets (325 mg) and aspirin/deglycyrrhizinated licorice tablets (325 mg aspirin and 175 mg DGL) appeared identical. Gastrointestinal blood loss was assessed by labeling erythrocytes from 15 ml blood with 70 μCi 51Cr, reinjecting the blood, and measuring the quantity of radioactive label excreted in the stool each day. To ensure daily stool samples, the volunteers were administered 3 g methylcellulose daily. Any nausea, flatulence, dyspepsia, or epigastric pain were noted. The protocol was: a control period (days 4 and 5 after blood labeling), a treatment period (days 6 to 10), a stool collecting period (day 8 to 11), a stool collection period for the second control (days 17 and 18), second treatment period (days 20 to 24), stool collection period (days 22 to 25), and stool collection for a final control (days 32 and 33).

Both aspirin preparations increased daily blood loss, to 5.3 ± 0.5 ml/d for aspirin alone over baseline ($p < .001$) and to 4.2 ± 0.5

ml/d for aspirin and DGL ($p < .005$). The blood loss was ~20% less with the aspirin and deglycyrrhizinated licorice preparation ($p < .05$). There was no difference in upper gastrointestinal symptoms between the 2 treatment periods (Rees et al. 1979).

Fuhrman et al. (1997) administered 0.1 g/d alcohol extract of licorice (Glycyrrhizinic Acid-free) to 10 healthy male volunteers aged 20 to 35 years in softgel capsules for 2 wk. Plasma cholesterol concentration and LDL-cholesterol concentration did not change during the study for the treatment group. No changes in blood count, coagulation tests or renal and liver function tests were observed. Mean body mass did not change. No adverse effects to the treatment group were reported.

Licorice Extract Mixture

Acharya et al. (1993) tested the use of licorice extract, in the form of Stronger Neo-Minophagen-C (SNMC), for the treatment of subacute hepatic failure. SNMC is an aqueous licorice extract that contains 0.2% glycyrrhizin, 0.1% cysteine , and 2.0% glycine amino acids in saline solution (Arase et al. 1997). Eighteen patients with subacute hepatic failure from viral hepatitis were intravenously administered 40 ml SNMC daily for 30 d followed by 40 ml 3x/week for 8 wk. For some the dose was increased to 100 ml. Before, after, and every 2 wk during treatment, blood was drawn to measure bilirubin, ALT, serum albumin and prothrombin time. If the patient died, these measurements were taken at time of death. Liver biopsies were performed before and after the treatment period. Postmortem liver biopsies were also performed. The results were compared to the historical data of 98 patients. Patients with complications such as encephalopathy, renal failure, sepsis and gatro-intestinal hemorrhage were excluded.

Thirteen of 18 (72.2%) of the treated patients survived compared to 31 of 98 (31.7%) of the historical patients ($p < .01$). Two patients who died had shown biochemical recovery. ALT levels, prothrombin time, and serum albumin in the survivors became normal after an average of 7.5, 5, and 8 wk of therapy, respectively. Serum bilirubin levels also became normal in all the survivors except in 4 patients at the end of treatment. These same patients reduced their serum bilirubin by 50% to 90%. Improvement in ascites was achieved in 3 to 8 wk (average of 4 wk). No adverse effects of the licorice extract were observed (Acharya et al 1993).

Dermal Irritation and Sensitization

Glycyrrhiza Glabra (Licorice) Root Extract

Laboratoires Phybiotex (1996) applied 0.02 ml of a test article (containing ~900 ppm Glycyrrhiza Glabra (Licorice) Root Extract) onto 50 mm2 of the back of 9 healthy female volunteers. The test article was placed on a disc of filter paper and held in place with an occlusive patch. After 48 h the patches were removed and the skin scored 30 min later. A very slight erythema was observed in 3 of the 9 volunteers.

Thomas J. Stephens & Associates, Inc. (2004a) tested 16 products containing Glycyrrhiza Glabra (Licorice) Root Extract (0.0001%; licochalcone was the marker) for contact sensitization on the healthy skin of Japanese subjects (n = 106). No other information on the composition of the products were provided. The products were occlusively patched 9 times at ~48- to 72-h intervals. Patches were removed after 48 h or 2 h prior to next patch application. After a 12- to 24-day rest, new patches were applied to naive locations for ~48 h. Sites were graded ~ 48 and 96 h after application. There were no signs of irritation or sensitivity except for 1 subject who showed signs of delayed contact hypersensitivity to 1 product. The test was repeated on this subject after a rest period. The subjects scores were again indicative of delayed contact hypersensitivity.

Thomas J. Stephens & Associates, Inc. (2004b) tested 3 products containing Glycyrrhiza Glabra (Licorice) Root Extract (0.0001%; licochalcone was the marker) for safety. The study was carried out at 2 sites, one in the United States with a mix of Caucasian, Vietnamese, Korean, and Chinese female subjects (n = 28) and the other in Japan with only Japanese female subjects (n = 30). All subjects had self-perceived sensitive skin. The subjects were instructed to continue their usual skin care. From day 1 to 14, the subjects added the first product by applying it over the entire face nightly. From day 15 to 28, the subjects in the United States were given the second product to apply and the subjects in Japan were given the third product to apply. Clinical grading was done at baseline, day 14 and day 28. There were no increases in objective or subjective irritation scores. Two subjects in the United States experienced noteworthy irritation from both products.

In a second study on Japanese women (n = 68), 3 other products containing Glycyrrhiza Glabra (Licorice) Root Extract (0.0001%; licochalcone was the marker) were tested for safety. From day 1 to 4, the subjects applied the first product to one eye area twice/day and applied the second product to the other eye area once/day. From day 5 to 18, subjects applied the first product to the entire face twice/day. From day 19 to 32, subjects applied the first product and the third product to the entire face morning and evening. Clinical evaluations were performed at baseline and days 2, 3, 4, 18, and 32; at baseline and on days 1 and 2, an ophthalmological examination with a slit lamp was performed on both eyes.

The ophthalmological examination showed only very mild changes that were not judged to be clinically significant. There was tarsal hyperemia. One product produced more bulbar and tarsal hyperemia on day 1 and more tarsal hyperemia on day 2 (product not specified). There was reported increases in burning and stinging on the facial skin at 2 wk (28/68 subjects, 41%). When the additional product was added, 5 subjects (7%) reported mild sensations. The authors conclude that one of the products is likely to produce mild subjective sensations on the face when used as directed. Individuals with sensitive or atopic skin may experience moderate to severe sensations. There were no objective irritation reported (Thomas J. Stephens & Associates, Inc. 2004b).

Stephens and Associates, Inc. (2005a) tested 2 products containing Glycyrrhiza Glabra (Licorice) Root Extract (0.001%; licochalcone was the marker) for safety around the eye area on Japanese women (n = 60; 47% wore contact lenses; 58% has self-

perceived sensitive skin). Group 1 (n = 30) applied the first product to the upper areas of both eyes and to the laugh-line area on both sides of the mouth each evening for 2 wk. The subjects then discontinued application to the mouth area and changed the area near the eye. Each application was left on for 15 min then massaged into the skin. Group 2 (n = 30) applied the same product to the upper areas of both eyes each evening in the same manner as above. The subjects also applied the other product to the crow's feet area, upper eyelid, and under the eye of both eyes each morning and after the application of the first product. Visual acuity was evaluated at baseline and week 4. An ophthalmalogical examination with a slit lamp was performed at baseline and week 4 (also at 30 min if subject reported eye irritation after first application). Clinical grading of irritation was performed at baseline and weeks 2 and 4. Subjective irritation questionnaires were filled out at baseline, 15 and 30 min post first application, and weeks 2 and 4.

Ophthalmological examination showed decreased (improvement) in tasal conjuctival hyperemia at week 4 for group 2. Clinical examination found no objective irritation but a decrease in facial scaling/dryness at week 2 in group 1. Group 1 reported increases in facial burning/stinging, itching, and tightness immediately and 15 min after the product was removed (~37%). The burning/stinging and itching persisted to week 4 (~17%). Subjects in group 2 reported facial burning/stinging and tightness (but not itching) after the product was removed (6 subjects, 20%). With the application of the second product, there was still burning/stinging and tightness (2 subjects, 6.7%). This persisted to week 4. Sensations in both groups lasted from 1 min to 1 h; most were mild with the exception of 1 subject on day 1. Irritation decreased as the study progressed (Stephens & Associates, Inc. 2005a).

Stephens & Associates, Inc. (2005b) tested the safety and effectiveness of a facial spot treatment containing Glycyrrhiza Glabra (Licorice) Root Extract (0.8%). Female subjects (n = 36; 15 with sensitive skin, 7 with melasma, 14 with hyper-pigmentation) applied the test substance to pigmented areas on the face. The control group (n = 14; 5 with sensitive skin, 2 with melasma, 7 with hyperpigmentation) applied a similar-appearing product (composition not provided). Clinical evaluations were performed at baseline and weeks 4, 8, and 12. There were no differences at any observation period for erythema and scaling/dryness between baseline or the control group. There were no increases in objective or subjective irritation. There were no adverse effects reported. There were improvements in the skin (i.e., pigmentation, skin tone, clarity) for the first 8 wk but not at 12 wk. The authors proposed that this was due to instructions to avoid sunlight and use sunscreen and the decreasing UV intensity from August to November.

TKL Research, Inc. (2005a) tested a product containing Glycyrrhiza Glabra (Licorice) Root Extract (0.0001%; lichochalcone was the marker) for dermal sensitization on healthy subjects (n = 104) with no dermatological disorders using a repeated insult patch test. Patches containing the product (20 µl) were applied Monday, Wednesday, and Friday for 2 wk. The patches were removed by the investigators and the site evaluated before a new patch was applied. After a 1- to 15 day rest period, a new patch on a naive site was applied for 48 h and evaluated 24 h after removal. There was a rechallenge if there were signs of possible sensitization. The authors concluded that there was no evidence of sensitization or irritation.

TKL Research, Inc. (2005b) conducted a repeated insult patch test of a product containing Glycyrrhiza Glabra (Licorice) Root Extract (0.8%) and 5 other botanicals in a carrier solution. The test substance was applied to the subjects (n = 106) by an occlusive Finn Chamber Monday, Wednesday, and Friday for 3 wk. After a rest period of 10 to 15 d, the test substance was applied to a naive site. The site was observed 48 h after application and 24 h after removal by a dermatologist. There was no evidence of sensitization or irritation by this product.

Licorice Extract

Saeedi et al. (2003) tested the effectiveness of licorice gel (1% and 2%) made from a methanol extract of G. glabra on atopic dermatitis. Eczema patients (90), 16 to 53 years old, 35 male and 55 female, with a duration of eczema ranging from 0.01 to 25 years were tested. The study was a randomized, double-blind, prospective, placebo-controlled trial. Severity of edema, itching, erythema, and scaling were scored on a four-point scale: absent = 0, mild = 1, moderate = 2, and severe = 3. The topical preparations (1%, 2%, and placebo; n = 30) were administered 3 times/d for 2 wk. Patients were followed up for 2 wk.

Treatment with 1% and 2% licorice gel resulted in reduction in the scores for erythema after 2 wk ($p < .05$) but not the first week. The 2% gel reduced the erythema scores more than the 1% gel ($p < .05$). The licorice extract treatment improved scores for edema and itching after 1 week ($p < .05$) and 2 wk ($p < .01$). Licorice gel at 2% was more effective than 1% a the end of the first and second week ($p < .05$). Both licorice gels were more effective than placebo for edema and itching after 1 and 2 wk ($p < .01$).

The reduction of erythema scores was 35.02% for 1% licorice gel and 60.76% for 2% licorice gel. The reduction of edema scores was 56.64% for 1% licorice gel and 83.76% for 2% licorice gel after 2 wk. The reduction in itching scores was 44.1% and 72.53% for 1% and 2% licorice gel, respectively. There were no ill effects to the patients reported (Saeedi et al. 2003).

In a safety data sheet submitted by CTFA (2004e), licorice, under the trade name oil soluble licorice extract P-T(40), was not a human skin irritant at 1% (n = 12) and not a skin sensitizer at 0.5% (n = 10). Under the trade name oil soluble licorice extract P-TH, licorice was not a skin irritant at 1% (n = 12). Under this trade name Glycyrrrhiza Glabra (Licorice) Leaf Extract, licorice was not a skin irritant at 10% (n = 41).

Thomas J. Stephens & Associates, Inc. (2006) tested the irritation, acnegenicity, and comedogenicity potential of a skin care regimen that includes an "essence" that contains Glycyrrhiza Glabra (Licorice) Root Extract (0.105%; glabridin was the marker) on women (n = 58; age 18 to 40; 22 with self-perceived acne-prone skin). The regimen used different products depending on the self-perception of oily or dry skin. Both regimens included the same

essence. The regimen was cleanser, toner, essence (containing licorice), moisturizer, then creme was followed twice daily for 6 wk. There were no increases in facial acne counts. There were no facial irritation changes except for a decrease in dryness/scaling. There was a increase in burning/stinging during the first 2 wk reported rated as "a slight increase". The authors stated that a "slight" stinging/burning is expected for this class of product under these circumstances. The regimen was judged to be non-acnegenic and non-comedogenic and found to be well tolerated.

TKL Research Inc. (2006) performed a repeat insult patch on human subjects (n = 105) of an "essence" containing oil soluble Glycyrrhiza Glabra (Licorice) Root Extract (0.105%; glabridin was the marker). The test substance was applied under an occlusive patch Mondays, Wednesdays, and Fridays for 9 applications. Patches remained in place until next application. After a 10 to 15 day rest, a challenge patch was applied to a naive site for 48 h. The challenge site was graded at patch removal and 24 h after removal. The authors reported no evidence of sensitization or significant irritation.

CTFA (2007a) submitted a closed patch test of Glycyrrhiza Inflata (Licorice) Root Extract (1% in 80% butylene glycol) applied for 24 h to the upper arms of 44 healthy males and females. All readings were negative 30 min after removal.

Thomas J. Stephens & Associates, Inc. (2007) tested the tolerance of a cleanser and an "essence" which contained oil soluble Glycyrrhiza Glabra (Licorice) Root Extract (0.105%; glabridin was the marker) on women (n = 35; age 22 to 55; 22 with self-perceived sensitive skin). Twice per day, the cleanser was lathered with water, applied to the face and massaged, then rinsed with water. The cleanser was followed by the application of the essence which was not washed off. The test lasted for 4 wk. There was improvement observed in cheek and whole face erythema ($p < .05$). The subjects reported no change in facial discomforts. There were no increases in objective or subjective irritation compared to baseline. The authors concluded that the test material were considered to be well tolerated by this test group, including subjects with sensitive skin.

Ulcer Treatment

Licorice Extract

Martin et al. (2008) tested the effectiveness of the aqueous extract of glycyrrhiza (species not specified) for the treatment of recurrent aphthous ulcers. Patients (n = 23), at the onset of an ulcer, were given a patch with aqueous extract of glycyrrhiza or a placebo with powdered star anise (having a similar taste). A third, non-treatment group was also recruited because star anise has mild antibiotic effects and was considered an active placebo. The patches were worn for 16 h/d and the ulcers were examined on days 1, 3, 4,and 8. The non-treatment group was examined on day 10. The treatment group had a 90% reduction in ulcer size by day 8 and the placebo group had a 68.5% reduction in size. The treatment group had less prestimulus by day 4 and poststimulus pain than the active control group by day 8 but not by a significant level. There was no difference in recovery time between the treatment group and the active control group. No adverse effects were reported. The authors concluded that the treatment with aqueous extract of glycyrrhiza is beneficial in the treatment of recurrent aphthous ulcers.

Deglycyrrhizinated Licorice Extract

Das et al. (1989) tested the effects of deglycyrrhizinated licorice on aphthous ulcers. Twenty patients with aphthous ulcers gargled with 200 mg deglycyrrhizinated licorice powder dissolved in 200 ml warm water daily. Fifteen patients had 50% to 75% relief of pain within 24 h. There was complete healing of ulcers after 3 d. One patient had a recurrence which healed within 2 d of retreatment. Of 4 patients who did not return for followup examinations, 2 were said to have stopped the treatment for lack of improvement. None of the patients experienced any side effects.

Phototoxicity and Photosensitization

Glycyrrhiza Glabra (Licorice) Root Extract

In a safety data sheet submitted by CTFA (2004e), Glycyrrhiza Glabra (Licorice) Root Extract, under the trade name oil soluble licorice extract P-TH, was not a human photosensitizer at 5% in 32 subjects.

Drug Interaction

Deglycyrrhizinated Licorice

Dalta et al. (1981) found that there was no difference in the excretion of nitrofurantoin (treatment for urinary infection) in the urine when administered with deglycyrrhizinated licorice to healthy volunteers (n = 7). There is a decrease in the excretion of nitrofurantoin in the urine when administered to patients with urinary tract infections when administered with deglycyrrhizinated licorice (41.95 ± 7.52 vs 36.98 ± 3.4 mg; $n = 6$; $p < .01$). Three of 6 patients experienced nausea in the deglycyrrhizinated licorice group; 5 of 6 patients experienced nausea in the non-DGL group.

Case Reports

The case reports summarized below are representative of those available in the open literature. Presenting symptoms are most often muscle weakness, but include dizzyness, fatigue, headache, heart palpitations, hypertension, and pain.

Oral

Conn et al. (1968) reported a case of a 58-year-old man who was admitted to the hospital with complaints of severe muscular weakness. Tests revealed that the patient had hypertension, hypokalemia, alkalosis, suppressed renin activity, and aldosteronopenia. Chlorothiozide (0.5 g twice daily) and chlorothidone (100 mg/day) failed to correct the hypertension. A careful history revealed that he had ingested 72 to 108 g of licorice candy daily for 6 to 7 years. The candy's manufacturer indicated that amount to contain about 0.5 g/day Ammonium Glycyrrhizate. The patient was put on a strict licorice-free diet with controlled sodium and supplements to replace lost potassium.

After recovery, the patient agreed to participate in an investigation to reproduce the clinical effects of Ammonium Glycyrrhizate. The patient was given 0.5 g Ammonium Glycyrrhizate every 12

hours for 2 d, followed by 1 g every 12 hours for 3 d, and then 2 g every 12 hours for 5 d. There was a prompt increase in body weight. Serum sodium and blood pressure increased, and serum potassium steadily declined. Aldosterone excretion fell from 5.1 µg/day before dosing to 1.7 µg/day by day 4 and to 0.3 µg/day by day 9. The data supported the investigators' suspicions that Ammonium Glycyrrhizate in the licorice candy that the patient had eaten for years caused a pseudoaldosteronism that contributed to electrolyte imbalance and hypertension. (Conn et al. 1968).

Chamberlain (1970) described a case of heart failure and pseudoaldosteronism in a 53-year-old man who had consumed 700 grams of licorice candy over a period of 9 d. The day after finishing the licorice candy, the patient suffered shortness of breath, ankle edema, increased abdominal girth, weight gain, headache, and weakness. No medications were given, but sodium and potassium were administered, and licorice was withheld. Over the next 4 d, the patent lost 12 lbs through diuresis, the EKG showed normal T- and U-waves, blood pressure returned to 128/80 mm Hg, and all other symptoms returned to normal. The patient was discharged on the fourth day.

Robinson et al. (1971) reported a 73-yr-old man who presented with sudden onset palpatations and dyspnea during moderate exertion along with pallor, diaphoresis, and fecal incontinence. The patient had been consuming ~124 g/d licorice for the past 3 months to help him quit smoking. He was treated with potassium chloride iv. Regular sinus rhythm returned. The patient was removed from potassium supplementation on the tenth day as symptoms abated. The patient had been abstaining from licorice.

Bannister et al. (1977) reported on a 58-year-old woman who had noticed tiredness and weakness in her limbs for a year. She began taking a bottle of tonic wine daily. After a mild attack of diarrhea, the weakness increased and she was found on the floor unable to move. Her only other medication at the time was monthly injections of B12. She was treated with 420 mmol of potassium chloride intravenously and 24 mmol of magnesium sulphate intramuscularly over the next 24 h. She was also treated with 115 mmol effervescent potassium chloride orally for 2 d. Muscle strength returned within 12 h. She revealed that she had been eating about 1.8 kg of licorice sweets per week. Three months after stopping licorice consumption she remained in good health.

Epstein et al. (1977b) studied the recovery of 4 female patients, aged 38 to 55 years, who were admitted to medical care for chronic licorice intoxication after 6 months to 5 years of consuming 25 to 200 g licorice daily. Upon admission, the patients were withdrawn from licorice and put on a 5-day low-sodium diet and then a normal hospital diet. Patients continued to have low urinary aldosterone levels and low PRA. Angiotensin-II and aldosterone levels in the plasma were normal under basal diet, but were abnormally low with low-salt alterations in the diet. All patients showed normal values of renin, aldosterone, angiotensin, and electrolyte balance 2 to 4 months after licorice withdrawal.

Werner et al. (1979) report on a 27-year-old woman presenting with monthly severe headache over the past 1.5 years. All tests were normal except prolactin, renin, and aldosterone levels were abnormal. It was discovered that she had been consuming large amounts of licorice for several years. After the discontinuation of licorice consumption, her BP returned to nornal within 2 wk; hormone levels were still abnormal after 1 month but normal at 6 months. She no longer had headache attacks.

Blachley and Knochel (1980) reported on an 85-year-old man with progressive and generalized weakness and inability to rise out of sitting position. The patient reported a 50 year history of chewing 8 to 12 3-oz (85.0 g) bags of chewing tobacco daily and swallowing the saliva. The tobacco was 8.3% (wt/wt) licorice paste with a Glycyrrhizinic Acid content of 0.15%. He had consumed between 680 and 1020 g of this product daily equaling 0.88 to 1.33 g Glycyrrhizinic Acid daily since he did not spit out the saliva, just the chewed leaves. After tobacco was withheld and treatment with 500 meq potassium chloride, intravenously and orally, for 10 d he had a BP of 140/80, body weight of 47.3 kg (104 lbs), and resolution of the muscle weakness. He was released on day 13. He was readmitted twice over the next 2 years for the same condition which resolved with treatment and withholding of tobacco.

Lai et al. (1980) reported the case of a 69-year-old woman presenting with increasing weakness of the proximal muscles over 3 months. The muscles were tender and the urine was tea-colored. BP was 110/50 mm Hg; potassium 107 mmol/l; and sodium, chloride, and bicarbonate were normal. Potassium was administered and her strength returned to normal in 3 wk. She had been consuming an over the counter medication for "women's problems" that contained licorice, with the result that she was ingesting 1.5 g licorice/d.

Corsi et al. (1983) reported the case of a 35-year-old man admitted to the hospital because of rapid onset flaccid quadriparesis with areflexia and paresthesias. A muscle biopsy performed 72 h after admission (serum potassium 2.1 mEq/l) was normal. The patient had been consuming 20 to 40 g of licorice daily since giving up smoking 2 years prior. The patient responded to a low salt diet and high doses of organic potassium salts. On the 15th day BP was 160/105 mm Hg and 140/90 on the 20th day. The patient was released and had normal BP, serum potassium, and sodium after 1 month.

Heidermann and Kreuzfelder (1983) reported the case of a 54-year-old man who was admitted to the hospital with acute rhabdomyolysis and myoglobinuria due to hypokalemia. The myoglobinemia led to a glomerulopathy and tubulopathy but there was no clinical evidence of acute renal failure. The patient had ingested 20 to 25 g of licorice/d for the last 2 years. Treatment with K returned the patient to normal functions within 7 to 10 d. The patient was advised to discontinue licorice ingestion and his hypertensive medications were stopped. BP, serum electrolytes and muscle enzymes were normal 3 months later.

Cibelli et al. (1984) reported on a 48-year-old man who presented with severe progressive subacute weakness that developed in the scapulohumeral muscles and spread, together with cramps, to the whole body. Intravenous potassium was perscribed. On the third

day after no change, it was revealed that the patient had been consuming 30 g/d of licorice for about 8 months. Within 48 h of discontinuing this practice, muscle strength improved and subsequently, plasma potassium and enzymes returned to normal. Several months later, the patient was symptom free and no longer took the drugs for hypertension that he had been taking before the illness.

Beretta-Piccoli et al. (1985) reported a case of a 68-year-old man, who had been ingesting licorice for 9 years, with recurrent abdominal pain with constipation. After licorice was discontinued BP became normal, exchangable sodium decreased, blood volume and plasma electrolytes were normal in 3 wk, PRA increased slowly, plasma aldosterone remained somewhat hyporesponsive, and plasma norepinephrine decreased but remained elevated.

Jamil et al. (1986) reported the case of a 46-year-old man who presented with a 1-week history of body aches and recurrent episodes of muscular cramps. Three d later he developed generalized weakness and stated that his legs gave out on 2 occasions. This episode was timed to the last week of a month of daytime fasting and the patient gave a history of increasing thirst and that he had been consuming approximately 2.5 l/day of licorice flavored soft drink over the preceding 3 wk. He was treated with a total of 280 mEq of parenteral potassium chloride over 5 d. There was a gradual and complete return of muscle power and reflexes. Blood pressure returned to normal on the third day (130-85 mm Hg). The authors pointed out that the levels of fasting serum glucose in this patient were consistent with the diagnosis of type II diabetes mellitus.

Farese et al. (1990) reported the case of a 70-year-old man presenting with a 5-year history of hypertension. He was hospitalized and revealed he had been eating 60 to 100 g licorice candies daily for the past 4 to 5 years until admittance. The Glycyrrhizic Acid content was 0.3%. He was treated with potassium chloride, sprionolactone, and verapamil. After full recovery, the patient was readmitted again due to reincorporation of licorice into his diet; he was consuming 100 g/d (300 mg Glycyrrhetinic Acid/d). Licorice consumption was discontinued after 1 week and he was provided a constant diet.

Scali et al. (1990) reported on 3 women (21, 53, and 62 years old) and 1 man (74 years old) who were hospitalized for hypertension and hypokalemia. The patients did not eat licorice candy but were taking laxatives containing licorice for chronic constipation resulting in consumption of 0.5 to 8 g/d for periods ranging from 3 months to 3 yr. Three patients were using a laxative formulated by a herbalist and the other was consuming a commercial preparation. All were treated with spironolactone (200 mg/d) for 2 wk which resulted in normal BP. Two months after treatment all patients had normal potassium levels and BP.

Caradonna et al. (1992) reported a clinical case of acute myopathy in a 45-year-old man with a history of chronic licorice intoxication. Infusion of potassium restored normal electrolyte, enzyme, and hormone pattern accompanied with complete disappearance of clinical symptoms. Muscle biopsies were taken from the right deltoid at the beginning of electrolyte therapy, 3 d later, and 1 month after admission. The first biopsy showed several areas of muscular necrosis with macrophage infiltration. Biochemical analysis showed absence of myoadenylate deaminase activity. Analysis of the biopsy taken 1 month after admission shows that all muscle structure and enzyme activity had returned to normal.

Megia et al. (1993) reported the case of a 34-year-old man presenting with edema, hypertension, and hypokalemia. One week prior the patient had noticed edema in the lower extremities. He was not taking any medications. It was discovered that he had been consuming licorice candies (40 g/d) for the past 2 to 3 years and had increased his intake to >100 g/d the week before symptoms appeared. He stopped consuming licorice and was seen for follow-up every 2 to 6 wk. BP returned to normal and the edema resolved in 10 d. PRA (<0.036 to 0.13 ng/ml/h), plasma aldosterone (<1.5 to 153 ng/dl), and urinary aldosterone (<1 to 1 µg/24 h) returned to normal by day 48.

Rosseel and Schoors (1993) reported the case of a 55-year-old man with atypical abdominal pain. He had a history of chronic neurosis, appendectomy, cholecystemectomy, and an inferior myocardial infarction in 1987. He was advised to quit smoking and had started chewing gum. Further investigation found that his chewing gum was flavored with licorice (24 mg Glycyrrhizinic Acid/16 g pack). After stopping chewing gum, the licorice-related symptoms disappeared in 3 months.

van der Zwan (1993) reported the case of a 15-year-old boy who ate 0.5 kg licorice and developed a serious headache, nausea, vomiting, and right-sided weakness 3 h later. The patient was treated with dextran for 3 d then 300 mg calcium acetylsalicylate for 3 wk. Aphasia resolved completely in 1 week. BP fell to 115/80 within 24 h. Full consciousness was regained in 3 d. Physiotherapy was necessary for the hemiparesis, which improved slowly. The patient could not walk after 3 wk and still has slight hemiparesis after 3 months. The patient returned to playing sports after 5 months.

Basso et al. (1994) reported the case of a patient with diabetes presenting with dizziness and postural hypotension. The patient was treated with commercial licorice with a calculated intake of Glycyrrhizic Acid of 3 g/d. Symptoms rapidly ameliorated. Within 7 d BP was 150/90 mmHg and all other parameters were normal for a diabetic. She was able to walk. All symptoms totally resolved in 3 months. Patient was removed from licorice therapy and the symptoms returned in a few days.

Brayley and Jones (1994) reported the case of a 29-year-old woman admitted to the hospital with a 2-week history of fluctuating weakness and muscle pain. She was unable to get out of bed or dress herself. She had a history of anorexia nervosa with bulemia and had in the past month increased her daily intake of licorice from 300 g/d to 600 g/d and had decreased her food and drink intake. She was administered saline and potassium intravenously until her potassium reached 3.1 mmol/l and she regained her strength. She ceased both her licorice intake and forosemide.

Heikens et al. (1995) reported the case of a 40-year-old female admitted for a leg fracture who presented with a BP of 200/110 mm Hg and potassium of 2.4 mmol/l. BP continued to be difficult to control even with oral potassium tablets, 200 mg metoprolol and 3 mg prazosine daily. The patient denied licorice consumption, but a candy intake of 100 to 200 g/d intake and actually not taking the medication were discovered. Withholding licorice returned her BP and plasma potassium to normal in 2 wk.

Barrella et al. (1997) reported the case of a 61-yr-old man presenting with 4 d of progressive asthenic deficit involving the distal muscles of the upper limbs, especially the hands. This was accompanied with worsening burning sensation in the palms and prehension difficulties. The patient was treated with potassium chloride iv. There was a slight recovery the next day which continured over the next 6 d. It was revealed that the patient had been eating licorice-based sweets every day for about a year (~50 g licorice/d). This had discontinued upon admission to the hospital. He completely recovered with the discontinuation of licorice consumption.

Chamberlain and Abolnik (1997) reported the case of a previously healthy 64-yr-old man presented after 2 d of dyspnea on exertion, orthopnea, and fatigue. He had eaten ~1020 g black licorice over the past 3 d (~3.6 g Glycyrrhizic Acid). The patient was administered 40 mg furosemide iv, several doses of oral enalapril, potassium, and oxygen. Complete resolution of pulmonary edema was accomplished in 2 d. No pathology was found in an exercise thallium test. Follow-up at 2 months revealed a normotensive patient without shortness of breath, normal potassium level (4.5 mmol/l), and a normal chest x-ray. The patient reported consuming no more licorice.

de Klerk et al. (1997) reported 2 cases of hyper-mineralocorticoidism resulting from licorice-flavored chewing gum. The first case was a 21-year-old woman presenting with a headache. She was consuming ~100 g of licorice daily and using oral contraceptives. Her historical BP was 110/70 mm Hg and was 190/70 mm Hg at examination. She was instructed to discontinue contraception and licorice. Her BP did not decline even with treatment with a combination of atenolol, lisinopril, hydrochlorothiazide, and amlodipine. After a more thorough history, it was found that she had replaced her licorice intake with 2 packets of sugar free chewing gum containing 585 mg licorice in each packet. Three wk after stopping chewing gum her BP was 110/80 mm Hg and her plasma potassium concentration was 5.3 mmol/l.

A 35-year-old presented with hypokalemia of 2.2 mmol/l. The patient stated that she did not consume licorice. She was hospitalized and infused with potassium chloride. The patient was found to be using about 3 packets/day of chewing gum flavored with 160 mg licorice in each packet. After stopping the gum chewing and intravenous and oral potassium supplements, the patient's clinical tests were normal after 3 wk (de Klerk et al. 1997).

Kageyama et al. (1997) reported the case of a 69-yr-old woman presenting with hypertension with hypopotassemia. After oral administration of potassium, serum electrolyte data indicated hypernatremia (150 mEq/l) and hypopotassemia (2.9 mE/l). The patient revealed that she had been taking 30 to 50 granules of a mouth freshener that contains licorice, cinnamon, ginger, and other spices. One granule contained 5.1 mg licorice (0.2 mg Glycyrrhizic Acid); it was estimated that she was consuming 6 to 10 mg Glycyrrhizic Acid/d. With the cessation of the breath freshener, her BP and serum potassium normalized. Four months later all biochemical markers and BP were in the normal range.

Eriksson et al. (1999) reported the case of a 44-year-old woman presenting with severe ventricular tachycardia of torsades de pointes type. She had been having episodes of palpitations over the past month and faintness over the past 4 d. She had recently been diagnosed with anemia from iron deficiency so was on supplements. After potassium and magnesium infusion, lidocain, and a blood transfusion, the arrhythmias ceased after a few hours. She was then treated for hypertension. Tests for endocrine disorders were negative. It was discovered that she had been ingesting ~40 to 70 g licorice candy daily for ~4 months prior to hospitalization.

Lozano et al. (2000) reported the case of a 34-year-old woman presenting with pain for the past 6 h, coldness, pallor, and paresia of her right forearm and hand. The patient had a 10-year history of licorice consumption. A brachial chather-ballon thrombo-embolectomy was performed with urokinase i.v. Radial and cubital pulses returned. With potassium and sprionolactone, her potassium level returned to normal in 4 d and she was discharged after 9 d with mild postischemic paretic signs in her hand.

Negro et al. (2000) reported the case of a 23-yr-old woman presenting with recent onset of widespread edema and rapid weight gain (9 kg over 3 wk). She also had profound weakness during the previous 2 wk. She had been taking sodium valproate for epilepsy until the previous year; otherwise, her history was unremarkable. She was found to have been ingesting 100 g/d extrapure commercial licorice for the past 10 yr. This had doubled over the past month. Licorice ingestion was discontinued. Over the next 2 wk urinary sodium excretion began to exceed sodium intake with progressive reduction to disappearance of edema and serosal effusion. The patient was completely recovered in 4 months.

Russo et al. (2000) reported on 2 cases of induced hypertension encephalopathy. The first case was a 42-year-old man presenting with a profound soporific state and 3 d of a worsening headache, nausea, and vomiting. The day before he had a sensitive neuropathy on the left side of his body for 30 min. He had a 6-month history of slight hypertension not treated with drugs. Treatment was full doses of calcium channel antagonist, angiotensin converting enzyme (ACE) inhibitor, diuretic, and β-blocker. His potassium remained low even with 600 mg potassium chloride 3 times/d. It was discovered that he was consuming 50 g of licorice/d (100 mg Glycyrrhizic Acid). After stopping licorice consumption, his BP was 120/85 mm Hg after 2 wk and his plasma potassium concentration was 4 mmol/l.

The second case was a 46-year-old man presenting with deep asthenia, headaches, and somnolence. Two wk prior he had hypokalemia (2.3 mmol/l) that remained low despite potassium supplementation (1,800 mg/d). He was treated with potassium and urapidil but his BP remained elevated even after nitroglycerin and sodium nitroprusside administration. His symptoms finally began to subside with the discontinuation of licorice. At discharge, his BP was 150/80 mm Hg. At 4 months, his BP was normal in the absence of drug treatment (Russo et al. 2000).

Woywodt et al. (2000) reported on a 38-year-old woman who presented with a worsening headache, having suffered from migraines for years, and decreasing appetite. It was discovered that the patient had been consuming large amounts of licorice candies (containing 200 mg Glycyrrhitinic Acid and 1.5 g sodium/100 g) and licorice lozenges (containing 200 mg Glycyrrhicinic Acid and 60 mg sodium chloride/100 g). She had increased the amount of lozenges consumed before admission. She was administered urapidil then metoprolol. Ramipril and hydrochlorothiazide were added. After some time on a licorice-free diet she became hypotensive and treatment was discontiued.

Elinav and Chajek-Shaul (2003) reported on a 36-year-old man with a 4-d history of rapidly progressing limb weakness, starting from the legs and hands, the arms, thighs, and torso. He could not lift his hands from the bed. The patient was administered potassium orally and intravenously. Serum potassium and creatine kinase levels were normal after 3 d. The patient then admitted eating ~25 g of licorice candy daily over the past year and had been drinking tea sweetened with licorice over the past 2 wk. He was instructed to avoid licorice. He had a normal check up 18 months later.

Hussain (2003) reported a case of a 56-year-old woman presenting with flu-like symptoms over 7 d. She had nausea, vomiting, and diarrhea and was generally weak to the point of incapacitation. She was administered potassium chloride and her serum potassium, CK concentrations, and liver function returned to normal in a few days and she regained motor function. She had been consuming "Pontefract cakes", a licorice sweet, to manage her chronic constipation at doses of 200 to 400 g/d, equating to 15 g licorice/d.

Lin et al. (2003) reported a case of a 76-year-old man presenting with muscular weakness that proceeded to paralysis. It was discovered he had been drinking tea flavored with ~100 g of licorice root (~2.3 g Glycyrrhizic Acid) daily for 3 years. Spironolactone was added to his treatment. With discontinuation of the tea his plasma potassium increased to 2.8 mmol/l 1 week later. At 2 wk, his plasma potassium and BP returned to normal and his body weight decreased from 78 to 74.0 kg.

Mumoli and Cei (2007) report a case of a 55-year-old man presenting with mild symptoms of depression and a 1-month history of leg cramps and fatigue. It was discovered that since he had quit smoking ~1 year earlier, he had been consuming a packet (25 g) of natural licorice root (2.3% Glycyrrhizic Acid) daily. The patient was treated with potassium chloride (i.v. and oral) which normalized his potassium levels after 3 d. He was discharged after 7 d with instructions to avoid licorice.

Yasue et al. (2007) report a case of a 93-year-old woman with a 20-year history of hypertension that had been taking a calcium antagonist (amlodipine, 5 mg/d). She presented with severe pain and swelling in both knees, inability to walk, fever of 38.4°C (101.1°F), and anorexia. The knees were aspirated, dexamethasone (4 mg) was injected and pseudo-gout was diagnosed. She was administered sodium and potassium by i.v. with oral antibiotics and anti-inflammatory drugs. It was found that the patient had been taking 2 herbal medicines, ninjinto and saikokeishito, prescribed by her doctor. Each contained licorice and she had consumed 5 g licorice daily for 7 years. She denied any previous similar episode. The herbal drugs were stopped and she was treated with Aspara K and sprionolactone. She had improvement within 2 wk and was normal in 3 wk.

Dermal

Nishioka and Seguchi (1999) reported the case of a 43-yr-old woman with itchy reddish eruptions on the face over a month. She had a history of Behçet's disease with macular amyloidosis on her back. A patch test of 15 cosmetics that she had been using, and 33 allergens of a cosmetic series resulted in positive reactions to facial cream, foundation, and essence. A further patch test of 7 ingredients present in 3 of these products resulted in a positive reaction to oil-soluble licorice extracts at 0.5%, 1%, and 5% in petrolatum after 2 and 3 d.

Vision Impairment

Dobbins and Saul (2000) reported 5 cases of temporary vision impairment or loss associated with licorice ingestion.

A 62-yr-old man with a history of hypertension but no history of transient vision loss presented with of vision OS (ocular sinister, left eye) for 3.5 h the previous day. He was treated with hyperbaric oxygen (90 min at 2.4 atmospheres, 100% O2). His visual acuity improved. The patient was found to have consumed 8 oz (~248 g) licorice-flavored jelly beans over the 3 d prior to symptoms. After 2 d of no licorice consumption, his BP was 145/75 mm Hg and his visual acuity remained 20/20 OS. Some "blotchiness" and occasional episodes of "...a piece of a jig-saw puzzle missing..." were reported at the 1 month and 4 month follow up examinations.

A 67-yr-old man with a history of benign prostatic hypertrophy and hypercholesterolemia presented with sudden onset scintillating peripheral scotomas; his central vision was minimally affected. The patient stated he was up late reading and consuming ~0.5 lb licorice. He remembered having similar episodes of shorter duration associated with consuming licorice. He was advised to stop licorice ingestion. After 9 yr of reduced licorice consumption, only 1 mild episode was reported after significant coffee consumption.

A 26-yr-old woman presented with 13 d of headaches and spells of vision loss and a "fuzzy" expanding spot in her left eye that became moon-shaped and spread into the right visual field. The spells would last for 30 min to several hours. The patient reported

eating 1 to 2 lbs (453.6 to 907 g) licorice/week and drinking ~20 cups of coffee/d and a pot of iced tea/d. She had visual acuity of counting fingers OD and 20/20 OS. The patient was advised to cease consuming licorice and lower her caffeine intake. Her visual field was improved after 4 d. After 7 yr she has stopped all licorice ingestion and decreased caffeine consumption resulting in only the occasional headache with only 2 associated with mild visual auras.

A 65-yr-old man presented with 4 months of intermittent left-sided headaches that would become generalized with some visual aberrations in the left eye, described as "pinwheels" that were red and white and fluctuating. The patient reported that he ate "a lot" of licorice and had consumed 0.25 lbs (113 g) licorice that night before presenting. Verapamil HCl RS 180 mg was prescribed and the patient was administered prochlorperazin iv. Headaches resolved in 24 h. The patient was advised to cease licorice consumption. After 2 yr, the headaches and visual problems had resolved.

A 39-yr-old man presented with a history of multiple episodes of binocular scintillating scotomas lasting 20 to 30 min. He was reported to be otherwise healthy. The patient reported ingesting "...about half of a bag..." of licorice-flavored candy during that morning and afternoon. The patient was advised to cease consuming licorice. After 13 yr of very little licorice consumption there were no further episodes of vision problems.

The authors suggested that licorice consumption caused the visual disturbances in these cases and are the result of retinal and occipital vasospasm and possibly vasospasm of vessels supplying the optic nerve (Dobbins and Saul 2000).

Epidemiology

Licorice Extract

Ibsen (1981) conducted a population study of 475 girls and 464 boys in the Copenhagen school system (ages 6 to 18 yr). The majority of the children were 16 and 17 years old. The children's weekly consumption of licorice was determined by questionnaire and then their BP was measured. A third of the children seldom or never ate licorice and were placed into the <50 g/week group. From the questionnaire, 358 were in the <50 g/week group, 457 in the 50 g/week group, 78 in the 100 g/week group, and 46 in the >100 g/week group. Only 3 (6%) had a BP higher than the 95 percent level. Of the 7 girls with BP greater than the mean plus one standard deviation, 2 were using contraception. The linear correlation coefficient between licorice consumption and BP was 0.05, not significant. The linear regression coefficient was 0.81, not significant. The author suggested that variation in response to licorice consumption may be genetically determined.

Strandberg et al. (2001) distributed questionnaires to Finnish mothers after the birth of singleton healthy births where both parents were of Finnish origin. Sick or preterm babies (<32 wk; n = 250) were not included; 1006 were included. Weekly licorice intake was used to calculate glycyrrhizin intake as the standard. Age of fetuses was determined by ultrasound. Regular consumption of licorice was reported by 46% of the mothers; 2% reported daily consumption. No licorice consumption was reported by 2.3% of the mothers during pregnancy. Low (< 250 mg/week), medium (250 to 499 mg/week 250), and high (≥ 500 mg/week) licorice consumption was found for 75%, 14%, and 11% of the mothers surveyed, respectively. Regression analysis showed a reduction in gestational age of 0.18 wk (1.26 d; 95% CI 0.31, 2.24; p = .009) for every 500 mg/week increase in glycyrrhizin intake. The high intake group had shorter gestation times than the low intake group by .036 wk (2.52 d; 95% CI 0.63, 4.34; p = .01). The difference remained significant after adjustments for age, smoking, parity, BP, and coffee consumption and the exclusion of the 87 augmented or induced births. The higher consumption of licorice resulted in an odds ratio of 2.5 (95% CI 1.1, 5.5; p = .03) of a delivery at < 38 wk.

Strandberg et al. (2002) distributed questionnaires to Finnish prenatal mothers in 3 maternity wards to determine any association with licorice consumption and preterm births (< 37 wk of gestation). The questionnaires asked detailed questions about consumption of licorice-containing foods and was used to determine glycyrrhizin intake. Preterm births associated with twins, elective Cesarean section, or induced delivery were excluded. The mothers were divided into 3 groups by Licorice consumption: low (< 250 mg/week), moderate (250 to 499 mg/week), and heavy (≥ 500 mg/week). There were 107 controls (mean gestation of 39.8 ± 1.3 wk) and 95 preterm cases (mean gestation of 33.3 ± 3.3 wk). Median glycyrrhizin intakes among the controls was 104.0 mg/week (range = 50.0 to 310.0 mg/week) and of the preterm mothers was 150.0 mg/week (range = 75.0 to 409.0 mg/week).

Of the heavy consumers of glycyrrhizin, 19 of 95 (20%) had premature births whereas 11 of 107 (10.3%) of the controls were heavy licorice consumers (p = .06) using the Mann-Whitney U test. The odds ratio of having a premature birth of < 37 wk and < 34 wk for those in the heavy consumption group was 2.18 (95% confidence interval 0.98, 4.86) and 2.37 (95% confidence interval 1.00, 5.57), respectively. The authors concluded that heavy licorice exposure was associated with preterm delivery (Strandberg et al. 2002) .

Cuzzolin et al. (2006) performed a survey of Italian women over a 5-month period in the outpatient ambulatories of an urban university to investigate the use of phytomedicine and any side effects or drug interactions. Of those completing the questionnaire (1,044), 491 (47%) reported taking at least one herbal compound. Of these, 47 reported side effects. Of these 47, 13 (27%) reported cardiovascular problems such as hypertension, ischemia, and tachycardia after licorice, ginseng, and green tea (this was not teased out further in the report); 5 reported adverse reactions to licorice (cardiovascular, neurological) while also taking non-steroidal anti-inflammatory drugs (NSAIDs) or contraceptives.

Other Evaluations

The National Toxicology Program (1974) stated that there is no evidence in the available information on licorice, glycyrrhiza, and ammoniated glycyrrhizin that demonstrates or suggests a hazard to the public when they are ingested at current levels and manner.

However, with the data reviewed it is not possible to determine whether a significant increase in consumption would constitute a dietary hazard. The maximum percentage reported for use of licorice root in food was 24.400% in hard candy, for licorice extract it was 8.020% in hard candy, for licorice extract powder it was 3.279% in soft candy and for ammoniated glycyrrhizin it was 0.228 in chewing gum.

Størmer et al. (1993) examined the case histories and clinical studies in the literature and concluded that the lowest observed adverse effect level (LOAEL) for regular intake of licorice is the amount that would equal 100 mg Glycyrrhetic Acid/day. Using a safety factor of 10, the safe dose for most healthy people was concluded to be 10 mg Glycyrrhizic Acid/day which would be about 5 g licorice sweets/day assuming 0.2% Glycyrrhizic Acid in licorice extract.

SUMMARY - Part I

Glycyrrhiza Glabra (Licorice) Rhizome/Root, Glycyrrhiza Glabra (Licorice) Leaf Extract, Glycyrrhiza Glabra (Licorice) Root, Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) Root Juice, Glycyrrhiza Glabra (Licorice) Root Powder, Glycyrrhiza Glabra (Licorice) Root Water, Glycyrrhiza Inflata Root Extract, and Glycyrrhiza Uralensis (Licorice) Root Extract are botanical cosmetic ingredients.

The extracts are prepared from various parts of *G. Glabra, G. Inflata,* and *G. Uralensis* plants in a single step using water or in multiple steps using water, ammonia, ethanol, ethyl acetate, and/or acetone.

A wide variety of chemicals have been reported in licorice extracts. Different constituents in the final product vary with solvent. Glycyrrhizic Acid and its derivatives, including Glycyrrhetinic Acid, were reviewed in a separate safety assessment and found to be safe. (See Part II for safety information on constituents of licorice extracts)

Impurities in licorice extracts may include methanol, ethanol, arsenic, and lead.

Glycyrrhiza Glabra (Licorice) Rhizome/Root, a flavoring agent and fragrance ingredient, was used in 8 cosmetic products; no concentration of use was reported. Glycyrrhiza Glabra (Licorice) Leaf Extract, a skin-conditioning agent - miscellaneous, was used in 2 cosmetic products; no concentration of use was reported. Glycyrrhiza Glabra (Licorice) Root Extract, an antioxidant, skin conditioning agent-miscellaneous, and skin conditioning agent-humectant, was used in 53 cosmetic products at concentrations ranging from. 0.0001% to 0.4%. The polyol-soluble Glycyrrhiza Glabra (Licorice) Root Extract , which contains a higher concentration of glabridin, was used at concentrations of 0.0001% to 0.06%. GlycyrrhizaGlabra (Licorice) Root Extract, an antioxidant, skin conditioning agent-miscellaneous, and skin conditioning agent-humectant, was used in 53 cosmetic products ranging from of 0.0001% to 0.4%. Glycyrrhiza Inflata (Licorice) Root Extract was reported to be used at 0.0001% to 0.5%.

Glycyrrhiza Glabra (Licorice) Root, Glycyrrhiza Glabra (Licorice) Root Juice, Glycyrrhiza Glabra (Licorice) Root Powder, Glycyrrhiza Inflata Root Extract, and Glycyrrhiza Uralensis (Licorice) Root Extract function in cosmetics as skin-conditioning agents - miscellaneous. Glycyrrhiza Glabra (Licorice) Root Water functions as an antioxidant, cosmetic astringent, and a skin-conditioning agent - emollient. There are no reported uses or concentration of use for these ingredients. Poly-ol Soluble Glycyrrhiza Inflata Root Extract is used at 0.001 to 0.05%.

Medical uses for Licorice Extracts include treating gastric ulcers, stomach and mouth ulcers, thirst, hepatitis, scabies, renal dysfunction, wounds, cough, inflammation, and constipation. Licorice Extracts are used as skin lighteners. Non-medical uses of Licorice Extracts include flavoring tobacco, chewing gums, and candies. They are used as sweetening agents. *G. glabra* and other species of Glycyrrhiza are generally recognized as safe by the Food and Drug Administration.

No data were available on the dermal penentration of the ingredients in this report. However, there is data on the constituents of these botanicals. Licochalcone A penetrated the horny layer, epidermis and dermis of pig skin but none was detected in the receptor fluid. Oral administration to rats of licorice extracts produced elevated blood levels of Glycyrrhetic Acid. The levels of Glycyrrhetic Acid in rats were not different as a function of roasting or not roasting the extract prior to oral exposure.

Ingestion of licorice extracts reportedly has a wide range of systemic effects. They included: reduction of ACTH, cortisol, and alderstone in rats and induction of liver oxygenase and testosterone hydrolase in mice and rats (linked to up- and down-regulation of genes).

Cerebral function and memory effects of licorice extract were determined using mice and maze performance (enhanced memory). Cognitive deficits in mice were reduced with ingestion of licorice extract; and brain cholineserase levels were reduced in mice given oral doses of licorice extract for 7 d.

Some convulsions were reduced by licorice extract i.p. injection in mice, in a dose-dependent manner.

A wide range of anti-effects have been reported, including: anti-inflammatory in mice in vivo and in several in vitro assays; anti-diabetic in rats and mice in vivo and in several in vitro assays; antioxidant in rats in vivo and in several in vitro assays; anti-viral in vitro (no effect on JEV and some inhibition of Epstein-Barr virus early antigen); anti-parasitic against Chinese river fluke; anti-microbial against C. albicans and other fungi, Propionibacerirum acnes, and Streptococcus mutans; and anti-hepatotoxic effects in rats.

Licorice extract reportedly increases the metabolism of acetaminophen, interacts with warfarin to increase bleeding, and may reduce effectiveness of cancer therapies that rely on potassium loss.

Glycyrrhiza Glabra (Licorice) Root Extract is not orally toxic to rats up to 5 g/kg and to Crj:CD-1(ICR) mice at 2250 to 5250 mg/kg; an intraperitoneal LD_{50} of > 5000 mg/kg was reported in

rats. The LD_{50} was > 2000 mg/kg in mice (p.o.) for the Glycyrrhiza Glabra (Licorice) Leaf Extract. The respective LD_{50} values of Licorice Extract in mice via intravenous and intraperitoneal routes are 251 and 641 mg/kg. There was no mortality to mice up to 1 g/kg intraperitoneally administered ethanolic extract of *G. glabra* root. Licorice Root Extract had a subcutaneous LD_{50} of 4200 mg/kg in rats and 4000 mg/kg in mice.

The effects of Glycyrrhiza Glabra (Licorice) Root Powder were similar to Ammoniated Glycyrrhizin and dichloroacetate in male Sprague-Dawley rats with regards to weight, growth, and organ weights. F344 rats fed powdered diets containing 0.38%, 1.5% or 3% (wt/wt) Licorice Extract ad libitum for 1 month or 3 months showed no effects at necropsy.

