Standardization, Quality Control and Pharmacological Review on *Glycyrrhiza glabra* L.

A Potential Medicinal Herb in Unani and Ayurvedic Systems of Medicine

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Glycyrrhiza glabra L. is a potential herbal drug used in Unani and Ayurvedic systems of medicine. In view of the medicinal and pharmacological importance of this species in different systems of medicine, an attempt has been made to provide comprehensive information on standardization and quality control of the herb based on WHO guidelines. The important parameters for standardization and quality control are sensory, macroscopic, microscopic and chemical characteristics along with the physico-chemical characteristics (moisture content, successive extractive value, ash value, acid insoluble ash, water soluble extractive value, acid value, iodine value) and TLC finger prints of different extractives. At the same time, chemical constituents, properties and its uses, pharmacology, precautions and toxicity are discussed based on literature review.

Keywords: *Glycyrrhiza glabra*, Standardization, Quality control, Chemical constituents, Properties and uses, Pharmacology, Precautions and toxicity.

Introduction

The medicinal plants are an important resource for all major systems

of medicine or health care and it is being globally recognized that medicinal plants play a significant role in providing health benefits to human beings. The medicinal plant-based drugs have the added advantages of being simple, effective and offering a broad spectrum of activities with an emphasis on the preventive action of drug. So, the demand of plant-based medicines and their eventual commercialization are increasing rapidly. With the increasing popularity of botanical medicine, the safety, efficacy and quality of the herbs are of major concern all over the world.

Glycyrrhiza glabra L. is a reputed herbal drug in Unani and Ayurvedic systems of medicine for thousands of years. In Chinese traditional medicine, it is a drug of the first class. In Greek and Roman medicine its root was frequently used, later expanded in Europe (Mohammed Said, 1996). This herb belongs to the family Fabaceae, and is commonly referred as *Jastimadhu* or *Yashtimadhu* in Bengali and liquorice or licorice in English. Similarly in Unani system of medicine, it is also referred by names 'Aslus-sus, Mulathee, Muleti, Mulhatti' and in Ayurvedic system of medicine; it is referred by name 'Madhuk' (BNUF, 2011; BNAF, 2011).

In Chinese and Indian traditional medicine, the root of this herb is considered as hot and dry, suppurative, demulcent and lenitive, relieving thirst and removing unhealthy humours; also diuretic and emmenagogue, useful in cough, hoarseness, asthma and irritable conditions of the bronchial passages, emollient, gentle laxative and local stimulant. Also recommended as a flavouring agent, and it enters into composition of many external cooling applications (Mohammed Said, 1996). This popular medicinal plant is also enlisted and described as an active ingredient in about 43 formularies of National Formulary of Unani Medicine and about 82 formularies of National Formulary of Ayurvedic Medicine of Bangladesh (BNUF, 2011; BNAF, 2011). Besides many Unani, Ayurvedic, other folk physicians also use it to treat various diseases and ailments. The medicinal properties and uses of *G. glabra* is also supported by various modern scientific studies.

In Bangladesh, there about 297 Unani, 204 Ayurvedic and 77 Homeopathic drug manufacturing industries (Rafiqul Islam, 2003), where *G. glabra* is used as active ingredient in producing medicines. However, as per published scientific literature review, no reliable experimental research documents for standardization and quality control methods of *G. glabra* in Bangladesh has been accomplished in broader extent. So, there is a wide gap regarding quality control of

medicinal herbs, particularly for *G. glabra* in Unani, Ayurvedic and Homeopathic drug manufacturing industries of Bangladesh. Beside this, due to its popularity for potential therapeutic value, there is a possibility for adulteration with other morphologically similar herbs like Indian Liquorice (*Abrus precatorius* L.), which is also known as *Kunch* or *Ratti* in Bengali.

Thus, the main urge behind this research and review is to establish quality standard methods for standardization and control the quality of *G. glabra* along with its safe use through supportive scientific studies on pharmacology and other medicinal properties.

In present paper an attempt has been made to identify, standardize and control the quality of this herb. An effort has been made to find out sensory, macroscopic, microscopic, chemical along with physico-chemical characteristics (moisture content, successive extractive value, ash value, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value) and TLC finger prints of different extractives of *G. glabra* based on WHO guidelines. At the same time chemical constituents, medicinal properties and uses, traditional uses, pharmacology, safety precautions, toxicity, storage and packaging of *G. glabra*, which are imperative for optimal and safe utilization of this herb based on literature review are discussed.

Botanical Description of the Herb

The plant is an herbaceous perennial. It is 1 to 2 m high and has a long sturdy primary taproot. The taproot is 15 cm long and subdivided into 3 to 5 subsidiary roots, 1.25 m in length. There are several horizontal woody stolons which may reach 8 m. New stems are produced every year. They are sturdy, erect, branched either from the base or from further up, and are generally rough at the top. The foliage leaves are alternate, odd pinnate and 10 to 20 cm long. The leaflets are in 3 to 8 pairs. The stipules are very small and drooping. The axillary inflorescences of flowers are upright, spike-like and 10 to 15 cm long. The individual flowers are 1 to 1.5 cm long, bluish to pale violet and short-pedicled. The calyx is short, bell shaped and glandular-haired. The tips of the calyx are longer than the tube, and are pointed lanceolate. Petals are narrow, the carina petals are not fused, and they are pointed but not beaked. The fruit is a pod, 1.5 to 2.5 cm long, and 4 to 6 mm wide. It is erect and splayed, flat with thick sutures, glabrous, somewhat reticulate-pitted, and usually has 3 to 5 brown, reniform seeds

(WHO, 1999) (Fig. 1). The Fig. 1 shows fresh plant, its flowers, fruits and small pieces of root used as drug.

