Glycyrrhiza glabra

Scientific Name

***Glycyrrhiza glabra* L.**

Synonyms

Glycyrrhiza brachycarpa Boiss., *Glycyrrhiza glabra* var. *caduca* X.Y. Li, *Glycyrrhiza glabra* var. *glabra*, *Glycyrrhiza glabra* subsp. *glandulifera* (Waldst. & Kit.) Ponert, *Glycyrrhiza glabra* var. *glandulifera* (Waldst. & Kit.) Regel & Herder, *Glycyrrhiza glabra* var. *glandulifera* (Waldst. & Kit.) Boiss., *Glycyrrhiza glabra* var. *glandulosa* X.Y. Li, *Glycyrrhiza glabra* var. *laxifoliolata* X.Y. Li, *Glycyrrhiza glabra* var. *typica* L., *Glycyrrhiza glabra* var. *violacea* (Boiss. & Noe) Boiss., *Glycyrrhiza glandulifera* Waldst. & Kit., *Glycyrrhiza hirsuta* Pall., *Glycyrrhiza pallida* Boiss. & Noe, *Glycyrrhiza pallida* Boiss., *Glycyrrhiza violacea* Boiss. & Noe

Family

Fabaceae

Common/English Names

Black Sugar, Common Liquorice, Licorice, Licorice-Root, Liquorice, Liquorice Root, Persian Licorice, Rhizoma Glycyrrhizae, Russian Licorice, Russian Liquorice, Si-Pei Licorice, Sinkiang Licorice, Spanish Juice, Spanish Licorice, Spanish Liquorice Sweet Root, Sweet Wood, Sweet Wood Liquorice, True Licorice, True Liquorice

Vernacular Names

Afrikaans: Drop
Albanian: Gliciriza E Shogët, Glicirizë
Arabic: Irq As-Sus, Irqu As-Sus, Irqu Al-Sus, Sous, Sus
Armenian: Madoodag, Matutak
Azeri: Biyanlıq
Basque: Erregaliz, Gotxerro, Makilgoxo
Belarusian: Lakryčnik
Brazil: Alcaçuz (Portuguese)
Breton: Regalis
Bulgarian: Sladnik, Sladuk Koren
Burmese: Noekiyu
Catalan: Regaléssia
Chinese: Gān Cǎo, Gam Chou, Guangguo–Gancao, Kan Tsau, Xi-Bei, Yang Gan Cao
Croatian: Sladki Korijen, Slatki Sladić
Czech: Lékořice, Lékořice Lysá, Sladký Dřevo
Danish: Glat Lakrids, Lakrids, Lakrids Rod, Lakridsrod, Lakridsplante
Dutch: Zoethout
Esperanto: Glicirizo

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Estonian: Lagrits, Lagritsa-Magusjuur, Magusjuur
Farsi: Shirin Bayan
Finnish: Lakritsi, Lakritsijuuri, Lakritsikasvi
French: Bois Sucré, Régalisse, Reglisse, Réglisse Glabre
Gaelic: Carra-Meille, Carrchan, Maide-Milis
German: Gemeines Süssholz, Kahles Süssholz, Lacrisse, Lakritz, Lakritze, Lakritzeholz, Lakritzenwurzel, Lakritzpflanze, Lakrizenwurzel, Spanisches Süssholz, Süsscholz, Süssholz, Süsswurz
Greek: Glikoriza, Glykoriza, Glykyrrhiza
Hebrew: Shush, Shush Kireah
Hungarian: Édesfa, Igazi Édesgyökér
Icelandic: Lakkrís, Lakkrísrót
India: Jesthimadhu, Yeshtmadhu, Zostimodhu (Assamese), Jaishbomodhu, Yashthimadhu (Bengali), Veymui (Dhivehi), Mulethi (Dogri), Jethimadha, Jethimard, Jethimadh, Jethimadhu (Gujarati), Jetimadh, Jethimadhu, Jethimadh, Jothi-madh, Mulathi, Mulethi, Mulaithi, Mulhathi, Yashtimadhu (Hindi), Atimadhura, Jestamadu, Jyeshtamadhu, Madhuka, Yasthimadhu (Kannada), Multhi (Kashimiri), Mauddh (Maithili), Etthimadhiram, Iradimadhuram, Irattimadhuram, Madhugam, Yashtimadhugam (Malayalam), Jestamadha (Marathi), Jatimadhu, Jasthimadhu (Oriya), Jethimadh, Malathi, Mulathi (Punjabi), Madhuuka, Madhuyasti, Yashti, Yastika, Yashtimadhu, Yashtomadhu (Sanskrit), Athimadhuram, Atimaduram (Tamil), Atimadhuramane, Atimadhuramu, Yastimadhu, Yashtimadhukkam (Telugu), Asl-us-sus, Mulhati, Mulethi (Urdu)
Indonesian: Akar Manis, Licorice
Irish: Liocras
Italian: Liquirizia, Liquirizia Commune, Regalizia, Regolizia
Japanese: Nankin-Kanzō, Nankin-Kanzo, Rikorisu, Kanzō, Kanzo
Kazakh: Mïya, Qızılmïya
Korean: Kamcho, Rikeorisu, Rikorisu
Laotian: Sa-Em, Sa-Em Thet
Latvian: Lakrica
Lithuanian: Saldymedis, Paprastasis Saldymedis
Macedonian: Sladok Koren
Malaysia: Akar Likuoris
Mongolian: Chiher Övs
Nepal: Istami, Jethimadhu (Nepali), Istivi (Nepalbhasa)
Norwegian: Lakrisplante, Lakrisrot
Pashto: Shireen Buya
Polish: Korzeń Lukrecji, Lukrecja Gładka
Portuguese: Alcaçuz
Provençal: Recalicé, Recalissi
Romanian: Lemn Dulce, Rădăcină Dulce, Reglisă
Russian: Koren Solodki, Lakrichnik, Lakritsa, Solodka
Serbian: Konjeda, Slacić, Sladić, Slatki Koren, Slatko Drvce
Slovak: Sladké Drievko, Sladovka Hladkoplodá
Slovenian: Golostebelni Sladki Koren, Sladki Koren, Sladki Koren Golostebelni
Spanish: Alcazuz, Orozus, Orozuz, Paloduz, Regaliz
Sri Lanka: Atimaduram, Valmi (Sinhala)
Swahili: Susu
Swedish: Äkta Lakrits, Lakrits, Lakritsrot, Lakritsväxt, Sötlakrits
Thai: Cha-Em Thet
Tibetan: Shi Na Ma Ngar, Shina ngar
Turkish: Biyam, Meyan, Meyankökü, Piyan, Tatlı Kök, Tatlı Meyan
Ukrainian: Lokrytsya, Solodkyj Korin, Solodka Hola
Uzbek: Miya, Qizilmiya
Vietnamese: Cam Thảo
Yiddish: Lakrets, Ziswortsl

Origin/Distribution

Glycyrrhiza glabra is native to Eurasia, in central and south-western Asia and the Mediterranean region (Plate 2). According to Hayashi (2009), Hayashi and Sudo (2009), *G. glabra* is found in South Europe (Spain, Italy), Turkey, Iran, Iraq, Central Asia and the north-western part of China, while *G. uralensis* is found in Central Asia, Mongolia and north-western and north-eastern parts of China, and *G. inflata* is found only in the north-eastern part, the Xinjiang Uygur Autonomous Region of China. *G. glabra* is divided into two varieties: *G. glabra* var. *typica* (Spanish licorice) and *G. glabra* var. *glandulifera*

(Russian licorice) (Hayashi and Sudo 2009). Three varieties of *G. glabra* have been reported; the Spanish and Italian licorice, assigned to *G. glabra* var. *typica*; Russian licorice to *G. glabra* var. *glandulifer*a; Persian and Turkish licorice to *G. glabra* var. *violace*a (Nomura et al. 2002).

Countries producing liquorice include Iran, Afghanistan, the People's Republic of China, Pakistan, Iraq, Azerbaijan, Uzbekistan, Turkmenistan and Turkey. In China, commercial licorice is produced from the three aforementioned species.

Agroecology

Licorice grows well in temperate, warm and subtropical climate. It thrives best in well-limed, well-drained, composted, loose, friable, deep soil, preferably in full sun. Licorice is not bothered by frosts, as it is dormant in winter, and actually benefits by the defined cold period, which induces the translocation of properties to the underground rhizomes. They are easily grown from divisions or root cuttings.

Edible Plant Parts and Uses

Fresh liquorice root when washed is externally of a bright yellowish-brown colour and is chewed fresh or dried as a mouth freshener, for teething in children and also as a tooth cleaner (Chiej 1984). Dried liquorice root can be chewed as a sweet. The extract of liquorice in rolls is glossy black in colour, often used in cough lozenges and pastilles. In Calabria a popular liqueur is made from pure liquorice extract. Liquorice is also very popular in Syria and Egypt, where it is sold as a drink, in shops as well as by the street vendors. Liquorice is used by brewers to flavour and colour porter classes of beers, and the enzymes in the root also stabilize the foam heads produced by beers brewed with it. Licorice powder used in sweets, baked goods, ice cream, soft drinks, etc., and the powdered root is also used as a sweetener in other herbal teas (Facciola 1990). The leaves are used as a tea substitute in Mongolia (Facciola 1990).

The licorice root contains glycyrrhizin, a substance that is 50 times sweeter than sucrose (Hill 1952; Facciola 1990; Bown 1995). Glycyrrhizin imparts a sweet taste to foods; moreover, it has salt-softening and flavour-enhancing properties and is also heat stable (Hayashi and Sudo 2009). Most Japanese people do not like the long-lasting sweet taste of glycyrrhizin; however, a more acceptable sweetness can be created by using a combination of glycyrrhizin and natural sugars or other sweeteners. Therefore, glycyrrhizin and licorice extracts are used as food additives in a variety of foods such as snacks, instant noodles, sausages and sauces. Glycyrrhizin is used in sweet foods such as sweet snacks, sweets and candies, ice creams and sherbets to enhance their sweetness. It is also used to reduce the saltiness of salty foods such as soy sauce, other sauces, savoury snacks, 'kamaboko' (boiled fish paste), tsukudani (fish boiled in soy sauce), 'tsukemono' (Japanese pickles) and sausages in Japan. In Japan, enzymatically modified licorice extract (a-glycosyl-glycyrrhizin) and enzymatically hydrolysed licorice extract (glycyrrhetinic acid 3-*O*-glucuronide) are also used as sweeteners (Hayashi and Sudo 2009). Most liquorice is used as a flavouring agent for tobacco. Licorice not only imparts a sweet taste but also an aroma of tobacco, which makes it mild (Nieman 1959). It also prevents the desiccation of tobacco. The licorice extracts used in the tobacco industry are supplied by an American company, namely, MAFCO. Licorice extracts were first used for flavouring confectionery products in England during the eighteenth century in Pontefract in Yorkshire; it was blended with sugar, flour and other ingredients to make Pontefract cakes (Nieman 1959). Nowadays, licorice confectionery is widely available in western countries, and large quantities of licorice are used in the confectionery industry. In the Netherlands, where liquorice candy ('drop') is one of the most popular forms of sweets, only a few of the many forms that are sold contain aniseed, although mixing it with mint, menthol or with laurel is quite popular. *Glycyrrhiza glabra* root is one of the common traditional Chinese medicines and used as flavouring and sweetening agents for tobaccos, chewing gums, candies, toothpaste and beverages (Dong et al. 2007).

Botany

A herbaceous perennial, with stem 0.5–1.5 m high, woody at base, densely scaly glandular punctuate with stoloniferous roots. Leaves imparipinnate, 7–15 cm long with 9–17 ovate-oblong, oblong-lanceolate, or elliptic leaflets 1.7–4.0 by 0.8–2.0 cm, abaxially densely scaly glandular punctate and pubescent on veins, adaxially glabrescent or pilose (Plate 1). Stipules caducous, linear, 1–2 mm. Inflorescence open, racemose, many flowered. Flowers 0.8–1.2 cm long. Calyx campanulate, 5–7 mm, 5-toothed, upper 2 teeth mostly joined; corolla purple or pale whitish blue, 9–12 mm, standard ovate or oblong, 1–1.1 cm, base clawed, wings 8–9 mm, keel straight, 7–8 mm; ovary glabrous. Fruit oblong, flat, glabrous or sparsely hairy legume, 2–3 cm long, containing 2–8, dark green, smooth seeds, 2 mm across.

Plate 1 Liquorice foliage

Plate 2 Liquorice plant label

Nutritive/Medicinal Properties

Root/Stolon Phytochemicals

Licorice is a powerful natural sweetener, 50–170 times sweeter than sucrose (Mukhopadhyay and Panja 2008). The chemical constituents of the roots include several bioactive compounds such as glycyrrhizin (~16 %), different sugars (up to 18 %), flavonoids, saponoids, sterols, starches, amino acids, gums and essential oils. Licorice roots were reported to contain 25–30 % starch, 3–10 % D-glucose and sucrose, 3–5 % glycyrrhizin and traces of flavonoids, saponoids, sterols, amino acids, etc. (Fenwick et al. 1990). Licorice root contained phenolic constituents (such as coumarin compounds, glycerol, glycerine, glycycoumarin, herniarin, umbelliferone, licopyranocoumarin, licoarylcoumarin and licocoumarone), amines (1–2 % asparagines, betaine and choline), amino acids, sterols (stigmasterol and β-sitosterol) and sugars (5–15 % as glucose, sucrose and mannitol), and starch about 20 % of dried root (Blumenthal 2000). Flavonoids, saponins and sugars were found in the methanol root extract, and sterols in the crude petroleum ether extract (Chopra et al. 2013). Alkaloids, proteins and tannins were not detected. The mineral elements found in the roots included K (0.66 %), Ca (1.87 %), S (0.09 %), Fe (0.14 %), P (0.06 %), Mg (0.17 %), Na (0.04 %), Si (0.12 %), Al (0.05 %), Sr (0.06 %), Mn (tr), Ti (tr) and AS (tr). The amount of total phenolics in Turkish *G. glabra* roots was 12.88 μgGAE/mg DW (Ercisli et al. 2008). The average composition (mg/100 g) of N, P, K, Ca, Mg, Fe, Mn, Zn, Na and Cu in licorice roots was 2.80 %, 175 mg, 1400 mg, 147 mg, 120 mg, 20 mg, 6 mg, 4.4 mg, 2.1 mg and 0.1 mg, respectively. Eight commercial licorice extracts used as food additive (sweetener) in Japan were found to contain 0.3–12.% ash, 10.9–77.4 % glycyrrhizin, 0.1–1.2 % sodium, 0.3–5 % potassium and 0.03–2.5 % ammonium nitrogen and pH of 4.1–6.8 (Iida et al. 2007).

The roots of *G. glabra* were reported to contain 1.6 % of water soluble polysaccharides consisting of rhamnose, arabinose, mannose, glucose and galactose, and also 9.7 % of total polysaccharides (Dzhumamuratova et al. 1978). Denisova et al. (2003) found that >50 % of the ethanol extract of licorice root consisted of monosaccharides and disaccharides (7–8 mass % of the dry raw material). The principal component was saccharose (46.78 %). Significant quantities of D-mannopyranose (9.06 %), β-D-glucopyranose (7.06 %) and 2-*O*-hydroxyethylglucose (12.84 %) and smaller quantities of sorbose (4.12 %), α-D-fructose (2.01 %), β-D-fructose (2.56 %) and β-D-galactofuranose (1.88 %) were observed among the identified sugars. The sugar alcohols mannopyranosyl-D-glucitol (3.49 %), ribitol (0.95 %), mannitol (1.33 %), and myo-inositol (0.33 %) were present in insignificant amounts. Rhizomes were reported to contain alkaloids, triterpenes, saponins, flavonoids, polysaccharides, steroids and tannins (Meena et al. 2010). An acidic polysaccharide, named glycyrrhizan GA, was isolated from the stolon of *Glycyrrhiza glabra* var. *glandulifera* (Shimizu et al. 1991). Its molecular mass was estimated to be 85,000 and it comprised L-arabinose: D-galactose: L-rhamnose: D-galacturonic acid: D-glucuronic acid in the molar ratio of 22:10:1:2:1, in addition to small amounts of *O*-acetyl groups. Glycyrrhizan GA, a representative polysaccharide with remarkable phagocytosis-enhancing activity, was isolated from the stolon of *Glycyrrhiza glabr*a var. *glandulifera* (Takada et al. 1992). The core structural features of glycyrrhizan GA included a backbone chain composed of β-1,3-linked D-galactose residues. Three-fifths of the galactose units in the backbone carry side chains composed of β-1,3- and β-1,6-linked D-galactosyl residues at position 6. Monosaccharide composition of the polysaccharides isolated, measured as alditol acetates, of Chinese and Hungarian *G. glabra* roots was very similar, but the Lithuanian *G. glabra* and the *G. echinata* were quite different (Gyémánt et al. 2001). All investigated samples contained glucuronic acid, the *G. echinata* contained also galacturonic acid. Although the yield of Hungarian origin species was found lower than the yield of eastern species, the uronic acid content was similar (Kiss et al. 1998). The water-extracted arabinogalactan protein enriched fraction of *Glycyrrhiza glabra* was found to con-

sist mainly of 3- and 3,6-linked galactopyranosyl, and 5- and 3,5-linked arabinofuranosyl residues (Saha et al. 2011). The hexane extract of *G. glabra* was found to contain 70 % neutral and 30 % polar lipids (Denisova et al. 2007). Among neutral lipids, the main components were sterol esters (SEs), which accounted for about half of this fraction. Triacylglycerides (TAGs), free fatty acids (FFAs) and free sterols (FSs) comprised this fraction in approximately equal proportions, amounting to 10.0, 10.5 and 11.5 %.

The contents (mg/g) of total phenols, total flavonoids and total tannins in licorice extracts of *G. glabr*a roots at different harvest times were determined to vary a from 72.10 to 107.93 mg/g, 18.42 to 44.2 mg/g and 4.8 to 12.78 mg/g, respectively (Cheel et al. 2013). Liquiritin and glycyrrhizin, the major components of licorice extract, varied in the range of 28.65–62.80 and 41.84–114.33 mg/g, respectively. The relative proportion of glycyrrhizin derivative, glabridin, glabrene and liquiritigenin derivative, varied in the range of 0.88–11.38 %, 1.86–10.03 %, 1.80–18.40 % and 5.53–16.31 %, respectively. Treatment of in-vitro cultured 65-day-old *G. glabra* plantlets with 0.1–2 mM methyl jasmonate and 0.1 and 1 mM salicylic acid enhanced the production of glycyrrhizin by 3.8 and 4.1 times, respectively, as compared to the controls (Shabani et al. 2009). Increasing amounts of glycyrrhizin in the roots treated with methyl jasmonate inhibited root growth, while salicylic acid increased the amount of glycyrrhizin without negative effects on growth.

Thirteen terpenoids, minor sapogenins were isolated from *G. glabra* roots by Canonica and co-workers (Canonica et al. 1966a, b, c, 1967a, b, c, 1968) glabrolide, isoglabrolide, 11-deoxoglabrolide, liquiritic acid, 11-deoxoglycyrrhetinic acid, 3β-hydroxy-ll, 13(18)-oleanadien-30-oic acid, glypallidifloric acid, glycyrretol (glycyrrhetol), 21α-hydroxyisoglabrolide, 24-hydroxyglycyrrhetic acid, 24-hydroxy-11-deoxoglycyrrhetic acid, 18α-hydroxyglycyrrhetic acid, liquiridiolic acid (glyyunnansapogenin B_1) and 24-hydroxyliquiritic acid. Glabric acid was isolated by Beaton and Spring (1956). Bogatkina et al. (1975) isolated 3,24-dihydroxy-ll, 13(18)-oleanadien-30-oic acid as a methyl ester. The following triterpenoid compounds were isolated from *G. glabra* roots, a pentacyclic triterpenoid, liquoric acid, was isolated as the methyl ester (Elgamal et al. 1965); another triterpenoid glabric acid (Elgamal and Fayez 1968), 7-hydroxy-4′-methoxy-isoflavone (formononetin) (Reiners 1966; Elgamal and Fayez 1972), another triterpenoid, 28-hydroxyglycyrrhetic acid (Elgamal and El-Tawil 1975); a carboxyliedialcoholic triterpenoid was isolated as the diacetate-methyl ester (Elgamal and Fayez 1975); five new pentacyclic triterpenoids 11-desoxoglycyrrhetic acid acetate methyl ester; 24-acetoxy-l 1-desoxoglycyrrhetic acid acetate methyl ester; 11-desoxo-glabrolide acetate; glabrolide acetate and 3 ß-acetyl-18 ß-hydroxy-1 l-keto-olean-12-en-30-oic acid, 30,18 ß-lactone were isolated as minor constituents together with 24-hydroxy-11-desoxoglycyrrhetic acid methyl ester, 3 ß, 18 ß-dihydroxy-11 -keto-olean-12-en-30-oic acid, 30,18 ß-lactone and glabrolide (Elgamal et al. 1990). *Glycyrrhiza glabra* root yielded two saponins named glabranin-A, a pentaglycoside of glycyrrhetic acid, and glabranin-B, both a heptaglycoside of glycyrrhetic acid (Varshney et al. 1983). Glabranin A on hydrolysis gave glucose and rhamnose (4:1) whereas glabranin-B gave glucose, xylose and rhamnose in the molar ratio (4:1:2).

About 70 phenolic compounds were isolated from the subterranean parts of *G. glabra* (Nomura and Fukai 1998). The flavanone glycoside 7,4′-dihydroxyflavanone, better known as liquiritigenin and liquiritin its aglycone were first isolated from *G. glabra* roots by Shinoda and Ueeda (1934a, b). Liquiritin and the corresponding chalcone, isoliquiritin were isolated from the dried root (Puri and Seshadri 1954); they also isolated isoliquiritin from fresh roots but not liquiritin. Litvinenko et al. (1963a, b) isolated liquiritigenin [7,4′-dihydroxyflavanone], liquiritin [liquiritigenin 4′-(β-D-glucopyranoside)], neoliquiritin [liquiritigenin 7-(β-D-glucopyranoside)], [lacroside [liquiritigenin7-(β-D-glucopyranosyl(1 → 2)-β-d-apiofuranoside)] and uraloside [liquiritigenin 4′-(β-d-glucopyranosyl(1 → 4)-β-d-apiofuranoside)]

from *G. glabra roots*. Liquiritoside, a flavonoside, was isolated from root of licorice, *G. glabra* (Paris and Guillot 1955).

Licuroside was first isolated from the roots of *Glycyrrhiza glabra* by Litvinenko (1964), Litvinenko and Obolentseva (1964), and was found not to be a homogenous compound (Miething and Speicher-Brinker 1989). It was separated into two isomeric glycosides: isoliquiritigenin-4-β-D-apiofuranosyl-2″-β-D-glucopyranoside with the proposed name neolicuroside and isoliquiritigenin-4′-β-D-apiofuranosyl-2″-β-D-glucopyranoside. Licoricidin was isolated from *G. glabra* root and its structure elucidated as 3′,6-diisopentenyl-2′,4′,5-trihydroxy-7-methoxyisoflavan (Shibata and Saitoh 1968). An isoflavan named glabridin and glabrol, a flavanone, were isolated from Russian *G. glabra* root (Saitoh et al. 1976b); glabranin and two flavonoids identified as 5,7-dihydroxyflavanone (= pinocembrin) and 4′,5-dihydroxy-7-methoxyisoflavone (= prunetin) (Kattaev and Nikonov 1972, 1974); flavonoids 7-hydroxy-2-methylisoflavone; 7-acetoxy-2-methylisoflavone and quercetin, kaempferol, apigenin, liquiritigenin isoliquiritigenin (Bhardwaj et al. 1976b), liqcoumarin with the assigned structure 6-acetyl-5-hydroxy-4-methylcoumarin (Bhardwaj et al. 1976a); and an isoflavone glyzarin (2-methyl-7-hydroxy-8-acetyl-isoflavone) (Bhardwaj et al. 1977). From Chinese licorice, Sipei or Xi-bei (Seihoku Kanzo in Japanese) and assigned to *G. glabra* var. *glandulifera* by Hattori et al. (1986), a new flavonol licoflavonol together with known compounds kumatakenin, glycerol and licoricone with the structure 6-γ, γ-dimethylallyl-kaempferol were isolated (Saitoh et al. 1976a). The Chinese licorice *G. uralensis* had been given the Japanese name Tohoku Kanzo (Saitoh et al. 1976a). Isoliquiritin, rhamnoisoliquiritin, liquiritin, liquiritin apioside were isolated from the roots of *Glycyrrhiza glabra* var. *glandulifera* grown in Iran (Afchar et al. 1980).

From the root of *Glycyrrhiza glabra* var. *typica* two new flavonoids were isolated (van Hulle et al. 1971). The flavanone was identified as 7,4′ dihydroxy–flavanone with a glucose–rhamnose moiety at the 4′–position. The other flavonoid was the corresponding chalcone. The structure of a new 3-arylcoumarin, glycerin, isolated from the root of *Glycyrrhiza* sp. (si-pei licorice = Seihoku Kanzo) was determined as 2′, 4′-dihydroxy-5, 7-dimethoxy-6-γ, γ-dimethylallyl-3-arylcoumarin (Kinoshita et al. 1978). Two new flavanone glycosides, liquiritigenin 4′-apiosyl(1 → 2)-glucoside and liquiritigenin 7,4′-diglucoside together with a known flavone, apigenin 6,8-di-*C*-glucoside, were isolated from licorice (Yahara and Nishioka 1984). The following isoflavonoids and related substances glabridin (1), glabrol (2), glabrene (3), 3-hydroxyglabrol (4), 4′-*O*-methylglabridin (5), 3′-methoxyglabridin (6), formononetin (7), phaseollinisoflavan (8), hispaglabridin A (9), hispaglabridin B (13), salicylic acid and O-acetyl salicylic acid were isolated from *Glycyrrhiza glabra* var. *typica* (Mitscher et al. 1980). Two compounds 9,12,13-trihydroxy-(10*E*)-octadecenoic and 9,12,13-trihydroxy-10,11-epoxy-octadecanoic acid were isolated from licorice (Panossian et al. 1988). A new prenylated isoflavan derivative, kanzonol R, was isolated from *G. glabra* (Fukai et al. 1994). Two new pyrano-2-arylbenzofuran derivatives named glabrocoumarones A and B were isolated from commercially available licorice of *Glycyrrhiza glabra* origin, and their structures were elucidated as 4′-6-dihydroxy-[6″, 6″-dimethylpyrano(2″,3″:2′,3′)]-2-arylbenzofuran and 2′, 6-dihydroxy-[6″, 6″-dimethylpyrano (2″,3″:4′,3′)]-2-aryl-benzofuran, respectively (Kinoshita et al. 1996b). Six known compounds were also obtained and identified as glabrol, 3-hydroxygrabrol, shinflavanone, [6″, 6″-dimethylpyrano(2″,3″:7,8)]-[6″,6″-dimethylpyrano (2″,3″:4′,3′)]-flavanone (xambioona), 3, 3′-di-γ, γ-dimethylallyl-2′-4,4′-trihydroxychalcone and [6″,6″-dimethylpyrano(2″, 3″:4,5)]-3′-γ,γ-dimethylallyl-2′,3,4′-trihydroxychalcone. A new isoflavan 8-prenyl-phaseollinisoflavan was isolated from *Glycyrrhiza glabra* root, together with five known isoflavans identified as glabridin, 4′-*O*-methylglabridin, hispaglabridins A, B and 3′-hydroxy-4′-*O*-methylglabridin (Kinoshita et al. 1996a). 2, 2′, 4′-

Trihydroxychalcone was reported from *G. glabra* (Zhu et al. 2010).

High-performance liquid chromatography (HPLC) profiles of ethyl acetate extract of underground parts *G. glabra* : Type A glabrene, glabridin; 3,4-dihydroglabridin; 3-hydroxyglabrol; glabrol, 4′-*O*-methylglabridin; shinflavanone, hispaglabradin A and hispaglabridin B; and Type B glabrene, parvisoflavone B, glabridin; 3,4-dihydroglabridin; 3-hydroxyglabrol; glabrol, 4′-*O*-methylglabridin; shinflavanone, hispaglabradin A and hispaglabridin B (Kusano et al. 2003). Two new prenylated isoflavanones were isolated from licorice roots along with the known compounds cetoleic acid, β-sitosterol, stigmasterol, lanast-5,24-dien-3 β-D-glucuronopyranoside and glucuronic acid (Suman et al. 2009). The structures of the prenylated isoflavanones were established as 8-isoprenyl-7,4′-dihydroxylicoisoflavanone (glabraisoflavanone A) and 7,3′-dihydroxy-8-isoprenyl-4′-cyclogeranioloxyisoflavanone (glabraisoflavanone B). The following flavonoids were isolated from the ethyl acetate extract of licorice root: glabridin, the principal component, 4′-*O*-methylglabridin, hispaglabridin B, the isoflavene glabrin, unsilylated glabridin and monotrimethylsilyl derivative of 4′-*O*-methylglabridin (Denisova et al. 2006). Twelve flavonoids, hispaglabridin A, glabrol, 4′-*O*-methoxy glabridin, glabridin, 4′,7-dihydroxy flavones, 7-hydroxy-4′-methoxy flavones, isoliquiritigenin, 3,3′,4,4′-tetrahydroxy-2-methoxychalcone, liquiritigenin, licuroside, isoliquiritoside and isoononin were isolated from *Glycyrrhiza glabra* roots (Birari et al. 2011). The ethyl acetate extract of the rhizome afforded 7-hydroxycoumarin (umbelliferone) (Kaur et al. 2012a).

One hundred and twenty six compounds including flavonoids, terpenoids, saponins, essential oils, amino acids, other nitrogen containing compounds, hydrocarbons, fatty acids and their esters were found in the ethanolic extract of licorice (*G. glabra*) root (Vijayalakshmi and Shourie 2013). The most abundant phytoconstituents identified were: 5-(hydroxymethyl)-2-furancarboxaldehyde (23.15 %), *N*-methyl-4-(4-methyl-1-phthalazinylamino)-benzamide (7.18 %), 2-phenyl-furo[b]benzopyran-4(4H)-one (5.69 %), 1, 2-benzenedicarboxylic acid (5.31 %), 4H-pyan-4-one, 2,6-dimethyl- (4.48 %), dicycloctanopyridazine (2.7 %), 4-methyl-herniarin (2.67 %), cyclotetracosane (2.28 %), glabridin (2.02 %), tetracosan-1-ol (1.95 %), 5,6,6a,6b,7,12,12a,12b-dodecahydrodicyclopent(a,c)-anthracene-7 (1.82 %), cycolongifolene, 9,10-dehydro- (1.08 %), cycloserine (1.08 %) and 3,4-furandimethanol (1.01 %). Other compounds (>1 %) included: 4,5-dimethyl-1,3-dioxol-2-one; 4-hydroxy-*N*-methylpiperidine; 2,5-dimethyl-3(2*H*)furanone; 2,4,5-trimethyl-1,3-dioxolane; *N*, 1-dimethyl-4-piperidinamine; glycerine monoacetate; 3-isopropoxy alanine; 2,8,4,6 (epoxyethanediylidenoxy)[1,3]dioxino [5,4-d]-1,3-dioxin; 2-methoxy-4-vinylphenol; *cis*-dimethyl morpholine; 2-chloro-4-formyl-6-methoxyphenyl 4-morpholine carboxylate; 3-methyl-3-[2-oxopropyl]amino-2-butanone; *N*-methyl- 3-hdroxymethylpyrrolidin-2-one; sarreroside, 6-methoxy-8-methyl-8-azabicyclo [3.2.1]octan-3-ol; 5-ethylfuran-2-carboxylic acid; 1-dodecanol; 4-allyl-4-hydroxyprolin; 3,7-dimethylimidazo[1,2-a]pyrimidine-2,5(1H,3H)-dione; 3,5-ditert-butylphenol; dodecanoic acid; acetic acid, (2-isopropenylcycl opentylidene)-, methyl ester; 7-methoxy-2-benzofuranyl methyl ketone; isonicotinic acid, 2-tetrahydrofurylmethyl ester; (3*Z*)-3-ethyl-2-methyl-1,3-hexadiene; 1-(4-amino-furazan-3-yl)-5-pyrrolidin-1-ylmethyl-1H-[1,2,3]triazole-4-carboxylic acid ethyl ester; 6-methoxy-2-hydroxyquinoxaline-4-oxide; 2-furan propionic acid; β-methyl-4-methoxycinnamic acid; 2-pyridine carboxylic acid; benzoic acid; tetradecanoic acid, 4-((1*E*)-3-hydroxy-1-propenyl)-2-methoxyphenol; 4-(7-methoxy-3,3,7-trimethyl-oxepan-2-ylidene)-butan-2-one; linalool; stypticin; 2,6-nonadienoic acid; eicosanoic acid, 1-tert-butyl-2-methoxy-4-methyl-3,5-dinitorbenzene; *o*-nitrocumene,2-isopropylnitrobenzene; 1,2-benzenedicarboxylic acid; salicylic acid; pseduosarsasapogenin-5,20-dien; 3-doxy-D-mannonic acid; ascorbic acid; mome inositol; benzenepropanoic acid, 2,5-dimethoxy-; hexadecanoic acid, 1-methylethyl ester; 9-octadecadienoic

acid, methyl ester; 9-octadecanoic acid; octadecanoic acid, methyl ester; linoleic acid, methyl ester; 22-tricosanoic acid; hystrene S-97; *N*-[3-[6-hydroxyhexyl]aminopropyl]aziridine; hydrazine carboxaldehyde, 2,2-diethyl-, diethyl hydrazone; undecanoic acid, 11-amino-; 1-phenanthrenecarboxylic acid; 5α-andro-7-ene; tetrahydropyran-4-carboxylic acid; 2-hydroxybenzoic acid (1-methyl-2-oxo-1,2-dihydroindol-3-ylidene)-hydrazide; *N*-(4-hydroxybutyl) phthalimide; 2-methyl-6-phenyl-2,3,4,5-tetrahydro-3-pyridazinone;4-(4-methoxyphenyl)-6-phenyl-2-pyrimidinol; 3(2H)-benzofuranone-3-18ol,6-methoxy-2-(3-phenyl-2-propenylidene); 1,2,3,4-tetrahydroisoquinolin; cis-9-tricosane;2-phenyl-furo[b]benzopyran-4(4H)-one-flavone; *N*-(2,4-dimethyl-phenyl)-3-oxobutyramide; 2,3-dihydro-7-methoxy-8-(3-methylbut-2-enyl)-2-phenyl-4H-1-benzopyran-4-one; licoisoflavone B; β-methylumbelliferone (hymecromone); 7-acetoxy-4-methylcoumarin; 7-hydroxy-8-(γ,γ-dimethylally); benzene, 1,3,5-trimethyl-2-(2-phenylethenyl)-,(*Z*)-; 4-(3,4-dimethoxyphenyl)-6-phenyl-2-pyrimidinol; 5,5-dimethyl-4-phenylcarbonyl-1,3,4-oxadoazoline; 3-quinoline carboxylic acid; 1-hexadecanesulfonyl chloride; 7-(ethylamino)-4,6-dimethyl-2H-chromenone; squalene, 2,6,10,14,18,22-tetracosahexaene; isocordoin; pyrimidine-2,4(1H,3H)-dione, 6-amino-1-(2-methylphenyl)-3-(2-phenylethyl)-; 4H,8H-benzo(1,2b:3,4b′)dipyran-4-one; 1-(4-[6-(4-acetylphenyl)hexyl]phenyl)ethanone; 2-(5-oxo-1,5-diphenyl-3-p-tolyl-pent-2-enylidene)-indan-1-one; 1-triacontanol; ethanone, 1-(4-cyclohexylphenyl)-, 4-cyclohexylacetophenone; liquiritigenin; coumarine, 8-allyl-7-hydroxy-6-ethy l-4-methyl-; 1,3-dioxolo[4,5-g]isoquinolin-5-ol,5,6,7,8-tetrahydro-6-methyl-; glycyrrhiza chalcone (licochalcone A); quinazolin-4(1H)-one,2,3-dihydro-2-methyl-3-(4-dimethylaminobenzylidenamino)-; cholest-5-en-3-ol(3β)-; olean-12-ene-28 al; dihydrocoumarin, 5,7,8-trimethyl-; 2H,8H-benzol [1,2-b:5,4-b′]dipyran-2-one; β-sitosterol; 3,4-heptadien-2-one, 3,5-dicyclopentyl-6-methyl-; 2,2-dimethyl-7-hydroxy-6-(2,4-dimethoxycinnamoyl)chromene; stigmasterol; 3-[2-(2-chloro-benzyloxy)-ethyl]-1H quinazolin 2H-dione; 2H,8H-benzo[1,2-b:5,4-b′]dipyran-2-one,4-hydroxy-5-methoxy-3-(4-methoxyphenyl)-8,8-dimethyl-; octadeamethyl-cyclononasiloxane; 4-(7-8- dihydro-tetrazolo[1,5-b][1,2,4]triazin-7-yl)-2,6-dimethyl-phenol; 2-tert-butyl-4,6-*bis*(3,5-di-tert-butyl-4-hydroxybenzyl)phenol; 7-methyl-1,2,3,4,4a,9a-hexahydro-9H-fluoren-9-one.

Glycyrol, glycyrin, isoglycyrol and glycycoumarin were isolated from the methanolic extract of licorice roots from northwest China, *G. glabra* var. *glandulifera* (Hattori et al. 1986). A new 2-arylbenzofuran derivative named licocoumarone with the structure 2-(2, 4-dihydroxyphenyl)-6-hydroxy-4-methoxy-5-(3-methyl-2-butenyl) coumarone was isolated from commercially available xibei licorice (seihoku kanzo) along with a known 3-arylcoumarin derivative, glycycoumarin (Demizu et al. 1988). An anti-HIV (human immunodeficiency virus) phenolic constituent, licopyranocoumarin, and two other new phenolics named licoarylcoumarin and glisoflavone together with glycyrrhisoflavanone, kaempferol 3-*O*-methyl ether and licocoumarone were isolated from Si-pei licorice (a commercial licorice; root and stolon of *Glycyrrhiza* sp. from the north-western region of China) (Hatano et al. 1989). Licuraside was isolated from *G. glabra* and on hydrolysis afforded liquiritigenin and isoliquiritigenin (Gorecki et al. 1991). From *G. glabra* (Xinjiang) roots the following were isolated five flavonoids and four coumarins, namely, liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin, glycycoumarin, isoglycycoumarin, licochalcone A, glycyrol and isoglycyrol (Zeng et al. 1990); glycyrrhizic acid, isoliquiritin, liquiritin, liquiritigenin, glycoumarin, isoglycoumarin and uralsaponin B (Zeng et al. 1991a); and three saponins glycyrrhizic acid (S-I), uralsaponin B and uralsaponin A (Zeng et al. 1991b). Five new flavonoid compounds named glucoliquiritin apioside (a flavonone bisdesmoside), prenyllicoflavone A (a bisprenylflavone), shinflavone (a prenylated pyranoflavanone), shinpterocarpin and 1-methoxyphaseollin (both pyranopterocarpans) were isolated together with eight known saponins (glycyrrhizin, licorice-saponins A3, C2, E2, G2, H2, apioglycyrrhizin and araboglycyrrhi-

zin), and seven known flavonoid glycosides (ononin, liquiritin, liquiritin apioside, isoliquiritin, neoisoliquiritin, luciraside and isoliquiritin apioside) from the aqueous fraction of the methanol extract of *G. glabra* dried roots ('Shinkyo-Kanzo' in Japanese) collected in Xinjiang province, China (Kitagawa et al. 1994). The ethyl acetate fraction yielded three known chalcones (licochalcone A and B, and echinatin), two new prenylated flavonoids (prenyllicoflavone A and shinflavanone) together with two known prenylated flavonoids (glabrol, licoflavone A), three known isoflavonoids (hispaglabridin A and B, and methylhispaglabridin B), two new pyranopterocarpans (shinpterocarpin and 1-methoxyphaseollin) and two known pterocarpans (medicarpin and *ent*-(−)-hemileiocarpin). A new prenylated isoflavan derivative, kanzonol R, was isolated from *G. glabra* (Fukai et al. 1994). A large amount of glabridin, a prenylated flavonoid, was detected exclusively in the cork layer and the decayed part of the thickening roots while large amounts of flavonoid glycosides, liquiritigenin glycosides and isoliquiritigenin glycosides were mainly distributed in the woody part of the thickening roots (Hayashi et al. 1996b). The isoflavans hispaglabridin A, hispaglabridin B, glabridin, and 4′-*O*-methylglabridin, the two chalcones, isoprenylchalcone derivative and isoliquiritigenin, and the isoflavone, formononetin were isolated from *G. glabra* roots (Vaya et al. 1997). Two new 3-arylcoumarin derivatives were isolated from *Glycyrrhiza glabra* root and their structures were elucidated as 2′,4′-dihydroxy-[6″, 6″-dimethylpyrano(2″,3″:7,8)]-3-arylcoumarin and [6′,6″-dimethylpyrano(2″,3″:7,8)]-2′-hydroxy-4′-methoxy-3-arylcoumarin (Kinoshita et al. 1997). Two known isoflavonoids glabrene and glabrone were also isolated. From the ether soluble fraction of the crude licorice root, licoricidin, 1-methoxyphaseollin, 6,8-diprenylgenistein and 1-methoxyphaseollidin were isolated (Nagumo et al. 1999). Isoflavan derivatives, glabridin, hispaglabridin A, hispaglabridin B, 4′-*O*-methylglabridin and 3′-hydroxy-4′-*O*-methylglabridin were isolated from *Glycyrrhiza glabra* (Haraguchi et al. 2000). Yuldashev et al. (2000b) isolated the following flavonoids from above ground parts of *G. glabr*a grown in Uzbekistan: 7-*O*-methylglabranin, glabranin (8-C-prenylpinocembrin) (5,7-dihydroxy-8-(γ,γ-dimethylallyl)-flavanone), pinocembrin, galangin and a new isoflavanoid glabrisoflavone with the structure (*E*)-5,7,3′-trihydroxy-6-(3-hydroxymethyl-2-butenyl)-isoflavone.