B6C3F1 mice fed diets containing 0 to 25% licorice root extract for 30 and 90 d had poor weight gain (at > 0.8%) that coincided with low food consumption. Clinical signs in the 8% and 25% groups included thinness, hunched posture, a lump near the prepuce, alopecia, swollen irritation and redness around the anus and tail, rough fur, lethargy/depression, and weakness. The NOEL for Licorice Root Extract in this study was 0.8%. Orally administered food grade licorice extract did not result in mortality in CD-1 mice (n = 30) for 5 d. There was no difference in mortality between the treated groups and control in the host resistance assay. There was no difference in the viability or activity of spleen cells between the treated groups and the control.

Rats orally administered Glycyrrhiza Glabra (Licorice) Root Powder for 50 d had a mortality rate of 9/14. The rats lost weight, had greater kidney weights, relative heart weights, and absolute and relative adrenal weights compared to controls. The rats had lower absolute pituitary weights. The kidneys had glomerular hyalinization, tubular dilations, hyaline casts, and proliferation of the interstitial tissue.

Glycyrrhiza Glabra (Licorice) Root Extract was found to be non-irritating to rabbit eyes. Glycyrrhiza Glabra (Licorice) Root Extract (1% in glycyerine product; 0.1 ml) was a slight ocular irritant in albino New Zealand rabbits. In a corneal model system consisting of normal, human-derived keratinocytes, Glycyrrhiza Glabra (Licorice) Root Extract (10%) was not irritating.

Glycyrrhiza Glabra (Licorice) Root Extract (0.15% in corn oil; 0.5 ml) applied to the intact and abraded skin of New Zealand white rabbits was not a primary dermal irritant. In a local lymph node assay Licorice Extract was a non-sensitizer when tested up to 10%. Licorice is not a primary irritant at 10% in rabbits (primary skin irritation test and repeated skin irritation test) and is not a skin sensitizer at 100% in guinea pigs. In a guinea pig maximization test on Glycyrrhiza Glabra (Licorice) Root Extract, there were no signs of sensitization. In an irritation test of Glycyrrhiza Glabra (Licorice) Root Extract (2%) on guinea pigs, there were no signs of irritation at any observation period.

The oral treatment with both wild and cultivated water extract of Glycyrrhiza Uralensis (Licorice) Root Extract (50, 100, or 200 mg/kg) of BALB/c mice reduced ear swelling after the mice were administered an intravenous (i.v.) injection of anti-dinitrophenol monoclonal antibody immunoglobulin E mAB-containing fluid 24 h before a dinitrofluorobenzene challenge.

Licorice Extract in a 3T3 Neutral Red (NR) Uptake Phototoxicity Test caused no cytotoxic effects (NR50 > 1,000 mg/l), but photocytotoxic effects (NR50 = 13.2 mg/l) were observed in this in vitro test with 5 J/cm2 UVA. Licorice Extract tested for phototoxicity using the EpiDermTM Phototoxicity Test with 6 J/cm2 UVA caused no cytotoxic and no photocytotoxic effects in this in vitro test. In a photosensitization test of Licorice Extract up to 2.5% in albino guinea pigs, there were no positive reactions to any of the exposures to Licorice Extract with or without UVA irradiation. Glycyrrhiza Glabra (Licorice) Leaf Extract was not phototoxic to guinea pigs at 10% and 100%. It was also not a photosentizer at 20% in guinea pigs.

Glycyrrhiza Glabra (Licorice) Root Extract was not a reproductive toxicant when orally administered at doses up to 2,000 mg/kg/d for 9 wk before mating, starting at 6 wk old, for males and 2 wk before mating, starting at 8 wk old, for females.

The estrogen and progestin bioactivity of a 50% ethanol/distilled water Licorice (*G. glabra*) Extract was tested on estrogen receptor (ER)/progesterone receptor (PR) positive (MCF7 and T47D) and ER/PR negative (MDA-468) breast carcinoma cell lines. The extract had similar effects on ER-binding of ER(-) breast cancer cells as the control. Licorice extract that demonstrated progestin antagonist properties by partially or totally blocked enzyme induction by progesterone in T47D cells were incubated in the extract. Licorice Extract (*G. glabra* L.) was not active when testing for ERα binding, ERβ binding, or alkaline phosphatase induction with Ishikawa cells. The results were negative in an uterotrophic assay for estrogenic activity of the alcohol extract of licorice. Water extract of Glycyrrhiza Glabra (Licorice) Root Extract used to treat MCF-7 human breast cancer cells stimulated cell growth at a range of 0.1 to 10 µg/ml. The alcohol extract of Licorice Root did not promote the proliferation of MCF-7 cells.

In the Ames testing the water and methanol extracts of *G. uralensis* were negative but, the methanol extract was cytotoxxic. In a rec-assay test using ethyl methanesulfonate (EMS; 4 µl/plate) as the mutagen, the ethanol Glycyrrhiza Glabra (Licorice) Root Extract (100 µg/plate) decreased the zones of inhibition when co-treated with the mutagen. Using 9-aminoacridine and acriflavine HCl (frameshift mutagens) in Ames tests, the extract reduced the number of revertants by a factor of 2. When F344 rats were fed Licorice Extract mixed in food (0.38%, 1.5%, and 3.0%), there were no cancer chemopreventive effects observed. There were no significant lesions or physiological changes related to dietary supplementation with licorice.

The water, ethyl acetate, DMSO, and ethanol extracts of *G. glabra* root on T47D (breast cancer), MCF-7 (breast cancer), and HL-60 (leukemia) cells were tested by Western blot analysis. All the extracts induced phosphorylation of Bcl-2 as demonstrated by slower migrating bands in contrast to the control (ethanol) except the water extraction. The licorice extracts induced G2/M cell cycle arrest similar to paclitaxel.

Trichloromethane and ethyl acetate extracts of licorice root inhibited the proliferation of MCF-7 cells in a dose- and time-dependent manner; 70% methanol and hexane extracts inhibited cell growth also in a dose- and time-dependent manner. All the test compounds induced chromatin condensation and nuclear fragmentation. MCF-7 cells treated in the exponential phase of growth accumulated in the sub G1 phase. MCF-7 cells treated with the methanol licorice extract had induced PARP degradation fragments.

Ehrlich ascites tumor cells injected intraperitoneally into Swiss albino mice followed by treatment with *G. glabra* crude extract or with methanolic or water extract, resulted in a 90% inhibition of growth of Ehrlich ascites tumor cells and formation of Ehrlich ascites tumor mass fluid. Petroleum ether, hexane, and benzene extract treatment resulted in inhibition to lesser extents.

BALB/c mice were orally administered ethanol extract of *G. inflata* with and without cisplatin. G. inflata ethanol extract (0.5, 1, and 2 mg/kg) inhibited tumor growth by 38%, 57%, and 71%, respectively, compared to cisplatin which inhibited tumor growth 92%.

A chorioallantoic membrane (CAM) assay showed that there was inhibition of growth of new blood vessels by G. glabra in chick chorioallantoic membranes.

In multiple clinical assessments of safety, oral administration of Licorice Extract resulted in various levels of hypokalemia; sodium retention; edema of the face, hands and ankles; headaches; lethargy; electrolyte imbalance; increased urinary unconjugated cortisol; reduced plasma renin activity; increased plasma atrial natriuretic peptide concentration; and hypertension. All symptoms resolved after termination of treatment with Licorice Extract.

In men, treatment with Licorice Extract resulted in a reduction of 25% in serum testosterone during licorice consumption. Serum luteinizing hormone (LH) increased. After treatment terminated, mean serum free and total testosterone returned to pretreatment levels. Licorice Extract also reduced serum testosterone in women.

Orally administered deglycyrrhizinized licorice effectively treated chronic duodental ulcers.

A very slight erythema was observed in 3 of 9 volunteers tested with a product containing ~900 ppm Glycyrrhiza Glabra (Licorice) Root Extract with an occlusive patch. In a repeated insult patch test of 16 products containing Glycyrrhiza Glabra (Licorice) Root Extract (0.0001%) there were no signs of irritation or sensitivity except for 1 subject who showed signs of delayed contact hypersensitivity to 1 product. Subjects applied 3 products containing Glycyrrhiza Glabra (Licorice) Root Extract (0.0001%) nightly for 14 d with no increases in objective or subjective irritation scores from baseline. When 3 other products containing Glycyrrhiza Glabra (Licorice) Root Extract (0.0001%) were applied near the eyes of volunteers, the ophthalmological examination showed only very mild changes that were not judged to be clinically significant. A product containing Glycyrrhiza Glabra (Licorice) Root Extract (0.8%) applied to the skin for 12 wk resulted in no differences at any observation period for erythema and scaling/dryness between baseline nor the control group. There were no increases in objective or subjective irritation. There were no adverse effects reported. In a dermal patch test of a product containing Glycyrrhiza Glabra (Licorice) Root Extract (0.0001%) there was no evidence of sensitization or irritation.

Oil soluble Licorice Extract was not a human skin irritant at 1% and not a skin sensitizer at 0.5%. Oil soluble licorice extract P-TH was not a skin irritant at 1%. Glycyrrrhiza Glabra (Licorice) Leaf Extract was not a skin irritant at 10%. When the irritation, acnegenic, and comedogenic potential of a skin care regimen that includes an essence that contains Glycyrrhiza Glabra (Licorice) Root Extract (0.105%) was tested, there was an increase in burning/stinging during the first 2 wk. A repeat insult patch on human subjects of an "essence" containing oil soluble Glycyrrhiza Glabra (Licorice) Root Extract (0.105%) resulted in no evidence of sensitization or significant irritation. A closed patch test of Licorice Extract (1% in 80% butylene glycol) applied for 24 h to the upper arms of 44 healthy males and females was negative.

Oil soluble Licorice Extract was not a human photosensitizer at 5%.

A higher consumption of licorice ($\geq$ 500 mg/week) by pregnant women resulted in an odds ratio of 2.5 of a deliver at < 38 wk for having premature births. In clinical testing, deglycyrrhizinated licorice was effective in treating apthous ulcers. No link was found between licorice consumption and BP in another study.

PART II - Constituent chemicals

Licorice Constituent Extraction

Licorice constituents are separated out of licorice extract by a series of steps that may involve solvent extraction, centrifugation, high performance liquid chromatography (HPLC), and other separation procedures.

Zayed et al. (1964) fractioned crude alcoholic extract of *G. glabra* root as diagrammed in **Figure 1**.

Hatano et al. (1991) isolated glicoricone and licofuranone from Chinese licorice by a multistep extraction. The plant was defatted with n-hexane and then extracted with ethyl acetate. The ethyl acetate extract was subjected to centrifugal partition chromatography and column chromatography.

Vaya et al. (1997) used a multi-step solvent approach to extract hispaglabridin A, hispaglabridin B, 4'-O-methylglabridin, isoprenylchalcone, formononetin, isoliquiritigenin and glabridin from powdered licorice root of *G. glabra*. Acetone extraction of the powdered root followed by solvent evaporation produced a brown solid. The organic fraction was suspended in hydrochloric acid and chloroform and further separated in methanol/hexane (90:10). The hexane fraction was further extracted with ether to produce isoliquiritigenin. The methanol fraction was further extracted through several steps to produce hispaglabridin A, hispaglabridin B, 4"-O-methylglabradin, isoprenylchalcone, fomonenetin, and glabridin.

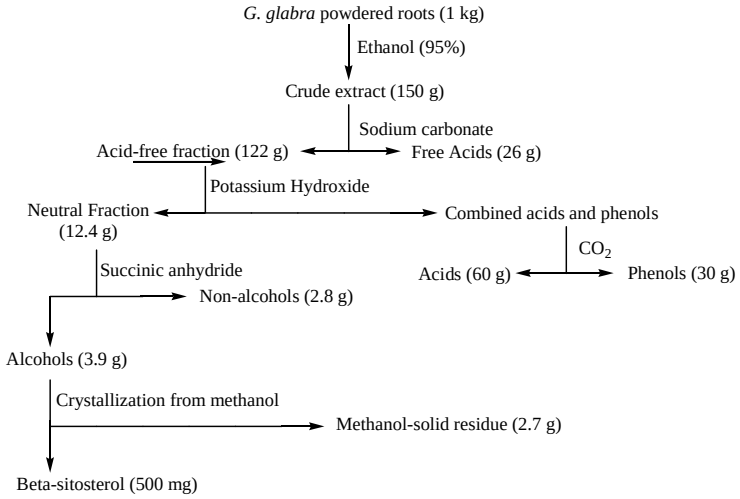


Figure 1. Fractionation procedure of crude alcoholic extract of *G. glabra* root (Zayed et al. 1964).

Licochalcone A is extracted from *G. inflata* by high-speed counter-current chromatography (Wang et al. 2004).

Cong and Lin (2007) used simulated moving bed technology to extract liquiritin from licorice root.

Sun et al. (2007) extracted Glycyrrhizic Acid and liquiritin from dried *G. uralensis* root using microwave-assisted micellar extraction with recoveries of 98.4% and 96.1%, respectively.

Analytical Methods

Methods for the analysis of the constituent chemicals of licorice-derived ingredients are listed in **Table 28.**

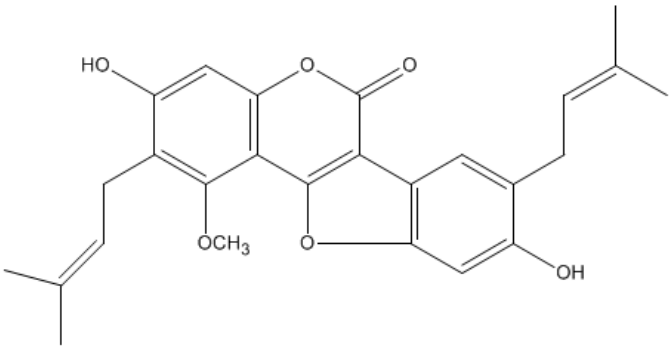
CHEMICAL STRUCTURES

Hatano et al. (1991), Fukai et al. (2000), Haraguchi et al. (2000), Singletary and MacDonald (200), Tanaka et al. (2001), and He et al. (2006) depicted structures for 52 chemical constituents of licorice extracts. These structures are shown in **Figure 2.**

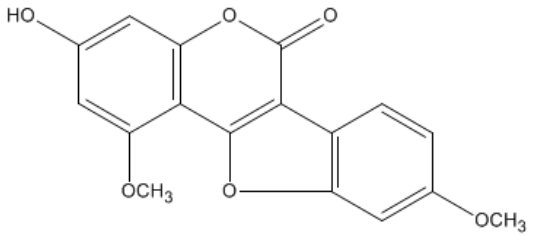
Table 28. Methods used to analyze chemical constituents of licorice-derived ingredients.

Method	Purpose	Reference
High-performance liquid chromatography (HPLC)	Determine the content of Glycyrrhizic Acid in Licorice-containing confectionary and health products	Spinks and Fenwick 1990
Mass and nuclear magnetic resonance (NMR) and by synthesis	Established the structure of licochalcone A	Chen et al. 1993
HPLC	purity	Chen et al. 1993
Gas chromatography (GC), mass spectra (MS) analysis, and HPLC	Purity of components extracted from licorice	Vaya et al. 1997
Low frequency electron paramagnetic resonance (EPR)	Measure free radicals in licorice root, powder, and sweets in the stomach after oral administration to mice	Gallez et al. 2000
Semi-micro-HPLC	Simultaneously detect Glycyrrhizic Acid, Glycyrrhetinic Acid, and glycyrrhetinic acid mono-glucuronide in a combination of Licorice root and peony root with rat feces	Okamura et al. 2001
MS and NMR spectra	Isolate and identify Glycyrrhizinic Acid, Glycyrrhetinic Acid and other constituents in a licorice extract	Liu et al. 2001a
Capillary electrophoresis	Assess the quality of compound licorice tablets	Sun et al. 2003
Capillary electrophoresis with electrochemical detection	Determine the amount of liquiritigenin and isoliquiritigenin in *G. uralensis* medical preparations, compound licorice tablets, and compound licorice mixtures to a detection limit of 4.7×10^{-7} and 2.9×10^{-7} mol/l	Cao et al. 2004
Capillary electrophoresis	Determine the presence of Glycyrrhetinic Acid, glycyrrhizin, and isoliquiritigenin in dried, dry roasted, and honey roasted *G. glabra* root	Sung and Li 2004
Capillary-zone electrophoresis (CZE)	Determine the content of licorice and distinguish species by the CZE peak area data of glycyrrhizin, glabridin, Glycyrrhetic Acid, liquiritin, and licochalcone A	Rauchensteiner et al. 2005
HPLC	Identify and quantify Glycyrrhizic Acid, glabridin, and lichochalcone in food grade and other types of licorice root estracts	Access Business Group 2007
Liquid chromatography tandem mass spectrometry (LC-MS/MS)-based method	Simultaneously quantify multiple active licorice flavonoids (including liquritin, apioside, liquiritin, liquiritigenin, isoliquiritin apioside, isoliquiritin, and isoliquiritigenin) in plasma	Li et al. 2007
LC with photodiode array detection	Determination of glycyrrhizic acid	Hennell et al. 2008
LC	Content verification	Korea Food & Drug Administration (KFDA) no date b

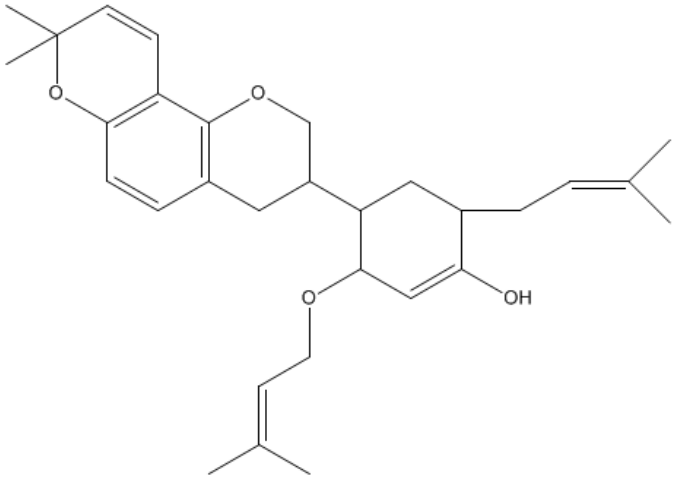
Figure 2. Structures for 52 chemical constituents of licorice extracts.



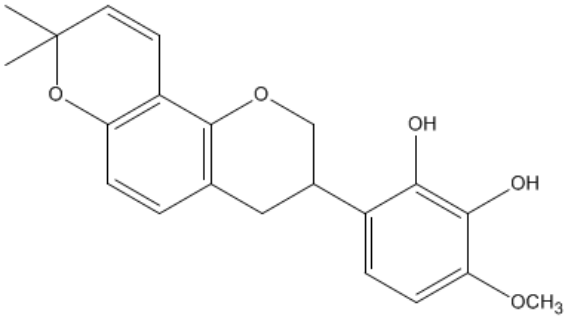
1-methoxyficifolinol



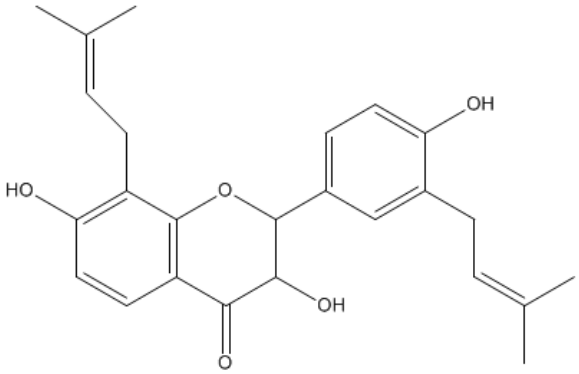
1-methoxyphaseollidin



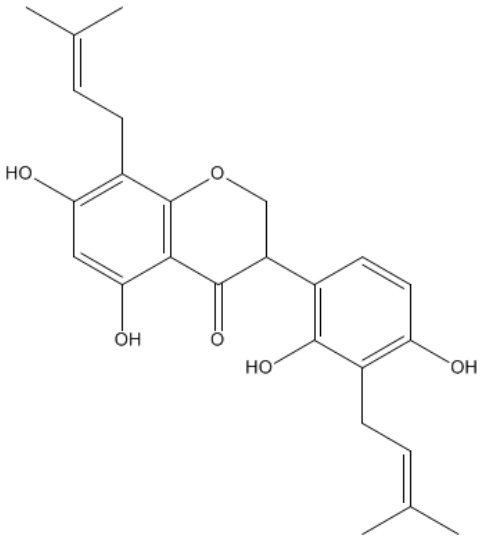
2'-*O*-5'-*C*-diprenylglabridin



3'-hydroxy-4'-*O*-methylglabridin

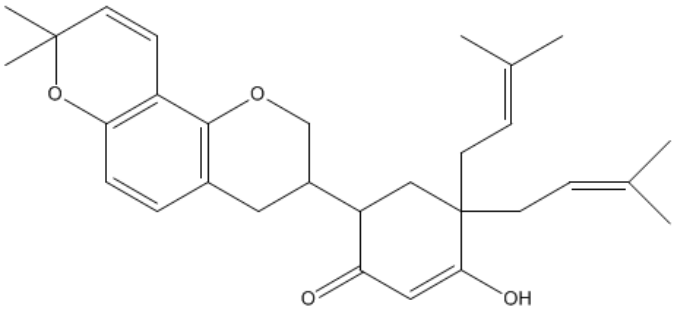


3-hydroxyglabrol

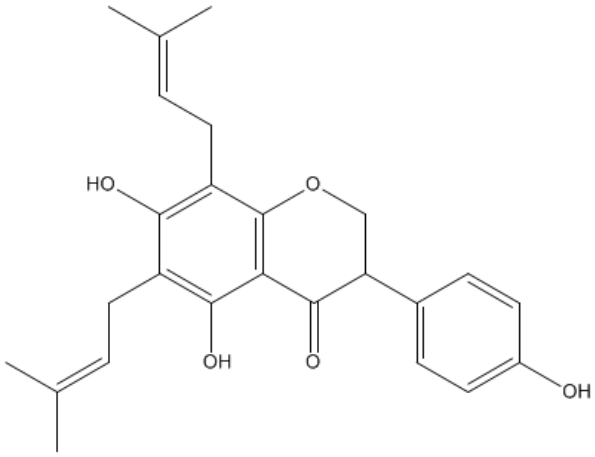


3'-prenylkievitone

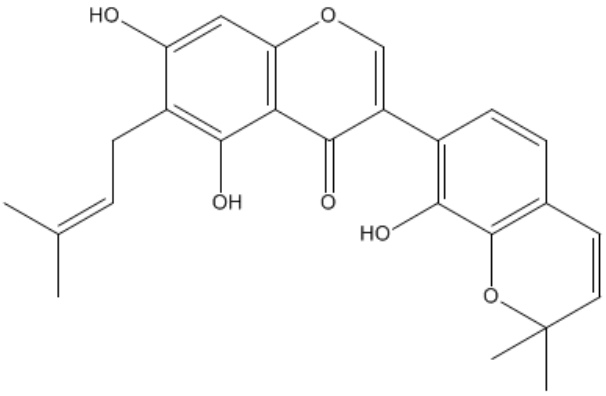
Figure 2 (continued). Structures for 52 chemical constituents of licorice extracts.



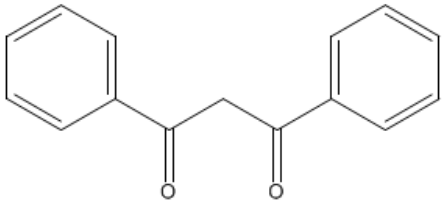
5,5'-diprenylglabridin



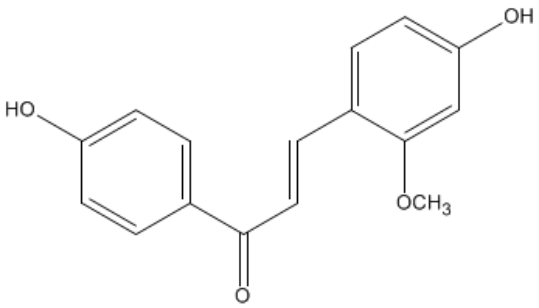
6,8-diisoprenyl-5,7,4'-trihydroxyisoflane



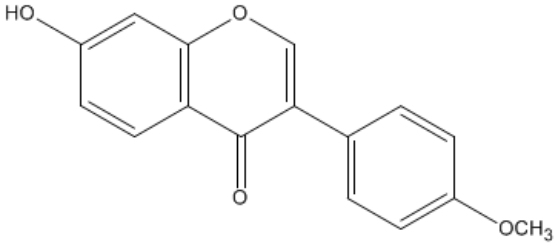
augustone



dibenzoylmethane

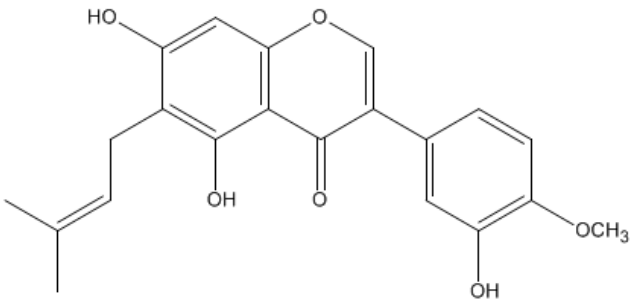


echinatin

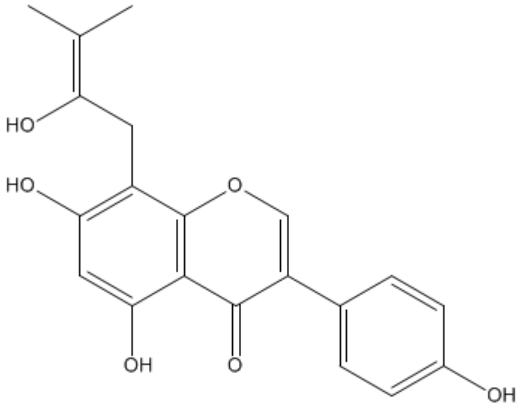


formononetin

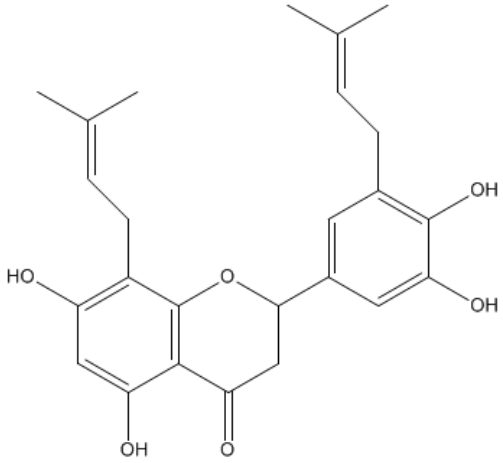
Figure 2 (continued). Structures for 52 chemical constituents of licorice extracts.



gancaonin B



gancaonin C



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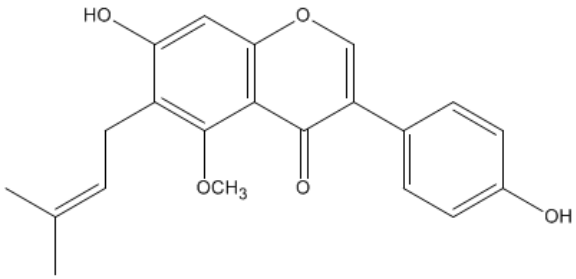
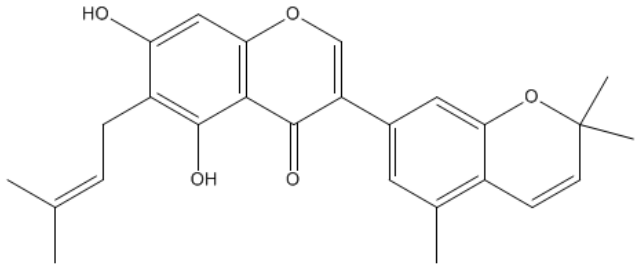
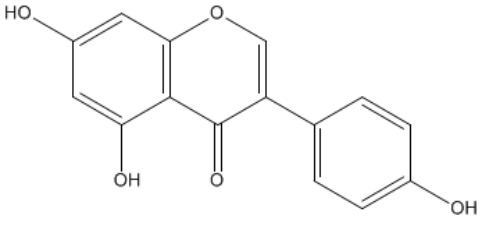


gancaonin G

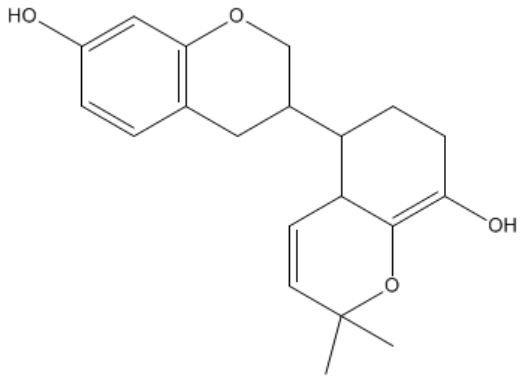


gancaonin H

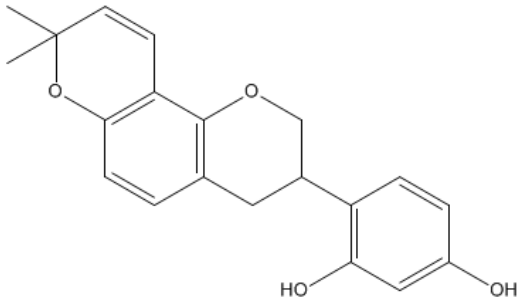


genistein

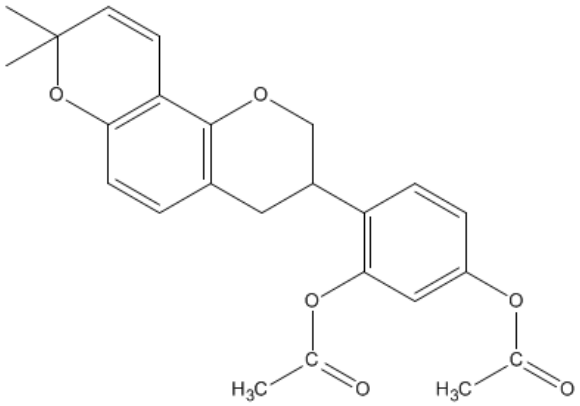
Figure 2 (continued). Structures for 52 chemical constituents of licorice extracts.



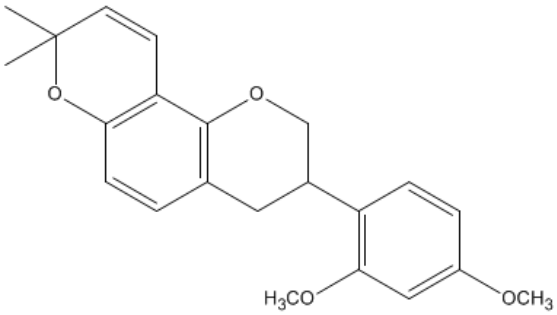
glabrene



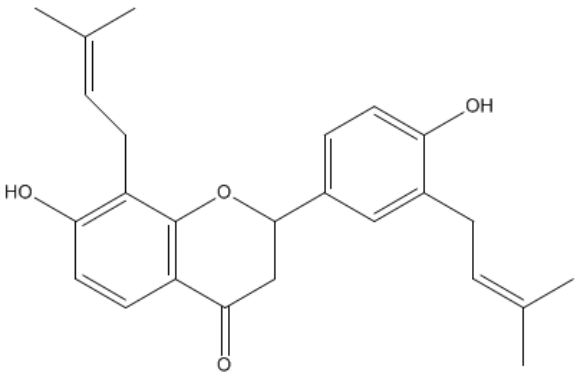
glabridin



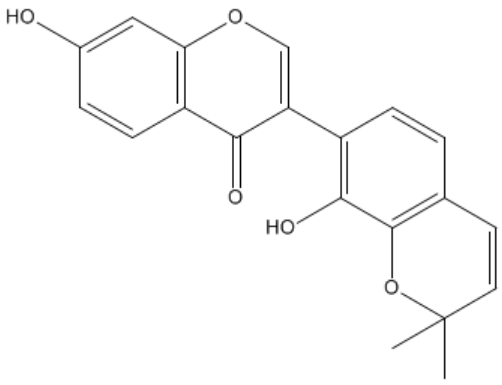
glabridin diacetate



glabridin dimethyl ether

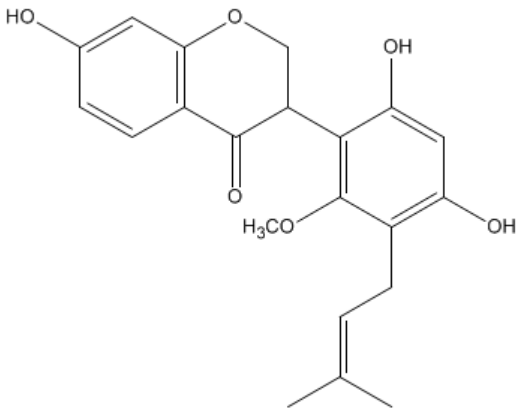


glabrol

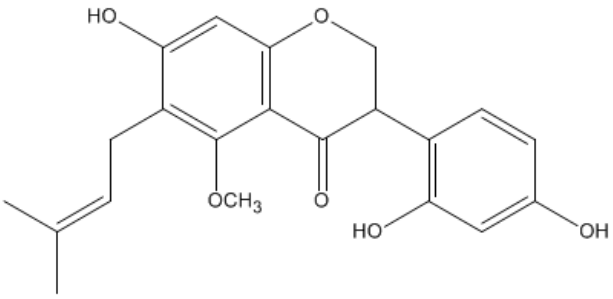


glabrone

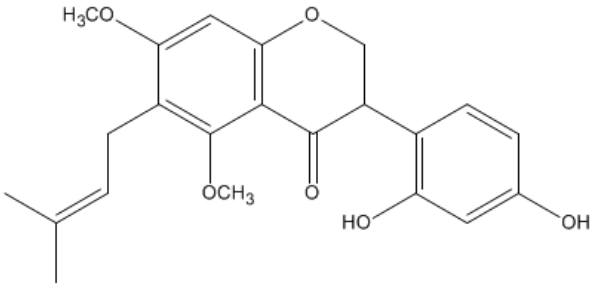
Figure 2 (continued). Structures for 52 chemical constituents of licorice extracts.



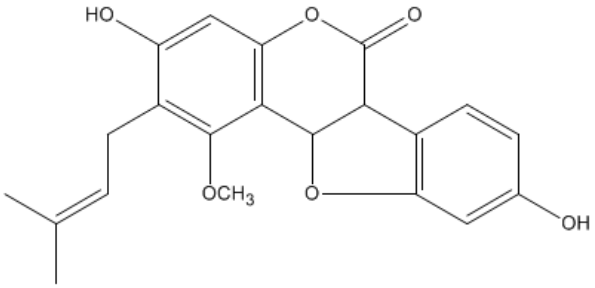
glicoricone



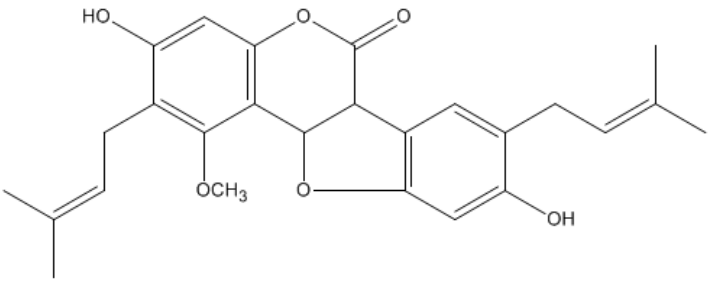
glycycoumarin



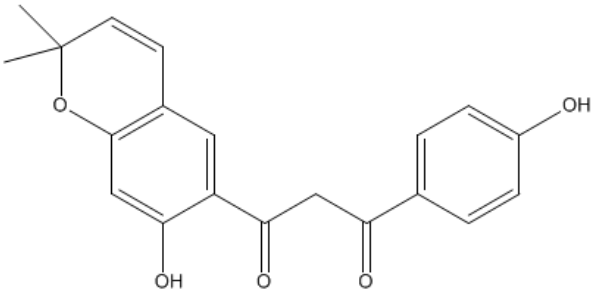
glycyrin



glycyrol

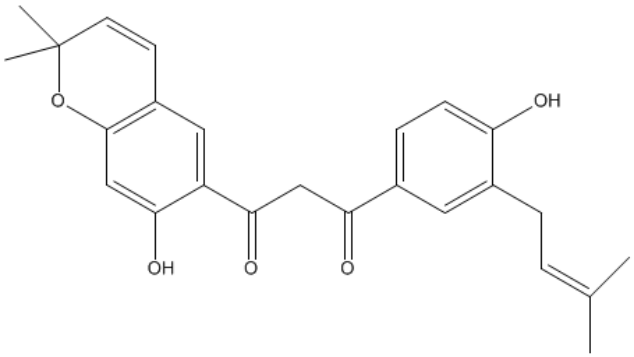


glycyrrhizol A

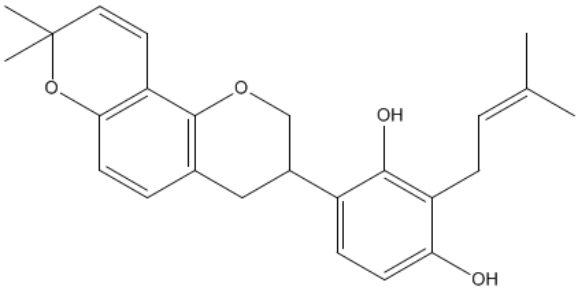


glyinflanin B

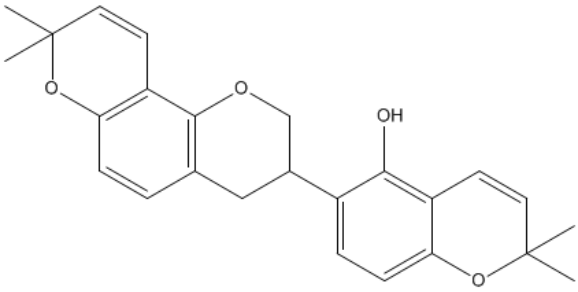
Figure 2 (continued). Structures for 52 chemical constituents of licorice extracts.



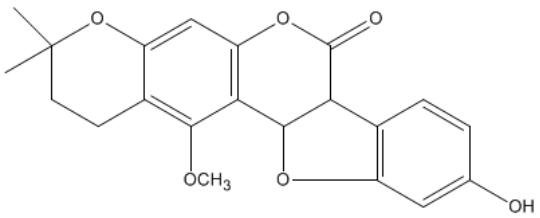
glyinflanin C



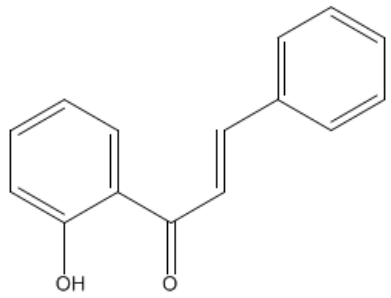
hispaglabridin A



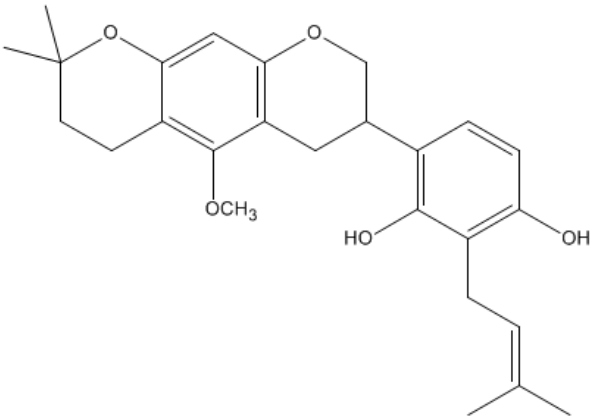
hispaglabridin B



isoglycyrol

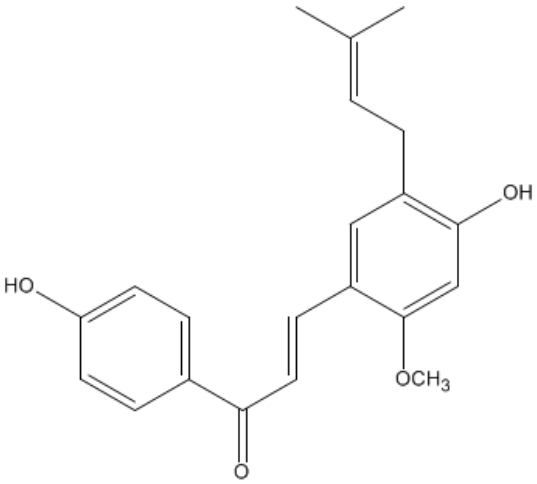


isoliquirtigenin

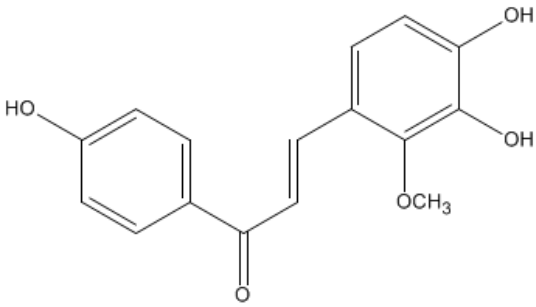


kanzonol

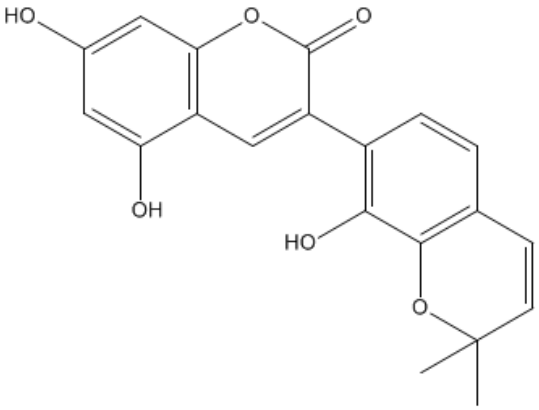
Figure 2 (continued). Structures for 52 chemical constituents of licorice extracts.



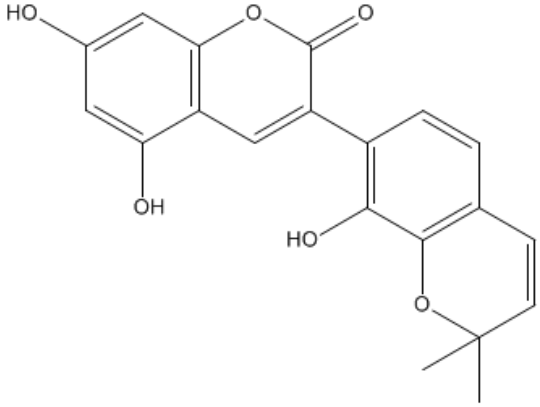
licochalcone A



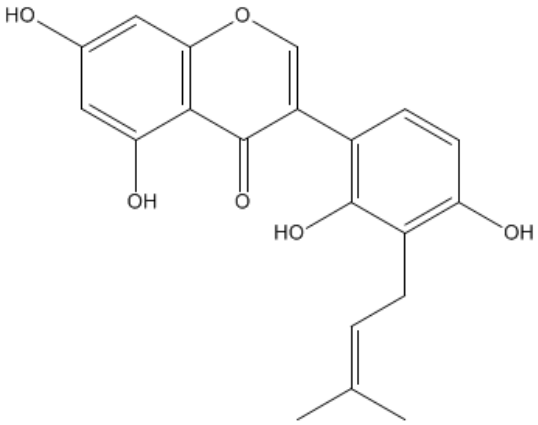
licochalcone B



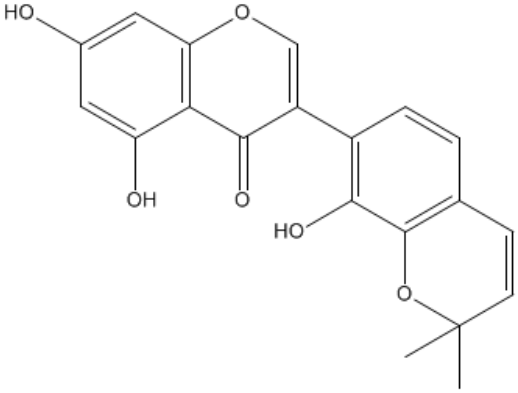
licofuranone



licoisoflavanone

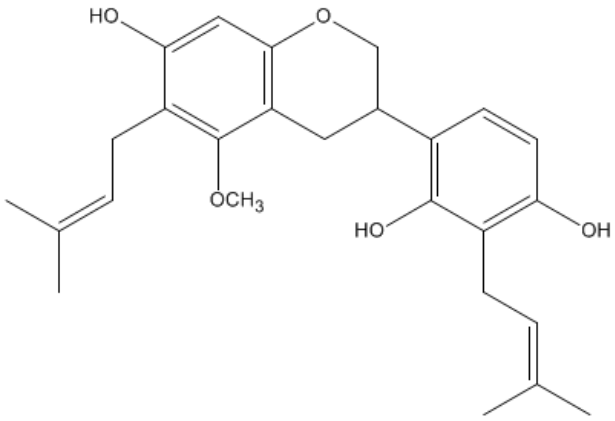


licoisoflavone A

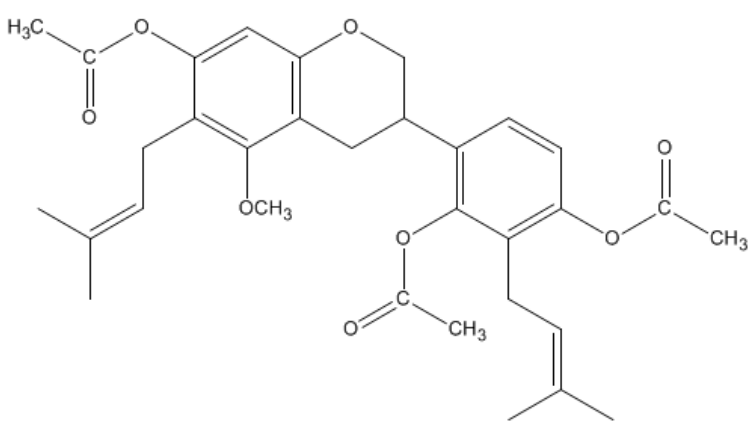


licoisoflavone B

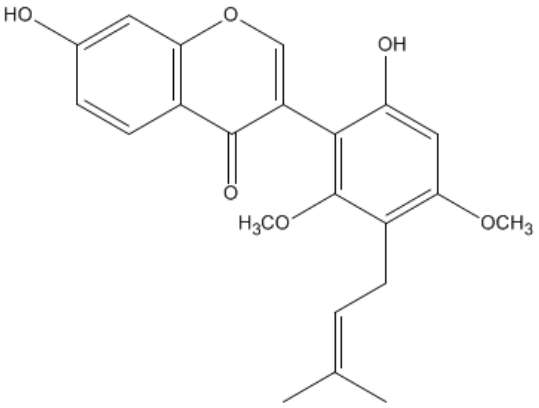
Figure 2 (continued). Structures for 52 chemical constituents of licorice extracts.



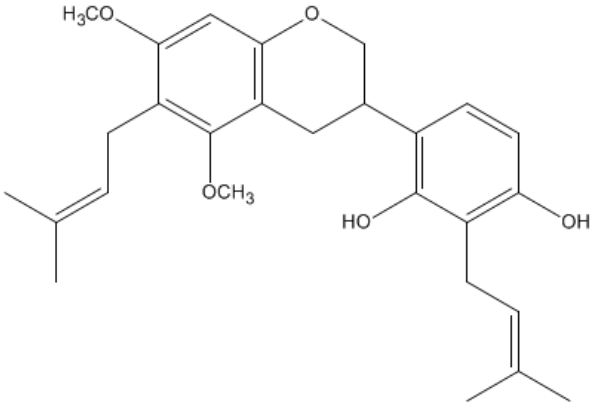
licoricidin



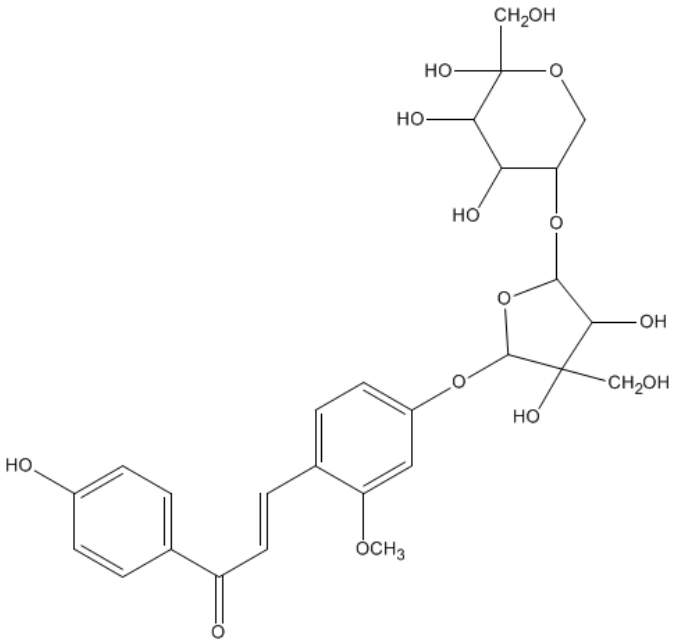
licoricidin triacetate



licoricone

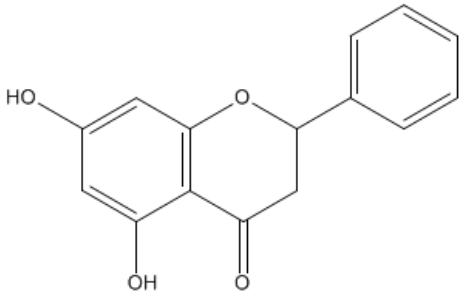


licorisoflavan A

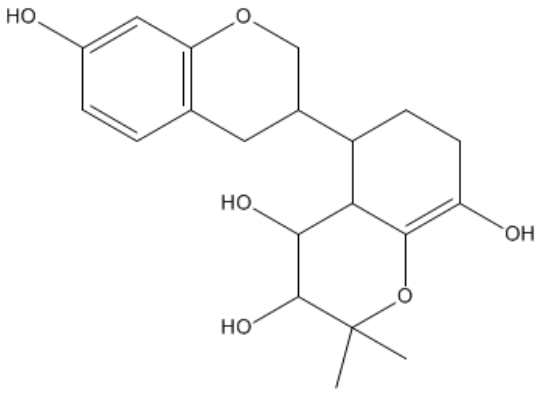


licuraside

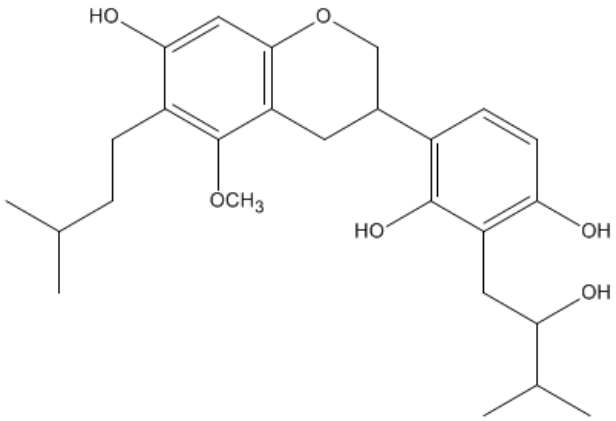
Figure 2 (continued). Structures for 52 chemical constituents of licorice extracts.



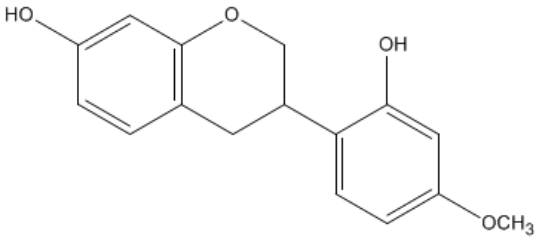
liquiritigenin



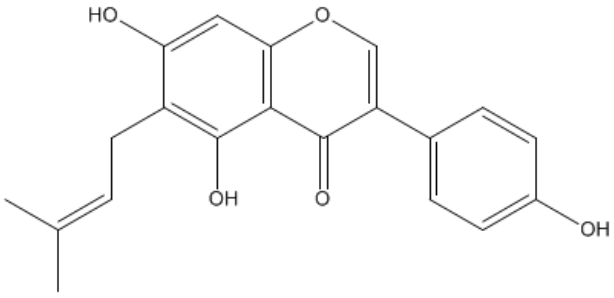
tetrahydroglabrene



tetrahydrolicoricidin



vestitol



wighteone

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Glycyrrhizic Acid and Derivatives

Glycyrrhizic Acid is poorly absorbed by the gastrointestinal tract, but it is hydrolyzed by an intestinal bacteria byproduct into Glycyrrhetinic Acid, which is absorbed and distributed through the circulatory system. Both Glycyrrhetinic Acid and Glycyrrhizic Acid bind extensively to rat and human plasma albumin, but are not absorbed well into tissues (CIR 2005).