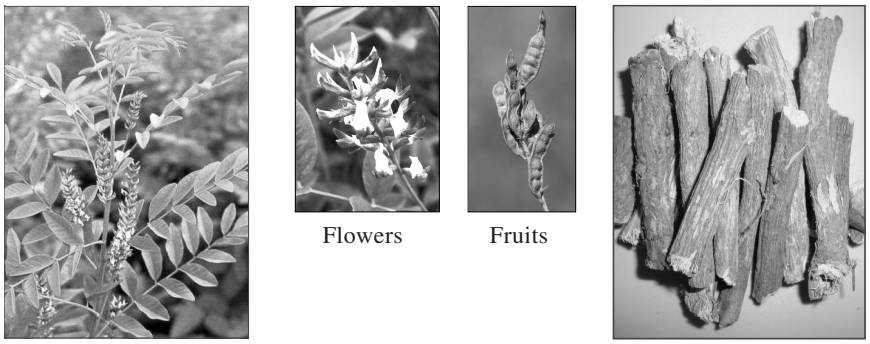


Fig. 1
Glycyrrhiza glabra L.

Distribution

The licorice bush is native to central and south western Asia and the Mediterranean region. It is cultivated in the Mediterranean is basin of Africa, in South Europe and in India (WHO, 1999).

Part/parts used as drug: Root (underground rhizome) (BNUF, 2011; BNAF, 2011; Mohammed Said, 1996; MUM, 2003).

Form and condition when received: Dried root (underground rhizome).

Temperament: Normal balanced; warm and dry/moist in first order (Mohammed Said, 1996; MUM, 2003).

Materials And Methods

The roots of *G. glabra* were collected from the raw materials section of Hamdard Laboratories (Waqf) Bangladesh. The specimen No. HMSRS-017, was preserved in the Herbarium of Hamdard Laboratories (Waqf) Bangladesh for record. The roots of the herb

dried under shade and processed using standard methods (Trease and Evans, 1996). For microscopic characterization, digital microscope was used. The powdered materials were used for chemical, physico-chemical characterization and successive extraction. Standard procedures from different pharmacopoeias were opted for physico-chemical studies (BHC, 1992; BHP, 1997; Chinese Herbal Pharmacopoeia, 1997; Hamdard Pharmacopoeia, 1997; WHO, 1993; WHO, 1998; IHP, 1999). All the extractives were evaporated by using a rotary evaporator. To develop Thin Layer Chromatogram, preparative TLC plates (precoated silica gel 60 F_{254}, Merck KGaA, Germany) were used. All the chemicals used were analytical grade and manufactured by Merck, Germany.

Preparation of Sample for TLC

A sample (15 g) of powdered root of *G. glabra* was weighed and poured into a round-bottom flask, approximately 100 ml of 95% (v/v) methanol was added, and the mixture refluxed on water bath for 30 min followed by filtration. Another 100 ml of 95% methanol was added to the mark, and the sample was refluxed for a further 30 min and then filtered. The alcoholic extracts were combined and evaporated on a rotary evaporator. To the resulting semi-solid, 01 ml of 50% (v/v) methanol was added and this sample solution was submitted for TLC analysis.

Result And Discussion

Sensory Characteristics

Colour : Greyish brown (inside bright yellow)
Odour : Characteristic
Taste : Sweet

Macroscopic Characters

The supplied root pieces are cylindrical, unbranched and hard. Externally, the bark is brownish grey to dark brown, longitudinally wrinkled. The peeled root is yellow, smooth and fibrous as depicted in Fig. 1.

Microscopic Characters

[*To make the root sample soft for section cuttings, it should be soaked into water for 6 hours*].

The transverse section of root samples of *G. glabra* were prepared and following microscopic characters were observed under the electronic microscope:

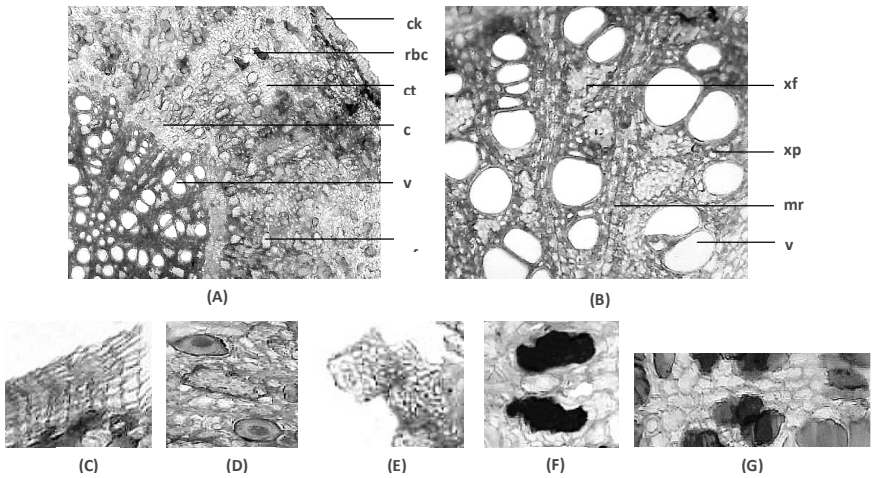


Fig. 2

Microscopy of *G. glabra* – (A) TS of root [4×] (B) Xylem with medullary rays [10×] (C) Multiple layer of cork cells (D) Reddish-brown contents (E) Yellow parenchyma cell with prisms of calcium oxalate (F) Medullary rays in outer cambial region and (G) Cell with starch grains.

[**ck**- cork, **ct**- cortex, **c**- cambium, **pf**- phloem fibre, **xf**- xylem fibre, **v**- vessel, **xp**- xylem parenchyma, **mr**- medullary ray, **rbc**- reddish-brown content].