The main phenolic compounds of licorice were reported as glycosides of liquiritigenin (4′,7-dihydroxyflavanone) and isoliquiritigenin (2′,4,4′-trihydroxychalcone), e.g., liquiritin, isoliquiritin, liquiritin apioside, etc. (Nomura et al. 2002). Minor phenolic compounds comprising many isoprenoid-substituted flavonoids, chromenes, coumarins, dihydrostilbenes and dihydrophenanthrenes were isolated from *Glycyrrhiza* species. Ninety phenolic compounds were isolated from *G. glabra*. Fifty were substituted isoprenoid groups, for example, in Type I licorice Spanish and Russian, the main isoprenoid-substituted flavonoid was a pyronoisoflavan, glabridin. The 5-position of most flavonoids was unsubstituted, for example, glabrene, glabrol and 3-hyrdoxyglabrol. Type 2 licorice comprising Chinese and Kirghiz *G. glabra*, both 5-unsubstituted flavonoids and 5-oxygenated flavonoids, for example, 3,8′-diprenylated dalbergioidin was isolated. Most flavonoids were 5-hyrdoxy-flavonoids or 5-methoxy-flavonoids. The main isoprenoid-substituted flavonoid of Kirghiz licorice was 3,8′-diprenylated dalbergioidin but glabridin had not been isolated. Fourteen flavonoids were isolated and purified from the benzene extract (33 g) of the roots of Kirghiz licorice (*G. glabra*): 3′,8-diprenyldalbergioidin (630 mg) 3′,6-diprenyldalbergioidin (67 mg), licoisoflavanone (19 mg), glyasperin A (25 mg), glyasperin C (20 mg), glyasperin D (49 mg), isoderone (1 mg), semilicoisoflavone B (3 mg), 8-(γ,γ-dimethylallyl)-wighteone (13 mg), gancaonin G (2 mg), gancaonin H (27 mg), 1-methoxyphaseollidin (53 mg), edudiol (3,9-dihydroxy-1-methoxy-2-prenypterocarpan) (12 mg) and glabrene (5 mg), 3′(γ,γ-dimethylallyl) kievitone, glisoflavanone (3′,6-diprenyl-2,4′,5,7-tetrahydroxyisoflavanone), isoderone (2,2-dimethylpyranol [4′,3′]-5,7-dihydroxyisofla

vone), 1-methoxyphaseollidin (Fukai et al. 2001). From European *G. glabra* cultivated in Japan the following flavonoids were isolated: glabrene, glabridin, 4′-*O*-methylglabridin, hispaglabridin A (3′-prenylglabridin), glabrol, 3-hydroxyglabrol, glabrone, medicarpin (3-hydroxy-9-methoxypterocarpan), shinpterocarpin, euchrenone a_5, glyinflanin K, glyinflanin G, two pyrano-2-arylbenzofurans, kanzonols U and V, a pyrano-3-arylcoumarin, kanzonol W, a diprenylated isoflavan, kanzonol X, a diprenylated α-hydroxydihydrochalcone kanzol Y ((*αR*)-3,5′-diprenyl-*α*,2′,4,4′-tetrahydroxydihydrochalcone), kanzol Z (prenylated 3-hydroxypyranoflavanone) and 3-hydroxyparatocarpin (Fukai et al. 1996a, 1998). A new isoprenoid-substituted isoflavone, kanzonol T, was isolated from Chinese licorice, *Glycyrrhiza glabra*, along with eight known flavonoids (Fukai et al. 1996b). The structure of glabrene was revised. Seven constituents, isolated from *Glycyrrhiza glabra*, were identified as the isoflavans hispaglabridin A, hispaglabridin B, glabridin and 4′-*O*-methylglabridin, the two chalcones, isoprenylchalcone derivative and isoliquiritigenin, and the isoflavone, formononetin (Vaya et al. 1997). Two new isoflavones named glabroisoflavones A and B, and a 3-arylcoumarin derivative glabrocurmarin and a known isoflavene derivative 3,4-didehdyroglabridin were isolated from the dichloromethane extract of commercially available licorice root (Kinoshita et al. 2005). Licochalcone-A, a novel flavonoid, was isolated from licorice root (*Glycyrrhiza glabra*) (Fu et al. 2004). Licocalchone-C was isolated from *Glycyrrhiza glabra* (Franceschelli et al. 2011). Two new flavonosides were isolated from *Glycyrrhiza glabra* roots and identified as 5,8-dihydroxy-flavone-7-*O*-β-D-glucuronide, glychionide A and 5-hydroxy-8-methoxyl-flavone-7-*O*-β-D-glucuronide, glychionide B (Li et al. 2005).

Licorice root was found to contain an estrogenic hormone in appreciable quantity (Costello and Lynn 1950). Eight phytoestrogenic compounds were found and assessed from the roots of *G. glabra* from Syria: daidzein (4′,7-dihydroxyisoflavone), daidzin (diadzein-7-glucoside), genistein (4′,5,7-trihydroisoflavone), genistin (genistein-7-glucoside), formononetin (7-hydroxy-4′-methoxyisoflavone), ononin (formononetin-7-glucoside), glycitein (4′,7-dihydroxy-6-methoxyisoflavone) and coumestrol (Khalaf et al. 2010). Phytosterols β-sitosterol, stigmasterol, campesterol and ergosterol were isolated from *G. glabra* roots from Syria (Khalaf et al. 2011).

Glycyrrhizin, a major bioactive compound in liquorice root, had been reported to be 50 times sweeter than sugar (Nitalikar et al. 2010). Licorice extracts had been widely used in pharmaceutical and confectionery industries because of the presence of glycyrrhizin. Glycyrrhizin was postulated to be most likely derived from the triterpene β-amyrin, an initial product of the cyclization of 2,3-oxidosqualene (Seki et al. 2008). *CYP88D6*, a cytochrome P450 monooxygenase (P450) gene, was successfully identified as a glycyrrhizin-biosynthetic gene. *CYP88D6* was characterized by in-vitro enzymatic activity assays and shown to catalyse the sequential two-step oxidation of β-amyrin at C-11 to produce 11-oxo-β-amyrin, a possible biosynthetic intermediate between β-amyrin and glycyrrhizin. *CYP88D6* expression was detected in the roots and stolons but not in the stem and leaves. A second relevant P450 (CYP72A154) was identified and shown to be responsible for C-30 oxidation in the glycyrrhizin pathway (Seki et al. 2011). CYP72A154 expressed in an engineered yeast strain that endogenously produced 11-oxo-β-amyrin (a possible biosynthetic intermediate between β-amyrin and glycyrrhizin) catalysed three sequential oxidation steps at C-30 of 11-oxo-β-amyrin supplied in situ to produce glycyrrhetinic acid, a glycyrrhizin aglycone.

The ethanol licorice (*G. glabra*) root extract afforded the following phenolic compounds: 5′-formylglabridin; (2*R*,3*R*)-3,4′,7-trihydroxy-3′-prenylflavane; echinatin; (3*R*)-2′,3′,7-trihydroxy-4′-methoxyisoflavan; kanzonol X; kanzonol W; shinpterocarpin; licoflavanone A; glabrol; shinflavanone; gancaonin L; glabrone; licochalcone B; morachalcone A; 2′3′,4′-trihydroxy- 3′γ,γ- dimethylallyl- 6″, 6″- dimethyl-pyrano[2″,3″:4,5]chalcone;1-(2′,4′-dihydroxphenyl)-

2-hydroxy-3-(4″-hydroxyphenyl)-1-propanone; kanzonol Y; (3*R*)- vestitol; glabridin; 4′-*O*-methylglabridin; 3′-hydroxyl-4′-*O*-methylgalbridin; hispaglabridin A; hispaglabridin B; glabrene; kanzonol W; kanzonol U; *O*-methyl-shinpterocarpin; licoagrocarpin; xambioona; 8′,8-dimethyl-3,4-dihydro-2*H*,8*H*-pyrano [2,3-*f*]chromone-3-ol; 3,3′,4,4′-tetrahydroxy-2′-methoxy-5- prenylchalcone; 3′,4,4′-tetrahydroxy- 3,5′-diprenylchalcone; 2′,3,4,4′, α-pentahydroxy- 3′,5-diprenyl-dihydrochalcone; 5′-formyl glabridin; (2*R*,3*R*)-3,4′,7-trihydroxy-3′-prenylflavanone; and 7,8-dihydroxy-4′-methoxy-6-prenylisoflavanone8-hydroxymethyl-8-methyl-3,4-dihydro-2*H*,8*H*-pyrano[2,3-*f*]-chromon-3-ol (Kuroda et al. 2010).

The essential oil yield from *G. glabra* root was 0.047 % by steam distillation, and 82 components of the volatile compounds were identified (Kameoka and Nakai 1987). The predominant constituent was hexanoic acid (31.57 %), followed by other components hexadecanoic acid (3.3 %), heptanoic acid (2.54 %), hexanol (1.7 %), octanoic acid (1.44 %), γ-nonalactone (1.33 %), 4-methyl-1-isopropyl-3-cyclohexen-1-ol (1.30 %) and *N*-methyl-2-pyrrolidone (1.07 %). Other compounds (<1 %) included methyl hexanoate, 2,3-dihydro-4-methyl-furan, 1-methoxy-4-isopropyl cyclohexane, 2-hexanal, 2-pentylfuran, 2-ethyl-1,4-dimethyl benzene, 3-methoxy-2-methyl propane, tridecane, 6-methyl-5-hepten-2-one, hexyl formate, camphor, 6-methyl-3-undecene, 3-methyl-hepten-2-one, tetradecane, acetic acid, furfural, 5-methyl-2-undecene, 9-methyl-3-undecene, linalool, 3,5-octadien -2-one, dihydro-5,5-dimethyl-2(3*H*)-furanone, myrtenal, 6-methyl-1-isopropyl-3-cyclohexen-1-ol, butanoic acid, estragole (methyl chavicol), 2-methyl-6-methylen-7-octen-2-ol, pentanoic acid, 2-methyl-5-isopropyl-2-cyclohexen-1-one, *o*-cresol, 2-methalyl phenol, 3-methyl-6-propryl phenol, anethole, cumic alcohol, pseudoionone, phenethyl alcohol, 5-pentylpyran-2-one, *o*-tolunitrile, 2-methyl-3-decen-5-one, 7-methoxy-3,7-dimethyl-octanal, 1-pentadecanol, 2-hydroxy-4-methyl benzaldehyde, 1-methoxy-4-isopropyl benzene, isobutyl adipate, nonanoic acid, eugenol, 2-methyl-5-isopropyl phenol, methyl hexadecanoate, decanoic acid, hexadecyl acetate, 2,3-dihydro benzofuran, indole dodecanoic acid and pentacosane. Those found in traces were dodecane decane, undecane, 2-methyl propanoic acid, pentadecane, phenyl acetaldehyde, acetophenone, heptadecane, octadecane, guaiacol, nonadecane, eicosane, heneicosane, docosane, tricosane, undecanoic acid, tridecanoic acid, tetradecanoic acid and pentadecanoic acid. Volatile compounds (%) found in *G. glabra* root essential samples from Egypt, Afghanistan and Syria were, respectively, 5-methyl furfural (3.6, 10.1, 9.4 %), *o*-guaiacol (2.2 %, tr, tr), 2-phenylethanol (0.5, 2.1 %, tr), *Z*-pinene hydrate (tr, 2.1 %, -), tetrahydro-lavandulol (-, 7.6, 4.1 %), terpinene-4-ol (tr, -, 3.6 %), (*E*)-linalool oxide (2.1 %, -, -), *p*-cymen-8-ol (-, 2.7, 3.0 %), α-terpineol (tr, 0, 2.4 %), methyl chavicol (-, -2.4 %), (4*E*)-decenal (5.3, 2.8, 5.4 %), cumin aldehyde (4.7, 1.8, 3.3 %), carvone (2.1, 0.2, 3.1 %), piperitone (9.4, 13.1, 7.2 %), (*E*)-cinnamaldehyde (3.6, 4.5, 6.2 %), (*E*)-anethole (1.3 %, - , -), thymol (27.2, 6.0, 5.5 %), indole (-, 7.4, 1.8 %), carvacrol (11.1, 1.4, 5.8 %), *p*-vinyl-guaiacol (8.5, 8.5, 9.5 %), unknown aldehyde (-, 3.7, 5.7 %), eugenol (9.4, 7.5, 8.8 %), γ-nonalactone (1.8, 2.5, 7.4 %), methyl eugenol (3.5, 0.2, 3.8 %), β-caryophyllene (0.5, 0.5, 1.1 %), himachalene epoxide (-, 8.8, 1.6 %) and β-caryophyllene oxide (1.1, -, 1, 1 %) (Farag and Wessjohann 2012). Total phenols (23.4–58.5 %) were most dominant in *G. glabra* followed by aldehydes (17.2–305), ketones (10.3–13.3 %), ether/epoxides (6.5–11.4 %) and alcohols (0.5–9.2 %). Two phenols, thymol and carvacrol, were found exclusively in essential oil and headspace samples of *G. glabra*, and with highest amounts for samples that originated from Egypt. Principal component and hierarchical cluster analyses clearly separated *G. echinata* and *G. inflata* from *G. glabr*a, with phenolics and aliphatic aldehydes contributing mostly for species segregation.

Compounds (and aglycone class) isolated from *G. glabr*a methanol root extract by LC-MS included: rhamanoliquiritin, isoviolanthin, liquiritin apioside, liquiritin, choerospondin, 5,7-dihydroxyflavone, licorice D2/D1,

3-hydroxyglabrol, glabrol (flavanone); neolicuroside, neolicuroside isomer, isoliquiritin, licochalcone B, licorice glycoside A, isoliquiritigenin (chalcone); glycyrrhizin, 22-acetoxyglyccyrrhizin, licorice saponin A3, licorice saponin G2, licorice saponin J2, glycyrrhizin isomer, licorice saponin C2, 11-deoxoglycyrrhetinic, glycyrrhetic acid (triterpene), glabrene (isoflavene), glabridin (isoflavan), kanzonol Y (dihydrochalcone) and kanzonol F (pterocarpan) (Farag et al. 2012). Chemical shift of constituents identified in *G. glabra* root included: glycyrrhizin, sucrose, liquiritin, isoliquiritin, liquiritigenin, iso liquiritigenin, 4-hydroxyphenyl acetic acid, fatty acids, licochalcone A and rhamnose (glycosides). Three new oleanane-type triterpene saponins, namely, licorice-saponin M3, licorice-saponin N4 and licorice-saponin O4, an artificial product, namely, ester of licorice-saponin G2, as well as five known triterpene glucuronides, namely, 24-hydroxylicorice saponin A3, licorice saponin G2, 22B-acetoxyl-glycyrrhizin, licorice saponin A3 and glycyrrhizin were isolated from *G. glabra* roots (Wei et al. 2014). Dibenzoylmethane (DBM; 1, 3-diphenyl-1, 3-propadinedione), a beta-diketone analogue of curcumin, had been reported to be constituent of licorice (Jackson et al. 2002; Thimmulappa et al. 2008; Khor et al. 2009; Liao et al. 2015) and was confirmed by Mancia et al. (2014) to be a natural constituent of Licorice root (*G. glabra*).

Based on nucleotide sequences of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (rbcL) sequences, six *Glycyrrhiza* species were divided into two groups: three, *G. glabra*, *G. uralensis* and *G. inflata*, producing glycyrrhizin as a major saponin, and the others, *G. echinata*, *G. macedonica* and *G. pallidiflora*, producing macedonoside C as a major saponin (Hayashi et al. 2000b). Among the three glycyrrhizin-producing species, only two nucleotide substitutions were observed between the rbcL sequences of *G. glabra* and *G. uralensis*, and the sequence of *G. uralensis* was identical to that of *G. inflata*, indicating *G. uralensis* and *G. inflata* to be closely related. Regarding six main constituents of licorice, glycyrrhizin, liquiritin, liquiritin apioside, isoliquiritin, isoliquiritin apioside and liquiritigenin, the constituent property of *G. glabra* was similar to that of *G. inflata*; both were dissimilar to *G. uralensis* which was characterized by a wide content variation of the six constituents compared to those of *G. glabra* and/or *G. inflata* (Kondo et al. 2007). The mean contents of liquiritin, isoliquiritin or liquiritigenin in *G. uralensis* were significantly higher than those of *G. glabra* or *G. inflata*. Additionally, glycycoumarin, glabridin and licochalcone A were reconfirmed as the species-specific typical constituents of *G. uralensis*, *G. glabr*a and *G. inflata*, respectively. Hatano et al. (1991a) found that the root and rhizome of *G. glabra*, and the licorice specimens imported from the Soviet Union and Afghanistan (Type B) contained glabridin and glabrene; the roots and rhizomes of *G. uralensis*, commercial licorice specimens from the north-western region of China (Seihoku-kanzo) and from the north-eastern region of China (Tohoku-kanzo) in Japanese markets, and also several licorice specimens from Chinese markets (Type A) contained licopyranocoumarin, glycycoumarin and/or licocoumarone, which were not found in *G. glabra* and *G. inflata*. Root sample of *G. inflata* (Type C) contained licochalcones A and B, which were not found in the samples of the other two *Glycyrrhiza* species. Extracts of some licorice specimens of types A and B, and all of the licorice specimens of type C inhibited 40–56 % of the xanthine oxidase activity at the concentration of 30 μg/ml. Extracts of some licorice specimens of types A and B also showed inhibitory effects on monoamine oxidase (44–64 % inhibition, at the concentration of 30 μg/ml), which were slightly weaker than that of harmane hydrochloride. Hatano et al. (1991b) isolated two new phenolic compounds glicoricone and licofuranone together with echinatin, genistein and licopyranocoumarin from a licorice species imported from the north-western region of China. Chinese *G. glabra* root was found to contain the highest levels of glycyrrhizic acid, followed by those from Italy (Calabria) (Montoro et al. 2011). *G. uralensis* was found to contain quercetin but *G. glabra* did not (Liao et al. 2012). Fifteen bioactive components were found in both

species. *G. glabr*a provided by Brion Research Institute of Taiwan contained uralsaponin B, but *G. glabr*a brought from herbal shops did not. The following mono- and di-prenylated flavonoids were extracted from the 70 % aqueous- ethanol, ethanol and ethyl acetate extracts of *Glycyrrhiza glabra* roots (Simons et al. 2009): liquiritin apioside, liquiritin, glabrol, 3-hydroxy-glabrol, isoliquiritin apioside, lichochalone B, glycyrrhizinic acid, glabrene, formononetin, glabrone, glabridin, 3′-hydroxy-4′-*O*-methylglabridin, 4′*O*-methylglabridin, hispaglabridin A and hispaglabridin B. Khalaf et al. (2012) found phenol carboxylic acids were present in larger quantities than flavonoids in *G. glabra* tincture. *p*-Coumaric acid, ferulic acid, luteolin and apigenin were found and quantified in both hydrolysed and unhydrolysed samples. Kaempferol, fisetin, myricetin, hyperoside, quercitrin, isoquercitrin and rutoside could not be found in the analysed samples. Regarding the phytoestrogens content, significant quantities of ononin and its aglycone formononetin were detected. Among sterols, the largest amount recovered in the tincture was β-sitosterol and the smallest ergosterol.

New esters of 18α-glycyrrhizic acid (18α-GA) namely 18 α-glycyrrhizic acid and its monopotassium salt; 18 α-glycyrrhizic acid pentasulphate sodium disodium salt; 18 α-glycyrrhizic acid di-*O*-nicotinate; 18 α-glycyrrhizic acid penta-*O*-nicotinate and penta-*O*-4-methoxycinnamate ester of 18 α-glycyrrhizic acid were synthesized; these were D/E-trans-isomers of natural 18α-GA, the major triterpene glycoside in roots of Spanish licorice and Urals licorice roots (Baltina et al. 2010). Drought stress was found to enhance the levels of secondary metabolites and key gene expression involved in the biosynthesis of triterpenoid saponins in liquorice (Nasrollahi et al. 2014). Due to osmotic stress, the gene expression levels of squalene synthase (SQS) and β-amyrin synthase (bAS) were increased, whereas those of cycloartenol synthase (CAS) were relatively unchanged at the seedling stage. At the adult plant stage, the expression levels of SQS and bAS were increased under drought stress conditions, whereas the gene expression level of CAS remained relatively constant. The glycyrrhizin content in stolons was increased only under severe drought stress conditions (28 days).

Derivatives were synthesized from isoliquiritigenin, a chalcone and liquiritigenin, a flavonoid found in *G. glabra* rhizomes (Gaur et al. 2014). 4,4′-Diacetoxy-2′-hydroxy chalcone; 2′,4′dimethoxy-4-hydroxychalcone; 4-acetoxy-2′,4′-dimethoxychalcone; 4-benzoyloxy-2′,4′-dimethoxychalcone and 2′,4′-dimethoxychalcone from isoliquiritigenin and liquiritigenin 7,4′-diacetate; liquiritigenin 4′-acetate; liquiritigenin 7,4′-dibenzoate and liquiritigenin-oxime from liquiritigenin.

Glycyrrhizin, 18β-glycyrrhetic acid and 18α-glycyrrhetic acid in liquorice roots were separated and quantified by means of capillary zone electrophoresis (Sabbioni et al. 2005). Linearity was found over the 5–200 and 2.5–100 μg/mL concentration ranges for glycyrrhizin and glycyrrhetic acid, respectively. Glycyrrhizin, glycyrrhetic acid, glabridin, liquiritin and licochalcone A and liquiritin apioside were found in samples of *Glycyrrhiza glabra*, *G. uralensis*, *G. inflata* and commercial licorice from Europe and China by means of capillary-zone electrophoresis (Rauchensteiner et al. 2005). The maximum recovery of mono-ammonium glycyrrhizate from licorice roots was achieved at 110 °C and 5 atm pressure with the ratio of 40 ml/g of 0.01 % (w/v) ammonia solution to powdered feed after 90 min of extraction (Mukhopadhyay and Panja 2008). Under the optimum extraction condition comprising a mixture of ethanol/water (30:70, v/v) and extraction time 60 min under 50 °C, 2.39 mg/g of glycyrrhizic acid and 0.92 mg/g of glabridin were extracted from Chinese licorice and the recoveries were 89.7 % and 72.5 %, respectively (Tian et al. 2008). The simultaneous HPTLC quantification of glycyrrhetinic acid and apigenin from *G. glabra* was 0.65 % and 0.0074 %, respectively (Rathee et al. 2010). The HPLC recoveries of 18β-glycyrrhetinic acid from *G. glabra* were 99.60–102.81 % (Esmaeili et al. 2010).

Highest concentrations of glycyrrhizinic acid were found in the main roots, lower concentrations in the lateral roots (Fuggersberger-Heinz and Franz 1984). The green parts of the plant

were shown to contain no glycyrrhizinic acid. Enzymatic hydrolysis of glycyrrhizinic acid with β-glucuronidase afforded the monoglucuronide of β-glycyrrhetinic acid as intermediate. Biosynthetic studies with licorice roots showed that acetate was specifically incorporated into the aglycone moiety of the triterpene saponin and glucuronic acid mainly in the sugar moiety of the diglucuronide, respectively. Glycyrrhizin was found to be localized exclusively in the woody parts of thickened roots but not in the leaf, seed, stem, rootlets, or root nodules while soyasaponins were detected in all parts of the plants examined, and the contents were higher in the seeds, rootlets and root nodules than in other parts (Hayashi et al. 1988, 1993a, 2004, Hayashi 2009). The contents of soyasaponins were higher in younger parts of a growing stolon, whereas those of glycyrrhizin tended to be higher in the older parts. As the primary roots grew and became thicker, the soyasaponin content tended to decrease, while the glycyrrhizin content increased. On the other hand, betulinic acid was localized to the rootlets, root nodules and the cork layer of thickening roots (Hayashi et al. 1988, 2004). Since both soyasaponins and betulinic acid were produced in the rootlets, root nodules and cultured cells, the triterpenoid metabolism of the cultured licorice cells was similar to that of the rootlets and root nodules, whereas glycyrrhizin was detected exclusively in the thickening root and the stolon (Hayashi et al. 1993a). An inverse relationship was found between the soyasaponins content and the glycyrrhizin content in the growing stolon and in the roots at different stages of growth. The glycyrrhizin content in 1-year-old roots rapidly increased from October to November, whereas the isoliquiritigenin glycoside content increased up to October (Hayashi et al. 1998a). In 3-year-old plants, although the isoliquiritigenin glycoside content rapidly increased from June to July, the glycyrrhizin content did not show any significant increase from May to August. The glycyrrhizin content increased during the senescence of the aerial parts as well as during the early stage of shoot elongation. The incorporation of [^{14}C]mevalonic acid into the glycyrrhizin fraction by the root segments was high in May, June and September, and low in August and winter

Leaf Aerial Plant Parts Phytochemicals

Nomura and Fukai (1998) listed the phenolic compounds found in all parts of *G. glabra* plant and suspension cell/hairy root cultures and callus tissues under the following categories: chalcone, flavanone, flavones, flavonol, isoflavanone, isoflavone, pterocarpan, coumestan, isoflavan, isoflav-3-ene, 2-arylbenzofuran, 3-arylcoumarin and miscellaneous phenolic compounds and also listed the saponins found. Ammosov and Litvinenko (2007) listed >250 phenolic compounds in all parts of plants of the genera *Glycyrrhiza* in their review paper. The classes of phenolic compounds reported in *G. glabra* plants included: phenols (2), hydroxycinnamic acids (5), coumarins (5), chalcones (7), chalcone glycosides (6), flavanones (9), flavan glycosides (6), hydroxyflavanones or flavanonols (2), flavones (4), glycoflavonoids or C-glycosides (4), hydroxyflavones or flavonols (6), flavonol glycosides (8), isoflavanones (3), isoflavones (17), 2-methylisoflavones (4), isoflavone glycosides (1), isoflavans (15), isoflavan-4-enes (isoflavenes) (2), ketoisofla-4-enes (3-arylcoumarins) (2), pterocarpans (7) and benzofurans (2-arylbenzofurans) (3).

Litvinenko (1966) isolated a new flavonoid glycoside named glyphoside with the chemical structure quercetin 3-β-D-glucopyranosyl-2′-acetate, kaempferol-3-*O*-diglucoside, and astragalin (kaempferol-3-*O*-glucoside) monoacetate from the aerial parts of Spanish *G. glabra.* Litvinenko and Kovalev (1967) isolated glycoflavonoids vitexin with the structure 5,7,4′-trihydroxyflavone 8-C-β-D-glucopyranoside (8-*syn*-isomer) and isovitexin (saponaretin) with the structure 5,7,4′-trihydroxyflavone 8-C-β-D-glucopyranoside (8-*anti*-isomer) from the aerial parts. Litvinenko and Nadezhina (1972) isolated *cis*-3-hydroxyflavanone folerogenin from the above ground parts. Kir'yalov et al. (1970) isolated methyl glycyrrhetate, two terpenoid com-

pounds not containing a conjugated keto group and presumed to be homo- and heteroannular dienes and uralenic acid which was identified by Belous et al. (1965) as 18α-glycyrrhetic acid. From the aerial parts, pinocembrin, 6-prenylpinocembrin and naringenin were isolated (Batirov et al. 1986).

An isoflavonoid phytoalexin isolated from *Glycyrrhiza glabra* leaves was characterized as 7,2′-dihydroxy-3′,4′-dimethoxyisoflavan (isomucronulatol) (Ingham 1977). Isomucronulatol was produced on inoculation with *Helminthosporium carbonum.* A new prenylated flavanone named licoflavanone was isolated, together with pinocembrin, from *Glycyrrhiza glabra* var. *typica* leaves (Fukui et al. 1988). Upon treatment with methanolic HCl, it was converted to cyclolicoflavanone. Coumarins xanthotoxin (8-methoxy(furano-7,6:2′,3′)coumarin) and bergapten (5-methoxy(furano-7,6:2′,3′)coumarin) were isolated from *G. glabra* leaves (Saleh et al. 1989). Flavonoids genistein, pinocembrin, prunetin, 6-prenylnaringenin, licoflavanone and wighteone were isolated from the leaves of *Glycyrrhiza glabra* collected on the west coast of Anatolia, whereas lupiwighteone was found only in the leaves of *G. glabra* growing in middle or east Anatolia (Hayashi et al. 1996d). Large amounts of flavonoids such as pinocembrin and licoflavanone were present on the outer surface of the young leaves, whereas isoquercitrin, a common flavonoid glycoside, was detected inside the leaves (Hayashi et al. 1996a). 7-*O*-Methylglabranin, 6-*C*-prenylpinocembrin, glabranin, pinocembrin, galangin and a novel isoflavonoid, (*E*)-5,7,4′-trihydroxy-6-(3-hydroxymethyl-2-butenyl)isoflavone (glabrisoflavone) were isolated from the aerial parts of *Glycyrrhiza glabra* (Yuldashev et al. 2000a). A new flavanonglycoside pinocembroside, 2(S)-7-*O*-*β*-D-glucopyranosyl-5-hydroxyflavanone, was isolated from the aerial part of *Glycyrrhiza glabra* (Yuldashev 2001). Five new prenylated dihydrostilbenes, α,α′-dihydro-3,5,4′-trihydroxy-4,5′-diisopentenylstilbene (1), α,α′-dihydro-3,5,3′,4′-tetrahydroxy-4,5′-diisopentenylstilbene (2), α,α′-dihydro-3,5,4′-trihydroxy-5′-isopentenylstilbene (3), α,α′-dihydro-3,5,3′-trihydroxy-4′-methoxy-5′-isopentenylstilbene (4) and α,α′-dihydro-3,5,3′,4′-tetrahydroxy-5′-isopentenyl stilbene (5), along with four known flavonoids, glabranin (6), pinocembrin, (7), licoflavone (8) and wighteone (9), were isolated from a lipid extract of the leaves of Sicilian *Glycyrrhiza glabra* (Biondi et al. 2003). HPLC profile of methanol extract from aerial parts of *G. glabra* (1–4) samples: *G. glabra*-1 schaftoside, isoquercitrin, astragalin, genistein, gancaonin C and pinocembrin; *G glabra*-2 vicenin 2, isoquercitrin, astragalin, ononin, genistein, gancaonin C, pinocembrin, licoflavanone, lupiwighteone and wighteone; *G. glabra*-3 vicenin 2, schaftoside, isoquercitrin, astragalin, genistein, gancaonin C, pinocembrin, licoflavanone, lupiwighteone and wighteone; *G. glabr*a-4 schaftoside, rutin, genistein, gancaonin C, pinocembrin, licoflavanone and wighteone (Kusano et al. 2003).

Licorice leaves of *G. glabra* var. *glandulifera* and *G. glabra* var. *glabra* were found to have the following proximate and monosaccharide compositions (% w/w), respectively: ash 10.3, 7 %; total lipid 10, 7.3 %; total carbohydrate 24.6, 11.8 %; protein 24.1, 19.8 %; monosaccharide – glucose 20, 50.4 %; galactose 21.7, 13.2 %; mannose traces 7.4 %; arabinose 15.8, 7 %; xylose 16.5, 6 %; and uronic acid 26, 16 % (Helmy et al. 2013).

Four new dihydrostilbenes – α,α′-dihydro-3,5-dihydroxy-4′-acetoxy-5′-isopentenylstilbene; α,α′-dihydro-3,3′,4′-trihydroxy-5-*O*-isopentenyl-6-isopentenylstilbene; α,α′-dihydro-3,5,3′-trihydroxy-4′-methoxystilbene; and α,α′-dihydro-3,3′-dihydroxy-5β-D-*O*-glucopyranosyloxy-4′-methoxystilbene – together with seven known flavonoids, glabranin isomer, naringenin, lupiwighteone, pinocembrin 7-*O*-glucoside, astragalin, isoquercitrin, vicenin II, and the inositol, pinitol, were isolated from the leaves of *Glycyrrhiza glabra* grown in Sicily (Biondi et al. 2005). Thirty compounds were isolated from *G. glabra* leaf ethyl acetate, *n*-hexane and methanol extracts: lutein, β-carotene; naringenin; α-α′-dihydro-3,5,3′,4′-tetrahydroxy-5′-isopentenylstilbene; α-α′-dihydro-3,5,3′-trihydroxy-4′-methoxystilbene; α-α′-dihydro-3,5,3′-trihydroxy-4′-methoxy-5′-isopentenylstilbene; pinocembrin; α-α′-dihydro-3,5-dihydroxy-4′-acethoxy-5′-

isopentenylstilbene; licoflavanone; α-α′-dihydro-3,5,4′-trihydroxy-5′-isopentenylstilbene; acetoxy derivative of α-α′-dihydro-3,5,4′-trihydroxy-5′-isopentenylstilbene; unknown, α-α′-dihydro-3,5,3′,4′-tetrahydroxy-4,5′-diisopentenylstilbene; α-α′-dihydro-3,5,4′-trihydroxy-4,5′-diisopentenylstilbene; α-α′-dihydro-3,5,4′-trihydroxy-5-*O*-isopentenyl-6-isopentenylstilbene; glabranin; glabranin isomer; kaempferol dihexoside; apigenin 6,8 di-C-glucoside (vicenin-2); aromadendrin dihexoside; pinocembrin hexoside-deoxyhexoside; apigenin di-C-hexoside-pentoside; quercetin rhamno-glucoside (rutin), quercetin hexoside (glucoside)-pentoside; isoquercitrin; quercetin 3-*O*-glucoside 6′acetate; α-α′-dihydro-3,3′-dihydroxy-5β-D-*O*′-glucopyranosyl-4′-methoxy-stilbene; kaempferol 3-*O*-glucoside (astragalin); kaempferol 7-*O*-glucoside and pinocembrin 7-*O*-glucoside (Siracusa et al. 2011).

Studies showed that at least three unknown ingredients were detected in rough bark (Cortex Glycyrrhizae) which were not in Fen Gancao (barked licorice root), and glycyrrhizic acid content in the Cortex Glycyrrhizae was higher than that in Fen Gancao (Rong et al. 2006). The results suggested that Cortex Glycyrrhizae could be used as the material not only to extract glycyrrhizic acid but also for making additives. Three oleanane-type monoglycosides along with glycyrrhizin and 3-*O*-[β-D-glucuronopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-18β-liquiritic acid were isolated from enzymatically hydrolysed licorice extract (EHLE) (Liut et al. 2001). The structures of the three compounds were determined to be 3-*O*-β-D-glucuronopyranosyl-24-hydroxy-18β-glycyrrhetinic acid; 3-*O*-β-D-glucuronopyranosyl-18β-glycyrrhetinic acid and 3-*O*-β-D-glucuronopyranosyl-18β-liquiritic acid. Six major constituents were isolated from enzymatically modified licorice extract: glycyrrhizin (major sweet constituent, 1), 3-*O*-[β-D-glucuronopyranosyl-(1 → 2)-β-D-glucuronopyranosyl] liquiritic acid (minor sweet component 2) and their derivatives glucosylated at the C-4 position of the terminal glucuronopyranose with additional one (compounds 3 and 4, respectively) and two (compounds 5 and 6, respectively) glucose moieties (Liu et al. 2000). Compound 2 was sweeter than compound 1. Compound 3, a monoglucosylated derivative of compound 1, was sweeter than compound 4. Compounds 5 and 6, with two additional glucose moieties, showed only slight sweetness.

Hayashi and co-workers (1988, 1995, 1998b, 2003a, b) conducted field surveys of *G. glabra* in Turkey, Italy, Spain, Kazakhstan and found that it could be divided into two types according to their major flavonol glycosides in leaves (Shibano et al. 1996; Hayashi et al. 2003a). The major leaf flavonol glycoside of *G. glabra* collected in Turkey, Italy and Spain was isoquercitrin (IQ), but that collected in Kazakhstan was rutin (RT) (Hayashi et al. 2003b). In addition to these, the three flavanones, pinocembrin, licoflavanone and glabranin, were recognized as major compounds common to both types. *G. glabr*a and *G. uralensis* were found growing together forming a mixed population, and intermediate-type plants between them were at three sites in Kazakhstan (Hayashi et al. 2003b). Although two nucleotide substitutions of the chloroplast rbcL gene were observed between *G. uralensis* and *G. glabra*, rbcL sequences of the intermediate-types were divided into *G. uralensis* -type (G-A type) and *G. glabra*-type (A-T type). Roots of *G. glabra* contained glabridin and no glycoumarin while that of *G. uralensis* glycycoumarin and no glabridin, but neither flavonoid was detected in underground parts of the intermediate-types. All three types contained glycyrrhizin, the rbcL genes for *G. glabra* plants having linear-oblong leaflets, and straight fruits from the three collection sites were identified as the A-T type sequence (*G. glabra*-type), and those of *G. uralensis* plants, having ovate leaflets and falcated fruits, were the G-A type sequence (*G. uralensis*-type). Leaves of *G. glabra* contained rutin, isoquercitrin, licoflavanone and pinocembrin, while *G. uralensis* contained rutin, isoquercitrin, 2 unidentified compounds and no licoflavanone and pinocembrin. Both *G. glabra*-specific and *G. uralensis*-specific compounds were detected in the leaves of the intermediate-type, thus suggesting that the intermediate plants were hybrids of *G. glabr*a and *G. uralensis*.

G. glabra plants collected in Uzbekistan could be divided into two types, RT-type and IQ-type, according to their major flavonol glycosides,

rutin or isoquercitrin, respectively (Hayashi et al. 2003a). In Uzbekistan, HPLC analysis of the underground parts (root, stolon) of *G. glabra* indicated that glycyrrhizin contents varied from 3.3 to 6.1 % of dry weight, and that glabridin, a species-specific flavonoid for *G. glabr*a, was detected in all underground samples (0.08–0.35 % of dry weight). All the *G. glabra* plants in Sicily and Spain were morphologically similar to each other, and their fruits had no glandular hairs on the capsule (Hayashi et al. 1998b). The glycyrrhizin content in the stolons ranged from 4.4 to 0.7 % of dry weight, and the stolons contained glabridin. A survey of the habitat of *Glycyrrhiza glabra* in the Mus district of East Anatolia, Turkey, revealed that *G. glabra* var. *glandulifera* and *G. glabra* var. *glabra* grew together forming a mixed population (Tabata et al. 1988). No significant morphological difference was observed between these two varieties except that the former had glandular hairs on the capsule. The glycyrrhizin content of the roots of these plants bearing fruits in early August was found to be from 0.6 to 3.5 % dw (dry weight), while that in the roots collected in spring in the same region was 3.0–6.1 % dw. In another study, HPLC analysis of leaves indicated a significant difference in the chemical composition between the *G. glabra* plants growing in the west and those in the other regions in Turkey (Hayashi et al. 1995). Pinocembrin (o.18–1.5 % dw), licoflavone 0.07–0.79 % dw and three unidentified compounds were found in the leaves. Root and stolon of all samples contained glabridin (0.15–0.70 % dw) and glycyrrhizin contents (1.1 to 8.0 % of dw). Nine samples of *Glycyrrhiza glabra* were collected in various sites of Calabria, Italy, which showed remarkable differences in chemical composition and biological activity (Statti et al. 2004).

Phytochemicals in Callus/Cell Suspension Cultures

The metabolites detected in licorice (*G. glabra*) single cell suspension culture included a volatile apple aroma, a polysaccharide pectin-like material, steroids and triterpenoids (Wu et al. 1974). The analyses of the licorice cell volatile apple aroma indicated the presence of ethanol and some related esters. The monosaccharides found in the pectin-like polysaccharide hydrolysate were glucose, fructose, galactose, arabinose, xylose, galacturonic acid and glucuronic acid. Glycyrrhizinic acid, the common licorice constituent found in the root, could not be detected in the suspension cultures. However, several other related compounds which gave typical steroid and triterpenoid reactions were found. Sorbitol, glucose and fructose were found to be the three major sugars which accumulated in free form in the licorice cell medium. Callus and cell suspension cultures of *Glycyrrhiza glabra* failed to produce detectable amounts of glycyrrhizin, the major oleanane-type triterpene glycoside of the thickening root, or of its 11-deoxoderivative (Hayashi et al. 1988). However, betulinic acid, a lupane-type triterpene, which was found in the root bark, and a small amount of β-amyrin, a possible precursor of oleanane-type triterpenes, were detected in cell suspension cultures in addition to lupeol, a fundamental form of lupane-type triterpenes. The biotransformation of papaverine with cell suspension cultures of G. *glabra* was not reported by Dorisse et al. (1988). The main metabolite of the transformation was a hydroxylated compound, papaverinol. *G. glabra* suspension cultures did not accumulate either glycyrrhizin of glycyrrhetic acid but did produce the isoflavonoid formononetin (Arias-Castro et al. 1993). The initial pH, sucrose concentrations, medium composition, auxin and cytokinins were found to affect the accumulation of formononetin. Mousa et al. (2007) reported regenerative callus and cell suspension system of licorice (*Glycyrrhiza glabr*a) to be a prerequisite for the production of the sweetener glycyrrhizin in cell suspension.

Soyasaponins I and II, oleanane-type triterpene glycosides, were shown to be produced by licorice cell suspension cultures (Hayashi et al. 1990b). The soyasaponin content in the licorice cell cultures varied from 0.017 to 1.1 % of the dry weight of cells depending on culture strains and was also greatly influenced by plant growth hormones.