Dermal Absorption

Licochalcone A

CTFA (2003) submitted a study on the dermal absorption and percutaneous penentration of licochalcone A in 2 licorice extract samples (1% and 0.05% Glycyrrhiza Inflata (Licorice) Root Extract; 0.012% and 0.019% licochalcone A) in o/w emulsions. The emulsions were applied topically to excised pig skin. Analytical determination of the test substances was carried out with HPLC-mass selective detector (MSD). There was 88% recovery of licochalcone A from the 1% sample and 68% from the 0.05% sample after 24 h. **Table 29** shows the amount and distribution of the absorption of the recovered licochalcone A. No licochalcone A was detected in the receptor fluid. In a second experiment using fine-cut pig skin for 24 h at 22°C, there was a 50% loss of licochalcone A that the authors stated was due to metabolic degradation (CTFA 2003).

Table 29. Dermal absorption and penetration through excised pig skin by recovered licochalcone A from 2 samples of Licorice Extract (CTFA 2003).

Parameter	Licorice sample 1%	Licorice sample 0.05%
Total dermal absorption	79%	86%
Dermally absorbed licochalcone A in the:		
Horny layer	80%	77%
Epidermis	15%	16%
Dermis	5%	7%
Receptor fluid	-[a]	-[a]

[a] not detectable

Intestinal Absorption

Glycyrrhizic Acid

Cantelli-Forti et al. (1994) and Wang et al. (1995) each did experiments to show that more Glycyrrhizic Acid is absorbed in the intestine after oral administration of pure Glycyrrhizic Acid than after an oral administration of Licorice Extract with equal Glycyrrhizic Acid content. The authors suggested that other constituents of licorice extract inhibit the intestinal absorption of Glycyrrhizic Acid.

Liquiritigenin, Liquiritin, Liquiritin Apioside and Davidigenin

Asano et al. (2003) used human colonic cells (Caco-2) as a model of human intestinal absorption in testing the absorption of liquiritigenin, liquiritin, and liquiritin apioside extracted from G. uralensis. Since liquiritin is converted to liquiritigenin and then to davidigenin by human intestinal flora (Zuo et al. 2002; Chung 1998), the absorption of davidigenin was also included in this experiment. Human colonic cells were grown in Eagle's minimum essential medium (E-MEM) with fetal bovine serum (FBS) and amino acids. Transepithelial electric resistance (TEER) values across the cell monolayers were measured with a voltohmmeter. The membrane used for the transport experiments showed TEER values over 600 Ωcm^2.

Transepithelial fluxes were measured for the 4 licorice constituents (1 mM in ethanol or 50% ethanol diluted to 50 µM with transport medium) and 2 transport marker substrates, [14C]mannitol (1.2 µM) and [3H]propanolol (100 µM).

The test substance was added to the apical (200 µl) or basolateral (500 µl) sides of the inserts and the opposite chamber filled with the appropriate volume of transport medium

Incubation was for 3 h at 37°C with samples extracted from both chambers every hour. Analysis for the flavonoids was by HPLC. Analysis for the paracellular transport markers and transcellular transport marker was by measuring radioactivity. Apparent permeability coefficients (P_{app}) were calculated.

The efflux of each flavonoid for both apical and basolateral sides was linear with time for up to 3 h. The P_{app} for the apical to basolateral flux of liquiritigenin and davidigenin were 16.0 ± 0.727 x 10-6 cm/s and 18.3 ± 1.67 x 10-6 cm/s, respectively. These values were higher than propranolol, 13.5 ± 0.34 x 10 -6 cm/s ($p < .01$). The reverse, basolateral to apical flux of liquiritigenin and davidigenin were nearly 2-fold higher, 39.3 ± 0.057 x 10 -6 cm/s and 41.4 ± 0.039 x 10-6 cm/s ($p < .001$), respectively.

The P_{app} for the apical to basolateral flux of liquiritin and liquiritin apioside were 0.26 ± 0.12 x 10-6 cm/s and 0.075 ± 0.005 x 10-6 cm/s, respectively. The P_{app} of liquiritin showed a tendency to rise compared to liquiritin apioside. These values were lower than mannitol, 0.64 ± 0.04 x 10-6 cm/s ($p < .01$ and .001, respectively). Higher P_{app} values were observed for liquiritin and liquiritin apioside in the basolateral to apical direction in comparison to those of the apical to basolateral direction, 2.4 ± 0.047 x 10-6 cm/s ($p < .001$) and 0.47 ± 0.26 x 10-6 cm/s (not significant), respectively.

Recoveries were based on the amount of each flavonoid recovered from both apical and basolateral chambers at 3 h; liquiritigenin was recovered at 42.0 ± 9.2% and 75.0 ± 10.7%, respectively. Recovery of davidigenin was 43.4 ± 1.8% and 81.3 ± 0.3%, respectively. Recovery was greater for the glycosides. The average recovery of liquiritigenin and liquiritin apioside was 97% or greater in apical and basolateral loaded experiments in all cases (Asano et al. 2003).

Distribution

Glabridin

Yu et al. (2007) connected adult male Sprague-Dawley rats (n = 8) to a perfusion system by the jugular veins. Glabridin was introduced at 0.5 ml/min up to 0.5 µM. The rats were decapitated and the brain removed at intervals. Cerebrospinal fluid was collected and the cerebral hemispheres and cerebellum homogenized and assayed. This experiment was repeated with the additions of verapamil (100 µM), nifedipine (100 µM), quinidine (50 µM), MK-571 (100 µM) or probenecid (200 µM). The blood-brain barrier permeability was calculated.

The uptake of glabridin into the cerebrum increased from 0.42 ± 0.09% at 1 min to 9.27 ± 1.69% (ml/100g tissue) at 30 min and was greater than that of sucrose at all time points. The values for the cerebellum were 2.02 ± 0.41% at 1 min and 6.43 ± 1.29% at 30 min. The cerebrospinal fluid values for glabridin were 1.10 ± 0.23% and 3.95 ± 0.86%, respectively. The effects of the drugs increased the tissue level/plasma level ratio by 33.6% to 142.9%.

In another experiment, the authors orally administered glabridin (5 mg/kg; 0.2% in DMSO) to male Sprague Dawley rats (n = 6). The rats were killed at 15 and 30 min and 1, 2, 4, 6, 8, 12 and 24 h and the plasma, brain, liver, heart, spleen, lung, and kidney were collected for glabridin analysis. This experiment was repeated with the oral administration of verapamil or quinidine (100 mg/kg) 2 h before glabridin administration.

Glabridin was found in the plasma and in all tissues; the greatest amount was found in the lung tissue as shown in **Table 30**. Both doses of verapamil and quinidine increased the AUC and maximum concentration in all tissues. Levels were 0 after 24 h in all tissues.

In plasma, glabridin alone peaked at 4 h at ~16 ng/ml, glabridin with 25 mg/kg verapamil peaked at 4 h at ~14 ng/ml, and glabridin with 100 mg/kg verapamil peaked at 2 h at ~26 ng/ml.

In brain tissue, glabridin alone peaked at 3 h at 2.8 ng/g tissue, glabridin with 25 mg/kg verapamil peaked at 1 h at ~4 ng/g tissue, and glabridin with 100 mg/kg verapamil peaked at 1 h at ~9.5 ng/g tissue.

In liver tissue, glabridin alone peaked at 4 h at 18.7 ng/ml, glabridin with 25 mg/kg verapamil peaked at 2 h at ~22 ng/g tissue, and glabridin with 100 mg/kg verapamil peaked at 2 h at ~22 ng/g tissue.

In kidney tissue, glabridin alone peaked at 4 h at 25.4 ng/ml, glabridin with 25 mg/kg verapamil peaked at 4 h at ~22 ng/g tissue, and glabridin with 100 mg/kg verapamil peaked at 2 h at ~33 ng/g tissue.

In spleen tissue, glabridin alone peaked at 4 h at 19.9 ng/ml, glabridin with 25 mg/kg verapamil peaked at 4 h at ~17 ng/g tissue, and glabridin with 100 mg/kg verapamil peaked at 2 h at ~24 ng/g tissue.

In heart tissue, glabridin alone peaked at 3 h at 25.8 ng/ml, glabridin with 25 mg/kg verapamil peaked at 2 h at ~30 ng/g tissue, and glabridin with 100 mg/kg verapamil peaked at 1 h at ~30 ng/g tissue.

In lung tissue, glabridin alone peaked at 3 h at 33.4 ng/ml, glabridin with 25 mg/kg verapamil peaked at 1 h at ~36 ng/g tissue, and glabridin with 100 mg/kg verapamil peaked at 1 h at ~36 ng/g tissue (Yu et al. 2007).

Metabolism and Excretion

Glycyrrhetinic Acid and Derivatives

Terasawa et al. (1986) measured the metabolism of Glycyrrhizic Acid into Glycyrrhetinic Acid in healthy human subjects. Five healthy male subjects (aged 20 to 24 years) each ingested 5 g of licorice dissolved in 100 ml water. The dose was determined by HPLC to contain 133 mg Glycyrrhizic Acid and no detectible glycyrrhetinic acid. Volunteers were prohibited from consuming alcohol but were not otherwise restricted in normal diet and activities. Blood was collected from the cubitus vein daily and urine every 10 h for 7 d after administration.Glycyrrhetinic Acid and Glycyrrhizic Acid levels in the serum were measured using an Enzyme Immuno-Antibody assay. Urinary levels were measured by HPLC.

Table 30. The pharmacokinetics parameters of glabridin in rats at 5 mg/kg administered to rats (Yu et al. 2007).

Tissue	Area under the curve (ng h/ml)	Maximum concentration (ng/ml tissue)	Time of maximum concentration	Elimination half-life	Tissue/plasma area under the curve for 24 h	Tissue/plasma area under the curve for 24 h corrected for blood
Plasma	97.4 ± 34.6	15.0 ± 4.6	3.13 ± 1.02	3.20 ± 0.93	1	-
Brain	18.3 ± 5.9	2.8 ± 0.8[a]	3.09 ± 0.96	3.56 ± 1.08	0.19	0.27
Lung	216.1 ± 65.7[a]	33.4 ± 9.9[a]	3.12 ± 1.11	3.73 ± 1.12[a]	2.22	2.15
Liver	155.0 ± 48.4[a]	18.7 ± 5.2[a]	4.11 ± 1.24[a]	4.93 ± 1.14[a]	1.59	1.57
Kidney	196.2 ± 65.8[a]	25.4 ± 7.7[a]	4.06 ± 1.23[a]	3.90 ± 1.07[a]	2.01	1.96
Spleen	165.8 ± 49.7[a]	19.9 ± 5.8[a]	4.08 ± 1.21[a]	4.95 ± 1.33[a]	1.70	1.60
Heart	150.3 ± 47.1[a]	25.8 ± 8.5[a]	3.10 ± 1.01	2.59 ± 0.82[a]	1.54	1.49

[a] $p < .05$ compared to plasma

The maximum serum Glycyrrhizic Acid occurred at 4 h and levels rapidly decreased afterwards. By the 96th hour, Glycyrrhizic Acid was undetectable in the sera of all subjects. Likewise, urinary Glycyrrhizic Acid peaked at the first 10-h collection after dose administration and rapidly decreased.

Serum glycyrrhetinic acid levels peaked at 30 ng/ml about 24 h after dosing and then declined. Four subjects had low but detectable serum glycyrrhetinic acid at 48 h, and 2 at 96 h.

Glycyrrhetinic acid appeared in the urine at the 20- and 30-hour time points and reduced at a rate consistent with serum levels. Total urinary excretion of glycyrrhetinic acid was about 2% of the administered dose (Terasawa et al. 1986).

Cantelli-Forti et al. (1997) studied the biliary excretion of Glycyrrhizic Acid and glycyrrhetinic acid after oral or intravenous (i.v.) administration of Licorice Extract or Glycyrrhizic Acid. Male Sprague-Dawley rats were given 480 mg/kg Glycyrrhizic Acid or 6278 mg/kg Licorice Extract by oral gavage (n = 6 rats per treatment group). The Glycyrrhizic Acid dose was selected to give the same amount of Glycyrrhizic Acid found in the Licorice Extract dose. Six control rats were given 10 ml/kg distilled water. After dosing, animals were anesthetized, and their abdomens were opened. The common bile duct of each animal was tied and cannulated with a polyethylene catheter, through which bile was steadily collected every 120 minutes for 16 h.

Bile samples were analyzed by HPLC to detect Glycyrrhizic Acid and glycyrrhetinic acid. After oral gavage of Glycyrrhizic acid, the excretion rate of Glycyrrhizic Acid via bile peaked (18.02 µg/min/kg) at 8 h to 10 h and then decreased sharply. After oral gavage with Licorice Extract (containing the same amount of Glycyrrhizic Acid), the biliary excretion rate of Glycyrrhizic Acid was significantly reduced compared to the i.v. dose and peaked (3.43 µg/min/kg) at 6 to 8 h. AUC analysis showed a significant 7-fold increase in biliary excretion of Glycyrrhizic Acid after oral Glycyrrhizic Acid, compared to Licorice Extract. Thus excretion of Glycyrrhizic Acid is higher after oral Glycyrrhizic Acid than oral Licorice Extract. Levels of Glycyrrhetinic Acid in the bile were below the detection limits in all bile samples.

In another experiment, the authors used rats that were anesthetized and their bile ducts were cannulated for bile collection. Baseline bile was collected for 1 h and then 32.7 mg/kg Licorice Extract or 2.5 mg/kg Glycyrrhizic Acid was intravenously injected over a period of 1 h by means of peristaltic pump. The Glycyrrhizic Acid dose was selected to give the same amount of Glycyrrhizic Acid found in the Licorice Extract dose. Bile samples were collected hourly for 6 h. Bile flow was increased after licorice extract injection, compared to Glycyrrhizic Acid injection. The authors did not compare the biliary Glycyrrhizic Acid after i.v. injection of licorice extract and Glycyrrhizic Acid (Cantelli-Forti et al. 1997).

Glabridin

Cao et al. (2007) investigated the role of P-glycoprotein in the intestinal absorption of glabridin. The authors orally administered (5 or 20 mg/kg) or injected i.v. (5 mg/kg) glabridin to healthy male Sprague-Dawley rats (200 to 260 g; n = 6) after overnight fasting. Blood samples were collected over the next 24 h and analyzed for glabridin. Oral availability of glabridin was dose-dependent with a proportional increase. The injected glabridin was available faster and at a higher level than oral administration and decreased linearly as shown in **Table 31.** The authors stated that the oral absorption and oral bioavailability of glabridin are low and dose-dependent.

The authors tested the in vitro metabolism of glabridin in rat intestinal and hepatic microsomes collected from healthy male Sprague Dawley rats (190 to 250 g). The hepatic (1.0 mg/ml) and intestinal (5.0 mg/ml) microsomes were incubated in the presence of nicotinamide adenosine dinucleotide phosphate (NADPH) or uridine diphosphate glucuronic acid (UDPGA) for 30 min. After cooling to stop the reaction, the cultures were centrifuged and the supernatant subjected to LC-MS and HPLC analysis. The experiment was repeated testing for the inhibition of of hepatic glucuronidation of glabridin (at various concentrations) by various compounds (verapamil, indomethacin, and dicofenac at 100 µM).

Table 31. The pharmacokinetic parameters of glabridin after oral and intravenous adminstration to rats (Cao et al. 2007).

Parameter	Oral adminstration at 5 mg/kg	Oral adminstration at 20 mg/kg	Intravenous administration at 5 mg/kg
T_{max} (h)	4.33 ± 1.86	4.50 ± 2.17	Not calculated
C_{max} (ng/ml)	15.10 ± 4.72	60.41 ± 18.87	422.96 ± 123.92
$T_{1/2\beta}$ (h)	2.38 ± 0.70	2.41 ± 0.74	1.92 ± 0.60
$AUC_{0\text{-}24h}$ (ng/ml/h)	96.49 ± 32.34	387.98 ± 137.28	1301.48 ± 375.79
$AUC_{0\text{-}\infty}$ (ng/ml/h)	103.54 ± 34.03	413.30 ± 127.72	1388.49 ± 400.22
CL (ml/min/kg)	Not calculated	Not calculated	59.01
V_d (l/kg)	Not calculated	Not calculated	2.72
Systemic Bioavailability (F)	7.45%	7.44%	Not calculated

No oxidative metabolites of glabridin were observed in the rat intestinal and hepatic microsomes when incubated in the presence of NADPH. Formation of glabridin glucuronide was observed in rat hepatic microsomes in the presence of UDPGA. The formation of glabridin glucuronide was linear up to 60 min in 50 µM of substrate. A minimal peak of glabridin glucuronide was observed using HPLC and a weak glabridin glucuronide ion signal was observed using LC-MS with rat intestinal microsomes. The rate of formation of glabridin glucuronide in rat intestinal microsomes was ~1/15 to 1/20 of that of rat hepatic microsomes.

Diclofenac and indomethacin inhibtied the formation of glabridin glucuronide in both microsomes by 68.5 ± 15.2% (hepatic) and 72.3 ± 14.3%. Verapamil did not inhibit glabridin glucuronidation in either microsome.

These authors also performed a single-pass intestinal perfusion experiment using male Sprague-Dawley rats. Under anesthesia the following procedures were performed: jugular vein cannulation for infusion of blood collected from the donor rats, isolation of an ileal segment for glabridin infusion, and cannulation of the mesenteric vein for continuous blood sample collection. Glabridin (0, 0.1, 0.5, or 2.0 µM) was infused with or without verapamil (a P-glycoprotein [PgP] inhibitor; 100 µM), probenecid (a multidrug resistance-associated protein (MRP) 1 inhibitor; 200 µM), 3-[[3-[2(7-chloroquinolin-2-yl)vinyl]phenyl]-(2-dimethylcarbamoylethylsulfanyl) methylsulfanyl] propionic acid (MK-571; an MRP1/2 inhibitor; 100 µM), or celecoxib (a MRP4 inhibitor, 100 µM). Glabridin was infused at 1.0 ml/min for 4 min then at 0.25 ml/min. The blood from the mesenteric vein was collected every 5 min over 60 min and analyzed.

There were no oxidative metabolites formed in the perfusates or mesenteric vein blood. No glabridin glucuronide was detected in perfusates or mesenteric vein blood using HPLC; a weak signal was observed using LC-MS. The authors stated that glucuronidation of glabridin by rat perfused ilium segment was minimal and gut metabolism had minor impact on the permeability coefficients of glabridin. The permeability of glabridin based on luminal disappearance values were 6.51 ± 0.72 x 10-4, 8.22 ± 0.91 x 10-4, and 11.54 ± 1.31 x 10-4 cm/s for 0.1, 0.5, and 2.0 µM glabridin, respectively. The appearance of glabridin in mesenteric vein blood increased at all concentrations and was 6.5-to 7.0-fold lower than the lumen values ($p < .05$). The authors stated that the concentration-dependent increase in lumen permeability of glabridin may be due to the relatively low to moderate intrinsic permeability of glabridin and possible involvement of a saturable active mechanism for its intestinal transport.

Verapamil, probenecid, MK-571, and celecoxib did not alter lumen permeability with regard to glabridin at any concentration. The appearance of glabridin in the blood at all 3 concentrations was increased by verapamil ($p < .05$); the glabridin in the lumen/blood ratio decreased from a range of 7.5 to 8.0 to 3.0 to 3.3. The authors stated that this shows that PgP-mediated efflux limited the absorption of glabridin across the intestinal wall which was reversed by verapamil.

To study the plasma pharmacokinetics of glabridin, male Sprague-Dawley rats (n = 6) were orally administered glabridin (5 mg/kg in water) or the same amount of glabridin 2 h after the oral administration of verapamil (50 or 100 mg/kg). Blood was collected and analyzed over 24 h. Verapamil increased the plasma glabridin in a dose-dependent manner (from 15.02 ± 4.59 [control] to 19.01 ± 5.38 [50 mg/kg] and 25.38 ± 7.62 ng/ml [100 mg/kg]; $p < .05$). The oral availability of glabridin increased from 7.55% to 9.02% and 13.19%, respectively. The maximum concentration of glabridin in the blood was 4.15 ± 0.97 h in the control, which decreased to 2.25 ± 0.52 and 2.15 ± 0.46 h, respectively.

The authors used FVB/NJ and mdr1a gene-deficient mice to further study glabridin pharmacokinetics. The mice (n = 4) were orally administered 5 mg/kg glabridin and killed at intervals over 24 h. The plasma phamacokinetics of glabridin in mdr1a(-/-) mice was different than the wild-type mice. The AUC was 3.77-fold higher and the maximum plasma concentration was 2.83-fold higher. The elimination half-life of the glabridin was longer in the mdr1a(-/-) mice compared to the wild-type mice (3.54 ± 1.14 vs. 2.97 ± 0.89 h). The authors stated that this indicates that PgP/MDR1 had an important impact on oral bioavailability and elimination in vivo.

The authors concluded that glabridin has a low oral bioavailability in rats. The authors suggested that first-pass metabolism also plays an important role in low oral bioavailability of glabridin (Cao et al. 2007).

Isoliquiritigenin

Guo et al. (2008a) investigated the hepatic metabolism of isoliquiritigenin isolated from *G. uralensis* using pooled human liver microsomes (0.17 nmol p450/mg protein). Liver microsomes (0.5 mg/ml microsomal protein) were incubated with isoliquiritigenin (10 or 50 µM) and NADPH (1 mM). NADPH (0.1, 0.01, or 0.02 mM) was added. After 5 min the reaction was stopped and the precipitate was analyzed. Four major (liquiritigenin, 3',4,4'-trihydroxyaurone, 2',3,4,4'-tetrahydroxychalcone, (Z)-6,4'-dihydroxyaurone) and 3 minor (2',4,4',5'-tetrahydroxychalcone, 2',4,4'-trihydroxy dihydrochalcone, (E)-6,4'-dihydroxyaurone) metabolites were observed.

These authors also incubated human hepatocytes and pooled human liver microsomes in isoliquiritigenin to determine the secondary metabolites. Five glucuronides were detected corresponding to monoglucuronides of isoliquiritigenin and liquiritigenin, but no sulfate conjugates were observed. The glucuronides were: isoliquiritigenin 4'-O-glucuronide (the most abundant), liquiritigenin 4-O-glucuronide, liquiritigenin 4'-O-glucuronide, isoliquiritigenin 4-O-glucuronide, and isoliquiritigenin 2'-O-glucuronide. Liquiritigenin 4-O-glucuronide and liquiritigenin 4'-O-glucuronide could not be conclusively distinguished from each other.

When this experiment was repeated with human liver, kidney, and intestine microsomes, liquiritigenin 4-O-glucuronide or liquiritigenin 4'-O-glucuronide and isoliquiritigenin2'-O-

glucuronide and isoliquiritigenin 4'-O-glucuronide were detected in all incubations. Liquiritigenin 4-O-glucuronide or liquiritigenin 4'-O-glucuronide was only in the liver and intestine microsome incubations. Isoliquiritigenin 4-O-glucruonide was formed only by liver and kidney microsome incubations (Guo et al. 2008a).

Licochalcone A

Nadelmann et al. (1997a) injected intraperitoneally 1 ml of a 10 mg/ml solution of licochalcone A into SPF Wistar male rats of the strain Mol:Wist. Urine samples were collected day and night and venous blood samples were collected from the orbital plexus. Urine analyzed by HPLC contained 1% to 3% of the administered dose as the cis- and trans-isomers of 4-phenolic glucuronide and a trace of licochalcone A. None of the expected trans-4'-phenolic glucuronide was detected. Only 2% to 3% of the total dose was excreted in urine. Only licochalcone A and trans-4-phenolic glucuronide was detected in the plasma; as the concentration of licochalcone A decreased, the concentration of trans-4-phenolic glucuronide increased.

Nadelmann et al. (1997b) injected intraperitoneally 1 ml of licochalcone A (10 mg/ml) into 8 male SPF Wistar rats. Two more rats were used as controls. Urine was collected for 3 d. The urine samples were diluted (1:1 v/v) with acetonitrile and centrifuged. The supernatants were analyzed by HPLC. The chromatograms of the urine samples showed that the metabolites passed in the urine with retention times of: Z-glucuronide, ~10 min; E-4-glucuronide, ~11 min and E-licochalcone A, ~23 min.

Systemic Renal Effects

Glycyrrhizic Acid and Derivatives

As reported in an earlier safety assessment (CIR 2005), moderate chronic or high acute exposure to licorice, Glycyrrhizic Acid, Ammonium Glycyrrhizate, and their metabolites have been demonstrated to cause several transient systemic alterations including increased potassium excretion, sodium and water retention, body weight gain, alkalosis, suppression of the renin-angiotensin-aldosterone system, hypertension, and muscular paralysis.

Ataxia was observed after a very high acute exposure to Glycyrrhizic Acid. These effects dissipated when exposure to Glycyrrhizic Acid was terminated.

Ataxia also was blocked by mineralocorticoid receptor antagonists spironolactone and eplerenone (CIR 2005).

Naringenin

Zhang and Wang (1997) isolated 11β-OHSD from the kidney cortexes of male Hartley guinea pigs. The activity of the 11β-OHSD was determined by measuring the rate of conversion of cortisol to cortisone. Five min prior to incubation, concentrated Triton DF-18 (2 µl) was added to each ml of the microsome suspension. The suspensions were incubated and the reaction terminated with 3 ml methylene chloride and 10 µl corticosterone (144 µmol/l). The enzyme inhibition constant for naringenin, a licorice constituent, was determined by adding naringenin (25 to 200 µmol/l) and cortisol at concentrations of 4, 8, and 16 µmol/l. The correlation coefficient (r) for naringenin was 0.99. The plotted lines of inhibition converged on the ordinate, indicating that the inhibition was competitive. The kinetic constants for the enzyme were Km = 3.30 µmol/l and Vmax = 26.21 µmol/h/g microsomal protein. The inhibitor constant, Ki, was 16.13 µmol/l, nearly 4-fold that of cortisol. The authors concluded that naringenin inhibited the 11β-OHSD in the renal cortexes at a similar potency as Glycyrrhizic Acid.

Antinephritic Activity

Glabridin

Fukai et al. (2003a) isolated glabridin from *G.glabra* extract and tested it for antinephritic effects on male BALB/c mice weighing 17 to 22 g. The mice were housed in a standard environment and fed under specific pathogen-free conditions. All the mice were treated with rabbit γ-globulins (1 mg/mouse) 5 d prior to the experiment. Antiglomerular basement membrane (GBM) nephritis was induced in mice by a single i.v. injection of rabbit antimouse GBM serum (0.2 ml/mouse). Oral treament with 30 mg/kg of glabridin for 10 d started immediately. Urinary protein excretion (UPX) was determined on days 5 and 10 (after last treatment) by the Coomassie Brilliant Blue dye binding method. Total cholesterol level in serum (TCL), serum creatinine level (SCL) and BUN in serum were measured on day 11 (24 h after last treatment) by automatic analyzer. Results are shown in **Table 32.**

Table 32. Effects of glabridin on urinary protein excretion (UPX), total serum cholesterol (TCL), serum creatinine level (SCL), and blood urea nitrogen (BUN) in a mouse glomerular disease model (Fukai et al. 2003a).

Treatment	Dose (mg/kg/d)	UPX (mg/d)	TCL (mg/dl)	SCL (mg/dl)	BUN (mg/dl)
Normal mice	-	18 ± 0.6	98 ± 9	0.41 ± 0.02	22 ± 3
Control (nephritis) mice	-	100 ± 23	357 ± 79	0.41 ± 0.04	31 ± 4
Glabridin treated mice (% inhibition vs control mice)	30	47 ± 4* (-53%)	209 ± 41* (-41%)	0.41 ± 0.02 (0%)	23 ± 1 (-26%)

* $p < .05$ vs. control, Student's t-test

UPX in mouse glomerular disease was reduced by 53% in glabridin treated animals. Serum TCL and BUN were decreased by 41% and 26%, respectively, in glabridin treated animals. The authors concluded that SCL was not affected by the treatment (Fukai et al. 2003a).

Licochalcone A, Licoricidin, and Licorisoflavin A

Fukai et al. (2003b) studied the antinephritic activity of licoricidin and licorisoflavan A (extracted from *G. uralensis*) and licochalcone A extracted from *G. inflata* on a mouse glomerular disease model (Masugi-nephritis). The mice (n = 6) were administered 30 mg/kg/d of 1 of the compounds orally for 10 d.

The results are shown in **Table 33.** Oral administration of glabridin in this study produced the same results as in the previous study. Oral administration of licochalcone A for 10 d reduced the amount of UPX by approximately 35% and BUN by 26% compared to nephritic mice ($p < .05$). There was not an effect on TCL or SCL levels. Licorisoflavan A reduced the amount of SCL and BUN ($p < .05$).

Hepatic Effects

Glycyrrhizic Acid and Derivatives

Patrick (1999) stated that Glycyrrhizic Acid extracted from licorice roots has been used as a treatment for chronic hepatitis in Japan for over 20 years. This author suggested that Glycyrrhizic Acid may inhibit the penetration of hepatitis virus into hepatocytes and/or work as a free radical scavenger modulating the immune system through interferon mechanisms and T-cells.

Glabridin

Kent et al. (2002) found that P450 3A4 (isolated from Escherichia coli), the major human drug metabolizing P450 enzyme, was inactivated by alcoholic licorice root extract (0.0125 to 6.25 µg/ml) and glabridin (0.625 to 40 µM), from G. glabra, in a time- and dose-dependent manner. The inactivation was NADPH-dependent and was not reversible by extensive dialysis. The loss in enzymatic activity correlated with a loss in the P450-reduced carbon monoxide (CO) spectrum and with a loss of the intact heme moiety. Incubations with glabridin and NADPH did not result in the destruction of the heme moiety as shown by HPLC analysis. The activity of P450 2C9 was competitively inhibited by glabridin; P450 2D6 and 2E1 were unaffected (Kent et al. 2002).

Various Compounds

Tsukamoto et al. (2005) reported that licopyranocoumarin, liquiritin, and liquiritin apioside, isolated from G. uralensis, exhibited CYP inhibitory activity at 97%, 98%, and 61%, respectively, at 0.25 mg/ml. The IC_{50} of these compounds were reported to be 3.6, 17, and 20 µM, respectively. 3-(p-Hydroxyphenyl)propionic acid, isoliquiritigenin, (3R)-vestitol, 4-hydroxyguaiacol apioglucodise, liquiritigenin 7,4-diglucoside, and glucoliquiritin apioside were poor CYP3A4 inhibitors.

Anti-hepatotoxic Activity

Glycyrrhizic Acid and Deriviatives

Shim et al. (2000) collected licorice extract from G. uralensis rhizomes with boiling water and glycyrrhizin by boiling water followed by 3 applications of ethyl acetate. Each extraction (100 mg/kg) was administered to male Wistar rats 30 min after inducing liver damage with 20% carbon tetrachloride diluted with olive oil (1 ml/100g). The licorice extract and the glycyrrhizin was administered either orally or intraperitoneally (n = 6). There was also a "normal" group of rats with no treatment and a control group that was administered the vehicle. Twenty-four h after dosing, blood samples were obtained and AST, ALT, LDH, and triglyceride (TG) values were measured.

Both the water extract and the glycyrrhizic acids (ethyl acetate extracts) demonstrated preventive effects for liver damage when administered orally but not when administered intraperitoneally as shown in **Table 34.** The same experiment was performed again with glycyrrhizin and 18β-glycyrrhetinic acid administered intraperitoneally only.

Glycyrrhizin showed no hepatic protective effects while 18β-glycyrrhetinic did have protective effects as shown in **Table 35.** The authors concluded that glycyrrhizin may be a natural prodrug with hepaptoprotective effects (Shim et al. 2000).

Glycyrrhizic acid and glycyrrhetinic acid have been shown to protect liver tissue from known hepatotoxins such as carbon tetrachloride, galactosamine, and retrosine. Glycyrrhizic Acid improved the viability of V79 cells also exposed to 5 mM acetaldehyde or 10 µM cadmium. Glycyrrhizic Acid (up to 200 µM) did not prevent cadmium- induced lipid peroxidation in these cells (CIR 2005).

Table 33. Effects of prenylated flavonoids on nephritic activity in nephritic mice (Fukai et al. 2003b).

	UPX (mg/d)		TCL in serum (mg/dl)	SCL (mg/dl)	BUN level (mg/dl)
Compound	**Day 5**	**Day 10**	**Day 11**	**Day 11**	**Day 11**
Licochalcone A	58 ± 5[a]	65 ± 14[a]	269 ± 65	0.45 ± 0.01	23 ± 3[a]
Licoricidin	78 ± 8	97 ± 10	510 ± 130	0.43 ± 0.03	53 ± 18
Licorisoflavan A	61 ± 2	129 ± 9	608 ± 58	1.04 ± 0.19[a]	25 ± 2[a]
Glabridin	59 ± 3[a]	47 ± 4[a]	209 ± 41[a]	0.41 ± 0.02	23 ± 1[a]

[a] significantly different from controls at $p < .05$

Table 34. The preventive effect of *G. uralensis* water extract and glycyrrhizin on carbon tetrachloride-induced hepatotoxicity in rats (Shim et al. 2000).

Group	CCl_4	Dose (mg/kg)	AST (Karmen unit)	ALT (Karmen unit)	LDH (Wroblewski unit)
Normal Control	-	0	608.5 ± 19.2	608.9 ± 33.5	1920.5 ± 205.9
CCl_4 Control	+	0	2306.3 ± 309.9[a]	1294.5 ± 261.4[a]	4936.3 ± 766.6[a]
Water Extract	+	100 p.o.	1428.5 ± 804.2[b]	790.3 ± 110.9[c]	2622.8 ± 1117.9[b]
Glycyrrhizin	+	100 p.o.	1560.8 ± 665.7[c]	738.8 ± 170.4[c]	2960.7 ± 1856.8[b]

[a] $p < .001$ from normal control data; [b] $p < .05$ from CCl_4 data; [c] $p < .01$ from CCl_4 data.

Gastrointestinal Protective Effects

FM100 Fraction

Ishii and Fujii (1982) tested the FM100 fraction of licorice root (methanol extract fractionally precipitated with sodium hydroxide and HCl) on serum gastrin concentration in rats and dogs to test if this may be the mechanism for the fraction's protective and healing effects on chronic ulcers. Male Sprague-Dawley rats (CRJ:CD; n = 7 or 8) were administered 400 or 800 mg/kg FM100 orally, an intramuscular (i.m.) injection of atropine sulfate (3 mg/kg), or cimetindine (50 mg/kg) after fasting overnight. After 1 h, the rats were killed and blood collected. The serum gastrin concentration was not affected by 400 mg/kg FM100 but it was increased by 800 mg/kg ($p < .05$). The serum gastrin concentration was increased by atropine sulfate ($p < .05$) and cimetidine ($p < .01$; positive controls).

In a second experiment, a small hairpin was used to separate the antrum from the fundus (the upper and lower sections of the stomach) to prevent the entry of gastric juice into the antrum in anesthetized rats. FM100 was administered intraduodenally (400 or 800 mg/kg; n = 8). The rats were killed 2 h later and the blood collected. The serum gastrin concentration was decreased by FM100 in a dose-dependent manner; 800 mg/kg of FM was lower than controls ($p < .01$).

After fasting overnight, capsules containing FM100 (400 mg/kg) were administered orally to beagles 60 min before feeding. Blood samples were drawn from the brachial vein 10 min before and at intervals after feeding. The serum gastrin concentration was increased upon feeding; there was no difference between treatment and controls.

In a second experiment using dogs, the antrum was separated from the fundus of anesthetized mongrel dogs and a metal fistula was installed to the fundus to collect gastric juice. The stomach was rinsed with saline until clean. Every 30 min, 200 ml of warmed saline flowed though the stomach and the acidity of the outflow was titrated. Ten ml of 40% peptone solution was administered to the antrum 3 times at 90 min intervals. FM100 was administered intraduodenally 30 min before the third dose of peptone solution. Blood was collected from the femoral vein.

The serum gastrin concentration and acid output were increased by intra-antral administration of peptone compared to pretreatment ($p < .05$). FM100 administration maintained the serum gastrin concentration and acid output at pretreatment levels. The authors suggested that the gastric anti-secretory action of FM100 may be due to the inhibition of endogenous gastrin release (Ishii and Fujii 1982).

Table 35. The preventive effect of glycyrrhizin and 18β-glycyrrhetinic acid on CCl_4-induced hepatotoxicity in rats (Shim et al. 2000).

Group	CCl_4	Dose (mg/kg)	AST (Karmen unit)	ALT (Karmen unit)
Normal Control	-	0	432.0 ± 91.2	351.0 ± 59.2
CCl_4 Control	+	0	3110.3 ± 668.4[a]	2052.3 ± 495.4[a]
Glycyrrhizin	+	50 i.p.	2784.6 ± 676.5	1802.5 ± 855.1
Glycyrrhizin		100 i.p.	2653.2 ± 465.2	1928.2 ± 1212.2
18β-glycyrrhetinic acid		50 i.p.	2446.4 ± 20.7[b]	1796.8 ± 231.6
18β-glycyrrhetinic acid		100 i.p.	2155.8 ± 98.1[c]	1120.8 ± 473.3[c]

[a] $p < .001$ from normal control data; [b] $p < .05$ from CCl_4 data; [c] $p < .01$ from CCl_4 data.

Glabridin

Kwon et al. (2008) tested the protective effects of glabridin on colonic inflammation using the dextran sulfate sodium (DSS)-induced mouse colitis model. Female BALB/c mice (n = 20; 6 wk old) were orally administered DSS (5%; 40,000 to 50,000 molecular weight [MW] in the drinking water) for 7 d. The control mice received water. Glabridin (10 or 50 mg/kg; in DMSO and PBS) or the vehicle was orally administered also for 7 d starting the same day as the DSS. The mice were observed daily for colitis development for 14 d. The mice were killed at 7 and 14 d and the mid-colon extracted. The colon was sectioned and incubated in culture plates. The TNF-α and IL-6 concentrations in the supernatant were measured. Some of the sections were then frozen, homogenized, and assayed for transcription-polymerase chain reaction for nitrous oxide synthesis. The rest of the sections were histologically examined. The experiment was run 3 times.

The survival of the mice was enhanced by glabridin. All of the controls survived for 14 d. The mice administered DSS only had a 50% survival rate. The mice administered 10 and 50 mg/kg glabridin had 67% and 84% survival rates, respectively. There was less weight loss in the glabridin administered mice ($p < .05$) and a lower disease activity index for the higher dosed mice ($p < .05$). The colon length (colitis is associated with decreased colon length) of the glabridin-treated mice were longer at day 7 for the 50 mg/kg dose and for both doses at day 14 than the DSS only-treated mice. Histological examination showed typical inflammatory changes in colonic architecture (ulceration, crypt dilation, globlet cell depletion, and cell infiltration of macrophages, lymphocytes, plasma cells, and granulocytes). The glabridin-treated mice showed reduced numbers of infiltrating cells, degree of mucosal injury, and edema. DSS administration induced increased levels of nitric oxide and PGE2.

Glabridin administration reduced the production of both nitrous oxide and PGE2 in a concentration-dependent manner at both day 7 and 14. Myeloperoxidase production was 4 times higher in the DSS-treated mice than the controls. This increase was reduced by glabridin at day 7 but not at day 14. The authors suggest that glabridin exerts anti-inflammatory effects by reducing neutrophil infiltration into the colonic mucosa.

iNOS and COX-2 immunoreactivity were evident throughout the gut wall in the DSS-treated mice, particularly in the cytoplasm of the epithelial cells. Glabridin administration decreased the iNOS- and COX-2 producing cells. DSS increased the TNF-α and IL-6 levels at both days 7 and 14. The higher dose of glabridin prevented the increase of TNF-α on both days 7 and 14 ($p < .05$). The IL-6 increase was prevented at both dose levels on both days 7 and 14 ($p < .05$). Treatment with glabridin reduced the increase in the mRNA levels of iNOS compared to the DDS-treated mice. mRNA levels of COX-2 were unaffected by glabridin treatment. The authors concluded that colitis caused by DSS administration is attenuated by glabridin due to anti-inflammatory effects on inflammatory mediators, nitric oxide, PGE2, and inflammatory cytokines (Kwon et al. 2008).

Isoliquiritigenin

Kim et al. (2006) orally administered saline, ranitidine (5 mg/kg), or isoliquiritigenin (5 and 10 mg/kg; in saline) to rats (n = 6) after fasting. After 1 h, the rats were anesthetized and the pylorus ligated. Indomethacin (40 mg/kg) was injected through the duodenum. The rats were killed and the stomachs removed and preserved. Hemorrhagic and ulcerative lesions were counted and measured by light microscopy. Sections were then examined by a pathologist. Ranitidine reduced the volume of gastric acid production and protected against gastric mucosal lesion formation. Isoliquiritigenin prevented gastric acid secretion ($p < .01$) and gastric ulcer formation ($p < .001$) similar to ranitidine.

Cerebral Effects

Glabridin

Cui et al. (2008) tested for the neuroprotective effects of glabridin, isolated from *G. glabra*, against scopolamine using male Kunming mice (SPF) using an elevated plus maze and the passive avoidance test. The mice were divided into 8 groups (n = 6) and were treated for 3 d. Control group 1 and scopolamine group 1 were administered carboxymethyl cellulose suspension (0.3%; p.o.). Control group 2 and scopolamine group 2 were administered the carboxymethyl cellulose suspension (i.p.). The piracetam group was administered the nootropic agent (400 mg/kg; i.p.). The glabridin groups were administered glabridin (1, 2, or 4 mg/kg; p.o.). On the third day, 60 min after the last dose, scopolamine (0.5 mg/kg; i.p.) was administered to all mice except for the control groups. Each mouse was placed on the open arm of the elevated maze 45 min later and the time it took for the mouse to go to a covered arm was timed. The mice were tested again 24 h later. There was no difference in the time the mice took to move to the closed arms between the scopolamine groups and the scopolamine with the low-dose glabridin groups. The mid- and high-dose glabridin groups had shorter times than the scopolamine groups ($p < .01$) and similar to the control groups.

In the passive avoidance test, 45 min after the scopolamine injection the mice were place in a box with an electrical grid on the floor and a wooden platform in the middle. The mice were timed for how long it took them to step onto the electrified grid. The test was repeated 24 h later. There was no difference in the time the mice took to move off the wooden platform onto the grid between the scopolamine groups and the scopolamine with the low-dose glabridin groups. The mid- and high-dose glabridin groups had times longer than the scopolamine groups ($p < .01$) and similar to the control groups.

The low dose of glabridin had no effect on cholinesterase activity in the mice. The mid- and high-doses of glabridin reduced the brain cholinesterase activity in the mice compared to the control group ($p < .01$) by 14.53% and 19.19%, respectively. The standard drug, metrifonate (50 mg/kg, i.p.) Reduced brain cholinesterase activity by 21.51% compared to controls. The authors concluded that the anticholinesterase, anti-inflammatory, and antioxidant effects of glabridin may be the sources of the memory-enhancing effect (Cui et al. 2008).

Yu et al. (2008) tested for the neuroprotective effects of glabridin (5 or 25 mg/kg in 0.2% DMSO i.p.) on male Sprague Dawley rat brains (n = 8) that had middle cerebral artery occlusion induced surgically. The rats were treated with glabridin for 1 week prior to and after surgery. Group 1 had a sham operation without artery occlusion and treatment with the vehicle; group 2 had a sham operation and treatment with glabridin (5 mg/kg); group 3 has a sham operation and treated with glabridin (25 mg/kg); group 4 had surgical cerebral artery occlusion and treatment with the control; group 5 had surgical cerebral artery occlusion and treatment with glabridin (5 mg/kg); group 6 had surgical cerebral artery occlusion and treatment with glabridin (25 mg/kg). The occlusions were removed after 2 h to allow reperfusion. Neurological functions of the rats were evaluated 24 h after reperfusion by induced and spontaneous motor activity by a blind evaluator. After 7 d, the rats were killed, the brains immediately removed, and the forebrains sectioned and fixed for examination. SOD activity, MDA levels, and glutathione levels were determined. Blood samples were taken before and after surgery.

Treatment with glabridin did not alter blood gasses, blood pressure, hematatocrit, and blood glucose before or after surgery in either the treatment or sham groups. Neurological functions were unaffected by the sham operations, with or without glabridin. Surgical cerebral occlusion with no glabridin resulted in marked neurological deficits (circling movements, postural abnormalities, less to no spontaneous movements and severe paw flections). Glabridin treatment at the high dose improved the neurological symptoms compared to the control; the low dose did not. Rats not treated with glabridin with surgical cerebral occlusion had brains with lesions present in both the lateral striatum and the overlying cortex (246.3 ± 20.8 mm3). Glabridin (5 mg/kg) reduced the infarct volume (238.2 ± 19.5 mm3; $p < .05$) as did the high dose (215.3 ± 15.8 mm3; $p < .05$).

Apoptosis in ischemic brain section for the controls was 9.73 ± 0.8%. The number of apoptotic cells was decreased in rats treated with glabridin ($p < .05$) at both the low (8.16 ± 0.6%) and high dose (7.03 ± 0.7%). Rat brain SOD and glutathione levels were decreased by surgical cerebral occlusion compared to the sham controls; MDA levels were increased. Treatment with glabridin increased SOD and glutathione levels in a dose-dependent manner ($p < .05$). Treatment with glabridin decreased MDA compared to the vehicle controls ($p < .05$ and .01).

In in vitro tests, cortical neuronal cultures from rat cerebral cortices collected on embryonic day 16 to 18 of Sprague-Dawley rats were used. The experiments were conducted on cultures 7 to 9 d old. Using the MTT assay, cells were exposed to glabridin (0.1 to 100 µM) and staurosporine (0 or 35 nM) for 24 h. Co-treatment of the neurons with glabridin for 24 h inhibited the cytotoxicity induced by staurosporine in a concentration-dependent manner (IC_{50} 4.1 ± 0.7 µM). (±)-α-Tocopherol was less potent (IC_{50} 6.9 ± 1.2 µM). Neurons incubated in staurosporine (35 nM) for 24 h were characterized by shrinkage of the cell body, membrane blebbing, chromatin condensation, nuclear pyknosis, and positive labeling of 3'-OH-DNA ends using the terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) assay. Co-treatment with glabridin (0.5 to 100 µM) inhibited the apoptosis of the rat neurons.

Staurosporine (35 nM) treatment induced time-dependent DNA laddering pattern in cell extracts prepared after treatment from 2 to 48 h. Co-incubation with glabridin (0.5 to 100 µM) reduced the DNA laddering in a concentration-dependent manner.

Staurosporine (35 nM) induced an increase in Bax protein and caspase-3 proenzyme but a decrease in bcl-2 protein in rat primary cortical neurons. Bax protein and caspase-2 proenzyme levels were reduced by glabridin in a concentration-dependent manner. Bcl-2 protein expression was recovered by glabridin co-incubation. Superoxide production in cortical neurons was enhanced by staurosponine (35 nM) over 24 h, with maximum production at 4 h, as labeled by ethidium. Co-incubation with glabrin (1 or 10 µM) inhibited superoxide production at 0.5, 2, 4, and 8 h but not at 24 h. The low concentration reduced the number of ethidium-labeled cells by 38.9%, 37.6%, 45.1%, and 9.3%, respectively. The high concentration of glabridin reduction values were 50.0%, 52.1%, 68.9%, and 26.5%, respectively. The authors concluded that glabridin exhibited neuroprotective effects in vitro and in vivo with inhibition of oxidative stress-induced neuronal damage (Yu et al. 2008).

Glycyrrhetinic Acid

Seckl et al. (1991) tested the effects of licorice, in the form of glycyrrhetinic acid extracted from licorice, on the brains of male Sprague-Dawley rats (300 g). Bilateral femoral arterial and venous cannulas were implanted under light anesthesia and the rats were allowed 3 h to recover. The rats were then injected s.c. with glycyrrhetinic acid (5 mg in ethanol; n = 5) or ethanol vehicle (n = 4) at time 0. At 45 min, the rats were injected again. Immediately after the second injection, the rats were injected s.c. with either glucocorticoid corticosterone (500 µg in ethanol) or the ethanol vehicle. Local cerebral glucose utilization was measured using the fully-quantitative [14C]2-deoxyglucose autoradiographic technique. Tracer was injected at 75 min and the animals killed at 120 min. Autoradiograms were prepared from coronal brain sections and analyzed densitometrically. Sixty forebrain regions were examined.

Administration of glycyrrhetinic acid, which inhibits 11β-OHSD, led to increased metabolic activity in the hypothalmic preoptic area (20%) and arcuate nuclei (26%) compared to controls ($p < .05$). The additional injection of glucocorticoid corticosterone elevated its level in the plasma to 603 ± 35 nmol/l (sampled at the time of the tracer injection) compared to 51 ± 6 nmol/l in the controls. Glucocorticoid corticosterone alone did not affect local cerebral glucose use in any forebrain region. Injection of glycyrrhetinic acid in the presence of elevated glucocorticoid corticosterone led to increases in glucose use in the arcuate nucleus (43%, $p < .01$); hypothalmic preoptic area (20%, $p < .05$); parietal cortex, layer IV (23%, $p < .01$); CA3 hippocampus (23%, $p < .01$); lateral hypothalmus (19%; $p < .05$); paraventricular nucleus (12%; $p < .05$); and zona incerta (40%; $p < .01$). There was a decrease in glucose use in the subfornical organ (9%; $p < .05$). The authors concluded that 11β-OHSD may play a role in

regulating the effects of corticosterone in the brain and is inhibited by glycyrrhetinic acid (Seckl et al. 1991).

Isoliquiritin and Liquiritin

Wang et al. (2008) tested the antidepressant effects of isoliquiritin and liquiritin extracted from *G. uralensis* on mice (sex and strain not specified). Fluoxetine (20 mg/kg; positive control), liquritin (10, 20, or 40 mg/kg), isoliquiritin (10, 20, or 40 mg/kg), or deionized water vehicle (control) was orally administered to the mice 30 min before testing. The mice performed either the forced swim test where they swam for 6 min in a glass cylinder and the time of immobility was recorded by an observer blind to treatment during the last 4 min. Other mice were suspended by the tail for 6 min and the time of immobility was recorded.

In a third test, liquiritin, isoliquiritin, and fluoxetine were administered at 20 mg/kg 30 min prior to the test. Four mice were placed in 4 cylinders at the same time and the total locomotor activity of each mouse was recorded for 5 min. All the mice administered 20 mg/kg of the phytochemicals, the controls, and a group that had not been treated or stressed were killed and the brain immediately dissected.

Immobility for mice administered liquiritin in both the forced swim test ($p < .001$) and tail suspension test ($p < .01$ and .001) was reduced compared to the control and similar to the positive control, fluoxetine, which indicated an antidepressant-like effect. Mice administered isoliquiritin in both the forced swim test ($p < .001$) and the tail suspension test ($p < .01$ and .001) had reduced immobility compared to the control. In all cases, the 20 mg/kg had the greatest effect.

There was no differences in locomotor activity for any treatment compared to the control in the locomotor activity test, indicating that the difference in mobility in the other tests were not due to possible central nervous system-stimulating effects.

Increases in the concentrations of the neurotransmitters 5-HT and NE in the mouse hippocampus, hypothalamus, and cortex from both the forced swim test and tail suspension test administered phytochemicals was similar to the positive control, fluoxetine, and greater than the control ($p < .05$, .01, and .001). There were no differences in the neurotransmitter DA in any brain region for any treatment. There was reduced 5-HIAA in the hippocampus and cortex with both treatments. The 5-HIAA/5-HT ratios were reduced in all brain regions tested in rats from both stress tests except for the hippocampus region for the liquiritin treatment. The authors concluded that isoliquiritin and liquiritin produced antidepressant-like activity (Wang et al. 2008).

Tyrosinase/Melanin Effects

Glycyrrhizic Acid

CIR (2005) reported that cellular melanin content and tyrosinase activity were dose-dependently increased with Glycyrrhizic Acid.

Various Compounds

Nerya et al. (2003), as part of a study of glabridin and related compounds (acetone extracts from *G. glabra* root) for skin lightening, tested the effects of these compounds on tyrosinase. Potassium phosphate buffer (0.07 ml, 50 mM) at pH 6.5, 0.03 ml tyrosinase (333 units/ml) and 2 µl of one of the tested compounds (licorice extract, 0 to ~30 µg/ml; glabridin, 0.03 to 3.5 µM; glabrene, 0.07 to 50 µM; or isoliquiritigenin, 2 to 500 µM) dissolved in absolute ethanol were inserted into 96-well plates. After a 5 min incubation at room temperature, 0.1 ml l-tyrosine (2 Mm) or 12 mM l-dihydroxyphenylalanine (DOPA) was added. The authors stated that the use of l-tyrosine and l-DOPA as substrates made it possible to distinguish between the ability of the extract to inhibit the o-hydroxylation of tyrosine and its further oxidation to o-diquinone. Ethanol was used as the control. Optical density at 492 nm was measured.