Transverse Section of Root

The TS of root showed following features:

1. Cork consists of several layers of flattened polygonal thin-walled cells arranged in regular rows
2. Cortex is wide and contains some reddish-brown contents. Numerous yellow parenchyma cells also contain isolated prisms of calcium oxalate
3. Phloem is wide, yellow and radially arranged with smaller bundles of fibers outside the cambial region
4. Xylem distinctly radiates with medullary rays and consists of strands of large vessels that are pitted and reticulately thickened walls
5. Xylem parenchyma is small, pale yellow and mixed with vessels
6. Groups of fibers are similar to those of the phloem but more lignified and surrounded by crystal-sheath
7. Some parenchyma cells contain small round or oval starch granules
8. Pith is absent

Characteristics of Powder

Macroscopic: The powder is light yellow in colour along with fibrous particle (Fig. 3).

Microscopic: When the powder of root is observed under microscope, it showed fragments of the fibers accompanied by crystal-sheath, vessel containing crystals of calcium oxalate and long, numerous simple oval, round or fusiform starch granules that are free or in parenchyma cells.

Fig. 3
G. glabra root powder

Reactions with Different Chemicals on Powder

Powder (200 mg) of dried root is treated with few drops (20-25) of different chemical reagents and it showed following characters:

Reagent	Reaction/Observation	Photograph
i) H_2SO_4 (conc.)	The original colour of powder changes from light yellow to reddish brown and produced dark foam on the of solution. Fig. 4.	
ii) HNO_3 (conc.)	The original colou powder changes from light yellow to or and produces foa the top of solutio..	
iii) NaOH (5%)	The original colour of powder changed from light yellow to greenish yellow and precipitation may also occur	
iv) $FeCl_3$ (5%)	The original colour of powder changes from light yellow to dark brown and precipitation may also occur	

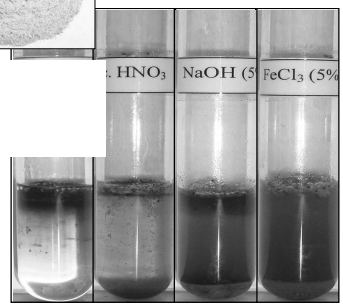


Fig. 4

Physico-chemical Analysis

i)	Moisture content (by loss on drying at 105°C)	: < 5.0% w/w
ii)	Ash content	: < 4.0% w/w
iii)	Acid insoluble ash	: < 2.0% w/w
iv)	Alcohol soluble extractive value	: > 25% w/w
v)	Water soluble extractive value	: > 20% w/w

Thin-layer Chromatography (TLC) of G. glabra

TLC Silica gel 60 $F_{254,}$ Merck KGaA (Germany) was used as absorbent. The dichloromethane: methanol (10:1) served as mobile phase.

At first UV light (at 254 nm and 366 nm) and than iodine vaporswere used to visualize the spots in the developed TLC plates or chromatograms.

R_f Value as Detected by TLC

In Fig. 5(A) the TLC examination of sample under UV light at 254 nm shows two major spots with R_f values of 0.45 and 0.55, respectively and under 366 nm shows four major spots with R_f values of 0.31, 0.4, 0.55 and 0.6 in Fig. 5(B) with tailing from base line. The sample in iodine vapors shows two major spots with R_f values of 0.45 and 0.55 in Fig. 5(C).

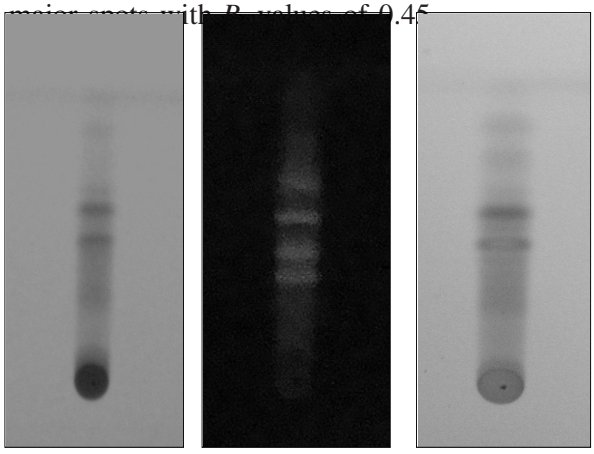
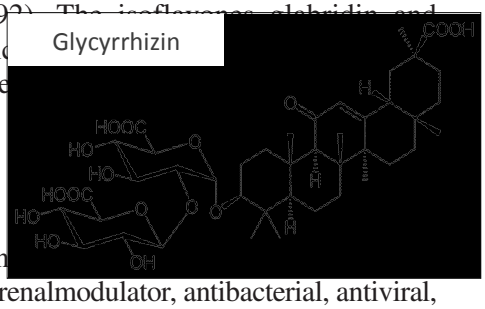


Fig. 5
TLC chromatograms of the root sample of *G. glabra*
(A) UV light at 254 nm; (B) UV light at 366 nm; and
(C) Iodine vapors

Major Chemical Constituents

A number of components have been isolated from *G. glabra* root including a water-soluble, biologically active complex that accounts for 40-50% of total dry material weight. This complex is composed of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts and various other substances (Obolentseva *et al*., 1999.). Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of *G. glabra* root. This compound represents a mixture of potassium-calcium-magnesium salts of glycyrrhizic acid that varies within a 2-25% range. Among the natural saponins, glycyrrhizic acid is a molecule composed of a hydrophilic part, two molecules of glucuronic acid, and a hydrophobic fragment, glycyrrhetic acid (Obolentseva *et al.*, 1999.). The yellow colour of licorice is due to the flavonoid content of the plant, which includes liquiritin, isoliquiritin (a chalcone) and other compounds (Yamamura *et al*., 199[illegible]. The isoflavanes glabridin and hispaglabridins A and B have signifi[illegible] 1997) and both glabridin and glabrene [illegible] *et al*., 2001).



Properties and Uses

Cooling, demulcent, expectoran[illegible] local stimulant, anti-inflammatory, adrenalmodulator, antibacterial, antiviral, antimutagen, anti-allergenic, nutritive, antioxidant, estrogenic, immunomodulator, antiulcer, antispasmodic, anti-tussive, antidote, hepatoprotective, antiarrhythmic, antiepileptic, antidepressant, emmenagogue, antiasthmatic, nervine tonic and antipyretic (Mohammed Said, 1996; MUM, 2003).