Two biotransformation products formed from 18β-glycyrrhetinic acid by cell suspension cultures of *Glycyrrhiza glabra* were isolated and their structures determined as 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-24-hydroxy-18β-glycyrrhetinic acid and 30-*O*-β-D-glycopyranosyl-18β-glycyrrhetinic acid (Hayashi et al. 1990a). The structures of seven triterpenoid metabolites including new compounds, 3-*O*-β-D-glucuronopyranosyl-24-hydroxy-18β-glycyrrhetinic acid and 24-hydroxy-18β-glycyrrhetinic acid 30-β-D-glucopyranosyl ester, derived from exogenous 18β-glycyrrhetinic acid administered to glycyrrhizin-free cell suspension cultures of *Glycyrrhiza glabra*, were determined (Hayashi et al. 1992a). 18β-Glycyrrhetinic acid 24-hydroxylase activity was detected in the microsomal fraction prepared from *Glycyrrhiza glabra* cell suspension cultures (Hayashi et al. 1993b). Cultured cells of *G. glabra* produced no detectable amount of glabridin, liquiritigenin glycosides, isoliquiritigenin glycosides, pinocembrin, licoflavanone and isoquercitrin but produced formononetin, an isoflavonoid (Hayashi et al. 1996b). Distribution of soyasaponin and betulinic acid triterpenoids was reported to be different in the intact plant of *G. glabra* (Hayashi et al. 1993a). Betulinic acid (0.01–0.17 %) was detected in all the callus cultures derived from various organs (hypocotyl, root, stem and leaf) of 1-month-old *G. glabra* seedlings. The presence of stigmasterol (0.04–0.07 % of cell dry weight), sitosterol (0.06–0.12 %) and small amounts of β-amyrin and lupeol were also detected. Both substrates [1-^{14}C]acetate and [2-^{14}C]mevalonate labelled β-amyrin, an oleanane-type triterpene, and cycloartenol and 24-methylenecycloartanol, both intermediates of phytosterol biosynthesis in plant organs and tissue cultures of *Glycyrrhiza glabra* var. *glandulifera* (Ayabe et al. 1990). The label in esterified triterpenes was distributed mainly in phytosterol intermediates, but not in β-amyrin. The ratio of β-amyrin formation among the three triterpenes from [2-^{14}C]mevalonate was relatively high in stolon segments and in root cultures, but negligible in callus cultures. Administration of a specific inhibitor of squalene-2, 3-epoxide:cycloartenol (lanosterol) cyclase caused a marked increase of β-amyrin synthesis in root suspension cultures, and of 24-methylenecycloartanol synthesis in cell suspension cultures, from [2-^{14}C]mevalonate. Echinatin and glabridin were isolated from *G. glabra* callus tissues (Oda et al. 1995)

UDP-glucuronic acid:triterpene glucuronosyltransferase activities for soyasapogenol B, soyasapogenol C, 24-hydroxyglycyrrhetinic acid and 24-hydroxyglycyrrhetinic acid methyl ester were detected in cultured licorice cells (Hayashi et al. 1996c). The pH dependency of the activity for soyasapogenol B was different from that for 24-hydroxyglycyrrhetinic acid methyl ester. Hayashi et al. (1996a) isolated two cDNAs (GgSQS1 and GgSQS2) encoding squalene synthase of *Glycyrrhiza glabra* by cross-hybridization with that of *Arabidopsis thaliana* squalene synthase. Their nucleotide sequences contained an open reading frame for a polypeptide of 413 amino acids (GgSQS1) and 412 amino acids (GgSQS2), respectively. The deduced amino acid sequence of GgSQS1 exhibits 88 %, 81 %, 78 %, 45–44 % and 45–41 % identity to those of the GgSQS2, *Nicotiana benthamiana*, *Arabidopsis thaliana*, mammal, and yeast squalene synthases, respectively. The cell-free extracts of *E.coli* transformed with the respective plasmid converted 3H-farnesyl diphosphate into squalene. Two cDNAs (GgSQS1 and GgSQS2) encoding squalene synthase were isolated from licorice, *Glycyrrhiza glabra* (Hayashi et al. 1999). Squalene synthase activity was found in the cell-free extracts of *Escherichia coli* transformed with the recombinant plasmids for GgSQS1 and GgSQS2, respectively. Northern blot analysis showed that GgSQS2 mRNA was mainly expressed during the exponential growth phase of the cultured licorice cells. A cDNA clone (GgCAS1) encoding cycloartenol synthase (CAS) was isolated from *Glycyrrhiza glabra* by cross-hybridization with that of *Pisum sativum* CAS as a probe (Hayashi et al. 2000a). Southern blot analysis suggested that at least two CAS genes exist in the licorice genome. An oxidosqualene cyclase cDNA, termed GgbAS1, was isolated from cultured cells of *Glycyrrhiza glabra* by heterolo-

gous hybridization with cDNA of *Arabidopsis thaliana* LUP1 lupeol synthase (Hayashi et al. 2001). It was shown that the level of β-amyrin synthase mRNA was drastically changed in the cultured licorice cells, whereas the mRNA level of cycloartenol synthase was relatively constant. Exogenously applied methyl jasmonate (MeJA) stimulated soyasaponin biosynthesis in cultured *Glycyrrhiza glabr*a cells (Hayashi et al. 2003c). mRNA level and enzyme activity of β-amyrin synthase (bAS), an oxidosqualene cyclase (OSC) situated at the branching point for oleanane-type triterpene saponin biosynthesis, were upregulated by MeJA, whereas those of cycloartenol synthase, an OSC involved in sterol biosynthesis, were relatively constant. Two mRNAs of squalene synthase (SQS), an enzyme common to both triterpene and sterol biosyntheses, were also upregulated by MeJA. In addition, enzyme activity of UDP-glucuronic acid: soyasapogenol B glucuronosyltransferase, an enzyme situated at a later step of soyasaponin biosynthesis, was also upregulated by MeJA. Accumulations of bAS and two SQS mRNAs were not transient but lasted for 7 days after exposure to MeJA, resulting in the high-level accumulation (more than 2 % of dry weight cells) of soyasaponins in cultured licorice cells. The cultured cells and intact plants of *Glycyrrhiza glabra* produced betulinic acid and oleanane-type triterpene saponins (soyasaponins and glycyrrhizin) (Hayashi et al. 2004). They found that the mRNA expression levels of lupeol synthase and β-amyrin synthase were consistent with the accumulation of betulinic acid and oleanane-type triterpene saponins, respectively. The transcript of lupeol synthase was highly expressed in the cultured cells and root nodules. The transcript of β-amyrin synthase, an OSC responsible for oleanane-type triterpene biosynthesis, was highly expressed in the cultured cells, root nodules and germinating seeds, where soyasaponin accumulated, and in the thickened roots where glycyrrhizin accumulated. Yeast extract promoted betulinic acid accumulation in cultured cells of *G. glabra* whereas soyasaponin accumulation was suppressed (Hayashi et al. 2005). The results indicated that soyasaponin and betulinic acid production were differently regulated in cultured cells of *G. glabra*. Differential pathway of soyasaponin and betulinic acid production in *G. glabra* were proposed as follows:

acetyl-CoA → mevalonic acid → squalene → squalene-2,3-epoxide → β-amyrin → glycyrrhetinic acid → glycyrrhizin;
acetyl-CoA → mevalonic acid → squalene → squalene-2,3-epoxide → β-amyrin → soyasapogenol B → Soyasaponin I (R = galactose) and soyasaponin II (R = arabinose)
acetyl-CoA → mevalonic acid → squalene → squalene-2,3-epoxide → lupeol → betulinic acid;
acetyl-CoA → mevalonic acid → squalene → squalene-2,3-epoxide → cycloartenol → β-sitosterol.

Kanzonol Y, 4-hydroxylonchocarpin, xambioona, glyinflanin K and several other unnamed compounds were isolated from hairy root cultures (Asada et al. 1997). A new compound named licoagrodione was isolated from *Glycyrrhiza glabra* hairy root cultures together with five known prenylated flavonoids (Li et al. 1998). Two new prenylated flavonoids, licoagrochalcone A and licoagrocarpin, were isolated from the hairy root cultures of *Glycyrrhiza glabra* along with eight known flavonoids (Asada et al. 1998). The structures of the new compounds were elucidated as 3-prenyl-2′,4,4′-trihydroxychalcone and (6a*R*, 11a*R*)-4-prenyl-3-hydroxy-9-methoxypterocarpan, respectively. A new prenylated biaurone, licoagrone, was isolated from the hairy root cultures of *Glycyrrhiza glabra* together with five known flavonoids, kanzonol D, afrormosin, odoratin, phaseol and echinatin (Asada et al. 1999). Asada et al. (2000) found that the biosynthesis of the hemiterpene moiety of glabrol, the main prenylated flavanone in the *Glycyrrhiza glabra* hairy root cultures proceeded via a glyceraldehyde/pyruvate non-mevalonate pathway.

An unusual biflavonoid named licoagrodin was isolated from *G. glabra* hairy root cultures along with three prenylated retrochalcones, licoagrochalcones B, C, D, a prenylated aurone, lico-

agroaurone and four known prenylated flavonoids, licochalcone C, kanzonol Y, glyinflanin B and glycyrdione A (Li et al. 2000). From the glycosidic fraction, a new isoflavone glycoside, licoagroside A, and a new maltol glycoside, licoagroside B were isolated together with known isoflavone glycosides onionin, calycosin 7-*O*-glucoside, wistin, afrormosin 7-*O*-(6″-malonylglucoside), vicenin-2, and isoschaftoside and three other known glycosides tachioside, isotachioside and dimethylallyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyanoside.

Pharmacological Activities

Licorice rhizome are considered to possess an expectorant and carminative, flavouring agent, depressant, antimicrobial, hypolipidemic, antiatherosclerotic, antiviral, antiulcerogenic, hypotensive, hepatoprotective, spasmolytic, antidiuretic, antimutagenic, antipyretic, anti-inflammatory (Isbrucker and Burdock 2006; Meena et al. 2010). Licorice had been reported to possesses many therapeutic properties as to potentiate the action of cortisol, to reduce testosterone synthesis, especially in women, to exert an estrogen-like activity and to reduce body fat mass (Armanini et al. 2002). Licorice flavonoid constituents mainly comprised flavones, flavonals, isoflavones, chalcones, bihydroflavones and bihydrochalcones (Xing et al. 2003). Pharmacological investigation concluded that they had antioxidant, antibacterial, anti-tumour and anti-HIV activities. Glycyrrhiza Radix is a commonly used Chinese herbal medicine, derived from the dried roots and rhizomes of Glycyrrhiza uralensis, *G. glabra* and *G. inflata* (Gao et al. 2009). The main bioactive constituents of licorice comprised triterpene saponins and flavonoids. Various pharmacological properties of liquorice had been reported including anti-ulcer, anti-inflammation, spasmolysis, anti-oxidative, contravariance, antiviral, anticancer activities, hepatoprotective, expectorant and memory enhancing effects. Glabridin, a prenylated isoflavonoid of *G. glabra* roots had been associated with a wide range of biological properties such as antioxidant, anti-inflammatory, anti-atherogenic, regulation of energy metabolism, estrogenic, neuroprotective, anti-osteoporotic and skin-whitening (Simmler et al. 2013). While glabridin is one of the most studied licorice flavonoids, both glabridin and standardized licorice extracts have significant impact on food, dietary supplements (DSs) and cosmetic markets, as evidenced by the amount of available patents and scientific articles since 1976, when glabridin was first described.

Antioxidant Activity

The highest antioxidant activity (β-carotene bleaching assay) of *G. glabra* root extract was 88.7 % at a concentration of 800 μg/mL (Ercisli et al. 2008). Aqueous and ethanol extract of Turkish *G. glabra* aerial parts and roots inhibited 87.9, 83.6, 88.6 and 80.1 % lipid peroxidation of linoleic acid emulsion at 30 μg/mL concentration, respectively (Tohma and Gulçin 2010). In contrast, α-tocopherol and trolox had inhibition of 68.1 and 81.3 %, respectively. The hydromethanolic *G. glabra* root extract displayed possessed potent hydroxyl radical scavenging activity with IC_{50} value of 80 μg/ml against the positive control standard ascorbic acid with IC_{50} value of 50 μg/ml (Varsha et al. 2013).

The organic extracts of two licorices, known in commerce as Russian licorice (*G. glabra* var. *glandulifera*) and Xinjiang licorice (*G. inflata*) exhibited potent antimicrobial and antioxidant activity (Okada et al. 1989). The bioassay-directed chemical investigation of both licorices revealed glabrene, glabridin and licochalcones A and B as active principles. Glabrene showed most potent antioxidant, three times as potent as vitamin E, glabridin showed no significant activity. The DPPH radical scavenging activity of *G. uralensis* and *G. glabra* achieved approximately 72–75 % if 10 mg/mL or more licorice extract was used (Liao et al. 2012). *G. uralensis* had slightly better radical scavenging activity than *G. glabra*. *G. uralensis* also showed higher reducing ability than *G. glabra*. Cheel et al. (2013) found that the chemical profile of licorice quantitatively varied at different harvest times and these fluctu-

ations determined changes in its bioactivities such as antioxidant and free radical scavenging activities. In general, the samples from May and November showed the most favourable free radical scavenging and antioxidant effects. Liquiritin and glycyrrhizin, the major constituents in the February and May licorice extract, appeared to contribute to the superoxide radical scavenging activity. Glabridin and glabrene, the compounds with the highest relative proportion in the November licorice extract, accounted for the antioxidant and DPPH scavenging activities of licorice.

Seven constituents with antioxidant capacity were isolated from *Glycyrrhiza glabra* (Vaya et al. 1997). The isolated compounds were identified as the isoflavans hispaglabridin A (1), hispaglabridin B (4), glabridin (3) and 4′-*O*-methylglabridin (2), the two chalcones, isoprenylchalcone derivative (5) and isoliquiritigenin (6), and the isoflavone, formononetin (7). The isoflavans (1–4) at a concentration of 50 μM inhibited *β*-carotene consumption, following 90 min of incubation at 50 °C, similar to the inhibitory effect of the whole licorice crude extract (at 16 mg/l). The chalcones (5 and 6) exhibited moderate inhibition and the isoflavone 7 was almost inactive, whereas vitamin E (50 μM) completely inhibited *β*-carotene consumption. Compounds 1–6 exhibited high inhibitory activity at a concentration of 30 μM on 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced LDL oxidation, but compound 7 and vitamin E were not active. A dose-dependent inhibitory effect of glabridin, on the formation of cholesteryl linoleate hydroperoxide (CLOOH), in an AAPH-induced LDL oxidation system was also shown. Glabridin, at 5 or 40–60 μM concentration, inhibited the CLOOH formation by 62 % and 90 %, respectively. The results suggested that constituents 1–6 were very potent antioxidants toward LDL oxidation with glabridin (11.6 % wet weight) being the most abundant and potent antioxidant.

Licorice root extract was tested for antioxidative activity in comparison with antioxidants (sodium metabisulphite and BHT) at 0.1 %, 0.5 %, 1.0 % and 2.0 % w/w in 2 % w/w hydroquinone cosmetic cream (Morteza-Semnani et al. 2002). After 3 months, at 25 °C and 45, the extract demonstrated more antioxidant activity than from two other commercial antioxidants at all concentrations, with about 43–53 % and 34–46 %, respectively, more hydroquinone remaining than in the control system. In the third month, the preparation containing 0.1 %, 0.5 %, 1.0 % and 2.0 % extract gave good physical formulation stability with about 72 %, 76 %, 78 % and 81 % hydroquinone remaining at 25 °C and 51 %, 55 %, 60 % and 63 % hydroquinone remaining at 45 °C respectively. Aqueous and ethanolic extracts of *Glycyrrhiza glabra* root demonstrated the dose-dependent scavenging activity against nitric oxide (IC_{50} = 72 and 62.1 μg/ml, respectively), superoxide (IC_{50} = 64.2 and 38.4 μg/ml, respectively), hydroxyl (IC_{50} = 81.9 and 63 μg/ml, respectively), DPPH (IC_{50} = 43.6 and 28.3 μg/ml, respectively) and ABTS•+ (IC_{50} = 77.3 and 57.2 μg/ml, respectively) radicals (Visavadiya et al. 2009). Further, both extracts showed strong reducing power and iron-chelating capacities. In the Fe^{2+}/ascorbate system, both extracts were found to inhibit mitochondrial fraction lipid peroxidation. In copper-catalysed human serum and low-density lipoprotein oxidation models, both extracts significantly lengthened the lag phase along with a decline in the oxidation rate, conjugated dienes, lipid hydroperoxides and thiobarbituric acid reactive substance formation. The findings showed *G. glabra* possessed considerable antioxidant activity and protective effect against the human lipoprotein oxidative system.

Isoflavans from *G. glabra* were shown to be effective in protecting mitochondrial function against oxidative stresses (Haraguchi et al. 2000). Mitochondrial lipid peroxidation linked to respiratory electron transport and that induced non-enzymatically were inhibited by these isoflavans. Hispaglabridin A strongly inhibited both peroxidations and 3′-hydroxy-4′-*O*-methylglabridin was the most effective at preventing NADH-dependent peroxidation. 3′-Hydroxy-4′-*O*-methylglabridin protected mitochondrial respiratory enzyme activities against NADPH-dependent peroxidation injury.

Dihydroxyfumarate-induced mitochondrial peroxidation was also prevented by this isoflavan. At the concentration of 0.10, 0.25 and 0. 5 mg/mL, licorice glabridin inhibited microsomal free radical (ROS) formation by 67 %, 73 % and 83 %, respectively (Ablise et al. 2007). At lower concentration, the ROS inhibitory activity of glabridin was the same with those of *Ginkgo biloba* extract EGB761. Administration of *Glycyrrhiza glabra* polysaccharides (GGP) dose-dependently and significantly enhanced immune and antioxidant enzyme activities in the GGP-treated high-fat mice (Hong et al. 2009). *Glycyrrhiza glabra* root samples irradiated with 20 and 25 kGy doses gamma irradiation as a method of decontamination for food and herbal materials, increased phenolic contents and DPPH scavenging activity (Khattak and Simpson 2010). Licorice infusion weakly scavenged DPPH, and its compounds liquiritin and glycyrrhizin showed negligible effects (Cheel et al. 2010). Both licorice infusion and glycyrrhizin substantially scavenged superoxide radicals. The β-carotene bleaching was inhibited by licorice infusion, but liquiritin and glycyrrhizin showed no effect. The licorice infusion, liquiritin and glycyrrhizin exhibited no meaningful activities against hypochlorous acid, and they showed pro-oxidant effects in the myeloperoxidase-chlorinating system.

The DPPH radical scavenging activity of *G. glabra* leaf extracts (SC_{50}) were determined as: methanol extract 35.01 μg/ml, ethyl acetate extract 52.02 μg/ml and n-hexane extract 92.01 μg/ml and the total phenols were 104.09 μg/mg, 297.25 μg/mg and 111.53 μg/gm, respectively (Siracusa et al. 2011). Chloroform fraction of licorice methanol extract was the most effective antioxidant in scavenging 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) with 87.7 % activity but the activity was less than the crude methanolic extract, that is, 90 % (Lateef et al. 2012). Chloroform fraction showed the same trend in reducing power as that in radical scavenging activity. Significant anti-urease activity, that is, 72 % was observed in the ethyl acetate fraction with respect to standard inhibitor thiourea. Umbelliferone isolated from *Glycyrrhiza glabr*a rhizome exhibited DPPH free radical scavenging potential of 43.88 % at 616.75 μM (Kaur et al. 2012a). The methanol extract of *G. glabra* roots was found to have good antioxidant activity of 67.22 % at 500 μg/mL with the IC_{50} value of 359.45 μg/mL (Chopra et al. 2013).

Incorporation of licorice flavonoids liquiritigenin (LQG) and liquiritin (LQ) into ceramide liposome-in-cellulose hydrogel complex system enhanced their skin permeability (Kim et al. 2014b). Encapsulation efficiencies for liquiritigenin and liquiritin-loaded liposome-in-hydrogel were 69.39 % and 64.71 %, respectively. Liposome-in-hydrogel complex systems (LQG: 56.55 %, LQ: 66.99 %) had greater skin permeability than control (LQG: 4.92 %, LQ: 5.30 %) or a single liposome systems (LQG: 43.34 %, LQ: 48.97 %) and hydrogel systems (LQG: 38.21 %, LQ: 55.07 %). Liposome-in-hydrogel system could be a potential drug delivery system for topical delivery of antioxidants such as licorice flavonoids to construct antioxidative skin barrier. Licorice had been shown to have antioxidant properties and may play a role in the treatment and prevention of photo-ageing and a natural ingredient used in cosmetics (Bowe and Pugliese 2014). When licorice extract (LE) was also used in emulsion preparation, a remarkable synergistic oxidation inhibition was observed with pea protein hydrolysates (PPH) (Zhang et al. 2013, 2014b). Remarkable synergistic effects were observed on both Flavourzyme (Fla-PPH) or Protamex (Pro-PPH) with licorice extract (LE) (Zhang et al. 2013). The presence of LE enhanced the antioxidant potential by producing a more compact network apparently via PPH-LE complexation. LE adsorbed onto oil droplets either directly or through associating with PPH to produce a thick and compact interfacial membrane enabling the defence against oxygen species (Zhang et al. 2014b). Liquiritin apioside, neolicuroside, glabrene and 18β-glycyrrhetic acid were the predominant phenolic derivatives partitioning at the interface and most likely the major contributors to the notable synergistic antioxidant activity when coupled with pea protein hydrolysates.

Five macroporous resins showed similar and effective adsorption and desorption properties for enriching flavonoids from licorice leaf (Dong

et al. 2015). Further column chromatography of two representative resins XAD-16 and SP825 showed that the total flavonoids (from 16.8 to 55.6 % by XAD-16 and to 53.9 % by SP825) and pinocembrin (from 5.49 to 15.2 % by XAD-16 and to 19.8 % by SP825) were enriched in 90 % ethanol fractions. Meanwhile, the antioxidant capacities and nitrite-scavenging capacities were 2–3 times higher than those of the crude extract. The fractions with high flavonoid and pinocembrin contents could be used as biologically active ingredients in functional food.

Anticancer Activity

In-Vitro Studies

Studies by Kobayashi et al. (1995) showed that the anti-angiogenic effect of licorice extract depended on the anti-tube formation effect of isoliquiritin. Isoliquiritin (0.31–3.1 mg/kg), a licorice-derived flavonoid, inhibited the carmine content of granuloma tissue 50-fold greater than licorice extract (Kobayashi et al. 1995). Glycyrrhizin (20–80 mg/kg), a licorice-derived saponin, inhibited carmine content with a weak potency. The licorice extract (0.01–1 mg/ml) also inhibited tube formation from vascular endothelial cells in a concentration-dependent manner. From the chemical structure-activities of used licorice-derived flavonoids (0.1–100 μM), their potencies for anti-tube formation were in the order isoliquiritigenin > isoliquiritin > liquiritigenin >> isoliquiritin-apioside. Glycyrrhizin (0.1–100 μM) and glycyrrhetinic acid (0.1–10 μM) increased tube formation. A glycyrrhizin (82 μg/ml)-induced increase in tube formation was inhibited by isoliquiritin. The combined effect of a mixture of 82 μg/ml glycyrrhizin and 4.2 μg/ml isoliquiritin, a similar concentration ratio to their yield ratio in the licorice extract, corresponded to the effect of 100 μg/ml extract. Various extracts of *G. glabra* and its constituents were found to be cytotoxic in-vitro (Rathi et al. 2009). The IC_{50} values of standard 18 β-glycyrrhetic acid was 0.412 μM and those for the three different extracts (chloroform, methanol and water) of *G. glabra* on MCF7 cancerous cell line were 0.4485, 0.9906 and 1.288 μM, respectively. HPTLC study indicated that the amount of 18 β-glycyrrhetic acid in three different extracts (chloroform, methanol and water extract) was 26.6, 12.5 and 5.6 μg/g, respectively. *Glycyrrhiza* extract exhibited antiangiogenic activity in the zebrafish antiangiogenic model (Li 2012). Of seven fraction form the ethyl acetate extract, Fr5 and Fr6 showed antiangiogenic activity. Results of studies suggested that licorice root extract could mitigate the tumorigenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a endocrine disrupting chemical in MCF-7 cell breast cancer cells by suppression of aryl hydrocarbon receptor (AhR), AhR nuclear translocator and cytochrome P450 1A1 in a dose-dependent manner and cell cycle arrest (Chu et al. 2014). Thus, licorice could be used as a potential toxicity-alleviating agent against endocrine disrupting chemical-mediated diseases.

Glycyrrhetic acid inhibited the action of tumour promoter in-vitro and in-vivo (Nishino et al. 1984). Glycyrrhetic acid inhibited the increased phospholipid metabolism of cultured cells induced by tumour promoters, 12-*O*-tetradecanoylphorbol-13-acetate or teleocidin, and it markedly suppressed the promoting effect of teleocidin on skin tumour formation in mice initiated with 7,12-dimethylbenz[a]anthracene. Application of glycyrrhetic acid, to Swiss 3 T3 mouse fibroblasts prior to the treatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a potent tumour promoter, showed a time- and dose-dependent inhibitory effect on the TPA-stimulated 3-*O*-methyl-glucose transport (Kitagawa et al. 1984). Glycyrrhetic acid inhibited the specific binding of TPA to mouse epidermal membrane fractions in a dose- and time-dependent manner (Kitagawa et al. 1986). Glycyrrhizic acid, a glycoside of glycyrrhetic acid, exerted no inhibitory effect on TPA binding. The results of kinetic analysis suggested that glycyrrhetic acid directly bound to the TPA receptor, resulting in the competitive inhibition of the binding of TPA to its receptor without affecting the number of binding sites. The authors suggested that the inhibitory effect of glycyrrhetic acid on TPA binding to the membrane

receptor may play a role in its antitumor-promoting activity in-vivo. Glycyrrhetic acid an anti-inflammatory agent isolated from licorice root inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-mediated tumour promotion in mouse skin (O'Brian et al. 1990). It was demonstrated that glycyrrhetic acid inhibited the Ca^{2+}- and phospholipid-dependent phosphotransferase activity of protein kinase C (PKC), the phorbol ester tumour promoter receptor. Therefore, inhibition of PKC may play a role in the anti-promoting activity of glycyrrhetic acid.

Studies showed that 18α-glycyrrhetinic acid (AGA) inhibited proliferation and growth of prostate cancer cell line DU-145 cells by inducing apoptosis (Shetty et al. 2011). Also it was shown that HUVEC tube formation was drastically reduced when cultured in conditioned medium of AGA-treated DU-145 cells. Additionally, AGA treatment prevented the invasion of DU-145 prostate cancer cells on matrigel coated transwells via downregulation of NF-κB (p65), VEGF and MMP-9 expression. Further, AGA treatment also downregulated the expression of pro-inflammatory cytokine/growth factor genes HMGB1, IL-6 and IL-8 in DU-145 cells. The results suggested that AGA may be a promising anticancer agent for the chemoprevention and treatment of prostate cancer. Non-polar compounds in the ethanol extract of roasted licorice (EERL) exerted potent anti-carcinogenic effects in inhibiting the growth of DU145 and MLL prostate cancer cells, as well as HT-29 colon cancer cells (Park et al. 2014a, b). In contrast aqueous extracts of un-roasted and roasted licorice showed minimal effects on cell growth. EERL potently inhibited growth of MCF-7 and MDA-MB-231 breast, B16-F10 melanoma, and A375 and A2058 skin cancer cells, whereas EERL slightly stimulated the growth of normal IEC-6 intestinal epithelial cells and CCD118SK fibroblasts.

18-α-glycyrrhetinic acid (AGA) caused more than 95 % rapid and reversible inhibition of intercellular gap-junctional communication at concentrations as low as 2 μM (Davidson et al. 1986). The related compounds 18-β-glycyrrhetinic acid and carbenoxolone also caused communication inhibition. Glycyrrhetinic acid was shown previously to inhibit intercellular gap-junctional communication between human fibroblasts (Davidson and Baumgarten 1988). Of 31 derivatives of glycyrrhetinic acid tested for their ability to inhibit communication, eight of the compounds inhibited communication with high potency (IC_{50} less than 3 μM) and showed low toxicity, properties which suggested they may be useful pharmacological probes for studies of gap-junction function. Endogenous and exogenous factors which modulate intercellular gap-junctional communication may be efficiently used to prevent potential cancer cells deviating from normal cell society and homeostasis (Yamasaki 1990). 2-cyano substituted analogues of glycyrrhetinic acid, namely, methyl 2-cyano-3,11-dioxo-18β-olean-1,12-dien-30-oate (β-CDODA-Me) and methyl 2-cyano-3,11-dioxo-18α-olean-1,12-dien-30-oate (α-CDODA-Me) were found to be more cytotoxic to colon cancer cells (SW480, HT-29, HCT-15) than their des-cyano analogues and to have selective peroxisome proliferator-activated receptor gamma (PPARgamma) agonist activity (Chintharlapalli et al. 2007). This selectivity in colon cancer cells was shown for the induction of two proapoptotic proteins, namely, caveolin-1 and the tumour-suppressor gene Krüppel-like factor-4 (KLF-4). The data suggested that PPARgamma agonists derived from glycyrrhetinic acid induced cell-dependent caveolin-1 and KLF-4 expression through receptor-dependent pathways.

Glycyrrhetinic acid, the metabolite of the natural product glycyrrhizin, had been found to be a nonselective inhibitor of 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1 and type 2 (Stanetty et al. 2010). 11β-HSD2 inhibitors may find therapeutic applications in chronic inflammatory diseases and certain forms of cancer, whereas 11β-HSD1 inhibitors may find use for treatment of metabolic diseases, such as obesity and diabetes. Several 3-amino and 29-hydroxamic acid derivatives of glycyrrhetinic acid (metabolite of glycyrrhizin) were synthesised and showed high selectivity for 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) (Stanetty et al. 2010). The most potent and selective compound was active

against human 11β-HSD2 in the low nanomolar range with a 350-fold selectivity over human 11β-HSD1. Kratschmar et al. (2011) found selected 18β-glycyrrhetinic acid derivatives potently inhibited 11β-HSD2 in intact SW-620 colon cancer cells, although the rank order of inhibitory potential differed from that obtained in cell lysates. The biological activity of compounds was further demonstrated in glucocorticoid receptor (GR) transactivation assays in cells co-expressing GR and 11β-HSD1 or 11β-HSD2. Earlier novel 18βa-glycyrrhetinic acid analogues, 7 *N*-(2-hydroxyethyl)-3β-hydroxy-11-oxo-18β--olean-12-en-30-oic acid amide (Su et al. 2004) and 5, *N*-(2-hydroxyethyl)-3β-hydroxy-11-oxo-18β-olean-12-en-30-oic acid amide (Vicker et al. 2004) were found to be very potent selective inhibitors of 11β-hydroxysteroid dehydrogenase 2 with IC_{50} values of 4pM. Gaware et al. (2011) described novel 29-urea- and 29-hydroxamic acid derivatives of glycyrrhetinic acid as well as derivatives with the Beckman rearrangement of the 3-oxime to a seven-membered ring, and the rearrangement of the C-ring from 11-keto-12-ene to 12-keto-9(11)-ene to have improved selective inhibition of 11β-HSD2 in the lower nanomolar range with up to 3600-fold selectivity. Glycyrrhetinic acid (GA) significantly suppressed the viability of NCI-H460 and A549 non-small lung cancer cells in-vitro (Song et al. 2014). Also, GA significantly increased the sub G1 population by cell cycle analysis and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) positive cells in a concentration dependent manner in NCI-H460 non-small lung cancer cells. The results suggested that GA induced apoptosis via inhibition of PKC α/βII and activation of JNK in NCI-H460 non-small lung cancer cells. 18β-Glycyrrhetinic acid (18β-GA) suppressed cell proliferation of non-small cell lung cancer by inhibiting thromboxane synthase and its ERK/CREB signalling (Huang et al. 2014).

Glycyrrhetinic acid (GA) was found to have potential as a chemotherapeutic agent for human non-small cell lung cancer (Zhu et al. 2015). GA suppressed the proliferation of A549 and NCI-H460 cells by inducing G1-phase cell cycle arrest by upregulation of cyclin-dependent kinase inhibitors (CKIs) (p18, p16, p27 and p21) and inhibition of cyclins (cyclin-D1, -D3 and -E) and cyclin-dependent kinases (CDKs) (CDK4, 6 and 2). GA also upregulated the unfolded proteins, Bip, PERK and ERP72 in the endoplasmic reticulum.

Isoliquiritigenin, one of the components in the root of *Glycyrrhiza glabra*, significantly inhibited the proliferation of DU145 and LNCaP prostate cancer cell lines in a dose-dependent and time-dependent manner (Kanazawa et al. 2003). Isoliquiritigenin induced S and G2/M phase arrest and enhanced the expression of GADD153 mRNA and protein associated with cell cycle arrest. Licochalcone from *G. glabra* roots inhibited growth and induced modest apoptosis of androgen-independent p53-null PC-3 prostate cancer cells but had more pronounced effect on cell cycle progression arresting cells in G2/M, accompanied by suppression of cyclin B1 and cdc2 (Fu et al. 2004). It also inhibited phosphorylation of Rb and decreased expression of transcription factor E2F concurrent with reduction of cyclin D1, downregulation of CDKs 4 and 6, but increased cyclin E expression. Kwon et al. (2009) found that isoliquiritigenin inhibited migration, and the metastatic and invasive capacity of human prostate cancer DU 145 cells possibly by decreasing Jun N-terminal kinase (JNK)/activator protein (AP)-1 signalling. Isoliquiritigenin (ISL), a flavonoid isolated from licorice, inhibited human promyelocytic leukaemia (HL-60) cell proliferation and decreased the iROS levels in a dose-dependent manner, while the treatment did not increase the lethality rate (Li et al. 2009). Isoliquiritigenin (ISL) triggered the mammalian target of rapamycin (mTOR)-dependent autophagic and apoptotic cell death in adenoid cystic carcinoma (ACC) (Chen et al. 2012). The results suggested that induction of mTOR-dependent autophagic and apoptotic cell death may be an important mechanism in cancer chemotherapy by ISL. Wang et al. (2013c) found that isoliquiritigenin (ISL) significantly inhibited the VEGF-induced proliferation of human umbilical vein endothelial cells (HUVECs) at non-toxic concentration. A series of angiogene-

sis processes including tube formation, invasion and migration abilities of HUVECs were also interrupted by ISL in-vitro (Wang et al. 2013b). Additionally, ISL suppressed sprout formation from VEGF-treated aortic rings in an ex-vivo model. In-vivo studies further showed that ISL administration could inhibit breast cancer growth and neoangiogenesis accompanying with suppressed VEGF/VEGFR-2 signalling, elevated apoptosis ratio and little toxicity effects. Studies by Kang et al. (2010) demonstrated that isoliquiritigenin blocked JNK- or p38 MAPK-responsive pathways leading to direct matrix metalloproteinases (MMPs) activation of PMA-exposed endothelial cells. The results suggested that isoliquiritigenin inhibition of MMP may boost a therapeutic efficacy during angiogenesis. Li et al. (2013) found that isoliquiritigenin induced growth inhibition and apoptosis through downregulating multiple key enzymes in arachidonic acid (AA) metabolic network and the deactivation of PI3K/Akt in human breast cancer cells. Isoliquiritigenin diminished cell viability, 5-bromo-2′-deoxyuridine (BrdU) incorporation, and clonogenic ability in both MCF-7 and MDA-MB-231 cells, and induced apoptosis. Furthermore, isoliquiritigenin inhibited mRNA expression of multiple forms of AA-metabolizing enzymes, including phospholipase A2 (PLA2), cyclooxygenases (COX)-2 and cytochrome P450 (CYP) 4A, and decreased secretion of their products, including prostaglandin E2 (PGE2) and 20-hydroxyeicosatetraenoic acid (20-HETE), without affecting COX-1, 5-lipoxygenase (5-LOX), 5-lipoxygenase activating protein (FLAP) and leukotriene B4 (LTB4). Isoliquiritigenin suppressed the migration of MDA-MB-231 cells by inhibiting upstream signalling pathways (Wang et al. 2013c). It reduced the secretions and protein levels of vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1-alpha (HIF-1α) and also inhibited the expression and gelatinolytic activity of matrix metalloproteinase MMP-2 and MMP-9. In another study, isoliquiritigenin inhibited breast cancer MDA-MB-231 cell metastasis by preventing anoikis resistance, migration and invasion through downregulating COX-2 and CYP 4A signalling (Zheng et al. 2014). The results suggested that isoliquiritigenin could be a promising multi-target agent for preventing breast cancer metastasis, and anoikis could represent a novel mechanism through which flavonoids may exert the anti-metastatic activities. Licorice flavonoid isoliquiritigenin diminished 7,12-dimethylbenz[α]anthracene (DMBA)-induced DNA (xenobiotic response element – XRE) binding of aryl hydrocarbon receptor (AhR) in MCF-7 breast cancer cells (Wong et al. 2014). Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay demonstrated that expressions of genes with XRE-containing promoters, including CYP1A1, 1A2 and 1B1, followed the same pattern of XRE transactivation. The present study illustrated that isoliquiritigenin might downregulate polycyclic aromatic hydrocarbon (PAH)-induced expressions through antagonizing AhR translocation. Isoliquiritigenin was found to be a promising chemopreventive agent against oral cancer (Hsia et al. 2015). It caused DNA damage and inhibited ataxia telangiectasia mutated (ATM) expression leading to G2/M phase arrest and apoptosis in oral squamous cell carcinoma.

Studies showed that licorice (*Glycyrrhiza glabra*) could induce caspase-dependent and autophagy-related cell death in human LNCaP prostate cancer cells (Yo et al. 2009). Licorice and its constituent licochalcone-A induced autophagy in LNCaP prostate cancer cells by suppression of Bcl-2 expression and the mTOR pathway. Licochalcone A showed the most cytotoxic activity (IC_{50} values of 40–92 μM) among all seven licorice compounds (lichochalone A, glycyrrhizic acid, glycyrrhetinic acid, isoliquiritigenin, glabridin, liquiritigenin, glycyrrhizic acid ammonium salt) tested against gastric cancer cell lines GES-1, MKN-28, SGC7901 AGS and MKn-45 (Xiao et al. 2011). Licochalcone A induced apoptosis of gastric cancer cell lines via the caspase-dependent mitochondrial pathway and induced G2 cell cycle arrest through regulation of G2/M phase check-point proteins. Studies by Kim et al. (2014a) demonstrated that licorice compound licochalcone-A-induced apoptosis in KB oral cancer cells was mediated by the extrinsic apoptotic signalling pathway, which involved

a caspase-dependent FasL-mediated death receptor pathway.

Animal Studies

The aqueous licorice root extract inhibited the in-vivo (mice) and in-vitro proliferation of Ehrlich ascites tumour cells (Sheela et al. 2006). The angio-inhibitory activity of *G. glabra* was confirmed by its inhibition of angiogenesis in in-vivo assays, peritoneal and chorioallantoic membrane assay. Licorice extract decreased VEGF production and the cytokine-induced neovascularization. The results suggested that licorice root extract may be a potential supplemental source for cancer therapy. Administration of the licorice extract inhibited the growth of mouse colon carcinoma in BALB/C mice inoculated with CT-26 colon cancer cells, without any adverse effects, and reduced the cisplatin-induced toxicity (Lee et al. 2007). In addition, the administration of the licorice extract significantly reduced the cisplatin-induced oxidative stress.

Glycyrrhetic acid suppressed tumour promoter-induced effects in-vitro, such as stimulation of 32Pi-incorporation into phospholipids of cultured cells and downregulation of the epidermal growth factor receptor (Nishino et al. 1986). Glycyrrhetic acid inhibited the promoting activity of both 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and teleocidin on skin tumour formation in mice initiated with 7,12-dimethylbenz[a]anthracene (DMBA). The percentage of tumour-bearing mice in the group treated with DMBA plus teleocidin was 88 % at week 18, whereas that in the group treated with DMBA plus teleocidin and glycyrrhetic acid (10 mumol/painting) was 6 %. Similarly, the percentage of tumour-bearing mice of the group treated with DMBA plus TPA was 97 % at week 20, whereas that of the group treated with DMBA plus TPA and glycyrrhetic acid was 40 %. Thus, glycyrrhetic acid was confirmed to inhibit the activity of two different tumour promoters, teleocidin and TPA, in mouse skin.

Stereoisomeric forms of glycyrrhetinic acid (GA) α-GA and β-GA 18α (α-GA) and 18β (β-GA) were found to inhibit the mutagenicity of benzo[*a*]pyrene (B[*a*] 2-aminoflourene) and aflatoxin B_1 in *Salmonella typhimurium* TA98 and TA100 (Wang et al. 1991). β-GA was more effective than α-GA as an antimutagen. In the two-stage skin tumorigenesis protocol using 7,12-dimethylbenz[*a*]anthracene (DMBA) as the tumour initiating agent followed by twice weekly applications of 12-*O*-tetradecanoylphorbol-13-acetate as tumour promoter, pretreatment of SENCAR mice with α-GA or β-GA resulted in significant protection against tumour initiation as well as tumour promotion. As an anti-tumour initiating agent, β-GA was found to be more effective than α-GA. Similarly, topical application of β-GA was found to be more effective than α-GA in inhibiting the binding of both [^{3}H]B[*a*]P and [^{3}H]DMBA to epidermal DNA.

Glycyrrhizin and caffeine inhibited the action of tumour promoter in mouse skin which was comparable to the effect of quercetin (Yasukawa et al. 1988). Glycyrrhizin and caffeine inhibited the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation, and these markedly suppressed the promoting effect of TPA on skin tumour formation in mice initiated with 7, 12-dimethylbenz[a]anthracene (DMBA). Agarwal et al. (1991) showed that oral feeding of licorice glycyrrhizin to Sencar mice resulted in substantial protection against skin tumorigenesis caused by DMBA initiation and TPA promotion. The latent period prior to the onset of tumour development was considerably prolonged in glycyrrhizin-fed animals compared with non-glycyrrhizin fed and resulted in significant decrease in the number of tumours per mouse, during and at the termination of the experiment. Oral feeding of glycyrrhizin in drinking water also resulted in inhibition in the binding of topically applied [3H]benzo[a]pyrene and [3H] DMBA to epidermal DNA.