The extract inhibited the oxidation of tyrosine by the enzyme to an IC_{50} of 0.9 µg/ml. The authors stated that the maximum inhibitory activity was higher than that expected from its glabridin content (~10% w/w). The extract also inhibited the oxidation of l-DOPA by the enzyme to an IC_{50} of 53 µg/ml. The inhibitory effect on the first step of oxidation was greater than that on the oxidation of l-DOPA. All the compounds tested behaved as inhibitors of the monophenolase activity of tyrosinase. The lag period depended on both enzyme and substrate concentrations in the reaction medium.

Glabridin, isoliquiritigenin and glabrene inhibited monophenolase activity with IC_{50} values of 0.09, 3.5 and 8.1 µM, respectively. The IC_{50} values for diphenolase activity were 15 to 1000 times higher: glabridin, 3.94 µM; isoliquriitigenin 47 µM; glabrene, 7600 µM. All effects were dose-dependent. Hispaglabridin A and B did not inhibit tyrosinase activity. Isoprenylchalcone, 2'- and 4'-O-methyl glabridin did not inhibit tyrosinase activity. By varying the concentration of l-tryrosine, the authors showed that glabridin is also a noncompetitive inhibitor of the monophenolase activity of tyrosinase, while glabrene and isoliquiritigenin exhibited mixed inhibition. The inhibition constant (Ki) values for glabridin, isoliquiritigenin and glabrene were 0.38, 0.18 and 0.99 mM, respectively. Noncompetitive inhibition for glabridin was Ki = 0.81 mM.

To test the effects these compounds (in DMSO) have on melanin content, human melanocyte (G361) cells were grown in DMEM supplemented with 2 µg/ml insulin, 1 mM sodium pyruvate, 1 mM nonessential amino acids, 4 mM glutamine, 10% fetal calf serum (FCS), and antibiotics. After 3 d in culture, the cells were collected by trypsinization and counted by means of trypan blue. The cells were analyzed for melanin content. Melanin content was determined at 405 nm. The IC_{50} of inhibited pigmentation was compared to the cytotoxic effects of the compounds as shown in **Table 36**.

Glabridin had an IC_{50} of ~ 2.4 µg/ml; isoliquiritigenin, 4.73 µg/ml and glabrene, 6.68 µg/ml. The authors stated that glabrene and isoliquiritigenin exert inhibiton on tyrosinase-dependent melanin biosynthesis, suggesting that isoflavones and chalcones may serve as skin-lightening agents (Nerya et al. 2003).

Table 36. Effects of glabridin, glabrene and isoliquiritigenin on melanin synthesis in G361 human melanocytes (Nerya et al. 2003).

Test compound (concentration)	% melanin	% survival
Glabridin (10 µM)	42.15 ± 13.1[a]	92.2 ± 19.5
Glabrene (10 µM)	73.6 ± 5.7[a]	71.9 ± 4.04
Isoliquiritigenin (10 µM)	87.9 ± 6.8	110.2 ± 7.9
Isoliquiritigenin (20 µM)	63.3 ± 4.8[a]	92.06 ± 2.6

[A] $p > .005$ compared to control

Neuromuscular Effects

Glycyrrhizic Acid and Derivatives

CIR (2005) reported that Glycyrrhetinic Acid and its derivatives reversibly blocked gap junction communication and that 18α-Glycyrrhetinic Acid and 18β-Glycyrrhetinic Acid mediated a concentration-dependent inhibition of junctional conductance by 60%. Complete blockage of the gap junction channels did not occur even at concentrations as high as 100 µM.

Glycyrrhetinic Acid (1250 mg/kg) administered ip to mice caused sedation, hypnosis, hypothermia, and respiratory depression. Glycyrrhetinic Acid administered ip (1250 mg/kg) or s.c. (625 mg/kg) did not stimulate or depress either the sympathetic or parasympathetic branches of the autonomic nervous system of mice. A cat administered 125 mg/kg Glycyrrhetinic Acid ip did not exhibit any changes in BP and had normal responses to stimulation of sympathetic and parasympathetic nerves (CIR 2005).

Glycycoumarin

Nagai et al. (2007) compared the ability of cultivated and wild *G. uralensis* root, due to its glycycoumarin content, to relax carbamylcholine (carbachol)-induced contraction of isolated mice jejunum. Glycycoumarin content was found to be 0.10 % for all 3 extract sources. Water extracts from 4-year-old and 5-year-old roots from cultivated plants and the roots from wild plants collected in Inner Mongolia were used. The jejunum was isolated from male ICR mice (7 to 10 wk old). Maximal contraction was induced with acetylcholine (1 µM) and the jejunum was washed then exposed to carbachol (1 µM). After the contraction came to equilibrium, samples (in methanol) were administered every 5 min in a cumulative manner. The potency of induced relaxation was expressed as the EC_{50}.

The extracts of cultivated roots (25 to 250 µg/ml) and collected roots induced relaxation in carbachol-induced contracted jejunal segments in a similar manner. The maximal relaxant effects of the cultivated roots (72.7 ± 3.2%) and the collected roots (68.5 ± 5.2%) were observed at 250 µg/ml. For comparison, the maximal relaxant effect of peppermint oil was 77.0 ± 9.6% at 40 µg/ml. The relaxant effects by all 3 extracts was in a dose-dependent manner (50 to 200 µg/ml). The EC_{50} was similar for the 4- and 5-year-old roots and the collected roots (134 ± 21, 149 ± 10, and 134 ± 16 µg/ml, respectively) but less than that of peppermint oil (13.4 ± 6.0 µg/ml; Nagai et al. 2006).

Sato et al. (2006) used the jejunums of male ICR mice to test the antispasmodic effects of glycycoumarin extracted from the roots of *G. uralensis*. The jejunum sections (10 to 15 mm) were attached to a strain-gauge transducer and allowed to come to a steady state. Carbamylcholine chloride (1 µmol/l), potassium chloride (60 mmol/l), barium chloride (3 mmol/l), or A23187 (calcium ionophoe III; 6 µmol/l) was added to induce a sustained contraction. Glycycoumarin (3 x 10-7 to 3 x 10-5 mol/l; in ethanol) was added cumulatively at 5 min intervals.

Verapamil, forskolin, 3-isobutyl-1-methylxanthine (IBMX), vinpocetine, milrinone, rilipram, and zaprinast were also tested for comparison. cAMP or cGMP content of the jejunum was measured by enzyme immunoassay.

Glycycoumarin inhibited the contraction of mouse jejunum induced by carbamylcholine chloride in a concentration-dependent manner with an IC_{50} of 2.93 ± 0.94 µmol/l (1.08 ± 0.16 µg/ml) similar to papaverine (1.64 ± 0.46 µmol/l; 0.57 ± 0.16 µg/ml). Carbamylcholine chloride-induced contraction of the ileum and colon were also inhibited (IC_{50} = 4.87 ± 1.27 µmol/l [1.79 ± 0.47 µg/ml] and 2.44 ± 1.06 µmol/l [0.90 ± 0.39 µg/ml], respectively). Glycyoumarin showed concentration-dependent inhibition of the contractions induced by potassium chloride (IC_{50} = 2.59 ± 0.58 µmol/l; 0.95 ± 0.29 µg/ml), barium chloride (IC_{50} 4.09 ± 1.82 µmol/l; 1.51 ± 0.67 µg/ml), and A23187 (IC_{50} = 7.39 ± 5.19 ümol/l; 2.72 ± 1.91 µg/ml), similar to values for papaverine. Pretreatment with glycycoumarin (10µmol/l) reduced the contraction caused by the cumulative addition of calcium in Ca++-free Tyrode solution (with a potency similar to verapamil). Forskolin (an adenylyl cyclast activator) inhibited the carbamylcholine chloride-induced contraction of the jejunum. Inhibition was potentiated by pre-treatment with glycycoumarin (10 µmol/l) or IBMX (a potent non-selective phosphodiesterase inhibitor). The authors suggest that glycycoumarin exerts its relaxant action through inhibition of phosphodiesterase.

To test phosphodiesterase inhibition in combination, carbamylcholine chloride was used to contract jejunum segments then milrinone (a selective inhibitor of phosphodiesterase 3) was added. Milrinone inhibited contraction in a concentration-dependent manner at less than 50% contraction at 10 µmol/l. Inhibition was not enhanced by the addition of glycycoumarin (µmol/l). However, relaxation by rolipram, a phosphodiesterase 4 inhibitor was enhanced by pretreatment with glycycoumarin. This was also the case with milrinone. Glycyroumarin did not affect the inhibition response of vinpocetine and zaprinast (selective inhibitors of phosphodiesterase 1 and 5, respectively).

Glycycoumarin (1 and 3 µmol/l) and forskolin (0.3 and 1 µmol/l) increased the cAMP content of mouse jejunum in the presence of carbamylcholine chloride (1 µmol/l) in a concentration-dependent manner. cGMP was not increased by glycycoumarin. The authors concluded that glycycoumarin, from G. uralensis roots, acts as a potent antispasmodic by means of intracellular accumulation of cAMP through the inhibition of phosphodiesterases, especially isozyme 3 (Sato et al. 2006).

Isoliquiritigenin and Related Compounds

Sato et al. (2007) isolated isoliquiritigenin from the roots of *G. uralensis*. Male ICR mice (8 wk old) were killed after overnight fasting and their jejunum, ileum, and rectums removed quickly, cleaned of connective tissues, and cut into rings of 10 to 15 mm in length. The sections were exposed to various stimulants and the isoliquiritigenin and the muscle tension measured. Isoliquiritigenin showed concentration-dependent inhibition of the tonic contraction of mouse jejunum induced by carbamylcholine (1 µM), potassium chloride (60 mM), and barium chloride (0.3 mM) with IC_{50} values of 4.96 ± 1.97 µM, 4.03 ± 1.34 µM, and 3.70 ± 0.58 µM, respectively. These values are similar to papavarine. Pretreatment with isoliquiritigenin inhibited the contraction cause by the cumulative addition of acetylcholine as strongly as papavarine and non-competitively. Inhibitory effect of butylscopolammonium bromide was competitive. Isoliquiritigenin also inhibited carbamylcholine-induced contraction of the mouse ileum and rectum with IC_{50} values of 5.55 ± 2.79 and 1.70 ± 0.07 µM, respectively.

The authors tested for phosphodiesterase inhibition by isoliquiritigenin. Forskolin, an adenylyl cyclase activator, inhibited carbamylcholine-induced contraction of mouse jejunum in a concentration-dependent manner (IC_{50} 0.25 ± 0.08 µM). The inhibition was not accelerated by pretreatment with isoliquiritigenin (10 µM) even though there was a shift in the IC_{50} to 0.16 ± 0.08 µM. Glycycoumarin (10 µM), a known phosphodiesterase inhibitor from licorice, had an IC_{50} 0.11 ± 0.04 µM. The inhibition of carbamylcholine-induced contraction with IBMX (IC_{50} 3.23 ± 0.72 µM) tended to decrease with pretreatment with isoliquiritigenin (1 µM), shifting the IC_{50} to 1.44 ± 0.35 µM. Isoliquiritigenin did not increase the cAMP or cGMP content in the mouse jejunum. The authors stated that these results suggest that the relaxant action of isoliquiritigenin on the mouse jejunum might not be related to its phosphodiesterase inhibition.

Other compounds from G. uralensis were tested for phosphodiesterase inhibition. Liquiritigenin was weak, liquiritin, liquirtitin apioside, and isoliquiritin were inactive. Davidigenin was a potent inhibitor with an IC_{50} of 5.07 ± 163 µM.

The authors tested the effect of the addition of nariginase on contractile inhibition. HPLC analysis of aqueous licorice extract did not show any peak of isoliquiritigenin, thus only a trace of this compound may be present; its glycosides, isoliquiritin, and isoliquiritin apioside were abundant. When the extract was treated with nariginase, the amounts of glycosides were decreased while those of their aglycones, such as liquiritigenin and isoliquiritigenin, were increased. The inhibition of carbamylcholine-induced contraction of mouse jejunum with aqueous extract of licorice was increased by treatment with nariginase, shifting the IC_{50} from 358 ± 104 to 150 ± 35 µg/ml (Sato et al. 2007).

Liu et al. (2008) tested the effects of isoliquiritigenin, extracted from *G. glabra* root, on guinea-pig tracheal smooth muscle as a possible treatment for airway inflammation and contraction. Dunkin Hartley guinea pigs were killed and the tracheas removed and cut into spiral strips. After the strips (n = 6) were precontracted with acetylcholine (10 µM), potassium chloride (40 mM), or histamine (10 µM), they were treated with isoliquiritigenin (1 to 1000 µM). The experiment was conducted with and without epithelium. The $-\log IC_{50}$ values for acetylcholine-, potassium chloride-, and histamine-treated trachea strips were 4.42 ± 0.6, 4.35 ± 0.59, and 4.49 ± 0.61, respectively, and statistically similar. Isoliquiritigenin relaxed the contractions in a concentration-dependent manner with and without epithelium. The authors stated that isoliquiritigenin-induced bronchiodialation was likely epithelium-independent.

To examine the possible mechanisms for the relaxant effects of isoliquirtigenin, the experiment was repeated and the tracheal strips were pretreated with Nω-nitro-l-arginine methyl ester (100 µM; nitric oxide synthase [NOS] inhibitor), oxyhemoglobin (10 µM; binds nitric oxide), or indomethacin (10 µM; cyclo-oxygenase inhibitor). There were no effects to the relaxations of acetylcholine-precontracted tracheal strips elicited by isoliquirtigenin. The authors suggest that nitric oxide and prostaglandin were not involved in the relaxation caused by isoliquiritigenin.

Further experiments were conducted with pretreatment with 1H-[1,2,4] oxandiazolo [4,3-a] quinoxalin-1one (10 µM; a soluble guanylyl cyclase inhibitor), 9-(terahydro-2-furanyl)-9H-purin-6-amine (100 µM; adenylyl cyclase inhibitor), or KT5823 (300 nM; cGMP protein kinase inhibitor). The adenylyl cyclase inhibitor had no effect on the relaxation effects of isoliquiritigenin. Pretreatment with the soluble guanylyl cyclase inhibitor and the protein kinase inhibitor attenuated relaxation by 62.5% and 46.6%.

An additional experiment using glibenclamide (1 µM; KATP channel blocker), 4-amino-pyridine (100 µM; voltage-dependent potassium channel blocker), apamin (1 µM; calcium-activated potassium channel blocker), or charybdotoxin (0.1 µM; another calcium-activated potassium channel blocker) for 30 min prior to treatment with isoliquiritgenin were conducted. Charybdotoxin inhibited the effect of isoliquiritigenin by 42.5%; the other chemicals had no effect.

In patch-clamp studies using tracheal smooth muscle cells, single-channel conductance by calcium-dependent potassium channels in the absence of isoliquirtigenin was not different from that measured after the application of isoliquiritigenin (100 µM). Average channel activity increased substantially (9-fold) from 0.035 to 0.31 within 20 s of exposure to isoliquritigenin (100 µM) and the magnitude of unitary current remained unchanged in the

presence of isoliquritigein. The average channel activity returned to control level (0.037) within 60 s of washout. The addition of KT5823 reduced the increased average channel activity of the channel in the presence of isoliquiritigenin to 0.04.

Pretreatment with isoliquiritigenin prior to stimulation with acetylcholine (10 µM) induced a dose-dependent reduction of actetylcholine-induced calcium contraction response. Pretreatment with KT5823 caused a reduction in the response to isoliquiritigenin in tracheal smooth muscle cells stimulated by acetylcholine.

Guinea pig tracheal smooth muscle cells were cultured with and without IBMX (100 µM; n = 6). The basal release of cAMP and cGMP was 71.8 ± 10.4 and 2.3 ± 0.4 pmol/mg protein. Isoliquiritigenin (30 or 100 µM) increased both cAMP (89.6 ± 7.8 and 105.2 ± 100 pM, respectively; $p < .05$) and cGMP (17.5 ± 2.8 and 23.7 ± 3.1 pmol/mg protein; $p < .05$) levels. Isoliquiritigenin alone had no effect on cAMP but increased cGMP ($p < .05$). The authors suggest that phosphodiesterase (PDE) inhibitory activity of isoliquritigenin is enough to prevent the cGMP breakdown, but has no effect on cAMP breakdown. Further testing showed that isoliquiritigenin and zaprinast (PDE5 inhibitor) had no effect on cAMP whereas IBMX, milrinone, and rolipram elevated cAMP.

Isoliquiritigenin's effect on PDE activity was tested using human platelets and U937 cells (n = 6). There were no increased in enzyme activity in any of the treated cells. The IC_{50} is > 300 µM.

The authors suggested that the relaxing activity of isoliquirtigenin in trachea might also attribute to accumulation of cyclic nucleotides and the resulting opening of potassium channels and diminution of agonists-induced calcium contraction response. Isoliquiritigenin relaxes guinea-pig trachea throuh a multiple of intracellular actions, including soluble guanylyl cyclase (sGC) activation, inhibition of PDE4, and associated activation of the cGMP/cAMP protein kinase signaling cascade leading to the opening of calcium-activated potassium channels and calcium contraction decrease through cGMP-dependent protein kinase (PKG)-dependent mechanism and thus to tracheal relaxation (Liu et al. 2008).

Licochalcone A

Nagai et al. (2007) used segments of ICR mice jejunum to measure the relaxant effects of licochalcone A isolated from *G. inflata* roots. The segments were contracted with acetylcholine (1 µM), allowed to equilibrate, then exposed to carbachol (1 µM), potassium chloride (60 mM), barium chloride (1 mM) or A23187 (6 µM). When contraction was at steady state, licochalcone A (0.3 to 30 µM) was added in a cumulative manner every 5 min. Licochalchone A demonstrated relaxant effects in a dose-dependent manner against all 4 stimulants. The EC_{50} against carbachol, potassium chloride, barium chloride, and A23187 were 5.64 ± 1.61, 5.12 ± 1.68, 1.97 ± 0.48, and 2.63 ± 2.05 µM, respectively.

The authors exposed the jejunum segments to acetylcholine (1 µM) or potassium chloride (60 mM). After a 45 min equilibrium period, licochalcone A (3.0 or 6.0 µM) was added. After 10 min, the jejunum segments were exposed to acetylcholine or potassium chloride again. Licochalcone A relaxed the tonic phase contraction induced by acetylcholine. The phasic contraction was only weakly affected and was not dose-dependent. Licochalcone A relaxed the tonic phase contraction induced by potassium chloride.

The phosphodiesterase activity of licochalcone A was determined using a cyclic nucleotide phosphodiesterase (PDE) assay kit. The IC_{50} was 22.1 ± 10.9 µM, similar to IBMX and papaverine (26.2 ± 7.4 and 31.8 ± 2.0 µM, respectively) and glycyoumarin (30.3 ± 8.0 µM) isolated from G. uralensis root. Isoliquiritigenin had no influence on PDE activity at > 200 µM. The authors suggested that licochalcone A exerted its relaxant effect on smooth muscle contraction through inhibition of cAMP PDE (Magai et al. 2007).

Blood Effects

Licochalcone A

Barfod et al. (2002) investigated the effect of licochalcone A on the activity of human peripheral blood mononuclear cell (PBMC) proliferation and cytokine production. Heparinized peripheral blood was collected from volunteers and PBMCs were isolated by density centrifugation and washed. The cells were placed in growth media or cryopreserved in liquid nitrogen. Incubation with 25 µg/ml of licochalcone A resulted in >93% viability. Licochalcone A showed a dose-dependent inhibition of the proliferation of the lymphocytes with ~80% inhibition at 25.00 µg/ml.

To further investigate the effect of licochalcone A on lymphocyte proliferation, staining and analysis by flow cytometry were performed. Licochalcone A demonstrated a dose-dependent anti-proliferative effect with a lower percentage of lymphocytes in the offspring generations. The proliferation index for the control was decreased with 25 µg/ml licochalcone A.

To measure cytokines in the culture supernatants after exposure to licochalcone A, frozen PBMCs were rapidly thawed and washed and stimulated (for 22 h) with either 10 ng/ml E. coli LPS or phenylmercuric acetate (PMA) and ionomycin with and without dilutions of licochalcone A (0 to 100 µg/ml). ELISA was used to measure the concentrations of interferon (IFN)-γ, TNF-α, IL-1β, IL-6, and IL-10. Licochalcone A reduced the release of all 4 cytokines in a dose-dependent manner. To investigate the effect of licochalcone A on IFN-γ secretion, PBMCs were stimulated with PMA and ionomycin in the presence of the chalcone. IFN-γ was reduced ($p < .05$) at 25.00 µg/ml.

To investigate the inhibitory mechanism of licochalcone A on cytokine production, T-cells and monocytes were stimulated with PMA (50 ng/ml), monensin (1.5 µM) and ionomycin (1 µM) while exposed to the chalcone. Licochalcone A reduced the frequency of TNF-α-producing monocytes after LPS stimulation in a dose-dependent manner. The mean level of TNF-α fluorescence was reduced in a dose-dependent manner. There was no inhibitory effect on the production of TNF-α or fluorescence of T-cells. The authors stated that licochalcone A and some analogues may have immunomodulatory effects and thus may be candidates for anti-microbial agents and treatment for other types of disease (Barfod et al. 2002).

Ziegler et al. (2004) investigated the effects of licochalcone A on the membranes of human erythrocytes. Erythrocytes were treated with 0, 0.2, 2.0, 10.0, and 25.0 µg/ml licochalcone A and examined under light microscopy after 5, 15, and 30 min and then 1, 2, 4, 24, 30 and 48 h. The erythrocytes had shape deformations within 5 min when treated with 25.0 µg/ml. At the lower concentrations, the deformations manifested in a time-dependent manner; discocytic or slightly stomatocytic forms were complete after 24 h. The researchers found that the resulting membrane loading was irreversible when cells exposed to the 25.0 µg/ml level were then exposed to 5 µg/ml and they did not return to the echinocyte III shape.

Isoliquiritigenin

Tawata et al. (1992) collected blood from healthy volunteers. The blood was centrifuged and suspended, washed, resuspended and adjusted to 4 x 105 platelets/mm in buffer. Ten ml of the suspension were incubated with 1 mCi of [32P]orthophosphate for 90 min. The labeled platelets were washed and exposed to isoliquiritigenin or aspirin (2, 5, and 20 µg/ml). The treatments were dissolved in ethanol (final concentration of ethanol was 0.1%). Platelets were exposed for 5 min. In an additional experiment, thrombin was added 5 min later. The reaction was terminated 2 min later with an equal amount of buffer containing sodium dodecyl sulfate (SDS) followed by boiling for 3 min. SDS-polyacrylamide gel electrophoresis and autoradiography were employed to assess the platelet aggregation.

Isoliquiritigenin reduced the rate of platelet aggregation from 82% to 64% (2 µg/ml) or to 52% (5 µg/ml). Aspirin reduced the platelet aggregation rate from 82% to 75% (2 µg/ml) or 64% (5 µg/ml). An inhibitory effect was also observed when thrombin was added to the platelets, ~50% reduced to ~15% and ~20% for isoliquiritigenin (5 µg/ml) and aspirin (5 µg/ml), respectively.

To explore the mechanism of the platelet aggregation inhibition, the researchers tested the effect of isoliquiritigenin on the production of 12(S)-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and 12-hydroxyeicosatetraenoic acid (12-HETE) by platelets. After exposure to isoliquiritigenin (1 and 10 µg/ml) and 5 min exposure to thrombin (0.5 U/ml), HHT production was reduced to 88.3 ± 1.0 and 24.7 ± 10.0%, respectively, and 12-HETE production was reduced to 89.9 ± 1.5% and 60.1 ± 10.7%, respectively.

Rats were orally administered 50 mg/kg isoliquiritigenin in 1% carboxymethyl cellulose (CMC) twice daily for 3 d. Control rats were administered the vehicle. Three h after the last dose, blood samples were collected in 3.8% sodium citrate. The plasma was centrifuged and the platelet aggregation was measured. The platelets from the rats administered isoliquiritigenin had a lower rate of aggregation compared to the control rats. Additionally, the amount of thrombin to achieve 50% aggregation of platelets from treated rats was 0.617 ± 0.016 U/ml; platelets from control rats required 0.504 ± 0.015 U/ml ($p < .05$). The authors stated that isoliquiritigenin may be an aldose reductase inhibitor (Tawata et al. 1992).

Licorice Flavonoid Oil

Nakagawa et al. (2008b) investigated the hemorrhagic effects of licorice flavonoid oil using 5-week-old male Crj:CD(SD) rats (SPF) as a follow-up study of short-term oral toxicity (see below). The rats were divided into 6 groups. Groups 1 (n = 5) and 5 (n = 3) were control groups fed a basal diet for 20 d. Groups 2 (n = 5), 3 (n = 5), and 6 (n = 3) had licorice flavonoid oil (5%) incorporated into the diet for 20 d. Group 4 (n = 5) had licorice flavonoid oil incorporated into the diet for 13 d then fed the basal diet for the remaining 7 d. The rats in group 3 were administered 2 vitamin K (70 mg/kg/d) injections i.p. on days 13 and 14. Blood samples were collected on day 11 for groups 5 and 6 and on day 15 for groups 1 to 4. Clotting measurements, prothrombin time and activated partial thromboplasin time were administered on days 15 and 20.

Group 2 had prolongation of prothrombin time and activated partial thromboplasin time compared to the control group. These levels in group 3 returned to normal within 2 d after the vitamin K injections; group 4 returned to normal within 2 d after cessation of licorice flavonoid oil in the feed. Groups 5 and 6 had prolonged prothrombin time and activated partial thromboplasin time. In group 2 at day 20, the activity of vitamin K-dependent coagulation factors II, VII, IX, and X were decreased; this decrease resolved after the vitamin K injections or cessation of the test diet. The concentration of fibrinogen, a vitamin K-independent factor, did not decrease, but increased. The effects of vitamin K were evident until the end of the experiment. The authors concluded that licorice flavonoid oil inhibited the synthesis of vitamin K related coagulation factors II, VI, IX, and X, similar to warfarin (Nakagawa et al 2008b).

Cardiac Effects

Glycyrrhizic Acid and Derivatives

Treatment with Glycyrrhizic Acid, but not glycyrrhetinic acid, reduced the size of myocardial infarcts in rabbits with a suture tied around the left coronary artery to occlude blood flow for 30 min (CIR 2005).

Cholesterol Effects

Glabridin

Rosenblat et al. (1999) examined the effect and mechanism of macrophage enrichment with glabridin on the capability of these cells to oxidize LDL. Glabridin was isolated from the acetone extract of G. glabra roots. Upon incubation of macrophages (J-774 A.1 cell line or mouse peritoneal macrophages (MPM)) with glabridin (20 µM) for 20 h at 30°C, cellular glabridin content increased from 0 to 1.8 ± 0.2 µg glabridin/mg cell protein (n = 3).

Incubation of MPMs or J-774 A1 macrophages with LDL in the presence of 2 µM copper sulfide ($CuSO_4$) for 5 h at 37°C resulted in a 90% or 82% inhibition of LDL oxidation, respectively, by the glabridin-enriched macrophages in comparison with the control cells (no inhibition). Extending the experiment to 20 h resulted in only 65% and 48% inhibition of LDL oxidation by these cells in comparison to controls. Glabridin was not cytotoxic to the cells since there was no effect on cell counts or on the release of

LDH. Cell fractionation revealed that ~60% of the glabridin was localized in the macrophage membrane and the rest in the cytosol. Repeating the experiment and varying the glabridin concentration from 0 to 20 µM demonstrated that the effect of glabridin was dose-dependent.

Apolipoprotein E-deficient (E0) mice (n = 10) received 20 µg/d glabridin in their drinking water or just water for 6 wk. Their MPMs were harvested and tested for cell protein. The treated mice had 1.6 ± 0.3 µg glabridin/mg cell protein. After 5 h LDL (10 µg protein/ml) incubation with the MPMs in the presence of 2 µM CuSO4, cell-mediated oxidation of LDL by the glabridin-enriched macrophages (0.6 ± 0.2 nmol MDA equivalents/mg of LDL protein) was reduced by 88% compared to untreated controls (cells from mice that just drank water; 5.6 ± 0.2 nmol MDA equivalents/mg of LDL protein). The mice were killed after 6 wk of glabridin consumption and their aortas dissected. There was a 50% reduction in lesion area (plaque accumulation) compared to controls (52 ± 8 and 106 ± 11 µm2 x 103, respectively). Analysis of the lesion oxysterol derivatives showed that 7 α-hydoxycholesterol, 7 β-hydroxycholesterol, α-epoxycholesterol and 7-ketocholesterol was reduced by 54%, 58%, 51%, and 56%, respectively (Rosenblat et al. 1999).

Glabrol

Choi et al. (2007) tested the effects of glabrol (from the ethanol extract of licorice root, species not identified) on atherosclerosis. Rat liver microsomal acyl-coenzyme A:cholesterol acyltransferase (ACAT) activity was measured after exposure to glabrol. ACAT activity was inhibited in a concentration-dependent manner with an IC_{50} of 24.6 µM in an enzyme assay. The positive control, pheylpyropene A, had an IC_{50} of 0.8 µM. The authors report that liquiritigenin, isoliquiritigenin, ononin, and glycycoumarin, also isolated from the same root, did not inhibit ACAT activity up 100 µM. The authors tested for the effect of glabrol on cellular cholesterol esterification on HepG2 cells. The inhibiton of cholesteryl[1-14C]oleate synthesis was concentration-dependent with an IC_{50} of 26.0 µM with no cytotoxicity up to 250 µM. The authors suggested that glabrol may be the source of antiathrerosclerotic activity of licorice in hypercholesterolemic patients.

Effects on cAMP Phosphodiesterase

Glabridin and Related Compounds

Kusano et al (1991) reported that flavanoids, including glabridin, isolated from the byproducts of glycyrrhizin extraction from licorice root for the medical industry were inhibitors of cAMP phosphodiesterase. The inhibitory activity of flavonoids is given in **Table 37**, with glycocoumarin being the most effective.

Cytotoxicity and Cellular Effects

Glycyrrhizic Acid and Derivatives

Glycyrrhizic Acid inhibited the enzyme activity of proteinase in an in vitro system and was a potent inhibitor of oxidative phosphorylation coupled to succinate oxidation in rat liver mitochondria. Glycyrrhizic acid administered to mice induced hepatic CYP3A-2B1 and 1A2-dependent microsomal monooxygenase; TH enzymes in the liver were also induced. Glycyrrhizic Acid binds to high mobility group proteins (HMGP1/2), caused a conformational change in the proteins, and, thus, inhibited the phosphorylation of HMPG1/2 by protein kinases (CIR 2005).

The neutral red (NR) cell viability assay gave an NR50 of 18α-glycyrrhetinic acid and 18β-glycyrrhetinic acid of 0.26 mM and 0.13 mM, respectively, using the neutral red assay with BALB/c mouse 3T3 fibroblasts as indicators (CIR 2005).

Glycyrrhetinic acid administered i.p. caused apoptosis in splenocytes and thymocytes in mice. The apoptosis of splenocytes was a result of the inhibition of 11β-OHSD by glycyrrhetinic acid, which increased the levels of corticosterone. Glycyrrhizic acid enhanced the production of IL-12, IL-2, and interferon (CIR 2005).

Glabridin

Cao et al. (2007) reports that glabridin (0.1 to 100 µM) was not cytotoxic to human Caco-2, Madin-Darby canine kidney (MDCK) II cells (control), or ultiple drug resistant (MDR) 1-MDCKII cells for up to 48 h in a MTT assay. There were no detectable metabolites produced by the cells as determined by HPLC and LC-MS analysis except for a minimal formation of glabridin glucuronides in Caco-2 cells.

The authors examined intracellular accumulation and efflux of glabridin in Caco-2, MDCKII and MDR1-MDCKII cells with regard to time and concentration. The uptake of glabridin was linear up to 60 min; the efflux of glabridin out of the cells was also linear up to 20 min. The accumulation and efflux of glabridin in Caco-2 cells increased in a concentration-dependent manner similar to the Michaelis-Menten kinetics with a one-binding site model as the best fit. The estimated Michaelis-Menten constant (Km) and maximum volume of distribution (Vmax)were 12.35 ± 1.41 and 4.68 ± 1.03 µM with 17.44 ± 0.76 ng/min/mg cellular protein and 0.67 ± 0.04 pg/min/mg cellular protein for uptake and efflux, respectively.

Table 37. Inhibitory activity of flavonoids on cAMP phosphodiesterase (Kusano et al. 1991).

Sample	IC_{50} (x 10^{-5} M)
Isoliquiritigenin-4'-*O*-apioglucoside	171
Isoliquiritigenin	18
Liquiritin	> 500
Liquiritigenin	108
Glabridin	8.2
Licoricidin	4.9
Licoarylcoumarin	1.0
Glycyrin	> 500
Glycycoumarin	0.7
Glycyrol	4.4
Isoglycyrol	> 500
Licoricone	2.3

When monitoring the intracellular concentration of glabridin at 1.0 µM over 120 min, it appeared that the efflux of glabridin from Caco-2 cells was monoexponential with a half-life of 9.84 min. The authors stated that this indicated a rapid exit of glabridin from the cells during the first 10 min followed by a slow exit requiring several hours. When the cells were preincubated with verapamil or nifedipine (100 µM) for 2 h, glabridin accumulation increased by 83.3 ± 13.3% and 116.9 ± 15.2% ($p < .05$), respectively. Addition of MK-571 (100 µM), celecoxib (100 µM), or probenecid (200 µM) did not change glabridin intracellular accumulation. When the cells were preincubated in verapamil or nifedipine for only 30 min, glabridin efflux was decreased by 27.8 ± 3.3% and 32.5 ± 4.2% ($p < .05$), respectively. There was no change with the addition of the other inhibitors. The authors stated that this indicates that glabridin is possibly a substrate for PgP/MDR1, but not for MRP1-4.

Intracellular accumulation and efflux of glabridin in both MDCKII and MDR1-MDCKII cells were linear up to 120 min. The accumulation and efflux amounts of glabridin (0.1 to 50 µM) in both cell lines increased in a concentration-dependent manner. The uptake of glabridin by MDCKII cells was ~3-fold higher than that of MDR1-MDCKII cells ($p < .05$). The efflux of glabridin by MDR1-MDCKII cells was 5- to 7-fold higher than the control MDCKII cells ($p < .05$ and .01). The cells remained viable for the 120 min of incubation. The authors state that these results suggest that PgP/MDR1 diminished the uptake increased by the efflux of glabridin by MDCKII cells and that glabridin is possibly a substrate for PgP/MDR1.

The authors examined the transport of glabridin in Caco-2 monolayers. Glabridin (0.1 to 100 µM) was loaded on the apical or the basolateral side and the receiving side analyzed with LC-MS. There were no detectable metabolites in either direction at all concentrations over 60 min. Glabridin was detected on the receiving side by 5 min. The flux rate in either direction was concentration-dependent and was linear up to 60 min. Transport from the basolateral side to the apical side (8.12 to 25.63 x 10-5 cm/s) was higher than the other direction (0.87 to 5.76 x 10-5 cm/s; $p < .05$). The permeability coefficient for both directions decreased with increasing glabridin concentrations. The authors state that this experiment demonstrated a polarization in the Caco-2 permeability toward glabridin and a predominantly secretory rather than absorptive transport. The efflux with the addition of verapamil suggested to the authors that glabridin was a substrate for PgP with high affinity.

To investigate the nature of the polarized transport of glabridin, transport studies were conducted using MDCKII and MDR1-MDCKII monolayers. MDR1-MDCKII cells over-express the human PgP/MDR1. Glabridin (0.1 to 50 µM) had ~2-fold greater permeability in MDCKII monolayers in the basolateral to apical side transport than in the other direction ($p < .05$ or .01). The transport of glabridin across MDR1-MDCKII monolayers was greater in the apical to basolateral side at all concentrations ($p < .01$ and .001).

Reducing the pH from 7.4 to a range of 5.5 to 6.5 caused an increase in glabridin flux by 24.5% to 56.8% at 0.1 to 1.0 µM in both directions. The authors state that lower pH may reduce the ionization of glabridin and thus increase its intestinal transport. Changing the medium from sodium salts to potassium salts had no effect on transport across the cells. The reduction of temperature from 37°C to 4°C reduced the flux of glabridin (0.1 and 1.0 µM) from the apical to basolateral side and the basolateral to apical side by 42.2% to 75.5% ($p < .05$). The absence of glucose in the medium did not affect transport from the apical to the basolateral side but did decrease transport of glabridin (0.1 and 1.0 µM) in the basolateral to the apical side direction by 50.5% and 65.2% ($p < .05$). The authors stated that glabridin transport across Caco-2 monlayers was pH-, energy-, and temperature-dependent but not sodium-dependent.

The authors tested the effects of ATP inhibitors and ATP-binding cassette (ABC) transporter inhibitors on glabridin (0.1 and 1.0 µM) transport in Caco-2 monolayers. The addition of the transport buffers sodium azide (10 mM), 2,4-dinitrophenol (1 mM), or verapamil (100 µM) increased the apical to basolateral transport ($p < .05$) by 52.8%, 38.6%, and 82.4%, respectively, for the low amount of glabridin. Similar results were reported for the higher amount. Probenecid, MK-571, and celecoxib did not alter the transport in either direction. The authors suggest the MRPs play a minor or negligible role in the intestinal transport of glabridin.

The authors tested for effects of glabridin on PgP-mediated transport of the probe digoxin in Caco-2 cells. Glabridin inhibited digoxin transport in a concentration-dependent manner with an IC_{50} of 2.56 ± 0.04 µM. Verapamil had potent inhibitory effects on PgP-mediated transport of digoxin, with an IC_{50} of 2.34 ± 0.03 µM. The authors stated that this indicates that glabridin was a potent PgP inhibitor in vitro.

The ATPase activity assay was used to assess glabridin's affinity for PgP/MDR1. Glabridin (0.1 to 100 µM) demonstrated a concentration-dependent stimulation of vanadate-sensitive PgP/MDR1 ATPase activity with the one binding site model as the best fit. Verapamil exhibited a stimulatory effect over 0.25 to 100 µM (Cao et al. 2007).

Yu et al. (2007) reported that glabridin (0.1 to 100 µM) was not cytotoxic (<10%) to cryopreserved primary rat brain microvascular endothelial cells incubated for up to 48 h in an MTT assay. There were no detectable oxidative metabolites and conjugates observed when primary rat brain microvascular endothelial cells were incubated with glabridin (0.1 to 100µM) for 2 to 48 h.

Rat brain microvascular endothelial cells incubated in glabridin (0 to 100 µM) for 120 min demonstrated a linear uptake of glabridin up to 30 min with a maximim uptake rate of 12.5 ± 1.3 ng/mg cellular protein. Uptake then gradually declined. The uptake of glabridin was ~0.5% to 0.75% of the loading dose. The estimated Km and Vmax were 10.87 ±1.46 µM and 4.81 ± 0.20 ng/min/mg cellular protein. The authors conclude that the uptake best fits the one-binding site Michaelis-Menten kinetics model without a passive diffusion component. They also state that there

may be 1 predominant transporter or multiple transporters with similar affinity for glabridin.

The efflux of grabridin by rat brain microvascular endothelial cells was linear up to 60 min then slightly declined to 120 min. The efflux increased with concentration of glabridin. The Km and Vmax were 15.48 ± 2.94 µM and 1.32 ± 0.08 pg/min/mg cellular protein.

Intracellular concentration of glabridin in rat brain microvascular endothelial cells (glabridin substrate 1.0 µM over 120 min) was bi-exponential with half-life values of 2.55 ± 0.37 and 14.62 ± 1.53 min, indicating a rapid exit of the drug from the cells in the initial 5 min followed by a relatively rapid exit. The authors stated that this suggested multiple transporters with differential affinities to glabridin.

Rat brain microvascular endothelial cells were pre-incubated for 2 h and co-incubated for 30 min with ATP and transporter inhibitors. Sodium azide (10 mM) increased the uptake of glabridin (0.1 and 1.0 µM) by 52.1 ± 5.8% and 57.3 ± 6.5% and 2,4-dinitrophenol (5 mM) increased uptake by 62.8 ± 7.8% and 64.5 ± 7.6%, respectively ($p < .05$). Verapamil (100 µM), nifedipine (100 µM), and quinidine (50 µM) increased the uptake of glabridin (0.1 µM) by 78.3 ± 8.9%, 87.2 ± 9.4%, and 91.4 ± 11.2%, respectively ($p < .05, .01$). Similar results were reported for glabridin at 1.0 µM. MK-571 (100µM) and probenecid (200 µM) increased glabridin uptake ($p < .05$). Celecoxib (100 µM) did not affect glabridin uptake. The authors concluded that glabridin uptake by these cells is ATP-dependent.

When testing the permeability of rat brain microvascular endothelial cells by glabridin, the authors reported that luminal to abluminal permeability of glabridin (0.10 to 100 µM) increased as did the abluminal to luminal permeabilty. The abluminal to luminal permeability was higher ($p < .05, .01$). There were no detectable metabolites in either direction. Permeability increased over time (60 min) in both directions; abluminal to luminal was greater than the opposite direction ($p < .05, .01$).

Rat brain microvascular endothelial cells were pre-incubated in sodium azide (10 mM), 2,4-dinitrophenol (5 mM), verapamil (100 µM), nifedipine (100 µM), quinidine (50 µM), probenecid (200 µM), MK-571 (100 µM) or celecoxib (100 µM) for 2 h. Glabridin (0.1 or 1.0 µM) was then added to either the luminal or abluminal side and incubated for an additional 30 min. All of the ATP and transporter inhibitors, except celecoxib, decreased luminal to abluminal permeability ($p < .05, .01$) and increased abluminal to luminal permeability ($p < .05, .01$) at both concentration of glabridin (Yu et al. 2007).

Glycyrrhizan GA

Shimizu et al. (1991) measured the effects on phagocytic activity of glycyrrhizan GA, as well as fractions from the extraction process, using a modified carbon clearance test. Five male mice (ICR-SPF) were administered glycyrrhizan GA (20 mg/kg i.p.) suspended in physiological saline daily for an unspecified period of time. The positive control was zymosan (an antigen that consists of a mixture of polysaccharides, proteins, and ash, of variable concentration, derived from the cell walls or the entire cell of yeast; 20 and 40 mg/kg). No further details of the experiment were provided. The phagocytic index was calculated. The values for and 5 fractions from the extraction process were greater than both the control doses ($p < 0.05, .01, .001$).

Isoliquiritigenin

Kim et al. (2006) investigated the cellular effects of isoliquiritigenin. When dimaprit (10 µM)-exposed U937 cells were incubated with isoliquiritigenin (10 µM), the histamine and cAMP induction by the dimaprit was inhibited in a concentration-dependent manner, similar to that of ranitidine. The IC_{50} was 2.3 ± 0.1 µM. The authors suggested that isoliquiritegenin inhibits histamine type 2 receptors (H2R)-mediated signals.

In the next experiment, the cells were exposed to forskolin (which directly activates adenylyl cyclase), isoproterenol (which activates Gs protein coupled β2-adrenoceptor), and cholera toxin (which evokes Gs protein activity). This exposure caused an increase in cAMP production. The pre-incubation of the cells with isoliquiritigenin did not effect the levels of cAMP. Based on these findings, the authors suggested that the site of inhibition is neither the adenylyl cyclase, Gs protein, or Gs protein-coupled β2-adrenoceptor indicating selective inhibition of H2R activation.

When histamine-trifluoromethyl toluidide derivative and (R)-α-methylhistamine were used as H1R- and H3R-selective agonists, respectively, isoliquiritgenin (10 µM) did not affect H1R-mediated calcium concentration nor H3R-mediated signaling. The authors also determined the ability of isoliquiritigenin to block histamine binding to H2R by testing its effects on [3H]tiotidine binding to H2R in undifferentiated HL-60 cells. Isoliquiritegenin blocked [3H]tiotidine binding in a concentration-dependent manner. Based on these findings, the authors suggested that isoliquiritigenin is a selective H2R antagonist that selectively and competitively inhibited histamine binding to the H2R.

MKN-45 gastric cancer cells, which express H2Rs and increased H2R expression with exposure to retinoic acid, were exposed to retinoic acid (5 µM). After exposure to isoliquiritigenin and dimaprit, cAMP production was inhibited. Isoliquiritigenin did not affect forskolin-induced adenylyl cyclase activation. Overall, these authors suggested that isoliquiritigenin has an inhibitory effect on H2Rs in gastric cells (Kim et al. 2006).

Kwon et al. (2007) tested several constituents of G.glabra for their effects on cell adhesion molecules (CAM). Western blot analysis of human umbilical vein endothelial cells (HUVEC) exposed to isoliquiritigenin, fomononetin, Glycyrrhizic Acid, or ononin was used to observe the effect of the licorice constituents on the TNF-α-activated expression of vascular cell adhesion molecule-1 (VCAM-1), endothelial leukocyte adhesion molecule-1 (E-selectin), and platelet endothelial cell adhesion molecule-1 (PECAM-1).

Unexposed control cells had weak expression of all 3 CAMs; TNF-α exposed cells prompted enhanced expression of the 3 CAMs. When treated with isoliquiritigenin, VCAM-1 and E-selectin expression was inhibited; the other constituents had no effect. Isoliquritigenin (1 to 50 µM) reduced VCAM-1 expression by TNF-α in a dose-dependent manner ($p < .05$). In a

second analysis, isoliquiritigenin- and TNF-α-exposed cells had inhibiton of PECM-1 expression but 18 β-Glycyrrhetinic Acid did not.

These authors also reported that HUVEC cells were cultured and plated at confluence. The cells were incubated with isoliquiritigenin, formononetin, Glycyrrhizic Acid, or ononin at ≥50 µM overnight and exposed to TNF-α activation (10 ng.nk) for 6 h. Cells from the human monocytic leukemic cells line THP-1 were labeled and added to the HUVEC cell culture. After incubation for 2 h. adhesion was measured.

Few monocytes adhered to unstimulated HUVEC free of TNF-α. There was increased adherence in the TNF-α-alone-exposed HUVED ($p < .05$). Treatment with TNF-α-exposed cells with isoliquiritigenin (50 µM) inhibited monocyte adherence in a dose-dependent manner. The other constituents had no effect.

Reverse transcriptase (RT)-PCR analysis showed that CAM mRNA was increased in TNF-α-stimulated HUVEC. The mRNA levels of VCAM-1 and E-selectin in isoliquiritigenin-treated cells were lower; the PECAM-1 mRNA level was also downregulated. The authors suggest that isoliquiritigenin inhibits expression by directly modulating gene transcription. Further RT-PCR analysis showed that isoliquiritigenin inhibits the CAM expression by blunting the degradation of IκB and translocation of NF-κB stimulated by TNF-α. The authors suggested that isoliquiritigenin has the potential capability to prevent the early events in atherosclerosis (Kwon et al. 2007).

Licochalcones

Dieck et al. (2005) tested the effects of licochalcone A (from G. inflata) on various human skin cells. Human neutrophils were stimulated with formyl-methionyl-eucyl-phenylalanine tripeptide (fMLP) or zymosan (antibody-coated yeast) to stimulate the production of ROS while exposed to various concentrations of G. inflata extract. ROS production was reduced by ~65% at ~7 ppm extract after exposure to fMLP and ~85% after exposure to zymosan when compared to controls. The reduction of reactive oxygen species (ROS) was in a dose-dependent manner.

PGE2 release was measured in full-thickness epidermal models after incubation with G. inflata extract containing 10 µg/ml licochalcone A, 50 ng/ml diclofenac (a cyclooxygenase inhibitor), or no additive. Some cultures were exposed to UVB (90 mg/cm2). UV-induced and basal PGE2 production were reduced in the presence of the extract by ~ 10% and ~65%, respectively.

Human dermal fibroblasts were incubated with various concentrations of licochalcone A- containing G. inflata extract or synthetic licochalcone A. The cells were stimulated with 25 ng/ml lipopolysaccharide and PGE2 was measured after 24 h. PGE2 production was reduced by both licochalcones in a dose-dependent manner. The IC_{50} for the extract was 283 ng/ml and 70 ng/ml for the synthetic licochalcone A.

Human neutrophils were incubated with various concentrations of G. inflata extract or licochalcone A then stimulated with fMLP. After 2 h, leukotriene B4 production was measured. Both the extract and licochalcone A had and IC_{50} of 200 ng/ml, similar to the IC_{50} for PGE2 release from LPS-stimulated fibroblasts.

In a final experiment, immature dendritic cells were incubated with various concentrations of G. inflata extract and stimulated with LPS (10 ng/ml). Both pro-inflammatory cytokines (IL-6 and TNFα) were suppressed in a dose-dependent manner. The authors noted that cytokine production can be reduced by the extract to the control, or below control, level of non-stimulated dendritic cells (Dieck et al. 2005).

Yoon et al. (2007) tested for cytotoxicity by licochalcone A and E, extracted from G. inflata roots, using the sulforhodamine B assay on A549 (lung), SK-OV-3 (ovarian), SK-MEL-2 (melanoma), and HCT-15 (colon) cell lines. After 24 h of incubation, the cells were further incubated in the test materials at various concentrations for an additional 48 h. The cells were fixed, stained and absorbance measured. The control was doxorubicin. Licochalcone A and E exhibited moderate cytotoxicity as shown in **Table 38.**

To test for the mechanism of the cytotoxicity, an assay for DNA topoisomerase I inhibition, which measured the relaxation of supercoiled DNA pBR322, was performed. The reaction mixture, calf thymus DNA topoisomerase I, and various concentrations of licochalcone A and E were incubated for 30 min. The reaction was terminated with a dye solution and the mixtures were subjected to electrophoresis for 1 h. The gel was stained and visualized under UV light and photographed. DNA relaxation induced by topoisomerase I was inhibited by both compounds in a concentration-dependent manner at concentrations of 6.25, 12.5, 25, 50, and 100 µg/ml (Yoon et al. 2007).

Table 38. Antiproliferative activities (IC_{50}) of licochalcones A and E (Yoon et al. 2007).

Treatment	Cell line			
	A549	SK-OV-3	SK-MEL-2	HCT-15
Licochalcone A (µg/ml)	4.83	4.56	2.68	3.43
Licochalcone E (µg/ml)	5.85	5.24	2.86	3.42
Doxorubicin (µg/ml)	0.24	0.26	0.24	0.02

Polyphenol Compounds

Fukai et al. (2000) tested for cytotoxicity of low molecular weight polyphenol compounds found in licorice against normal human gingival fibroblast (HGF) cells, tumor (human squamous cell carcinoma [HSC-2]) cells, and human salivary gland (HSG) tumor cells. The cells were incubated with and without the test compounds as given in **Table 39** for 4 h. The 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve.

These licorice derived compounds had higher cytotoxic activity against oral HSC-2 and HSG cell lines than against normal human gingival fibroblasts HGF. Glabridin's CC_{50} was < 13 µg/ml and licochalocone A was < 20 µg/ml against HSC-2 cells. Figures 3 - 7 show the structures of the compounds tested. **Table 39** lists all compounds tested, their source licorice species (when provided) and the results of the cytotoxicity tests (Fukai et al. 2000).

In the RAW 264.7 cells, there was 33% inhibition at 3 µM of glabridin and 67% inhibition at 10 µM. Again, there was no inhibition for the lower 2 concentrations. The presence of glabridin had no effect on the viability of isolated peritoneal macrophages and RAW 264.7 cells. It was observed that this inhibition is mediated, at least in part, by inhibition of gene expression of iNOS.

Monoamine Oxidase Effects

Various Compounds

Tanaka et al. (1987b) reported that isoliquiritigenin isolated from G. uralensis root inhibits rat liver mitochondrial monomine oxidase, with an IC_{50} of 2.09 mM and that liquiritigenin (also isolated from G. uralensis root) inhibits rat liver mitochondrial monomine oxidase with an IC_{50} of 17.3 mM.

Hatano et al. (1991) tested the inhibitory effects of licorice constituents on monomine oxidase from bovine plasma. IC_{50} ranged from $>2.0 \times 10^{-4}$ nM to 5.7×10^{-5} nM, which may be compared to 6.4×10^{-5} for quinine sulfate. Complete results are provided in **Table 40.**

Anti-Inflammatory Effects

Glabridin

Kang et al. (2005) measured nitrite accumulation for 24 h during LPS treatment to assess the blocking effect of glabridin on nitrous oxide (NO) production in LPS-stimulated isolated peritoneal macrophages and RAW 264.7 cells (a mouse macrophage-like cell line). Peritoneal macrophages were harvested from virus-free female BDF1 mice by sterile peritoneal lavage with PBS. The cells were washed and plated (5×10^6). RAW 264.7 cells (ATCC TIB71) were also grown and plated (5×10^5). The cells were stimulated with LPS (200 ng/ml from *Salmonella typhosa)* in the presence or absence of glabridin (0.3, 1, 3 or 10 µM) as well as grown without LPS or glabridin.