It is prescribed for the treatment of asthma, bronchitis, hepatitis, peptic-ulcer, irritation of the larynx; useful for piles, fevers, lung affections, administered for the treatment of hoarseness. It is also prescribed for the treatment of duodenal ulcer, respiratory infections, dry cough, catarrh, tuberculosis, urinary tract infections; regarded as cancer remedy in a number of countries. It has been used to treat adrenal insufficiency (MUM, 2003).

The root is prescribed for the treatment of urine retention, in incipient

loss of sight, in diseases of eyelids; removes biliousness, improves taste, heals ulcers, wounds, lessen thirst, useful for leprosy, anaemia and fatigue. It is prescribed in combination with other drugs for the treatment of respiratory, spleen and hepatic disorders; being nerve stimulant also prescribed for the treatment of nervous disorders; regarded as useful for impotency and piles (MUM, 2003).

Traditional Uses

In Greco-Arab medicine it is reputed to cure hoarseness of voice, asthma, irritation of the larynx; largely employed for relieving sore throat. It is much used for flavouring medicinal decoctions and as base for pills. In coughs and catarrhal affections of the throat and pulmonary mucus membrane, also in dysuria and oedema of the belly due to urinary trouble, it proves useful. The compound liquorice powder is a mild laxative owing to senna and sulphur contents (Mohammed Said, 1996).

Pharmacology

Peptic Ulcer

Licorice has been used as a demulcent and emollient for 2000 years to promote the healing of ulcers by acting on the mucosal layer. Glycyrrhizin (as carbenoxolone sodium) speeds healing of gastric ulcers and protects against aspirin-induced damage to the gastric mucosa. In a double-blind, placebo-controlled study, 70 patients with endoscopically-confirmed gastric or duodenal ulcers were given carbenoxolone sodium 300 mg or placebo daily during the first seven days, followed by 150 mg daily over the next 3-5 weeks. The authors concluded the carbenoxolone group increased pH at the stomach antrum from 1.1-6.0 and a reduction in basal and histamine-induced gastric acid at pH 3 and 5. Overall, 70 percent of ulcers in the glycyrrhizin group healed within 3-5 weeks of beginning therapy, compared to 36% employing placebo (Longinov *et al*., 1980).

A two year follow-up trial comparing the two therapies in the prevention of gastric ulcer recurrence noted the outcomes were similar, with a reported relapse rate of 29% (9/31) in the Caved S group and 25% (8/32) in the cimetidine group (Morgan *et al*., 1985).

Other clinical trials demonstrated the effectiveness of DGL for gastric ulcer (Turpie *et al*., 1969; Glick, 1982). A four week clinical trial by Turpie *et al.* demonstrated a statistically significant greater reduction in ulcer size in patients receiving 760 mg of a DGL preparation compared to placebo (Turpie *et al*., 1969).

Helicobacter pylori infection is prevalent in individuals with peptic ulcer and is also a known risk factor for gastric cancer (Parsonnet, 1996; Peterson, 1991). Consequently, effects of licorice flavonoid components showed promising anti *H. pylori* activity against clarithromycin- and amoxicillin-resistant strains. As the antimicrobial property seems to be attributed to the flavonoid constituents of licorice, DGL preparations may provide therapeutic benefit against *H. pylori* infection (Fukai *et al*., 2002).

Other studies have also demonstrated DGL's beneficial effects in healing of duodenal ulcers. In a trial of 40 patients receiving either 3.0-4.0 g DGL daily for eight weeks, showed significant improvement after 5-7 days. Patients were assessed for relief from epigastric pain, nausea, vomiting, x-ray of ulcer craters to determine changes in size, and frequency of relapse (return of ulcer pain for two days per week). Patients receiving the higher DGL dose showed more improvement (Tewari *et al*., 1973). In a larger study of 874 patients with chronic duodenal ulcers, patients received DGL, cimetidine or antacids. Regardless of treatment type 91% of all ulcers healed. Differences among treatment groups were not statistically significant, but patients in DGL group experienced fewest relapses (Kasir, 1985).

Aphthous Ulcers

In a double-blind, placebo-controlled trial, 24 patients with recurrent aphthous ulcers were randomly allocated to consume 2 g glycyrrhizin (carbenoxolone sodium) in 30 ml of warm water or a placebo three times daily following meals for four weeks. In contrast to the placebo group, the use of the oral licorice mouthwash significantly reduced the average number of ulcers per day, pain scores and also the development of new ulcers (Poswillo *et al*., 1984). In another study on 20 patients using DGL mouthwash four times daily, 15 experienced around 50-75% clinical improvement after only one day with complete healing of canker sores after 3 days (Das *et al*., 1989).

Chronic Hepatitis

In Japan, glycyrrhizin has been used for more than 60 years as a treatment for chronic hepatitis C. Stronger Neo-Minophagen C (SNMC), a glycyrrhizin preparation, has been extensively used with considerable success. In two clinical trials, it significantly lowered aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyltransferase (GGT) levels, while simultaneously ameliorating histological evidence of necrosis and inflammatory lesions in the liver

(Van Rossum *et al*., 2001; Tsubota *et al*., 1999). In recent years, several studies supported this action (Van Rossum *et al*., 1999; Su *et al*., 1984). Presently, interferon (IFN) therapy is a predominant treatment for chronic hepatitis however, its efficacy is limited, therefore an alternative treatment is desirable. SNMC has profound effects on the suppression of liver inflammation and is effective in improving chronic hepatitis and liver cirrhosis with considerably fewer side effects than IFN.

In a double-blind, randomized, placebo-controlled trial investigating IV infusions of SNMC, short-term efficacy of licorice was confirmed with regard to ALT levels. The study demonstrated the need for daily administration of SNMC, which may be impractical for patients. It was noticed that after cessation of therapy the ALT-decreasing effect of licorice disappeared, suggesting the need for its long-term administration (Van Rossum *et al*., 2001).