A topical application of a chalcone derivative, 4,2′,4′-trihydroxychalcone (isoliquiritigenin) inhibited epidermal ornithine decarboxylase (ODC) induction and ear oedema formation, that is, inflammation, caused by a topical application of TPA in CD-1 mice (Yamamoto et al. 1991). Also, isoliquiritigenin potently inhibited DMBA-initiated and TPA-promoted skin papilloma formation and epidermal ODC induction and

skin tumour promotion caused by 7-bromomethylbenz[alpha]anthracene (BrMBA), a non-TPA type of tumour-promoting agent, in DMBA-initiated mice. It was found that isoliquiritigenin exerted its anti-tumour-promoting action through the lipoxygenase inhibition by acting on cells other than the target epidermal cells. Intragastric administration of isoliquiritigenin (ISL) for 12 weeks in mice significantly decreased azoxymethane (AOM) induced colon cancer incidence, multiplicity and tumour size by 60 %, 55.4 % and 42.6 %, respectively (Zhao et al. 2014). Moreover, ISL inhibited M2 macrophage polarization in colitis-associated tumorigenesis through downregulating PGE2 and IL-6.

Licorice liquiritigenin effectively inhibited the growth of tumours xenografted in nude mice from human cervical cancer cell line HeLa cells (Liu et al. 2012). Also microvascular density (MVD) of the tumour exposed to liquiritigenin was reduced in a dose dependent manner, especially in the high dose group. Moreover, the expression and secretion of VEGF were downregulated by liquiritigenin in-vivo and in-vitro. Liquiritigenin markedly reduced cell viability, enhanced apoptotic rate, induced lactate dehydrogenase over-release, and increased intracellular reactive oxygen species (ROS) level and caspase 3 activity in both hepatocellular carcinoma PLC/PRL/5 and HepG2 cells (Wang et al. 2014b). It was found that liquiritigenin induced tumour cell death through mitogen-activated protein kinase- (MPAKs-) mediated pathway. This antitumor activity was further confirmed in PLC/PRL/5-xenografted mice model. In another study, liquiritigenin inhibited cell viability, caused G1 phase arrest and initiated apoptosis in both pituitary adenoma MMQ and GH3 cells via Ras/ERKs and ROS-dependent mitochondrial pathways, suggesting it to be a potential suppressor of pituitary adenoma (Wang et al. 2014c). In mice with GH3 cells xenograft, liquiritigenin markedly reduced tumour size without affecting bodyweight.

Dibenzoylmethane (DBM), a minor β-diketone constituent of licorice and sunscreens, had been shown to exhibit anti-neoplastic effects in chemically induced skin and mammary cancers in several animal models (Jackson et al. 2002). They found that DBM inhibited the growth of LNCaP, DU145 and PC-3 prostate carcinoma cell lines by induction of cell cycle deregulation. Frazier et al. (2004) investigated the proteomic changes induced by treatment of human prostatic LNCaP cancer cells with DBM in order to develop a model for the mechanism to elucidate how DBM induced cell cycle arrest and repressed androgen receptor expression. Dibenzoylmethane was reported to be a promising chemopreventive agent for colon, breast and skin cancer; it mediated the induction of phase II enzymes by Nrf2 activation and inhibited benzo[a]pyrene induced DNA adducts by enhancing its detoxification in lungs (Thimmulappa et al. 2008). Khor et al. (2009) demonstrated that DBM-fed TRAMP mice had a lower incidence of palpable tumour and high-grade prostatic intraepithelial neoplasia. Their findings suggested that DBM blocked the growth and progression of prostate cancer in TRAMP mice via modulation of tumour cell cycle regulation. Dibenzoylmethane (DB), a minor constituent of the root extract of licorice, hydroxydibenzoylmethane and hydroxymethyldibenzoylmethane with an identical structure to DB, inhibited phorbol-12-myristate 13-acetate-induced breast carcinoma cell migration and invasion through the protein kinase PI3K/PKCδ-mediated matrix metalloproteinase (MMP)-9 pathway (Liao et al. 2015).

Antimutagenic Activity

For all the compounds tested namely *Glycyrrhiza glabra* extract, glycyrrhizinic acid, 18 α- and 18 β-glycyrrhetinic acids, no desmutagenic activity was observed against ethyl methanesulfonate and N-methyl-N′-nitro-N-nitrosoguanidine; only *Glycyrrhiza glabra* extract showed antimutagenic activity against ethyl methanesulfonate (Zani et al. 1993). On using the ribose-lysine mutagenic browning mixture, the desmutagenic activities of the *Glycyrrhiza glabra* extract, glycyrrhizinic acid, 18 α- and 18 β-glycyrrhetinic

acids were observed. 18 β-Glycyrrhetinic acid was the most active compound. *Glycyrrhiza glabra* extract also exhibited antimutagenic activity against ribose-lysine. *Glycyrrhiza* extract and one of its components, glycyrrhizin, inhibited the mutagenicities of 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 (Tanaka et al. 1987). *Glycyrrhiza* extract and glycyrrhizin also inhibited the mutagenicities of benzo[a]pyrene, 3-methylcholanthrene, 2-naphthylamine, 2-amino-6-methyldipyrido [1,2-a:3′,2′-d]-imidazole, dimethylnitrosamine and dimethylaminoazobenzene. The mutagenicity of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) was inhibited by the *Glycyrrhiza* extract but not by glycyrrhizin. A standardized licorice extract GutGard™ did not show significant increase in number of revertant colonies in *Salmonella typhimurium* strains (TA98 and TAMix) with/without S9 fraction (Chandrasekaran et al. 2011b). In chromosome aberration and micronucleus studies, GutGard™ did not show clastogenic effect at 4 and 18 h treatments with and without S9 fraction. The results indicated that GutGard™ was not mutagenic in a battery of genotoxicity tests.

Antigenotoxic Activity

The antimutagenic and genoprotective activities of *Glycyrrhiza glabra* root extracts were demonstrated both on plant test systems – *Allium fistulosum*, *Allium cepa*, *Vicia faba* and on animals – Wistar rats (Agabeĭli 2012). Various genotoxic studies had indicated that glycyrrhizin was neither teratogenic nor mutagenic, and may possess anti-genotoxic properties under certain conditions (Isbrucker and Burdock 2006). Studies showed that licorice flavonoid oil (LFO) a functional food ingredient appeared not to present any genotoxic hazard to humans on dietary consumption (Nakagawa et al. 2008a) in a reverse mutation assay using four *Salmonella typhimurium* strains and *Escherichia coli*, LFO did not increase the number of revertant colonies in any tester strain with or without metabolic activation by rat liver S9 mix. In a chromosomal aberration test using Chinese hamster lung (CHL/IU) cells, LFO did not induce any chromosomal aberrations either in the short period test without rat liver S9 mix or in the continuous treatment (24 or 48 h) test. No significant or dose-dependent increases in the frequency of micronucleated polychromatic erythrocytes (MNPCE) were observed and the high dose suppressed the ratio of polychromatic erythrocytes (PCE) to total erythrocytes in the bone marrow micronucleus test using male F344 rats. No micronuclei induction either in hepatocytes or PCE was observed even at the highest dose of 5000 mg/kg/day. In-vitro studies found that isoliquiritin apioside, a chalcone oligoglucose isolated from *G. glabra* exhibited marked potential to combat oxidative stress-induced genotoxicity (Kaur et al. 2009). It exhibited modulatory effect against hydrogen peroxide and 4-nitroquinoline-N-oxide induced genotoxicity in *Escherichia coli* PQ37 using SOS chromotest and in human peripheral blood lymphocytes using the Comet assay. Umbelliferone isolated from *Glycyrrhiza glabr*a rhizome exhibited moderate response by reducing the induction factor of mutagens hydrogen peroxide by 68.99 % (IC_{50} 223.44 μM) and that of 4NQO (4-nitroquinoline-N-oxide) by 59.71 % (IC_{50} 280.74 μM) in the SOS chromotest using PQ37 strain of *Escherichia coli* (Kaur et al. 2012a). In comet assay in human blood lymphocytes, it exhibited good activity by inhibiting the genotoxicity of both hydrogen peroxide and 4NQO by 61.64 % (IC_{50} 330.02 μM) and 50.66 % (IC_{50} 577.83 μM), respectively. None of licorice leaf (methanol, ethyl acetate and n-hexane) extracts exhibited genotoxic effects in the SOS (short term bacterial) chromotest assay used at doses up to 100ug/assay both in the presence and absence of enzymatic metabolization (Siracusa et al. 2011). Licorice fraction comprising glycyrrhizic acid inhibited the genotoxicity of oxidative mutagens, namely, H_2O_2 and 4NQO quite efficiently (Kaur et al. 2012b). In SOS chromotest, using *Escherichia coli* PQ37 tester strain, it inhibited induction factor induced by H_2O_2 and 4NQO by 75.54 % and 71.69 % at the concentration of 121.46 μM, respectively. In Comet assay, it reduced the tail moment induced by H_2O_2 and

4NQO by 70.21 % and 69.04 %, respectively, at the same concentration in human blood lymphocytes. The isolated fraction also exhibited DPPH free radical scavenging activity and was able to scavenge 85.95 % radicals at a concentration of 120 μM. The results showed glycyrrhizic acid to be a potential modulator of genotoxins as well as efficient scavenger of free radicals. In-vitro study demonstrated that *G. glabra* extracts protected against cadmium-induced genetic and oxidative damage in human lymphocytes (Dirican and Turkez 2014). Co-application of *G. glabra* extract (5, 10 and 20 ppm) and $CdCl_2$ resulted in decreases of micronucleus and sister chromatid exchange formations as compared to the group treated with CdCl2 alone.

Antimicrobial Activity

In-Vitro Studies

The methanol extracts of *G. glabra* roots showed antibacterial activities against *Agrobacterium tumefaciens*, *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas syringae* pv. *tomato*, but none of the water extracts showed any antibacterial activity against the microorganisms (Ercisli et al. 2008).

Of the isoflavanoids and related substances isolated from *Glycyrrhiza glabra* var. *typica* hispaglabridin A, hispaglabridin B, 4′-*O*-methylglabridin, glabridin, glabrol and 3-hydroxyglabrol exhibited significant antimicrobial activity in-vitro (Mitscher et al. 1980). Both glycoumarin and licocoumarone inhibited the growth of Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* as strongly as streptomycin. Both also inhibited yeasts *Saccharomyces cerevisiae*, *Candida utilis* and *Pichia nakazawa*e, while streptomycin was inactive (Demizu et al. 1988). Pinocembrin, licoflavanone (from licorice leaves) and its converted product cyclolicoflavanone were weakly active against *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*, but were inactive against *Escherichia coli* (Fukui et al. 1988). Glabrene and glabridin, from Russian licorice (*G. glabra* var. *glandulifera*) showed potent antimicrobial activity against Gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* with IC_{50} values of 1.95–7.81 μg/ml, yeast – *Saccharomyces cerevisiae* and *Candida utilis* (IC_{50} values of 7.81–31.3 μg/ml) and the fungus, *Mucor pusillus* (IC_{50} values of 3.91–15.6 μg/ml) (Okada et al. 1989). Seven licorice phenolic compounds showed induction activities of DNA damage in a recombinationless mutant of *Bacillus subtilis* M45 (Fukai et al. 1998). *Glycyrrhiza glabra* rhizome extracts exhibited antifungal activity against *Candida albicans* in-vitro with MIC values of 1.56 mg/ml (Motsei et al. 2003). *Glycyrrhiza glabra* rhizome extract exhibited antifungal activity against *Candida albicans* in-vitro with MIC values of 1.56 mg/ml (Motsei et al. 2003). *G. glabra e*xtracts from samples collected in various sites of Calabria, Italy, exhibited antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Micrococcus luteus* and the fungus *Trichophyton mentagrophytes*, some samples inhibited *Pythium ultimum* (Statti et al. 2004). The hydroalcoholic extract of *G. glabra* exhibited antifungal activity in-vitro against *Candida albicans* and *Aspergillus niger* (Tharkar et al. 2010). The ethanolic extract of *Glycyrrhiza glabra* showed good antifungal activity in-vitro against *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans*, *Mucor* sp. and *Penicillium marneffei* (Geetha and Roy 2013). The in-vitro growth of the *Candida albicans* strains was markedly reduced, in a pH-dependent manner, by relatively low doses (6.2 μg/mL) of 18-β glycyrrhetinic acid, from *Glycyrrhiza glabra* root (Pellati et al. 2009). The results demonstrated 18-β glycyrrhetinic acid to be a promising biological alternative for the topical treatment of recurrent vulvovaginal candidiasis.

Of various oriental herb extracts tested only *Glycyrrhiza glabra* showed a remarkable antibacterial activity against *Propionibacterium acnes*, resulting in negligible induction of resistance, in comparison with a marked development of resistance in the bacteria treated with erythromycin (Nam et al. 2003). The ethanol extract (0.01 %) of *Angelica dahurica* markedly suppressed neutrophil chemotaxis, comparable to

the effect of erythromycin (0.01 %), whereas a strong antilipogenic effect was obtained with rhizoma coptidis (*Coptis chinensis*) extract (0.01 %), leading to a higher efficacy than that of retinoic acid (0.01 %). The authors suggested that an appropriate formulation containing *A. dahuric*a, *Coptis chinensis* rhizome and *G. glabra* could be helpful for the prevention and treatment of acne lesions. The mixture of *Capsella bursa-pastoris* and *Glycyrrhiza glabra* extracts was more effective against all the oral pathogenic bacteria *Streptococcus mutans*, *S. sanguis*, *Actinomyces viscosus*, *Enterococcus faecalis* than the separate individual extracts indicating synergistic effects between the two plant extracts (Soleimanpour et al. 2013).

Fukai et al. (2002b) found that glabridin exhibited antibacterial activity in-vitro against methicillin sensitive *Staphylococcus aure*us, methicillin resistant *S. aureu*s (MRSA) and licochalcone A exhibited antibacterial activity against MRSA with MIC values ranging from 6.25 to 16 μg/mL depending on the strain of microorganism. *G. glabr*a extract at concentration higher than 7.5 % exhibited inhibitory effects in-vitro against *Salmonella typhi*, *S. paratyphi B*, *Shigella sonnei*, *S. flexneri* and enterotoxigenic *Escherichia coli* (Shirazi et al. 2007). Antimycobacterial activity of *Glycyrrhiza glabra* root was found at 500 μg/mL concentration (Gupta et al. 2008). Bioactivity guided phytochemical analysis identified glabridin as potentially active against both *Mycobacterium tuberculosis* H_{37}Ra and H_{37}Rv strains at 29.16 μg/mL concentration. It exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria. Raw polysaccharides from *Glycyrrhiza glabra* were shown to have strong anti-adhesive effects against *Porphyromonas gingivalis*, a major pathogen for induction of periodontal inflammations (Wittschier et al. 2009). Glabridin, from licorice roots, was found to be active against both yeast and filamentous fungi (Fatima et al. 2009). Glabridin also showed resistance modifying activity against drug-resistant mutants of *Candida albicans* at a minimum inhibitory concentration of 31.25–250 μg/mL. The vegetative cell growth of *Bacillus subtilis* was inhibited dose-dependently by licochalcone A and was completely inhibited at a concentration of 3 μg/ml (Tsukiyama et al. 2002). Licochalcone A did not inhibit the germination of heat-treated spores of *B. subtilis* induced by L-alanine. Licochalcone A showed effects against all Gram-positive bacteria tested and was especially effective against all *Bacillus* spp. tested, with MICs of 2–3 μg/ml, but was not effective against Gram-negative bacteria or eukaryotes at 50 μg/ml. Glabridin and licochalcone A showed antifungal activity on *C. albicans* while glycyrrhizic acid had no effect (Messier and Grenier 2011). Biofilm formation was inhibited by 35–60 % in the presence of licochalcone A (0.2 μ/ml/). A strong inhibitory effect (>80 %) on hyphal formation was observed with licochalcone A or glabridin (100 μg/ml).

Licorice root and leaf extracts showed activity against *Candida albican*s, and tested Gram-positive bacteria *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus* in a dose dependent manner (Irani et al. 2010). The ethanolic extract of the leaves was the most active extract against Gram-positive bacteria. Ether, chloroform, acetone extracts of liquorice roots showed significant antibacterial activities against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria (Nitalikar et al. 2010). The hydromethanolic *G. glabra* root extract displayed antibacterial activities in-vitro against *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexneri*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis* (Varsha et al. 2013). *Shigella flexneri* was the most sensitive. Aqueous and ethanolic licorice extracts were found to have antimicrobial activity (Ajagannanavar et al. 2014). MIC of aqueous and ethnolic licorice root extract against oral pathogens, *Streptococcus mutans* and *Lactobacillus acidophilus* were 25 % and 12.5 %, respectively. The methanol licorice root extract exhibited moderate antimicrobial activity (Chopra et al. 2013). The extract was most potent against *Staphylococcus aureus* at 500 μg/mL (inhibition zone 13 mm) among bacteria and showed maximum potency against

Rhizopus spp. at 500 μg/ml (inhibition zone 11 mm) among fungi. It was least active against *Aspergillus awamori*. Diethyl carbonate extracts of *Glycyrrhiza glabra* root from Astrakhan region (Russia) exhibited maximum activity against test bacterial strains *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*; activity of Astrakhan licorice was superior among 50 % ethanol extracts from Astrakhan (Russia) and Calabria (Italy) (Astaf'eva and Sukhenko 2014). Antibacterial activity was directly proportional to the content of glycyrrhizin and 18β-glycyrrhetinic acid in the extracts. The content of these chemical components in *Glycyrrhiza glabra* root from Astrakhan region was higher than in licorice growing in Italy.

Clinical Studies

Recent research suggested that licorice and its bioactive ingredients such as glycyrrhizin, glabridin, licochalcone A, licoricidin and licorisoflavan A possessed potential beneficial effects against oral microbial pathogens and the host immune response involved in common ora-dental diseases (dental caries, periodontitis, candidiasis and recurrent aphthous ulcers) (Messier et al. 2012). A 1989 split-mouth clinical study on 21 dental subjects found that glycyrrhizin had a tendency toward a statistically significant effect for controlling dental plaque formation caused by cariogenic bacteria after just a few days (Steinberg et al. 1989). However, another pilot study involving 40 male and female volunteers showed that tooth brushing for up to 42 days with a toothpaste containing glycyrrhizin had no effect on the plaque index compared with using a control toothpaste (Goultschin et al. 1991). Possible explanations for the lack of efficiency in improvement of plaque, gingival and bleeding indices may have been an insufficient glycyrrhizin concentration and/or chemical incompatibility in a toothpaste containing a mixture of an anionic detergent and an organic antibacterial surface agent. In a more recent pilot study of pre-school children, clinical reduction of *Streptococcus mutans* was obtained by using a licorice root lollipop intervention twice daily for 3 weeks (Peters et al. 2010). High-risk children showed the steepest early decrease in mean log-*Streptococcus mutan*. At the end of a follow-up (9 week) period, the log *Streptococcus mutans* decrease moved the high-risk group down to moderate-risk level. Glycyrrhizol A extracted from licorice roots was found to have strong antimicrobial activity against cariogenic bacteria like *Streptococcus mutans* (Hu et al. 2011). In two pilot human studies they found that a brief application of sugar-free licorice lollipops (twice a day for 10 days) led to a marked reduction of cariogenic bacteria in oral cavity among most human subjects tested. This herbal lollipop could be a novel tool to promote oral health through functional foods. Jain et al. (2013) conducted as a double-blind pilot study of paediatric patients ($N=60$), aged 7–14 years, equally divided by randomization into three groups, namely, Group 1 using aqueous liquorice mouthwash (15 %), Group 2 using ethanolic liquorice mouthwash (3.75 %) and Group 3 using chlorhexidine gluconate (0.156 %) as positive control. They found the mean *Streptococcus mutans* colony counts in all three groups decreased significantly immediately after the oral rinsing. The reduction in colony counts was significant in ethanolic liquorice group as compared to the control. Liquorice extracts also led to an immediate rise in salivary pH. The study affirmed that both aqueous and ethanolic liquorice extracts were potent cariostatic agents and were palatable by child patients.

Antiviral Activity

Glycyrrhizin exhibited a concentration-dependent inhibition of the replication of hepatitis A virus (HAV) in PLC/PRF/5 cells (Crance et al. 1994). It was shown to inhibit an early stage of the HAV replication. In in-vitro studies, glycyrrhizin suppressed the secretion of hepatitis B surface antigen (HBsAg) dose-dependently in PLC/PRF/5 cells (Takahara et al. 1994). It was found that glycyrrhizin suppressed the intracellular transport of HBsAg at the trans-Golgi area after O-linked glycosylation and before its sialylation. Glycyrrhizin administered intravenously in guinea pigs might bind to hepatocytes at the con-

centration at which glycyrrhizin could modify the expression of chronic hepatitis B virus (HBV)-related antigens on the hepatocytes and suppress sialylation of HBV surface antigen (HBsAg) (Sato et al. 1996). Glycyrrhizin and its diastereoisomers were found to be inhibitory against two new human herpes virus: HHV-6 and HHV-7 (Ceremelli et al. 1996). Randomized controlled trials confirmed that the *Glycyrrhiza glabra* derived compound glycyrrhizin and its derivatives reduced hepatocellular damage in chronic hepatitis B and C (Fiore et al. 2008). In hepatitis C virus-induced cirrhosis the risk of hepatocellular carcinoma was reduced. Animal studies demonstrated a reduction of mortality and viral activity in herpes simplex virus encephalitis and influenza A virus pneumonia. In-vitro studies revealed antiviral activity against HIV-1, SARS related coronavirus, respiratory syncytial virus, arboviruses, vaccinia virus and vesicular stomatitis virus. Mechanisms for antiviral activity of *Glycyrrhiza* spp. include reduced transport to the membrane and sialylation of hepatitis B virus surface antigen, reduction of membrane fluidity leading to inhibition of fusion of the viral membrane of HIV-1 with the cell, induction of interferon gamma in T-cells, inhibition of phosphorylating enzymes in vesicular stomatitis virus infection and reduction of viral latency (Fiore et al. 2008). Matsumoto et al. (2013) demonstrated that glycyrrhizin treatment of hepatitis C virus (HCV)-infected Huh7 cells caused a reduction of infectious HCV production. The results suggested that glycyrrhizin inhibited the release of infectious HCV particles via its inhibitory effect on 1B phospholipase A2. Also combination treatment with glycyrrhizin augmented IFN-induced reduction of virus in the cell culture-produced HCV system.

Ito et al. (1987) found that glycyrrhizin completely inhibited human immunodeficiency virus (HIV)-induced plaque formation in molt-4 (MT-4) cells at a concentration of 0.6 mM, the 50 % inhibitory dose being 0.15 mM. Glycyrrhizin completely inhibited the cytopathic effect of HIV and the HIV-specific antigen expression in MT-4 cells at a concentration of 0.3 and 0.6 mM, respectively. Further, glycyrrhizin inhibited giant cell formation of HIV-infected Molt-4 clone No. 8 cells. The anti-HIV-1 activity of glycyrrhizin may be attributed to its inhibition of protein kinase activity and partly to an interference with virus-cell binding (Ito et al. 1988). Five phenolics licochalcone A, isolicoflavonol, glycycoumarin, glycyrrhisoflavone and licopyranocoumarin isolated from licorice inhibited the cytopathic activity of a human immunodeficiency virus (Hatano et al. 1988). An anti-HIV (human immunodeficiency virus) phenolic constituent, licopyranocoumarin was isolated from Si-pei licorice (a commercial licorice; root and stolon of *Glycyrrhiza* sp. from the north-western region of China) (Hatano et al. 1989). Betulinic acid and dihydrobetulinic acid derivatives were reported to be potent anti-HIV agents (Kashiwada et al. 1996). The results of in-vitro studies indicated that glycyrrhizin had the potential to inhibit a non-syncytium-inducing variant of HIV (NSI-HIV) replication in HIV-infected patients peripheral blood mononuclear cell cultures by inducing the production of β-chemokines CCL4 and CCL5 (Sasaki et al. 2002–2003). 18α-glycyrrhizic acid exhibited anti-HIV activity based on primary infection of MT-4 lymphoid cells with HIV (an acute HIV infection model) using strain HIV/EVK but its esters 18α-glycyrrhizic acid pentasulphate sodium disodium salt (III), 18α-glycyrrhizic acid di-*O*-nicotinate (IV) exhibited lower activity (Baltina et al. 2010). Changes in the stereochemistry of the18α-glycyrrhizic acid aglycone led to significant decreases in its anti-HIV-1 activity.

Glycyrrhizic acid inactivated herpes simplex virus particles irreversibly (Pompei et al. 1979). Studies by Utsunomiya et al. (1995) found that glycyrrhizin improved the resistance of thermally injured mice to opportunistic infection of herpes simplex virus type 1 through the induction of CD4+ contrasuppressor T cells. Glycyrrhizin was found to reduce morbidity and mortality of mice infected with lethal doses of influenza virus through stimulation of IFN-gamma production by T cells (Utsunomiya et al. 1997). In-vitro studies showed that soyasaponin II was more potent than soyasaponin I against herpes simplex virus type 1 (HSV-1) as shown by reduction of

HSV-1 production (Hayashi et al. 1997). When acyclovir and soyasaponin II were evaluated in combination for anti-HSV-1 activity, additive antiviral effects were observed for this virus. Soyasaponin II was also found to inhibit the replication of human cytomegalovirus, influenza virus and human immunodeficiency virus type 1. The action was not due to inhibition of virus penetration and protein synthesis, but might involve a virucidal effect. Ghannad et al. (2014) found that aqueous licorice extract pretreatment of Vero cells infected with HSV-1 was efficacious. There was significant difference in the efficacy of the extract with regard to incubation period between 1 and 4 h, 1 and 8 h, 4 and 12 h and 8 and 12 h. When glycyrrhizic acid and rapamycin were added to HeLa cells together with the viruses, glycyrrhizic acid demonstrated a strong anti-herpes simplex virus type 1 (HSV1) activity, whereas rapamycin had no activity (Laconi et al. 2014). However, if the compounds were added to the cells 24 h before the viruses, glycyrrhizic acid induced the production of an even higher amount of Beclin 1 and showed an improved antiviral effect; under these conditions, rapamycin was also able to exert a significant anti-HSV1 activity. The results suggested glycyrrhizic acid to be a strong inducer of the autophagy activator Beclin 1.

The study of Omer et al. (2014) showed that *Glycyrrhiza glabra* extract (60 mg/100 ml) inhibited replication of Newcastle disease virus and was non-toxic in the embryonated eggs. Glycyrrhizic acid from *Glycyrrhiza glabra* root inhibited growth and cytopathology of several unrelated DNA and RNA viruses: Vaccinia, Newcastle disease, vesicular stomatitis, herpes simplex type 1, influenza, etc., in-vitro, while not affecting cell activity and the ability to replicate (Pompei et al. 1979). Glycyrrhizic acid was also found to have antiviral activity against enterovirus 71 (EV71) of foot and mouth disease and coxsackievirus A16 (CVA16) on Vero cells (Wang et al. 2013a). Treatment with glycyrrhizin enhanced the production of interferon-γ in human peripheral lymphocyte-macrophage cultures by concanavalin A (Con A) (Shinada et al. 1986). Collaboration between enriched T-lymphocytes and macrophages, both treated with glycyrrhizin, was needed for the enhancement of interferon-γ production. A smaller increase in interferon production was also observed in the glycyrrhizin-treated peripheral lymphocyte-macrophage cultures derived from an asymptomatic carrier of hepatitis B virus, in response to Con A and surface antigen of hepatitis B virus.

Glycyrrhizin exhibited antiviral activity against varicella-zoster virus (VZV) in-vitro (Baba and Shigeta 1987). When human embryonic fibroblast (HEF) cells were treated with glycyrrhizin after inoculation of virus (post-treatment), the average 50 %-inhibitory dose (ID_{50}) for five VZV strains was 0.71 mM, and the selectivity index (ratio of ID50 for host-cell DNA synthesis to ID50 for VZV replication) was 30. Glycyrrhizin was also effective against VZV replication when HEF cells were treated 24 h before the inoculation (pretreatment). In addition, at a concentration of 2.4 mM glycyrrhizin inactivated more than 99 % of virus particles within 30 min at 37 °C. In combination with other anti-herpes drugs (acyclovir, adenine arabinoside, bromovinyldeoxyuridine and phosphonoformate) or human native β-interferon, glycyrrhizin had an additive or slightly synergistic effect on VZV replication. Glycyrrhizin, licorice and ammonium salt of glycyrrhizic acid exhibited antiviral activity on three strains of Japanese encephalitis virus (JEV), Nakayama, P-20778 and 821564XY48 (Badam 1997). Purified glycyrrhizin inhibited plaque formation in all three strains of JEV at a concentration of 500 μg/ml at 96 h. Similar effect was observed at 1000 μg/ml concentration with licorice and ammonium salt of glycyrrhizic acid. The minimal inhibitory concentrations were not toxic to porcine stable kidney (PS) and human cervical carcinoma (HeLa) cell lines. Glycyrrhizin inhibited the viral antigen expression of human cytomegalovirus (HCMV) in human monocytic cell line U-937 and human embryonic lung cell line MRC-5 in-vitro (Numazaki et al. 1994). Glycyrrhizin (GA) and primary metabolite 18β-glycyrrhetinic acid (GRA) pharmacologically active components of the medicinal licorice root had both been shown to have antiviral and immunomodulatory proper-

ties (Hardy et al. 2012). However, GRA, but not GA, exhibited significant antiviral activity against rotavirus replication in-vitro. GRA treatment reduced rotavirus yields by 99 % when added to infected cultures post-virus adsorption. In in-vivo studies, they showed that GRA delivered orally to mice with rotavirus infection augmented lymphocyte recruitment to the intestinal mucosa and induced maturation of B cell-rich ILF independently of ectopic antigenic stimulus (Hendricks et al. 2012). GRA reduced the duration of viral antigen shedding, and endpoint serum antibody titers were higher in GRA-treated animals.

Glycyrrhizic acid, a component of licorice root was active against Epstein Barr virus replication in superinfected Raji cells in a dose-dependent fashion (Lin 2003). The IC_{50} values for viral inhibition and cell growth were 0.04 and 4.8 mM, respectively. The selectivity index (ratio of IC_{50} for cell growth to IC_{50} for viral DNA synthesis) was 120. Of the antiviral compounds ribavirin, 6-azauridine, pyrazofurin, mycophenolic acid and glycyrrhizin assessed against two clinical isolates of SARS-associated coronavirus (FFM-1 and FFM-2) from SARS patients, glycyrrhizin was the most active in inhibiting replication of the SARS-associated virus (Cinatl et al. 2003). Aqueous *Glycyrrhiza glabra* root extracts were found to have strong significant antiviral activity against rhesus rotavirus with a 50 % inhibitory concentration (IC_{50}) < 300 µg/ml; its pure constituent, 18ß-glycyrrhetinic acid, was found to have the strongest antiviral activity (IC_{50} 46 µM) (Knipping et al. 2012).

Antihyperglycaemic/Antidiabetic Activity

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitors had been investigated as potential treatments for metabolic diseases, such as diabetes mellitus type 2 or obesity (Su et al. 2007; Classen-Houben et al. 2009; Stanetty et al. 2010). Su et al. (2007) reported the inhibition of human and rat 11β-hydroxysteroid dehydrogenase type 1 by 18β-glycyrrhetinic acid (18β-GA) derivatives. The 11-modified 18β-GA derivatives 2 and 3 with apparent selectivity for rat 11β-HSD1 showed a high percentage inhibition for human microsomal 11β-HSD1 at 10 µM and exhibited IC_{50} values of 400 and 1100 nM, respectively. Classen-Houben et al. (2009) compared the biological activity of 18β-GA and its diastereomer 18α-GA against the two enzymes in lysates of transfected HEK-293 cells and showed that 18α-GA selectively inhibited 11β-HSD1 but not 11β-HSD2. This was in contrast to 18β-GA, which preferentially inhibited 11β-HSD2. The side chain modified 18β-GA derivatives 4 and 5, although showing selectivity for rat 11β-HSD1 inhibited human microsomal 11β-HSD1 with IC_{50} values in the low micromolar range.

Administration of 18β-glycyrrhetinic acid to streptozotocin-induced diabetic rats reduced hyperglycaemia and hyperlipidaemia related to the risk of diabetes mellitus (Kalaiarasi et al. 2009). All elevated levels of total cholesterol, triglyceride, free fatty acid and phospholipids and reduced HDL cholesterol level in the diabetic rats were reverted back to normalcy. Results of the study by Sawada et al. (2010) indicated that glabridin, a prenylated isoflavone in licorice, may possess a therapeutic effect on metabolic disorders, such as diabetes and hyperglycaemia, by modulating glucose metabolism through adenosine monophosphate-activated protein kinase (AMPK)-dependent GLUT4 translocation pathway in the plasma membrane of mice skeletal muscle cells. Licorice rhizome isoliquiritigenin and its derivatives 4,4′-diacetoxy-2′-hydroxy chalcone; 2′,4′dimethoxy-4-hydroxychalcone; 4-acetoxy-2′,4′-dimethoxychalcone; 2′,4′-dimethoxychalcone and liquiritigenin derivatives liquiritigenin 4′-acetate; and liquiritigenin 7,4′-dibenzoate showed significant blood glucose lowering effect in normal Swiss albino male mice (Gaur et al. 2014). Isoliquiritigenin, 2′,4′dimethoxy-4-hydroxychalcone and liquiritigenin 7,4′-dibenzoate were selected for in-vivo antidiabetic activity and found to be potential candidates for treatment of diabetes

Studies found that oral administration of male Sprague–Dawley rats with high-fat/high-sucrose (HF/HS) diet elevated the fasting blood glucose level and insulin resistance index which was pre-

vented by licorice root glycyrrhizic acid (GA) supplementation (Cheng et al. 2014). GA treatment significantly lowered the circulating advanced glycation end product (AGE) independent of its glucose-lowering effect. HF/HS diet also triggered receptor for advanced glycation end product (RAGE) upregulation in the abdominal muscles while GA administration downregulated RAGE expression in the abdominal muscles, aorta and subcutaneous adipose tissues. It was concluded that HF/HS diet could cause glucose intolerance, insulin resistance and upregulation of RAGE expression while GA ameliorated the metabolic dysregulation besides exhibiting inhibitory effects on the AGE-RAGE axis.

Antihyperlipidemic/ Hypocholesterolaemic/Anti-obesity Activity

Animal Studies

Administration of licorice root extract to hypercholesterolaemic rats reduced the elevated levels of triglycerides and total lipids and augmented the low level of phospholipids in the rat serum and most organs (Sitohy et al. 1991). Licorice exhibited hypocholesterolaemic action and improved the impaired function of both liver and kidney. Administration of *Glycyrrhiza glabra* root extract to male rats at oral doses of 200, 400 and 800 mg/kg for 4 weeks induced a significant decrease in food intake and significant increases in body weight gain and feed efficiency ratio as compared to the control (Shalaby et al. 2004). At doses of 400 and 800 mg/kg, the extract caused significant decreases in total cholesterol and triglycerides associated with non-significant reductions in HDLc, LDLc and VLDLc concentrations in the serum. The extract at all doses produced significant decreases in the levels of serum liver enzymes (AST and ALT) and urea nitrogen, while the creatinine concentration significantly decreased by the high dose only. Studies showed that feeding obese diabetic KK-A(y) mice with diets containing licorice flavonoid oil (LFO) suppressed body weight gain, weights of abdominal adipose tissues and blood glucose levels (Nakagawa et al. 2004). Furthermore, LFO and licorice ethanolic extract stimulated human adipocyte differentiation in-vitro. The results indicated that licorice hydrophobic flavonoids possessed abdominal fat-lowering and hypoglycaemic effects, possibly mediated via activation of peroxisome proliferator-activated receptor-gamma (PPAR-gamma).

A 4-week administration of licorice root powder (5 and 10 gm% in diet) to hypercholesterolaemic rats resulted in significant reduction in plasma, hepatic total lipids, cholesterol, triglycerides and plasma low-density lipoprotein and VLDL-cholesterol accompanied by significant increases in HDL-cholesterol levels (Visavadiya and Narasimhacharya 2006). Furthermore, significant increases in faecal cholesterol, neutral sterols and bile acid excretion along with an increase in hepatic HMG-CoA reductase activity and bile acid production were observed in these animals. The root powder administration to hypercholesterolaemic rats also decreased hepatic lipid peroxidation with a concomitant increase in superoxide dismutase (SOD) and catalase activities and total ascorbic acid content. The antioxidant status of these animals also was improved upon treatment. Animal studies showed that *G. glabra* extract significantly decreased total cholesterol, LDL cholesterol and triglycerides levels, increased HDL cholesterol and reduced atereosclerotic lesion in the aorta of hypercholesterolemic rabbits (Asgary et al. 2007). This effect was ascribed to the effect of licorice on plasma lipoproteins and its antioxidant and anti-inflammatory properties.

Administration of ethanolic extract and its ethyl acetate soluble, water soluble and hexane soluble fractions to dyslipidaemic hamsters fed a high-fat diet (containing g/kg fructose 500 g, casein 190 g, Dalda Vansapati Ghee 110 g, wheat/corn/gram flour 150 g, cholesterol 5 g, methionine 3 g, vitamin mix 3 g, mineral mixture 40 g), decreased serum level of total cholesterol by 25.9, 38.0, 39.0 and 26.3 %, respectively (Maurya et al. 2009). The ethanolic extract, ethyl acetate soluble, water soluble and hexane soluble fraction increased the serum HDL-cholesterol level

by 14.8, 34.3, 27.3 and 17.2 %, respectively. The ethanolic extract, ethyl acetate fraction, aqueous fraction and hexane fraction decreased the triglyceride level by 31.3, 37.2, 41.2 and 28.9 %, respectively. The reduction in LDL-cholesterol level by ethanolic extract, ethyl acetate soluble fraction and water soluble fraction were 43.9, 31.0, 33.4 and 24.6 %, respectively. Compared with obese mice in the control group, those fed a high-fat diet containing1% and 2 % licorice flavonoid oil (LFO) presented reductions in the weight of abdominal white adipose tissues and body weight gain (Aoki et al. 2007a). A histological examination revealed that the adipocytes became smaller and the fatty degenerative state of the hepatocytes was improved in the 2 % LFO group. The findings suggested that LFO prevented and ameliorated diet-induced obesity via the regulation of lipid metabolism-related gene expression in the liver. Their findings suggested that the decrease in abdominal adipose tissue weight by LFO was mediated by the transcriptional regulation of sterol regulatory element-binding protein-1c (SREBP-1c) (a transcription factor promoting hepatic fatty acid synthesis), and peroxisome proliferator-activated receptor-alpha (PPAR-alpha) (a transcription factor promoting hepatic fatty acid oxidation) in the liver (Honda et al. 2009). Among the isolated phenolic compounds from *G. glabra* root ethanol extract, 5′-formylglabridin; (2*R*,3*R*)-3,4′,7-trihydroxy-3′-prenylflavane; echinatin; (3*R*)-2′,3′,7-trihydroxy-4′-methoxyisoflavan; kanzonol X; kanzonol W; shinpterocarpin; licoflavanone A; glabrol; shinflavanone; gancaonin L; and glabrone all exhibited significant PPAR-γ ligand-binding activity (Kuroda et al. 2010). The activity of these compounds at a sample concentration of 10 μg/mL was three times more potent than that of 0.5 μM troglitazone. Among 12 flavonoids isolated from *G. glabra* roots, isoliquiritigenin; 3,3′,4,4′-tetrahydroxy-2-methoxychalcone; licuroside and isoliquiritoside showed strong inhibition against pancreatic lipase in-vitro with IC_{50} values of 7.3 μM, 35.5 μM, 14.9 μM and 37.6 μM, respectively (Birari et al. 2011). In high fat diet (HFD) fed rats supplemented with isoliquiritigenin, the body weight increase was only 23.2 g as compared to 64.2 g in the HFD control group while rats treated with licuroside showed 23.2 g weight gain only. Isoliquiritigenin decreased the levels of plasma total cholesterol (TC) to 84.6 mg/dl and plasma total triglycerides (TG) to 128.8 mg/dl. Licuroside also lowered the plasma TC and TG levels considerably. The results indicated the potential of the chalcone scaffold as a source of pancreatic lipase inhibitors for preventing obesity.

Glabridin, an isoflavan isolated from licorice, effectively inhibited adipogenesis in 3T3-L1 cells and glabridin-rich supercritical fluid extract of licorice (LSC) showed inhibitory effect on adipogenesis in a dose-dependent manner (Ahn et al. 2013). LSC significantly reduced weight gain by high-fat diet mice in a dose-dependent manner. The reductions of the hypertrophy of white adipose tissue and of fat cell size were also observed. In the liver, LSC supplementation effectively inhibited high-fat diet-induced hepatic steatosis through downregulation of gluconeogenesis related phosphoenolpyruvate carboxykinase and glucose 6-phosphatase and upregulation of the β-oxidation related carnitine palmitoyltransferase 1. The results suggested that glabridin and glabridin-rich licorice extract would be effective anti-obesity agents.

Clinical Studies

In a study of 15 normal-weight subjects (7 males, age 22–26 years, and 8 females, age 21–26 years), consumption of 3.5 g a day of a commercial preparation of licorice for 2 months was found to reduce body fat mass (BFM) and plasma renin activity and aldosterone were suppressed, without any change in body mass index (BMI) (Armanini et al. 2003). It was suggested that licorice could reduce fat by inhibiting 11β-hydroxysteroid dehydrogenase Type 1 at the level of fat cells. In another study of 18 healthy women (age range 20–33 years) with normal BMI, topical application of a cream containing 2.5 % glycyrrhetinic acid to the thigh for a month reduced the thickness of subcutaneous layer of thigh fat in comparison to the contralateral untreated thigh and to control subjects treated with the placebo cream (Armanini et al. 2005).