At the lower 2 concentrations of glabridin there was no difference in nitrite production in the peritoneal macrophage cells. At 3 µM there was 34% inhibition and at 10 µM there was 67% inhibition. In the RAW 264.7 cells, there was 33% inhibition at 3 µM of glabridin and 67% inhibition at 10 µM. Again, there was no inhibition for the lower 2 concentrations. The presence of glabridin had no effect on the viability of isolated peritoneal macrophages and RAW 264.7 cells. It was observed that this inhibition is mediated, at least in part, by inhibition of gene expression of inducible nitric-oxide synthase.

More mice were exposed to LPS and glabridin i.p. (n = 4). Whole blood samples were withdrawn from the mice and the plasma concentrations of nitrite/nitrate (NOx) and TNF-α were determined. Peritoneal macrophages were isolated 6 h after LPS treatment and cultured for 24 h. Culture supernatants were then isolated and analyzed for nitrite production. There was inhibition of plasma NOx at 3 and 10 mg/kg of glabridin, plasma TNF-α at 10 ng/ml and nitrite at 3 and 10 mg/kg of glabridin as shown in **Table 41.**

The authors suggested that glabridin inhibits NO production and iNOS (inducible nitric oxide synthase) gene expression by blocking activation, in part, by inhibiting reactive oxygen species generation. The authors also suggested that glabridin is a possible therapeutic agent in inflammatory disease (Kang et al. 2005).

Table 39. CC_{50} of polyphenols against HSC-2, HSG and normal HGF cells (Fukai et al. 2000).

	HSC-2		HSG		HGF	
Compound (source species)	**(µg/ml)**	**(µM)**	**(µg/ml)**	**(µM)**	**(µg/ml)**	**(µM)**
Glabrol (*G. glabra*)	9	23	9	23	19	49
Glabridin (*G. glabra*)	13	40	13	40	24	74
Licorisoflavan A (*G. uralensis*)	18	41	15	34	29	66
Wighteone (*G. glabra*)	20	59	13	39	43	130
Isoglycyrol (*G. uralensis*)	29	79	43	120	54	150
1-Methoxyficifolinol (*G. uralensis*)	5	12	4	9.5	21	50
Licochalcone B (*G. inflata*)	5	18	8	28	210	730

Table 40. The inhibitory effects (IC_{50}) of licorice constituents on MAO from bovine plasma (Hatano et al. 1991).

Licorice constituent/control	IC_{50} (nM)
Genistein	9.5×10^{-5}
Glicoricone	1.4×10^{-4}
Glycycoumarin	$>2.0 \times 10^{-4}$
Glycyrrhisoflavone	9.5×10^{-5}
Harmane hydrochloride	5.7×10^{-5}
Isoliquiritigenin	$>2.0 \times 10^{-4}$
Licochalcone a	$>2.0 \times 10^{-4}$
Licochalcone b	$>2.0 \times 10^{-4}$
Licocoumarone	6.0×10^{-5}
Licofuranone	8.7×10^{-5}
Licopyranocoumarin	1.41×10^{-4}
(–)-medicarpin	$>2.0 \times 10^{-4}$
Quinine Sulfate	6.4×10^{-5}

Glycyrrhizic Acid

CIR (2005) reported that oral administration of Glycyrrhizic Acid resulted in smaller ankle diameters of adrenalectomized rats injected with formaldehyde to induce arthritis.

Licochalcones A and B

Kimura et al. (1988) tested the effects of licochalcones A and B on leukotriene biosynthesis in human polymorphonuclear neutrophils (PMN) to explore possible inflammation treatments. The cells were incubated in the test substances at various concentrations for 5 min at 37°C then for 5 min more with [1-14C]AA and 2 M calcium ionophore A 23187. Calcium ionophore A 23187 induces arachidonate metabolism in human PMNs. The samples were prepared for thin-layer chromatography (TLC), GC-MS, and HPLC. Licochalcone A and B, extracted from G. inflata, inhibited calcium ionophore A 23187-induced leukotriene B4 and C4 formation in human PMNs at concentration of 10-3 to 10-6.

The authors also incubated human PMNs with the test compounds at various concentrations for 5 min at 37°C then for 5 more min in 2 M A 23187.

The reactions were stopped, the samples were centrifuged, and the supernatant analyzed for leukotriene B4 (LTB4) biosynthesis. Licochalcones A and B completely inhibited A 23187- induced leukotriene B4 biosynthesis. For licochalcone A, the IC_{50} of LTB4 and LTC4 formation was 4.6 x 10-7 and 4.2 x 10-6 M, respectively. For licochalcone B, the IC_{50} was 1.2 x 10-6 and 2.0 x 10-6 M, respectively.

Human PMNs were again incubated in various concentrations of the test substances for 2 min to test for effects on cAMP production. The reaction was stopped and the samples centrifuged. The supernatant was tested for cAMP. Licochalcones A and B slightly increased the cAMP levels at concentrations from 10-4 to 10-3 M.

Human PMNs were incubated with various concentrations of the test substances after the loading of fura 2-acetoxymethylester, a calcium indicator, for 20 min at 35°C in the presence of 1 mM calcium chloride ($CaCl_2$) or buffer. The samples were washed and a fluorimeter was used to measure fluorescence. Licochlacones A and B reduced the elevation of cytosolic free calcium concentration induced by A 23187 dose-dependently.

The authors suggested that the possible mechanism of the inhibitory effects of licochalcones A and B on A 23187-induced leukotriene biosynthesis may be the inhibition of Ca^{2+}-influx into the cells and Ca^{2+} mobilization from the intracellular pools. This and the effects on cAMP may be related to these compounds' therapeutic effects on inflammation and allergic conditions (Kimura et al. 1988).

Shibata et al. (1991) tested the effects of licochalcone A, extracted from a G. inflata root, on the healing of damaged skin. Licochalcone A was extracted from the root with EtOAc and chromatogrphy and purified with HPLC. The ears of 6-week old mice were either treated with various amounts of licohalcone A 30 min before treatment with AA (2 mg/ear) or 30 min after treatment with 12-o-tetradecanoylphorbol 13-acetate (TPA, 2 g/ear). Ear thickness was determined 1 h after AA treatment and 5 h after TPA treatment. The control was the vehicle, acetone. Licochalcone A inhibited both TPA- and AA-induced edema in a dose dependent manner as shown in **Table 42**

Table 41. Effect of in vivo exposure of glabridin on the plasma levels of NO_x and TNF-∝ and the nitrite production by peritoneal macrophages (Kang et al. 2005).

Treatment	Plasma NO_x (μM)	Plasma TNF-∝ (ng/ml)	Nitrite (nmol/10^6 cells)
Vehicle	0.38 ± 0.13	0.11 ± 0.02	1.13 ± 0.52
LPS (200 μg/kg)	6.03 ± 0.66	1.60 ± 0.39	31.87 ± 1.02
LPS (200 μg/kg) + glabridin (1 mg/kg)	5.69 ± 1.09	1.23 ± 0.44	30.25 ± 3.03
LPS (200 μg/kg) + glabridin (3 mg/kg)	3.60 ± 0.49*	1.08 ± 0.06	109.33 ± 2.35*
LPS (200 μg/kg) + glabridin (10 mg/kg)	1.51 ± 0.67*	0.73 ± 0.09*	4.37 ± 0.81*

* $p < .05$

Table 42 Inhibitory effects of licochalcone A, derived from *G. inflata* root, on TPA- and AA induced mouse ear edema (Shibata et al. 1991).

Licochalcone A topical dose (mg/ear)	Increase in ear thickness ($x10^{-2}$ mm)	
	TPA-induced edema	AA-induced edema
Control	36.0 ± 0.4	32.5 ± 1.5
0.1	Not tested	27.7 ± 3.1
0.5	23.7 ± 3.8[a]	25.2 ± 1.8[b]
1.0	13.0 ± 1.8[c]	18.3 ± 2.6[b]
3.0	12.8 ± 1.7[c]	Not tested

[a] $p < .05$; [b] $p < .01$; [c] $p < .001$

Various Constituents

Kobayashi et al. (1995) investigated the effects of G. uralensis extract, glycyrrhizin, glycyrrhitinic acid and the flavonoids isoliquiritin, isoliquiritin apioside, isoliquiritigenin, and liquiritigenin on granuloma angiogenesis by measuring carmine content in newly formed blood vessels. Male ddY mice (6 to 7 wk old) were injected with 3 ml air s.c. into the dorsum under anesthesia to produce a regular ellipsoid air sac. FCA emulsion (with 0.1% croton oil and 2 mg heat-killed Mycobacterium tuberculosis; 0.5 ml) was injected into the air pouch under anesthesia 24 h later. Two h later, isoliquiritin (n = 22), glycyrrhizin (n = 23) or licorice extract (n = 21) was injected i.p. daily for 4 d. The mice were injected with 10% carmine solution containing 5% gelatin into the tail vein on day 5 then killed. The weights of granuloma tissues and pouch fluid isolated from the pouch were measured. Optical density was used to determine the carmine content; the carmine content is an index of newly formed blood vessels in the pouch granuloma.

Isoliquiritin (0.31 to 1.3 mg/kg) inhibited carmine content in granuloma tissues in a dose-dependent manner; the inhibitory dose (ID_{50}) was 1.46 mg/kg (0.824 - 2.58 95% confidence interval). Isoliquiritin inhibited granuloma weight by <50% of controls. Isoliquiritin inhibited the weight of pouch fluid and had an ID_{50} of 0.771 mg/kg (0.513 - 1.18). The control values for carmine content, granuloma weight, and pouch fluid weight were 0.256 ± 0.017 mg, 353 ± 15 mg, and 144 ± 26 mg, respectively. The authors stated that these results show the potency ratio of isoliquiritin to licorice extract was greater than fluid exudation for angiogenesis.

To test the effect of licorice constituents on angiogenisis by measuring tube formation, the authors also cloned endothelial cells (EC) from the thoracic aorta of male Wistar rats in DMEM supplemented with 10% heat-inactivated FBS, benzyl penicillin potassium and streptomycin sulfate. Two to 11 wk after confluence (5th to 14th passage) the cells were washed and replated (2.6 x 104 cells/well). The DMEM was supplemented with various concentrations of isoliquiritin apioside, liquiritigenin, related to these compounds' therapeutic effects on isoliquiritinigenin, or isoliquiritin except for controls. The cells were cultured for 4 d with the medium changed every other day. Tube formation was measured and total tube length was quantified. The experiment was repeated 3 times.

Isoliquiritin (1 to 100 µM) inhibited tube formation in a concentration-dependent manner; the IC_{50} was 28.3 µM (20 to 40). Isoliquiritigenin (0.1 to 10 µM) and liquiritigenin (0.1 to 100 µM) inhibited tube formation in a concentration-dependent manner; their IC_{50} values were 7.39 µM (4.78 to 11.4) and 39.2 µM (17.1 to 90.1), respectively. Isoliquiritigenin apioside had no effect on tube formation. Glycyrrhizin and glycyrrhetinic acid (1 to 10 µM) increased tube formation in a concentration-dependent manner; glycyrrhetinic acid was 10-fold more potent than glycyrrhizin.

The previous experiment was repeated with isoliquiritin and glycyrrhizin in various combinations. The tube formation promotion of glycyrrhizin (82 µg/ml) was inhibited by isoliquiritin (0.42 to 42 µg/ml) in a concentration-dependent manner. The resulting tube formation at a isoliquiritin/glycyrrhizin ratio of 0.05:1 was similar to licorice extract alone (100 µg/ml) in which their yield ratio is estimated to be similar to their mixture. The authors concluded that the anti-angiogenic effect of licorice extract could be attributed to the anti-tube formation properties of isoliquititin (Kobayashi et al. 1995).

Kim et al. (2008) tested isoliquiritin and isoliquiritigenin, extracted from G. uralensis, for anti-inflammatory effects using RAW 264.7 macrophages (n = 3). The macrophages were preincubated in isoliquiritin or isoliquiritigenin (0.4, 0.8, or 1.6 µM) or with l-N6-(1-iminoethl)lysine or NS-389 (positive controls) then stimulated with LPS (1µg/ml).

Isoliquiritin and isoliquiritigenin inhibited LPS-induced NO and PGE2 production in a concentration-dependent manner. Isosquiritigenin ($p < .05, .01, .01$ for 0.4, 0.8 and 1.6 µM, respectively) was more effective than isoliquiritin ($p < .05$ for 1.6 µM). Treatment with isoliquiritin and isoliquiritigenin at 1.6 µM reduced LPS-induced PGE2 production by 86% and 49%, respectively. Neither substance affected viability of the cells.

Further testing using Western blotting and RT-PCR determined that preincubation with isoliquiritigenin inhibited the upregulated iNOS and COX-2 proteins by LPS in a concentration-dependent manner. Isoliquiritigenin had no effect on the expression of the

house-keeping gene β-actin. Preincubation of RAW264.7 macrophages with isoliquiritigenin reduced the production of TNF-α and IL-6 in a concentration-dependent manner; changes in TNF-α and IL-6 production were similar to the observed reduction in their RNA expressions.

NF-κB activation, critical for the expression of iNOS, COX-2, TNF-α, and IL-6 by LPS, was measured with and without exposure of nuclear extracts obtained from the cells to isoliquiritigenin using a DNA binding assay of NF-κB. Treatment with LPS (1 µg/ml) increased NF-κB-DNA binding; pretreatment with isliquiritigenin decreased NF-κB-DNA binding in a concentration-dependent manner. RAW 264.7 cells, transiently transfected with pNF-κB-Luc plasmid and stimulated with LPS (with or without pretreatment with isoliquiritigenin (0.4, 0.8, or 1.6 µM) reduced LPS-induced increases in NF-κB-dependent luciferase activity ($p < .05$ and .01 for 0.8 and 1.6 µM, respectively). Pretreatment with isoliquiritigenin prior to LPS exposure attenuated the translocation of the levels of subunits of NF-κB, P65 and p50, nuclear fractions. LPS-induced IκB-α degradation was blocked and LPS-induced IκB-α phosphorylation was reduced in a concentration-dependent manner by isoliquiritigenin pretreatment.

Isoliquiritigenin reduced LPS-induced phosphorylation of ikappa-α/β kinases (IKK) of IκB in the NF-κB signal pathway; isoliquiritigenin did not affect the total amounts of IKK-α and IKK-β. Isoliquiritigenin suppressed the LPS-induced activations of IRD1/2 and p38 MAP kinases in RAW 264.7 cells but did not affect the phosphorylation of JNK1/2. Non-phosphorylated ERK, JNK, and p38 kinase expression were unaffected by LPS or by LPS plus isoliquiritigenin.

The authors concluded that isoliquiritigenin inhibits LPS-induced iNOS and COX-2 protein expressions and iNOS, COX-2, TNF-α, and IL-6 transcriptions by inhibiting the degratdation and phosphorylation fo Iκ-α, and by blocking the activation of NF-κB in RAW 264.7 macrophages. The authors suggested that the anti-inflammatory effects of isoliquiritigenin may be a treatment for inflammatory diseases (Kim et al. 2008).

Anti-gout Effects

Various Compounds

Hatano et al. (1989) isolated the licorice constituents shown in Table 43. Each constituent was tested for xanthine oxidase inhibition. A solution of xanthine oxidase (from cow's milk) was added to a solution of test compound in water/DMSO (8:2 v:v; 360 µl) and preincubated for 10 min at 37°C. An aqueous solution of xanthine was added to the mixture and the resulting solution was incubated for 30 min. The enzyme reaction was stopped and the absorbance was measured. The blank was prepared the same way except the solution of xanthine oxidase was added after stopping" the reaction. Each compound was run in triplicate.

Licochalcone B and A, for example, inhibited xanthine oxidase at 3.0×10^{-5} M and at 5.6×10^{-5} M, respectively. Complete results for all of the isolated constituents are given in **Table 43**.

The authors concluded that these licorice constituents inhibited oxidative stress (Hatano et al. 1989).

Anti-diabetic Effects

Glycyrrhizic Acid

In an earlier safety assessment (CIR 2005), Glycyyrrhizic Acid was reported to have an anti-diabetic effect in genetically diabetic mice; i.e., the mice had lower blood glucose levels after oral doses of glucose.

Glycyrin

Kuroda et al. (2003) tested the ameliorative effects of glycyrin (fractioned from the EtOAc extract of *G. uralensis* root) on genetically diabetic KK-Ay mice. The mice were housed and fed and watered ad libitum for 7 wk. At 15 wk of age, the blood glucose levels were > 470 mg/dl. The mice were divided into 3 groups (n = 5). The control group was fed a commercial diet. The treatment groups were fed the same diet containing 0.1% glycyrin (w/w), 0.1% glycyrol (w/w; also isolated from G. uralensis root), or 0.02% pioglitazone (w/w; a potent synthetic PPAR-γ angonist). Diet and water were available ad libitum for 10 d. Blood was sampled from tail veins on day 4 and glucose concentrations measured. On day 10 the mice were fasted overnight and administered 0.5% CMC-Na (w/v), glycyrin (100 mg/kg), or pioglitazone (20 mg/kg) orally. After 30 min, 40% sucrose solution was orally administered at 2 g/kg. Blood glucose levels were measured at 30, 60, and 120 min.

It was calculated that the mice consumed ~100 mg/kg/d glycyrin or glycyrol for those groups and 23 mg/kg/d pioglitazone for the pioglitazone group. There were no differences in feed intake or body weight between the groups.

Table 43. Inhibitory effects of licorice phenolics on xanthine oxidase, a gout inducer (Hatano et al. 1989).

Compound	IC_{50} (M)
Licochalcone B	3.0×10^{-5}
Licochalcone A	5.6×10^{-5}
Glycyrrhisoflavone	5.3×10^{-5}
Glisoflavone	$>1.0 \times 10^{-4}$
Glycyrrhisoflavanone	$>1.0 \times 10^{-4}$
Licopyranocoumarin	$>1.0 \times 10^{-4}$
Licoarylcoumarin	$>1.0 \times 10^{-4}$
Glycyroumarin	$>1.0 \times 10^{-4}$
Licocoumarone	1.3×10^{-5}
Kaempferol 3-*O*-methyl ether	$>1.0 \times 10^{-4}$
Allopurinol	1.6×10^{-7}

After 4 d, blood glucose levels decreased in both the glycyrin and pioglitazone groups compared to controls, but not in the glycyrol group. In the oral glucose tolerance test, glycyrin and pioglitazone suppressed the usual increase in blood glucose levels at 30 and 60 min ($p < .01$); glycyrol did not (Kuroda et al. 2003).

Isoliquiritigeinin and Related Compounds

Aida et al. (1990) isolated compounds (echinatin, narigenin, GU-12, licoricone, glycyrin, isoliquiritigenin) from G. uralensis root extract to test for aldose reductase inhibition qualitites. In the first experiment, rat lenses were homogenized and centrifuged; the supernatant was used as an enzyme fraction. Assays conducted on the isolates and {(E)-3-carboxymethyl-5-[(2E)-methyl-3-phenyl-propenylidene]}-rhodanine (ONO-2235; an aldose reductase inhibitor) used dl-glyceraldehyde (0.1 mM) or glucose (20 mM) as substrates to determine the increase of fluorescence of NADP in the presence of 6 N NaOH containing 10 mM imidazole at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Blanks contained all reagents except a substrate. Isoliquiritgenin was the only isolate to have any effect. Isoliquiritigenin inhibited rat lens aldose reductase in a concentration dependent manner. The IC_{50} was 3.2 x 10-7 M for dl-glyceraldehyde as a substrate and 1.6 x 10-6 M for glucose and 0.9 x 10-8 and 6.5 x 10-8, respectively, for ONO-2235. According to these authors, analysis of the kinetic effects of isoliquiritigenin resulted in parallel slopes on Lineweaver-Burk plots, characteristic of non-competitive inhibition; ONO-2235 had a mixed type of uncompetitive and non-competitive inhibition.

The authors then employed human RBC from healthy subjects to test for the accumulation of sorbitol, which accumulates in the non-insulin dependent tissues in diabetic humans. RBCs were incubated for 60 min in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 28 mM glucose with or without various concentrations of isoliquiritigenin or ONO-2235. The RBCs were washed with cold saline, treated with 6% cold perchloric acid and centrifuged to deproteinize. The supernatant was neutralized and the sorbitol content assayed enzymatically using sorbitol dehydrogenase. The IC_{50} of isoliquiritigenin for sorbitol accumulation was 2.0 x 10-6 M and 6.2 x 10-6 M for ONO-2235.

In a third experiment, the authors induced diabetes in male Wistar rats (220 to 250 g) by a single ip injection of STZ (80 mg/kg) in sodium citrate buffer. Control rats ($n = 6$) were injected with vehicle only. The animals were fed standard diet and water ad libitum. Two weeks after the injection, the rats were administered isoliquiritigenin, ONO-2235, or vehicle (saline) alone by gavage twice/d (100 mg/kg/d) for 2 wk ($n = 7$). The rats were killed and the sorbitol content of the rat RBC, sciatic nerves, and lenses were determined enzymatically.

There was no difference in body weights or blood glucose levels between the treated rats and the diabetic control. In the diabetic control rats, the sorbitol content of the RBC (161.8 ± 5.4 nmol/g Hb) was higher than in normal rats (75.2 ± 4.2 nmol/g hg, $p < .01$). In diabetic rats treated with isoliquiritigenin or ONO-2235, accumulation of sorbitol in the RBC was inhibited by 44.3% ($p < .01$) and 54.5% ($p < .01$), respectively. The sorbitol accumulation in the sciatic nerves increased to 0.47 nmol/mg wet wt from 0.14 nmol/mg wet wt. ($p < .01$). ONO-2235 inhibited sorbitol accumulation in diabetic sciatic nerves by 12.1% (not significant [ns]) and isoliqiritigenin lowered the sorbitol content by 63.6% ($p < .01$). Isoliquiritigenin and ONO-2235 reduced the sorbitol content by 13.7% and 15.6%, respectively. The authors concluded that isoliquiritigenin may be effective in preventing diabetic complications (Aida et al. 1990).

Antioxidant Effects

Various Compounds

Demizu et al. (1988) tested the antioxidant properties of glycycoumarin and licocoumarone extracted from licorice by dissolving a sample of each in a minimal amount of ethanol mixed with 18 g of lard at the concentration of 100 ppm then placed in a fat stability test apparatus. One g of mixed lard was taken at time intervals and mixed with saturated potassium iodine solution to quench the peroxide formed. Generated iodine was titrated with 0.01 n sodium thiosulfate. Glycycoumarin was found to have some antioxidative properties while licocoumarone was found to have little to none.

Okada et al. (1989) tested for the antioxidant properties of glabridin and glabrene from G. glabra and licochalcones A and B from G. inflata by the active oxygen method. Both licochalcones A and B exhibited potent antioxidant activity comparable to that of vitamin E. Glabrene showed the most antioxidant activity among the compounds tested, being 3 times as potent as vitamin E, while glabridin exhibited no significant activity in spite of its structural similarity to glabrene. The authors noted that since the content of glabridin, glabrene and licochalcones A and B in each licorice plant were estimated at 9.1%, 2.8%, 18.2%, and 2.7%, respectively, by HPLC, these compounds should account for much of the antioxidant activities of licorice extracts.

Vaya et al. (1997) evaluated the antioxidation properties of 7 extracted compounds of G. glabra root powder (hispaglabridin A, 4'-O-methylglabridin, glabridin, hispaglabridin B, isoprenylchalcone, isoliquiritigenin, and fomononetin) by measuring the oxidation of β-carotene. The test concentrations of linoleic acid and β-carotene were 1.4 and 5.6 x 10-3 mM, respectively. Each of the licorice extracts and vitamin E were tested at 50 µM. Whole Licorice Extract (16 mg/l, equivalent to the molar concentration of glabridin) was also tested. The control/blank was 20 mg linoleic acid, 200 mg Tween 20 and 50 ml distilled water.

The highest antioxidative effect was obtained with vitamin E, which completely inhibited β-carotene oxidation. The relative inhibition of of β-carotene oxidation after 90 min by glabridin, hispaglabridin A and hispaglabridin B were 93%, 93% and 91%, respectively. At the same time of incubation, Licorice Extract inhibited β-carotene consumption by 87%, 4'-O-methylglabridin by 78%, and isoprenylchalcone derivative by 57%. Isoliquiritigenin and formononetin were less effective at 26% and

14%, respectively. Oxidation inhibition decreased over the remaining time for all test compounds except vitamin E.

The authors used human plasma derived LDL to test the protection of LDL against lipid peroxidation by these licorice compounds. Oxidation of LDL at 37°C was performed. LDL was incubated for 2 h with 5mM of a water soluble azo compound that thermally decomposes to produce peroxyl radicals at a constant rate. The licorice compound (30 M), Licorice Extract (10 µM), and vitamin E as the control (30 µM; n = 3in all cases) were examined. LDL oxidation was determined by measuring TBARS (532 nm vs. water blank) and the amount of lipid peroxides with a commercially available kit (cholesterol color reagent, CHOD iodide; 365 nm vs color reagent as a blank).

All the licorice compounds except formononetin protected LDL from oxidation (65 - 85%). Glabridin was the most effective at 85.5% by TBARS and 75.3% by measuring LPO. The licorice extract was less active (32% and 41% inhibition). Vitamin E had no antioxidative effect in this assay.

The researchers tested the effect of glabridin on cholesteryl linoleate hydroperoxide (CLOOH) formation in LDL during oxidation and on cholesteryl linoleate (CL) consumption by HPLC.

The inhibition of CLOOH formation and CL consumption was concentration dependent for glabridin. Using 5 or 10 µM of glabridin, 62% or 75% inhibition in LDL-associated CLOOH formation was obtained, respectively. On increasing the concentration of glabridin to 40 and 60 µM, CLOOH formation was inhibited by 90%. After the oxidation of LDL without glabridin, about 57% of the initial concentration of CL was consumed. Using 5 or 10 µM glabridin, 64% or 78% inhibition in LDL-associated CL consumption was obtained, respectively. Maximum inhibition of CL consumption was obtained at 20 µM glabridin (Vaya et al. 1997).

Fuhrman et al. (1997) isolated glabridin from the alcohol extract of G. glabra root. LDL (200 mg protein/l) was incubated with 1 mmol AAPH/l in the presence of 3 mg glabridin/l (9.2 µmol/l). There was a 100-min prolongation of the lag phase and an 87% and 70% reduction in the formation of TBARS and lipid peroxides in LDL, respectively.

The experiment was repeated with E0 mice; their LDL is highly susceptible to oxidation. The mice were divided into 3 groups (n = 20): group 1 received a placebo (2% alcohol in water; control), group 2 received 200 µg extract/d in 2% alcoholized water, and group 3 received 20 µg purified glabridin/d in 2% alcoholized water. Blood was drawn and LDL isolated before and after 6 wk of treatment. The E0 mice were killed and the heart and entire aorta were rapidly removed and fixed.

Licorice and glabridin treated mice had decreased susceptibility to the LDL to copper ion-induced oxidation by 68% and 22%, respectively, as measured by TBARS formation. Of the placebo-treated mice, 6 of 10 had well-defined atherosclerotic lesions including 3 grade 2 lesions (small lesions only, areas < 2500 µm2), 2 grade 3 lesions (larger, more extensive lesions with well-defined fatty streaks and disruption of elastic lamina, areas ≤ 20,000 m2) and 1 advanced grade 4 lesion (areas ≤ 50,000 m2). Only 2 of 10 licorice extract-treated mice had atherosclerotic lesion of the aortic arch at grade 3. The remaining 8 mice had grade 1 lesions (Fuhrman et al. 1997).

Belinky et al. (1998a) tested the effects of glabridin, isolated from the acetone extract of G. glabra roots, on LDL during its oxidation. E0 mice (4 wk old) were administered alcoholized water (1.1% alcohol; control); glabridin (50 µg/day) in alcoholized water; quercetin (50 µg/d) in alcoholized water; or catechin (50 µg/d) in alcoholized water in their drinking water for 6 wk. Blood was collected after 2 and 6 wk. LDL (100 µg protein/ml) was isolated and incubated with the free radical initiator AAPH (5 mM) or copper ions (10µM) for 2 h. Formation of TBARS was measured.

Glabridin or quercetin consumption resulted in a 95% and 83% reduction in AAPH-induced oxidation, respectively, and a 53% and 54% reduction in copper ion reduction oxidation, respectively.

LDL (750 µg protein/ml) was incubated with glabridin (0 to 60 µM) for 3 h at 37°C in the presence of 5 mM AAPH or 10 M CuSO4. Oxidation of LDL was determined by measuring LDL associated with TBARS, lipid peroxides, and CLOOH.

With AAPH, glabridin inhibited oxidation of LDL by all 3 parameters measured at all concentrations. Glabridin at 20 µM inhibited TBARS, lipid peroxides, and CLOOH formation in LDL by 73%, 66%, and 82%, respectively. The amount of CLOOH formed by LDL oxidation with AAPH (control) was 85% of the total lipid peroxides formed. When 10 µM glabridin was added only 62% of the lipid peroxides produced during AAPH-induced LDL oxidation originated from CLOOH. At increasing concentrations of glabridin (40 to 60 µM) less CLOOH was produced, and at 60 µM, oxidation was only 24% of the total peroxides originated from CLOOH.

The effects of licorice extract (13 µg/ml) and vitamin E (40 µM) on the AAPH-induced LDL oxidation were also tested and compared to glabridin. Licorice extract inhibited LDL associated TBARS, lipid peroxide, and CLOOH formation by 96%, 65%, and 92%, respectively, and vitamin E by 83%, 61%, and 70%, respectively.

Glabridin (20 µM) inhibited the formation of TBARS and lipid peroxides with copper ions by 43% and 34%, respectively, but no inhibition of CLOOH was observed. An increase of 125% in the formation of CLOOH over CLOOH formed by LDL oxidation in the absence of glabridin was observed. Glabridin (5 to 10 µM) increased lipid peroxide formation by up to 16%; at 40 µM glabridin, TBARS, lipid peroxide, and CLOOH formation were inhibited by 85%, 92%, and 71%, respectively. At 40 to 60 µM, glabridin inhibited the formation of LDL associated lipid hydroperoxides induced by copper ions, 4-fold lower than that observed for AAPH-induced LDL oxidation.

When licorice extract (13 µg/ml) was added to the lipoprotein, LDL was inhibited, similar to that caused by glabridin. The reduction of TBARS, lipid peroxides, and CLOOH formation was 90%, 97%, and 68%, respectively.

To test the chelating capacity of glabridin, a solution of glabridin (50 µM) in PBS was scanned with and without CuSO4 or iron sulfate (50 µM) in a diode array spectrophotometer. The spectra were compared. Glabridin's maximum absorption was 280 nm. Adding copper or ferrous ions did not change the absorption for up to 18 h.

The effect of glabridin on the formation of oxidized sterols during AAPH- and copper ion-induced LDL oxidation was tested. After 6 h of incubation of LDL with AAPH, the amount of total LDL cholesterol decreased from 3100 nmol/mg to 2880 nmol/ml LDL protein, and to 2200 nmol/ml after 24 h. Glabridin (30 µM) inhibited the production of 7-hydroxycholesterol, 7-ketocholesterol, and 5,6α-epoxycholesterol by 55%, 80%, and 40%, respectively, and 46%, 42%, and 29% after 24 h, respectively. After 6 h of incubation with copper ions, total LDL cholesterol decreased to 2766 nmol/mg LDL protein and 1543 nmol/mg after 24 h. Glabridin (30 µM) inhibited the production of 7-hydroxycholesterol, 7-ketocholesterol, and 5,6α-epoxycholesterol by 73%, 94%, and 52%, respectively, and 32%, 51%, and 32% after 24 h incubation, respectively.

The authors concluded that the reduction of LDL oxidation obtained with the exposure to glabridin may be related to the absorption or binding of glabridin to the LDL particle and subsequent protection from oxidation by inhibiting the formation of lipid peroxides and oxysterols also by protecting LDL associated carotenoids (Belinky et al 1998a).

Belinky et al. (1998b) isolated glabridin, hispaglabridin A and B, and 2',4'-O-dimethylglabridin from the acetone extract of G. glabra roots and tested their effects on LDL oxidation. These compounds have similar structures and by comparing them an evaluation can be made of the contribution of the different parts of the isoflavan molecule to its antioxidant activity. Oxidation of LDL (100 mg protein/l) was carried out in a shaking water bath at 37° in the absence or presence of 1 of the 3 compounds (at various concentrations) with copper ions (10 M) for 3 h. TBARS and total lipids were measured with commercial kits.

The lag phase for the initiation of LDL oxidation was ~90 min and the slope of the propagation phase was 3.0. Glabridin inhibited the formation of conjugated dienes, and the lag phase of LDL oxidation was retarded by ~165 min. The slope of the propagation phase with glabridin was 1.3. This effect was similar to that of vitamin E at the same concentration (5 µM). Hispaglabridin A and B retarded the lag phase of LDL oxidation by ~ 160 and ~ 110 min, respectively. Hispaglabridin A inhibited the formation of conjugated dienes in the propagation phase (curve slope = 2.5) less than glabridin. Hispaglabridin B was less active against LDL oxidation (curve slope = 2.2). Since none of the isoflavan derivatives nor the isoflavene compound chelated iron or copper ions, the authors suggested that the antioxidant properties of glabridin on LDL oxidation may reside mainly in the 2' hydroxyl group and that the hydrophobic moiety of the isoflavan is essential. The authors stated that the position of the hydroxyl group on the B ring affects the inhibitory efficiency of the isoflavan derivatives on LDL oxidation but not their ability to donate an electron to DPPH (Belinky et al. 1998b).

Fuhrman et al. (2000) tested the synergistic antioxidative effect of glabridin and lycopene on LDL. The authors found that lycopene (5 µmol/l) alone inhibited LDL oxidation, measured as TBARS and as lipid peroxides formation, by 5% and 9%, respectively, by carrying out LDL oxidation in a shaking water bath or by the lipid peroxides assay. The addition of glabridin (1 µmol/l) exceeded the calculated additive effect of inhibition by 32%. The IC_{50} of glabridin against the copper ion-induced LDL oxidation measured in TBARS formation was 1.8 µmol/l; a maximal inhibition of 94% was measured with the addition of 6.75 µmol/l for glabridin. Lycopene (5 µmol/l) alone inhibited the AAPH-induced LDL oxidation by 9%; the addition of glabridin (1 µmol/l) exceeded the expected additive inhibition by 72%. β-Carotene (5 µmol/l) alone inhibited copper ion-induced LDL oxidation by 4.5%; the addition of glabridin (1 µmol/l) exceeded the expected additive inhibition by 9%. The calculated degree of synergism in the inhibition of LDL oxidation by β-carotene in combination with glabridin was 1.16.

Haraguchi et al. (2000) found that isoflavans isolated from G. glabra by alcohol extraction were potent inhibitors against mitochondrial lipid peroxidation induced by Fe3+-ADP/NADH. The IC_{50} (concentration required to inhibit lipid peroxidation by 50%) for glabridin was 23.4 µM. The IC_{50} values for the other isoflavans were 4.9 µM for hispaglabrindin A, 19.8 µM for hispaglabridin B, 22.4 µM for 4'-O-methylglabridin and 0.1µM for 3'-hydroxy-4'-O-methylglabridin. These isoflavans are shown in Figure 9.

Fuhrman et al. (2002a) tested the effects of glabridin, isolated from G. glabra, on cellular oxidative stress in E0 mice. Three groups of 10 2-month old mice were administered water (control), 50 mg vitamin E/d or 25 µg purified glabridin/d through their drinking water. The experiment lasted 2 months. MPM were harvested from the peritoneal fluid 4 d after i.p. injection of 3 ml thioglycolate into each mouse. The macrophage, vitamin E, or glabridin content were determined in cell lipid extract by HPLC.

The levels of vitamin E and glabridin in the harvested MPM increased 10- and 34-fold over the control, respectively. The effect was accompanied by a 31% and 53% reduction in the cellular lipid peroxide content, respectively, in comparison to control Eo mice. In parallel, macrophage uptake of oxidized low-density lipoprotein (Ox-LDL) measured as Ox-LDL degradation decreased by 26%, when measured by cell-association, it decreased by 56% compared to control in macrophages derived after vitamin E consumption. Macrophage uptake of Ox-LDL and cell association was reduced by 25% and 27%, respectively, after consumption of glabridin, compared to controls. The binding of Ox-LDL to MPM harvested from Eo mice after consumption of vitamin E or glabridin decreased by 36% (0.28 ± 0.02 µg of Ox-

LDL protein/mg from 0.44 ± 0.03 µg of Ox-LDL protein/mg for the control) and 27% (0.32 ± 0.02 µg of Ox-LDL protein/mg), respectively. The authors stated that administration of dietary antioxidants (vitamin E and glabridin) to E0 mice resulted in reduced cellular uptake of Ox-LDL and may be related to the inhibition of cellular lipid peroxide formation (Fuhrman et al. 2002a).

Rosenblat et al. (2002) found that male E0 mice administered 25 µg/kg/d glabridin in their drinking water (n = 30) for 2 months accumulated glabridin in their peritoneal macrophages and increased MPM GSH content by 80%. MPM lipid peroxide content was reduced by 60% and was accompanied by a 60% decrease in cell-mediated oxidation of LDL.

Georgetti et al. (2003) found that glabridin (in the form of Aqua Licorice extract PT) showed a large chemiluminescence inhibition at time 0. The authors state that this result suggests that the extract may inhibit luminol radical formation by HRP enzyme inhibiton, by H_2O_2 scavenging or by reducing the oxidized HRP enzyme rather than luminol. The antioxidant activity was confirmed by the DPPH free radical assay and iron-induced lipid peroxidation.

Fukai et al. (2003a) mixed 5 mM sodium ascorbate with the same volume of glabridin at various concentrations (0.002 to 10 mg/ml) in DMSO and 0.2 M Tris-HCl buffer at pH 8.0. Artonin E was used as the control. The intensity of ascorbate radical was measured after 1 min. Glabridin from G. glabra had no effect on the radical intensity of sodium ascorbate.

Chin et al. (2007) tested the antioxidant activities of various compounds extracted from G. glabra roots by monitoring the oxidation of non-fluorescent dihydrorhodamine 123 to highly fluorescent rhodamine 123. A compound was considered anti-oxidative if the IC_{50} was < 20 µM. As shown in **Table 44**, IC_{50} values ranged from 2.3 to >20 for the extract components, compared to a value of 3.1 for DL-penicillamine.

Table 44. Anti-oxidant activities of compounds isolated from *G. glabra* roots (Chin et al. 2007).

Compound	IC_{50} (mg/ml)
1,2-Dihydroparatocarpin A	19.8
Neolignan lipid esters	>20
Formononetin	12.4
Glabridin	12.9
Hemileiocarpin	19.7
Hispaglabrin B	3.2
Isoliquiritigenin	9.3
4'-*O*-Methylglabridin	18.5
Paratocarpin B	2.3
DL-penicillamine (control)	3.1

Račková et al. (2007) tested the anti-inflammatory/anti-oxidation effects of glycyrrhizin extracted from G. glabra. In a DPPH assay, the extract, dissolved in ethanol (0.001 to 0.06 mg/ml) was added to an ethanol solution of DPPH (54 µm/l). The absorbance was measured after 30 min and the IC_{50} calculated. The IC_{50} for the glycyrrhizin was > 0.6 mg/ml. The IC_{50} for Trolox was 0.0028 ± 0.00005 mg/ml.

Unilamellar dioleoyl phosphatidylcholine liposomes were used to evaluate the antioxidant activity of glycyrrhizin and Trolox. The IC_{50}s were > 0.6 and 0.0182 ± 0.0049 mg/ml, respectively.

In a chemilunimescence assay using human blood, whole blood was stimulated with opsonizied zymosan (0.5 mg/ml) or receptor-bypassing stimuli (0.5 µmol/l) was measured. Formation of chemiluninescence signal was initiated by addition of blood (50 µl), stimulus, methanol extract of G. glabra, and phosphate buffer. Chemiluminescence was measured continuously. There was no significant inhibition of antioxidant activity by glycyrrhizin (Račková et al. 2007).

Licochalcone A and Related Compounds

Haraguchi et al. (1998a) tested for the antioxidant and superoxidant activities of licochalcone A, B, C and D and echinatin isolated from the roots of G. inflata. The tests used were a) scavening activity of free radicals based on chemical trapping; b) inhibitory activity on superoxide anion generation by both the xanthine-xanthine oxidase system and rat liver microsomes; c) inhibitory activity on LPO in rat liver microsomes and mitochondria; and d) protective activity of RBC from peroxidation-induced hemolysis.

All 4 licochalcones and echinatin showed antioxidant and superoxidant activities; licochalcones B and D were the most effective. Licochalcones B and D strongly inhibited superoxide anion production in the xanthine-xanthine oxidase system and exhibited potent scavenging activity on DPPH radical (~90%).

Microsomal LPO induced by Fe(III)-adenosine diphosphate (ADP)/NADPH was inhibited almost completely by 3 µg/ml of licochalcones B and D. Mitochondrial LPO induced by Fe(III)-ADP/NADH was completely inhibited by all licochalcones tested (10 µg/ml), the inhibition was most sensitive to licochalcones B and D. Licochalcones B and D also protected RBC against oxidative hemolysis (Haraguchi et al 1998a).

Fukai et al. (2003b) tested for autooxidation (radical intensity under alkaline conditions) effect on radical intensity of sodium ascorbate and superoxide anion radical (O2-) scavenging activity using electron spin resonance (ESR) spectroscopy for licoricidin and licorisoflavan A extracted from G. uralensis and licochalcone A extracted from G. inflata. All 3 compounds exhibited a weak scavenging activity against the superoxide anion radical.

Antiviral Effects

Glycyrrhizic Acid

In an earlier safety assessment of Glycyrrhizic Acid and its derivatives (CIR 2005), the acid was found to have anti-viral

effects on vaccinia, herpes simplex 1, Newcastle disease, vesicular stomatitis, JEV, and influenza virus A2. Polio type 1 virus was unaffected by Glycyrrhizic Acid up to 8 mM.

Anti-parasitic Effects

Licochalcone A

Chen et al. (1993) reported that the 50% inhibition of growth of logarithmic and stationary-phase promastigote of Leishmania major and L. donovaini were seen at 4 and 2.5 µg/ml licochalcone A, respectively, as measured by [3H]thymidine uptake. The growth of L. major promastigotes was totally inhibited after a 20-h incubation with 5 µg/ml licochalcone A. A 1:200 dilution of licochalcone A inhibited the motility of the promastigotes by more than 85%.

Licochalcone A was found to inhibit the in vitro growth of L. major in human peripheral blood monocyte-derived macrophages (MDMs) and U937 cells. No amastigotes were found in macrophages incubated with licochalcone A at 5 and 10 µg/ml and a decrease in the number of amastigotes was observed at 1 and 0.5 µg/ml ($p < .05$) compared to controls. The parasite growth in both human MDMs and U937 cells was decreased by >95% with 1 µg/ml (Chen et al 1993).

Chen et al. (1994a) tested the antileishmanial activity of licochalcone A in female BALB/c mice infected with L. major and male golden hamsters (Mesocricetus auratus) infected with L. donovani in the footpad. On day 0, the footpad thickness of the left hind foot of each mouse was measured then injected subcutaneously with 107 stationary-phase L. major promastigotes. The footpads were measured again on day 7 and every 3 d after that. Also on day 7, the mice (n = 2) were intraperitoneally or intralesionally injected with licochalcone A (2.5 or 5.0 mg/kg) or PBS; this was continued daily. After 39 d of licochalcone A injections, the mice were killed and the livers and spleens removed. The parasite load was measured by the [3H]thymidine uptake method. Impression smears of the footpads, spleens, and livers were also prepared and examined by light microscopy.

Intraperitoneal administration of licochalcone A at 2.5 and 5 mg/kg/d completely prevented lesion development in the mice compared to control ($p < .05$).

Intralesional administration at 1 and 2.5 mg/kg/d inhibited lesion size by ~50% compared to control ($p < .05$).

Parasite loads in the footpads were reduced by 80% and 75% by i.p. doses of 2.5 and 5 mg/kg/day, respectively. Intralesional administration of licochalcone A at 1 and 2.5 mg/kg/d inhibited lesion size by ~50% ($p < .05$). Licochalcone A decreased the parasite load in the footpads, spleens, and livers of the mice treated both intraperitoneally and interlesionally as shown in **Table 45.**

In the hamster experiment, licochalcone A was administered either intraperitoneally or orally to the hamsters. On days 0 and 11 the hamsters were administered intracardiac injections of 109 stationary-phase L. donovani promastigotes in 0.1 ml PBS. One h later, 1 hamster was killed and the liver and spleen removed and weighed. Impression smears were made and parasite loads determined by light microscopy. From day 1, the hamsters were administered 10 mg/kg licochalcone A in 0.1 ml PBS intraperitoneally twice daily for 6 d (n = 5) or the same volume of PBS as control (n = 5). Another group of hamsters was orally administered 5, 50, or 150 mg/kg licochalcone A or 0.5 ml synthetic licochalcone A suspended in 1% CMC daily for 6 d (n = 5). The control group was administered 1% CMC in PBS orally. On day 8, the hamsters were killed and the livers and spleens removed, weighed, and impression smears prepared. The slides were examined by light microscopy. Parasite load was determined by [3H]thymidine uptake.

Parasite load of L. donovani in the liver was decreased by 98% by intraperitoneally administered licochalcone A at 20 mg/kg ($p < .05$). In the spleen, the load was decreased by 99% as determined by [3H]thymidine uptake and 96% by light microscopy. Synthetic licochalcone A decreased the parasite load in the spleen by >70% ($p < .05$) and in the liver by 65% ($p < .05$) (Chen et al. 1994a).

Chen et al. (1994b) used a [3H] hypoxanthine uptake assay to study the effects of licochalcone A (0.1 to 10 µg/ml) on both chloroquine-susceptible (3D7) and chloroquine-resistant (Dd2) strains of *Plasmodium falciparum*. Growth inhibition was dose-dependent, similar in both strains, and greater than controls (infected and uninfected erythrocytes with no licochalcone A; $p < .05$).

Table 45. Effect of licochalcone A on the numbers of amastigotes in the footpads, spleens, and livers of mice (n = 2) infected with *L. major* (Chen et al. 1994a).

	Parasitic load score		
	Footpad	**Spleen**	**Liver**
Buffer			
Intraperitoneal	500 to 1000	100 to 500	100 to 500
Intralesional	500 to 1000	100 to 500	100 to 500
Licochalcone A			
1 mg/kg, intralesional	< 100	< 100	< 100
2.5 mg/kg, intralesional	< 100	< 100	< 100
2.5 mg/kg, intraperitoneal	< 100	–	0
5 mg/kg, intraperitoneal	< 100	–	0

In a life stage study, licochalcone A (0.1 to 10 µg/ml) decreased the number of parasites moving on to the next life stage at each stage ($p < .05$). Licochalcone A, (5, 10, and 15 mg/kg; twice/d; for 3 d) injected i.p. in mice, decreased the level of parasitemia in mice infected with *P. yoilii* YM. All of the control animals died after 8 d of infection; most of the treated mice (5 and 10 mg/kg) survived up to 3 week. Four of 5 in the 15 mg/kg group cleared the parasites.

Orally administered licochalcone A (50, 150, and 450 mg/kg/d) given for 6 d (starting 3 h after infection as above) almost completely cleared the parasitic infection from the mice and they survived to day 21+. All of the untreated mice died between days 7 and 9. The authors concluded that licochalcone A has strong antimalarial activity (Chen et al. 1994b).

Chen et al. (1997) reported that an analog of licochalcone A, 2,4-dimethoxy-4'-butoxychalcone, protected mice from the lethal effects of *Plasmodium berghei* and *P. yoelii* and rats from *P. berghei* after oral, i.p., and s.c. dosing. This compound inhibited the growth of both a chloroquine-susceptible (3D7) and chloroquine-resitant (Dd2) strain of *P. falciparum* in a [3H]hypoxanthine uptake assay.

Chen et al. (2001) reported that when promastigotes of *L. major* incubated in various concentrations of licochalcone A, nicotinamide adenine dinucleotide (NAD)-fumarate reductase and succinate dehydrogenase were inhibited in a concentration-dependent manner (IC_{50} = 1.2 and 19 µM, respectively). When licochalcone A was exposed to the crude mitochondria of the parasite, fumarate reductase reduced in an time- and concentration-dependent manner. Succinate dihydrogenase, NADH dehydrogenase (NDH), succinate-cytochrome c reductase, and NADH-cytochrome c reductase in the crude mitochondria of *L. major* were inhibited in a concentration-dependent manner (IC_{50} = 593, 460, 1519, and 1985 µM, respectively). When human PBMC cells were treated with licochalcone A, succinate-cytochrome C reductase and NADH dehydrogenase were inhibited in a time- and concentration-dependent manner (IC_{50} = 1.4 mM for both).

When a mouse macrophage cell line (J774) was treated, the same result was observed (IC_{50} = 1.4 and 0.94 mM, respectively). Both cell types had much higher values than did the parasitic mitochondria. Licochalcone A also had a time- and concentration-dependent inhibitory effect on fumarate reductase when *L. major* promastigotes were exposed to it (IC_{50} = 32 µM). The authors concluded that fumarate reductase might be the target for antileishmanial drugs (Chen et al. 2001)

Other Constituents

Brøgger-Christensen et al. (1994) stated that E-1-[2-,4-dihydroxy-3-(3-methyl-2-butenyl)phenyl(-3-(4-hydroxy-3-(3-methyl-2-butenyl) phenyl]-2-propen-1-one, a chalcone extracted from the roots of *G. inflata*, has antileishmaial effects.

Anti-microbial Effects

Gancaonin

Fukai et al. (2004) reported that gancaonin I, a hydrophobic 2-arylbenzofuran extracted from *G. uralensis*, had substantial antibacterial activity against vancomycin-resistant enterococci (VRE) and vancomycin-sensitive enterococci (VSE) using an agar dilution method. The MIC against *E. faecalis* JMC 7783 (VSE), JU 1856 (VRE), JU 1782 (VRE), JU 1858 (VRE), JU 1777 (VRE), *E. gallinarum* JU 2786, *Staphylococcus aureus* subspecies aureus JCM 2874, and *S. aureus* (10 strains) was 3.13 µg/ml. The MIC against *E. faecium* JCM 5804 (VSE) was 6.25 µg/ml. The MIC value for *E. coli* NIHJ HC-2, *Klebsiella pneumoniae* PLI 602, *Pseudomonus aeruginosa* JCM 6119 was >25 µg/ml.

Glabridin and Related Compounds

Mitscher et al. (1980) tested glabridin and other isoflavonoids extracted from *G. glabra* for antimicrobial properties. As shown in **Table 46**, glabridin and most of the 10 other flavonoids exhibited antimicrobial activity against *S. aureus* and *Mycobacterium smegmalis*. There was little effect against *E. coli*, *S. gallinarum*, *K. penumoniae* and *C. albicans*.

Table 46. In vitro MIC (µg/ml) of compounds extracted from *G. glabra* (Mitscher et al. 1980).

Substance	*S. aureus*	*E. coli*	*S. gallinarum*	*Klebsiella penumoniae*	*M. smegmalis*	*C. albicans*
Glabridin	6.25	x[a]	x	x	6.25	25
Glabrol	1.56	x	x	x	1.56	x
Glabrene	25	x	x	x	25	x
3-Hydroxyglabrol	6.25	x	x	x	6.25	x
4'-*O*-methylglabrindin	6.25	x	x	x	3.12	x
3'-Methoxyglabridin	50	x	x	x	x	x
Formononetin	x	x	x	x	x	x
Phaseollinisoflavan	25	x	x	x	12.5	x
Hispaglabridin A	3.12	x	x	x	3.12	x
Hispaglabridin B	6.25	x	x	x	3.12	x
Positive control (Streptomycin Sulfate)	5	5	50	2.5	1.25	x

[a] x = no inhibition at 100 µg/ml

Gupta et al. (2008) reported the anti-microbial activity of glabridin against various gram negative and gram positive bacteria and compared its effectiveness to streptomycin as shown in **Table 47**.

Licochalcone a and Related Compounds

Haraguchi et al. (1998b) used the broth dilution method to determine that licochalcone A through D and echinatin, retrochalcones isolated from G. inflata, inhibited the growth of gram-positive bacteria as shown in **Table 48.** The authors noted that Licochalcone A and C had a small effect on fungal growth and that Licohchalcone A showed the most potent antibacterial activity.

The authors also found that licochalcone A inhibited the incorporation of radioactive thymidine, uracil and leucine into DNA, RNA, and protein, respectively, in *M. luteus* cells.

The authors suggested that licochalcone A interfered with energy metabolism in the same manner as several respiratory inhibiting antibiotics.

In further studies by these authors, suspensions containing *M. luteus*, *E. coli* or *S. aureus* were incubated with licochalcone A. Oxygen consumption was inhibited for *M. luteus* and *S. aureus* but not in *E. coli* as shown in **Table 49.** The authors suggested that licochalcone A cannot permeate into gram-negative bacterial cells.