Oral Lichen Planus

Patients with chronic hepatitis C often experience oral lichen planus, an inflammatory disease characterized by lymphocytic hyperkeratosis of the oral mucosa. It is rarely cured and effective treatments are limited. In an open clinical trial, 17 hepatitis C-positive patients with oral lichen planus were given either routine dental care or 40 ml glycyrrhizin daily for one month. Among nine patients taking glycyrrhizin, six (66.7%) showed improved clinical symptoms, such as decreased redness, fewer white papules and less erosion of the mucosa. In the non-glycyrrhizin group of eight patients, only one (14.3%) reported any improvement (Da Nagao *et al*., 1996).

Other Viral Illness

The licorice reported to inhibit growth and cytopathology of many unrelated DNA and RNA viruses, while not affecting cell activity or cellular replication (Pompei *et al*., 1979).

Hepatitis A virus (HAV) causes acute hepatitis, a major public health concern in numerous countries. In human hepatoma cell line glycyrrhizin completely suppressed the expression of the HAV antigen. In comparison to ribavirin (an antiviral agent used to treat hepatitis), glycyrrhizin appeared to be in 10 times more potent in reducing infectivity of HAV, as measured by reduction in viral titers. Glycyrrhizin also exhibited 5-fold greater cell selectivity than ribavirin and was less cytotoxic to the hepatoma cells. These results indicates glycyrrhizin may be a potential therapeutic adjunct in fighting HAV infections (Crance *et al*., 1990).

Licorice and its constituents, specifically glycyrrhizin, showed antiviral activity against *Herpes simplex* and capable of irreversibly inactivating the virus (Partridge *et al*., 1984; Hirabayashi *et al*., 1991; Pompei *et al*., 1980). Glycyrrhizin also inhibited viral replication and infectivity of HIV (Ito *et al*., 1988; Pompei *et al*., 1980), herpes zoster (Aikawa *et al*., 1990), *Varicella zoster* (Baba *et al*., 1987) and CMV (Numazaki *et al*., 1994; Numazaki *et al*., 1993; Numazaki *et al*., 1998).

In a case report a 2% topical application of glycyrrhizic acid cream (carbenoxolone sodium) applied six times daily in 12 patients with acute oral hepatic (*Herpes simplex*) infections resolved pain and dysphasia within 24-48 hours. Moreover, the accompanying ulceration and lymphadenopathy gradually healed within 24-72 hours (Partridge *et al*., 1984).

A clinical study of three HIV patients with hemophilia investigated the effect of glycyrrhizin on HIV replication. The HIV p24 antigen was detected in all patients at the beginning of treatment courses. At the end of one month glycyrrhizin (400-1600 mg) on six separate occasions, p24 antigen level either decreased significantly or became negative. Tapering of the glycyrrhizin dose resulted in an immediate elevation in p24 antigen level, suggesting that at higher doses it is responsible for reducing antigen levels, probably via suppressing viral replication (Hattorri *et al*., 1989).

In a clinical trial of 31 patients with severely painful herpes zoster lesions, 12 patients were given glycyrrhizin (20 mg) on six separate occasions. The remaining 19 patients received zoster immune gamma-globulin, recombinant interferon-˜, or acyclovir. Glycyrrhizin ranked next to acyclovir for pain resolution at the end of a month therapy (Aikawa *et al*., 1990).

CMV is the most common cause of congenital and perinatal viral infections throughout the world. It manifests with profound liver dysfunction and poor weight gain. In a series of studies, preparations of licorice (SNMC) were administered to infants with CMV. Liver dysfunction and weight gain improved in nearly all cases compared to groups without treatment (Numazaki *et al*., 1994; Numazaki *et al*., 1993; Numazaki *et al*., 1998).

Hepatocellular Carcinoma

In a retrospective study, long-term licorice administration for hepatitis C infection was effective in preventing hepatocellular carcinoma (HCC). Four hundred fifty-three patients diagnosed with hepatitis C divided into three groups and received either licorice (SNMC) at a dose of 100 ml daily for two month, or other natural treatments, such as vitamin K. The

remaining group of patients was treated with a wider number of agents including SNMC, corticosteroids and immunosuppressive agents; as a result of the mixed medication regimen, this group was excluded from the study. After 10 years, analysis of the results showed 30/84 patients (35.7%) employing SNMC had normalized AST levels, compared with seven patients (6.4%) not treated with IV SNMC. Moreover, the 10 and 15 year appearance rate of HCC was 7 and 12% in the treated group compared to 12 and 25% in the untreated group, respectively (Arase *et al*., 1997). The literature on HCC and the use of SNMC confirmed that glycyrrhizin not only decreases ALT levels but also improves liver histology and decreases incidence of hepatic cirrhosis (Kumada *et al*., 2002).

Antitussive and Demulcent

The liquorice powder and extract was found to be useful for the treatment of sore throat, cough and bronchial catarrh. It is antitussive and expectorant loosening and helping to expel congestion in the upper respiratory tract as it accelerates tracheal mucus secretion (Hikino, 1985). The demulcent action is attributed to glycyrrhizin. It has been recently found that liquiritin apioside is an active compound in the methanolic extract of liquorice that inhibited capsaicin-induced cough (Kamei *et al*., 2003).

Thrombin Inhibitor

Glycyrrhizin, an already known anti-inflammatory compound, is the first plant based inhibitor of thrombin. It prolonged the thrombin and fibrinogen clotting time and increased plasma recalcification duration. The thrombin-induced platelet aggregation was inhibited but platelet aggregating factor (PAF) or collagen-induced agglutination was not affected by glycyrrhizin (Mendes-Silva *et al*., 2003; Mauricio *et al*., 1997).