The effect of glycyrrhetinic acid on the thickness of subcutaneous fat was likely related to a block of 11βa-hydroxysteroid dehydrogenase type 1 at the level of fat cells; therefore, glycyrrhetinic acid could be effectively used in the reduction of unwanted local fat accumulation.

Hepatoprotective Activity

In-Vitro Studies

Fractionated extracts of *Glycyrrhiza glabra* and *Schisandra chinensis* inhibited the action of acetaminophen or D-galactosamine in small scale rat hepatocyte primary culture model (Nakagiri et al. 2003). It was concluded that the hepatocyte microculture system presented was suitable for the screening of hepatoprotective substances. Glycyrrhizic acid (GA) exhibited protective effects against aflatoxin B_1 (AFB_1)-induced cytotoxicity in human hepatoma cell line (HepG2) (Chan et al. 2003). Both CYP1A1 and glutathione *S*-transferase (GST) activities were increased in cells after treatment with the GA. For cells without GA pretreatment, cell injury was implicated as indicated by the decrease in cell viability. It was found that GA pretreatment provided protective effects in terms of the enzyme activity and increase in cell viability. GA also protected against aflatoxin-induced oxidative stress. In a hepatocyte model of cholestatic liver injury in a hepatocyte model of cholestatic liver injury, pre-incubation with licorice glycyrrhizin exerted pro-apoptotic properties, whereas pre-incubation with its metabolite 18β-glycyrrhetinic acid potently inhibited bile acid-induced apoptosis and necrosis in a manner consistent with its antioxidative effect (Gumpricht et al. 2005). Pretreatment with glycyrrhizic acid (GA) (4 μg) protected the hepatocytes against t-BHP induced oxidative injury and the results were comparable to the pretreatment with positive control, that is, silymarin (Tripathi et al. 2009). The protective potential against cell death was achieved mainly by preventing intracellular GSH depletion, decrease in ROS formation as well as inhibition of mitochondrial membrane depolarization. GA was found to modulate critical end points of oxidative stress-induced apoptosis and could be beneficial against liver diseases.

Animal Studies

Oral administration of *G. glabra* extract (200 mg/kg, bodyweight) protected against paracetamol induced liver damage in rats (taju et al. 2011). All altered levels of biochemical markers were restored to the near normal levels in the dose dependent manner. Histological examination of the liver tissues confirmed the hepatoprotective effect of *G. glabra.*

Studies by Jeong et al. (2002) showed that protective effects of 18β-glycyrrhetinic acid (GA) against the carbon tetrachloride-induced hepatotoxicity in mice may be due to its ability to block the bio-activation of carbon tetrachloride, primarily by inhibiting the expression and activity of P450 2E1, and its free radical scavenging effects. Biotransformation of 18β-glycyrrhetinic acid by *Absidia pseudocylinderospora*, *Gliocladium viride* and *Cunninghamella echinulata* afforded seven metabolites, including three new ones 15α-hydroxy-18α-glycyrrhetinic acid; 13β-hydroxy-7α,27-oxy-12-dihydro-18β--glycyrrhetinic acid and 1α-hydroxy-18β-glycyrrhetinic acid and known metabolites 7β, 15α-dihydroxy-18β-glycyrrhetinic acid; 7β-hydroxy-18β-glycyrrhetinic acid; 5α-hydroxy-18 β-glycyrrhetinic acid and 3-oxo derivative of glycyrrhetinic acid (Maatooq et al. 2010). Two major metabolites 7β, 15α-dihydroxy-18β-glycyrrhetinic acid and 13β-hydroxy-7α,27-oxy-12-dihydro-18β-glycyrrhetinic acid displayed significant hepatoprotective activity against CCl_4-induced hepatotoxicity in albino mice. Pretreatment of Wistar rats with 18-β Glycyrrhetinic acid (18β-GA) at two different doses (45 and 75 mg kg^{-1} b.w.) significantly ameliorated 2-acetylaminofluorene-induced increased lipid peroxidation, alanine transaminase and aspartate transaminase, xanthine oxidase activities and activities of phase-II detoxifying enzymes along with the levels of glutathione content (Hasan et al. 2015). Administration of 18β-GA also significantly restored the expressions of proliferating cell nuclear antigen, cyclooxygenase 2, inducible

nitric oxide synthase and nuclear factor κB. Furthermore, histological observations also supported the preventive effects of 18β-GA. Their findings suggested that pretreatment with 18β-GA showed potential hepatoprotective effects via attenuation of oxidative stress, inflammation and hyperproliferation.

In-vivo studies demonstrated that liquiritigenin, an aglycone of liquiritin in licorice root, efficaciously protected the liver from acute injuries induced by acetaminophen-induced or from acetaminophen plus buthionine sulfoximine-induced severe injuries in rats (Kim et al. 2006). Liquiritigenin pretreatments significantly reduced the potentiated liver necrosis, decreasing mortality. Another animal study demonstrated that liquiritigenin had a choleretic effect and the ability to induce transporters and phase-II enzymes in the rat liver, which may be associated with a hepatoprotective effect against galactosamine/LPS (Kim et al. 2009). Liquiritigenin treatments attenuated galactosamine/LPS-induced hepatitis in rats, as supported by decreases in the plasma alanine aminotransferase, liver necrosis and plasma TNF-alpha. Intragastric administration of liquiritigenin (20 mg/kg) for 15 days effectively inhibited the growth of transplanted H(22) hepatocarcinoma in mice (Zhou et al. 2010). It also decreased malondialdehyde content and increased thymus weight. The study by Kim et al. (2010) demonstrated that isoliquiritin, a licorice antioxidant flavonoid, had the ability to repress liver X receptor-α (LXRα)-dependent hepatic steatosis through JNK1 inhibition and protected hepatocytes from oxidative injury inflicted by fat accumulation. In mice fed a high-fat diet, isoliquiritin treatment inhibited hepatic steatosis, as shown by a decrease in fat accumulation and repression of lipogenic genes. The results of blood biochemistry and histopathology confirmed attenuation of high-fat diet-induced liver injury by isoliquiritin. Moreover, isoliquiritin inhibited oxidative stress, as indicated by decreases in thiobarbituric acid-reactive substance formation, iNOS and COX2 induction, and nitrotyrosinylation.

Studies showed that compared to glycyrrhizin (Gly) and matrine (Mat) alone, a combination of Gly + Mat reduced the mortality of acetaminophen overdosed mice more effectively, attenuated acetaminophen-induced hepatotoxicity, and reduced the number and area of γ-GT positive foci, thus protecting liver function and preventing hepatocellular carcinoma from occurring (Wan et al. 2009). Further, Gly + Mat had a protective effect on immunosuppression, a strong non-specific anti-inflammatory effect, and an effect of reducing the incidence of sodium and water retention. Glycyrrhizin (Gly) in combination with matrine (Mat) had a better effect in inhibiting the proliferation of activated rat hepatic stellate cell (HSC) line and diminishing collagen I and HA levels secreted by HSC as compared with Gly or Mat alone (Zhao et al. 2012a). The combination significantly reduced serum hexadecenoic acid and laminin and procollgan type-III levels in the rat model of CCl_4-induced liver fibrosis compared with Gly or Mat alone. Hepatic histological analysis also confirmed that Gly + Mat administration exhibited the recovery effect remarkably and could improve liver fibrosis in-vitro and in-vivo more effectively. El-Tahawy et al. (2011) found that pretreatment with glycyrrhizin protected against lipopolysaccharide/D-galactosamine-induced acute hepatitis in albino rats by its anti-inflammatory and anti-apoptotic effects. Studies by Tu et al. (2012) found that mice treated with glycyrrhizin prevented concanavalin A-induced liver inflammation and fibrosis. Glycyrrhizin was found to alleviate liver injury and fibrosis progression via regulation of $CD4^+$T cell response in JNK, ERK and PI3K/AKT-dependent pathways. Results of studies by Tsai et al. (2013) suggested that glycyrrhizin pretreatment decreased total parenteral nutrition-associated acute liver injury factors in rats by suppressing endoplasmic reticulum stress and reactive nitrogen stress. Results of animal studies by Kuroda et al. (2014) revealed that a single injection of LPS/GalN (lipopolysaccharide/D-galactosamine) might stimulate apoptosis of mouse hepatocytes through the binding of HMGB1 (high mobility group box 1) protein to Gsto1(Glutathione S-transferase omega-1) promoter region and that glycyrrhizin-treatment might prevent the apoptosis and inflammatory

infiltrates caused by LPS/GalN-injection in mouse liver by disturbing the binding of HMGB1 protein to Gsto1 promoter sequence. Intraperitoneal (i.p.) administration of 200 mg/kg glycyrrhizin from licorice significantly protected lithocholic acid (LCA)-induced liver damage in mice, indicated by alleviated histology alteration and prevention of the alanine transaminase elevation (Han et al. 2014). Glycyrrhizin treatment significantly prevented LCA-induced reduction of the three phospholipid compounds, lysophosphatidylcholine LPC 16:0, LPC 18:0 and LPC 18:2. Glycyrrhizin and omega-3 fatty acids (ω-3) alone or in combination protected rat liver from thioacetamide-induced hepatotoxic effects as they significantly decreased serum aspartate aminotransferase activity and serum total bilirubin level; they also significantly increased serum albumin and total protein levels (El Magd et al. 2015). The hepatoprotective effects of glycyrrhizin and ω-3 were confirmed by the histopathological analysis as they significantly reduced the necroinflammatory scores and the extent of fibrosis. GL and ω-3 significantly decreased liver malondialdehyde level.

Results of in-vivo studies indicated that glycyrrhizic acid effectively protected against titanium dioxide nanoparticles (NTiO2) in rats (Orazizadeh et al. 2014). Pretreatment of glycyrrhizic acid significantly decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), attenuated the histopathology of hepatic injury, decreased apoptotic index, ameliorated oxidative stress in hepatic tissue and increased the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Clinical Studies

In a clinical trial of 18 patients with subacute hepatic failure due to viral hepatitis, thrice weekly intravenously treatment of the interferon stimulator named Stronger Neo Minophagen-C (SNMC) derived from the plant *G. glabra* for 8 weeks increased survival rate (Acharya et al. 1993). The survival rate among these patients was 72.2 %, as compared to the earlier reported rate of 31.1 % in 98 patients who received supportive therapy. In a retrospective study, long-term administration of the Japanese medicine 'Stronger Neo-Minophagen C' (SNMC), which contained 0.2 % glycyrrhizin (GL), 0.1 % cysteine and 2.0 % glycine in physiologic saline solution, used for the treatment of chronic hepatitis C, was effective in preventing liver carcinogenesis (Arase et al. 1997). The 10th-year rates of cumulative hepatocellular carcinoma (HCC) incidence for Group A (SNMC-treated) and Group B (vitamin K-treated) were 7 % and 12 %, respectively, and the 15th-year rates were 12 % and 25 %. HCC is caused by hepatitis C virus. By Cox regression analysis, the relative risk of HCC incidence in patients not treated with SNMC (Group B) was 2.49 compared with that of patients treated with SNMC (Group A). SNMC was also reported to be particularly helpful in patients who failed to respond to interferon and in patients who could not be treated with it for various reasons (Kumada 2002). In a prospective randomized controlled trial of 170 patients glycyrrhizin and glycyrrhizin plus ursodeoxycholic acid were compared for efficacy against chronic hepatitis C virus infection (Tsubota et al. 1999). It was found that the combined therapy with ursodeoxycholic acid and glycyrrhizin was safe and effective in improving liver-specific enzyme abnormalities and may be an alternative to interferon in chronic hepatitis C virus infection, especially for interferon-resistant or unstable patients. In a double-blind, randomized, placebo-controlled phase I/II trial of 57 patients with chronic hepatitis C, intravenous glycyrrhizin administration at a dose of 240 mg, thrice weekly for 4 weeks, lowered serum alanine aminotransferase (ALT) during treatment, but had no effect on hepatitis C virus (HCV)-RNA levels (van Rossum et al. 1999). The drug appeared to be safe and is well tolerated. The mechanism by which glycyrrhizin improved liver biochemistry and histology are not well elucidated (van Rossum and De Man 1998). In another clinical study of 69 European patients with chronic hepatitis C, glycyrrhizin treatment induced a significant decrease in ALT. Six times per week glycyrrhizin treatment for 4 weeks appeared more effective than three times per week. In another randomized phase II

trial, HCV-RNA-positive patients with elevated ALT and marked fibrosis or necro-inflammation who were not eligible for interferon therapy were treated for 4 weeks with six infusions weekly of glycyrrhizin (Orlent et al. 2006). Seventy two patients with an ALT response at week 4 were randomized to continue treatment for 22 weeks in three dose frequency groups: 6×, 3× or once weekly. They found that ALT responses induced by 4 weeks glycyrrhizin therapy could be maintained in a subset of chronic hepatitis C patients receiving at least three injections weekly. The observed ALT response did not translate in a significant histological improvement after 6 months treatment.

Ikeda (2007) retrospectively analysed 1249 patients with chronic hepatitis with or without cirrhosis and found that long-term glycyrrhizin injection therapy significantly decreased the incidence of hepatocellular carcinoma in patients with interferon-resistant active chronic hepatitis C, whose average aminotransferase value was twice or more of the upper limit of normal after interferon.

Yasui et al. (2011) treated 17 patients defined with acute onset autoimmune hepatitis with intravenous glycyrrhizin and 17 patients of severe disease with intravenous glycyrrhizin and corticosteroids. The alanine aminotransferase level could be controlled at an early stage using glycyrrhizin with no significant difference compared with the combined treatment. Recovery rate was higher in the glycyrrhizin group than in the glycyrrhizin + corticosteroid group. Glycyrrhizin could be used safely and be useful for patients with difficult-to-diagnose acute liver disease as an 'initial' treatment tool to improve liver inflammation before starting disease-specific treatment. In a randomized, double-blind, placebo-controlled, intravenous administration of glycyrrhizin, 5×/or 3×/week, and 5×/week placebo for 12 weeks to 379 patients, followed by a randomized, open comparison of glycyrrhizin i.v. 5×/versus 3×/week for 40 weeks was found to be effective in treating chronic hepatitis C patients who failed to respond to interferon-based therapies (Manns et al. 2012). Glycyrrhizin exhibited a significantly higher ALT reduction compared to placebo after 12 weeks of therapy and an improvement of necro-inflammation and fibrosis after 52-weeks treatment. Generally, glycyrrhizin treatment was well tolerated.

In a study of 38 patients with non-severe aplastic anaemia (NSAA), the combination therapy of glycyrrhizin and cyclosporine was found to be an effective treatment for NSAA in terms of improvement of response rate, reduction in cyclosporine-related liver injury, and attenuation of severity of nausea and other adverse events (Ren et al. 2013).

Neuroprotective Activity

In-Vitro Studies

The results of studies by Kao et al. (2009) suggested that glycyrrhizic acid may protect PC12 cells from ischemic injury caused by 6-hydroxydopamine (6-OHDA)-induced cytotoxicity via modulation of the intracellular antioxidant system and mitochondria-induced apoptosis. Moreover, glycyrrhizic acid and 18β-glycyrrhetinic acid may modulate the ratio of the mitochondrial Bcl-2 family and influence PI3K/Akt signalling. Glycyrrhizin and its metabolite 18β-glycyrrhetinic acid in *Glycyrrhiza*, a constituent herb of yokukansan, a traditional Japanese medicine, ameliorated thiamine deficiency-induced dysfunction of glutamate transport in cultured rat cortical astrocytes in a dose-dependent manner (Kawakami et al. 2010). Studies indicated that extracellular signal-regulated kinases (ERKs) and mitochondria-related pathways were essential for the neuroprotective effect of glycyrrhizic acid against glutamate-induced toxicity in DPC12 cells (Wang et al. 2014a). Glycyrrhizic acid pretreatment enhanced activation of ERKs but not AKT (protein kinase B). This was further confirmed by Teng et al. (2014a) who demonstrated the involvement of the ERK pathway in the neuroprotective effects of glycyrrhizic acid against the 1-methyl-4-phenylpyridinium (MPP+)-induced apoptosis of dopaminergic neuronal cells. Pretreatment with glycyrrhizic acid had no

effects on the expression of phosphorylated AKT (p-AKT) and total AKT (t-AKT).

Studies found that liquiritin exhibited neuroprotective effect against glutamate toxicity in differentiated PC12 (DPC12) rat pheochromocytoma cells, predominantly through the extracellular signal-regulated kinase (ERK) and protein kinase B (AKT)/glycogen synthase kinase-3β (GSK-3β) pathways, indicating the potential of liquiritin for the treatment of neurodegenerative diseases (Teng et al. 2014b).

Animal Studies

In in-vivo studies, gerbils treated with roasted licorice but not raw licorice exhibited significant neuroprotection against ischemic damage in the hippocampus after transient forebrain ischemia (Hwang et al. 2006). It was found that non-polar compounds containing glycyrrhizin-degraded products, such as glycyrrhetinic acid and glycyrrhetinic acid monoglucuronide, were increased in roasted licorice. In an in-vitro study, both raw and roasted licorice significantly reduced acetate dehydrogenase release from PC12 cells exposed to hypoxia. *G. glabra* root extract (250 and 500 mg/kg) promoted the locomotor activity and spatial behaviour significantly, which was impaired in hypoxic rats (Muralidharan et al. 2009). The extract administration restored the decreased levels of brain enzymes such as glutamate and dopamine and decreased acetylcholinesterase (AchE) activity significantly. Levels of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase were reduced due to hypoxia and were restored to near normalcy by administration of *G. glabra* extract. Oral administration of aqueous licorice root extract in the dose of 150 and 225 mg/kg to 1-month-old male Wistar albino rats showed a significant enhancement of dendritic arborization (dendritic branching points) and dendritic intersections along the length of both apical and basal dendrites in hippocampal (CA3) pyramidal neurons (Chakravarthi and Avadhani 2014). The results indicated that constituents present in aqueous licorice root extract had neuronal dendritic growth stimulating properties.

Pretreatment of rats with isoliquiritigenin (ISL), flavonoid constituent of *G. glabra* root, significantly reduced the cerebral infarct volume and oedema and produced significant reduction in neurological deficits (Zhan and Yang 2006). ISL pretreatment increased the brain ATP content, energy charge and total adenine nucleotides in a dose-dependent manner. ISL significantly inhibited the increases of brain malondialdehyde content and prevented the decline in activities of brain superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) caused by cerebral ischemia–reperfusion.

Yu et al. (2008) showed that glabridin, a major flavonoid of *Glycyrrhiza glabra*, at 25 mg/kg by intraperitoneal injection, significantly decreased the focal infarct volume, cerebral histological damage and apoptosis in stroked rats induced by cerebral artery occlusion middle (MCAO) compared to sham-operated rats. Glabridin significantly attenuated the level of brain malonyldialdehyde (MDA) in MACO rats while it elevated the level of two endogenous antioxidants in the brain, that is, superoxide dismutase (SOD) and reduced glutathione (GSH). Co-treatment with glabridin significantly inhibited the staurosporine-induced cytotoxicity and apoptosis of cultured rat cortical neurons and DNA laddering caused by staurosporine in a concentration-dependent manner. Glabridin also suppressed the elevated Bax protein and caspase–3 proenzyme and decreased bcl–2 induced by staurosporine in cultured rat cortical neurons, facilitating cell survival. Glabridin also inhibited superoxide production in cultured cortical neurons exposed to staurosporine. The findings indicated that glabridin had a neuroprotective effect via modulation of multiple pathways associated with apoptosis.

In-vivo studies by Zhang et al. (2014a) showed that glycyrrhizin had neuroprotective efficacy against brain ischemia–reperfusion injury in mice through high mobility group box 1 (HMGB1)-Toll-like receptor 4 (TLR4) signalling pathway. Administration of glycyrrhizin (10 mg/kg) intravenously in rats at 3 or 6 h after middle cerebral artery occlusion reduced infarct volumes to 12.9 % and 46.2 %, respectively (Kim

et al. 2012). This neuroprotective effect was accompanied by improvements in motor impairment and neurological deficits and suppressions of microglia activation and pro-inflammatory cytokine induction. The results indicated that glycyrrhizin had neuroprotective efficacy in the post-ischemic brain via its anti-inflammatory, anti-excitotoxic and anti-oxidative effects and in particular, it exerted anti-inflammatory effect, at least in part, by inhibiting HMGB1 (high mobility group box 1) secretion. Song et al. (2013) found that oral administration of glycyrrhizin alleviated neuro-inflammation and memory deficit induced by systemic lipopolysaccharide (LPS) treatment in mice. Glycyrrhizin significantly reduced TNF-α and IL-1β mRNA, COX-2 and iNOS protein expressions and the elevated Iba1 protein expression and the average cell size of Iba1-expressing microglia induced by LPS. In the Morris water maze test, glycyrrhizin significantly prolonged the swimming time spent in the target and peri-target zones. Glycyrrhizin also significantly increased the target heading and memory score numbers. The neuroprotective effect of glycyrrhizin was found to be mediated through the inhibition of pro-inflammatory mediators and microglial activation in the brain tissue. Glycyrrhizin exhibited neuroprotective effect on ischemia-reperfusion injury in rat brains through the inhibition of inflammation, oxidative stress and apoptotic injury by antagonizing the cytokine activity of high mobility group box 1 (HMGB1) (Gong et al. 2014). Pretreatment with glycyrrhizin significantly reduced infarct volume and improved the accompanying neurological deficits in locomotor function. The expression levels of inflammation- and oxidative stress-related molecules including TNF-α, iNOS, IL-1β and IL-6, which were over-expressed in I/R, were decreased by glycyrrhizin. Glycyrrhizin was found to reduce secondary brain injury and improved outcomes in rat following traumatic brain injury (TBI) by downregulation of high-mobility group box 1 (HMGB1)/HMGB1 receptors (toll-like receptor 4 (TLR4) and receptor for advanced glycation end products (RAGE))/NF-κB-mediated inflammatory responses in the injured rat brain (Gu et al. 2014). Beam walking performance impairment and brain oedema were significantly reduced in TBI + glycyrrhizin group compared with TBI group. In separate studies, Okuma et al. (2014) found glycyrrhizin to be a novel for TBI through its interference with HMGB1 and RAGE interaction in rats. The beneficial effects of glycyrrhizin on motor and cognitive functions persisted for 7 days after injury.

The anti-inflammatory and anti-excitotoxic effects of glycyrrhizic acid were verified in LPS-treated primary microglial cultures and in N-methyl-D-aspartate (NMDA)-treated or kainic acid-treated primary cortical cultures (Luo et al. 2013). Also they found that the neuroprotective effect of glycyrrhizic acid in the kainic acid-injected mouse brain might be attributable to the inhibitions of HMGB1 induction and release, which in turn mitigated the inflammatory process.

Results of studies by Shi et al. (2011) suggested that pinocembrin provided neuroprotection against global cerebral ischemic injury in rats with a wide therapeutic time window, which may be attributed to its anti-oxidative, anti-inflammatory and anti-excitotoxic effects. Studies by Meng et al. (2011) demonstrated that pinocembrin alleviated blood-brain barrier injury induced by global cerebral ischemia/reperfusion (GCI/R) in rats. Pinocembrin decreased neurological score and lessened brain oedema induced by GCI/R. Pinocembrin also alleviated the ultrastructural changes of cerebral microvessels, astrocyte end-feet and neurons, and improved cerebral blood flow in the GCI/R rats.

Central Nervous System (CNS) Activity

In in-vivo studies in dogs, *G. glabra* extract was found to have anti-cholinergic action as it blocked the stimulatory effect of acetylcholine; the extract produced inhibition of the intestinal movements (Shihata and Elghamry 1963b). Studies with licorice-derived enzyme inhibitors indicated functional effects for 11β-hydroxysteroid dehydrogenase (11β-HSD) in the adult brain, notably in the periventricular hypothalamus and limbic

system (Seckl 1997). 11β-HSD catalyses the conversion of the active glucocorticoids corticosterone and cortisol to inert 11 keto-products (11-dehydrocorticosterone, cortisone), thus regulating access of glucocorticoids to receptors. Thus, 11beta-HSD represents a novel and potentially important level of control of glucocorticoid action in the CNS. Enzyme modulation by pharmacological or other agents may provide a useful means to target increased or attenuated glucocorticoid action to specific sites in the brain. Administration of *Glycyrrhiza glabra* aqueous extract (150 mg/kg) significantly improved learning and memory of mice in the elevated plus-maze and passive avoidance test (Dhingra et al. 2004). Furthermore, this dose significantly reversed the amnesia induced by diazepam (1 mg/kg i.p.) and scopolamine (0.4 mg/kg i.p.). Anti-inflammatory and antioxidant properties of liquorice may be contributing favourably to the memory enhancement effect (Parle et al. 2004). As scopolamine-induced amnesia was reversed by liquorice, it was suggested that the beneficial effect on learning and memory may be because of facilitation of cholinergic transmission in brain. The results showed *G. glabra* to have promise as a memory enhancer in both exteroceptive and interoceptive behavioural models of memory. Administration of 150 mg/kg aqueous licorice extract significantly reduced the immobility times of mice in both forced swim test (FST) and tail suspension test (TST), without any significant effect on locomotor activity of mice (Dhingra and Sharma 2006). The efficacy of extract was found to be comparable to that of imipramine (15 mg/kg i.p.) and fluoxetine (20 mg/kg i.p.). Liquorice extract reversed reserpine-induced extension of immobility period of mice in FST and TST. It was found that the antidepressant-like effect of liquorice extract appeared to be mediated by increase of brain norepinephrine and dopamine, but not by increase of serotonin (Cui et al. 2008). Oral administration of the higher doses (2 and 4 mg/kg) of glabridin and piracetam to mice significantly antagonized the amnesia induced by scopolamine in both the elevated plus maze test and passive avoidance test. Furthermore, glabridin (2 and 4 mg/kg per os) and metrifonate (50 mg/kg intraperitoneally), used as a standard drug, both remarkably reduced the brain cholinesterase activity in mice compared to the control group. The results suggested glabridin to be a promising candidate for memory improvement.

Zhu et al. (2010) reported that the natural product 2,2′,4′-trihydroxychalcone (TDC) from *Glycyrrhiza glabra* functioned as a specific non-competitive inhibitor against β-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1) enzyme, and potently repressed β-cleavage of APP and production of amyloid-β (Aβ) in human embryo kidney cells-APPswe cells. In APP-PS1 double transgenic mice, treatment with 9 mg/kg/day of TDC markedly decreased Aβ production and Aβ plaque formation, while efficiently improving the memory impairment based on Morris water maze test. Their findings demonstrated that the natural product TDC as a new BACE1 inhibitor could ameliorate memory impairment in mice and could have potential as a lead compound for further anti-Alzheimer's disease reagent development. Studies showed that liquiritigenin treatment improved the behavioural performance of Abeta (25–35) hippocampal-injected rats and attenuated neuronal loss in the brain (Liu et al. 2010a). More importantly, liquiritigenin treatment decreased mRNA levels and protein expression of Notch-2, an effect that could promote the generation of new neurons. They found that treatment of brain-derived progenitor cell cultures with liquiritigenin increased the number of cells that differentiated into neurons; but the treatment did not alter the growth of astrocytes (Liu et al. 2010b). In addition, treatment with liquiritigenin decreased Notch-2 mRNA and protein expression, which could promote the growth of new neurons. In a subsequent study, they found that treatment with liquiritigenin improved the behavioural performance of transgenic mice and it attenuated the protein expression of oligomeric form of amyloid β-peptide (Aβ) (Liu et al. 2011). Furthermore, treatment with liquiritigenin inhibited astrocytosis in the hippocampus, through its inhibitory activities on Notch-2, an important molecular regulating neural proliferation and dif-

ferentiation. These findings provided evidence for beneficial activity of liquiritigenin in a mouse model of Alzheimer's disease.

Glycyrrhiza glabra ethanol extract (GGE) dose-dependently potentiated pentobarbital-induced sleep and increased the amount of non-rapid eye movement sleep in mice without decreasing delta activity (Cho et al. 2012). The major flavonoid glabrol was isolated from the flavonoid-rich fraction of GGE; it inhibited [^{3}H] flumazenil binding to the $GABA_A$-BZD receptors in rat cerebral cortex membrane. Glabrol increased sleep duration and decreased sleep latency in a dose-dependent manner (5, 10, 25 and 50 mg/kg); its hypnotic effect was also blocked by flumazenil a well-known γ-aminobutyric acid type A-benzodiazepine ($GABA_A$-BZD) receptor antagonist. The results implied that GGE and its flavonoid glabrol induced sleep via a positive allosteric modulation of $GABA_A$-BZD receptors. Results of animal studies demonstrated that the anti-amnestic dose of *Glycyrrhiza glabra* extract (150 mg/kg for 7 days) resulted in prepulse inhibition (PPI) disruption; it augmented cortical, hippocampal and striatal monoamine levels in mice (Michel et al. 2013). It was concluded that liquorice extract (150 mg/kg)-induced PPI deficit was mediated through augmenting monoaminergic transmission in the cortex, hippocampus and striatum.

A panchagavya Ayurvedic formulation (300, 500 mg/kg, po) containing *Emblica officinalis*, *G. glabra*, and cow's ghee produced a significant prolongation of pentobarbital-induced sleeping time and reduced spontaneous locomotor activity (Achliya et al. 2004). The formulation also significantly antagonized the amphetamine induced hyper-locomotor activity (500, 750 mg/kg, po) and protected mice against tonic convulsions induced by maximal electroshock (500, 750 mg/kg, po). The formulation slightly prolonged the phases of seizure activity but did not protect mice against lethality induced by pentylenetetrazole. The formulation did not show neurotoxicity. Combination drug therapy of *G. glabra* and *Piper nigrum* in rats exerted better antidepressant effects than the individual dosage as evaluated by the force swim and tail suspension tests (Sohi et al. 2013).

Anti-inflammatory/Anti-allergic Activities

In-Vitro Studies

Liquiritigenin and 18β-glycyrrhetinic acid most potently inhibited the degranulation of RBL-2H3 cells induced by IgE with the antigen (DNP-HSA) and rat peritoneal mast cells induced by compound 48/80 (Shin et al. 2007). Liquiritigenin and 18β-glycyrrhetinic acid potently inhibited the passive cutaneous anaphylactic reaction as well as the scratching behaviour in mice induced by compound 48/80. These components inhibited the production of IgE in ovalbumin-induced asthma mice but liquiritigenin had little effect. The results suggested that the anti-allergic effects of licorice were mainly due to glycyrrhizin, 18β-glycyrrhetinic acid and liquiritigenin, which could relieve IgE-induced allergic diseases such as dermatitis and asthma. At non-toxic ≥10 μM concentration, isoliquiritigenin blocked the induction of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin on activated human umbilical vein endothelial cells (HUVEC) and markedly interfered with THP-1 monocyte adhesion to TNF-alpha-activated endothelial cells (Kwon et al. 2007). Isoliquiritigenin abolished TNF-alpha-induced mRNA accumulation of VCAM-1 and E-selectin. Additionally, isoliquiritigenin attenuated platelet endothelial cell adhesion molecule-1 (PECAM-1) expression induced by TNF-alpha. In contrast, other components found in licorice, 18β-glycyrrhetinic acid, glycyrrhizin, formononetin and ononin did not downregulate the expression of VCAM-1 and/or PECAM-1 activated by TNF-alpha, implying that these components are inactive in modulating adhesion of leukocytes to stimulated endothelial cells. Isoliquiritigenin downregulated CAM proteins in TNF-alpha-activated HUVEC at the transcriptional levels by blocking degradation of IkappaBalpha and nuclear translocation of NF-kappaB. The results demonstrated that the induction blockade of VCAM-1 and E-selectin

by isoliquiritigenin was directly mediated by its interference with the cell adhesion molecules (CAM) mRNA transcription through NF-kappaB-dependent mechanisms under inflammatory conditions. In lipopolysaccharide (LPS)-treated RAW 264.7 macrophages, isoliquiritigenin (ILG), more potently inhibited LPS-induced nitric oxide (NO) and prostaglandin E(2) (PGE(2)) production than isoliquiritin (ILT) (Kim et al. 2008a). ILG dose-dependently reduced the LPS-induced expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) at the protein and mRNA levels via suppression of the transcription activity of nuclear factor-kappa B (NF-kappaB). The results suggested that the anti-inflammatory properties of ILG are caused by iNOS, COX-2, TNF-alpha and IL-6 downregulation due to NF-kappaB inhibition via the suppression of IKK, ERK1/2 and p38 phosphorylation in RAW 264.7 cells. Isoliquiritigenin and glycyrrhizin inhibited lipopolysaccharide (LPS)-induced NF-κB activation and interleukin IL-6 production in dose-dependent manners in RAW264.7 cells (Honda et al. 2012). They both modulated LPS sensor toll-like receptor 4/MD-2 complex at the receptor level, leading to suppress LPS-induced activation of signalling cascades and cytokine production, but their effects were exerted at different steps of TLR4/MD-2 signalling.

G. glabra crude extract displayed remarkable reactivity with free stable 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radical, inhibitory efficacy in peroxidatively damaged unilamellar dioleoyl phosphatidylcholine (DOPC) liposomes and inhibition of ROS chemiluminescence, generated by whole blood, induced by both receptor-bypassing stimuli (PMA) and receptor operating stimuli (Opz) in the ranking order of stimuli PMA > Opz (Račková et al. 2007). These activities were postulated to be attributed to phenolic antioxidants involving isoflavan derivatives, coumarins and chalcones. Nonetheless, triterpene saponin glycyrrhizin exhibited no efficacy in the system of DPPH reaction and peroxidation of liposomal membrane, and negligible inhibition of chemiluminescence generated by inflammatory cells. These results indicate that the mechanism of anti-inflammatory effect of glycyrrhizin most probably did not involve ROS and this major constituent was not responsible for the inhibition effects of liquorice extract on neutrophil functions. The results of studies in a lipopolysaccharide (LPS)-stimulated macrophage model indicated that licorice glycyrrhizic acid and 18β-glycyrrhetinic acid may provide an anti-inflammatory effect by attenuating the generation of excessive NO, PGE(2) and ROS and by suppressing the expression of pro-inflammatory genes through the inhibition of NF-κB and PI3K activity (Wang et al. 2011). Kao et al. (2010) demonstrated that both glycyrrhizic acid (GA) and 18β-glycyrrhetinic acid (18βGA) reduced inflammatory cytokine production and its resulting anti-inflammation (Kao et al. 2010). GA acted via PI3K/Akt/GSK3β to reduce cytokine production, while 18βGA caused the dissociation of a glucocorticoid receptor (GR)-HSP90 complex to block inflammation. They proposed that GA and 18βGA may be valuable biological inhibitors of lung inflammation. In-vitro studies using murine macrophages (J774A.1) and human neutrophil (HL-60) cells revealed that *G. glabra* and glabridin significantly inhibited prostaglandin E_2 (PGE_2), thromboxane (TXB_2), cyclooxygenase (COX), leukotriene (LTB_4), leukotriene (LTB_4) and lipoxygenase (LOX), while isoliquiritigenin exerted inhibitory effect only against COX products but failed to suppress LOX products (Chandrasekaran et al. 2011a). However, glycyrrhizin at the tested concentrations failed to exhibit inhibitory effect on both COX and LOX products. Umbelliferone isolated from *Glycyrrhiza glabra* rhizome exhibited 95.68 % inhibition of COX-2 at 10 μM concentration with $IC_{50} < 1$ μM (Kaur et al. 2012a). In the xylene-induced ear oedema and ovalbumin-induced mouse paw oedema assay, extracts of the cell culture of *Glycyrrhiza* exhibited similar anti-inflammatory effects to those of its field cultivated equivalent, through the enhancement of the SOD activity of plasma and liver tissues (Man et al. 2013).

Studies by Thiyagarajan et al. (2011) showed that the inhibitory effect of *G. glabra* extract on

lipopolysaccharide (LPS)-induced pro-inflammatory mediators was influenced by glabridin and isoliquiritigenin and was not contributed by glycyrrhizin. *G. glabra* and isoliquiritigenin significantly inhibited LPS stimulated NO, IL-1 β and IL-6 production. Glabridin showed significant inhibition of NO and interleukin IL-1 β release, but failed to attenuate IL-6 levels at the tested concentrations. In addition, glycyrrhizin did not exhibit inhibitory response towards any of the LPS-induced pro-inflammatory mediators at the tested concentrations. Treatment of THP-1 (human myelomonocytic leukaemia) cells with licochalcone C attenuated lipopolysaccharide (LPS)-IFN-γ-induced inflammatory response by significantly decreasing the expression and activity of inducible nitrate synthase (iNOS) via NFκB (nuclear factor kappa-B), by influencing extracellular O_2^- production, and by modulating the antioxidant network activity of SOD (superoxide dismutase), CAT (catalase) and GPx (glutathione peroxidase) activity. It was hypothesized that licochalcone C had antioxidant properties since it reduced the production of superoxide radicals and consequently reduced the activity of iNOS (Franceschelli et al. 2011). Licorice extract inhibited nitric oxide (NO) production and inducible NO synthase (iNOS) expression in lipopolysaccharide (LPS)-stimulated RAW264 murine macrophage cells (Uto et al. 2012). However, treatment of glycyrrhizin alone could not show the suppression of NO production and iNOS expression. The combined treatment with glycyrrhizin and glycyrrhizin-removed extract (GC–KO) extract enhanced the attenuated inhibition. The ethyl acetate leaf extract showed good dose-dependent ability to inhibit release of both thromboxane B_2 (TxB_2) and prostaglandin E_2 (PGE_2) in whole blood (Siracusa et al. 2011). The methanol leaf extract appeared to inhibit only the PGE2 release, suggesting a selective action on the cyclooxygenase COX-2 pathway. No effect on TxB2 and PGE_2 was observed with *n*-hexane leaf extract.

Studies by Bhattacharjee et al. (2012) showed that glycyrrhizic acid (GA) treatment caused an enhanced expression of iNOS2 along with inhibition of Cox-2 in *Leishmania donovani*-infected macrophages. GA treatment in infected macrophages enhanced the expression of interleukin IL-12 and tumour necrosis factor TNF-α, concomitant with a downregulation of interleukin IL-10 and transforming growth factor TGF-β. GA increased macrophage effector responses via inhibition of Cox-2-mediated prostaglandin E2 release in *L. donovani*-infected macrophages. Studies showed that monoammonium glycyrrhizate (MAG) derived from licorice suppressed TNF-α-induced chemokine (including CXCL8, CX3CL1 and CXCL16) mRNA expression in human dermal microvascular endothelial cell line (HMEC-1) cells, in a dose-dependent manner, and reduced the secretion of these chemokines in culture supernatant (Cao et al. 2014). MAG also suppressed TNF-α-induced chemokine production in HMEC-1 cells. The results revealed MAG to be a potential anti-inflammatory agent capable of improving inflammatory skin diseases.

Treatment of licochalcone A, from *G. glabra* root, suppressed polyinosinic-polycytidylic acid (poly-IC)-induced thymic stromal lymphopoietin (TSLP) in BEAS 2B cells and primary bronchial epithelial cells in a dose dependent manner (Kim et al. 2015). The poly-IC-induced mRNA expression of other pro-inflammatory mediators such as MCP-1, RANTES and interleukin IL-8 was suppressed by licochalcone A. Licochalcone A inhibited the Iκβ kinase (IKK)/nuclear factor kappa (NF-κB) signalling pathway, which might be involved in the pathogenesis of virus-exacerbated asthma.

Animal Studies

The anti-inflammatory activity of aqueous licorice extract in rat foot arthritis model was ascribed to glycyrrhizin and glycyrrhetic acid (Gujral et al. 1959). Effect at doses tested was comparable to that of hydrocortisone and butazolidin. The *Streptococcus* LJ-22-transformed product, 18β-glycyrrhetinic acid-3-*O*-β-D-glucuronide (GAMG), of glycyrrhizin (18β-glycyrrhetinic acid-3-*O*-β-D-glucuronopyranosyl-(1 → 2)-β-D-glucuronide, GL) exhibited anti-allergic activity with IC_{50} values of 0.28 mM (Park et al. 2004). GAMG, which was sweeter than glycyrrhizin, and 18β-glycyrrhetinic acid, a GAMG metabolite

by human intestinal bacteria, also inhibited the passive cutaneous anaphylaxis and skin contact inflammation. Licorice root extract lipids produced statistically significant suppression of inflammatory oedema growth induced by 1 % carrageenan and 3 % formalin solutions in mice compared to that in the untreated control (Denisova et al. 2007) and their antiphlogistic action was comparable with that of the reference drug ortophen. In animal studies, glyderinine, a derivative of glycyrrhizic acid isolated from *Glycyrrhiza glabr*a, was found to exert a pronounced anti-inflammatory effect exceeding that of hydrocortisone and amidopyrine (Azimov et al. 1988). Oral administration of *Glycyrrhiza glabra* root extract to rats caused a significant reduction in pedal inflammation and swelling induced by formalin compared to the control group (Shalaby et al. 2004). However, the anti-inflammatory effect was less marked than that produced by tenoxicam (a standard anti-inflammatory drug). Glycyrrhizin, a major constituent of *G. glabra* (5 mg/kg) markedly inhibited ovalbumin (OVA)-induced immediate airway constriction, airway hyper-reactivity to methacholine, lung inflammation and infiltration of eosinophils in the peribronchial and perivascular areas in BALB/c mice (Ram et al. 2006). It prevented the reduction of interferon IFN-γ and decreased interleukin IL-4, IL-5 and eosinophils in the bronchoalveolar lavage fluid. Also, it reduced OVA-specific IgE levels and prevented the reduction of total IgG2a in serum. Studies by Ma et al. (2013) demonstrated that glycyrrhizic acid exerted anti-asthmatic effects via modulation of Th1/Th2 cytokines and enhancement of CD4 + CD25 + Foxp3+ regulatory T cells in ovalbumin (OVA)-sensitized mice. Glycyrrhizic acid inhibited OVA-induced increases in Raw and eosinophil count; interleukin (IL)-4, IL-5, IL-13 levels were recovered in bronchoalveolar lavage fluid; and increased IFN-γ level in bronchoalveolar lavage fluid. Separate studies demonstrated that liquiritigenin exerted anti-inflammatory effects, through inhibition of NF-kappaB activation in Raw264.7 macrophages, thereby decreasing production of iNOS and pro-inflammatory cytokines (Kim et al. 2008b). In rats, liquiritigenin treatment inhibited the formation of paw oedema induced by carrageenan. Administration of glycyrrhizin at 10 mg/kg i.p. 5 min prior to carrageenan exerted potent anti-inflammatory effects in mice (Menegazzi et al. 2008). Injection of carrageenan into the pleural cavity of mice elicited an acute inflammatory response and carrageenan-induced pleurisy which were attenuated by glycyrrhizin. It was found that prevention of the activation of NF-κB and STAT-3 by glycyrrhizin reduced the development of acute inflammation. 18β-Glycyrrhetinic acid ameliorated acute *Propionibacterium acnes*-induced liver injury in C57BL/6 mice through reduced macrophage inflammatory protein (MIP)-1α expression in Kupffer cells by downregulating MyD88 expression and inhibiting NF-κB activation (Xiao et al. 2010).