Licochalcone A and C were found to be effective inhibitors of bacterial NADH-oxidase as shown in **Table 50.** The authors suggested this demonstrates effects on the respiratory chain in the bacterial membrane (Haraguchi et al. 1998b).

Table 47. Antibacterial activity of glabridin against gram-positive and gram-negative bacterial strains (Gupta et al. 2008).

Bacterial strain	**Streptomycin MIC (µg/ml)**	**Glabridin MIC (µg/ml)**
S. aureus (MTCC96)	12.71 ± 2.82	3.9 ± 0.45
Staphylococcus epidermidis (MTCC435)	6.35 ± 1.41	7.5 ± 0.89
S. mutans (MTCC890)	6.35 ± 1.41	7.5 ± 0.89
B. subtilis (MTCC121)	12.71 ± 2.82	15.6 ± 1.79
Enterococcs faecalis (MTCC439)	0.36 ± 0.04	31.25 ± 3.61
Klebsiella pneumoniae (MTCC109)	2.71 ± 0.36	250 ± 28.86
Salmonella typhi (MTCC733)	2.71 ± 0.36	125 ± 14.43
Yersinia enterocolitica (MTCC861)	12.71 ± 2.82	250 ± 28.86
Enterobacter aerogens (MTCC111)	2.71 ± 0.36	250 ± 28.86
E. coli (MTCC723)	1.35 ± 0.18	250 ± 28.86

Table 48. MIC (µg/ml) of licorice constituents from *G. inflata* (Haraguchi et al. 1998b).

Microorganism	Licochalcone A	Licochalcone B	Licochalcone C	Licochalcone D	Echinatin
Bacillus subtilis IFO 3060	3.13	100	12.2	50	25
Staphylococcus aureus IFO 3007	1.56	100	6.25	50	25
Micrococcus luteus IFO 3333	1.56	50	6.25	50	25
Escherichia coli HUT 215	>100[a]	>100	>100	>100	>100
Pseudomonus aeruginosa JCM 2776	>100	>100	>100	>100	>100
Saccharomyces cerevisiae IFO 0203	>100	>100	50	>100	>100
Candida albicans TIMM 0134	>100	>100	>100	>100	>100
Mucor pusillus HUT 1185	12.5	>100	100	>100	>100
Asperigillus niger IFO 4343	>100	>100	>100	>100	>100

[a] no effect at 100 µg/ml

Table 49. Effect of licorice constituents extracted from *G. inflata* on respiratory activity (IC_{50} in µM) on bacterial cells (Haraguchi et al. 1998b).

Microorganism	Licochalcone A	Licochalcone B	Licochalcone C	Licochalcone D	Echinatin
M. luteus IFO 3333	3.0 ± 0.4	> 100	53.3 ± 3.5	> 100	> 100
S. aureus IFO 12732	22.7 ± 1.6	> 100	58.6 ± 2.9	74.9 ± 5.2	> 100
S. aureus 11D 671	23 ± 2.1	> 100	55.9 ± 4.1	> 100	> 100
E. coli IFO 3545	> 100	> 100	> 100	> 100	> 100

Table 50. Effect of licochalcone A and C from *G. inflata* on NADH oxidase (IC_{50} in µM) on bacterial membranes (Haraguchi et al. 1998b).

Microorganism	Licochalcone A	Licochalcone C
M. luteus	6.2 ± 0.5	3.0 ± 0.2
S. aureus	8.0 ± 0.7	23.4 ± 2.0
P. aeruginosa	18.9 ± 1.3	13.9 ± 1.4
E. coli	9.5 ± 0.7	7.4 ± 0.5

Tsukiyama et al. (2002) determined that licochalcone A, isolated from G. inflata, exhibited a concentration-dependent inhibition of B. subtilis growth. At 2 µg/ml, licochalcone A inhibited growth for 12 h before cell growth started. At 3 µg/ml, cell growth was completely inhibited. The authors also determined the MICs of licochalcone A against several food contaminant microorganisms as shown in **Table 51.**

Friis-Moller et al. (2002) reported that licochalcone A, from G. inflata, had cytotoxic effects on human pathogenic Mycobacteria species. *M. tuberculosis, M. bovis, M. kansasii, M. xenophii, M. marnum,* and 3 tuberculosis strains were inhibited by locochacone A (MIC ≤ 20 mg/l). All non-M. tuberculosis complex mycobacteria, including *M. szulgai, M. avium/intracellulare, M. scrofulaceum, M. malmoense, M. terrae/triviale, M. smegmatis, M. nonchromogenicum, M. flavescens, M. fortuitum,* and *M. chelonae* (except *M. kansasii, M. xenophii,* and *M. marinum*) were resistant to ≥20 mg/l licochalcone A.

Nineteen strains of *M. tuberculosis* (MIC 5 - 10 mg/l), 8 strains of M. bovis (MIC 10 - 20 mg/l), and 3 Bacillus of Calmette and Gurin tubercular strains (MIC 5 - 10 mg/l) were inhibited by licochalcone A. All *M. avium/intracellulare* strains were inhibited by ≥20 mg/l licochalcone A and most were killed by >80 mg/l. The minimal bactericidal concentration of licochalcone A against both *M. instracellulare* (n = 2) and the Bacillus of Calmette and Gurin tubercular strains (n = 3) was 40 mg/l.

Various Compounds

Fukui et al. (1988) tested the antimicrobial effects of cycloflavanone, pinocembrin, and licoflavanone isolated from the air-dried leaves of *G. glabra* by the paper disk method. The authors conclude that these compounds were minimally active against *B. subtilis, S. aureus,* and *C. albicans* and not active against *E. coli.*

Demizu et al. (1988) extracted glycycoumarin and licocoumarone from licorice root and tested their antimicrobial activity. There was no significant antimicrobial activity by either of the test compounds on *S. mutans* IFO 13922, *S. aureus* IFO 3060, *B. subtilis* IFO 3007, *E. coli* IFO 3366, *S. cerevisiae* IFO 0306, *C. utilis* IFO 1086, *Pichia nakazawai* IFO 1668, *Rhizopus formosaensis* IFO 4756, or *A. niger* IFO 4407.

Table 51. Activity of licochalcone A against food contaminant microorganisms (Tsukiyama et al. 2002).

Microorganism	MIC (µg/ml)
Escherichia coli K-12 IFO 3301	>50[a]
Pseudomonas aeruginosa IFO 3923	>50
Bacillus cereus IFO 3514	3.0
Bacillus subtilis IFO 3007	2.0
Bacillus coagulans IFO 12583	2.0
Bacillus stearothermophilus IFO 12550	2.0
Clostridium sporogenes IFO 13950	8.0
Enterococcus faecalis IFO 3989	6.0
Lactobacillus acidophilus IFO 13951	5.0
Lactobacillus plantarum IFO 12519	5.0
Leuconostoc mesenteroides IFO 3832	15.0
Staphylococcus aureus 209P IFO 12732	3.0
Streptococcus lactis IFO 12546	8.0
Streptococcus mutans IFO 13955	5.0
Aspergillus niger IFO 6341	>50
Saccharomyces cerevisiae IFO 0203	>50

[a] no effect at 50 µg/ml

Okada et al. (1989) tested the antimicrobial properties of extracts from *G. glabra* and *G. inflata* on various microbial strains. Glabrene and glabradin was extracted from *G. glabra* and licochalcone A and B were extracted from *G. inflata*. MICs were determined for *S. aureus* IFO 3060, *B. subtilis* IFO 1668, *E. coli* IFO 3366, *P. aeruginosa* JCM 2776, *S. cerevisiae* IFO 0306, *C. utilis* IFO 1086, *M. pusillus* HUT 1186 and *A. niger* IFO4407. All of the compounds inhibited the growth of gram-positive bacteria such as *S. aureus* and *B. subtilis* as shown in **Table 52**, but not the gram-negative strains. The authors noted that potencies of the antimicrobial activity of licochalcone A and glabridin were comparable to streptomycin. Glabridin exhibited significant growth-inhibition against yeast and fungi while the other compounds and streptomycin were inactive. The authors speculated that since the content of glabridin, glabrene, and licochalcones A and B in each licorice plant were estimated at 9.1%, 2.8%, 18.2% and 2.7%, respectively, by HPLC, these compounds should account for much of the antimicrobial activities of licorice extracts.

Hatano et al. (2000) examined the antimicrobial effects of 40 compounds isolated from G. uralensis. The compounds were tested on 4 strains of methicillin-resistant S. aureus (MRSA), one strain of methicillin-sensitive S. aureus (MSSA), P. aeruginosa (PAO1), and E. coli K12. Two of these compounds, 8-(γ,γ-dimethylallyl)-wighteone and 3'-(γ,γ-dimethylallyl)-kievitone, had MICs of 8 µg/ml on the MRSA strains and MSSA. Licochalcone A, gancaonin G, isoangustone A, glyasperins C and D, glabridin, licoricidin, glycycoumarin, and licocoumarone showed antibacterial effects on the MRSA strains with MIC values of 16 µg/ml.

None of the compounds demonstrated any effect on E. coli or P. aeruginosa as shown in **Table 53.** The authors suggested that the antibacterial properties of various licorice compounds may be useful in the treatment of diseases caused by for S. aureus, at least in the intestines.

To test if the presence of certain of these licorice compounds decreased the resistance of the MRSA strains against oxacillin, the above experiment was repeated at concentrations lower than each compound's MIC value in combination with the lowest concentrations of oxacillin which did not cause turbidity due to bacterial proliferation.

Oxacillin in the absence of the phenolic compounds showed MICs of 64 to 512 µg/ml for the 4 MRSA strains, while the MIC for MSSA 209P was < 0.5 µg/ml. In the presence of 16 µg/ml of glicophenone, the MICs of oxacillin for the MRSA strains decreased to 1/2 to 1/8 of the values in the absence of glicophenone. Isowightenone and isoangustone A also reduced the MICs of oxacillin. Licoricidin (8 µg/ml) decreased the MICs of oxacillin to lower than 1/128 to 1/1000 of the MICs without licoridicin. The growth curve of the MRSA OM481 strain after 24 h of incubation in the presence of both oxacillin (10 µg/ml) and licoricidin (8 µg/ml) was ~1/100 of that in the absence of them (control); oxacillin alone (10 µg/ml) or licoricidin alone (8 µg/ml) did not cause such an inhibition of bacterial growth.

A mechanistic study suggested to the authors that licoricidin restored the antibacterial effect of oxacillin without inhibition of the formation of penicillin-binding protein 2' (PBP2'), but affected the enzymatic function of PBP2'. The authors further suggested that licoricidin may work well in decreasing the MIC of oxacillin (Hatano et al. 2000).

Table 52. MIC (µg/ml) of the anitmicrobial activity of constituents of licorice (Okada et al. 1989).

Microorganisms	Licochalcone A	Licochalcone B	Galbrene	Glabridin	Streptomycin
Gram-positive bacteria					
Staphylococcus aureus	1.95	31.3	7.81	1.95	1.95
Bacillus subtilis	3.91	31.3	7.81	3.91	1.95
Gram-negative bacteria					
Escherichia coli	>250[a]	>250	>250	>250	7.81
Pseudomonas aeruginosa	>250	>250	>250	>250	31.3
Yeasts					
Saccharomyces cerevisae	>250	>250	15.6	7.81	>250
Candida utilis	>250	>250	31.3	7.81	>250
Fungi					
Mucro pusillus	>250	>250	15.6	3.91	>250
Apergillus niger	>250	>250	>250	31.3	>250

[a] no inhibition observed up to 250 µg/ml

Table 53. MICs (µg/ml) of *G. uralensis* phenolics for 4 strains of methicillin-resistant *S. aureus* (MRSA), one strain of methicillin-sensitive *S. aureus* (MSSA), *Pseudomonas aeruginosa* (PAO1), and *E. coli* K12 (Hatano et al. 2000).

Compounds	MRSA OM481	MRSA OM505	MRSA OM584	MRSA OM623	MSSA 209P	*E. coli* K12	*P. Aeruginosa* PA01
Flavanones							
Liquiritigenin	>128[a]	>128	>128	>128	>128	>128	>128
Liquiritin	>128	>128	>128	>128	>128	>128	>128
6"-*O*-Acetylliquiritin	>128	>128	>128	>128	>128	>128	>128
Naringenin	>128	>128	>128	>128	>128	>128	>128
Chalcones							
Isoliquiritin apioside	>128	>128	>128	>128	>128	>128	>128
Isoliqiritin	>128	>128	>128	>128	>128	>128	>128
Tetrahydroxymethoxychalcone	>128	>128	>128	>128	>128	>128	>128
Echinatin	128	64	64	64	64	>128	>128
Licochalcone A	16	16	16	16	16	>128	>128
Licochalcone B	128	128	128	128	128	>128	>128
Isoliquiritigenin	128	128	128	128	128	>128	>128
Isoflavones							
Glycyrrhisoflavone	64	64	32	32	32	>128	>128
Semilicoisoflavone B	64	64	64	32	32	>128	>128
Genistein	>128	>128	>128	>128	>128	>128	>128
Glicoricone	64	64	64	64	64	>128	>128
Gancaonin G	16	16	16	16	16	>128	>128
8-(γ,γ-Dimethylallyl)-wighteone	8	8	8	8	8	>128	>128
Isowighteone	32	32	32	32	16	>128	>128
Glisoflavone	64	64	64	64	64	>128	>128
Isoangustone A	16	16	16	16	16	>128	>128
Isoflavanones							
Glycyrrhisoflavanone	64	64	32	32	32	>128	>128
Glyasperin F	64	64	64	64	32	>128	>128
Licoisoflavanone	32	32	32	32	32	>128	>128
Glicoisoflavanone	64	64	32	32	32	>128	>128
3'-(γ,γ-Dimethylallyl)-kievitone	8	8	8	8	8	>128	>128
Isoflavans							
(3*R*)-Vestitol	128	128	128	128	128	>128	>128
Glyasperin C	16	16	16	16	16	>128	>128
Glyasperin D	16	16	16	16	16	>128	>128
Glabridin	16	16	16	16	16	>128	>128
Licoricidin	16	16	16	16	16	>128	>128
3-Arylcoumarins							
Glycycoumarin	16	16	16	16	16	>128	>128
Licopyranocoumarin	>128	>128	>128	>128	>128	>128	>128
Licoarylcoumarin	32	32	32	32	16	>128	>128
Glycyrin	128	128	128	128	128	>128	>128
Isolicopyranocoumarin	>128	>128	>128	>128	>128	>128	>128
Glycyrin permethyl ether	>128	>128	>128	>128	>128	>128	>128
Others							
Licocoumarone	16	16	16	16	16	>128	>128
Glicophenone	32	32	32	32	32	>128	>128
Licoriphenone	32	32	32	16	16	>128	>128
Glycyron	>128	>128	>128	>128	>128	>128	>128
Isoglycyrol	>128	>128	>128	>128	>128	>128	>128

[a] no inhibition observed up to 128 µg/ml

Tanaka et al. (2001) tested the antimicrobial effects of chemicals extracted from *G. uralensis*, including licoricidin, liquiritigenin, liquiritin; glycyrol, glycyrin, and glycycoumarin; and vestitol (see Figure 10), on upper airway respiratory tract bacteria (*S. pyogenes*, *Haemophilus influenzae* ATCC 33391), and *Moraxella catarrhalis* (ATCC 25238)). The MIC was measured by the 2-fold serial broth dilution method. As shown in **Table 54**, most of the fractions showed antibacterial activity; and licoricidin exhibited the highest activity with an MIC of 12 mg/ml for all 3 strains.

Fukai et al. (2002) tested fractions and isolates extracted from G. uralensis for anti-*Helicobacter pylori* activity. *H. pylori* ATCC 43526 was incubated for 3 d then colonies were suspended in Brucella broth to give the turbidity equivalent to optical density (OD)570 = 0.25. The suspensions contained ~2 x 107 colony forming units (cfu)/ml. The final concentration was 4 x 105 cfu/ml. Samples were dissolved in EtOAc and diluted to 1 mg/ml with the solvent. Air-dried 8 mm paper disks charged with 50 µl of the sample solution were placed on agar plates. Diameters of inhibition zones were determined after 3 d incubation under microaerophilic conditions.

The antibacterial activities of these fractions were not as strong as the positive control, amoxicillin (5 µg/ml) with a diameter of 37.0 mm as shown in **Table 55.**

MICs of the extracts were determined by the agar dilution method. Four strains of H. pylori were incubated as above and inoculated on plates containing the test compounds.

The compounds of *G. uralensis* were confirmed by TLC. The test compounds were initially dissolved in DMSO and further diluted in a two-fold series with DMSO before placement on the plates. MICs were determined after 3 d of incubation at 37°C under microaerophilic conditions. MIC was defined as the lowest concentration of the test compound inhibiting visible bacterial growth. Anti-*H. pylori* activity is shown in **Table 56.**

The authors concluded that anti-*H. pylori* activity compounds in licorice are isoprenoid-substituted flavonoids, and suggested that the inhibitory activity of licoisoflavone B (a pyranoisoflavone) against strain GP98, which is resistant to amoxicillin, may be of therapeutic value.

Table 54. MICs (µg/ml) of constituents of *G. uralensis* against oropharyngeal bacteria (Tanaka et al. 2001).

Constituent	*S. pyoenes* (µg/ml)	*H. influenza* (µg/ml)	*M catarrhalis* (µg/ml)
Licoricidin	12	12	12
Vestitol	50	100	100
Glycyrol	50	100	>100
Glycyrin	25	25	50
Glycycoumarin	25	25	100
Liquiritigenin	>100	>100	>100
Liquiritin	>100	>100	>100

[a] no inhibition observed up to 100 µg/ml

Table 55. Anti-*Helicobacter pylori* activity (paper disk method) of semi-purified fractions of *G. uralensis* (Fukai et al. 2002).

	Diameter of inhibition zone	
Fraction	**10 mg/ml (mm)**	**1 mg/ml (mm)**
Methanol	19.0	negative
Chloroform soluble	21.5	14.0
Chloroform insoluble	15.5	negative
Benzene	20.0	15.0
Fr A	21.5	negative
Fr B	32.5	15.0
Fr C	29.0	16.0
Fr D	16.0	11.5
Fr E	11.5	negative
Fr F	negative	negative

Table 56. Anti-*Helicobacter pylori* activities (MIC in µg/ml) of the characteristic compounds of licorice species *G. glabra*, *G. uralensis* and *G. inflata* (Fukai et al. 2002).

Compound (source)	cfu [a]	ATCC 43504	ATCC 43526	ZLM 1007	GP98
Glycyrrhizic acid (all species)	a	> 100	> 100	> 100	> 100
	b	> 100	> 100	> 100	> 100
Glycyrrhitic acid (all species)	a	50	50	50	50
	b	25	25	25	25
Licorice-saponin G2 (all species)	a	> 100	> 100	> 100	> 100
	b	> 100	> 100	> 100	> 100
Liquiritigenin (all species)	a	50	50	50	50
	b	50	50	50	50
Liquiritin (all species)	a	> 50	> 50	> 50	> 50
	b	> 50	> 50	> 50	> 50
Formononetin (all species)	a	> 100	> 100	> 100	12.5
	b	12.5	12.5	12.5	12.5
Glabridin (*G. glabra*)	a	12.5	12.5	25	12.5
	b	12.5	12.5	25	12.5
Glabrene (*G. glabra*)	a	12.5	12.5	12.5	12.5
	b	12.5	12.5	12.5	12.5
Licochalcone A (*G. inflata*)	a	25	25	25	25
	b	25	25	12.5	12.5
Licochalcone B (*G. inflata*)	a	> 50	> 50	> 50	> 50
	b	> 50	> 50	> 50	> 50
Licoricidin (*G. uralensis*)	a	12.5	12.5	6.25	12.5
	b	12.5	12.5	6.25	6.25
Glycyrol (*G. uralensis*)	a	> 50	> 50	> 50	> 50
	b	> 50	> 50	> 50	> 50
Isoglycyrol (*G. uralensis*)	a	> 100	> 100	> 100	> 100
	b	> 100	> 100	> 100	> 100
Licoisoflavone B (*G. uralensis*)	a	6.25	6.25	6.25	6.25
	b	6.25	6.25	6.25	3.13
Amoxicillin	a	0.05	0.05	0.05	0.20
	b	0.025	0.025	0.025	0.10

[a] a corresponds to an inoculum of 2 x 10^7 colony forming units (cfu) and b corresponds to 2 x 10^5 cfu.

Because licoisoflavone B is a minor constituent of licorice plants and licoricidin, with not as high inhibitory activity, is present in higher concentrations in the plants, the authors suggested that licoricidin may be the main anti-*H. pylori* agent of *G. uralensis* (Fukai et al. 2002).

He et al. (2006) performed an extract procedure on the roots of *G. uralensis* with 95% ethanol followed by solvent-solvent partitioning. Two new pterocarpenes were identified: glycyrrhizol A and glycyrrhizol B. These 2 compounds along with 5-O-methylglycyrol, isoglycyrol, 6,8-diisoprenyl-5,7,4'-trihydroxyisoflaone and gancacaonin G (also isolated from G. uralensis root in this process) were tested for antimicrobial activity against S. mutans with the MIC protocol. Chlorhexidine (0.12%) was the positive control and the untreated suspension was the negative control.

The MIC values were: glycyrrhizol A, 1 g/ml; glycyrrhizol B, 32 µg/ml; 5-O-methylglycyrol, isoglycyrol, 500 µg/ml; isoglycyrol, 500 µg/ml; 6,8-diisoprenyl-5,7,4'-trihydroxyisoflaone, 2 µg/ml and gancaonin G, 125 µg/ml (He et al. 2006).

Drug Interactions

Glycyrrhetinic Acid

In an earlier safety assessment of Glycyrrhetinic Acid and related chemicals, the acid, but not the dipotassium salt, enhanced drug absorption (CIR 2005). Hydrocortisone and Glycyrrhetinic Acid combined produced a dose-dependent increase in degree of blanching (vasoconstriction) where neither substance alone had an

effect on the forearms of humans. Glycyrrhetinic acid inhibited the production of 3H-11-dehydrocorticosterone in a dose-dependent manner when applied to protein extracted from the dorsal skin of nude mice. A formulation containing 0.1% Glycyrrhizic Acid produced a 10-fold increase in the flux of diclofenac sodium across a rat skin barrier in Franz type diffusion cells.

Glabridin

Since glabridin has been found to be a mechanism-based inhibitor of CYP (Kent et al. 2002; Zhou et al. 2004), Zhou et al. (2005) stated that glabridin may cause licorice-drug interactions as a result.

Isoliquiritigenin

Jang et al. (2008) reported that isoliquiritigenin (5, 10, and 20 mg/kg; p.o.), administered to adult Sprague-Dawley rats 1 h prior to an injection of cocaine inhibited cocaine-induced extracellular dopamine levels in the nucleus accumbens in a dose-dependent manner. This experiment was also conducted on Glycyrrhiza Glabra (Licorice) Root Extract; see the main report. The inhibition of dopamine release by isoliquiritigeinin resulted inattenuation of the expression of c-Fos (an immediately early gene induced by cocaine). The effect of isoliquiritigenin was completely prevented by a gamma-aminobutyric acid-B (GABAB)receptor antagonist. The authors concluded that G. glabra extract and isoliquiritigenin inhibit cocaine-induced dopamine release by modulating the GABAB receptor, suggesting that isoliquiritigenin might be effective in blocking the reinforcing effects of cocaine.

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Glycyrrhetinic Acid

The oral LD_{50} of Glycyrrhetinic Acid was reported to be 610 mg/kg in rats (CIR 2005).

Acute Parenteral Toxicity

Glycyrrhetinic Acid

The i.p. LD_{50} of glycyrrhetinic acid in mice was reported to be 308 mg/kg (CIR 2005).

Short-term Oral Toxicity

Licochalcone A

In a preliminary experiment on the antileishmanial activity of licochalcone A, an unspecified number of rats were administered up to 1,000 mg licochalcone A/kg in 1% CMC orally once/day for 2 wk. The rats did not exhibit any signs of toxicity (Chen et al. 1994a).

Short-term Parenteral Toxicity

Glabridin

Kang et al. (2005) tested glabridin for its anti-inflammatory effects against LPS isolated from *Salmonella typhosa* when injected into female BDF1 mice. The test injections consisted of, vehicle (5% Tween 80 v/v and 2% ethanol), no injection, 1 mg/kg glabridin or 10 mg/kg glabridin (n = 7). The injections were made 24 and 2 h before an i.p. injection of 15 mg/kg LPS. Survival was noted daily for 4 d. All of the mice injected with the vehicle only survived the experiment. On days 2 to 4, 43% of the mice injected with just LPS survived. The mice injected with the low dose of glabridin had 57% survival while the mice injected with the high dose of glabridin had 86% survival.

Glycyrrhetinic Acid

Glycyrrhetinic Acid injected i.m. into young rats 3 times/week for 4 wk resulted in no tissue damage except for a slight thinning of the lipid in the zona glomerulosa of the adrenal glands (CIR 2005).

Licochalcone A

Daily i.p. injections of licochalcone A dissolved in DMSO in doses up to 100 mg/kg administered to rats and 150 mg/kg to hamsters did not show any toxicity (Chen et al. 1994a).

Subchronic Oral Toxicity

Licorice Flavanoid Oil

Nakagawa et al. (2008b) orally administered licorice flavonoid oil (consisting of licorice hydrophobic pholyphenols in medium-chain triglycerides; 400, 600, 800, or 1600 mg/kg) to 6-week-old, male and female Crj:CD(SD) rats (SPF) (n = 10; 6 groups of each sex) for 90 d. Controls were the vehicle and corn oil. Ophthalmoscopic examinations were conducted prior to and at the end of treatment. Urine and blood were collected prior to and at the end of treatment. The rats were killed and necropsied.

In the 1600 mg/kg group, 1 female and 8 males died or were killed due to adverse effects attributed to treatment. Hematological analyses at necropsy revealed a decrease in RBC, hemoglobin, and hematocrit and a prolongation of clotting time by prothrombin time and activated partial thromboplastin time. There were traces of hemorrhage in several organs in the animals that died and the surviving males in the high-dose group. Clinical observations of these rats include staining of the nose, bleeding in the oral cavity, swelling of the hindlimb, rough fur/and or prone position. Accidental deaths occurred to 1 female in the 400, 800, and 1600 mg/kg treatment groups due to gavage accidents; 2 males in the 800 mg/kg also died. Clinical signs among the surviving rats were spontaneous formation of a skin crust occurring on the neck of 1 female in the 400 mg/kg group and on the face of 1 male in the 800 mg/kg group.
There were no changes in body weights except for the males in the 1600 mg/kg group from week 9. There were no changes in feed consumption. The authors noted a trend toward increased water consumption in the female 800 mg/kg group from week 4, and in the female 1600 mg/kg group from week 2. There was a trend of increased water consumption in the males in the 600, 800, and 1600 mg/kg groups from weeks 1 or 2. There was no statistical analysis of the water consumption. There was elevated urine output for females in the 800 and 1600 mg/kg groups and for males in the 600, 800, and 1600 mg/kg groups. There were no

other changes observed in the urinalysis.

RBC decreased in the male 600 and 800 mg/kg groups ($p < .05$, .01). Hematocrit levels decreased in the male 400, 600, and 800 mg/kg groups ($p < .05$, .01). Mean corpuscular hemoglobin levels were increased in the male 800 mg/kg group ($p < .05$) and mean corpuscular hemoglobin concentrations were increased in the male 600 and 800 mg/kg group ($p < .01$). Similar tendencies were noted but not significant in the male 1600 mg/kg group. The clotting measurement prothrombin time was prolonged in the female 1600 mg/kg group ($p < .05$) and the male 400 and 800 mg/kg groups ($p < .01$). Activated partial thromboplastin time was prolonged in the female 1600 mg/kg group ($p < .01$) and in the male 800 mg/kg group ($p < .01$).

AST decreased in the female 1600 mg/kg group ($p < .05$) and the male 800 mg/kg group ($p < .01$). ALT was increased in the female 600, 800, and 1600 mg/kg groups ($p < .05$, .01) and male 400, 600, and 800 mg/kg groups ($p < .05$, .01). Alkaline phosphatase and total bilirubin were increased in the female 1600 mg/kg group ($p < .05$). Creatinine was increased in the male 800 mg/kg group ($p < .05$). Glucose was decreased in the female 600, 800, and 1600 mg/kg groups ($p < .05$, .01). Inorganic phosphates were elevated in the female 1600 mg/kg group ($p < .05$). Magnesium was decreased in the female 1600 mg/kg group ($p < .05$). Potassium was decreased in the male 600 and 800 mg/kg group ($p < .05$, .01) with a similar trend in the 1600 mg/kg group.

Absolute spleen weight was decreased ($p < .05$) and relative kidney weight was increased in the female 1600 mg/kg group ($p < .01$). The absolute ($p < .01$) and relative ($p < .01$) weights of abdominal fat around the uterus were decreased in the female 1600 mg/kg group. The absolute and relative weights of abdominal fat around the kidneys were decreased in the female 800 and 1600 mg/kg groups ($p < .05$, .01).

Of the animals that died, traces of hemorrhage, such as dark red discoloration, were observed in several organs macroscopically. One surviving male in the 1600 mg/kg group had dark discoloration of the musculature. Histopathological examination revealed hemorrhage in several organs and tissues and hematopoiesis in the bone marrow in the male 1600 mg/kg group and in the female that died. Inflammatory lesions, apoptosis, necrosis, and atrophy were also observed. Ophthalmosopic examinations were unremarkable.

A followup study was conducted to investigate the hemorrhagic effects (see above). The authors concluded that the NOAEL for licorice flavonoid oil was 800 mg/kg/d for female rats and 400 mg/kg/d for male rats; and the NOEL was < 400 mg/kg/d (Nakagawa et al 2008b).

Ocular Irritation

Glycyrrhetinic Acid

Glycyrrhetinic Acid (6%) in water was classified as "slightly irritating" using a chick embryo chorio-allantoic membrane in vitro assay (CIR 2005).

Dermal Irritation and Sensitization

Glycyrrhetinic Acid

Glycyrrhetinic Acid applied to the shaved backs of albino rabbits caused no edema or erythema on intact or abraded skin after 24 h of exposure (CIR 2005).

Liquiritin

Sansho Seiyaku, Co., Ltd. (2004) conducted a primary irritation test of liquiritin, extracted from *G. glabra* root, on guinea pigs ($n = 5$). The test sample was a 0.15% ethanol solution of liquiritin. The test sample was applied on the shaved backs of the animals and the sites were covered by occlusive dressings for 24 h. No reactions, including erythema and edema, were observed in any animals at 2 and 24 h after removal of the test sample.

In a further test, a skin sensitization study on guinea pigs ($n = 8$) was performed using a 0.15% ethanol solution of liquiritin with Freund's adjuvant. The test sample was applied to the shaved backs of the animals (animals pretreated with adjuvant) with induction patches and then with occlusive challenge patches. Although mild erythema was observed in 1 of the 8 animals, no reaction was seen in the remaining animals (Sansho Seiyaku, Co., Ltd. 2004).

Treatment of Immediate Hypersensitivity

Glycyrrhizic Acid and Derivatives

Shin et al. (2007) tested glycyrrhizin, 18β-glycyrrhetinic acid, isoliquiritin, and liquiritigenin, isolated from *G. glabra* roots, for antiallergic effects. In a histimine release study, male Sprague-Dawley rats (200 ± 20 g) were killed then injected i.p. with 20 ml physiological solution. The abdominal regions were massaged and the peritoneal exudate cells collected, centrifuged, and washed. The cells (5 x 104/ml) were mixed with various concentrations of the isolates in 0.5 ml physiological solution for 5 min. Compound 48/80 (0.5 ml) was added and the cells cooled. The released histamine from the rat peritoneal exudate cells was then measured. This was repeated with RBL-2H3 cells induced by IgE with DNP-human serum albumin.

Liquiritigenin, the most potent compound tested, inhibited the release of β-hexosaminidase from RBL-2H3 cells (IC_{50} = 86 µM) followed by 18β-glycyrrhetinic acid. The IC_{50} of azelastine, the control, was 35 µM. These isolates also inhibited compound 48/80-induced histamine release from peritoneal cells. Glycyrrhizin and isoliquiritin did not inhibit degranulation.

The authors also performed an IgE-dependent passive cutaneous anaphylactic reaction assay on male ICR mice. The mice were intradermally injected with anti-DNP IgE in 2 shaved, dorsal sites that had been marked. The licorice isolates were administered orally or i.p. after 23 h. The mice were injected i.v. 48 h later with 3% Evans Blue in PBS with 200 µg DNP-human serum albumin (200 µl). The mice were killed 30 min later, the skin excised, and the pigment determined colorimetrically. All the isolates demonstrated inhibition, both orally and i.p. as shown in **Table 57**.

Table 57. Effect of compounds isolated from *G. glabra* root on the mouse passive cutaneous anaphylactic reaction assay induced by IgE-antigen complex (Shin et al. 2007).

Treatment	Dose (mg/kg)	Administration route	Inhibition (%)
Glycyrrhizin	5	i.p.	48 ± 7
	10	p.o.	67 ± 11
	50	p.o.	97 ± 12
Isoliquiritin	5	i.p.	63 ± 9
	10	p.o.	60 ± 21
	50	p.o.	83 ± 18
Liquiritigenin	5	i.p.	54 ± 5
	10	p.o.	80 ± 16
	50	p.o.	84 ± 15
18β-Glycyrrhetinic Acid	5	i.p.	63 ± 5
	10	p.o.	83 ± 8
	50	p.o.	96 ± 6
Azelastine	5	i.p.	87 ± 12
	10	p.o.	89 ± 6

In a scratching behavior assay, the authors placed male BALB/c mice (n = 6)` in acrylic cages after orally administering the isolates 1 h earlier. Compound 48/80 was injected interdermally on the rostral part of the skin of the back which had been clipped. The controls received saline. Scratching behavior was recorded. Liquirititigenin and 18β-glycyrrhetinic acid (both at 50 mg/kg) inhibited scratching behavior by 51% and 52%, respectively. Glycyrrhizin (50 mg/kg) inhibited scratching behavior by ~25% and isoliquiritin (50 mg/kg) by ~30%.The authors used BALB/c mice for a sensitization and antigen challenge experiment. On day 1, the mice were injected with ovalbumin (5 mg in 1 ml aluminum hydroxide gel). The controls were administered alum or betamethasone. On day 10 the mice were administered a booster injection, the same as the first injection. The mice were challenged on day 17 with aerosolized ovalbumin (5%) by ultrasonic nebulizer for 1 h over 5 d.

The licorice isolates were orally administered 1 h before the challenge. The day after the last challenge, day 22, the mice were killed and IgE was measured in the blood. Glycyrrhizin and 18β-glycyrrhetinic acid inhibited the ovalbumin-induced production of IgE ($p < .05$), but not as much as betamethasone. Liquiritigenin and isoliquiritin did not inhibit IgE production.

The authors concluded that orally administered licorice extract containing glycyrrhizin, 18β-glycyrrhetinic acid, and liquiritigenin can be use to treat IgE-induced allergic disease (Shin et al. 2007).

Phototoxicity and Photosensitization

Glabridin

Two areas of the shaved backs of 3 Hartley guinea pigs were irradiated once with 150 mJ/cm2 UVB. Immediately after UVB treatment, 0.5 % glabridin solution (ethanol:propylene glycol:water = 6.5:2.5:1) was applied to 1 of the irradiated areas, and the base solution without glabridin was applied to the other irradiated site. Erythema was measured with a Minolta CR-100 chromameter at 6, 24, and 48 hours after UVB irradiation. The degree of redness was reduced ($p < 0.01$) in the glabridin-treated sites at all 3 time points (Yokota et al. 1998).

Depigmentation

Glabridin

Yokota et al. (1998) tested glabridin for its skin depigmentation effects under UVB. Two areas of the shaved backs of 3 brownish guinea pigs were irradiated with 250 mJ/cm2 UVB/day for 4 consecutive days. For 3 wk after the last UVB irradiation, 0.5% glabridin solution (ethanol:propylene glycol:water = 6.5:2.5:1) was applied to 1 of the irradiated sites on each guinea pig, while the base solution without glabridin was applied the other site. The UVB-induced skin depigmentation of the treated sites was measured by a Minolta CR-100 chromameter. The gabridin-treated sites were significantly lighter in color ($p < 0.05$) than the control sites; glabridin reduced UVB-induced pigmentation.

Hormonal Effects

Glabridin

Tamir et al. (2000) tested the effect of glabridin extracted from *G. glabra* on 3 lines of human breast cancer cells (T-47D, MCF-7 and MDA-MB-468) for estrogenic effects. The cells were seeded into 96-well tissue culture plates and incubated for 48 h. The medium was replaced with fresh medium plus various concentrations of glabridin or estradiol after 3 d. The medium was changed every 3 d thereafter. Growth was measured by spectrometry at 450 nm.

Glabridin stimulated growth of the cancer cells over a range of 0.1 to 10 µM, maximum stimulation was at ~ 10 µM. Glabridin then inhibited cell growth at concentrations > 15 µM. The maximum growth stimulation by glabridin was equal to that of estradiol at 0.1 to 10 nM.

To differentiate the estrogenic agonist activities from antiproliferative effects, the authors performed a dose-response experiment of glabridin with ER- (MDA-MB-468) and ER+ (T47D) human breast cancer cells.

The effects of glabridin was biphasic. Glabridin did not increase cell growth in ER-cells but at 25 µM it inhibited growth. The growth of the ER+ cells was above that of the controls with ~0.1 to 10 µM glabridin and then abruptly inhibited at ~25 µM.

To test the effects of glabridin on colony formation in soft agar by breast cancer cells, MCF-7 cells were plated onto soft agar plates in the presence of various concentrations of glabridin. Plates were fed weekly and after 3 wk, colony formation was assessed. Colonies > 0.15 mm in diameter were scored.

The effects were biphasic as in the cell proliferation experiment. Cells formed large colonies in the presence of 10 µM of glabridin and anchorage independent growth was inhibited at 25 µM. This

experiment was repeated in the presence of estradiol. There was no effect on the anchorage-independent, growth-promoting effects of estradiol at 10 nM; the growth inhibition of glabridin at ≥ 25 M was not affected by estradiol as shown in Table 58 (Tamir et al. 2000).

To test the estrogenic effects of glabridin and compare them to estradiol, Tamir et al. (2001) injected 25 Wistar-derived, prepubertal female rats (25 d old, ~ 60 g) with 0.5 ml PBS containing 2.5, 25, 200, or 250 µg glabridin or 5 µg estradiol. After 24 h, the rats were killed, the uterus was removed, and the wet weight taken. The aorta, left ventricle, diaphysis and epiphysis of the femur were removed and frozen for later analysis. Each type of organ was homogenized and centrifuged. The supernatant was tested for creatine kinase activity (a known marker for estrogen responsive genes).

Estradiol (5 µg) stimulated creatine kinase activity at the same level as 2.5 µg of glabridin in the diaphysis and aorta and at 25 µg in the uterus and left ventricle. Glabridin had a weaker effect on the stimulation of creatine kinase activity in the left ventricle (1.43 ± 0.13 experimental (E)/control (C)) than estradiol (3.36 ± 0.7 E/C). Glabridin (200 µg) and estradiol (5 µg) caused an increase in uterus wet weight to 78.6 ± 19 and 90.5 ± 19 g. respectively, compared to 57.8 ± 5 mg for the control (Tamir et al. 2001).

Glabridin and Glabrene

Ofir et al. (2003) reported that both glabridin and glabrene inhibit the re-uptake of radioactive 5-hydroxytryptamine (5-HT) expressed in human serotonin transporter (hSERT) when assayed in HEK-293 cells. Cells were incubated for 20 min with 50 µM of glabradin or glabrene. Radioactivity was measured in scintillation liquid. By combining 4 experiments, it was found that glabridin's inhibition was 59.5 ± 5.8% and glabrene's was 45.7 ± 3.0%. Further experimentation showed that glabridin's inhibition effects were dose-dependent; the optimal effect was found at ~50 µM. The authors suggested that the effects of glabridin and glabrene on the serotonergic system and the inhibition of serotonin re-uptake may be beneficial for mild to moderate depression in pre- and postmenopausal women.

Somjen et al. (2004a) tested for estrogen activity of glabridin and glabrene in rat and human bone cells. Female bone cells from pre- and post- menopausal women (from the trabecular surface of the iliac crest or long bones) were incubated with 30 nM estradiol-17β (control) or 3000 nM glabridin or glabrene and creatine kinase (CK) specific activity was measured. An increase in CK specific activity compared to controls was found in both pre- and post-menopausal cells treated with glabridin ($p < 0.01$ for pre-menopausal; $p < 0.05$ for post-menopausal).

The same finding was reported for glabrene (0.05 for pre-menopausal; $p < 0.01$ for post-menopausal). The basal activity of creatine kinase was 0.020 ± 0.005 µmol/min/mg protein (pre-menopausal) and 0.016 ± 0.004 µmol/min/mg protein (post-menopausal). The increase was greater in the case of glabridin in pre-menopausal cells but lower than glabrene in post-menopausal cells.

Table 58. Effect of increasing concentrations of glabridin on anchorage-independent growth of MCF-7 cells (Tamir et al. 2000).

Treatment	Colonies
Estradiol, 10 nM (control)	11 ± 4
Galbridin, 10 µM	7 ± 3
Galbridin, 25 µM	3 ± 1
Galbridin, 35 µM	1 ± 0.8
Estradiol, 10 nM + Galbridin, 10 µM	8 ± 2
Estradiol, 10 nM + Galbridin, 25 µM	4 ± 2
Estradiol, 10 nM + Galbridin, 35 µM	0

The experiment was repeated with the human female bone cells being pre-incubated with less-calcemic vitamin D analogs, CB 1093 (1 nM), and non-calcemic JK 1624 F2-2 (1 nM), daily for 3 d and then treated for 24 h with either estradiol (30 nM), glabridin (3000 nM) or glabrene (3000 nM). There was enhancement of the stimulation of CK synthesis by estradiol (E2) ($p < 0.01$ for both pre- and post-menopausal for both vitamins) and glabridin ($p < 0.001$ for both pre- and post menopausal for CB 1093 and pre-menopausal for JKF; $p < 0.01$ for post-menopausal for non-calcemic JK 1624 F2-2). The response of pre-menopausal cells to glabrene with CB was not different from the control; with non-calcemic JK 1624 F2-2 the response was increased ($p < 0.05$). The response of post-menopausal cells to glabrene with CB and non-calcemic JK 1624 F2-2 was increased, but not as much as with glabridin ($p < 0.05$). The effects of estradiol 17β and glabridin was enhanced by pretreatment with the vitamin D analog CB 1093.

Immature female Wistar rats (25 d old; n = 4) were administered a single injection of glabridin or glabrene, ranging from 3 to 300 µg/rat. The rats were killed and CK measured 24 h after injection. In the immature rats, CK at both the diaphyseal bone and the epiphyseal cartilage showed an increase relative to vehicle-treated controls at 3 µg/rat ($p < 0.05$). The effects reached near plateau between 25 and 300 µg/rat ($p < 0.01$). Ovariectomized female rats (2 wk after surgery; n = 10) were injected with 100 µg glabridin, killed 24 h after injection and assayed for CK. The specific activity of CK was stimulated in diaphyseal bone ($p < 0.01$) and epiphyseal cartilage ($p < 0.01$) compared to controls.

The authors also fed female rats (n = 6) by direct stomach tube daily for 4 d at doses of 2.5 to 250 µg/day glabridin or E2 at 0.5 to 5 µg/d. The CK specific activity in diaphyseal bone ($p < 0.01$ - 0.001) and epiphyseal cartilage ($p < 0.05$) was stimulated when compared to controls.

Ovariectomized female rats (n not given) were injected (i.p.) with glabridin at 100 µg/rat. The specific activities of CK in

diaphyseal bone ($p < 0.01$) and epiphyseal cartilage ($p < 0.01$) were stimulated.

The authors concluded that glabridin is more similar to estradiol-17β than glabrene and has greater potential, with or without vitamin D, to modulate bone disorders in post-menopausal women (Somjen et al. 2004a).

Somjen et al. (2004b) tested for estrogen activity of glabridin and glabrene extracted by multiple solvents from *G. glabra* powdered root by measuring CK specific activity in the left ventrical. A single i.p. injection of estradiol, glabradin, or glabrene (0.3 - 300 µg) was administered to ovariectomized female Wistar rats 2 wk after ovariectomization (n = 5 for each group) or in 25-day-old immature female rats (n = 5). Matched control rats (n = 5) were injected with vehicle (0.05% ethanol in PBS). E2 was injected at 5 µg for immature and 10 µg for ovariectomized female rats. The rats were killed 24 h after the injection and the organs removed and stored at -20°C. Glabridin (2.5 - 250 g/d) and estradiol (0.5 - 5 µg/d) were administered to additional rats (n = 5 for each dose level) by feeding the rats directly into the stomach daily for 4 d. The rats were killed and the organs stored as above. The specific activity of CK was measured in the left ventricle of the heart and the aorta using a coupled spectrophotometric assay. Protein was determined by Coomasie blue dye binding.

In immature female rats, stimulation of CK by glabridin and glabrene in both organs was maximal at 300 µg. The increase in activity was evident at 30 µg when compared to controls. In ovariectomized rats, the increase in CK activity was significant when compared to controls; in both organs, the effect of a single injection of glabridin was similar to a single injection of estradiol. The CK activity for glabridin is shown in **Table 59**.

Table 59. Maximal stimulation of CK activity (µmols/min/mg protein) in ovariectomized and immature female rats injected with glabridin and estradiol (Somjen et al. 2004b).

	Aorta [a]	Left ventricle [a]
Ovariectomized female rats		
Basal	0.36 ± 0.05	1.51 ± 0.39
Control	1.00 ± 0.16	1.00 ± 0.22
Estradiol	2.00 ± 0.19[c]	1.59 ± 0.10[c]
Glabridin	1.65 ± 0.19[c]	1.51 ± 0.16[c]
Immature female rats		
Basal	0.73 ± 0.07	0.65 ± 0.29
Control	1.00 ± 0.06	1.00 ± 0.11
Estradiol	1.50 ± 0.15[b]	1.37 ± 0.13[b]
Glabridin	1.38 ± 0.10	1.78 ± 0.17[c]
Glabridin + estradiol	2.29 ± 0.13[c]	2.08 ± 0.16[c]

[a] mean ± SEM of ratios of the specific activities of CK in hormone-treated and control animals - 8 assays from 2 experiments

[b] $p < 0.05$; [c] $p < 0.001$

Daily feeding of glabridin, estradiol, or both for 3 d to immature female rats stimulated CK specific activity in the left ventricle and the aorta. The effects on the aorta were much stronger than on the left ventricle. When the daily feeding with 2.5 µg/d of E2 or 25 µg/d of glabridin or both were extended for 2 wk, the effects of glabridin were the same as those of estradiol in the aorta and both were additive as shown in **Table 60**. The effects on the left ventricle were lower than on the aorta. In the left ventricle, glabridin was more effective than E2 and was not significantly different than the mixture.

The authors also incubated vascular smooth muscle cells (VSMC; which contain both ER α and β) and ECV-304 (E304) cells (an endothelial cell line derived from a human umbilical vein containing both ER α and β) in glabridin and glabrene at increasing concentrations.

Twenty-two h after exposure, [3H]thymidine was added for 2 h. After treatment with 10% trichloroacetic acid and washing, the cellular layer was dissolved in 0.3 ml of 0.3 N NaOH and aspirated. The amount of [3H]thymidine incorporated into the DNA was determined as well as the CK specific activity. In VSMC, glabridin and glabrene stimulated DNA synthesis at low concentrations (up to ~100 nM) but at high concentrations glabridin had an inhibitory effect while glabrene appeared to have none on DNA synthesis. In E304 cells, the dose-dependent stimulation of DNA synthesis was observed in both compounds. For both glabidin and glabrene, there was a dose-dependent stimulation of CK specific activity in BSMC and E304 cells.

The authors suggested that the use of glabradene, with or without estradiol, may modulate vascular injury and atherogenesis for the prevention of cardiovascular diseases in post-menopausal women (Somjen et al. 2004b).

Choi (2005) reported that glabridin (1 to 10 µM) can increase cell growth, alkaline phosphatase activity, collagen sythesis and osteocalcin secretion in osteoblastic MC3T3-E1 cells in vitro. These effects were blocked by the presence of cycloheximide, an inhibitor of protein synthesis. The effects of glabridin in increasing alkaline phosphatase activity and collagen synthesis in osteoblastic cells were blocked by the anti-estrogen tamoxifen.

Table 60. Stimulation of CK specific activity (µmols/min/mg protein) by repeated oral administration of glabridin (25 µg), estradiol (0.5 µg), or both in immature female rats (Somjen et al. 2004b).

	Aorta [a]	Left Ventrical [a]
Basal	0.73 ± 0.07	0.65 ± 0.29
Control	1.00 ± 0.06	1.00 ± 0.11
E_2	1.50 ± 0.10[b]	1.37 ± 0.13[b]
Glabridin	1.38 ± 0.10[b]	1.78 ± 0.17[c]
Glabridin + E_2	2.29 ± 0.13[c]	2.08 ± 0.16[c]

[a] mean ± SEM of ratios of the specific activities of CK in hormone-treated and control animals - 8 assays from 2 experiments

[b] $p < 0.05$; [c] $p < 0.001$

Isoliquiritigenin

Maggiolini et al. (2002) tested the estrogenic and antiproliferative effects of isoliquiritigenin in MCF7 breast cancer cells. Endogenous ERα was transfected into MCF7 cells (which contain no ER β) with the gene reporter XETL. A plasmid that expresses renilla luciferase enzymatically was cotransfected and used as an internal transfection control. Isoliquiritigenin induced maximal XETL expression at a concentration of 1 µM; 10 nM was sufficient to achieve 8-fold induction. The authors stated that isoliquiritigenin bound to the Erα receptor.

The authors investigated whether the levels of ER α were sensitive to isoliquiritigenin in MCF7 cells. After 24 h of exposure to 10 nM isoliquiritigenin, the receptor protein content was down-regulated compared with 10 nM estradiol ($p < .01$). The authors stated that this is evidence of isoliquititigenin ERα autoregulation.

The authors tested whether the expression of pS2 mRNA is sensitive to isoliquiritigenin in MCF7 cells. The mRNA levels were compared with semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and standardized using the mRNA levels of the house-keeping gene 36B4. A 24 h exposure to 10 nM isoliquiritigenin up-regulated the mRNA of pS2 overlapping the expression induced by 10 nM E2 ($p < .01$). The authors stated that isoliquiritigenin is an estrogenic agonist.

Proliferation of MCF7 and HeLa cells were treated with increasing concentrations of isoliquiritigenin for 5 d then counted and compared to untreated cells on the sixth day. Concentrations up to 1 µM induced MCF7 cell growth and the stimulation observed with 10 nM was inhibited by 1 µM of the anti-estrogen hydroxytamoxifen, confirming that an estrogen receptor α-mediated mechanism is involved in cell proliferation. The addition of 10 µM isoliquiritigenin induced a drop in cell number due to cytotoxicity demonstrating that the effects of isoliquiritigenin is biphasic.

The authors suggested that the cytotoxic effect is not estrogen receptor dependent (Maggiolini et al. 2002)..

Licochalcone A

Rafi et al. (2000) tested for ER-binding activity of licochalcone A on the yeast Saccharomyces cerevisaiae strain PL3, with a URA3 gene under the control of the hER. In this assay system, trascription of URA3, which is required for the growth of the cells on media lacking uracil, is dependent on the activation of the hER by estrogen. Cells were seeded (5,000 or 1,000 yeast cells) in medium lacking uracil and treated with 50 µM licochalcone A. Incubation was 72 h. The vehicle control was ethanol. Licochalcone A at 50 µM supported growth at both cell concentrations. The positive control, estradiol, supported growth at 0.1 nM.

Multiple Licorice Constituents

Wang and Kurzer (1997) tested the effects of phytoestrogens from licorice on estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-231) breast cancer cells. In a dose-response experiment, 1 to 1.5 x 105 cells/well were seeded and incubated for 24 h with 0.01, 0.1, 1.0, 10, 50, and 100 µM of each of the phytoestrogens, except for chrysin at 0.01, 0.1, 1.0, 10, 20, and 30 µM. Cell viability was similar to controls.

All phytoestrogens tested stimulated DNA synthesis in MCF-7 cells at low concentrations compared to controls; at high concentrations (50 to 100 µM), the isoflavonoids genistein, biochanin A and coumestrol inhibited DNA synthesis, as did the flavones chrysin and apigenin, and the lignan enterolactone. Rutin stimulated DNA synthesis to 250% of control at 100 µM and showed no inhibition effects at the tested concentrations.

Most of the compounds inhibited DNA synthesis at high concentrations in MDA-MB-231 cells as shown in **Table 61.**

Table 61. Effects of isoflavonoids, flavones, rutin, and enterolactone on DNA synthesis in MCF-7 and MDA-MB-231 cells (Wang and Kurzer 1997).