Antimicrobial

Multi-drug resistant micro-organisms pose a serious global threat in clinical medicine today due to the rapid spread as well as chronic infections caused by them. Each species of the genus *Glycyrrhiza* is characterized by isoprenoid phenols, which have selective antimicrobial spectrum. Glabridin, glabrene and glabrol are the main phenols residing in *G. glabra* root. Glicophenone and glicoiso-flavanone are two new phenolic compounds having potential activity to control methicillin resistant

Staphylococcus aureus (MRSA) (Li *et al*., 1998; Hatano *et al*., 2000). Effective minimal inhibitory concentration (MIC) of licochalcone A is (16 µg/ml) for MRSA and (3 µg/ml) for food borne pathogens like *Clostridium* spp. and *Bacillus subtilis* (Hansen *et al*., 2001). Some compounds like glabridin, glabrene, licochalcone A, licoricidin and licoisoflavone B inhibited the growth of clarithromycin and amoxicillin resistant *H. pyroli* (Fukai *et al*., 2002). Further the mechanism of antimicrobial activity of licochalcone A is via inhibition of NADH cytochrome C reductase in the bacterial respiratory electron transport chain (Tsukiyama *et al*., 2002). *G. glabra* extracts have also been evaluated and implicated in oral mouth washes against oral pathogens and oral candidal thrush (Katsura *et al*., 2001; Motsei *et al*., 2003). A variety of compounds isolated from *Glycyrrhiza* include glabridin, gabrin, glabrol, glabrene, hispaglabridin A, hispaglabridin B; 40-methylglabridin and 3-hydroxyglabrol exhibited potential *in vitro* antimicrobial activity (Mitscher *et al*., 1980; Haraguchi *et al*., 1998). Glycyrrhizinic acids have been used as a cure for atopic dermatitis, pruritis and cysts due to parasitic infestations of skin (Seedi *et al*., 2003).

Antiviral

There is an ever increasing database of natural products in treating and preventing medical problems. Plant purified chemicals have been subjected to extensive screening but a few studies have been initiated on crude plant materials and herbal preparations to be used in healthcare systems. Glycyrrhizin has a prominent antiviral activity, as it does not allow the virus cell binding (Agarwal *et al*., 1991; Badam *et al*., 1997; Cinatl *et al*., 2003). β-Glycyrrhizic acid has been found to inhibit HIV-1 reproduction in MT-4 cells (Badam *et al*., 1994; Pliasunova *et al*., 1992). Replications of flaviviruses like the Japanese encephalitis virus, yellow fever virus were inhibited by high non-cytotoxic concentrations. Recently, antiviral activities of ribavirin, 6-azauridine, pyraziofurin, mycophenolic acid and glycyrrhizin against two clinical isolates of SARS (Severe Acute Respiratory Syndrome) virus (FFM-1 and FFM-2) from patients with SARS, admitted to clinical center of Frankfurt University, Germany were evaluated and glycyrrhizin was most effective in controlling viral replication and hence could be used as a prophylactic agent (Cinatl *et al*., 2003). Glycyrrhizin has been previously used to treat patients suffering from HIV-1 and chronic hepatitis C virus (Badam *et al*., 1997; Badam *et al*., 1994; De Clercq *et al*., 2000). Thus, it would valuable to design pharmacophores of glycyrrhizin for use as an antiviral drug.

Antioxidant and Anti-inflammatory

Glycyrrhiza root powder exhibited a marked hepatoprotective action by antioxidant activity against ascorbate dependent oxidation of endogenous polyenic lipids in rat liver (Konovalova *et al*., 2000). A variety of phytochemicals present in root extracts of *Glycyrrhiza* exhibit a potential antioxidant activity. Licochalcones B and D exhibited a potential activity by inhibiting the microsomal lipid peroxidation. Retrochalcones exhibit mitochondria lipid peroxidation and prevented red blood corpuscles from oxidative hemolysis. Isoflavones like glabridin, hispaglabridin A and 3-hydroxy-4-O-methylglabridin present in *Glycyrrhiza* demonstrated potential antioxidant activity in NADH dependent peroxidation injury (Haraguchi *et al*., 1998; Belinky *et al*., 1998). More recently dehydrostilbene derivatives like α, α-Dihydro-3, 5,4-trihydroxy-4, 5-diiodopentenylstilbene isolated and reported to possess antioxidant activity (Biondi *et al*., 2003).

Antidiabetic

Type 2 (non-insulin dependent) diabetes mellitus, an insulin resistant syndrome, is a growing heath concern in the modern society. Peroxisome proliferation activated receptors (PPAR's) are ligand dependent transcriptional factors regulating the expression of a group of genes that play an important role in glucose and lipid metabolism. The PPAR receptors are classified as PPAR-α, γ and δ. The PPAR-α in liver, muscle and kidney. PPAR-γ is associated with adipose tissue, adrenals and small intestine, whereas PPAR-δ is expressed ubiquitously. PPAR-γ serves as a predominant target for insulin sensitizing drugs like pioglitazone and roziglitazone. Ethyl acetate extract of licorice using GAL-4-PPAR-γ chimera assay, exhibited a significant PPAR-γ binding activity which was attributed to six phenolic compounds, viz. dehydroglyasperin, glyasperin B, glyasperin D, glycycoumarin, glycyrin, glycrol and isolglycyrol (Kuroda *et al*., 2004). Pioglitazone and glycyrin suppressed the increased blood glucose level in mice after sucrose loading during the oral sucrose tolerance test. Pioglitazone, a potent PPAR-γ ameliorated the insulin resistance and type-2 diabetes mellitus. Similarly glycyrin also exhibited a potent PPAR-γ ligand binding activity and therefore reduced the blood glucose level in knockout diabetic mice (KK-A^y). This finding is of much significance as licorice has also been traditionally used as an artificial sweetening agent and could be helpful in insulin resistance syndrome prevalent in the modern society. Glycyrrhizin also exhibited anti-diabetic activity in non-insulin dependent diabetic model (Takii *et al*., 2000).