Isoliquiritigenin (ILG) was found to be a potent inhibitor of NLRP3 inflammasome activation (Honda et al. 2014). In-vivo, analyses revealed that ILG potently attenuated high-fat diet (HFD)-induced obesity, hypercholesterolemia and insulin resistance. Further ILG treatment improved HFD-induced macrovesicular steatosis in the liver. Additionally, ILG markedly inhibited diet-induced adipose tissue inflammation and IL-1β and caspase-1 production in white adipose tissue in ex-vivo culture. The results suggested ILG to be a potential drug target for treatment of NLRP3 inflammasome-associated inflammatory diseases.

Oral administration of Saiboku-To, a herbal medicine comprising *G. glabra*, *Magnolia officinalis* and *Suctellaria baicalensis* and their constituents medicarpin (*G. glabra*), baicalein, magnolol (*M. officinalis*) and baicalin (*S. baicalensis*) (100 mg/kg), inhibited picryl chloride-induced ear swelling significantly by 23.5, 40.1, 30.5, 23.6 and 20.9 %, respectively, though the effects were weaker than that of 5 mg/kg of prednisolone (52.9 %) (Taniguchi et al. 2000). Medicarpin derived from *Glycyrrhiza glabra*,magnololand8,9-dihydroxydihydromagnolol from *Magnolia officinalis*, baicalein, wogonin and oroxylin A from *Suctellaria baicalensis* inhibited concanavalin A-induced human lymphocyte blastogenesis in dose-dependent fashion

with IC_{50} values ranging from 3.0 to 7.7 μg/mL. The results suggested that flavonoids and lignans tested in the present study were implicated in anti-asthmatic effect of Saiboku-To through suppression of type IV allergic reaction.

Clinical Studies

In double-blind clinical trial of 30 patients with atopic dermatitis, treatment with licorice extract prepared as a 2 % licorice topical gel was more effective than 1 % in reducing the scores for erythema, oedema and itching over 2 weeks (Saeedi et al. 2003). The quantity of glycyrrhizinic acid was determined 20.3 % in the extract and 19.6 % in the topical preparation. Kolbe et al. (2006) conducted a prospective randomized vehicle-controlled clinical trial to assess the anti-irritative efficacy of cosmetic formulations containing licochalcone A in a post-shaving skin irritation model and on UV-induced erythema formation. Topical licochalcone A caused a highly significant reduction in erythema relative to the vehicle control in both the shave- and UV-induced erythema tests, demonstrating the anti-irritative properties of licochalcone A. further, licochalcone A was found to be a potent inhibitor of pro-inflammatory in-vitro responses, including N-formyl-MET-LEU-PHE (fMLP)- or zymosan-induced oxidative burst of granulocytes, UVB-induced PGE(2) release by keratinocytes, lipopolysaccharide (LPS)-induced PGE(2) release by adult dermal fibroblasts, fMLP-induced LTB(4) release by granulocytes, and LPS-induced IL-6/TNF-alpha secretion by monocyte-derived dendritic cells.

Cardiovascular Protective/Anti-atherosclerotic and Haemodynamic Activities

In-vitro studies showed that glycyrrhizin inhibited saponin-induced haemolysis in washed erythrocytes (Segal et al. 1977). The saponins inhibited included digitonin, tomatin, saponin A and cscin. Licorice extract and its major polyphenol glabridin protected low-density lipoprotein (LDL) against lipid peroxidation: in-vitro and in ex-vivo dietary supplementation studies in humans and in atherosclerotic apolipoprotein E-deficient mice (Fuhrman et al. 1997). These results could be related to the absorption and binding of glabridin to the LDL particle and subsequent protection of the LDL from oxidation by multiple modes as shown in humans and in E zero mice. They also found that glabridin or quercetin consumption by atherosclerotic apolipoprotein E-deficient mice resulted in a 53 and 54 % reduction in copper ion induced oxidation, respectively, and a 95 and 83 % reduction in 2,2′-azobis′2-amidino propane hydrochloride (AAPH) induced LDL oxidation, respectively (Belinky et al. 1998a). In the in-vitro oxidation of LDL induced by AAPH (5 mM), glabridin inhibited the formation of TBARS, lipid peroxides and cholesteryl linoleate hydroperoxide (CLOOH) at all the concentrations tested (5–60 μM), while in oxidation induced by copper ions (10 μM), glabridin exhibited a pro-oxidant activity at concentrations lower than 20 μM, and a clear antioxidant activity at concentrations greater than 20 μM. Glabridin (30 μM) inhibited the formation of cholest-5-ene-3,7-diol (7-hydroxycholesterol), cholest-5-ene-3-ol-7-one (7-ketocholesterol) and cholestan-5,6-epoxy-3-ol (5,6-epoxycholesterol) after 6 h of AAPH-induced LDL oxidation, by 55, 80 and 40 %, respectively, and after 6 h of copper ion induced LDL oxidation, by 73, 94 and 52 %, respectively. Glabridin also inhibited the consumption of β-carotene and lycopene by 38 and 52 %, respectively, after 0.5 h of LDL oxidation with AAPH, but failed to protect vitamin E. The in-vivo and in-vitro reduction of the susceptibility of LDL to oxidation obtained with glabridin may be related to the absorption or binding of glabridin to the LDL particle and subsequent protection of LDL from oxidation by inhibiting the formation of lipid peroxides and oxysterols, and by protecting LDL associated carotenoids. Results of further studies suggested that the antioxidant effect of glabridin on LDL oxidation appeared to reside mainly in the 2′ hydroxyl, and that the hydrophobic moiety of the isoflavan was essential to obtain this effect. It was also shown that the position of the hydroxyl group at B ring

significantly affected the inhibitory efficiency of the isoflavan derivatives on LDL oxidation, but did not influence their ability to donate an electron to DPPH or their peak potential values (Belinky et al. 1998b). Both mouse peritoneal macrophages (MPMs) and the J-774 A.1 macrophage-like cells accumulated up to 1.5 μg of glabridin/mg of cell protein after 2 h (Rosenblat et al. 1999). In parallel, in glabridin-enriched cells, macrophage-mediated oxidation of LDL was inhibited by up to 80 % in comparison with control cells. In glabridin-enriched macrophages, protein kinase C activity was reduced by approximately 70 % and in in-vivo studies, using the atherosclerotic apolipoprotein E-deficient (E0) mice, glabridin reduced capability to oxidize LDL by 80 % in comparison with placebo-treated mice. This latter phenomenon was associated with a reduction in the lesion oxysterols and a 50 % reduction in the aortic lesion size. It was concluded that glabridin accumulation in macrophages was associated with reduced cell-mediated oxidation of LDL and decreased activation of the NADPH oxidase system and these phenomena could be responsible for the attenuation of atherosclerosis in E0 mice, induced by glabridin.

Animal studies demonstrated that glycyrrhizin protected rat heart against myocardial ischemia-reperfusion-induced injury via directly inhibiting extracellular HMGB1 cytokine activity and blocking the phospho-JNK/Bax pathway (Zhai et al. 2012). Intravenous administration of glycyrrhizin (10 mg/kg) significantly reduced the infarct size, but did not change the hemodynamic parameters at different time points during reperfusion. Glycyrrhizin significantly decreased the levels of serum HMGB1, TNF-α and IL-6. Glycyrrhizin changed the distribution of Bax and cytochrome c expression between the mitochondrial and cytosolic fractions in the heart tissue, resulting in inhibition of myocardial apoptosis. Studies showed that *Glycyrrhiza glabra* protected rats from myocardial ischemia–reperfusion injury by improving hemodynamic, biochemical, histopathological and ventricular function (Ojha et al. 2013). Pretreatment with *G. glabra* significantly prevented the depletion of the antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and myocyte injury marker enzymes: creatine phosphokinase-MB isoenzyme and lactate dehydrogenase. It also prevented depletion of glutathione (GSH) and inhibited lipid peroxidation in the heart. In addition to improving biochemical indices of myocardial function, *G. glabr*a also significantly reinstated mean arterial pressure, heart rate, (±) LVdP/dtmax and attenuated abrupt rise in left ventricular end diastolic pressure. Histopathological preservation evidenced by reduced infiltration of cells and myonecrosis depicted the myocardial salvaging effect of *G. glabra*. Basso et al. (1994) reported a case of a 63-year-old patient with severe postural hypotension caused by autonomic diabetic neuropathy who recovered after licorice (equivalent to 3 g/day of glycyrrhizic acid) treatment for 7 days. The results suggested that licorice could be used for the therapy of postural hypotension attributable to autonomic diabetic neuropathy.

Haemodynamic changes induced by liquorice consumption in 20 subjects versus 30 controls with average blood pressures of 120/68 and 116/64 mmHg, respectively, were investigated by Leskinen et al. (2014). Two weeks of daily liquorice consumption increased extracellular volume, amplified pressure wave reflection from the periphery and elevated central systolic and diastolic blood pressure. Heart rate, systemic vascular resistance, cardiac output and pulse wave velocity did not differ between the groups. Licorice active constituent, glycyrrhetinic acid (GA) effectively restored vascular contractility in the model of lipopolysaccharide (LPS)-treated rat aorta (Muller et al. 2014). GA was as effective as the NO synthase inhibitor N(G)-nitroarginine methylester. The results suggested GA to be an interesting alternative to NO synthase inhibitors in sepsis-associated vascular dysfunction.

The polyphenols glabridin (derived from licorice), rosmarinic acid or carnosic acid (derived from rosemary), as well as garlic (which contains a mixture of natural antioxidants) inhibited LDL oxidation in a dose-dependent manner (Fuhrman et al. 2000). In-vivo, a meal of lycopene in com-

bination with other natural antioxidants reduced susceptibility to oxidation by 21 % in four healthy subjects. It was concluded that lycopene acted synergistically, as an effective antioxidant against LDL oxidation, with several natural antioxidants such as vitamin E, the flavonoid glabridin, the phenolics rosmarinic acid and carnosic acid, and garlic. The results suggested a superior anti-atherogenic characteristic to a combination of different natural antioxidants over that of an individual one.

Anticoagulation/Fibrinolytic Activities

Aqueous licorice leaf extracts exhibited no anticoagulation activities at 2000 µg/ml concentration but addition of sulphate group to the aqueous extracts enhanced the anticoagulation activity (Helmy et al. 2013). The highest activity was found by using both the sulphated alkaline and neutral extract of *G. glabra* var. *glabra* at a concentration of 400 µg/ml. Most of the aqueous extracts and their sulphated extracts of licorice leaves (*G. glabra* var. *glabra* and var. *glandulifera*) exhibited fibrinolytic activities higher than standard (Hemoclar) preparation at concentration of 2000 µg/ml.

Anti-ulcer/Gastroprotective Activity

In-Vitro Studies

Among the chemical constituents of licorice, glabridin and glabrene, licochalcone A, licoricidin and licoisoflavone B exhibited inhibitory activity against the growth of *Helicobacter pylori* in-vitro (Fukai et al. 2002a). These flavonoids also showed anti-*H. pylori* activity against a clarithromycin (CLAR) and amoxicillin (AMOX)-resistant strains. Glycyrrhetinic acid was as the most potent compound ($MIC_{50}=50$ mg/L, $MIC_{90}=100$ mg/L) inhibiting 79.3 % *Helicobacter pylori* strains at 50 mg/L or <. Krausse et al. (2004) exhibited rapid, concentration and strain-dependent bactericidal activity. Clarithromycin-resistant strains of *H. pylori* were susceptible at 12.5 and 25 mg/L, and metronidazole-resistant strains at 25–50 and at 200 mg/L. The MIC distribution (mg/L) of the lipophilic acetylated derivative of glycyrrhetinic acid monoglucuronide was ≤6.25 (29.2 %), 50 (4.2 %), 100–200 (12.5 %) and ≥400 (54.1 %). Extractum liquiritiae and glycyrrhizic acid were less active (MICs >400 mg/L). Studies by Malek Jafarian and Ghazvini (2007) showed that therapeutically administered concentrations of licorice extract (100–400 mg/ml) could have growth-inhibiting effect on *H. pylori* in-vitro. Cheel et al. (2013) found that the chemical profile of licorice quantitatively varied at different harvest times and these fluctuations determined changes in its bioactivities including gastroprotective activity. Licorice samples in May gave the best gastroprotective effect. Liquiritin and glycyrrhizin, the major constituents in the February and May licorice extract, appeared to contribute to the superoxide radical scavenging and gastroprotective effects. GutGard®, a flavonoid rich extract of *Glycyrrhiza glabra*, exhibited anti-*Helicobacter pylori* activity in both agar dilution and microbroth dilution methods possibly by inhibiting protein synthesis, DNA gyrase and dihydrofolate reductase (Asha et al. 2013). Glabridin, the major flavonoid present in GutGard® exhibited superior activity against *Helicobacter pylori* while glycyrrhizin did not show activity even at 250 µg/ml concentration.

Animal Studies

Gastric mucosal damage induced by giving 60 mg aspirin orally to rats was reduced by simultaneous administration of 100–500 mg deglycyrrhizinated liquorice (Rees et al. 1979). Studies demonstrated that deglycyrrhinized liquorice (DGL) exerted a protective effect against aspirin and aspirin plus bile acid-induced gastric mucosal damage, and aspirin absorption in rats (Russell et al. 1984). In the first study, lesion scores were increased from 6(3;10) with aspirin alone to 12(5;16) by taurodeoxycholic acid (TDC) and reduced by DGL to 1(0;3.5) for aspirin alone and 3.5(0;6) for aspirin plus TDC. In the second study a slight reduction of aspirin absorption was found only at 20 min with

a median level of 0.9 mmol/l for the DGL treated rats and 1.2 mmol/l for the aspirin alone group. Although DGL diminished aspirin (128 mg/kg)-induced gastric mucosal damage from 17(12;25) to 8(3;14) when the two were given together it did not do so significantly when DGL was given before aspirin -15(20;22). Intraperitoneal deglycyrrhinized licorice reduced lesion scores from aspirin (128 mg/kg) from 14(11;24) to 7(1;19), thus indicating a systemic as well as a local effect of deglycyrrhinized licorice. Bennett et al. (1985) concluded that in rats, carbenoxolone and deglycyrrhized liquorice may exert their anti-ulcer effect by a non-prostaglandin mechanism. This contrasted with the mechanism occurring in man with carbenoxolone. Aspirin coated with liquorice was found to reduce the number and size of gastric ulcers, reducing the ulcer index from 1.5 to 0.5 and the incidence from 96 to 46 % in rats (Dehpour et al. 1994). Coating with derivatives including the deglycyrrhized form, a high glycyrrhized form, carbenoxolone and enoxolone was less effective (ulcer index, 0.70–0.94; incidence 62–76 %). GutGard dose dependently decreased gastric content, total acidity, ulcer index and increased the pH of gastric fluid in pylorus ligated ulcer rats (Mukherjee et al. 2010). In cold-restraint stress and indomethacin induced ulcer rats all the doses of GutGard decreased the ulcer index and increased the pH of gastric fluid. GutGard exhibited potent antioxidant activity with high hydrophilic and lipophilic oxygen radical absorbance capacity (ORAC) value.

Oral administration of *Glycyrrhiza glabra* root extract caused a significant reduction in the length of gastric ulcer induced by ethanol in rats (Shalaby et al. 2004). The curative ratios from gastric ulceration were 40.0, 65.9 and 67.3 % in groups of rats given the extract at 200, 400 and 800 mg/kg, respectively. Licorice root extract lipids markedly reduced the degree of damage stomach ulceration in mice caused by indomethacin and voltaren and was more efficacious than the well-known reference drugs – licurazide and liquiriton (Denisova et al. 2007). Special licorice extracts (containing low glycyrrhizin and high licochalcone A) afforded significant attenuation of either *Helicobacter pylori*-induced gastritis or tumorigenesis in interleukin-deficient mice through its potent antioxidative, anti-inflammatory and antimutagenic actions (Park et al. 2014a, b).

Clinical Studies

The double-blind, cross-over design study involving patients with chronic ulcer disease failed to demonstrate any healing effect of deglycyrrhizinated liquorice extract (Caved-S) 760 mg three times daily for 4 weeks on gastric ulcer (Engqvist et al. 1973). In another clinical trial of 96 patients with gastric ulcer, after 4 weeks no differences were found between placebo or treatment with deglycyrrhizinated liquorice whether assessed by gastroscopy or radiology, or in the percentage reduction in ulcer area, or in clinical improvement (Bardhan et al. 1978). In a clinical trial, retrospective endoscopic examination in 32 patients of chronic duodenal ulceration treated with deglycyrrhizinated liquorice tablets showed that healing of the ulceration had occurred and in the majority the mucosa appeared normal (Larkworthy and Holgate 1975). The authors asserted that for optimum effect the preparation in adequate dosage should be well chewed and swallowed on an empty stomach in the ambulant patient. Human faecal blood loss from gastric mucosal damage induced by 975 mg aspirin orally three times a day was less when 350 mg deglycyrrhizinated liquorice was given with each dose of aspirin (Rees et al. 1979).

Aqueous extract (1 mg/mL) of *Glycyrrhiza glabra* significantly inhibited the adhesion of *Helicobacter pylori* to human stomach tissue (Wittschier et al. 2009). This effect was related to the polysaccharides isolated from the extract, with one purified acidic fraction (0.25 SPB) as main active polymer. Purified polysaccharides did not exhibit direct cytotoxic effects against *Helicobacter pylori* and did not influence hemagglutination. In a double-blind clinical trial study of 60 patients with peptic ulcer disease, 4 weeks treatment with licorice was found to be as effective as bismuth in *Helicobacter pylor*i eradication (Momeni et al. 2014). They suggested that in patients whom bismuth was contraindicated, licorice could be used safely instead. In a double-

blind study of 40 patients with peptic ulcer, licorice was found to be a good a replacement for bismuth sub-nitrate in treatment of peptic ulcer in a quadruple therapy comprising amoxicillin (500 mg, 3 times/day after diet for 15 days), metronidazole (250 mg, 4 times/day after diet for 15 days), omeprazole (20 mg, 2 times/day ½ h before the diet for 30 days) and licorice (250 mg, 3 times/day ½ h before the diet for 30 days). In a randomized, double-blind clinical trial, the use of an over-the-counter licorice medicated intraoral adhesive patch for treatment of recurrent aphthous ulcers significantly reduced ulcer size and pre-stimulus pain in treated subjects compared with placebo (Martin et al. 2008). In a double-blind, randomized prospective trial of 60 patients, Ghalayani et al. (2014) found both triamcinolone (30 patients) and licorice (30 patients) mucoadhesive films were effective in the management of oral mucositis during head and neck cancer radiotherapy. Furthermore, comparison of the pain scores between two groups demonstrated no meaningful difference, although an overall trend to reduced oral discomfort was seen in the licorice group.

Anti-dyspepsia Activity

In a randomized, double-blind, placebo-controlled study of patients with functional dyspepsia, administration of GutGard, an extract of *Glycyrrhiza glabra*, exerted a significant decrease in total symptom scores on day 15 and day 30, respectively, compared to placebo (Raveendra et al. 2012). The GutGard group also showed a significant decrease in the Nepean dyspepsia index on day 15 and 30, respectively, when compared to placebo. GutGard was generally found to be safe and well tolerated by all patients.

Immunomodulatory Activity

Glycyrrhizan GA, an acidic polysaccharide isolated from the stolon of *Glycyrrhiza glabra* var. *glandulifera* showed remarkable reticuloendothelial system-potentiating activity in a carbon clearance test (Shimizu et al. 1991). Glycyrrhizan GA, a representative polysaccharide with remarkable phagocytosis-enhancing activity was isolated from the stolon of *Glycyrrhiza glabra* var. *glandulifera* (Takada et al. 1992). Smith degradation product obtained from glycyrrhizan GA showed markedly higher values of anti-complementary and alkaline phosphatase-inducing activities than that from glycyrrhizin UA, the main polysaccharide from *G. uralensis* root. Kroes et al. (1997) found β-glycyrrhetinic acid to a potent inhibitor of the classical complement pathway ($IC_{50} = 35$ μM), whereas non-inhibitory activity was observed towards the alternative pathway ($IC_{50} > 2500$ μM). The anti-complementary activity of β-glycyrrhetinic acid was dependent on its conformation, since the α-form was not active. Detailed mechanistic studies revealed that β-glycyrrhetinic acid acted at the level of complement component C2. Crude polysaccharide fraction from *G. glabra* shoot was found to have immunomodulatory action; the fraction induced nitric oxide production by murine peritoneal macrophages in-vitro (Nose et al. 1998).

Studies demonstrated that glycyrrhizin could promote phenotypic and functional maturation of murine dendritic cells and this adjuvant-like activity may have potential therapeutic value (Hua et al. 2012). Also glycyrrhizin increased the production of interleukins IL-12, IL-10 and decreased the production of tumour necrosis factor alpha (TNF-α). Glycyrrhizin was able to upregulate the expression of CD40, CD86 and MHC-II maturation markers on dendritic cells (DCs) (Bordbar et al. 2012). DCs treated with glycyrrhizin enhanced proliferation of allogeneic T cells along with the production of IFN-γ and IL-10 cytokines and reduced IL-4 production. The data indicated that glycyrrhizin had the capacity to upregulate allostimulatory activity of professional antigen presenting DCs and conduct immune responses toward a T helper 1 response.

Granulocytes and NK cells were markedly activated by licorice infusion, whereas liquiritin and glycyrrhizin were inactive (Cheel et al.

2010). The results suggested that licorice infusion could be used as a potential non-specific immune stimulator. Leukocyte count and phagocytic index (carbon clearance) in sheep red blood cells was increased significantly with the treatment of aqueous liquorice extract (1.5 g/kg) compared to control (Mazumder et al. 2012). Zinc (45 mg/kg) in combination with licorice extract (0.75 g/kg) showed highly significant increase of leukocyte count and phagocytic index compared to control. The combination of zinc (45 mg/kg) and licorice (0.750 g/kg) showed significant increase in haemagglutinin titre and antibody secreting cells of mouse spleen value compared to vehicle control. In systemic anaphylaxis reaction test, results indicate a positive effect on anaphylaxis with the treatment of licorice in both doses and in combination with zinc. The results indicated that *G. glabra* in combination with zinc had shown potentiation of immunomodulatory activity.

In the in-vitro phagocytosis test with human granulocytes, Revitonil® (a phytopharmaceutical containing an extract of *Echinacea purpurea* and *Glycyrrhiza glabra* root) showed a 44–53 % immunostimulating effect at a concentration of 100 µg/ml (Wagner and Jurcic 2002). Whereas in the chemiluminescence test at a concentration of 1.25 µg/ml, Revitonil® exhibited a moderate enhancing effect only, a remarkable stimulating activity (30–50 %) was observed in the T-lymphocyte CD69 bioassay at a concentration of 100 µg–1 µg/ml. The highest immunological efficacy could be assigned to Revitonil® as revealed by the in-vivo carbon-clearance model in mice. With RCt/RCc-values of 2.0, Revitonil® exhibited a very high carbon elimination rate at oral administration. Because the *Echinacea* and *Glycyrrhiza* mono-extracts alone showed lower RCt/RCc-values (1.3–1.7) than Revitonil®, a potentiating synergistic effect of the extract mixture in Revitonil® could be postulated. Vikhe et al. (2013) reported that neutrophils when treated with *G. glabra* and *Tinospora cordifolia* plant extracts showed increase in phagocytic activity.

Adaptogenic Activity

Two anti-stress compounds were isolated from licorice 9,12,13-trihydroxy-(10*E*)-octadecenoic and 9,12,13-trihydroxy-10,11-epoxy-octadecanoic acid (Panossian et al. 1988). Rabbits were treated (orally) with a preparation of *Glycyrrhiza glabra* for 30 days and concurrently were exposed to vibration stress (30 days) (Oganesyan 2002). The licorice preparation reduced catalase activity in the peripheral blood and increased animal resistance to vibration stress. Active substances of licorice root accelerated metabolism in rabbit peripheral blood red cells of the bone marrow erythroid stem, enhanced compensatory reserve of the organism and increased animal's resistance to stress (Adamyan et al. 2005). Minasian et al. (2007) found that biological active substances of licorice accelerated metabolism processes of rabbit bone marrow stem cells, enlarged the animal compensatory abilities, thus providing its resistance to vibration.

Incorporation of *Glycyrrhiza glabra* powder to the feed media of *Drosophila melanogaster* was found to reduce stress in *D. melanogaster* induced by methotrexate at different concentration (Sowmya and Sathish Kumar 2010). Stress-related enzymes like catalase (CAT) and super oxide dismutase (SOD) were reduced by licorice powder. The exposure of mice to chronic fatigue stress for 15 days demonstrated an increased immobility time, increased anxiety, impaired memory, reduction in muscle co-ordination, reduced activity and increased pain perception (Trivedi and Sharma 2011). These altered behavioural parameters were attenuated significantly by the treatment of *Glycyrrhiza glabra* (100 and 200 mg/kg p.o.) comparable to fluoxetine (10 mg/kg, i.p.).

Estrogenic Activity

Glycyrrhiza glabra was confirmed to have a high estrogenic activity as proven by the effects of its alcoholic extract (25 mg dose) on mouse uterine response and vaginal opening which was compa-

rable to estradiol monobenzoate (Shihata and Elghamry 1963a). A higher dose of 50 mg daily for 3 days proved to possess a lower estrogenic activity when compared with estradiol monobenzoate. Upon uterine motility, licorice extract manifested an inhibitory influence upon the spontaneous movement of the organ at di-oestrus, oestrus and pregnancy. In in-vivo studies in dogs, *G. glabra* extract caused relaxation of the uterine musculature of both pregnant and non-pregnant bitches (Shihata and Elghamry 1963b).

Beta-sitosterol was isolated as an estrogenic principle from Egyptian *G. glabra* (Zayed et al. 1964). Studies demonstrated glabridin to be a phytoestrogen, binding to the human estrogen receptor and stimulating creatine kinase activity in rat uterus, epiphyseal cartilage, diaphyseal bone, aorta and left ventricle of the heart (Tamir et al. 2000). The stimulatory effects of 2.5–25 μ g/animal glabridin were similar to those of 5 μg/animal estradiol. Glabridin was found to be three to four times more active than 2′-*O*-methylglabridin and 4′-*O*-methylglabridin, and both derivatives were more active than 2′,4′-*O*-methylglabridin. The effect of increasing concentrations of glabridin on the growth of breast tumour cells was biphasic. Glabridin showed an estrogen receptor-dependent, growth-promoting effect at low concentrations (10 nm–10 μm) and estrogen receptor-independent antiproliferative activity at concentrations of >15 μm. Glabridin and its derivatives exhibited varying degrees of estrogen receptor agonism in different tests and demonstrated growth-inhibitory actions on breast cancer cells.

Animals fed with licorice extract, compared with estradiol and glabridin, showed an increase in creatine kinase (CK) activity, a known marker for estrogen responsive genes, which was higher than expected from the levels of glabridin in the extract indicating the possible presence of other components that may contribute to this strong estrogen agonist activity (Tamir et al. 2001). Results indicated that glabrene and isoliquiritigenin, (2′,4′,4-3 hydroxy chalcone) (ILC) in the licorice extract could bind to the human estrogen receptor with higher affinity (IC_{50}, 1 and 0.5 μM) than glabridin (IC_{50}, 5 μM). The stimulatory effects of glabrene in-vivo were tissue specific and similar to those of estradiol. The effect of increasing concentrations of glabrene and ILC on the growth of breast tumour cell were biphasic. Both showed an estrogen receptor-dependent growth-promoting effect at low concentrations (10 nM–10 μM), and estrogen receptor-independent antiproliferative activity at concentrations >15 μM.

Licorice root constituents: glabridin and glabrene exhibited estrogen-like activity in-vitro and in-vivo (Somjen et al. 2004b). Similar to estradiol-17β (E2), glabridin stimulated DNA synthesis in human endothelial cells (ECV-304; E304) and had a bi-phasic effect on proliferation of human vascular smooth muscle cells. In animal studies, in intact females or after ovariectomy, glabridin similar to E2 stimulated the specific activity of creatine kinase in aorta and in left ventricle of the heart. Raloxifene inhibited glabridin as well as E2 activities. Glabrene, on the other hand, had only the stimulatory effect on DNA synthesis in vascular cells, with no inhibition by raloxifene, suggesting a different mechanism of action. The authors suggested the use of glabrene with or without E2 as a new agent for modulation of vascular injury and atherogenesis for the prevention of cardiovascular diseases in post-menopausal women. In pre-menopausal human bone cells, the response to estradiol-17β and glabridin (at higher concentration) was higher than in post-menopausal cells; whereas, glabrene (at higher concentration) was more effective in post-menopausal cells (Somjen et al. 2004a). At both ages, the response to estradiol-17β and glabridin was enhanced by pretreatment with the less-calcemic Vitamin D analogue CB 1093 (CB) and the demonstrably non-calcemic analogue JK 1624 F(2)-2 (JKF). The response to glabrene was reduced by this pretreatment. Both glabridin and glabrene stimulated creatine kinase specific activity in diaphyseal bone and epiphyseal cartilage of prepubertal female rats. Daily feeding (3–14 days) of prepubertal female rats with glabridin, estradiol-17β or their combination also stimulated creatine kinase specific activity. Glabridin, similarly to estradiol-17β, also stimulated creatine kinase specific activity in

ovariectomized female rats. Raloxifene, in combination with glabridin or estradiol-17β, demonstrated the phenomenon of mutual annihilation of stimulation of creatine kinase specific activity in both epiphysis and diaphysis. Glabrene activity was not inhibited by raloxifene. They found that glabridin showed greater similarity to estradiol-17β and thus greater potential, with or without Vitamin D, to modulate bone disorders in postmenopausal women. In a study, nine healthy women 22–26 years old, in the luteal phase of the cycle were given 3.5 g of a commercial preparation of licorice (containing 7.6 % w.w. of glycyrrhizic acid) daily for two cycles (Armanini et al. 2004). It was found that licorice could reduce total serum testosterone probably due to the block of 17-hydroxysteroid dehydrogenase and 17–20 lyase. It was concluded that licorice could be considered an adjuvant therapy of hirsutism and polycystic ovary syndrome.

Studies by Dong et al. (2007) found that activation of rapid signalling pathways, including Erk1/2 and Akt, and the subsequent transcriptional regulation were involved in the proliferation of MCF-7 cells induced by the extract of *G. glabra* root. The extract had similar activity to that induced by 17β-estradiol (E_2), although glycyrrhizin did not show such an activity. Furthermore, the extract had estrogenic activity and a distinguishable profile of gene expression, suggesting the presence of potentially useful components other than glycyrrhizin in *G. glabra* root for hormone and anti-cancer therapies. In in-vivo studies, administration of alcoholic *G. glabra* extract at doses of 150 and 300 mg/kg exerted significant reduction of prostate weight, total serum testosterone and ventral prostate epithelium/stroma ratio in immature male rats (Zamansoltani et al. 2009). Increase in testosterone metabolism, downregulation of androgen receptors or activation of estrogen receptors could be the mechanisms involved. Most fraction of the ethyl acetate *G. glabra* root extract showed some estrogenicity on both human estrogen receptors (ER) α and β (Simons et al. 2011). A compound was considered a phytoestrogen when it activates the ER at concentrations $\leq 10^4$ times than that of estradiol (E_2). The main flavonoids in the fractions were glabrene, glabrone, glabridin, glabrol 4′-mehtyl-glabridin, 3′-hydroxy-4-*O*-methyl-glabridin, hispaglabridin A and hispaglabridin B. Fractions F16-20, rich in glabrene, showed a predominant estrogenic activity on the ERα. Several fractions displayed higher responses than the maximum response obtained with the reference compound, the natural hormone 17β-estradiol (E_2). In addition to glabrene, the estrogenic activity of licorice roots extract had been ascribed to the presence of glabridin and its derivatives. Glabridin did not exert agonistic activity to both ER subtypes. Prenylation of isoflavonoids had been suggested to induce antagonism towards the ERα. The estrogenic activities of all fractions, including this so-called superinduction, were clearly ER-mediated, as the estrogenic response was inhibited by 20–60 % known ER antagonists, and no activity was found in yeast cells that did not express the ERα or ERβ subtype. Prolonged exposure of the yeast to the estrogenic fractions that showed superinduction did not, contrary to E_2, result in a decrease of the fluorescent response. Glabridin displayed ERα-selective antagonism, similar to the ERα-selective antagonist RU 58668. Whereas glabridin was able to reduce the estrogenic response of E_2 by approximately 80 % at 6×10^{-6} M, glabrene-rich fractions only exhibited agonistic responses, preferentially on ERα.

Pulmonoprotective Activity

In-vivo studies showed that ovalbumin-induced asthmatic mice treated with glycyrrhizin ameliorated all established chronic histopathologic lung parameters (Hocaoglu et al. 2011). When the glycyrrhizin and dexamethasone groups were compared, there was no statistically significant difference between the two groups in the histopathologic parameters, including thickness of basement membrane, subepithelial smooth muscle, and epithelium and number of mast and goblet cells. Oral administration of glycyrrhizic acid (GA) (50 and 100 mg/kg b.wt.) significantly protected against benzo(a)pyrene-induced debilities in lungs of Wistar rats against B(a)P induced

debilities in lungs of Wistar rats (Qamar et al. 2012). GA protected lung epithelium by suppression of caspases activities in lung tissue and reduction of total protein, total cells, elastase activity, lactate dehydrogenase, alkaline phosphatase activities along with fortification of phospholipids in bronchoalveolar lavage fluid. Studies by Dai et al. (2013) found that liquiritigenin could protect human lung cells (A549) from *Staphylococcus aureus* α-haemolysin-mediated injury. Low concentrations of liquiritigenin remarkably decreased *Staphylococcus aureus* α-haemolysin production in a dose-dependent manner.

Antiplatelet/Antithrombotic Activity

Butanolic extract of *G. glabra* inhibited human platelet aggregation induced by adrenaline with an IC_{50} of 1.66 mg/mL (Sajid et al. 1991). The ether soluble fraction of the crude licorice root produced a 27.3 % inhibition of lysoPAF (platelet-activating factor) acetyltransferase in-vitro at a concentration 10 μg/ml (Nagumo et al. 1999). From this fraction, licoricidin, 1-methoxyphaseollin, 6,8-diprenylgenistein and 1-methoxyphaseollidin were isolated as active components, with IC_{50} values of 7.7, 57, 19 and 48 μM, respectively. Glycyrrhizin, isolated from *G. glabra*, was identified as a new thrombin inhibitor: (a) It prolonged plasma recalcification and thrombin and fibrinogen clotting times, and (b) it inhibited thrombin-induced, but not collagen-, PAF- or convulxin-induced platelet aggregation; but glycyrrhizin did not block thrombin's amidolytic activity upon S-2238 (Francischetti et al. 1997). Furthermore, the fluorescence emission intensity of dansyl-thrombin was increased upon glycyrrhizin binding. Moreover, glycyrrhizin displaced hirudin as an inhibitor of thrombin-catalysed hydrolysis of S-2238. The data indicated that glycyrrhizin was a selective inhibitor of thrombin and that it was able to exert its anti-thrombin action by interacting with the enzyme's anion binding exosite 1.

Intravenous administration of rats with glycyrrhizin caused a dose-dependent reduction in thrombus size on a venous thrombosis model that combines stasis and hypercoagulability (Mendes-Silva et al. 2003). It was observed that glycyrrhizin doses of 180 mg/kg body weight produced 93 % decrease on thrombus weight. Glycyrrhizin was also able to prevent thrombosis using an arteriovenous shunt model. Glycyrrhizin doses of 180 and 360 mg/kg decreased the thrombus weight by 35 and 90 %, respectively. In addition, glycyrrhizin doses above 90 mg/kg caused significant haemorrhagic effect. In contrast with heparin, glycyrrhizin did not potentiate the inhibitory activity of anti-thrombin III or heparin cofactor II towards thrombin.

GU-7, a 3-arylcoumarin derivative, from glycyrrhizae radix, inhibited platelet aggregation, phosphorylation of 40 K and 20 K dalton proteins, inositol 1,4,5-trisphosphate production, intraplatelet calcium increase and phosphodiesterase activity in-vitro (Tawata et al. 1990). The data indicated that GU-7 inhibited platelet aggregation by increasing intraplatelet cAMP concentration. Isoliquiritigenin, a new aldose reductase inhibitor purified from licorice (Glycyrrhizae radix), inhibited platelet aggregation (Tawata et al. 1992). It significantly inhibited the phosphorylation of 40,000- and 20,000-Da proteins, and inhibited the formation of 12 (S)-hydroxy-5,8,10-heptadecatrienoic acid, 12-hydroxyeicosatetraenoic acid and thromboxane B2. The inhibitory effect of isoliquiritigenin on platelet aggregation in-vitro was comparable to that of aspirin. It was suggested that isoliquiritigenin elicited an anti-platelet action by inhibiting not only cyclooxygenase but also lipoxygenase or peroxidase activity in platelets. Since the hyperaggregability of platelets had been implicated in the pathogenesis of diabetic complications, isoliquiritigenin may offer a unique benefit as an aldose reductase inhibitor. Isoliquiritigenin, glabridin, licoarylcoumarin, glycycoumarin, glycyrol, licoricone and licoricidin were identified as strong inhibitors of adenosine 3′, 5′-cyclic monophosphate (cAMP) phosphodiesterase in waste materials which were obtained during the industrial extraction of glycyrrhizin from licorice roots (Kusano et al. 1991). Isoliquiritigenin-4′-*O*-apioglucoside and liquiritigenin were weakly

inhibitory while liquiritin, glycyrin and isoglycerol were not inhibitory. (cAMP) phosphodiesterase inhibitors had also been reported to be cardiotonic.

Xanthine Oxidase Inhibitory/ Antigout Activity

Of 10 licorice phenolics, sinkiang licorice (licochalcone and licochalcone A) and si pei (licorice glycyrrhisoflavone, glycyrrhiza flavanone, 3-arylcoumarin, licopyranocoumarin, licoarylcoumarin, glisoflavone, kaempferol 3-*O*-methyl ether, 2-arylbenzofuran, licocoumarone and glycycoumarin), 4 phenolics licochalcone B, glycyrrhisoflavone, licocoumarone and licochalcone A showed 50 % inhibition on xanthine oxidase at the concentration of $1.3–5.6 \times 10^{-5}$ M (Hatano et al. 1989). However, all were weaker than allopurinol a remedy for gout. Among 12 licorice constituents examined, six compounds namely glicoricone, licofuranone, genistein, licopyranocoumarin, licocoumarone and glycyrrhisoflavone, inhibited the enzyme with the IC_{50} (concentration required for 50 % inhibition of the enzyme activity) values of 6.0×10^{-5}–1.4×10^{-4} M. Glycyrrhizin also inhibited monoamine oxidase with the IC_{50} value of 1.6×10^{-4} M (Hatano et al. 1991b).

Antiangiogenic Activity

Using various experimental models of ocular neovascularization, namely (1) silver nitrate cauterization-induced corneal neovascularization in BALB/c mice, followed by topical isoliquiritigenin (ISL) (0.2–50 μM) and CD31 immunofluorescence of corneal blood vessels; (2) argon laser photocoagulation-induced choroidal neovascularization in C57BL/6 mice, followed by intravitreal ISL (10–200 μM) and fundus fluorescein angiography and immunofluorescence with *Griffonia simplicifolia* isolectin-B4 (GSA I-B4); and (3) oxygen-induced retinopathy in C57BL/6 J mice pups, followed by intravitreal ISL (1–100 μM) and GSA I-B4 immunofluorescence mice. Jhanji et al. (2011) demonstrated that ISL from licorice extract had an antiangiogenic effect. The authors suggested that ISL may be a potential antiangiogenic agent in the development of therapy for neovascularization diseases.

Anti-tyrosinase Activity

Studies showed that glabridin, from licorice extract, at concentrations of 0.1–1.0 μg/ml, inhibited tyrosinase activity of cultured B16 murine melanoma cells and guinea pig skins and had no detectable effect on their DNA synthesis (Yokota et al. 1998). It was also shown that UVB-induced pigmentation and erythema in the skins of guinea pigs were inhibited by topical applications of 0.5 % glabridin. Anti-inflammatory effects of glabridin in-vitro were also shown by its inhibition of superoxide anion productions and cyclooxygenase activities. By replacing each of the hydroxyl groups of glabridin with others, it was revealed that the inhibitory effect of 2′-*O*-ethyl glabridin was significantly stronger than that of 4′-*O*-ethyl-glabridin on melanin synthesis in cultured B16 cells at a concentration of 1.0 mg/ml. The 50 % tyrosinase-inhibitory concentration of the *Glycyrrhiza glabra* methanol extract was 21.2 μg/ml (Khanom et al. 2000). A glabridin derivative, 3″,4″-dihydroglabridin exhibited higher tyrosinase inhibitory activity than glabridin (IC_{50} value = 11.40 μM), which was probably due to the 4-substituted resorcinol skeleton and the lacking of double bond between carbon atom 3″ and 4″ on its structure giving more conformational flexibility to interact with the enzyme more effectively (Jirawattanapong et al. 2009). In addition, various acylated derivatives were synthesized as glabridin prodrugs. The chemical and enzymatic hydrolysis of prodrugs revealed that the diacetate ester was rapidly hydrolysed by porcine liver esterase with the half-life of 2.36 min, while that of the dihexanoate was 14.8 h.