	MCF-7			MDA-MB=231		
Compound	**Max stimulation (% of control)**	**Concentration (µM)**	**IC_{50} (µM)**	**Max stimulation (% of control)**	**Concentration (µM)**	**IC_{50} (µM)**
Isoflavonoids						
Genistein	215[a]	1.0	41.0			26.7
Daidzein	210[a]	10				81.2
Biochanin A	235[a]	1.0	37.7	110[a]	0.01	35.5
Coumestrol	185[a]	0.1	42.5			24.4
Flavones						
Chrysin	120[a]	10	23.0			>30.0
Apigenin	170[a]	1.0	27.7			22.5
Flavone			61.0	110[b]	0.1	28.2
Other Flavonoid						
Rutin	250[a]	100				>100
Lignans						
Enterolactone	210[a]	10	82.0			>100

[a] $p < .001$; [b] $p < .05$

The isoflavonoids genistein, daidzein, biochanin A, and coumestrol inhibited DNA synthesis, as did the flavones chrysin, apigenin, and flavone and the flavonols quercetin, and kaempferol. Rutin, catechin, and enterolactone were weak inhibitors. Biochanin A and flavone showed slight stimulation to 110% of control at 0.01 to 1.0 and 0.1 to 1.0 µM, respectively.

For a genistein incubation and withdrawal experiment, 1.5 x 105 MCF-7 cells/well were seeded, genistein added (0.01 to 100 µM), and the cells incubated for 48 h with the medium being changed at 24 h. The medium was then changed to the control medium and the cells were incubated for another 48 h with the medium being changed at 24 h. DNA synsthesis was determined every 24 h.

DNA synthesis data are shown in **Table 62.** Incubation of MCF-7 cells at 0.01 to 10 µM genistein for 24 h stimulated DNA synthesis to 130% to 184% of control ($p < .01$) and 144% to 263% of control ($p < .01$) at 48 h. Incubation at 50 to 100 µM inhibited DNA synthesis to 39% to 55% of control ($p < .01$) at 24 h and 13% to 42% ($p < .01$) at 48 h.

After removal of the genistein, DNA synthesis levels returned to control levels in 24 h for the 0.1 and 1.0 µM treatments; the other elevated levels remained elevated ($p < .01$).

The 50 µM treatment group elevated DNA synthesis to 186% of controls ($p < .01$) 24 h after removal. All treatment groups were elevated at 48 h ($p < .01$) except for the 0.1 µM groups which inhibited DNA synthesis ($p < .05$) and the 1.0 group which was similar to the control.

To explore the long-term exposure effects of genistein and coumestrol to MCF-7 cells, 5 to 7 x 104 cells were seeded and exposed to one of these compounds at various concentrations for 10 d. In this study, DNA synthesis was inhibited by genistein at the higher concentrations of genistein (50 and 100 µM; $p < .05$ and .01) for the entire 10 d with cytotoxicity occuring after day 4. At 0.1 and 1.0 µM genistein, there was stimulation up to >300% control ($p < .05$ and .01); 0.1 µM stimulated DNA synthesis to almost 200% by day 10 ($p < .05$ and .01).

Results for exposure to coumestrol were similar to genistein. DNA synthesis was inhibited by coumestrol at 50 and 100 µM and was stimulated by 0.01 to 10 µM for the 10 d ($p < .01$ for all values).

The authors suggested that the inhibition of estrogen-dependent and estrogen independent breast cancer cells may be because of mechanisms independent of the estrogen receptors and that the biphasic effects of the phytoestrogens need to be taken into account if using them for cancer prevention (Wang and Kurzer 1997).

Nomura et al. (2002) reported that compounds isolated from Glycyrrhiza species have weak binding affinities (IC50 < 1 µg/ml) in an estrogen receptor binding assay as shown in **Table 63.**

Hillerns et al. (2005) tested the binding ability of phytoestrogens from G. glabra root. The uteri from freshly killed Sprague-Dawley rats were homogenized and centrifuged. The supernatant containing the estrogen receptors was mixed with buffer, [3H]-estradiol (4 pM to 20 µM), or a phytoestrogen (0.05 nM to 0.5 mM) and incubated for 4 h. Incubation was terminated with dextran coated charcoal. Unbound [3H]-estradiol was removed by centrifugation. The supernatant was mixed with scintillation cocktail and radioactivity was measured in a liquid scintillation counter.

The relative binding affinity of 17β-estradiol (E_2) and licorice phytoestrogens for rat uterine estrogen receptors are listed in **Table 64.** Displacement of estradiol was not detected for enterolactone and enterodiol up to 500 µM. The authors also assayed datiscetin, galangin, fisetin, 4',7-OCH3-quercetin, chrysin, pinocembrin, and liquiritigenin; only liquiritigenin had a weak binding to ER.

To test for binding affinity of G. glabra root phytoestrogens, the authors also treated human plasma with dextran coated charcoal to remove endogenous steroids then bound plama glycoproteins.

Table 62. Time course of genistein-stimulated DNA synthesis from licorice incubation and removal on DNA synthesis in MCF-7 breast cancer cells (Wang and Kurzer 1997).

Genistein concentration (µM)	Genistein treatment (%)		Genistein removal (%)	
	24 h	48 h	24 h	48 h
0.01	130 ± 12[a]	144 ± 12[a]	220 ± 31[a]	282 ± 20[a]
0.1	184 ± 13[a]	239 ± 17[a]	92 ± 9	84 ± 13[b]
1.0	183 ± 24[a]	263 ± 24[a]	110 ± 14	87 ± 12
5.0	154 ± 14[a]	242 ± 16[a]	192 ± 19[a]	138 ± 8[a]
10	141 ± 13[a]	252 ± 21[a]	257 ± 15[a]	127 ± 9[a]
25	89 ± 5	123 ± 11[b]	186 ± 31[a]	128 ± 20[a]
50	55 ± 12[a]	42 ± 12[a]	186 ± 31[a]	128 ± 20[a]
100	39 ± 13[a]	13 ± 17[a]	66 ± 9[a]	368 ± 13[a]

[a] $p < .001$; [b] $p < .05$

Table 63. Estrogen receptor binding affinities of phenolic compounds found in licoirce plants (Nomura et al. 2002).

Test Compound	Relative binding affinity [a]	Source(s)
Gancaonis R	0.016	*G. uralensis (aerial part)*
Glabrene	0.0022	*G. glabra* (root)
Glycyrol	0.0011	*G. uralensis* (underground part)
Sigmoidin B	0.00077	*G. uralensis* (aerial part)
Liquiritigenin	0.00038	*Glycyrrhiza* sp. (root and aerial part)

[a] binding of test compound conpared to 100 for 17β-estradiol

The plasma was centrifuged and the pellet washed resulting in bound sex hormone-binding globulins (SHBG). The SHBG was incubated with 400 µl [3H]-5α-dihydrotestosterone (DHT; 6 nM) or [3H]-E2, DHT cold ligand, or a phytoestrogen (10nM to 1mM) for 60 min with periodic stirring (n = 3). The pellet was centrifuged and washed. The pellet was mixed with scintillation cocktail as above and radioactivity was measured with a liquid scintillation counter.

Most of the tested compounds displaced estradiol 2 to 30 times more effectively than DHT as shown in **Table 65.** Some phytoestrogens were specific for estradiol or DHT. E2 was displaced with weak relative binding affinity (RBA) between 0.02 and 0.14 by prunetin, daidzein, formononetin, liquiritigenin, datiscetin, fisetin, and isoliquiritigenin. The authors concluded that several of the phytoestrogens in G. glabra root compete with 17β-estradiol for binding to SHBG (Hillerns et al. 2005).

Table 64. Relative binding affinity (RBA) values of 17β-estradiol (E_2) and licorice phytoestrogens for rat uterine estrogen receptors (Hillerns et al. 2005).

Compound	RBA
17β-Estradiol (E_2)	100.00
Coumestrol	4.199
Genistein	0.696
Phloretin	0.206
Daidzein	0.080
Isoliquiritigenin	0.059
NDGA	0.046
Prunetin	0.038
Apigenin	0.037
Formononetin	0.036
Biochanin A	0.026
Liquiritigenin	0.002

Lee et al. (2007) tested the effect of glycyrrhizin, glycyrrhetinci acid, liquiritic acid, glabrolide, liquiritigenin, isoliquiritigenin, and formononetin, isolated from G. glabra or G. uralensis, on the release of growth hormone in rat pituitary gland cells. When the rat pituitary cells were incubated with rat growth hormone releasing hormone (0.1, 0.3, 0.5, or 1.0 µM), each of the licorice constituents, glycyrrhetinic acid (10 µg/ml; n = 5) and glycyrrhizin (10 µg/ml; n = 5), increased growth hormone release by 1.42 times ($p < .05$; 0.25 ± 0.02 nM) and 1.43 times ($p < .05$; 0.26 ± 0.02 nM) the control, respectively. The other constituents had no significant effect. When the constituents were injected into an anesthetized rat and blood samples taken at timed intervals, isoliquiritigenin, formononetin, liquiritigenin, liquiritic acid, glabrolide, glycyrrhetinic acid, and glycyrrhizin increased adrenocorticotropic hormone release by 11.6, 13.2, 13.8, 13.4, 14.3, 13.0, and 14.7 times the control ($p < .01$), respectively.

Chalcones

De Vincenzo et al. (1995) determined the effect of the chalcone 2',4,4,-trihydroxychalcone on the proliferation of both established and primary ovarian cancer cells expressing type II estrogen binding sites (type II EBS). Controls cells were treated with the medium alone. Treatment was repeated at 24 h intervals. After 3 d of treatment hemocytometer counts were performed. The IC_{50} was 5.0 µM.

The authors also tested the ability of 2',4,4,-tryhydroxychalcone to displace [3H]estradiol ([3H]E2) binding to type II EBSin OVCA 422 cells. The concentration resulting in a 50% displacement was 3.2 µM. The authors concluded that this chalcone demonstrated growth inhibition effects on ovarian cancer cells (De Vincenzo et al. 1995).

Licorice Extract Fractions

Zayed et al. (1964) used fractionation techniques to break down 1 kg dry root of G. glabra as shown in Figure 1 (in Book 1 of this assessment). The resulting fractions were tested for estrogenic activity using the mouse uterotrophic assay (n not given).

Table 65. EC_{50}, K_a, and RBA values for E_2, 5α-dihydrotestosterone (DHT), and phytoestrogens for binding to SHBG (Hillerns et al. 2005).

	E_2 (SHBG)[a]				5α-DHT (SHBG)[b]			
Compound	EC_{50} (μM)	K_a x 10^{-8}	± SEM	RBA	EC_{50} (μM)	K_a x 10^{-8}	± SEM	RBA
E_2 0.101	9.794	2.264	100.000	-	-			
DHT	-	-		-	0.017	41.933	11.540	100.00
Coumestrol	13.010	1.057	0.358	0.776	21.660	0.652	0.232	0.078
Chrysin	28.460	0.454	0.108	0.355	nd	-	-	-
Galangin	32.650	0.394	0.138	0.309	64.770	0.216	0.135	0.026
Genistein	67.450	0.186	0.063	0.150	162.200	0.086	0.042	0.010
NDGA	71.880	0.175	0.045	0.141	43.730	0.320	0.116	0.039
Prunetin	72.780	0.172	0.106	0.139	nd	-	-	-
Daidzein	78.230	0.160	0.087	0.129	nd	-	-	-
Apigenin	80.190	0.156	0.038	0.126	260.400	0.053	0.031	0.006
Pinocembrin	95.560	0.131	0.037	0.106	540.600	0.026	0.009	0.003
Phloretin	106.900	0.117	0.041	0.094	249.400	0.056	0.015	0.007
Liquiritigenin	125.700	0.099	0.052	0.080	nd	-	-	-
Formononetin	133.900	0.093	0.049	0.075	nd	-	-	-
Enterolactone	201.900	0.061	0.015	0.050	175.000	0.080	0.036	0.010
Datiscetin	274.600	0.045	0.012	0.037	nd	-	-	-
Biochanin A	444.100	01028	0.016	0.023	132.100	0.105	0.051	0.013
Isoliquiritigenin	552.800	0.022	0.011	0.018	nd	-	-	-
Fisetin	669.300	0.018	0.009	0.015	nd	-	-	-
Enterodiol	nd	-	-	nd	-	-	-	-
4',7OCH_3-Quercetin	nd	-	-	nd	-	-	-	-

[a] Diluted plasma 1/10; [b] Diluted plasma 1/20; nd = not detected

The fractions having estrogenic activity are shown in **Table 66.** Fractions that showed no estrogenic activity up to 200 μg/d were: non-alchohols, acids, phenols, and methanol-solid residue.

Gene Expression

Flavonoids

Aoki et al (2007a) tested the effects of incorporating licorice flavonoids into the food of mice on the expression of fat producing genes. The mice (8 wk old) were fed a high fat diet for 8 wk. They were then divided into 4 groups (n = 10). Licorice flavonoid oil extracted from *G. glabra* roots (medium-chain triglyceride mixture 88%; 1.2% was glabridin; solid fraction ~12%) was fed to the mice at 0, 0.5%, 1.0%, or 2.0% incorporated into their high-fat diet for an additional 8 wk. They were allowed food and water ad libitum, weighed weekly, and food intake per cage calculated weekly. At the end of the treatment period, unfasted mice were killed and blood, mesenteric, perirenal, and periuterine white adipose tissues, liver, kidney, and spleen samples were collected. Serum was collected to measure insulin, leptin, and adiponectin levels. Livers and mesenteric white adipose tissue were prepared for microscopic evaluation. RNA (7 mice/group) and DNA (3 mice/group) from the liver samples were analyzed by microarray. The relative expression levels of the control and 2% groups were calibrated and all differences greater than 2-fold were categorized by gene ontology.

The body weight of the controls increased to a mean of 33.1 ± 2.3 g; the mean body weights of the 1% and 2% groups were reduced ($p < .01$). Feed consumption was not affected by the addition of the flavonoids. Daily licorice flavonoid oil consumption for the 0.5%, 1%, and 2% groups were 386, 804, and 1640 mg/kg/d, respectively. The weights of the mesenteric, perirenal and periuterine white adipose tissue were reduced in the 1% and 2% groups ($p < .05$ or .01).

There were no weight differences among the livers, kidney and spleens. Motor activity was not affected. Histological examination showed reduced diameters of adipocytes in the 2% group compared to controls. Lipid droplets in the hepatocytes were abundant in the control group but not the 2% group; there were no adverse histological findings in the liver or mesenteric white adipose tissue.

Table 66. The estrogenic activity of different fractions obtained by fractionation of dried *G. glabra* root in a mouse uterotrophic assay (Zayed et al. 1964).

Fraction	Daily Dose (µg)	Average uterine weight (mg/100 mg body weight)		Equivalent dosage of estradiol (µg)[a]	Estrogenic activity comparison (Standard:fraction)
		Control	Experimental		
Crude extract	20,000	58.2	162.5	0.0105	1:5,714,200
Free acids (semi-solid)	5,000	56.1	132.3	0.0093	1:1,612,900
Free acids (solidified)	500	59.2	70.1	0.0072	1:208,300
Acid-free fraction	600	52.1	62.9	0.0071	1:253,500
Neutral fraction	200	51.7	74.8	0.0074	1:80,000
Alcohols	125	49.1	66.7	0.0071	1:53,500
Crystallized compound (impure)	10	48.3	62.4	0.0070	1:4,200
β-Sitosterol	1	53.1	73.1	0.0074	1:400

[a] Weight of estradiol monobenzoate (standard) necessary to give the same response produced by 3 daily dosed of tested fractions.

The authors suggested that the lack of differences in the liver, kidney, and spleen weights suggest that the effects of licorice flavonoid oil were not due to toxicity. Based on the results from an additional experiment, where the same type of mice were fed either a normal or a high-fat diet, which resulted in an 18.8% higher weight increase in the high-fat diet mice compared to the high-fat plus 2% licorice flavonoid oil mice group, the authors concluded that the treatment resulted in the suppression of the high-fat diet-induced obesity.

There were 9 up-regulated and 13 down regulated genes. The up-regulated genes were involved in beta-oxidation and acyl-CoA degradation (enoyl-CoA; hydratase/3-hydroxyacyl CoA dehydrogenase, 2.09-fold change; acyl-CoA thiesterase 1, 2.72-fold change). Genes for acetyl-CoA synthesis (ATP citrate lyase, -2.11-fold change; acetyl-CoA synthetase 2 (-2.06-fold change) were down regulated.

Pyruvate kinase in the liver and RBC (a rate-limiting enzyme involved in glycolytic lipogenesis) was down regulated. Polymerase chain reaction (PCR) analysis showed gene expression of hydratase/3-hydroxyacyl CoA dehydrogenase increased by 2.49-fold compared to controls ($p < .001$).

The expression levels of ATP citrate lyase and acetyl-CoA were decreased by -2.51- and 2.25-fold (p .001), respectively. Serum insulin decreased in a dose-dependent manner with the 2% group measuring less than half that of the control group. The authors concluded that the change in fatty acid metabolism in the liver is due to the consumption of licorice flavonoid oil and the resulting change in expression of the above mentioned genes (Aoki et al. 2007a).

GENOTOXICITY

Licorice Flavonoid Oil

Nakagawa et al. (2008a) tested the genotoxicity of licorice flavonoid oil, extracted from *G. glabra*. In a reverse mutation assay using *S. typhimurium* (strains TA98, TA100, AT1535, and TA1537) and *E. coli* (WP2uvr/pKM101), licorice flavonoid oil (0 to 5000 µg/plate, with and without metabolic activation) was tested. The positive controls were sodium azide, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, 2-aminoanthracene, and B[a]P. Licorice flavonoid oild did not induce more than double the number of revertant colonies compared to the negative control.

Licorice flavonoid oil was tested using the chromosomal aberration assay with Chinese hamster lung cells. The cells were incubated for 6 h (0.1 to 0.5 mg/ml), with and without activation, and for 24 and 48 h (0.2 to 0.8 mg/ml) without activation. The final slides were examined blind. In the 6-h test, the results without activation were < 5%, the threshold for a positive test. However, with activation, there was a structural aberration induction rate of 10.5% at 0.6 mg/ml, above the 10% threshold.

In a confirmation test, structural aberrations induction rates of 11.0%, 17.0%, and 30.0% were observed at 0.6, 0.65, and 0.7 mg/ml licorice flavonoid oil, respectively. The growth index was 62%, 48%, and 34%, respectively. In the 2 longer studies, structural chromosomal aberrations and the polyploidy induction rates were < 5% at all concentrations. The authors concluded that licorice flavonoid oil induced structural chromosomal aberrations only in the presence of metabolic activation, therefore it was considered genotoxic for mammalian cells in vitro.

In a bone marrow micronucleus test using F344/DuCrlCrlj rats (n = 5), licorice flavonoid oil (625, 1250 2500, or 5000 mg/kg/d for 2 d) showed no significant or dose-dependent increases in the frequency of micronucleated polychromatic erythrocytes.

In a peripheral blood and liver micronucleus test using rats (n = 4), licorice flavonoid oil was administered orally (2500 of 5000 mg/kg/d in olive oil) twice per day on days 1, 2, and 6. The controls were the vehicle or a single injection (i.p.) of mitomycin C (1 mg/kg) on day 2. Peripheral blood was collected on day 4. On day 5, two/thirds of the liver was collected under anesthesia. There were no increases in micronuclei induction in either the peripheral blood or livers of the licorice flavonoid oil-treated rats

compared to the negative control. Cell division of hepatocytes after partial hepatectomy was thought to be adequately induced because micronuclei induction in the liver in the negative control group was within the historical control data. Mild diarrhea was observed in almost all treated rats, but the authors suggested that this did not have any influence on hepatic regeneration (Nakagawa et al 2008a).

Anti-mutagenicity

Various Compounds

Mitscher et al. (1986) fractioned Glycyrrhiza Glabra (Licorice) Root Extract to obtain a semipurified polar-lipid fraction which demonstrated activity against spontaneous mutations only at near-toxic doses to *S. typhimurium* TA100 in an Ames test. In further Ames tests, glabrene was antimutagenic against spontaneous and EMS-induced mutations up to 1.0 µg/plate; it was toxic at 10 µg/plate. Glabrol, fromononet, and glabridin were not antimutagenic. Glabridin was toxic at 1.0 µg/plate. Hispaglabridin A was not antimutagenic up to 1.0 µg/plate.

Nomura et al. (2002) evaluated the rec-assay for detection of bioactive compounds in phenolic compounds obtained from licorice. Sixty-nine compounds in 108 Glycyrrhiza phenols showed inhibitory activity for the growth of both B. subtilis H17 and 45. Among the active compounds, 7 compounds (isoliquiritigenin, semilicoisoflavone B, gancaonin C(6-(E)-3-hydroxymethyl-2-butenylgenistein), 6- and 8-prenylated eriodictylos, licoisoflavone B, and licoisoflavone (2,3-dihydrolicoisoflavone B)) showed positive results in the rec-assay.

Cancer Inhibition and Tumor Suppression

Glycyrrhizic Acid and Derivatives

In an earlier safety assessment, CIR (2005) reported that topical application of glycyrrhetinic acid inhibited tumor initiation and promotion by 7,12-dimethylbenz[a]antracene (DMBA) and TPA in mice. Topical application of 18α- or 18β-glycyrrhetinic acid inhibited the binding of radiolabeled DMBA and B[a]P to epidermal DNA; they also inhibited the TPA-induced increases in ornithine decarboxylase activity and lipoxygenase activity in vitro. Glycyrrhetinic acid inhibited the tumor-promoting activity of TPA and teleocidin. Glycyrrhetinic acid dose-dependently inhibited the binding of TPA to the TPA receptor in mouse skin.

Glycyrrhizic acid (0.05%) in the drinking water of mice reduced the number of tumors in mice that were administered DMBA topically. Pre-treatment or post-treatment of mice with glycyrrhizic acid did not protect from lethal tumors, however, combined pre- and post-treatment delayed the 50% mortality date from day 15 to 18-19 when Erlich ascites tumor cells were transplanted (CIR 2005).

Dibenzoylmethane (DBM)

Singletary and MacDonald (2000) tested the inhibition capacity of DBM (Figure 12) on the formation of DNA adducts following exposure to B[a]P and 1,6-dinitropyrene (1,6-DNP) and stimulation effects on the expression of GST and NAD(P)H-quinone reductase (QR) proteins in the human mammary epithelial cell line MCF-10F. Cells (1×10^5) were added to T150 flasks containing medium and supplements. After overnight attachment and before confluence, either DBM (98%) or sulforaphane (97%) were added (0.1 to 2 µM) for 7 d with medium changes every 2 d. On day 7, the cells received fresh medium with B[a]P (0.3 µM) for 12 h or 1,6-DNP (10 µM) for 22 h. Cells were harvested and DNA isolated or the cytosolic fraction prepared.

Aliquots of DNA were analyzed for either B[a]P-DNA or 1,6-DNP adducts by 32P-postlabeling. For quantitation of adducts, adduct spots were removed from TLC plates and radioactivity was determined by scintillation counting. Immunoblot (Western) analysis was also performed using either QR antibody (rabbit anti-NQO1E. coli) or GSTP-1 antibody. Bands were detected by enhanced chemiluninescence and quatitated by densitometry.

Neither DBM nor sulforaphane had toxic effects toward the MCF-10F cells at concentrations and periods of exposure in this experiment. B[a]P-adduct formation increased linearly when cells were exposed to B[a]P for 12 h at concentrations from 0.08 to 0.8 µM. The formation of B[a]P-DNA adducts was inhibited by 63%, 62%, and 81% for cells treated with 0.1, 0.5, and 2.0 µM DBM, respectively, compared to controls. For comparison, cells exposed to 1,6-DNP had increased adduct spot formation when cells were incubated for 22 h at 5 and 10 but not 50 µM. Total 1,6,-DNP-DNA adduct spot formation was inhibited by 46%, 61%, and 50% for samples treated with 0.1, 0.5, and 2.0 µM sulforaphane, respectively, compared to controls.

DBM increased QR protein expression, compared to controls, but only at the 2.0 µM concentration. DBM had no effect on GSTP-1 protein levels. For comparison, sulforaphane increased QR protein and GSTP-1 protein levels by 3- to 5-fold and 3- to 4-fold, respectively, at concentrations from 0.5 to 2.0 µM (Singletary and MacDonald 2000).

Lin et al. (2001) tested the DMBA-induced mammary cancer preventive effects of DBM on female Sencar mice. The mice were fed a diet of laboratory chow with AIN-76A or 1% DBM in AIN-76A. Two weeks into the diet, the mice were intubated with 1 mg DMBA (0.1 ml corn oil) once/week for 5 wk. The sizes of palpable mammary tumors were measured; mice with tumors >15 mm were killed. All mice were killed at 20 wk after the last dose of DMBA.

The mice in the control group (DMBA for 5 wk) developed 0.025, 0.50, 0.73, and 1.08 palpable mammary tumors/mouse at 3, 9, 13, and 20 wk after last DMBA dose, respectively. Mice in the treatment group (1% DBM) did not have any palpable tumors until 18 wk. Tumor incidence in the 1% DBM treated mice was 97 to 100%.

In a second feeding experiment, female mice were fed the control diet (n = 21) or diet plus 1% DBM (n = 20) for 4 to 5 wk. All mice were killed at 50 to 60 d during the estrus phase of the estrous cycle 1 h after injection with bromodeoxyruidine (BrdU) for DNA labeling. The mice were necropsied.

The 1% DBM diet inhibited the proliferation rate of mammary gland epithelial cells by 53% ($p < .05$), uterine epithelium by 23%, and uterine stoma by 77% ($p < .05$). Uterine wet weight was decreased by 43% ($p < .05$). Liver weight increased by 48% ($p < .05$). There was no difference in total body weight.

Eight groups (n = 9) of mice (5 wk old) were fed various amounts of DBM in AIN-76A: groups 1 and 5 were controls and fed only AIN-76A; groups 2 and 6 were fed 0.2% DBM; groups 3 and 7 were fed 0.5% DBM; and groups 4 and 8 were fed 1% DBM. Two weeks into the diet the mice were orally administered 1 mg DMBA in 0.1 ml corn oil once/week for 2 wk for groups 1 through 4 and for 5 wk for groups 5 through 8. The mice were killed 24 h after the last dose of DMBA and DNA adducts were determined using 32P-postlabeling.

The quantity of total adducts in the treatment groups 2 through 4 (DMBA for 2 wk) were less than controls ($p < .05$) for all treatment levels of DBM. The quantity of total adducts in treatment groups 6 through 8 (DMBA for 5 wk) was not different from control except for group 8 (1% DBM; $p < .05$) (Lin et al. 2001).

Jackson et al. (2002) exposed human prostate carcinoma cells (LNCaP, DU145, and PC-3), after reaching 70% confluence, to various concentrations (5, 10, 25, and 50 µM in DMSO; n = 3) of DBM. The viable cells were determined microscopically by exclusion of trypan blue. This experiment was repeated using MTT uptake to monitor growth. The cells were seeded (9 x 103 cells/well) to flatbottomed 96-well plates and allowed to grow overnight. The culture medium was replaced with medium containing DBM at the above concentrations and allowed to run for 3 d. The growth of all cell lines was inhibited by DBM. The estimated IC_{50} ranged from 25 to 100 µM; growth was inhibited in a dose-dependent manner and continued to have an effect for over 3 d of exposure without retreatment.

For cell cycle analysis, flow cytometry was performed on the prostate cancer cells after treatment with DBM (0 and 50 µM) in media for 72 h. Ten thousand events were recorded, stored, and analyzed by computer. The deregulation of the cell cycle correlated with the observed cytostatic effects of DBM. There was accumulation of cells in the G1 phase for the LNCaP and DU145 cell lines. There was an increase of cells in the S phase in the PC-3 cell line. There was no evidence of apoptosis by morphologic assessment (Jackson et al. 2002).

Pan et al. (2003) studied apoptosis of DBM. COLO 205 (CCL-222) cell line, developed from a poorly differentiated human colon adenocarcinoma, was used in a cell viability test. The cells were plated at 1 x 105 cells/ml in 24 well plates and incubated overnight. The cells were then pretreated with various concentrations of DBM for 48 h. MTT (30 µl) was added and the cells incubated an additional 4 h. Cell viability was measured by ELISA at 490 nm. DBM had an IC_{50} of 31 µM.

COLO 205 cells were incubated with various concentrations of DBM for 48 h. The genomic DNA from the cells was subjected to agarose gel electrophoresis. Cells treated with 50 µM DBM had DNA ladders that were just visible 48 h after treatment.

To test the caspase activity of DBM, 6 flurogenic peptide substrates were employed. Caspase activities were measured following treatment of COLO 205 cells with various concentrations of DBM for 48 h. DBM only slightly induced caspase-3 activity but none for caspase -2, -6, -8, or -9.

Activation of caspase-3 leads to the cleavage of several proteins, one of which is PARP. The cleavage of PARP is the hallmark of apoptosis. The authors determined the cleavage of PARP in COLO 205 cells with DBM at 24 and 48 h to be time-dependent and weak (Pan et al. 2003).

Frazier et al. (2004) performed 2 flourescence 2-D difference gel electrophoresis (2-D-DIGE) on human prostate carcinoma LNCaP cells where the lysates were treated with 0, 10 or 50 µM DBM for 72 h and labeled with N-hydroxy succinimidyl ester derivatives of Cy2, Cy3, an Cy5 CyDyes before placement on the gels. Analysis of protein was done by computer analysis.

The 10 µM DBM-treated lysates were not different from controls. The 25 and 50 µM treatments had greater than 2-fold increases in protein expression compared to controls. Twenty spots of interest were noted, 17 spots were up-regulated and 3 were down-regulated. The proteins were identified by MS analysis and HSP70 (up-regulated by 3.71-fold by 25 µM and 2.99-fold by 50 µM DBM) was singled out for further testing.

The authors also performed a Western blot analysis on LNCaP cells incubated in DBM to investigate its effect on HSP 70 expression. After seeding onto 100 mm plates for 24 h, the cells were then cultured for 72 h in 0, 10, 25, or 50 µM DBM. After lysis on ice, the cells were centrifuged and the protein content was quantified using a modified Bradford assay. The absorbance of each band was quantified using image analysis software.
There was an increase of 200% and 160% (compared to control) in HSP 70 and actin expression in the carcinoma cells treated with 50 µM DBM. There was a lower expression of HSP 70 at the 10 and 25 (< 20-fold) µM concentrations than at the 50 µM concentration of DBM (Frazier et al. 2004).

1-(2,4-Dihydroxyphenyl)-3-Hydroxy-3-(4'-Hydroxyphenyl)-1-Propanone

Rafi et al. (2002) reported that the active compound that induced apoptosis, G2/M cell cycle arrest, and Bcl-2 phosphorylation in various cancer cells was 1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4'-hydroxyphenyl)-1-propanone (aka β-hydroxy-DHP), isolated from licorice extract. β-Hydroxy-DHP was isolated through HPLC, MS, and NMR.

Flavones

Sakagami et al. (2000) tested for cytotoxicity in oral tumor cell lines using various flavones including gancaonin O and P from *G. uralensis* and glyasperin A from *G. glabra*. Human oral tumor cells (HSC-2 and HSG) were maintained and incubated with and without test substances in monolayers in DMEM. HGF cells were isolated from healthy gingival biopsies of a 10-yr-old female.

Cytotoxic activity was assayed by incubation of near confluent

cells for 24 h with and without the test compounds. The CC_{50} was determined by the dose-response curve. Flavones were found to be more cytotoxic to tumor cells than to normal cells (HGF) as shown in **Table 67.**

Apoptosis in cultured HSG cells was demonstrated by visualizing DNA fragmentation using an in situ apoptosis detection kit. After exposure to 0.141 nM of gancaonin O for 6 h, the cells were fixed and counterstained. Gancaonin O induced apoptotic cell death characterized by DNA fragmentation and caspase activation and resultant degradation of cytokeatin 18 in the cytoplasmic fractions.

The authors concluded that gancaonin is potentially cytotoxic to human oral tumor cells (Sakagami et al. 2000).

Glabridin

Yokota et al. (1998) studied the ability of glabridin, extracted from *G. glabra* root extract, to inhibit melanogenesis and inflammation of the skin by inhibiting tyrosinase activity. B16 F0 murine melanoma cells were grown in culture and exposed to 0, 0.1, 1.0, or 10.0 µg/ml glabridin for 72 hours. The cells were then exposed to [3H]-tyrosine (1.0 µCi/well) for 72 hours. The 3H2O released was measured with a liquid scintillation counter. 3H2O release was concentration-dependently decreased with increasing doses of glabridin, with the lowest exposure of 0.1 µg/ml showing reduction ($p < 0.05$) in 3H2O release. The authors suggested that these data show that glabridin inhibits tyrosinase activity in B16 melanoma cells. An SDS-PAGE assay of control and 1.0 µg/ml glabridin showed that B16 cells treated with glabridin had significantly reduced ($p < 0.01$) T1 and T3 tyrosinase activities, compared to control cells.

The effect of glabridin on DNA synthesis was measured by assessing [3H]-thymidine incorporation by B16 melanoma cells exposed to 0, 0.1, 1.0, or 10.0 µg/ml glabridin for 72 hours. Then the cells were exposed to 1.0 µCi/well [3H]-thymidine for 6 hours. Cells were then lysed and neutralized, and radioactivity was determined with a liquid scintillation counter. [3H]-Thymidine incorporation was inhibited ($p < 0.01$) by 10 µg/ml glabridin, but not at the lower concentrations.

Glabridin at 33.3 µg/ml significantly inhibited ($p < 0.05$) superoxide anion production by xanthine oxidase. Lower concentrations of 3.33 or 0.333 µg/ml glabridin had no effect, but the positive control (superoxide dismutase) was effective. Also, 6.25 µg/ml glabridin decreased cyclooxygenase activity. The authors suggested that glabridin may be an anti-inflammatory agent.

Finally, B16 melanoma cells were grown in cell culture flasks and exposed to control medium, 1.0 µg/ml glabridin or 1.0 µg/ml of one of three glabridin derivatives: 2', 4' -O-diethyl glabridin, 4'-O-ethyl glabridin, or 2'-O-ethyl glabridin. Glabridin or glabridin derivative exposure lasted 3 d before the cells were processed, and melanin contents were measured by spectrophotometer at 400 nm. Treatment with glabridin, 4'-O-ethyl glabridin, and 2'-O-ethyl glabridin each caused a decrease ($p < 0.01$ or 0.05) in melanin production, compared to control treatment, and melanin content in the glabridin treatment was lower ($p < 0.01$) than in the 4'-O-ethyl glabridin and 2'-O-ethyl glabridin treatments. The 2', 4' -O-diethyl glabridin treatment did not affect melanin content.

The authors concluded that glabridin may inhibit melanin synthesis (Yokota et al. 1998).

Isoliquiritigenin

Shibata (1994) tested for anti-tumor-promoting activities of synthesized chalcones, one of which was isoliquiritigenin, in vitro by their inhibitory activities against phosphorylation promoted by TPA in HeLa cells at a concentration of 5 µg/ml. Isoliquiritigenin's inhibition was 76.8%. The authors concluded that isoliquiritigenin has tumorigenesis inhibiting properties.

Ma et al. (2001) exposed human gastric cancer MGC-803 cells to various concentrations (range not specified) of isoliquiritigenin during log phase growth. For microscopic observation, the cells were centrifuged, resuspended, and co-stained with propidium iodide (PI) and Hoechst 33342 for 30 min. The cells were centrifuged again to remove the supernatant. The pellet was suspended in the remaining culture medium and smeared for fluorescence microscopy.

Cells treated with 0.1125 g/l isoliquiritigenin exhibited apoptotic features as in cell shrinkage, turned round, and smaller volume than control cells. Some cells floated. Chromatin DNA was condensed. The integrity of the plasma membrane was not compromised.

Table 67. Cytotoxicity to tumor and normal cells of flavonoids found in licorice plants (Sakagami et al. 2000).

Flavonoid (source plant)	HSC-2 CC_{50} (µg/ml)	HSG CC_{50} (µg/ml)	HGF CC_{50} (µg/ml)
Gancaonin O (*G. uralensis*)	20	31	43
Gancaonin P (*G. uralensis*)	14	41	45
Glyasperin A (*G. glabra, G. aspera*)	<8	20	19

Analysis of DNA ladder fragmentation (the biochemical marker of apoptosis) in MGC-803 cells was performed by terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL). An obvious DNA ladder was observed in a dose-dependent manner demonstrating DNA damage. Flow cytometry was used to detect apoptosis. Apoptosis was shown to be dose-dependent in the range 0.1125 g/l to 0.15 g/l.

Flow cytometry was used to measure mitochondrial transmembrane potential and intracellular free calcium concentration. The transmembrane potential dropped and the intracellular free calcium concentration increased with exposure to isoliquiritigenin compared to controls.

The authors concluded that isoliquiritigenin is potentially useful as an anti-cancer agent (Ma et al. 2001).

Yamazaki et al. (2002) injected murine renal cell carcinoma cells (1 x 10 4 cells in 0.2 ml RPMI-1640 culture medium) from a BALB/c mouse into the tails of healthy mice. Starting the next day, the treated mice were intraperitoneally injected with isoliquiritigenin (isolated from *G. glabra*) in 100 µl 0.1% Tween 80 solution for 10 d (n = 15; 0.1, 0.5, 2, and 10 mg/d; based on an LD_{50} estimated to be 3000 mg/kg body weight). Control groups received only vehicle (negative control) or 20 mg/kg 5-fluorouracil (positive control). On day 21, the lungs were excised under anesthesia and stained. The metastatic nodules were counted. The experiment was repeated.

In the first run of the experiment, the number of nodules for 2 and 10 mg/d isoliquiritigenin (34.2 ± 32.0 and 27.1 ± 31.1, respectively) were reduced compared to control (85.2 ± 47.8; $p < .001$). In the second run of the experiment, the number of nodules in the lungs were decreased in the 0.1 and 0.5 mg/d groups (60.6 ± 39.3 and 55.5 ± 31.3, respectively) compared to the control group (102.0 ± 23.9).

In a cytotoxicity experiment, the authors exposed the murine renal carcinoma cells (5 x 103 cells/ml) in FBS to various concentrations of isoliquiritigenin in ethanol for 24 h. An equal amount of ethanol was the control. Viability was measured with an Alamar Blue assay kit. Isoliquiritigenin decreased cell viability in concentrations > 12.5 µg/ml.

The authors then tested if the effect of isoliquiritigenin on nitric oxide production by macrophages was a possible mechanism to prevent metastasis. Mice were injected intraperitoneally with 2 mg isoliquiritigenin in 100 µl saline daily for 10 d. Control was just saline. On day 11, the mice were killed and macrophages collected by peritoneal lavage. Macrophages were plated and stimulated with LPS. The macrophages were incubated for 2 d then the nitric oxide production was measured with a micro plate reader after the addition of 100 µl Griess' reagent. Isoliquiritigenin induced no change in basal nitric oxide production but increased production (56 ± 0.12 µM; $p < .001$) compared to control (7.5 ± 0.01 µM) when macrophages were stimulated.

To examine the effect of isoliquiritigenin on splenic lymphocyte activation, cytotoxicity against leukemia RL male 1 cells was used as an indicator of lymphocyte activity. Activation of cytotoxicity of splenic lymphocytes with isoliquiritigenin was as low as 0.049 µg/ml when the ratio of splenic lymphocyte:RL male 1 cell was higher than 12.5:1 ($p < .05$ to .001).

Murine renal carcinoma cells were grown to 80% confluency in PRMI-1640 in a 12-well flat bottom plate (2 ml/well). Various concentrations of isoliquiritigenin in 20 µl ethanol were added to each well and incubated for 24 h. Control was just vehicle. VEGF secretion was measured by mouse VEGF immunoassay. Isoliquiritigenin decreased the amount of VEGF in the cell culture medium in a dose-dependent manner, but it was paralleled with the decrease in cell viability. The authors stated that VEGF is unlikely to be a mechanism of action with regard to isoliquiritigenin.

Murine renal carcinoma cells (1 x 104 cells/0.2 ml) were injected into tail veins of mice. Isoliquiritigenin (0.1, 0.5, 2, and 10 mg/d) was injected intraperitoneally in 0.1 ml 0.1% Tween 80. Additional groups received isoliquiritigenin (2 mg/d) with or without 5-fluorouracil (20 mg/kg d-1). Additional mice did not receive the cells but were injected with the test substance (2 mg/d) or vehicle. On day 11 manual leukocyte differential counts were performed.

White blood cells were affected in a dose-dependent manner by isoliquiritigenin as shown in **Table68**. The authors stated that isoliquititigenin prevented severe leukocytopenia caused by 5-fluorouracil

The authors suggested that activation of macrophages, activation of cytotoxicity of lymphocytes, and direct cytotoxicity are possible mechanisms of metastasis suppression by isoliquiritigenin. (Yamazaki et al. 2002).

Table 68. The effect of isoliquiritigenin on leukocytosis (Yamazaki et al. 2002).

Treatment	White blood cell count (mm^3)
Murine renal carcinoma cells (+)	
Vehicle	8546 ± 1336
Isoliquiritigenin 0.1 mg/d	8800 ± 2154
Isoliquiritigenin 0.5 mg/d	12,635 ± 1922[a]
Isoliquiritigenin 2 mg/d	13,407 ± 3061[a]
Isoliquiritigenin 10 mg/d	15,037 ± 3283[a]
Murine renal carcinoma cells (-)	
Vehicle	7832 ± 1306
Isoliquiritigenin 2 mg/d	8841 ± 945
Murine renal carcinoma cells (+)	
Vehicle	7409 ± 1646
5-FU	1290 ± 561[a]
5-FU + isoliquiritigenin	6183 ± 933

[a] $p < .001$

Kanazawa et al. (2003) tested for the anti-tumor activity of isoliquiritigenin from *G. glabra*. Human prostate cell lines DU145 and LNCaP were seeded in 2 cm dishes (4 x 104 cells/dish) for 24 h. Isoliquiritigenin at various concentrations (n = 3) were added and cells counted at 24, 48, and 72 h using a trypan blue dye exclusion assay.

Isoliquiritigenin inhibited DU145 cell growth (50% inhibition at 11 µM) and LNCaP cell growth (50% inhibition at 13 µM), both in a dose-dependent manner. The authors suggested that isoliquiritigenin inhibits the cell growth of both androgen-dependent and androgen-independent prostate cancer.

The authors then tested the effects of isoliquiritigenin on the cell cycle of DU145 cells. The cells were cultured (1 x 106) for various periods of time with isoliquiritigenin or DMSO for the control then subjected to fluorescence-activated cell sorting analysis. DNA flow cytometric analysis indicated that S and G2/M phase arrest was induced dose-dependently; no apoptosis was observed. Isoliquiritigenin induced the arrest at 4 h and the effect maintained for 24 h.

The authors tested the effect of isoliquiritigenin on the mRNA expression of cell cycle-regulating genes by incubating DU145 cells cultured with 20 µM isoliquiritigenin or DMSO for the control then using Atlas cDNA Expression Array. Three upregulated (GADD153 (4.1-fold), CDC10 (2.6-fold), SOD, and 2 downregulated (CDK5 activator p391 (0.4-fold), and MDM2-like p53-binding protein) genes were detected. Isoliquiritigenin had no effect on cycline-dependent kinases. A real-time RT-PCR using LightCycler™ confirmed that GADD153 was upregulated; the mRNA expression was enhanced more than 8-fold at 24 h. The other genes were not confirmed. Western blot analysis showed that GADD153 in DU145 cells was low and was increased by isoliquiritigenin dose-dependently and time-dependently. The induction was seen at 4 h and peaked at 12 h after treatment.

The authors concluded that isoliquiritigenin is a possible agent for the treatment of prostate cancer and GADD153 may play a role in isoliquititgenin-induced cell cycle arrest and cell growth inhibition (Kanazawa et al. 2003).

Ii et al. (2004) tested the effects of isoliquiritigenin on the human lung cancer cell line A549. In a cell growth experiment, the cells were seeded at 4 x 104 cells/2 ml medium in 35 mm dishes. Isoliquiritigenin was added at various concentrations 1 d later. The number of viable cells was scored using trypan blue dye exclusion method on days 2, 3, and 4.

Isoliquiritigenin inhibited cell proliferation in a dose- and time-dependent manner. Three d after the addition of 10, 20, and 30 µM isoliquiritigenin the cell growth decreased by 17%, 56%, and 89% of controls (DMSO), respectively ($p < .05$). The IC_{50} was calculated to be 18.5 µM.

The authors tested the effects of isoliquiritigenin on cell cycle by seeding 1 x 106 A549 cells/10 ml medium in 100 mm dishes. Isoliquiritigenin was added at various concentrations 24 h later. Cells were harvested at time intervals by trypsinization and centrifugation. DNA content in stained nuclei was analyzed with a flow cytometer. The percentage of cells in each cell cycle was determined by software.

Isoliquiritigenin increased the population of G2/M cells in a dose-dependent manner at 24 h. The accumulation of cells in G2/M was observed at 4 h after the addition of 40 µM isoliquiritigenin and the effects continued for 24 h. Pre-G1 apoptotic populations were not observed in this condition.

The authors assayed the effect of isoliquiritigenin on the mRNA expression of cell cycle regulating genes using a cDNA expression array. The exposure of 15 µM isoliquiritigenin up-regulated 3 genes (p21CIP1/WAF1, 4.1-fold; GADD45, 436-fold; and GTPase-activating protein, 4.0-fold) and down-regulated 1 gene (MDM2-like p53-binding protein, 0.3-fold). Isoliquritigenin had little effect on p53 (1.5 fold) and cycline-dependent kinases cdc2 (1.5-fold), dck2 (1.3-fold), and cdk4 (0.8-fold).

The authors further analyzed the cDNA using real-time quantitative RT-PCR. In the case of p21CIP1/WAF1, up-regulation by isoliquiritigenin was confirmed, observed as early as 4 h, and was 21-fold greater at 24 h for 40 µM.

The authors suggested that isoliquititigenin is a possible agent for chemoprevention or therapeutics against lung cancer (Ii et al. 2004).

Takahashi et al. (2004) tested isoliquiritigenin for its abilities as a suppressor of colon carcinoma cells in a series of experiments on rat and human cancer cell lines. In the first experiment, RAW264.7 cells, known to express COX-2 and iNOS on LPS stimulation resulting in accumulation of prostaglandins such as PGE2, were used. Measurements were performed in triplicate. Treatment with isoliquiritigenin resulted in a decrease of PGE2 in RAW264.7 cells (IC_{50} = 1.47 µM; $p < .005$). Assays of NO (as nitrite) demonstrated a clear inhibitory influence of isoliquiritigenin on NO production (IC_{50} = 7.78 µM; $p < .05$). The decreases were dose-dependent.

The remaining RAW264.7 cells from the above experiment were treated with solubilization buffer and lysate to be used in a western blot and reverse transcription-polymerase chain reaction analysis. The protein concentration of each lysate was measured using a protein assay kit. Rabbit anti-mouse COX-1, rabbit anti-mouse COX-2, rabbit anti-mouse iNOS, and rabbit anti-actin were used as primary antibodies. Goat anti-rabbit IgG (H+L)-HRP was used as the secondary antibody. Dilutions for anti-COX-1, COX-2, iNOS, and actin were 1:250, 1;2000, 1:2000, and 1:5000, respectively. The dilution of the secondary antibodies was 1:100,000 in .01% Tween-PBS containing 1% bovine serum albumin.

RT-PCR analysis of the mRNA levels of the 3 enzymes did not show any alteration. The protein of COX-1 was unchanged; COX-2 and iNOS protein expression were suppressed by isoliquiritigenin in a dose-dependent manner correlating with the levels of NO. The down-regulation for COX-2 and iNOS in the

25 µM isoliquiritigenin treatment group were 52% and 86%, respectively. Similar results were observed for the positive controls, cyclosporin and genistein.

The effect of isoliquiritigenin on cell growth was tested on Colon 26, RCN-9, and COLO-320DM cells. To test for time-dependency the cells were exposed to isoliquiritigenin at 5, 25, and 100 µM and incubated for 12, 24, and 36 h (colon 26 and RCN-9 cells) or 24, 48, and 72 h (COLO-320DM cells). 5-Fluorouracil (50 µM) was used as a positive control in both schedules. Cell viability was determind by the MTT cell proliferation assay based on the values of OD550; n = 6 for all cases.

The concentrations of isoliquiritigenin giving 50% viability were 14.6, 19.5, and 17.9 µM for Colon 26, RCN-9, and COLO-320DM cells, respectively. At 25 µM values of OD550 were similar at all timepoints for all cell types so there was no time-dependency. The researchers found obvious cytotoxic effects at 100 µM for all cell types.

Apoptosis was assessed by flow cytometric analysis using aliquots of 1 x 106 cells exposed to 5, 25, and 100 µM isoliquiritigenin for 48 h (Colon 26 and RCN-9 cells) or 72 h (COLO-320DM cells). Both attached and floating cells were collected and centrifuged. DNA content in the G0/G1 phase assessed in duplicate.

Caspase-3 activity was determined for Colon 26 and COLO-320DM cells. Colon 26 and COLO-320DM cells exposed to isoliquiritigenin underwent apoptosis in a dose-dependent manner up to 25 µM with up to 40% of the cells affected. RCN-9 cells were resistant to apoptosis. Colon 26 cells underwent apoptosis only after 48 h of exposure to isoliquiritigenin. COLO-320DM cells underwent apoptosis in a time-dependent manner. Induction of caspase-3 activation in Colon 26 and COLO-320DM cells was found after 24 h.

The final experiment was performed on 45 6-week-old male F344 rats divided into 5 groups over 5 wk. All of the rats except group 5 were injected with the carcinogen azoxymethane (AOM; 15 mg/kg) weekly for 3 wk (days 0, 7, 14). Isoliquiritigenin was incorporated into their diet at 100 ppm and fed to the rats in following protocols: Group 1 ate the augmented diet during week 1 and 2 then the unaugmented diet for 3 wk; Group 2 was fed unaugmented diet for 2 wk then the augmented diet for 3 wk; Group 3 was fed the augmented diet for 5 wk; Groups 4 and 5 were fed unaugmented food the entire 5 wk. After 5 wk the rats were killed; the colons excised, fixed, and stained; and the numbers of aberrant crypts and crypt foci (ACF) were counted. Body and organ weights were also measured.

Oral administration of isoliquiritigenin inhibited the induction of total and large (≥ 4 crypts) ACF in all 3 treatment groups compared with the control group ($p < .01$). Singlet and triplet ACF were reduced in Groups 3 and 2, respectively. Only treatment on Group 3 inhibited the yield of aberrant crypts; there was no difference between Groups 4 and 1 or 2 with regard to aberrant crypts. Isoliquiritigenin did not have any toxic effects demonstrated by organ or body weight differences (Takahashi et al. 2004).

Jung et al. (2006) tested the effects of isoliquiritigenin on prostate cancer cells lines MAT-LyLu (rat) and DU145 (human). Both types of cells were plated (20,000 cells/plate) and treated with various concentrations of isoliquiritigenin for 24 and 48 h, respectively. The cells were fixed and stained then examined by fluorescence microscopy. Isoliquiritigenin (5, 10, and 20 mol/l) increased apoptosis of the prostate cancer cells of both lines in a dose-dependent manner ($p < .05$). The test was repeated on normal intestinal epithelial cells (ICE-6 cells) with no effect on their growth. The number of apoptotic cells was analyzed by flow cytometry after staining with phycoerythrin-conjugated annexin V and 7-amino-actinomycin.

There were dose-dependent increases in both phycoerythrin-conjugated annexin V positive/7-amino-actinomycin-negative cells when both prostate cancer cell lines were treated with isoliquiritigenin ($p < .05$).

DU145 cells were incubated with isoliquiritigenin (0 or 20µmol/l) to measure caspase-9 activation in a time-related manner. The cleaved form of caspase-9 was observed within 6 h. The cells were stained with JC-1 and the percentages of green-positive and red-negative fluorescence were scored as depolarized cells. The number of cells with depolarized mitochondrial membranes increased in a concentration-dependent manner (0, 5, 10, 20 µmol/l; $p < .05$) (Jung et al. 2006).

Chin et al. (2007) tested the chemopreventive properties of isoliquiritigenin using female Swiss-Webster mice (9 wk old; n = 25 for treatment groups; n = 44 for control). The mice were administered 1,2-dimethylhydrazine by i.p. injection twice/week for 8 wk. Seven d after the last injection, the mice were fed a diet incorporated with isoliquiritigenin (0, 50, 100, or 300 mg/kg). The mice were killed after 36 wk on the diet and necropsied. One mouse in the 100 mg/kg group died before the end of the treatment. There were no toxic effects from the isoliquiritigenin. All but one mouse in the 50 mg/kg group had at least 1 lung tumor; > 97.5% of the tumors in the treatment groups were adenomas, the rest adenocarcinomas. The percentage of tumors did not vary between treatment groups (1.46 to 2.91% of the tumors). All the mice had at least 1 colon tumor. The high-dose group had a reduced number of tumors (lung and colon) compared to the control ($p < .05$).