Hepatoprotective

Chronic hepatitis (viral as well as non-viral) is a slowly progressive liver disease that may evolve into cirrhosis with its potential complications of liver failure or hepatocellular carcinoma. Current therapy with the alpha-interferon is directed as viral clearance, but sustained response is only achieved in 20-40% of patients without cirrhosis and is less than 20% in patients with cirrhosis who have greatest need of therapy. In Japan, glycyrrhizin has been used for more than 60 years as treatment for chronic hepatitis under the name of Stronger Neo-Minophagen C (SNMC) clinically as an anti-allergic and anti-hepatitis agent (Acharaya *et al*., 1993). Glycyrrhizin induced a significant reduction in serum aminotransferases and improved the liver histology compared with the placebo. It has also been implicated that long-term usage of glycyrrhizin prevented development of hepatocellular carcinoma in chronic hepatitis C. *In vitro* studies have indicated that glycyrrhizin modifies the intracellular transport and suppressed hepatitis B virus (HBV) surface antigen (HbsAg) (Sato *et al*., 1996; van Rossum *et al*., 1998). The 18 β-glycyrrhetinic acid (GA), an aglycone of glycyrrhizin decreased the expression of P450 E1 thereby protecting the liver (Jeong *et al*., 2002). GA also prevented the oxidative and hepatic damage caused by aflatoxins by increasing the CYP1A1 and glutathione-S-transferase activities and may also contribute to anti-carcinogenic activity by metabolic deactivation of the hepatotoxin (Chan *et al*., 2003). The glycyrrhizin and its analogues showed mitogenic effect via epidermal growth factor receptors subsequently stimulating the MAP (mitogen activated protein) kinase pathway to induce hepatocyte DNA synthesis and proliferation (Kimura *et al*., 2001).

Anticancer

Herbal therapies are being looked at with much hope to combat cancers despite little understanding of the biological diversity underneath. *G. glabra* extract used in herbal formulations for combating cancers like PC-SPES, a polyherbal composition used against prostrate cancer. The licorice extract induced Bcl2 phosphorylation and G2/M cycle arrest in tumour cell lines similar to clinically used anti-microtubule agent paclitaxel. The 1-(2,4-dihydroxyphenyl)-3-hydoxy-3-(4-hydroxyphenyl) 1-propanone (β-hydroxy-DHP) was identified in the licorice extract, induced Bcl2 phosphorylation in breast and prostate tumour cells, G2/M cell cycle arrest and apoptosis using Annexin V and TUNEL assay along with reduced cell viability by tetrazolium (MTT) assay, and also altered microtubule structure (Rafi *et al*., 2002). Methanol soluble fraction (70%)

of licorice acetone extract induced apoptosis in human monoblastic leukaemia U937 cells. The compound was identified to be licocoumarone also responsible for antioxidant and antimicrobial activity (Watanabe *et al*., 2002). Activator protein-1 (AP-1) a nuclear transcription factor. Blocking of tumour promoter induced AP-1 activity could be used to arrest the induced cellular transformation. The glycyrrhizin induced AP-1 activity in untreated cells, whereas inhibited TPA (12-O-tetradecanoylphorbal-13-acetate) induced AP-1 activity in TPA treated cells. This mechanism could serve as a model for development of new chemo-protective agents (Hsiang *et al*., 2002).

Drug Delivery Agent

Transdermal delivery of drugs is an attractive proposition as it has several advantages over the intravenous or oral administration due to sensitivity of drugs. The principal barrier is the stratum corneum , the outermost layer of the skin comprising keratin rich cells embedded in multiple layers. A common or simple approach would be to enhance the permeability of the skin by use of penetration enhancers or accelerators, which could induce a reversible permeability of stratum corneum. The glycyrrhizin extracted from *Glycyrrhiza glabra* var. *glandulifera* (roots) enhanced the percutaneous absorption of diclofenac sodium using excised abdominal rat skin. The results showed that the efficiency of glycyrrhizin as an enhancer agent is greater in gel formulations than in the emulsions. The enhancer with the concentration of 0.1% w/w in gel increased diclofenac sodium flux value to 10 fold compared with the control gel (Nokhodchi *et al*., 2002). Liquiritigenin and davidigenin reported to be drug delivery agents due to appreciable transepithelial flux through human intestinal epithelial cell lines (Caco-2) (Asano *et al*., 2003).

Other Therapeutic Considerations

In a trial of 15 normal-weight subjects (7 males, 8 females, age between 22-26), of a commercial licorice preparation (3.5 mg) daily for two months reduced body fat mass. Plasma renin activity and aldosterone were also suppressed with no changes in body mass index were noted. These results indicate licorice and its constituents can reduce body fat by inhibiting 11-β-hydroxysteroid-dehydrogenase in fat cells (Armanini *et al*., 2003).

The total serum testosterone in nine healthy women, age between 22-26, was decreased from 27.8±8.2 to 9.0±9.4 ng/dl after one month, and further declined to 17.5±6.4 ng/dl after the second month of therapy

with licorice. This is likely due to inhibition of 17-hydroxysteroid dehydrogenase; indicating licorice may be of benefit in treating women with hirsutism and polycystic ovary syndrome (Armanini *et al*., 2004).

Several animal and *in vitro* studies indicated glycyrrhizin and its constituents possess anticarcinogenic activity against a variety of cancers, warranting further investigation in clinical trials (Tamir *et al*., 2000; Shiota *et al*., 1999; Liu *et al*., 1998; Nishino *et al*., 1984).

Studies also showed licorice constituents to be effective in the treatment of eczema (Evans, 1958), melasma (Amer *et al*., 2000), eosinophilic peritonitis (Takeda *et al*., 1991), postural hypotension (Basso *et al*., 1994), erosive gastritis (Kolarski *et al*., 1987) and as anti-malarial (Chen *et al*., 1994) and anti-leishmanial agents (Christensen *et al*., 1994). More recently, aqueous extracts of *G. glabra* demonstrated memory-enhancing activity via reversal of chemically-induced amnesia, as measured by maze and passive avoidance testing in mice (Parle *et al*., 2004).