The cellular levels of tyrosinase mRNA, protein, enzyme activities and melanin contents in B16 murine melanoma cells were increased by glycyrrhizin in a dose-dependent manner (Jung et al. 2001). Expression of tyrosinase-related pro-

tein-2 (TRP-2) mRNA was also increased by glycyrrhizin, however, no significant change was observed on TRP-1. Glycyrrhetinic acid showed no effect on melanogenesis at the equivalent non-toxic concentrations, indicating that glycoside structure is important in the stimulatory effect of glycyrrhizin on melanogenesis. Liquiritin was the main compound in licorice root extract and exhibited strong inhibitory effect on mushroom tyrosinase (Dong et al. 2014). Pinocembrin, the main compound in the leaf extract showed good antioxidant activity and nitrite scavenging capacity, but moderate inhibitory effect on mushroom tyrosinase. Both compounds exhibited significant protection effect on H_2O_2-injured PC12 cells at a low concentration.

Wound/Burn Healing Activity

Licorice root extract (LRE) lipid fraction effectively stimulated the reparative regeneration of damaged burnt skin caused by hydrochloric acid (I), skin layer removal (II), thermal burns (III) and in guinea pigs and rats (Denisova et al. 2007). An analysis of the results of these tests showed that the healing of model wounds I and II treated with LRE lipids was comparable to the action of rosehip oil and more effective than the natural healing in the control. In series burn model III RE lipid fraction also reliably reduced the area of burned skin and this effect was more pronounced than the action of rosehip oil. Intraperitoneal administration of glycyrrhizin (Gly) (60 mg/kg) significantly reduced the levels of elevated serum TNF-α and IL-1β caused by severe skin scald burn injury in rats (Shen et al. 2015). Gly treatment reduced these biochemical indices accompanied by lower level of HMGB1 protein and mRNA expression.

Antitussive Activity

Glycyrrhetinic acid and its derivatives were active in antitussive activity in experiments using chemical stimulation in the un-anaesthetized guinea-pig and electrical stimulation in the lightly anaesthetized cat indicating a central antitussive effect (Anderson and Smith 1961). Several derivatives had approximately the same potency as codeine when given subcutaneously to guinea-pigs; one of these, dicholine glycyrrhetinic acid hydrogen succinate, exhibited the same degree of activity after oral administration. The water-extracted arabinogalactan protein enriched fraction of *Glycyrrhiza glabra*, when administered orally in a dose of 50 mg/kg body weight decreased the number of citric acid induced cough efforts in guinea pigs more effectively than codeine (Saha et al. 2011). It did not induce significant change in the values of specific airway resistance or provoked any observable adverse effects.

Renoprotective Activity

Malekinejad et al. (2011a) found that liquorice plant extract could reduce ochratoxin A-induced nephrotoxicity in rats. Liquorice plant extract like melatonin could alleviate an ochratoxin A-reduced antioxidant power of serum and lower the toxin-induced malondialdehyde generation. They also found that *Glycyrrhiza glabra* **e**xtract and melatonin could protect against ochratoxin A-induced damages on testes in mature rats through their antioxidative actions (Malekinejad et al. 2011b).

Oral administration of glabridin, a pyranoisoflavan isolated from *Glycyrrhiza glabra* (30 mg/kg/day) for 10 days to mice with glomerular disease (Masugi-nephritis) reduced the amount of urinary protein excretion from control level (100 mg/day) to a significantly lower level (47 mg/day) (Fukai et al. 2003). Pretreatment with DHC-1, a herbal formulation derived from *Bacopa monnieri*, *Emblica officinalis*, *Glycyrrhiza glabra*, *Mangifera indica* and *Syzygium aromaticum* protected arts against isoproterenol-induced myocardial infarction and cisplatin-induced renal damage (Bafna and Balaraman 2005). This beneficial effect may be attributed, at least in part, to its antioxidant activity. Glycyrrhizin was demonstrated to attenuate renal I/R injury in mice via administration of

glycyrrhizin, which suppressed the serum levels of creatinine and blood urea nitrogen 6 h following reperfusion; furthermore, the superoxide anions as well as the activity of superoxide dismutase within renal tissues was reduced by glycyrrhizin pretreatment (Ye et al. 2014). Further, the protein level of cleaved caspase-3, as well as its activity in renal tissue, was suppressed as a result of the glycyrrhizin pretreatment, indicating that glycyrrhizin inhibited I/R-induced renal cell apoptosis. Additionally, glycyrrhizin pretreatment appeared to ameliorate I/R-induced renal injury via inhibition of inflammatory cell infiltration, as well as the production of pro-inflammatory cytokines, including tumour necrosis factor-α, interferon-γ, interleukin (IL)-1β and IL-6. The results suggested that glycyrrhizin provided significant protection against I/R-induced renal injury in mice by inhibiting inflammatory responses and renal cell apoptosis.

Licorice and its compounds had been found to have anti-hyperkalaemia action. Hyperkalaemia is a common life-threatening problem in haemodialysis patients. Licorice treatment could provide an important tool to maintain predialysis [K(+)] within safe limits in some dialysis patients at risk of hyperkalaemic arrhythmias (Ferrari 2009). In a prospective, double-blind, cross-over, placebo study of seven patients with anuria or chronic haemodialysis, administration of glycyrrhetinic acid, active compound of licorice (1 g/day for 2 weeks) decreased plasma potassium concentration (Serra et al. 2002). The decline in potassium level was paralleled by an increase in plasma cortisol/cortisone ratio indicating the inhibition of renal 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2). The data indicated that extra-renal 11beta-HSD activity influenced serum potassium concentrations but did not regulate blood pressure independently of renal sodium retention. In another prospective, double-blind, placebo-controlled crossover study of 10 haemodialysis patients, administration of cookies or bread rolls supplemented with glycyrrhetinic acid for 6 months significantly lowered serum potassium levels (Farese et al. 2009). No differences were found in parameters reflecting sodium retention.

Hair Promoting Activity

Studies showed that petroleum ether extract of *G. glabra* roots had potentials as a hair growth promoting agent for female rats (Upadhyay et al. 2013). Female rats treated with *G. glabra* had longer hair than those treated with either minoxidil or control. A maximum of 76 % of hair follicles were in anagenic stage (active growth phase of hair) in licorice extract-treated animals, compared to 66 and 45 % in minoxidil-treated and control groups, respectively. In another study in male Wistar albino rats, they found that petroleum ether extract of *G. glabra* possessed antiandrogenic alopecia activity which was comparable to that of standard drug finasteride (Upadhyay and Singh 2013).

Antiosteoporotic Activity

In a clinical study, it was found that licorice 3.5 g of a commercial preparation of licorice (containing 7.6 %, w/w of glycyrrhizic acid) administration daily for 2 months could increase serum parathyroid hormone and urinary calcium levels from baseline value in nine healthy women (22–26 years old, in the luteal phase of the cycle) after only 2 months of treatment (Mattarello et al. 2006). The effect of licorice on calcium metabolism was postulated to be influenced by several components of the root, which showed aldosterone-like, estrogen-like and antiandrogen activity.

Treatment with glabridin (1–10 μM) prevented apoptosis induced by TNF-alpha in murine MC3T3-E1 osteoblastic cells (Choi 2005). Moreover, glabridin (50 μM) decreased the TNF-alpha-induced production of prostaglandin E2 and nitric oxide in osteoblasts. The data indicated that the enhancement of osteoblast function by glabridin may result in the prevention for osteoporosis and inflammatory bone diseases. Liquiritigenin protected osteoblastic MC3T3-E1 cells from antimycin A-induced cell death (Choi et al. 2014). It was found that modulation of PI3K, antioxidant effects and the attenuation of mitochondrial dysfunction by liquiritigenin rep-

resent an important mechanism for its protection of osteoblasts against cytotoxicity resulting from mitochondrial oxidative stress. Glabridin attenuated 2-deoxy-D-ribose(dRib)-induced oxidative cell damage in MC3T3-E1 mouse osteoblastic cells (Kim et al. 2013). Treatment with glabridin resulted in a significant elevation of alkaline phosphatase (ALP) activity, collagen contents and osteoblast differentiation genes [ALP, collagen, osteopontin (OPN), osteoprotegerin (OPG) and osteocalcin (OC)] and bone morphogenetic protein (BMP) genes (BMP2, BMP4 and BMP7). Glabridin activated dRib-induced decreased expression of phosphatidylinositol 3′-kinase (PI3K) and protein kinase B 2 (AKT2) genes, master regulators of survival-related signalling pathways. Glabridin also upregulated the gene expression of antioxidant enzymes, superoxide dismutase 1 (SOD1) and glutathione peroxidase 4 (GPX4), which were inhibited by dRib. The results suggested that glabridin may be useful for the treatment of diabetes-related bone disease.

Ming et al. (2014) found that 0.35 mg/L licochalcone A (L-A) had a strong effect in increasing the osteogenic differentiation and mineralization of bone marrow-derived mesenchymal stem cells (BMSCs) both in-vivo and in-vitro by upregulating FasL and further playing a role in regulating the ERK and GSK-3β-catenin systems. It was also demonstrated that the administration of L-A could restore the biological function of the damaged BMSCs differentiation by recovering or protecting bone mass in a disease state through activating the endosteal bone formation and partially inhibiting bone resorption in acute estrogen deficiency model.

Radioprotective Activity

G. glabra root extract was found to protect microsomal membranes from gamma irradiation, as evident from reduction in lipid peroxidation, and could also protect plasmid DNA from γ-radiation-induced strand breaks (Shetty et al. 2002).

Cytoprotective Activity

Incubation of H4IIE murine hepatoma cells with *Glycyrrhiza* radix extract (GRE) inhibited cell death induced by 10 μM cadmium (Kim et al. 2004). The results demonstrated that GRE blocked Cd-induced cell death by inhibiting the apoptotic processes involving translocation of Bad into mitochondria, decreases in mitochondrial Bcl_{xL} and cytochrome *c*, and poly(ADP-ribose)polymerase cleavage. Among the major components present in GRE, liquiritigenin, but not liquiritin, isoliquiritigenin or glycyrrhizin exerted cytoprotective effect.

Choleretic Activity

Recent studies indicated that licorice extract, when administered per os or i.v., caused a marked choleretic effect in rats (Raggi et al. 1995). Umbelliferone (7-hydroxycoumarin) and glycyrrhizin were found to be the bioactive constituents with choleretic effects. Unlike the glycyrrhizin, which was present in a fairly large amount, umbelliferone was present at a very low concentration (traces), both in licorice and in bile. Licorice extract presented a significant choleretic effect after both oral and i.v. administration in rats, which increased the excretion rate of glycyrrhizin (Cantelli-Forti et al. 1997). Intravenous administration of liquiritigenin, a flavonoid aglycone from licorice, was found to have a choleretic effect and the ability to induce hepatic transporters and phase-II enzymes in rats (Kim et al. 2009).

Anti-convulsant Activity

G. glabra var. *glandulifera* leaf ethanol extract and dichloromethane fraction showed anticonvulsant effect in pentylenetetrazol seizure test in mice (Yazdi et al. 2011). The ED_{50} value of 2.11 g/kg and 1.30 g/kg was obtained for the

crude extract and dichloromethane fraction, respectively. The anticonvulsant activity of the extract and dichloromethane fraction could be mainly attributed to the compounds of triterpenes/sterols class present in the leaves. *G. glabra* ethanol extract at three doses, namely, 100, 200 and 400 mg/kg i.p. delayed the onset of pentylenetetrazole (PTZ)-induced seizure in rat but the duration of convulsion was reduced only in higher dose level (200 and 400 mg/kg) (Chowdhury et al. 2013). Pretreatment with the extract attenuated lipid peroxidation due to increase in antioxidant enzymes in the rat brain tissues.

Aldose Reductase Inhibitory Activity

Isoliquiritigenin was found to have potent aldose reductase inhibiting activity (Aida et al. 1990). Isoliquiritigenin inhibited rat lens aldose reductase with an IC_{50} of 3.2 x 10^{-7} M, using DL-glyceraldehyde as a substrate. It inhibited sorbitol accumulation in human red blood cells in-vitro, with an IC_{50} of 2.0×10^{-6} M. Isoliquiritigenin, when administered via an intragastric tube to diabetic rats, suppressed sorbitol accumulation in the red blood cells, the sciatic nerve and the lens as effectively as ONO-2235. The results suggested that isoliquiritigenin may be effective in preventing diabetic complications.

Vasorelaxant Activity

Isoliquiritigenin was found to have vasorelaxant activity (Yu and Kuo 1995). Isoliquiritigenin caused endothelium-independent relaxation of phenylephrine-precontracted rat aortic rings. Relaxation of phenylephrine-precontracted rat aorta and carbachol-precontracted guinea-pig trachea by rolipram (phosphodiesterase, PDE IV inhibitor) was markedly enhanced by isoliquiritigenin, while response to cilostamide (PDE III inhibitor) was not significantly changed by isoliquiritigenin. It was concluded that isoliquiritigenin exerted a vasorelaxant effect by activating soluble guanylate cyclase and increasing cyclic GMP.

Anti-metrorrhagia Activity

In a study of 32 women with polycystic ovary syndrome (PCOS), mean blood pressure was significantly reduced during spironolactone treatment, while it was unchanged in women receiving spironolactone plus licorice. Twenty percent of women treated with spironolactone and none treated with the addition of licorice complained of symptoms related to volume depletion. Consistently, the activation of the renin-aldosterone system was significantly lower during spironolactone plus licorice than with spironolactone alone. The prevalence of metrorrhagia was lower in the combined therapy. The results suggested that in patients with PCOS the mineralocorticoid properties of licorice could reduce the prevalence of side effects related to the diuretic activity of spironolactone.

Cosmetic Application Property

Both α-glycyrrhizin and β-glycyrrhizin exhibited similar considerable interfacial activity for cosmetic applications (Kondo et al. 1986). However, the aqueous solution of β-glycyrrhizin formed an extremely rigid gel in acidic media, whereas α-glycyrrhizin showed no sign of gelation. β-glycyrrhizin could emulsify various oily materials over a wide range of required HLB (hydrophilic-lipophilic balance) values, while α-glycyrrhizin had solubilizing ability for several perfume materials. Results from a series of experiments on the solubilizing, emulsifying and gelling mechanisms of α- and β-glycyrrhizin suggested that the β-glycyrrhizin molecule which was found to be cyclically constructed constituted the micelies which in turn orient anisotropically to form a rigid gel and to stabilize the emulsion. Based on the already described properties, α-glycyrrhizin should be utilized as a solubilizer, and β-glycyrrhizin as an emulsifier and a

stabilizer in cosmetics. Baltina et al. (1996) separated *cis* (18β) and *trans* (18α) isomers of glycyrrhizic acid (GA) from *G. glabra* root. The two GA isomers possess close physicochemical compositions, but *trans*-GA differed from the *cis*-isomer by higher solubility in water, higher stability of the aqueous solutions and lack of gel formation in these solutions. *Trans*-GA was of interest as a surfactant and solubilizing agent for the production of cosmetics and stabilized water-soluble medication forms.

Postoperative Sore Throat (POST) Attenuation Activity

In a prospective, randomized, single blind study of 40 adults (18–60 year), ASA physical status I and II of either sex, undergoing elective lumber laminectomy, licorice gargle performed 5 min before anaesthesia was found effective in attenuating the incidence and severity of postoperative sore throat (Agarwal et al. 2009). In a randomized, double-blind study of 236 patients having elective thoracic surgery, it was found that preoperative gargling with licorice solution rather than sugar water prevented postoperative sore throat and post-extubation coughing in patients intubated with double-lumen tubes (Ruetzler et al. 2013). Licorice gargling halved the incidence of postoperative sore throat.

Antiparasitic Activity

In in-vivo studies in dogs, *G. glabra* extract was found to have an anthelmintic influence upon Taenia worms but not on Ascaris worms (Shihata and Elghamry 1963b). Glycyrrhizic acid (GA) decreased hepatic and splenic *Leishmania donovani* parasite burden and increased T-cell proliferation in *Leishmania*-infected BALB/c mice (Bhattacharjee et al. 2012). When treated with GA at 75 mg/kg/day, *L. donovani*-infected mice exhibited a 95 % and 92 % reduction of the parasite burden in the liver and spleen, respectively. GA significantly enhanced the cell-mediated immune response.

Licorice glycyrrhetinic acid (GA) was found to be effective against microfilariae in-vitro (LC_{100}: 12.5 μM; IC_{50}: 1.20 μM), but was inactive against adult *Brugia malayi* worms (Kalani et al. 2013b). Of six GA analogues, the benzyl amide analogue killed adults and microfilariae at 25 and 50 μM concentration, respectively, and inhibited 49 % MTT reduction potential of the adult parasites. The IC_{50} values were found to be 8.8 and 2.2 μM for adults and microfilariae, respectively. In contrast, the octylamide analogue required much higher concentration to adversely affect the parasites. In-vivo using *B. malayi*-jird model, the benzyl amide analogue exhibited promising macrofilaricidal activity at 100 mg/kg, s.c. ×5 days and around 40 % of the treated animals showed calcified masses of worm fragments in the peritoneal cavity of the animals. The in-vitro studies against *Plasmodium falciparum* showed significant (IC_{50} 1.69 μg/ml) anti-malarial potential for licorice 18β-glycyrrhetinic acid (Kalani et al. 2013a). In-vivo evaluation showed a dose dependent anti-malarial activity ranging from 68 to 100 % at doses of 62.5–250 mg/kg on day 8.

Pharmacokinetic Studies

The pharmacokinetics of glycyrrhizin had been described and indicated its reduced bioavailability when consumed as licorice and based on available in-vivo and clinical evidence. Isbrucker and Burdock (2006) proposed an acceptable daily intake of 0.015–0.229 mg glycyrrhizin/kg body weight/day.

It was found that the time required for a maximum concentration (Tmax) of glycyrrhizin (G) in the rat plasma was 8 h after oral administration of licorice extract (Ozaki et al. 1990). In contrast, glycyrrhizin reached a maximum plasma concentration at less than 6 h after administration of glycyrrhizin. The plasma level of glycyrrhizin fell slowly within 24 h after their oral administration, and it was still detected in the plasma even after 24 h. Glycyrrhizin in the rat plasma, bile and urine could be precisely determined in concentrations as low as 1, 1 and 2.5 μg/ml, respectively, in a 0.1-ml sample using selective high-performance

liquid chromatographic method (Yamamura et al. 1991). The equivalent values for the glycyrrhetic acid-3-*O*-glucuronide were 1, 2.5 and 2.5 μg/ml, respectively. After oral administration of glycyrrhizin (100 mg) to three normal subjects, the major metabolite of glycyrrhizin (glycyrrhetic acid) appeared in plasma (<200 ng/mL), but glycyrrhizin was not found (Yamamura et al. 1992). In contrast, glycyrrhizin was found in urine, and the amount excreted was 1.1–2.5 % of the dose. The finding suggested that glycyrrhizin was partly absorbed in the intact form from the gastrointestinal tract. The concentration of glycyrrhizin in plasma after intravenous administration of glycyrrhizin (40, 80 and 120 mg) showed bi-exponential profiles during the 24-h period after administration of each dose. The glycyrrhizin metabolites, glycyrrhetic acid and glycyrrhetic acid-3-*O*-glucuronide, were not detected in either plasma or urine. Glycyrrhizin was not detected in plasma after oral administration of the usual therapeutic dose of glycyrrhizin, and no dose dependency of the drug was observed in the dose range of 40–120 mg.

The pharmacokinetics of glycyrrhetinic acid and glycyrrhizic acid humans and experimental animals could be described by a biphasic elimination from the central compartment with a dose-dependent second elimination phase (Krähenbühl et al. 1994). Depending on the dose, the second elimination phase in humans had a half-life of 3.5 h for glycyrrhizic acid and between 10 and 30 h for glycyrrhetinic acid. The major part of both glycyrrhetinic acid or glycyrrhizic acid was eliminated by the bile. While glycyrrhizic acid could be eliminated unmetabolized and underwent enterohepatic cycling, glycyrrhetinic acid was conjugated to glycyrrhetinic acid glucuronide or sulphate prior to biliary excretion. Orally administered glycyrrhizic acid was almost completely hydrolysed by intestinal bacteria and reached the systematic circulation as glycyrrhetinic acid. HPLC methods were found suitable in terms of precision and accuracy for the glycyrrhizin and glycyrrhetic acid determination in plasma and urine of human volunteers and in bile, plasma and urine of rats (Raggi et al. 1994a). Raggi et al. (1994b) found that after oral administration of licorice extract or glycyrrhizin to rats and humans, glycyrrhizin showed significantly reduced bioavailability when administered as licorice extract compared to when administered as glycyrrhizin. Significantly lower glycyrrhizin and glycyrrhetic acid plasma levels were found in rats and humans treated with licorice extract compared to the levels obtained with those in which glycyrrhizin alone was administered (Cantelli-Forti et al. 1994). The pharmacokinetic curves showed significant differences in the areas under the plasma-time curve (AUC), Cmax, and Tmax parameters. The data obtained from urine samples are in agreement with the above results and confirm a reduced bioavailability of glycyrrhizin present in licorice extract compared to pure glycyrrhizin. Glycyrrhizic acid was mainly absorbed after presystemic hydrolysis as glycyrrhetic acid (Ploeger et al. 2000, 2001). Once absorbed, glycyrrhetic acid was transported, mainly taken up into the liver by capacity-limited carriers, where it was metabolized into glucuronide and sulphate conjugates. These conjugates were transported efficiently into the bile possibly by the hepatic transfer protein 3α-hydroxysteroid dehydrogenase. After outflow of the bile into the duodenum, the conjugates were hydrolysed to glycyrrhetic acid by commensal bacteria; glycyrrhetic acid was subsequently reabsorbed, causing a pronounced delay in the terminal plasma clearance.

After oral administration of *Glycyrrhiza* extract to rats, glycyrrhizin and glycyrrhetic acid were detected in the plasma (Wang et al. 1995). However, the plasma concentration-time curves of glycyrrhizin and glycyrrhetic acid after *Glycyrrhiza* extract oral administration were much lower than those of pure glycyrrhizin, indicating the marked reduction in bioavailability of glycyrrhizin and as glycyrrhetic acid after this administration. It was found that the lipophilic components of *Glycyrrhiz*a extract reduced the gastric emptying rate and the absorption of glycyrrhizin from the small intestine, while these effects were not observed in the hydrophilic components. In contrast, the bioavailability of glycyr-

rhizin as glycyrrhetic acid was increased by the hydrophilic components, but not the lipophilic ones.

The results of toxicological and glycyrrhetic acid analyses showed significantly lower concentrations of glycyrrhizin in bile samples from rats treated with pure aqueous liquorice extract (LE) compared to pure glycyrrhizin (Cantelli-Forti et al. 1997). Furthermore, LE presented a significant choleretic effect after both oral and i.v. administration, which increased the excretion rate of glycyrrhizin. In case of glycyrrhetic acid, all the concentrations were very low, often below the detection limit.

Ruminococcus sp. PO1-3 and *Clostridium innocuum* ES24-06 intestinal bacteria isolated from human faeces were found capable of metabolizing glycyrrhizin (Hattori et al. 1985). *Ruminococcus* sp. had the ability to hydrolyse GL to glycyrrhetic acid (GA) and to reduce 3-dehydroglycyrrhetic acid (DGA) to GA while the *Clostridium innocuum* had the ability to reduce DGA to 3-epiglycyrrhetic acid (EGA). A mixture of the two strains could not only reduce DGA to both GA and EGA, but also epimerized GA to EGA and vice versa, possibly through a 3-dehydro intermediate. A bacterial strain capable of hydrolysing glycyrrhizin (GL) to glycyrrhetic acid (GA) was isolated from human faeces and identified as *Eubacterium* sp. (Akao et al. 1987). The GL-hydrolysing activity increased in parallel with the growth of this bacterium, which also produced β-D-glucuronidase acting on β-D-glucuronides of phenolic compounds such as phenolphthalein mono-β-D-glucuronide. Akao and Kobashi (1988) found that *Eubacterium* sp. strain GLH, isolated from human faeces, produced two kinds of β-D-glucuronidase, one enzyme specific for glycyrrhizin (GL) and the other for phenyl β-D-glucuronides. GL or p-nitrophenyl-mono-β-D-glucuronide (pNPG) stimulated the production of GL or pNPG β-glucuronidases and the growth of strain GLH in a basal medium lacking carbohydrate. D-Glucuronic acid also stimulated the growth of the bacterium, but glycyrrhetic acid did not. During mixed cultivation of the *Eubacterium* strain with *Streptococcus faecalis*, which does not produce GL β-glucuronidase, GL β-glucuronidase was also increased by GL or pNPG, but not by glycyrrhetic acid and p-nitrophenol. It was suggested that GL stimulated the growth of strain GLH even in the mixed culture. Glycyrrhizin (GL), a main constituent of liquorice, was hydrolysed to 18β-glycyrrhetic acid mono-β-D-glucuronide (GAMG, glycyrrhetyl monoglucuronide) by rat liver homogenate, and the hydrolytic activity was localized in the lysosomes among the same subcellular fractions as acid β-D-glucuronidase activity (p-nitrophenyl β-D-glucuronide (pNPG)-hydrolysing activity) (Akao et al. 1991). Rat liver lysosomes hydrolysed GAMG to 18β-glycyrrhetic acid (GA) at only 30 % rate compared with the rate of GL to GAMG. GA was also produced slowly from GL after time lag by the lysosomes. *Ruminococcus* sp. PO1-3, metabolized glycyrrhizin (GL) to glycyrrhetic acid (GA) and GA to 3-oxo-glycyrrhetic acid (3-oxo-GA) and possessed GL β-D-glucuronidase and 3β-hydroxysteroid dehydrogenase (3β-HSD) involved in the metabolism of GL (Akao 1999). This bacterial growth was enhanced by GL at a concentration of 0.4 mm and was suppressed by GA at a concentration of 1.0 mM. Chenodeoxycholic acid, deoxycholic acid and lithocholic acid among the bile acids added to this bacterium suppressed the growth and GL β-D-glucuronidase activity and 3β-HSD activity incident to it at a concentration of 1.0 mM, while cholic acid, hyodeoxycholic acid and glycine and taurine conjugates of cholic acid, chenodeoxycholic acid, deoxycholic acid and lithocholic acid had almost no effect on this bacterium at a concentration of 0.2–1.0 mm

Glycyrrhizin (18β-glycyrrhetic acid β-D-glucuronyl α-D-glucuronic acid, GL) and baicalin (baicalein β-D-glucuronic acid) were metabolized to glycyrrhetinic acid and baicalin, respectively, by human intestinal bacteria (Kim et al. 1996). However, α-glucuronidase of *Bacteroides* JY-6 isolated from human intestinal bacteria, hydrolysed GL or 18β-glycyrrhetinic acid α-D-glucuronic acid to 18β-glycyrrhetic acid but not to baicalin. However, *E. coli* β-glucuronidase from human intestinal bacteria hydrolysed baicalin to baicalein, but did not hydrolyse GL.

β-Glucuronidase of mammalian tissues hydrolysed both GL and baicalin. Glycyrrhizin (18β-glycyrrhetinic acid-3-*O*-[β-D-glucuronopyranosyl-(1 → 2)-β-D-glucuronopyranoside], GL) was metabolized to 18β-glycyrrhetinic acid (GA) in the main pathway by glucuronidases of *Bacteroides* J-37 and *Eubacterium* sp., human intestinal bacteria (Kim et al. 1999). In the minor pathway GL was metabolized to 18β-glycyrrhetinic acid-3-*O*-β-D--glucuronide (GAMG) by β-glucuronidase of *Streptococcu*s LJ-22. In a study of 20 healthy subjects, in-vivo 11β-hydroxysteroid dehydrogenase type 2 was investigated by administration of a 30- or 180-mmol/day of sodium diet or 500 mg/day of glycyrrhetinic acid for a week and measuring the urinary cortisol metabolite ratio (tetrahydrocortisol [THF] + 5alpha-THF)/tetrahydrocortisone (THE) (Ferrari et al. 2001). Absolute glycyrrhetinic acid-related increase in (THF + 5alpha-THF)/THE but not in the ratio of urinary free glucocorticoids UFF/UFE was higher in salt-sensitive than salt-resistant subjects and correlated with changes in mean blood pressure. Together with blood pressure responses to glycyrrhetinic acid, these findings supported a pivotal role of 11bβ-HSD-2 in salt sensitivity.

3β-hydrosteroid dehydrogenase activity of *Ruminococcus* sp. PO1-3 and *Ruminococcus* sp. PO1-3 with *Eubacterium* sp. GLH was suppressed greater in the presence of glycyrrhizin than without glycyrrhizin (Akao 2000b). Both bacteria and their mixture and intestinal flora metabolized 1.0 mM GL to glycyrrhetic acid (GA) in yields of about 10, 70, 40 and 100 %, respectively, with 24 h culture. Moreover, GA at a concentration of 1.0 mM suppressed growth of *Ruminococcus* sp. and *Eubacterium* sp. and the mixture of both and intestinal flora, which metabolized 1.0 mM GA to a negligible amount of 3-oxo-glycyrrhetic acid, indicate the accumulation of unchanged GA. Glycyrrhizin beta-D-glucuronidase activity of intestinal flora was enhanced by GA, which stimulated bacteria possessing particularly this characteristic. Also, Akao (2000a) found that there was competition in the metabolism of glycyrrhizin (GL) with glycyrrhetic acid mono-glucuronide (GAMG) by mixed *Eubacterium* sp. GLH and *Ruminococcus* sp. PO1-3. It was found that the metabolism of GAMG was faster than that of GL. GL with GAMG added to mixed *Eubacterium* sp. and *Ruminococcus* sp. cultured led to a lower level of these enzyme (GL β-D-glucuronidase and GAMG β-D-glucuronidase) activities and the consumption of GAMG more quickly, not GL. Low GAMG β-D-glucuronidase had the ability to hydrolyse GAMG well.

Results of studies indicated excellent absorption of liquiritigenin and davidigenin through the human intestinal epithelial cell line (Asano et al. 2003). In contrast, poor absorption of liquiritin and liquiritin apioside was found due to the little transepithelial flux of these compounds in the human colonic cell line Caco-2. Glabridin was extracted from human plasma by solid-phase extraction and LC–MS/MS (Aoki et al. 2005). Glabridin was recovered >90 %. In a caco-2 cell monolayer model of intestinal absorption, glabridin (active principle from licorice) was found to be easily incorporated into the cells and released to the basolateral side examined (Ito et al. 2007). After oral administration to rats, glabridin showed a maximum concentration 1 h after the dose of 87 nmol/L for standard glabridin and 145 nmol/L for licorice flavonoid oil (LFO) glabridin, and decreased gradually over 24 h after the dose. The level of incorporation into the liver was about 0.43 % of the dosed amount 2 h after the dose. These detected glabridins were in the aglycone form and not conjugated forms. The bioavailability was calculated to be AUC(inf) of 0.825 and 1.30 μM.h and elimination T(1/2) of 8.2 and 8.5 h for standard glabridin and LFO, respectively. The systemic bioavailability of glabridin was approximately 7.5 % in rats, but increased when combined with verapamil (Cao et al. 2007). In single-pass perfused rat ileum with mesenteric vein cannulation, the permeability coefficient of glabridin based on drug disappearance in luminal perfusates (P_{lumen}) was approximately 7-fold higher than that based on drug appearance in the blood (P_{blood}). The transport of glabridin in both apical (AP) and basolateral (BL) direction was significantly higher in MDCKII cells overexpressing P-glycoprotein

PgP/MDR1 than in the control cells. Glabridin inhibited PgP-mediated transport of digoxin but stimulated PgP/MDR1 ATPase activity. The findings indicated glabridin to be a substrate for PgP and that both PgP/MDR1-mediated efflux and first-pass metabolism contributed to the low oral bioavailability of glabridin. They also found that PgP limited the brain penetration of glabridin through the blood-brain barrier and that PgP may cause drug resistance to glabridin (licorice) therapy for CNS diseases and potential drug-glabridin interactions (Yu et al. 2007). Glycyrrhizin had been reported to have low oral bioavailability due to its impermeability across the gastrointestinal mucosa and studies by Jin et al. (2012) found that the formulation of glycyrrhizin as sodium deoxycholate/phospholipid-mixed nanomicelles could enhance glycyrrhizin absorption in gastrointestinal tract and pharmacodynamic effect in the treatment of acute liver injury caused by CCl_4. The formulation reduced aminotransferase (ALT), aspartate aminotransferase (AST) and improved the pathological changes of liver tissue.

Licorice/Drug Interactions

Pretreatment of male Sprague–Dawley rats with the methanol extract of *Glycyrrhiza glabra* roots (1 g/kg, p.o.) for 6 days significantly increased the cumulative biliary (156 %) and urinary (132 %) excretions of acetaminophen-glucuronide conjugate within 120 min after the administration of acetaminophen (150 mg/kg, i.v.) without affecting thioether and sulphate conjugates (Moon and Kim 1997). The findings suggested that licorice root might enhance the glucuronidation pathway of acetaminophen. Also, administration of licorice root or glycyrrhizin caused increases in specific activities of UPD-glucuronosyltransferase (UGT1A) by 111 % and 96 %, respectively. The concentration of UDP-glucuronic acid was increased 257 % by licorice root and 484 % by glycyrrhizin. The data indicated that licorice root and glycyrrhizin activated glucuronidation, thus suggesting that licorice may influence detoxification of xenobiotics in rat liver.

Skin permeation experiments using excised abdominal rat skin showed that the efficiency of glycyrrhizin as an enhancer agent was greater in gel formulations than it was in the emulsions (Nokhodchi et al. 2002). The enhancer with the concentration of 0.1 % w/w in gel increased diclofenac sodium flux value to 10-fold compared with the control sodium carboxymethyl cellulose gel.

P450 3A4, the major human drug metabolizing cytochrome P450 enzyme, was inactivated by licorice root extract and by glabridin in a time- and concentration-dependent manner (Kent et al. 2002). The inactivation was NADPH-dependent and was not reversible by extensive dialysis. P450 2B6 was also inactivated by glabridin in a time- and concentration-dependent manner. The activity of P450 2C9 was competitively inhibited by glabridin, whereas P450 2D6 and P450 2E1 were virtually unaffected. The data showed that glabridin could serve as a substrate for at least three human P450 enzymes and that depending on the isoform, metabolism of glabridin could lead to mechanism-based inactivation or inhibition of the P450. Haem and reduced CO spectral analysis also indicated that glabridin inactivated P450s 2B6 and 3A4 by different mechanism.

Nitrofurantoin alone and with liquorice were given to healthy volunteers and patients suffering from urinary tract infections (Datla et al. 1981). The excretion rates of nitrofurantoin were significantly higher in patients receiving the drug with liquorice and also side effects were minimal. There was no significant difference in the excretion rates of the drug with addition of liquorice in healthy volunteers. Lin et al. (2009) caution the licorice or its component glycyrrhetic acid with methotrexate, the immunosuppressant. They found the AUC (area under the curve) and MRT (mean residence time) of methotrexate were significantly increased by licorice and glycyrrhetic acid in rats. Hou et al. (2012) found that licorice and glycyrrhizin significantly decreased the peak blood concentration and the areas under the curves of the immunosuppressant cyclosporine in rats. It was concluded that liquorice significantly reduced the oral bioavail-

ability of cyclosporine through activating P-glycoprotein and cytochrome P450 3A4 (CYP3A4). In a two-phase randomized crossover design, placebo controlled study of 16 healthy male subjects, volunteers were given placebo or glycyrrhizin for 14 days and midazolam on the 15th day (Tu et al. 2010a). Glycyrrhizin reduced the AUC of imidazole in blood plasma, indicating a modest induction of CYP3A by glycyrrhizin. In another two-phase randomized crossover study of healthy Chinese male volunteers with different CYP2C19 genotypes, eighteen healthy subjects (six CYP2C19*1/*1, five CYP2C19*1/*2, one CYP2C19*1/*3, five CYP2C19*2/*2 and one CYP2C19*2/*3) were given placebo or glycyrrhizin salt tablet 150 mg twice daily for 2 weeks followed by omeprazole on the 15th day (Tu et al. 2010b). After 14-day treatment of glycyrrhizin, plasma omeprazole significantly decreased, and those of omeprazole sulfone significantly increased. However, plasma concentrations of 5-hydroxyomeprazole did not significantly change. The ratio of AUC(0-infinity) of omeprazole to omeprazole sulfone decreased by 43.93 % in CYP2C19*1/*1; 44.85 % in CYP2C19*1/*2 or *3 and 36.16 % in CYP2C19*2/*2 or *3 while those of omeprazole to 5-hydroxyomeprazole did not change significantly in all three genotypes. No significant differences in glycyrrhizin response were found among CYP2C19 genotypes. Glycyrrhizin induced CYP3A4-catalysed sulfoxidation of omeprazole leading to decreased omeprazole plasma concentrations, but had no significant impact on CYP2C19-dependent hydroxylation of omeprazol. Glycyrrhetinic acid was found to be a mixed inhibitor of the enzyme cytochrome P450 3A (CYP3A), with an IC_{50} of 7.25 μmol/l, a K_m of 4.3 μmol/l and a K_i of 6.4 μmol/l (Li et al. 2010). CYP3A activity was also affected by intragastric administration of glycyrrhetinic acid, which resulted in increases in area under the plasma concentration-time curve $(AUC)_{0\text{-}t}$ and in apparent elimination half-time ($t_{1/2}$) and significant decreases in body clearance, as well as in the formation of 1-hydroxy-midazolam after intragastric or intravenous administration of midazolam. The results suggested the likelihood of an interaction between glycyrrhetinic acid and CYP3A-metabolized drugs in humans and indicated that liquorice root should be used with caution when taken concomitantly with other drugs that interact with CYP3A.

In a study of 17 patients with Addison's disease on stable cortisone acetate therapy, two 3-day periods of co-administration of licorice or grapefruit juice significantly increased the median AUC for serum cortisol (Methlie et al. 2011). Licorice increased the median urinary cortisol/cortisone ratio (0.43 vs 0.21), whereas GFJ increased the (allo-tetrahydrocortisol + tetrahydrocortisol)/tetrahydrocortisone ratio (0.55 vs 0.43). The study of Zhao et al. (2012b) indicated CYP3A4 to be likely the major enzyme responsible for glycyrrhetinic acid metabolism in human liver microsomes while CYP2C9 and CYP2C19 were considerably less active. The results suggested that glycyrrhetinic acid had the potential to interact with a wide range of xenobiotics or endogenous chemicals that were CYP2C9, CYP2C19 and CYP3A4 substrates. The inhibitory action of glycyrrhetinic acid was observed in CYP2C9 for 4-hydroxylation of diclofenac, CYP2C19 for 4′-hydroxylation of (S)-mephenytoin and CYP3A4 for 1′-hydroxylation of midazolam. However, glycyrrhetinic acid showed relatively little inhibitory effect (IC_{50} > 400 μm) on phenacetin O-demethylation, dextromethorphan O-demethylation and chlorzoxazone 6-hydroxylation.

Toxicology/Toxicity Studies

Walker and Edwards (1994) demonstrated that a daily oral intake of 1–10 mg of glycyrrhizin, corresponding to 1–5 g licorice, had been estimated to be a safe dose for most healthy adults. Bernardi et al. (1994) administered graded daily doses of dried, aqueous extract of licorice root, containing 108, 217, 380 and 814 mg of glycyrrhizin, to 4 groups of 6 healthy volunteers of both sexes for 4 weeks. No observed-adverse-effect level (NOAEL) based on the study report was 217 mg/person/day. At higher dose levels, sodium reten-

tion and depression of plasma renin and aldosterone levels were observed. Female participants were slightly more sensitive to glycyrrhizinic acid than male participants. In a subsequent double-blinded randomized placebo-controlled study by Bijlsma et al. (1996), four groups of 10 healthy female volunteers received orally 0, 1, 2 or 4 mg of pure glycyrrhizinic acid/kg/day for 8 weeks. In this study the NOAEL for glycyrrhizinic acid was 2 mg/kg/day. The European Commission Scientific Committee on Food (2003) deemed that the NOAEL obtained in the study by Bijlsma and co-workers was more appropriate as the study encompassed a larger sample of volunteers (40 volunteers in contrast to 24 volunteers), a longer period of exposure (8 weeks in contrast to 4 weeks) and inclusion of a placebo control group.

A single-dose and two multiple-dose studies at low (300 mg), moderate (600 mg) and high (1200 mg) daily doses of licorice flavonoid oil (LFO) in healthy human subjects using a placebo-controlled single-blind design showed LFO to be safe when administered once daily up to 1200 mg/day (Aoki et al. 2007b). In these human studies at three dose levels, there were no clinically noteworthy changes in haematological or related biochemical parameters. All clinical events observed were mild and considered to be unrelated to LFO administration even after repeated administration for 4 weeks. The multiple-dose studies with healthy male and female subjects for 1 week and 4 weeks suggested that plasma glabridin reached steady state levels within 2 weeks with a single daily administration of 300–1200 mg/day LFO.