Licochalcone A

Shibata et al. (1991) shaved the backs of 7-week old female ICR mice. After 2 d, a single application of 100 µg of DMBA was applied. One µg of TPA in 100 µg DMSO plus acetone was painted twice a week on the shaved area of the control animals (n = 15) starting 1 week after initiation. One mg of licochalcone A, isolated from *G. inflata* root , mixed with 1 µg of TPA in 100 µg of DMSO plus acetone solution, was topically applied twice a week to the test animals (n = 15) for 20 wk. The number and size of the tumors appearing on the backs of the mice were determined once a week and the body weight every 4 wk.

The first tumor appeared at week 6 in the control group and week 11 in the group treated with licochalcone A. At week 20, the percentage of mice with tumors was 73% for the control and

26.7% for the treated group ($p < .02$). Licochalcone A also decreased the average number of tumors/mouse, 10.8 tumors/mouse for the control and 0.8 tumors/mouse for the treated group ($p < .001$). Body weight for the treated group had a larger increase than in the control group ($p < .02$) (Shibata et al. 1991).

Park et al. (1998) exposed human and mouse cancer cells to licochalcone A and found the compound to be cytotoxic as shown in **Table 69.** The researchers also found that licochalcone A had an IC_{50} of 30.4 ± 1.5 µM against PI-PLCγ1 enzyme activity. In the cell differentiation assay licochalcone A induced expression of neuron-specific enolase activity, a marker of macrophage (monocyte) formation (ED_{50} = 1.32 x 10-5 M) in a dose-dependent manner. Licochalcone A did not show activity in the nitroblue tetrazolium reduction assay, an indicator of granulocyte formation. The authors concluded that the compound seemed to be an inducer of differentiation into monocytes rather into granulocytes.

Rafi et al. (2000) tested the effects of licochalcone A on cell viability using the tetrazolium dye method. Three human cancer cell lines, MCF-7, PC-3, and DUPro-1, were treated with various concentrations of licochalcone A and incubated for 72 h. Absorbance was measured at 570 nm.

Licochalcone A decreased viability in a dose-dependent manner in all 3 cancer cells. Total mortality was reached at 100 µM for MCF-7 cells and 45 µM for PC-3 and DUPro-1 cells. It was also found that the compound enhanced the effect of chemotherapy (paclitaxel and vinblastine chemotherapy) against PC-3 cells and decreased bcl-2 expression in MCF-7 cells over 96 h. The ratio of bcl-2 to bax decreased from 1.0 to 0.3, favoring tumor cell apoptosis (Rafi et al. 2000).

Fu et al. (2004) tested the effect that licochalcone A has on PC-3 prostate cancer cells. Human PC-3 CaP cells were seeded at 5 x 104/ml in T25 flasks overnight. They were then incubated with 0, 12.5, or 25 µM licochalcone A.

Table 69. Effect of licochalcone A on ED_{50} against human and mouse cancer cell lines (Park et al. 1998).

Cell line	ED_{50} (µg/ml)
Human cancer cell lines	
A549 (lung cancer)	4.6 ± 0.46
MCF7 (breast adenocarcinoma)	9.2 ± 1.37
HCT-15 (colon adenocarcinoma)	8.8 ± 2.22
SK-OV-3 (ovary adenocarcinoma)	>20
Malme-3M (malignant melanoma)	>20
Mouse cell lines	
P388 (leukemia)	3.6 ± 0.36
NIH3T3 (embryo)	>20

Cells were harvested daily for 3 d by trypsinization, stained with trypan blue, and cell number and viability were determined by a hemocytometer. The cells were also fixed, washed and stained to be examined by light microscopy for abnormal morphology. There was no anti-proliferative effects of licochalcone A on day 1 ($p = 0.4423$ for 12.5 µM; $p = 0.1805$ for 25 µM). On day 2 there was 65 to 80% growth inhibition ($p = 0.0472$ and 0.0230). On day 3 there was 55 to 83% growth inhibition ($p = 0.0242$ and 0.0015). Licochalcone A was not cytotoxic; the cell viability was greater than 90%. The authors concluded that there was little evidence of morphological changes in the PC-3 cells.

These authors tested the growth inhibition effects of licochalcone A on the cell cycle of PC-3 cells (prostate cancer cells). The cells were treated with 0, 12.5, or 25 µM licochalcone A and were harvested periodically for 3 d. The harvested cells were washed with PBS and stained with 1.0 µg/ml of 4,6-diamidion-2-phenylindole in a solution containing 100 mM NaCl, 2 mM MgCl2, and 0.1% Triton X-100. Cell cycle phase distribution and the percentage of apoptotic cells were determined using flow cytometry.

Cells treated with 25 µM licochalcone A had an increased percentage of cells in the G2/M phase at all time points. Cells treated with 12.5 µM had the same result only on day 3. The number of apoptotic cells, based on the appearance of a "sub-G1" peak, was observed in a time- and dose-dependent manner. The magnitude of change was relatively small in comparison to the observed decrease in cell proliferation. A 3-day treatment with 25 µM licochalcone A only had a 5 to 6% increase in apoptotic cells. The authors stated that this indicates that at these concentrations of licochalcone A growth inhibitions in PC-3 cells can be primarily attributed to induction of G2/M block, with only minor contributions from the induction of apoptosis.

The researchers also treated PC-3 cells in buffer (50 µl/106 cells) with 25 µM licochalcone A for 3 d. The cells were harvested, washed, and lysed by repeated freezing and thawing. The cells were centrifuged and the supernatant stored. Protein concentrations were measured with Pierce protein assay reagent then analysis was also performed by Western blot. The membranes were probed for the level of expression of cyclin B1, D1, E, cdc2, CDK 2, 4,6, Rb, PCNA, and β-actin. Site-specific phosphorylated retinoblastoma (Rb) antibodies (antibody to DNA polymerase δ(78FS)) were used to demonstrate specific immunoreactivity.

There was inhibition of the level of cyclin B1 for all 3 d. Reduction in its catalytic partner, cdc2, was time-dependent and was not evident until d 2 and 3. There was a time-dependent reduction in the expression cyclin D1 and a down-regulation of CDK 4 along with a delayed decrease in DCK6 on day 3. A time-dependent increase in cyclin E without commensurate change in CDK2 was observed.

The authors reported that the changes in D1 and E were in concordance with time-dependent transition of the cell population from G1 to S. The licochalcone A exposure caused a decrease in

the expression of Rb (>60% inhibition), particularly the hyperphosphorylated form of Rb. A reduction of phosphorylation was found in the site specific antibody S780 but not T821. The authors suggested that licochalcone A may be a chemopreventive agent (Fu et al. 2004).

Licocoumarone

Watanabe et al. (2002) tested licocoumarone, extracted from *G. glabra*, for the inducement of apoptosis of human monoblastic leukemia U937 cells. The cells were treated with licocoumarone at various concentrations and the controls were incubated without the test substance. After 16 h the numbers of viable cells were determined by Alamar blue assay. Proliferation of U937 cells was reduced to ~65% at 0.0125 mg/ml, ~45% at 0.025 mg/ml, ~25% at 0.05%, and ~22% at 0.10 mg/ml.

Agarose gel electrophoresis of DNA extracted from the treated cells incubated with and without Z-Asp-Ch2-DCB (a general caspase inhibitor; 200 µM) demonstrated that licocoumarone induced DNA fragmentation in a nucleosome unit; this DNA fragmentation was inhibited by Z-Asp-CH2-DCB. The authors stated that these data indicate that licoumarone caused apoptosis in U937 cells (Watanabe et al. 2002).

Liquiritin

Kawamori et al. (1995) tested the preventive effects of liquiritin (extracted from *G. glabra*) on colorectal cancer using male F344 rats (5 wk old). Over the 5-week study, the rats were treated with 1) injections of azoxymethane at wk 1, 2, and 3 and fed a basal diet (positive control; n = 16); 2) injections of azoxymethane at weeks 1, 2, and 3 and fed the basal diet with 200 ppm (0.02%) liquiritin (n = 16); 3) no injections of azoxymethane and fed the basal diet with 200 ppm liquiritin (n = 6); or 4) no injections of azoxymethane and fed the basal diet (negative control). At the end of week 5, the rats were killed and necropsied.

The mean body weight, liver weight, and relative liver weight of the rats injected with azoxymethane and fed liquiritin were lower than the those without the liquiritin ($p < .0001$) but not different from the negative control. The number of aberrant crypt foci, number of aberrant crypts/colon, and number of aberrant crypts/focus were similar between rats injected with azoxymethane and rats injected with azoxymethane than fed liquiritin. Ornithine decarboxylase activity was lower in rats injected with azoxymethane and fed liquiritin than those just injected with azoxymethane (1.9 ± 1.9 vs 22.1 ± 19.4 pmol 14CO2/h/mg protein; $p < .05$). Liquiritin alone was similar to azoxymethane alone (42.1 ± 16.3 pmol 14CO2/h/mg protein).

When measuring polyamine levels, liquiritin lessened the effects of the azoxymethane for the spermidine levels (2.49 ± 0.58 vs 3.11 ±0.31 nmol/mg protein; $p < .05$) but not the diamine, spermine, or total levels; there were no differences between the positive and negative controls. Liquiritin had no effect on the number of cells/crypt in azoxymethane-treated rats but did lower the number of silver-stained nuclear organizer region proteins per nucleus (1.44 ± 0.28 vs 1.01 ± 0.29; $p < .05$).

The authors suggested that liquiritin has antipromoting effects by inhibiting azoxymethane-induced colonic mucosal ornithine decarboxylase activity, silver-stained nuclear organizer region protein number, and/or polyamine levels in the colonic epithelium (Kawamori et al. 1995).

Oleanolic Acid

Kawamori et al. (1994) also tested the preventive effects of oleanolic acid (extracted from *G. glabra*) on colorectal cancer (using the same procedure above).

The mean body weight, liver weight, and relative liver weight of the rats injected with azoxymethane and fed oleanolic acid were lower than the those without the oleanolic acid ($p < .0001$) but not different from the negative control. The number of aberrant crypt foci were lower in oleanolic acid-treated rats (152.5 ± 30.6 vs 97.6 ± 11.1; $p < .005$). The number of aberrant crypts/colon were lower in oleanolic acid -treated rats (222.9 ± 39.9 vs 171.0 ± 28.2 ($p < .05$). The number of aberrant crypts/focus were similar between rats injected with azoxymethane and rats injected with azoxymethane than fed oleanolic acid. Ornithine decarboxylase activity was lower in rats injected with azoxymethane and fed oleanolic acid than those just injected with azoxymethane (4.3 ± 3.0 vs 22.1 ± 19.4 pmol 14CO2/h/mg protein; $p < .05$). Oleanolic acid alone was similar to azoxymethane alone (9.1 ± 13.9 pmol 14CO2/h/mg protein).

When measuring polyamine levels, oleanolic acid had no effects on the diamine, spermidine, spermine, or total levels; there were no differences between the positive and negative controls. Oleanolic acid had no effect on the number of cells/crypt in azoxymethane-treated rats but did lower the number of silver-stained nuclear organizer region proteins per nucleus (1.44 ± 0.28 vs 0.85 ± 0.25; $p < .01$).

The authors suggested that oleanolic acid may be a candidate for a chemopreventive agent in colon tumorigenisis (Kawamori et al. 1995).

Various Compounds

Lee et al. (2008) extracted ammonium glycyrrhizinate, liquiritigenin, ononin, glycyrol, and cabenoxolone disodium salt from *G. uralensis* and pretreated HEK293T tumor cells with each compound (10 µg/ml) for 30 min prior to treatment with phorbol 12-myristate 13-acetate (PMA) for 16 h. The control was untreated. Glycyrol decreased NF-κB luciferase activity; the other compounds had no effect. The above experiment was repeated with the pretreatment of glycyrol (0, 10, 50, 100 nmol/l) for 30 min then treatment with PMA (100 nmol/l) for 24 h. NF-κB activity was decreased in a concentration-dependent manner. The cells were treated with just glycyrol for 24 h (n = 3) using a MTT assay.

Viability was not affected up to 20 µg/ml; concentrations >50 induced cytotoxicity.

The effects of these licorice compounds (10 µg/ml), with and without PMA, on DNA expression was investigated using DNA microarrays with HED293Tcells (n = 3). Only glycyrol had any

effect. Glycyrol highly modulated inflammation-related genes to inhibit inflammation, including immunity and defense-related genes, by modulating NF-κB-dependent pathways. Of 789 apoptosis-related genes investigated, all were involved in the induction of the glycyrol-modulated apoptosis in tumor cells. The authors concluded that glycyrol may be a candidate for a tumor suppressor (Lee et al. 2008).

Nabekura et al. (2008) tested for inhibition against p-glycoprotein and mutidrug resistance protein 1 by phytochemicals including glycyrrhizin, Glycyrrhitinic Acid, glabridin, genistein, and isoliquiritigenin. KB-C2 cells were cultured and incubated with daunorubicin (50 µM) in the presence or absence of a phytochemical for 2 h. Fluorescence in the treated samples compared to the control was used as a measure of accumulation of daunorubin in the cells.

Glycyrrhetinic Acid and glabridin increased the cellular accumulation of daunorubicin ($p < .01$), which indicated inhibition of the p-gp-mediated efflux of daunorubicin.

The above experiment was repeated on Glycyrrhitinic Acid and glabridin and other phytochemicals with KB/MRP cells using the accumulation of calcein, a fluorescent substrate of multidrug resistance protein 1(MRP1) transfected into these cells.

Glycyrrhitinic Acid (50 µM) increased the accumulation of calcein in KB/MRP cells ($p < .01$) but glabridin did not. Glycyrrhitinic Acid had the strongest inhibitory effect on the functions of P-gp and MRP1.

The effect of Glycyrrhitinic Acid on the resistance of KB-C2 cells to vinblastine and KB/MRP cells to doxorubicin was examined. The cells were incubated with the chemotherapeutic agents with or without Glycyrrhitinic Acid.

In the presence of Glycyrrhitinic Acid, DB-C2 and DK/MRP cells were more susceptible to the cytotocitiy of vinblastine (a P-gp substrate) and doxorubicin (an MRP1 substrate) as compared to chemotherapeutic agents alone ($p < .01$). The authors stated that this indicated the inhibition of P-gp or MRP1-mediated efflux from cells and an increase in intracellular concentrations of anticancer agents in response to Glycyyrrhetinic Acid.

The effects of Glycyrrhitinic Acid on the basal and verapamil-stimulated P-GP ATPase activity was examined. Verapamil, a substrate of P-GP, stimulated ATPase activity of P-GP.

Glycyrrhetinic Acid alone had no effect on the basal P-GP ATPase activity. Glycyrrhetinic Acid inhibited the ATPase hydrolysis by verapamil stimulated P-GP ATPase. Glycyrrhetinic Acid alone stimulated the basal MRP1 ATPase activity and further enhanced the N-ethylmaleimide-glutathione-stimulated MRP1 ATPase activity. The authors suggested that Glycyrrhetinic Acid may be a lead to a more efficacious and less toxic chemosensitizing agents to enhance the efficacy of cancer chemotherapy (Nabekura et al 2008).

Polysaccharides

Cheng et al. (2008) isolated the polysaccharides from *G. uralensis* and tested them for effects on nitric oxide and iNOS production in peritoneal macrophages. The macrophages were from the peritoneal fluid of male BALB/c mice. Adherent macrophages were incubated with the polysaccharides (0, to 400 µg/ml) and/or LPS (2 µg/ml)/interferon-γ (1, 10, or 100 U/ml) for 48 h.

The polysaccharides increased nitric oxide and iNOS production at 50 to 400 µg/ml without LPS ($p < .05$) compared to the control in a concentration-dependent manner. The polysaccharides increased nitric oxide production at 100 to 400 µg/ml and iNOS production at 200 to 400 µg/ml with LPS ($p < .05$). The polysaccharides increased nitric oxide production at all concentration with IFN-γ at all concentrations ($p < .05$) and iNOS production at all concentrations with IFN-γ at all concentrations ($p < .05$) in a concentration-dependent manners.

In another experiment, the macrophages were incubated with the polysaccharides (0 to 400 µg/ml) and monitored by RT-PCR analysis. Unstimulated cells had no detectable iNOS mRNA whereas exposed cells induced the accumulation of iNOS mRNA in a dose-dependent manner. The authors suggested that the increased production of nitric acid in macrophages was mediated by the expressionof the iNOS gene. Control β-actin was constitutively expressed and not affected by the treatment with the polysaccharides.

The authors concluded that the *G. uralensis* polysaccharides stimulated macrophages to produce nitric oxide through the induction of iNOS gene expression. The authors suggested that polysaccharides may be useful immunopotentiating agents (Cheng et al. 2008).

CLINICAL ASSESSMENT OF SAFETY

Oral

Glycyrrhizic Acid and Derivatives

CIR (2005) reported that Glycyrrhizic Acid, administered orally to hemophilia HIV patients (200 to 800 mg/d) for 8 wk, resulted in increased lymphocyte count in all 9 patients; OKT4 (T-cell helper)-positive lymphocytes increased in 8 of the patients, and OKT4/OKT8 ratio increased in 6 patients. Four patients with signs of liver dysfunction had clear improvement in hepatic function parameters (i.e., GOT and GPT). Serum electrolytes, lipids, protein, and kidney function were not affected by Glycyrrhizic Acid.

Another study was described in which Glycyrrhizic Acid (0, 1, 2, 4 mg/kg/d) was administered orally to 39 volunteers for 8 wk. The aldosterone concentration and renin activity in the serum of the 4 mg/kg/day group were significantly lower ($p < 0.001$) than control after 2, 4, 6, and 8 wk of dosing. Aldosterone levels and renin activities in the 1 and 2 mg/kg/day groups were similar to controls. The concentration of ANP was significantly decreased ($p < 0.001$) in the 4 mg/kg/day group, but not in the 1 and 2 mg/kg/day groups. Systolic and diastolic blood pressure measurements remained the same throughout the study in the 2

and 4 mg/kg/day groups but decreased slightly in the control group. Thus, relative to the control group, the 4 mg/kg/day group had significantly higher ($p = 0.018$) blood pressure than the control group. Body weight was similar between all treatment groups. Volunteers in the 4 mg/kg/day group had a significantly lower ($p < 0.01$) blood potassium concentration than those in the control group at 2 and 4 wk into the dosing period. In daily questionnaires of physical conditions, volunteers in the 4 mg/kg/day group described headache, nausea, vomiting, change in defecating pattern, swollen face, and tickling in the arms and legs. However, the number of complaints decreased as the study progressed.

The authors of this study determined that the no effect level of Glycyrrhizic Acid in this study was 2 mg/kg/day. They proposed an acceptable daily intake of 0.2 mg/kg/day, using a safety factor of 10. This corresponds to 6 g of licorice per day would be safe for a 60 kg person, assuming that licorice contains 0.2 % Glycyrrhizic Acid. They also noted that the Dutch Nutritional Council advises a limit of 200 mg Glycyrrhizic Acid per day.

A linear dose-response relationship between Glycyrrhizic Acid (75, 270, 540 mg/d) and increased blood pressure was demonstrated in humans (CIR 2005).

FM100 Fraction

Shiratori et al. (1986) used a licorice extract produced by precipitating the methanol licorice extract with sodium hydroxide and hydrochloric acid (Fm100) to test the effect of licorice on the release of endogenous secretin (polypeptide hormone, produced in the small intestine, that activates the pancreas to secrete pancreatic juice) in humans. Seven volunteers (average age 35) were fasted overnight. A quadruple-lumen duodenal tube was passed orally beyond the ligament of Treitz. Polyethylene glycol 4000, a nonabsorbable marker, was infused into the second portion of the duodenum at 3 ml/min by peristaltic pump. The contents of the duodenum was aspirated continuously from the distal end, 10 cm distal to the perfusion site. The tube in the jejunum was used for intrajejunal perfusion with either Fm100 or 0.5% CMC. The Fm100 was in the CMC at 0.2%, adjusted for pH 6.5 with 0.1 N sodium hydroxide. Another double-lumen tube was placed at the most dependent portion of the stomach for aspiration of gastric juice. The aspirations took place for 45 min before the test started.

The test solutions were continuously adminstered in the upper jejunum at 10, 20, and 40 ml for 30 min with final doses of 200, 400, and 800 mg, respectively. The control, 10 ml CMC, was administered over 30 min. In the 30 min between doses of the test substance, CMC solution was infused at the same rate as the Fm100 to be infused during the following 30 min. Duodenal contents were continuously collected in iced glass tubes. The concentration of bicarbonate was determined immediately. The amount of protein, amylase, and polyethylene glycol were also determined. Blood samples were taken every 10 min for radioimunoassay of secretin.

Intrajejunal infusion of Fm100 solution resulted in increases in both plasma secretin concentration in a dose-dependent manner; the correlation coefficient between Fm100 dose and plasma secretin concentrations was $r = 0.737$ ($p < .001$) and for pancreatic bicarbonate outputs was $r = 0.483$ ($p < .01$). The mean values of plasma secretin concentrations and pancreatic bicarbonate output at 400 and 800 mg were greater than the control and 200 mg doses ($p < .03$ and .01, respectively).

The authors concluded that endogenous secretin is released in humans treated with Fm100 and the increased pancreatic bicarbonate secretion is caused by the increase in plasma secretion (Shiratori et al. 1986).

Glabridin

Fuhrman et al. (1997) isolated LDL (by discontinuous density gradient ultracentrifugation) from plasma samples from healthy normal volunteers before, after 1 week, and after 2 wk of ingestion of 0.1 g/d of alcoholic extraction of *G. glabra* root. The extract contained 7 materials, the major one being glabridin (11.6 % by wt). LDL oxidations were determined by the TBARS assay. The amount of lipid peroxides was determined continuously with a commercial kit (cholesterol color reagent, CHOD iodide method) by measuring absorbance at 365 nm.

Ingestion of the licorice extract inhibited the onset of LDL oxidation by 50 ± 7 min compared to controls in a dose-dependent manner. The licorice extract required to inhibit formation of MDA equivalents and lipid peroxides in LDL by 50% (IC_{50}) was 1.8 and 2.2 mg/l, respectively. The licorice extract inhibited the onset of LDL oxidation by the water-soluble radical generator AAPH for 260 min, at a concentration as low as 1.5 mg/l. AAPH-induced LDL oxidation was inhibited in a dose-dependent manner; the IC50s that inhibited formation of MDA equivalents and lipid peroxides were 0.3 and 1.2 mg/l, respectively.

Glabridin was isolated from the extract. LDL (200 mg protein/l) was incubated with 1 mmol AAPH/l in the presence of 3 mg glabridin/l (9.2 µmol/l). There was a 100-min prolongation of the lag phase and an 87% and 70% reduction in the formation of TBARS and lipid peroxides in LDL, respectively (Fuhrman et al. 1997).

Aoki et al. (2007b) tested the safety of licorice flavonoid oil, containing glabridin (1%), medium-chain triglycerides (90% w/w) and a solid fraction derived from licorice ethonolic extract (~10% w/w). The polyphenol content is ~ 8%. The contents of licorice flavonoids other than glabridin in the licorice flavonoid oil is ~0.2% glabrene, 0.2% glabrol, and 0.1% 4'-O-methylglabridin. The glycerrhizin content is < 0.005%. The oil was adminstered in gelatin capsules with 300 mg licorice flavonoid oil and 22 mg beeswax. The control was medium-chain tryglycerides.

The first study was a single dose of licorice flavonoid oil orally administered to male subjects ($n = 5$) at 300, 600, or 1200 mg after breakfast. Blood and urine samples were collected at baseline and up to 7 d after dosing. The mean time to the concentration peak was 4.27 h and was eliminated linearly with a

mean half life of 10.7 h. The area under the curve and maximum concentration increased linearly with dose.

The licorice flavonoid oil (300, 600, and 1200 mg in capsules) was administered to subjects (n = 10, 5/sex) daily after breakfast daily for 1 week. The placebo control (n = 12, 6/sex) was administered at the same time. Blood samples were collected at baseline, at hour 4 and 24 on days 1 and 7.

The mean plasma glabridin level at each time point was increased linearly ($r > .96$) with the dose levels.

The accumulation ratios of glabridin were 1.35 (range 1.04 to 1.67). The authors suggested this finding indicates a slight elevation of plasma glabridin due to multiple doses.

The above study was repeated for a 4-week period (n = 14, 7/sex; control n = 16, 9 males, 7 females). Blood samples were collected at baseline and at weeks 2 and 4. Plasma glabridin level increased linearly ($r < .99$) with dose level. Plasma levels of glabridin at weeks 2 and 4 were similar at each dose level, indicating a steady state. There were no sex-related differences in plasma glabridin levels in the second and third study. The authors stated that no clinical or noteworthy abnormalities were observed in any of the studies and that all blood chemistry values remained within normal parameters in all test groups (Aoki et al. 2007).

Dermal

Glycyrrhetinic and Glycyrrhizic Acid

CIR (2005) reported that in dermal sensitization studies, Glycyrrhetinic Acid at 0.6%, 3%, and 6% produced no evidence of irritation or sensitization in human subjects.

An eye gel containing 0.1% Glycyrrhizic Acid applied to the area around the eyes of 20 women for 21 d resulted in 8 subjects reporting discomfort sensations. Another test resulted in 1 subject out of 232 women with well-defined erythema with pruritus after the first application that reappeared at the challenge. No other treatment-related observations were reported. A repeated insult patch test of an eye gel with 0.1% Glycyrrhizic Acid on 100 humans (applied 3/week for 3 wk) resulted in 6 subject with mild erythema with/without edema at the challenge 20 d after last application but no evidence of induced contact hypersensititvity (CIR 2005).

Licochalcone A

Weber et al. (2005) tested the tolerance and efficacy of 4 skin care products containing licochalcone A on female patients with mild to moderate erythematotelangiectanic rosacea (n = 32) and red facial skin not attributed to rosacea (n = 30). The patients used a cleanser, an SPF 15-day lotion, a spot concealer, and a night cream (all with licochalcone A; concentrations not provided) daily for 8 wk. Clinical grading was done at baseline and weeks 4 and 8. There was improvement in the patient's erythema scores at weeks 4 and 8 ($p < .05$). Overall improvement was noted at weeks 4 and 8 ($p < .05$). There were no adverse effects reported.

Licorice Flavonoid

Sansho Seiyaku Co., Ltd. (2004) applied a cream containing 0.65% licorice flavonoid derived from *G. glabra* to 20 healthy volunteers. A 48-h closed patch test was performed. Although a single volunteer showed a slight erythema at 1 h after removal of the patch, it disappeared 24 h later. No reaction, including erythema and edema, was observed in the remaining volunteers.

Skin Lightening Effects

Liquritinin

Amer and Metwalli (2000) examined the effects of liquiritin on melasma. Women (n = 20; 18 to 40 years old) with bilateral melasma on the face applied a liquiritin (2%) cream (1 g/d) to 1 side of the face and the vehicle control to the other side twice daily for 4 wk. The authors stated that an excellent response was observed in 16 women (80%); i.e., progressive lightening of the pigmented area and reduction in lesion size, sometimes with a discernible outline. A good response was observed in 2 (10%) of the women. Mild skin irritation (erythema and burning sensation) was observed in 2 patients (10%) treated with the liquiritin cream which disappeared with continuation of treatment. Analysis of blood samples taken before and after treatment were within normal values. During the follow-up period of 10 wk, 1 patient showed a mild degree of pigmentation.

Phototoxicity and Photosensitization

Glycyrrhizic Acid

Glycyrrhizic Acid at 0.1% and 5% applied to human skin (in vivo) and exposed to UV irradiation did not result in photosensitization or phototoxicity (CIR 2005).

Licochalcone A

Two areas of the backs of subjects (n = 12) were exposed to 1.4 minimal erythema dose of UV radiation. The areas were then treated with a licochalcone A-containing lotion or the vehicle. After 5 and 24 h, the areas were graded for erythema. Erythema was reduced at the 5-h observation ($p < .05$) but not at the 24-h observation. These authors concluded that licochalcone A has topical anti-inflammatory effects (Immeyer, et al. 2005).

Epidemiology

Glycyrrhizic Acid

An epidemiology study of 1,049 women who consumed Glycyrrhizic Acid and birth statistics for their offspring showed that birth weight and maternal BP were not affected by maternal licorice consumption. Babies with heavy exposure to Glycyrrhizic Acid ($\geq$ 500 mg/week) were more likely to be born before 38 wk (CIR 2005).

Food Use Evaluation

The European Commission's Scientific Committee on Food (SCF; 2003) released an opinion on the safety of Glycyrrhizic Acid and Ammonium Glycyrrhizate in foods. The Committee had previously reviewed Glycyrrhizic Acid in 1991 and concluded that the data were inadequate to derive an acceptable daily intake

(ADI). However, they advised that regular daily ingestion from all food products should not exceed an upper use level (UUL) of 100 mg/day.

In 2003, the SCF reviewed new data on Glycyrrhizic Acid and considered the safety of Ammonium Glycyrrhizate as a flavoring substance. After reviewing the new data, the SCF concluded that there is a stronger basis for the limit of 100 mg/day, but they did not conclude that the human toxicity data were sufficient to develop an ADI. They noted that there are subgroups of people for whom a limit of 100 mg/day may not provide sufficient protection. These groups include people with decreased 11--OHSD activity, people with prolonged gastrointestinal transit time, and people with hypertension or electrolyte-related or water homeostasis-related medical conditions (SCF 2003).

SUMMARY - Part II

A wide variety of chemicals may be found in licorice extracts. Individual chemical constituents vary in their dermal penetration and intestinal absorption, but once absorbed are metabolized and widely distributed. For example, while licochalcone A was not dermally absorbed; liquiritigenin, davidigenin, liquiritin, and liquiritin apioside were dermally absorbed. Most of the licochalcone A injected into rats metabolized into 4'-phenolic glucuronides in the plasma and urine.

The metabolic relationship between Glycyrrhizic Acid and Glycyrrhetinic Acid were described in an earlier safety assessment. Intravenously administered glabridin to rats was detected in the cerebellum and spinal fluid. Oral absorption and oral bioavialability were low and dose-dependent for glabridin, but it was found in all tissues with the greatest amount in the lungs.

Glycyrrhizic Acid and its derivatives and metabolites may cause several transient systemic alterations including: increased potassium excretion, sodium and water retention, body weight gain, alkalosis, suppression of renin-angiotensin-aldosterone system, hypertension, and muscular paralysis.

Extensive information was described regarding protective effects of these chemical constituents of licorice. For example, glabridin, licochalcone A, and licorisoflavan A decreased the effects of induced nephritis in TCL and BUN in mice. Hepatic protection has been reported for Glycyrrhizic Acid, glabridin, isoliquiritigenin, licopyranocoumarin, liquiritin, liquritin and glycyrrhizin. Deglycyrrhizinized licorice had gastrointestinal protective effects from ulcers in rats and dogs as did isoliquiritigenin in rats.

Neurological effects were reported for several constituents, Glycyrrhizic Acid and its derivatives reversibly blocked gap junction communication. Glycyrrhetinic Acid administered ip to mice caused sedation, hypnosis, hypothermia, and respiratory depression. Glycyrrhetinic Acid administered ip did not stimulate or depress either the sympathetic or parasymathetic branches of the autonomic nervous system of mice. The relaxant effects of glycycoumarin from cultivated and wild G. uralensis were similar in mice.

Muscular contraction was reported for glycycoumarin, while inhibition of contraction was reported for isoliquiritigenin and davidigenin inhibited induced jejunum contractions; liquiritigenin was a weak inhibitor and liquiritin, liquirtitin apioside, and isoliquirtin were inactive.

Erythrocytes had shape deformations when incubated with licochalcone A. Isoliquiritigenin reduced platelet aggregation in human and rat blood.

Treatment with Glycyrrhizic Acid, but not glycyrrhetinic acid, reduced the size of myocardial infarct in rabbits with a suture tied around the left coronary artery to occlude blood flow. Glabridin in drinking water reduced lesion area in mice aortas. Glycyrrhizic Acid combined with hydrocortisone increase the degree of vasoconstricion in humans.

Several flavonoids, including glabridin, were inhibitors of cAMP. Glycyrrhizic Acid inhibited the enzyme activity of proteinase and was a potent inhibitor of oxidative phosphorylation coupled to succinate oxidation in rat liver mitochondria. Glycyrrhizic acid administered to mice induced hepatic CYP3A-2B1 and 1A2-dependent microsomal monooxygenase and TH enzymes in the liver.

Glycyrrhetinic acid administered i.p. caused apoptosis in splenocytes and thymocytes in mice. Glabradin was not cytotoxic to human, canine kidney, rat brain, or multiple drug-resistant cells. Glycyrrhizan GA enhances phagocytic activity in some cells. Licochalcone A and E were not cytotoxic to human lung, ovarian melanoma, and colon cell lines. Polyphenol compounds from licorice had higher cytotoxicity toward oral HSC-2 and HSG cell lines than against normal human gingival fibroblasts.

Isoliquiritigenin inhibits histamine mediated signals and is a selective antagonist in U937 cells. Isoliquiritigenin inhibited monocyte adhesion in a dose-dependent manner. Licochalcone A reduced induced ROS, PGE2, and cytokine production in human skin cells in a dose-dependent manner. Induced DNA relaxation was inhibited by licochalcones A and E. Isoliquiritgenin inhibited MAO of rat livers. MAO inhibitory effects were found in glicoricone and licofouranone. Anti-inflammatory effects were found with glabridin, Glycyrrhizic Acid, licochalcone A and B, and various other licorice constituents.

Glycyrrhizic Acid had an anti-diabetic effect in genetically diabetic mice; the mice had lower blood glucose levels after oral doses of glucose. Glycyrin suppressed blood glucose levels in genetically diabetic mice; glycyrol did not. Isoliquiritigenin may prevent diabetic complications through aldose reductase inhibition in rat lenses, inhibition of sorbitol accumulation in human RBCs, and rat RBCs, sciatic nerves, and lenses.

Antioxidant effects were found for licochalcone A and B, glabridin, hispaglabridin A and B, formononetin, hemileiocarpin, paratocarpin B, and 4'-O-methylglabridin in in vivo and in vitro assays. Glycycoumarin had antioxidative properties while licocoumarone did not.

Glycyrrhizic Acid was found to have anti-viral effects on vaccinia, herpes simplex 1, Newcastle disease, vesicular stomatitis, JEV, and influenza virus A2. Polio type 1 virus was unaffected by Glycyrrhizic Acid up to 8 mM.

Anti-parasitic activity was demonstrated by licochalcone A by the inhibition of growth of Leishmania major and L. donovaini in culture and in human peripheral blood moncyte-derived macrophages and U937 cells.

Anti-microbial effects towards multiple species of microbes were demonstrated by many licorice constituents including gancaonin, glabridin, and licochalcone A.

Glycyrrhizic Acid increased the flux of diclofenac sodium across rat skin.

The oral LD_{50} of Glycyrrhitinic Acid in mice is 610 mg/kg; the i.p. LD_{50} is 308 mg/kg. Rats orally administered 1000 mg licochalcone A daily for 2 wk did not exhibit any signs of toxicity. Glycyrrhetinic Acid injected into rats 3 times/week for 4 wk had no tissue damage except slight thinning of the lipids in the zona glomerulosa of the adrenal glands. Daily injections of licochalcone A at 100 mg/kg to rats and hamsters showed no toxicity.

Glycyrrhetinic Acid is slightly irritating at 6% in the chorio-allantoic membrane assay. Glycyrrhetinic Acid was not irritating or sensitizing to the intact or shaved skin of albino rabbits. Liquritin was not or mildly irritating to guinea pigs. In an IgE-dependent passive cutaneous anaphylactic reaction assay on mice, 18β-glycyrrhetinic acid, isoliquiritinin, and liquiritigenin demonstrated inhibition both orally and i.p. as well as by a scratching behavior assay. In a sensitization and antigen challenge, liquirititigenin and isoliquiritin did not inhibit IgE production. Glycyrrhizin and 18β-glycyrrhetinic acid inhibited IgE production.

Glabridin was not phototoxic to guinea pigs with UVB exposure. Glabridin-treated (0.5%) sites on brownish guinea pigs were lighter in color after UVB irradiation.

Gancaonis R, glabrene, glycyrol, gismoidin B and liquiritigenin have weak binding affinties in an estrogen receptor binding assay. E2 was displaced with weak relative binding affinity by prunetin, daidzein, formononetin, liquiritigenin, datiscetin, fisetin, and isoliquiritigenin in human plasma. Glabridin stimulated the growth of human breast cancer cells up to 10 µM, but inhibited cell growth at concentrations >15 µM. Uterine weights were increased after rats were injected with glabridin, but not as much as estradiol. Glabradin and glabrene demonstrated estrogen activity when incubated with human female bone cells from pre- and post menopausal women.

In estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-231) breast cancer cells isoflavonoids genistein, biochanin A and coumestrol inhibited DNA synthesis, as did the flavones chrysin and apigenin, and the lignan enterolactone. Rutin stimulated DNA sythesis to 250% of control at 100 µM and showed no inhibition effects at the tested concentrations. Most of the compounds inhibited DNA synthesis at high concentrations in MDA-MB-231 cells. The isoflavonoids genistein, daidzein, biochanin A, and coumestrol inhibited DNA synthesis, as did the flavones chrysin, apigenin, and flavone and the flavonols quercetin, and kaempferol. Rutin, catechin, and enterolactone were weak inhibitors. Biochanin A and flavone showed slight stimulation.

In an Ames tests, glabrene was antimutagenic against spontaneous and EMS-induced mutations up to 1.0 µg/plate; it was toxic at 10 µg/plate. Glabrol, fromononet, glabridin, and Hispaglabridin A were not antimutagenic.

Glycyrrhizic Acid and its derivatives demonstrated tumor inhibition properties. Chalcones inhibited the proliferation of ovarian cancer cells. Human prostate carcinoma cells exposed to DBM had inhibited growth in a dose-dependent manner. The flavones gancaonin O and P and glyasperin A were more cytotoxic to oral tumor cells than normal cells. Isoliquiritigenin has tumorigenesis inhibiting properties in in vitro and in vivo assays for several types of cancer cells. Licochalcone A decreased number and size of DMBA-induced tumors in mice. Licochalcone A was not cytotoxic to PC-3 prostate cancer cells but caused growth inhibition. Licocoumarone caused apoptosis in U937 cells.

Glycyrrhizic Acid increased lymphocyte count in HIV patients and improved liver function. Licorice flavonoid oil orally administered to humans daily for 1 and 4 wk resulted in a slight elevation of plasma glabridin with accumulation but no clinical abnormalities.

Dermal application of Glycyrrhizic Acid (0.1%) showed irritation but not sensitization in humans. Skin care products containing licochalcone A applied to the face for 8 wk resulted in no adverse effects.

The offspring of women with a high daily consumption of Glycyrrhizic Acid had normal birth weights, but birth was more likely to be before 38 wk gestation than for controls.

DISCUSSION (Parts I and II)

The CIR Expert Panel recognized that the available data do not address all licorice-derived ingredients. The available data on the constituents of licorice, however, were consistent across species, demonstrating that the composition of the plants were similar enough that toxicity data on *G. glabra* extracts could be extrapolated to the others, and vice versa. The Panel also recognized that different constituents could be present in a licorice extract depending on the method of extraction.

In this regard, the Panel considered the available data on a wide range of individual chemical constituents to consistently demonstrate an absence of significant toxicity, with many studies reporting protective effects. These data on chemical constituents also are consistent with the safety test data for the individual extracts. The Panel concluded that the variation in constituents with different extraction solvents did not present different toxicity

issues regarding the use of licorice-derived ingredients in cosmetics.

The Panel recognized that constituent glabridin can have depigmentation properties. The concentration of glabridin is < 42% in polyol-soluble Glycyrrhiza Glabra (Licorice) Root Extract, which has the greatest concentration of glabridin. When used in a formulation, such a low concentration of glabridin should not have any dipigmentation activity. Cosmetic formulators should only use licorice extracts in products in a manner that does not cause depigmentation.

The Expert Panel expressed concern regarding pesticide residues and heavy metals that may be present in botanical ingredients, including licorice derivatives. They stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulation.

The cosmetic industry should also use the necessary procedures to limit mycotoxins before blending into cosmetic ingredients and that all these ingredients should be free of methanol.

The Panel believes that aflatoxin should not be present in Licorice and botanical ingredients that are derived from G. glabra, G.inflata, and G. uralensis; the Panel adopted the USDA designation of ≤ 15 ppb as corresponding to "negative" aflatoxin content.

The CIR Expert Panel noted the estrogen receptor and neurotoxic effects of the extracts. When applied dermally, licorice extracts are unlikely to significantly penetrate the skin, precluding exposure that could present any hazard in these regards.

The CIR Expert Panel recognizes that there are data gaps regarding use and concentration of these ingredients. However, the overall information available on the types of products in which these ingredients are used and at what concentrations indicate a pattern of use, which was considered by the Expert Panel in assessing safety.

In the absence of inhalation toxicity data, the Panel determined that the Glycyrrhiza Glabra (Licorice) Leaf Extract, Glycyrrhiza Glabra (Licorice) Rhizome/Root, Glycyrrhiza Glabra (Licorice) Root, Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) Root Juice, Glycyrrhiza Glabra (Licorice) Root Powder, Glycyrrhiza Glabra (Licorice) Root Water, Glycyrrhiza Inflata Root Extract, and Glycyrrhiza Uralensis (Licorice) Root Extract can be used safely in hair sprays, because the ingredients particle size is not respirable. The Panel reasoned that the particle size of aerosol hair sprays (~38 μm) and pump hair sprays (>80 μm) is large compared to respirable particulate sizes (≤10 μm).

CONCLUSION (Parts I and II)

Based on the available information, Glycyrrhiza Glabra (Licorice) Rhizome/Root, Glycyrrhiza Glabra (Licorice) Leaf Extract, Glycyrrhiza Glabra (Licorice) Root, Glycyrrhiza Glabra (Licorice) Root Extract, Glycyrrhiza Glabra (Licorice) Root Juice, Glycyrrhiza Glabra (Licorice) Root Powder, Glycyrrhiza Glabra (Licorice) Root Water, Glycyrrhiza Inflata Root Extract, and Glycyrrhiza Uralensis (Licorice) Root Extract are safe as cosmetic ingredients in the practices of use and concentration given in this safety assessment.[1]

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[1]Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in the group.

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Acronyms and Abbreviations

3T3 NRU PT - 3T3 Neutral Red Uptake Phototoxicity
5-HT - 5-hydroxytrptamine

AA - arachidonic acid
AAP - acetaminophen
AAPH - 2,2'-azobis'2-amidino propane hydrochloride
ABC - ATP-binding cassette
ACE - angiotensin converting enzyme
ACAT - acyl-coenzyme A:cholesterol acyltransferase
ACF - aberrant crypt fociACTH - adrenocorticotrophic hormone
ADH - antidiuretic hormone
ADI - acceptable daily intake
ADP - adenosine diphosphate
ALT - alanine aminotransferase
ANP - atrial natriuretic peptide
AOM - azoxymethane
ARS - Agricultural Research Service
ATHF - allotetrahydrocortisol
AUC - area under the curve
AST - aspartate aminotransferase

BFM - body fat mass
BHI - brain-heart infusion
BIA - bioelectrical impedance analysis
BMI - body mass index
B[a]P - benzo[a]pyrene

BP - blood pressure
BrdU - bromodeoxyruidine
BUN - blood urea nitrogen

CaCl2 - calcium chloride
Caco-2 - colonic adenocarcinoma cells
CAM - cellular adhesion molecule
cAMP - adensoine 3',5'-cyclic monophosphate
CC_{50} - 50% cytotoxic concentration
CCl4 - carbon tetrachloride
CD - conjugated dienes
cGMP - guanosine 3',5'-cyclic monophosphate
CIR - Cosmetic Ingredient Review
CK - creatine kinase
CL - cholesteryl linoleate
Cl- - chloride
CLOOH - cholesteryl linoleate hydroperoxide
CMC - carboxymethyl cellulose
CO - carbon monoxide
CO2 - carbon dioxide
Council - Personal Care Products Council
COX-2 - cyclooxygenase-2
CPS - calcium polystyrene sulfonate
CS - chondroitin sulfate
CTFA - Cosmetic, Toiletry, and Fragrance Association
CuSO4 - copper sulfide
CYP - cytochrome P450
CZE - capillary-zone electrophoresis

DBM - dibenzoylmethane
DCA - dichloroacetate
DES - diethylstilbestrol
DGL - deglycyrrhizinized licorice
DHT - 5α-dihydrotestosterone
DMBA - 7,12-dimethylbenz[a]anthracene
DMEM - Dulbecco's Modified Eagle's Medium
DMSO - dimethyl sulfoxide
DNFB - dinitrofluorobenzene
DNP - dinitrophenol
1,6-DNP - 1,6-dinitropyrene
DOPA - dihydroxyphenylalanine
DOPC - 1-alpha-dioleylphosphatidyl choline
DPPH - 2,2'-Diphenyl-1-picrylhydrazyl
DPPH• - 1,1-diphenyl-2-picrylhydrazyl
DSS - dextran sulfate sodium

E0 - apolipoprotein E-deficient
E2 - estradiol
EBSS - Earle's buffered salt solution
EBV-EA - Epstein-Barr virus antigen
EC - endothelial cells
EC_{50} - 50% effective concentration
ECG - electrocardiogram
ECW - extra cellular water
ED_{50} - effective dose
EDTA - ethylene diamine triacetic acid
EEG - electroencephalogram
EKG - electrocardiogram
ELISA - enzyme linked immunosorbent assay
E-MEM - Eagle's minimum essential medium
EMS - ethyl methanesulfonate
EPR - electron paramagnetic resonance
ER - estrogen receptor
E-selectin - endothelial leukocyte adhesion molecule-1
ESR - electron spin resonance
EtOAc - ethyl acetate

FBS - fetal bovine serum
FCA - Freund's complete adjuvant
FCS - fetal calf serum
FDA - U.S. Food and Drug Administration
fMLP - formyl-methionyl-leucyl-pheny-lalanine tripeptide

GABAB - gamma-aminobutyric acid-BGBM - antiglomerular basement membrane
GC - gas chormatography
GIHM - glycyrrhizin (licorice)-induced hypokalemic myopathy
GOT - glutamate-oxaloacetate transaminase
GPT - glutamate-pyruvate transaminase
GRAS - generally recognized as safe
GS - genetic similarity coefficient
GSH - glutathione
GSH-Px - glutathione peroxidase
GST - glutathione-S-tranferase

H2R - histamine type 2 receptors
-HCG - β-human chorionic gonadotrophin
HDA - histidine decarboxylase activity
HDL - high density lipoprotein
hER - human estrogen receptor
12-HETE - 12-hydroxyeicosatetraenoic acid
HGF - human gingival fibroblast
HHT - 12(S)-hydroxy-5,8,10-heptadecatrienoic acid
HIV - human immunodeficiency virus
HMG-CoA - 3-Hydroxy-3-Methyl-Glutaryl-CoA
H_2O_2 - hydrogen peroxide
HPLC - high performance liquid chromatography
HRP - horseradish peroxidase
HSC - human squamous cell
hSERT - human serotonin transporter
HSG - human salivary gland
HT - hypertensive
HUVEC - human umbilical vein endothelial cells
β-hydroxy-DHP - 1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4'-hydroxyphenyl)

IBMX - 3-isobutyl-1-methylxantine
IC_{50} - median inhibition concentration
ICAM-1 - intercellular adhesion molecule-1
ID_{50} - inhibitory dose
IFN - interferon
IgE - immunoglobulin E
IKK - ikappa-α/β kinases
IL - interleukin
i.m. - intamuscular
INCI - International Nomenclature Cosmetic Ingredient
iNOS - inducible nitric oxide synthase
i.p. - interperitoneal
i.v. - intravenous

JEV - Japanese encephalitis virus

KFDA - Korea Food & Drug Administration
Ka - association constant

Ki - inhibition constant
Km - Michaelis-Menten constant
LC-MS/MS - liquid chromatography tandem mass spectrormetry

LDH - lactic acid dehydrogenase
LDL - low density lipoproteinLH - leuteinizing hormone
LLNA - local lymph node assay
LOAEL - lowest observed adverse effect
LPO - lipid peroxidation
LPS - lipopolysaccharides
LTB4 - leukotriene B4

mAb - monoclonal antibody
MAO - monoamine oxidase
3-MC - 3-methylcholanthrene
MD-571 - 3-[[3-[2[(7-chloroquinolin-2-yl)vinyl]phenyl]-(2-dimethylcarbamoylethylsulfanyl)methylsulfanyl] propionic acid
MDA - malondialdehyde
MDCK - Madin-Darby canine kidney
MDM - monocyte-derived macrophages
MDR - multidrug resistance
MHLW - Ministry of Health, Labor and Welfare (Japan)
MIC - minimum inhibitory concentration
MNNG - N-methyl-N'-nitro-N-nitrosoguanidine
MPM - mouse peritoneal macrophages
MPO - myeloperoxidase
MRP - multidrug resistance-associated protein
MRSA - methicillin-resistant S. aureus
MS - mass spectra analysis
MSD - mass selective detector
MSSA - methicillin-sensitive S. aureus
MTT - 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
MW - molecular weight

NaCl - sodium chloride
NADH - nicotinamide adenine dinucleotide
NADPH - nicotinamide adenosine dinucleotide phosphate
NDH - NADH dehydrogenase
NF - nuclear factor
NMR - nuclear magnetic resonance
NO - nitrous oxide
NOEL - no observed effects level
NOS - nitric oxide synthase
NR - neutral red
ns - not significant
NSAID - non-steroidal anti-inflammatory drugs
NT - normotensive

OD - optical density
17-OHP - 17-hydroxyprogesterone
11β-OHSD - 11β-hydroxysteroid dehydrogenase-2
ORAC - oxygen radical absorbance capacity
Ox-LDL - oxidized low-density lipoprotein

Papp - apparent permeability coefficients
PARP - poly ADP ribose polymerase
PBMC - peripheral blood mononuclear cells
PBP2' - penicillin-binding protein 2'
PBS - phosphate-buffered saline
PCR - polymerase chain reaction
PDE - phosphodiesterase
PECAM-1 - platelet endothelial cell adhesion molecule-1
PgP - P-glycoprotein
PGE2 - prostaglandin (with 2 double bonds)
PI - propidium iodide
PIF - photo irritation factor
PKG - cGMP-dependent protein kinase
PMA - phenylmercuric acetate
PMN - polymorphonuclear neutrophil
PMS - phenylmercuric acetate
p.o. - per os
PON1 - paraoxonase
PPAR - peroxisome proliferator-activated receptor
PR - progesterone receptor
PRA - plasma renin activity
PTZ - pentylenetetrazol

QR - quinone reductase

RAA - renin-angiotensin-aldosterone
Rb - retinoblastoma
RBA - relative binding affinity
RBC - red blood cells
RBMVEC - cryopreserved primary rat brain microvascular endothelial cells
RL - ribosine-lysine
ROS - reactive oxygen species
RT-PCR - reverse transcriptase-polymerase chain reaction

SARS - severe acute respiratory syndrome
s.c. - subcutaneously
SCL - serum creatinine level
SDL - step-down latency
SDS - sodium dodecyl sulfate
sGC - soluble guanylyl cyclase
SHBG - sex hormone-binding globulins
SI - stimulation index, selective index
SNMC - Stronger Neo-Minophagen-C
SOD - superoxide dismutase
STZ - streptozotocin

TBARS - thiobarbituric acid reactive substances
TCL - total cholesterol level
TDC - taurodeoxycholic acid
TEER - transepithelial electric resistance
TG - triglyceride
TH - testosterone hydroxylase
THE - tetrahydrocortisone
THF - tetrahydrocortisol
TL - transfer latency
TLC - thin-layer chromatography
TNF - tumor necrosis factor
TPA - 12-o-tetradecanoylphorbol-13 (acetate)
TRP-2-1 - 3-amino-1,4-dimethyl-5 H-pyrido[4,3-b]indole (Trp-P-1)
TRP-2-2 - 3-amino-1-methyl-5 H-pyrido-[4,3-b]indole
TUNEL - terminal deoxynucleotidyl transferase mediated dUTP nick end labeling

UDP - uridine diphosphate
UDPGA - uridine diphosphate glucuronic acid
UFE - free cortisone
UFF - free cortisol
UGT - uridine diphosphoglucuronosyltransferase
UPX - urinary protein excretion

UUL - upper use level
UV - ultraviolet

Vmax - maximum volume of distribution
VCAM-1 - vascular cell adhesion molecule-1
VCRP - Voluntary Cosmetic Ingredient Reporting Program
VEGF - vascular endothelial growth factor
VLDL - very low density lipoprotein
VRE - vancomycin-resistant enterococci
VSE - vancomycin-sensitive enterococci
VSMC - vascular smooth muscle cells