Mechanism of Action

The beneficial effects of licorice can be attributed to a number of mechanisms. Glycyrrhizin and glycyrrhizic acid shown to inhibit growth and cytopathology of numerous RNA and DNA viruses, including hepatitis A (Crance *et al*., 1990) and C (Van *et a*l., 1999), herpes zoster (Su *et al*., 1984), HIV (Hattori *et al*., 1989; Ito *et al*., 1988), Herpes simplex (Pompei *et al*., 1979; Partridge *et al*., 1984) and CMV (Numazaki *et al*., 1994).

Glycyrrhizin and its metabolites inhibited hepatic metabolism of aldosterone and suppressed 5-β-reductase, responsible for the well documented pseudoaldosterone syndrome. The similarity in structure of glycyrrhetic acid to hormones secreted by the adrenal cortex accounts for the mineralocorticoid and glucocorticoid activity of glycyrrhizic acid (Armanini *et al*., 1983).

Licorice constituents also exhibited steroid-like anti-inflammatory activity, similar to the action of hydrocortisone. This is due, in part, to inhibition of phospholipase A2 activity, an enzyme critical to numerous inflammatory processes (Okimasu *et al*., 1983). *In vitro* research also demonstrated glycyrrhizic acid inhibits cyclo-oxygenase activity and prostaglandin formation (specifically prostaglandin E_2), as well as indirectly inhibiting platelet aggregation, all factors involved in the inflammatory process (Okimasu *et al*., 1983; Ohuchi *et al.,* 1982).

Certain licorice constituents possess significant antioxidant and hepatoprotective properties. Glycyrrhizin and glabridin inhibited the generation of reactive oxygen species by neutrophils at the site of

inflammation (Akamatsu *et al*., 1991; Wang *et al.*, 2001). *In vitro* studies have demonstrated licorice isoflavones, hispaglabridin A & B, inhibit Fe^{3+}-induced mitochondrial lipid peroxidation in rat liver cells (Haraguchi *et al*., 2000). Other research indicates that glycyrrhizin lowers lipid peroxide levels in animal models of liver injury caused by ischemia reperfusion (Nagai *et al*., 1991). Licorice constituents also exhibited hepatoprotective activity by lowering serum liver enzyme levels and improving tissue pathology in hepatitis patients (Van *et al*., 2001).

Glycyrrhizin and other licorice components appear to possess anticarcinogenic properties as well. Although the exact mechanisms are still under investigation, research has demonstrated that they inhibit abnormal cell proliferation, tumor formation and growth in breast (Tamir *et al*., 2001), liver (Shiota *et al*., 1999) and skin (Nishino *et al*., 1984; Liu *et al*., 1998) cancers.

Deglycyrrhizinated licorice formulations used in the treatment of ulcers do not suppress gastric acid release like other anti-ulcer medications. Rather, they promote healing by increasing mucous production and blood supply to the damaged stomach mucosa, thereby enhancing mucosal healing (Van *et al*., 1981; Goso *et al*., 1996).

Names of Hamdard Products Containing G. glabra

Alvasin: Effective natural bronchodilator, expectorant and antitussive, indicated for the treatment of general cold and cough, dry cough as well as congestion of lung.

Jernide: An effective natural remedy for spermatorrhoea, burning micturition and excessive nocturnal emission.

Sualin: A proven remedy for influenza, bronchitis, tonsillitis, sore throat, cold and cough.

Qarhin: A herbal remedy for hyperacidity, stomach ulcer, duodenal and intestinal ulcer.

Joshina: An effective herbal tea. Relieves cold and cough magically. Highly effective in the treatment of catarrh, headache, bronchitis, influenza, tonsillitis, hoarseness of voice and rhinitis.

Susi: A proven herbal remedy for hyperacidity, peptic ulcer and abdominal pain.

Maul Hayat: A herbal remedy for pneumonia, fever associated with tuberculosis, palpitation, general weakness and stranguary.

Laooq Sapistan: An effective natural remedy for chronic cough, headache due to cold, sinusitis, influenza and bronchitis.

Tonalax: An effective preparation, indicated for constipation, colic due to obstruction and chronic headache.

Hamdard Aswagandharista: An effective nervine tonic, indicated for epilepsy, syncope (fainting), arthritis, insanity, nervous debility, memory disorder, insomnia and leanness.

Suput: An effective herbal remedy for hyperacidity, gastritis and peptic ulcer. (Yousuf Harun Bhuiyan, 2015).

Precautions

Pregnant women should keep their licorice intake less than what is usually prescribed as a medicine. Studies indicate that intake of large doses (>50 g/day) of the drug for extended periods (>6 weeks) may cause sodium retention and potassium loss which lead to the sudden rise in blood pressure levels, water retention and severe electrolyte imbalance or cause hormonal imbalance that may, in turn affect the fetus (Mohammed Said, 1996).

Toxicity

Its major dose-limiting toxicities are corticosteroid in nature, due to the inhibitory effect its chief active constituents (glycyrrhizin and enoxolone) have on cortisol degradation that includes oedema, hypokalaemia, weight gain or loss, and hypertension (Olukoga *et al*., 2000; Armanini *et al*., 2002). The amount of licorice ingested daily by patients with mineralocorticoid excess syndromes appears to vary over a wide range, from as little as 5.5 gram day to as much as 250 gram day (Stormer *et al*., 1993).

Packaging and Storage

The dried root of *G. glabra* should be packaged in clean, dry boxes, sacks, bags or other containers in accordance with Standard Operating Procedure (SOP) and stored in clean, well ventilated area protected from light, moisture and against attack by insects and rodents (Hamdard, 2006; Rafiqul Islam, 2003, 2005).

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