In a 90-day repeated-dose toxicity study, licorice flavonoid oil (LFO) induced an anticoagulation effect in both sexes of rats, although there was a clear sex difference (Nakagawa et al. 2008b). It was concluded that the no-observed-adverse-effect level (NOAEL) for the LFO concentrate solution is estimated to be 800 mg/kg/day for female rats, and approximately 400 mg/kg/day for male rats. Based on findings obtained from the genotoxicity assays performed including reverse mutation assay using four *Salmonella typhimurium* strains and *Escherichia coli*, chromosomal aberration test using Chinese hamster lung cells, bone marrow micronucleus test, liver and peripheral blood micronucleus tests in male F344 rats, Nakagawa et al. (2008a) concluded that the consumption of LFO would not present any genotoxic hazard to humans.

The results of sub-chronic inhalation studies in rats with various forms of licorice extract applied to cigarette tobacco suggested that adding licorice extract to cigarette tobacco at levels of ≤5 % did not discernibly alter the smoke chemistry or biological effects normally associated with mainstream cigarette smoke (Carmines et al. 2005). Female rats in the 12.5 % block licorice extract exposure group displayed an increased incidence and severity of epithelial hyperplasia in the nose (level 2), with no relevant respiratory tract changes in the 8 % powder licorice extract exposed rats.

Studies by Canciu-Dobrea et al. (2012) showed that there were no surface-active impediments in using *Glycyrrhiza glabra* and *G. echinat*a as possible ingredients in parenteral formulation as they were sources of low haemotoxic saponins as assessed by the foam index and haemolysis capacity. *G. echinata* (FI (foam index) 400; HD_{50} = 9153 μg/ml), *G. glabra* (FI 250, HD_{50} = 123,822 μg/ml) and the tested saponins (ammoniacal glycyrrhizin HD50 = 63.25 μg/ml and *G. echinata* saponins HD_{50} = 42.5 μg/ml) had low haemolytic capacity.

According to Omar et al. (2012) licorice is a US Food and Drug Administration (FDA) approved food supplement used in many products without precise regulations to prevent toxicity. They hoped that FDA would start regulating the use of licorice.

Adverse Health Issues

Tamura et al. (1970) demonstrated that glycyrrhetinic acid (GA) and its derivatives inhibited 5β-reduction to a much greater extent than 5α-reduction of cortisol, aldosterone and testosterone in rat liver preparations. When GA or glycyrrhizin (GL) were administered, 5β-reductase activity was significantly sup-

pressed. In contrast, 5α-reductase was markedly increased. In humans 5β-reductase is quantitatively the major enzyme and plays an important role in the regulation of cortisol and aldosterone metabolism. The authors suggested that the suppression of 5β-reductase activity by GA or GL administration may delay the clearance of corticosteroids and prolong the biological half-life of cortisol resulting in the synergism of these steroids and GA or GL. Latif et al. (1990) demonstrated that glycyrrhetinic acid (GA) did not affect either microsomal 5α-reductase or cytosolic 3α-hydroxysteroid dehydrogenase activity. However, GA was a potent inhibitor of cytosolic 5β-reductase. GA specifically inhibited microsomal 3β-HSD enzyme activity by apparently a competitive inhibition mechanism, causing a build-up of the intermediate, 5α-dihydroaldosterone (DHAldo). The results indicated that GA had a profound effect on hepatic ring A-reduction of aldosterone. Inhibition of 5β-reductase and 3β-HSD resulted in decreased synthesis of both 3α, 5β-tetrahydroaldosterone (THAldo) and 3β, 5α-THAldo and, hence, accumulation of aldosterone and 5α-DHAldo, both potent mineralocorticoids. The ingestion of liquorice, and/or its active metabolites, could sometimes produce an acquired form of apparent mineralocorticoid excess (AME) syndrome, expressed as sodium and fluid retention, potassium loss and suppression of the renin-angiotensin-aldosterone system, in addition to clinical consequences such as raised blood pressure (hypertension) and oedema (Sigurjónsdóttir et al. 1995; Olukoga and Donaldson 2000). Excessive ingestion of licorice had been known to induce a syndrome of hypokalaemia and hypertension reflecting increased activation of renal mineralocorticoid receptors by cortisol (Walker and Edwards 1994). A similar syndrome of cortisol-dependent mineralocorticoid excess had been reported to occur in congenital deficiency of the enzyme 11β-hydroxysteroid dehydrogenase. Liquorice had been reported to induce pseudohyperaldosteronism by inhibiting the 11β-hydroxysteroid dehydrogenase type 2 and was also known to inhibit the renin-angiotensin-aldosterone system (RAAS) (Sigurjónsdóttir et al. 2006). The continuous, high level exposure to glycyrrhizin compounds could produce hypermineralocorticoid-like effects in both animals and humans as biochemical studies indicated that glycyrrhizinate inhibited 11β-hydroxysteroid dehydrogenase, the enzyme responsible for inactivating cortisol (Isbrucker and Burdock 2006). These effects were reversible upon withdrawal of licorice or glycyrrhizin. Consumption of large amounts of liquorice could cause hypertension and hypokalaemia as liquorice contains glycyrrhetinic acid, which inhibits the enzyme 11β-hydroxysteroid dehydrogenase type 2, and ultimately leads to an apparent mineralocorticoid excess syndrome (Nielsen et al. 2012). Excessive intake of licorice may cause a hypermineralocorticoidism-like syndrome characterized by sodium and water retention, hypertension, hypokalaemia, metabolic alkalosis, low-renin activity and hypoaldosteronism (Celik et al. 2012), and generally the onset and severity of symptoms depend on the dose and duration of licorice intake, as well as individual susceptibility (Mumoli and Cei 2008). Glycyrrhizic acid, contained in licorice, possessed a mineralocorticoid-like effect and chronic excess intake of licorice could induce the rare syndrome of 'apparent mineralocorticoid excess', due to the inhibitory effect of glycyrrhizic acid on 11β-hydroxysteroid dehydrogenase type 2 determining clinical/biochemical manifestations as resistant hypertension, metabolic alkalosis and severe hypokalaemia (Bisoni et al. 2014). They emphasized the importance of anamnesis in the diagnosis so as to avoid unnecessary and expensive investigations, and reduce the duration of hospitalization. Liquorice intoxication could be confirmed by the shut-off of the renin-angiotensin-aldosterone axis, and by the increase of the urinary ratio of [cortisol metabolites (5α tetrahydrocortisol + 5β tetrahydrocortisol)]/[cortisone metabolite (5β tetrahydrocortisol)] together with increase of urinary free cortisol excretion (Luchon et al. 1993).

Case Reports

Gross et al. (1966) reported an obese patient striving to reduce weight ingested excessive

amount of licorice leading to severe potassium depletion and acute myopathy and myoglobinuria. Hypertension, initially normokalaemic, was eventually associated with hypokalaemia, alkalosis, suppressed plasma renin activity and aldosteronopenia in a 58-year-old man who had ingested two to three 36-gm licorice candy bars daily for 6 years to 7 years (Conn et al. 1968). Metabolic balance studies recorded complete recovery upon cessation of licorice ingestion. Holmes et al. (1970) reported a case of a 63-year-old man with pseudohyperaldosteronism induced by habitual ingestion of liquorice (3.5 lb licorice for the last 15–20 years). Chamberlain (1970) described a case of a previously healthy 53-year-old male patient who presented with fulminant congestive heart failure (CHF) after ingesting large quantities of licorice for a week. Kuriyama et al. (1975) described a patient who developed marked hypokalaemia due to chronic administration of glycyrrhizin (150 mg daily). Bannister et al. (1977) reported a case of a 58-year-old women with cardiac arrest associated with hypokalaemia caused by ingesting 1.8 kg of liquorice a week. Three months after stopping liquorice, she remained well and all laboratory values were normal. Takeda et al. (1979) reported the natural recovery from the aggravated hypertension, hypokalaemia and suppression of the renin-aldosterone axis after the glycyrrhizin discontinuation in two mild hypertensive women aged 71 and 68 years, who had been administered 273–546 mg glycyrrhizin daily for 1.5 and 6 months, respectively, for the treatment of liver disease. About one month after the glycyrrhizin discontinuation, acceleration of hypertension, hypokalaemia and suppression of the renin-aldosterone system still continued in both patients. About one and a half months later, the improvements of aggravated hypertension, hypokalaemia and suppressed renin-aldosterone system gradually occurred in both patients. The results demonstrated that both patients had a prolongation of the syndrome resembling primary aldosteronism except the low plasma aldosterone level about 1 month after the glycyrrhizin discontinuation. A man 85 years old, who habitually swallowed saliva while chewing tobacco leaf containing about 8.3 % (w/w) liquorice paste with a glycyrrhizinic acid content of 0.15 %, developed the classical syndrome of exogenous mineralocorticoid excess: hypokalaemia, hypertension, renal potassium wasting, metabolic acidosis, sodium retention and low plasma rennin (Blachley and Knochel 1980). Cuspidi et al. (1981) reported four female subjects with a form of severe systo-diastolic hypertension, recalcitrant to previous anti-hypertensive treatment, accompanied by marked hypokalaemia caused by habitual licorice ingestion. Abstinence from licorice led to normalization of kalemia in a period varying from 6 to 15 days, while arterial pressure values and all other essential parameters examined (plasma renin activity, etc.) recovered their balance more slowly. A 54-year-old man was admitted to hospital with acute rhabdomyolysis and myoglobinuria due to hypokalaemia caused by chronic licorice ingestion and diuretic treatment (Heidemann and Kreuzfelder 1983). The myoglobinaemia led to a glomerulopathy and tubulopathy, however there was no clinical evidence of acute renal failure. Four cases of pseudohyperaldosteronism due to chronic ingestion of liquorice-containing laxatives were described (Scali et al. 1990). All patients had hypertension and hypokalaemia with suppression of plasma renin activity and aldosterone.

Böcker and Breithardt (1991) reported two cases of licorice-induced arrhythmias. In both cases the ingestion of large amounts of licorice caused a marked hypokalaemia. Okada et al. (1987) reported a case of Sjögren's syndrome associated with hypokalaemic myopathy due to glycyrrhizin. Chubachi et al. (1992) reported the case of a 72-year-old man who developed acute renal failure (ARF) following severe hypokalaemic rhabdomyolysis; the hypokalaemia was due to chronic glycyrrhizin (glycyrrhizic acid) administration. Caradonna et al. (1992) reported a patient with acute rhabdomyolysis and absence of myoadenylate deaminase (MADA) associated with chronic licorice intoxication. These were completely reversed with potassium supplementation and licorice withdrawal. A case of a 55-year-old man of hypertension and hypokalaemia was reported by Kageyama (1992). He had been administered glycyrrhizin from 1 year

before admission for the treatment of chronic hepatitis. His blood pressure, potassium, plasma renin activity and plasma aldosterone concentration returned to normal within about 4 weeks after discontinuation of the glycyrrhizin. Re-administration of glycyrrhizin caused increases in plasma aldosterone concentration (PAC) and plasma renin activity (PRA). His urinary cortisol excretion was increased and urinary cortisone excretion decreased, while his serum cortisol level remained unchanged. The results suggested that increased renal cortisol as a result of decreased conversion to cortisone might play an important role in the development of pseudoaldosteronism as well as in its own mineralocorticoid activity. Corsi et al. (1983) reported a 35-year-old man who had been ingesting one or two bags of tablets of pure licorice daily (20–40 g/day) for about 2 years, developed an acute myopathy with high levels of serum muscle enzymes and the typical features of mineralocorticoid excess: serious hypokalaemia, hypertension and metabolic alkalosis. Both plasma renin and serum aldosterone were below the normal values. Van der Zwan (1993) reported a 15-year-old boy who developed a hypertension encephalopathy after ingestion of 0.5 kg liquorice candy. He recovered completely in the course of 5 months.

Luchon et al. (1993) reported a case of chronic intoxication with glycyrrhizinic acid, at a dosage of 1000–1500 mg per month over a period of 11 months, in a former alcoholic. This intoxication was revealed by profound hypokalaemia and rhabdomyolysis but blood pressure remained constantly normal. A 78-year-old Japanese man hospitalized because of muscular weakness and acute renal failure was diagnosed to suffer from licorice-induced pseudoaldosteronism that produced hypokalaemic rhabdomyolysis, resulting in acute renal failure and profound deposition of calcium into the damaged skeletal and cardiac muscles (Saito et al. 1994). Brayley and Jones (1994) described a 29-year-old female patient who presented with acute severe hypokalaemia after increasing her licorice consumption from 300 to 600 g/day. She had a history of anorexia nervosa with bulimia. Berlango Jiménez et al. (1995) presented the case of a 36-year-old patient who, as the result of the intake of five daily licorice sticks (25 g/day) for a month, developed analytical and clinical signs of acute rhabdomyolysis characterized by typical disorders of mineralocorticoid excess, such as severe hypokalaemia, arterial hypertension and metabolic alkalosis. Heikens et al. (1995) described of a 40-year-old female with severe hypertension and hypokalaemic metabolic alkalosis, due to prolonged liquorice ingestion. They suggested that glycyrrhetinic acid, the hydrolytic metabolite of glycyrrhizic acid, was the active component of liquorice which caused inhibition of the peripheral metabolism of cortisol. Cortisol had been found to bind with the same affinity as aldosterone to the mineralocorticoid receptor resulting in a hyperminer-alocorticoid condition. Ingestion of liquorice may therefore result in retention of sodium and water, hypertension, hypokalaemia, alkalosis and suppression of the renin-aldosterone system.

Seelen et al. (1996) reported a case of 38-year-old woman who was hospitalized because of hypertension and hypokalaemic alkalosis caused by the intake of liquorice (200 g per day). De Klerk et al. (1997) reported the case of a 21-year-old woman with hypertension associated with chewing of licorice-flavoured chewing gum. Chamberlain and Abolnik (1997) described a case of a 64-year-old previously healthy person who developed pulmonary oedema after a binge of black licorice sweet consumption. Cataldo et al. (1997) described a 6 1/2-year-old child with pseudohyperaldosteronism due to excessive and prolonged liquorice ingestion and its unusual association with haemorrhagic gastritis never observed in the course of liquorice intoxication. A case of a 69-year-old women with pseudoaldosteronism characterized by hypertension with hypokalaemia induced by a mouth refresher containing licorice, cinnamon, ginger and other spices was reported by Kageyama et al. (1997). Dilated cardiomyopathy during hypokalaemic myopathy resulting from excessive use of licorice and glycyrrhizin for gastritis had been reported by Hasegawa et al. (1998). Doeker and Andler (1999) reported an 11-year-old boy who had hypoparathyroidism and Addison's disease.

The boy reported an excessive daily intake of 300–400 g liquorice corresponding to 600–800 mg glycyrrhizic acid because of salt craving. After complete withdrawal of liquorice, all symptoms of hypermineralocorticoidism diminished and growth velocity increased. Ishikawa et al. (1999) reported a patient with a history of anorexia nervosa who developed licorice-induced hypokalaemic myopathy and hypokalaemic renal tubular damage. With potassium replacement, high creatinine phosphokinase blood level and myopathic signs returned to normal.

Lozano et al. (2000) reported a young woman with right upper limb ischemia induced by chronic licorice ingestion. The patient had a long lasting history, longer than 10 years, of continuous licorice ingestion. Blood samples showed severe hypokalaemia that caused EKG changes. Transoesophageal echocardiogram discovered mild mitral valve prolapse. Russo et al. (2000) described two cases of hypertension encephalopathy (in addition to the classical symptoms of hypertension, hypokalaemia and suppression of the renin-aldosterone system) which resulted in pseudohyperaldosteronism syndrome due to the regular daily intake of low doses of liquorice. Glycyrrhizic acid, a component of liquorice, had been known to produce both hypermineralocorticism and the onset of encephalopathy through the inhibition of 11βa-hydroxysteroid dehydrogenase. Harada et al. (2002) reported an 84-year-old man with congestive heart failure caused by digitalis toxicity after taking Chinese herbal laxative containing licorice. Cartier et al. (2002) described the case of a 33-year-old woman herbalist who developed occupational asthma due to liquorice roots as confirmed by specific inhalation challenges. Elinav and Chajek-Shaul (2003) described a patient who suffered life-threatening hypokalaemic paralysis caused by consumption of licorice in the form of a tea sweetener superimposed on long-term consumption of licorice candy. Aggressive fluid and potassium replenishment produced complete and lasting recovery. Lin et al. (2003) reported an elderly Asian man with hypokalaemic muscle paralysis cause by chronic licorice ingestion. Campana et al. (2003) described a case of cardiac arrest due to 'torsade de pointes' resulting from a marked hypokalaemia caused by the patient's habit of eating daily of an appreciable quantity of licorice. Elinav and Chajek-Shaul (2003) described a patient who suffered life-threatening hypokalaemic paralysis caused by consumption of licorice in the form of a tea sweetener superimposed on long-term consumption of licorice candy. Aggressive fluid and potassium replenishment produced complete and lasting recovery. Ishiguchi et al. (2004) described a 90-year-old woman with hypertension who developed metabolic alkalosis and myoclonus from ingestion of antacid medication containing licorice. A provisional diagnosis of licorice-induced metabolic alkalosis was established and the patient was successfully treated after correction of serum pH and cessation of the medications.

Cooper et al. (2007) described the case of a 42-year-old woman who self-treated undiagnosed Addison's disease for several years with soy sauce and liquorice sticks consuming around 46 g of salt per week. She presented with a 4-week history of decreased energy, malaise and postural dizziness. Mumoli and Cei (2008) described a patient with hypokalaemia caused by long-term consumption of natural licorice root after quitting smoking. Sontia et al. (2008) described a patient with long-standing hypokalaemia and uncontrolled hypertension related to excessive ingestion of liquorice. Yaguchi et al. (2008) reported a case of an 86-year-old female with licorice-induced hypokalaemic myopathy; she had been taking two kinds of Chinese medicines containing licorice for 4 years. The patient presented with difficulty in holding her head up and proximal-dominant tetraparesis with significant laterality. The general reflexes were decreased, and the bilateral Chaddock's reflexes were repeatedly positive. They found that the pathologic reflex was caused by the aggravation of cervical spondylotic myelopathy due to neck weakness and that tetraparesis with laterality was caused by hypokalaemic myopathy. Tacconi et al. (2009) reported one patient presented with carpal tunnel syndrome with nerve conduction studies revealing bilateral median neuropathies likely attributed to licorice-induced water

retention caused by excessive licorice consumption.

Johns (2009) reported a 49-year-old female physician who presented with peripheral oedema, weight gain and relative hypertension caused by the consumption of licorice candy cigars containing glycyrrhizic acid (GZA) found in natural licorice extract. Yorgun et al. (2010) reported a 50-year-old woman who was admitted to the emergency department with an aborted cardiac arrest due to ventricular fibrillation and electrocardiographic changes consistent with Brugada syndrome due to liquorice-induced hypokalaemia. Licorice consumption was found to be the cause of a posterior reversible encephalopathy syndrome (PRES) in a 49-year-old woman admitted to hospital because of thunderclap headache and blurred vision (van Beers et al. 2011). The combination of sequential computed tomography (CT) and the triad of hypertension, hypokalaemia and metabolic alkalosis in this patient suggested the diagnosis. Omar et al. (2012) reported a 35-year-old man from Egypt, with no past medical history, who presented to the emergency room with progressive weakness that started in his lower extremities and quickly progressed to involve the upper limbs. He was diagnosed with hypokalaemic myopathy due to excessive licorice ingestion. Celik et al. (2012) reported a case of an association of hypokalaemia, oedema and thrombocytopenia that was developed due to the excessive intake of licorice. Nielsen et al. (2012) described a 50-year-old woman with hypertension and hypokalaemia-induced limb paresis due to chronic liquorice ingestion. The patient was treated with potassium supplementation and spironolactone. Her blood pressure and electrolyte status normalized within a month after cessation of liquorice intake. A 47-year-old woman was admitted to the emergency department with a history of asthenia, periorbital and lower limbs oedema, associated with hypokalaemia and increased blood pressure levels (Robles et al. 2013). It was revealed that she had been consuming several sachets of raw liquorice lollies obtained from a herbalist a month ago and clinical tests established the cause to liquorice poisoning. During the patient's stay at the hospital, liquorice was stopped and potassium supplements were started. Subsequently, a week after, the patient fully recovered without any significant sequelae. O'Connell et al. (2014) reported a case of a 56-year-old lady who presented with thunderclap headache, visual disturbance and a generalized tonic–clonic seizure, diagnosed with hypokalaemia and posterior reversible encephalopathy syndrome (PRES) associated with regular liquorice consumption.

Nugmanova and Kalitina (1979) reported a case of contact dermatitis caused by licorice. Sailler et al. (1993) reported three cases of diffuse acute oedema caused by licorice. Dobbins and Saul (2000) reported five cases of transient visual loss after licorice ingestion. The visual symptoms were similar to one with ocular migraine without headache. According to the authors, the underlying pathogenesis may involve vasospasm of the optic nerve blood vessels leading to transient monocular or binocular visual loss/aberrations. The occurrence of adverse ocular side effects related to licorice ingestions were reported by Hall and Clemett (2004), Fraunfelder (2004) and Santaella and Fraunfelder (2007).

Preclinical and Clinical Studies

Epstein et al. (1977) studied the electrolyte status and renin-angiotensin-aldosterone axis after withdrawal of licorice in four sick women aged 35–55 years admitted with chronic licorice intoxication. They had been consuming 25–200 g licorice daily for 6 months to 5 years. All patients showed normal renin, angiotensin and aldosterone values 2–4 months later, however, indicating that long-term suppression of the renin-angiotensin-aldosterone axis was uncommon despite several years of liquorice ingestion. Ingestion of licorice, 100 g daily for 8 weeks, caused a rise of 81 % in plasma atrial natriuretic peptide (ANP) concentration in 12 healthy subjects (Forslund et al. 1989). The plasma concentrations of antidiuretic hormone, aldosterone and plasma renin activity decreased. Blood pressure increased transiently and two subjects developed reversible hypertension. The rise in plasma ANP concentration during ingestion of licorice may be

considered a physiological response to prevent fluid retention and development of hypertension.

Gomez-Sanchez and Gomez-Sanchez (1992) found that intracerebroventricular (icv) administration of the infusion of both glycyrrhizic acid, an active principle of licorice, and carbenoxolone, a synthetic analogue, into a lateral ventricle of the brain of a rat, at a dose less than that which had an effect when infused subcutaneously, produced hypertension. Oral administration of carbenoxolone or glycyrrhizic acid caused saline polydipsia and polyuria typical of chronic systemic mineralocorticoid excess, and the icv licorice derivatives produced hypertension without affecting saline appetite. The findings provided additional evidence for a central role in blood pressure control by mineralocorticoids that was distinct from their renal effects. They also suggested that more was involved in licorice-induced hypertension than only inhibition of 11β-hydroxysteroid dehydrogenase. Hayashi et al. (1992b) reported two patients with hypokalaemic myopathy induced by the administration of glycyrrhizin, 270–273 mg per day for a period of 2 and 8 months, respectively. Myotonic and repetitive discharges were observed when the serum chloride level fell below 90 mEq/l, and these discharges disappeared following administration of KCl. The findings supported the causal role of hypochloremia in myotonic discharges. Shintani et al. (1992) reported 59 cases of glycyrrhizin (licorice)-induced hypokalaemic myopathy (GIHM). In many cases, conditions leading to the onset of GIHM were habitual licorice ingestion, ingestion of antituberculosis agents containing licorice and long-term ingestion of licorice-containing agents for chronic gastritis, chronic hepatitis or chronic dermatitis. The main clinical symptom was flaccid quadriplegia in almost all cases, with muscle pain in 32.2 % and peripheral dysesthesia in the extremities, manifested mainly by numbness (27.1 %). Muscle biopsy was performed in 17 of the 59 cases with resultant findings of myopathic changes consisting mainly of phagocytosis, necrotic fibres, vacuolar degeneration, together with sporadic neurogenic changes. Complete cure was attained in 57 of the 59 cases of GIHM by discontinued ingestion of glycyrrhizin (licorice) and potassium supplement.

According to Schambelan (1994) studies had demonstrated that a paste prepared from succus liquiritiae, a dried watery extract of the roots of *Glycyrrhiza glabra*, could prevent the formation of gastric ulcers in experimental animals and confirmed the salutary effects in patients, but found that approximately 20 % of patients so treated developed facial and dependent oedema, often accompanied by headache, shortness of breath, stiffness and pain in the upper abdomen. Glycyrrhizic and glycyrrhetinic acids administration to rats in-vivo (75 mg/kg. day for 5 days) resulted in inhibition of 11β-hydroxysteroid dehydrogenase (11β HSD) activity, but also a significant reduction in steady state 11 β HSD mRNA levels in both predominantly mineralocorticoid (kidney and distal colon) and glucocorticoid (liver and pituitary) target tissues (Whorwood et al. 1993). In-vitro, 11βHSD mRNA and activity were present in rat pituitary GH3 cells (81 % conversion of corticosterone to 11-dehydrocorticosterone/4 × 10^6 cells after 24-h incubation) and inhibited by glycyrrhizic and glycyrrhetinic acids. Oral administration of a water freeze-dried extract of *Glycyrrhiza glabra* (liquorice) at doses of 100, 250 and 500 mg/kg in rats induced dose-dependent and mostly significant decreases in the plasma concentration of cortisol, adrenocorticotropic hormone, aldosterone and potassium (K) (Al-Qarawi et al. 2002). The results suggested a strong and dose-dependent suppression of the adrenal-pituitary axis, accompanied by stimulation of renin production from the kidney. Calò et al. (2004) reported a direct, mineralocorticoid-mediated effect on the protein expression of two markers of oxidative stress after incubation of mononuclear leukocytes with 1 × 10^{-8} M aldosterone (p22(phox)/β-actin = 1.38 and PAI-1/β-actin = 1.80). The same effect was also found with 3 × 10^{-5} M glycyrrhetinic acid, the principal constituent of licorice root (p22(phox)/β-actin = 1.37 and PAI-1/β-actin = 1.80). The

findings confirmed an involvement of mononuclear leukocytes in the pathogenesis of the oxidative stress induced by hyperaldosteronism.

Studies by Kato et al. (1995) suggested that licorice-induced pseudoaldosteronism was due to an increased concentration of 3 β-D-(monoglucuronyl)18β-glycyrrhetinic acid (3MGA), but not 18β-glycyrrhetinic acid (GA), in the circulating blood of these patients. They found an increased concentration of 3MGA in 10 patients with licorice-induced pseudoaldosteronism, but not in 11 patients without pseudoaldosteronism.

Ingestion of regular moderate liquorice consumption of 100 g of liquorice daily by 30 normotensive subjects caused a significant rise in systolic blood pressure (SBP) and a fall in plasma potassium (Sigurjónsdóttir et al. 1995). In a subgroup of 13 women the consumption of 50 g of liquorice daily also caused a significant rise in SBP of 5.6 mmHg ($P<0.001$) and diastolic blood pressure of 3.4 mmHg. A significant change in the cortisol/cortisone ratio in urine was observed during 100 g liquorice consumption indicating inhibition of 11β-hydroxysteroid dehydrogenase in kidneys. In another study of healthy Caucasian volunteers, consumption of licorice in various doses, 50–200 g/day, for 2–4 weeks, corresponding to a daily intake of 75–540 mg glycyrrhetinic acid, was found to increase systolic blood pressure in a dose–response relationship (Sigurjónsdóttir et al. 2001). They found even doses as low as 50 g of liquorice (75 mg glycyrrhetinic acid) consumed daily for 2 weeks can cause a significant rise in blood pressure. They also found that patients with essential hypertension were more sensitive to the inhibition of 11β-hydroxysteroid dehydrogenase (11 beta-HSD) by liquorice than normotensive subjects, and that this inhibition caused more clinical symptoms in women than in men although the difference in the effect on the blood pressure was not significant (Sigurjónsdóttir et al. 2003).

In another study of hypertensive patients (eight men and three women, mean age 40.7 years) and healthy controls (13 men and 12 women, mean age 31.2 years), licorice consumption of 100 g liquorice (containing 150 mg glycyrrhetinic acid) daily for 4 weeks, inhibited aldosterone secretion, the degree of licorice induced inhibition of aldosterone secretion differed between the genders and was not influenced by the blood pressure levels (Sigurjónsdóttir et al. 2006). In a clinical study, six male volunteers taking daily 7 g of a commercial preparation of licorice for 7 days, corresponding to an intake of 500 mg/day of glycyrrhizic acid developed pseudohyperaldosteronism during the treatment characterized by increase of body weight, suppression of plasma renin activity and plasma aldosterone and reduction of serum potassium (Armanini et al. 1996). The authors concluded that the pseudohyperaldosteronism from licorice was initially related to decreased activity of 11β-hydroxysteroid-dehydrogenase and afterwards also a direct effect of licorice derivatives on mineralocorticoid receptors becomes evident in some cases. Results of studies by Ohtake et al. (2007) suggested that accumulation of 3-monoglucuronyl-glycyrrhetinic acid (3MGA), a metabolite of glycyrrhizin, in the plasma may be involved in the pathogenesis of pseudoaldosteronism induced by chronic glycyrrhizin treatment. Studies by Makino et al. (2012) suggested that 3MGA was actively transported into tubules through organic anion transporters (OATs), resulting in the inhibition of type 2 11β-hydroxysteroid dehydrogenase (11β-HSD2). As the plasma level of 3MGA depended on the function of hepatic transporters, monitoring 3MGA levels in plasma or urine may be useful for preventing pseudoaldosteronism when licorice or GL was prescribed to patients. Celik et al. (2012) presented a case report on an association of hypokalaemia, oedema and thrombocytopenia that was developed due to the excessive intake of licorice.

In a study of a sample of 1049 Finnish women and their healthy singleton infants in 1998, Strandberg et al. (2001) found that heavy glycyrrhizin exposure during pregnancy did not significantly affect birth weight or maternal blood pressure, but it was significantly associated with shorter gestation. Heavy glycyrrhizin intake was sufficient to double the risk of being born before 38 weeks. In another study conducted in 2000–2001 of 95 Finnish women who delivered preterm singletons, heavy licorice consumption was found to be associated with a twofold to threefold increase in the risk of pre-

term (<37 weeks) birth (Strandberg et al. 2002). However, these results were later questioned due to the retrospective collection of data and the possibility of confounding factors that might have biased the results (Hughes et al. 2003).

In a study of nine healthy women 22–26 years old, in the luteal phase of the cycle, after 2 months of administration of licorice, serum parathyroid hormone, 25-hydroxycholecalciferol and urinary calcium were increased significantly from baseline values, while 1,25-dihydroxy Vitamin D and ALP did not change during treatment (Mattarello et al. 2006). All these parameters returned to pretreatment levels 1 month after discontinuation of licorice. Plasma renin activity and aldosterone were depressed during licorice therapy, while blood pressure and plasma cortisol remained unchanged. In a clinical study of 321 Finnish children 8.1 years of age born in 1998 as healthy singletons at 35–42 weeks of gestation, Räikkönen et al. (2009) found that prenatal exposure to licorice dose-dependently predicted poorer verbal and visuospatial abilities and narrative memory as well as increased risk of externalizing symptoms, attention, rule-breaking and aggression problems in children aged 8.1 years. Their findings supported adverse foetal 'programming' by overexposure to glucocorticoids and counsel concern against consuming excessive amounts of foodstuffs containing licorice during pregnancy. Further they found that maternal prenatal licorice consumption altered hypothalamic-pituitary-adrenocortical axis (HPAA) function in children (Räikkönen et al. 2010) and may increase risk of adult disease. Their findings lend support to prenatal 'programming' of HPAA function by overexposure to glucocorticoids.

Miscellaneous Adverse Issues

All analysed samples of licorice root and derived products (licorice-confectionery, licorice block and licorice extract) were found to contain ochratoxin A, and some of them showed extremely high concentrations up to 252.8 ng/g of ochratoxin A (Arino et al. 2007). Highest levels of ochratoxin A were found in dry licorice root, averaging 63.6 ng/g, while mean contents in fresh licorice root were 9.2 ng/g. Licorice-confectionery (sweets) contained 3.8 ng/g of ochratoxin A. Ochratoxin A was also abundant in two licorice derivatives, liquid licorice extract (16.0 ng/g) and solid licorice block (39.5 ng/g). The ochratoxin levels found in licorice and derived products are higher than those reported in the literature for other food commodities. The experiments of ochratoxin A transfer into the tea beverages showed that almost 5 % of the ochratoxin A present in dry licorice root is transferred to the corresponding decoction tea, whereas only 1 % of ochratoxin A remains in infusion tea.

Traditional Medicinal Uses

Liquorice is one of the most commonly used herbs in Western herbal medicine and has a very long history of use, both as a medicine and also as a flavouring to disguise the unpleasant flavour of other medications, in cough medicines and also in the treatment of catarrhal infections of the urinary tract (Grieve 1971). Licorice is deemed emollient, expectorant, laxative, moderately pectoral and tonic (Grieve 1971; Launert 1981; Uphof 1968). Liquorice root is taken internally in the treatment of Addison's disease, asthma, bronchitis, coughs, peptic ulcer, arthritis, allergic complaints and following steroidal therapy (Bown 1995). Liquorice should be used in moderation and should not be prescribed for pregnant women or people with high blood pressure, kidney disease or taking digoxin-based medication (Bown 1995). Prolonged usage raises the blood pressure and causes water retention (Chiej 1984; Bown 1995). Externally, the root is used in the treatment of herpes, eczema and shingles (Bown 1995).

Licorice root extracts have been used in traditional Chinese, Tibetan and Indian medicine for the treatment of pulmonary diseases and inflammatory processes (Kwon et al. 2007). It can be used in the folk medicine at different parts of the world to treat many diseases including bacterial infection, cough suppression, 4 gastric ulcer treatment 5, treatment of early Addison disease

6, 7, treatment of liver disease 8, 9 and as a laxative (Nitalikar et al. 2010). Licorice is clinically used for the treatment of stomach ulcers 10, 11. Its preparations are used as a conditioning and flavouring agent in tobacco products. Aqueous extracts from the roots of *Glycyrrhiza glabra* are widely used for treatment of stomach ulcer (Wittschier et al. 2009). Glycyrrhizin has long been used in China in the treatment of various liver diseases to lower transaminases (Ren et al. 2013). Glycyrrhizin, a major component of a herb (licorice), has been widely used to treat chronic hepatitis B in Japan (Takahara et al. 1994). Licorice is the most common ingredient of traditional Japanese Kampo medicines (Hayashi and Sudo 2009). The minimum content of glycyrrhizin in these medicines should be 2.5 % according to the standards of the Japanese Pharmacopeia. Glycyrrhizin is a prescription drug used in the treatment of liver and allergic diseases in Japan. It is manufactured as an injectable preparation (Stronger Neo-Minophagen® C) and in a tablet form (Glycyron®) by a Japanese company, namely, Minophagen Pharmaceutical Co. Ltd. Stronger Neo-Minophagen® C has been available in the Japanese market for over 60 years. Glycyrrhizin, glycyrrhetinic acid and licorice extracts are used in various over-the-counter drugs, including anti-allergic and anti-inflammatory drugs. In addition, in England, the glycyrrhetinic acid derivative glycyrrhetinic acid 3-β-*O*-hemisuccinate (carbenoxolone) is a prescription drug used in the treatment of peptic ulcers.

Licorice is used for many ailments including asthma, athlete's foot, baldness, body odour, bursitis, canker sores, chronic fatigue, depression, colds and flu, coughs, dandruff, emphysema, gingivitis and tooth decay, gout, heartburn, HIV, viral infections, fungal infections, ulcers, liver problems, Lyme disease, menopause, psoriasis, shingles, sore throat, tendinitis, tuberculosis, ulcers, yeast infections, prostate enlargement and arthritis (Khalaf et al. 2010). Glycyrrhizic acid coupled with glycyrrhetinic acid and 18-β-glycyrrhetic acid was developed in China or Japan as an anti-inflammatory, antiviral and anti-allergic drug for liver disease (Li et al. 2014).

Glycyrrhizin (GL) has been used in Japan to treat patients with chronic viral hepatitis (Matsumoto et al. 2013). In Japan, glycyrrhizin therapy is widely used for more than 20 years for chronic hepatitis C and reportedly reduces the progression of liver disease to hepatocellular carcinoma (van Rossum and De Man 1998; van Rossum et al. 1999).

The roots are sweet, refrigerant, emetic in large dose, tonic, mild, laxative, aphrodisiac, haemostatic (Meena et al. 2010). They are useful in hyperdipsia, cough, bronchitis, ulceration of urinary tract, pharyngitis, epilepsy and anaemia. In the Ayurvedic system of medicine it is used in the preparations of yashtyadi churna, Yashtimadhvadya taila, Brihatashwagandha hrita, Pippalyadi taila and Vridhihara lepa.

Other Uses

Liquorice extracts (in pharmacy called succus liquiritiae) are currently used mainly in the tobacco, pharmaceutical and confectionery industries (Fenwick et al. 1990). Licorice plant yields a substance that is used for etching steel sections in photomicrographic work (Hill 1952). Extracts from the root are used as a foaming agent in beers and fire extinguishers (Bown 1995). A fibre obtained from the roots is used for insulation, wallboard, boxboard, etc. (Hill 1952; Grieve 1971). The fibres can be used after the medicinal and flavouring constituents of the root have been extracted (Grieve 1971).

The world's leading manufacturer of liquorice products is M&F Worldwide, which manufactures more than 70 % of the worldwide liquorice flavours sold to end-users. Mafco Worldwide produces a variety of licorice products from licorice root, intermediary licorice extracts produced by others and certain other ingredients (M & F Worldwide Corp 2010). Approximately 63 % of Mafco Worldwide's licorice product sales are to the worldwide tobacco industry for use as tobacco flavour enhancing and moistening agents in the manufacture of American blend cigarettes, moist snuff, chewing tobacco and pipe tobacco. Mafco

Worldwide also sells licorice products to food processors, confectioners, cosmetic companies and pharmaceutical manufacturers for use as flavouring or masking agents, including its *Magnasweet* brand flavour enhancer, which is used in various brands of chewing gum, energy bars, non-carbonated beverages, lip balm, chewable vitamins, aspirin and other products. In addition, Mafco Worldwide sells licorice root residue as garden mulch under the name *Right Dress*. Mafco Worldwide also sells licorice products worldwide to food processors, confectioners, cosmetic companies and pharmaceutical manufacturers for use as flavouring and masking agents, including its *Magnasweet* brand flavour enhancer, which is used in various brands of chewing gum, lip balm, energy bars, non-carbonated beverages, chewable vitamins, aspirin and other products.

Licorice extracts and many glycyrrhizin derivatives are widely used in the preparation of cosmetics in Japan (Hayashi and Sudo 2009). Glycyrrhizin as well as powdered *Glycyrrhiza* roots, licorice extracts, glycyrrhetinic acid, stearyl glycyrrhetinate, pyridoxine glycyrrhetinate and glycyrrhetinic acid 3-β-*O*-hemisuccinate (carbenoxolone) are used in cosmetics for their anti-inflammatory action. Furthermore, glabridin-containing glycyrrhiza flavonoids isolated from *G. glabra* are used in cosmetic preparations owing to their skin-whitening, anti-sensitizing and anti-inflammatory properties (Yokota et al. 1998).

Glycyrrhiza glabra was liquefied by ethanol and acetone in an autoclave under high pressure using potassium hydroxide or sodium carbonate as the catalyst, as well as without catalyst at various temperatures (250, 270 and 290 °C) for producing bio-oil (Durak 2014). The maximum bio-oil yield was obtained in acetone (79 %) at 290 °C without catalyst. GC–MS identified 131 and 147 different compounds in the bio-oils obtained at 270 and 290 °C, respectively.

G. glabra was found to be a good carbon source in the biological denitrification of drinking water (Ovez et al. 2006). Complete denitrification was achieved with *G. glabra*. It was found that the nitrate removal rate of *G. glabra* was 6.96 mg/L/day.

Mohammadi et al. (2014) prepared high surface area-activated carbon from *Glycyrrhiza glabra* residue by ZnCl2 activation for removal of Pb(II) and Ni(II) from water samples. High values of intra-particle rate constants calculated show the high tendency of activated carbon for removal of lead and nickel ions. Studies showed that *G. glabra* root could be used as an adsorbent of toluene vapour from gaseous media (Mohammadi-Moghadam et al. 2013). Licorice adsorbent is a waste material with a sorption capacity of 2.2 mg/g. In comparison with other natural sorbents (e.g., compost, diatomaceous earth and chaff), licorice root appeared to be a cost-effective sorbent in the removal of toluene vapour.

G. glabra foliage has potential as animal feed. Kamalak (2006) found that *G. glabra* leaves harvested at the proper stage of maturity offered considerable potential as a high quality forage for ruminant during critical period in the semi-arid and arid regions. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and condensed tannin (CT) contents of *G. glabra* leaves increased with increasing maturity whereas the crude protein decreased. Gas production, dry matter (DM) and crude protein (CP) disappearance and estimated parameters also decreased with increasing maturity. CP, ADF and CT contents ranged from 16.19 to 26.93 %, from 20.74 to 29.07 % and from 1.57 to 10.83 %, respectively. The potential gas production and metabolizable energy ranged from 65.34 to 72.12 ml/0.200 g of DM and from 10.14 to 12.12 MJ/kg DM, respectively. The effective DM degradability (EDMD) and effective CP degradability (ECPD) ranged from 58.70 to 70.59 % and from 57.32 to 73.72 %.

Comments

In Chinese Pharmacopoeia, three *Glycyrrhiza* species *Glycyrrhiza uralensis*, *G. glabra* and *G. inflata* are listed as licorice. While in Japanese Pharmacopoeia, two species *G. uralensis* and *G. glabra* are prescribed as licorice. Three varieties of *G. glabra* have been reported the Spanish and Italian licorice, assigned to *G. glabra var. typica*, Russian licorice to *G glabra var. glandulifera*, Persian and Turkish licorice to *G. glabra var. violacea* (Nomura et al. 2002).

Commercial licorice is derived from three *Glycyrrhiza* species, *G. glabra* L., *G. uralensis*, Fisch., and *G. inflata* Batal. in the family Fabaceae, which are indigenous to Asia and the Mediterranean region (Shibata 2000).

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