



AMMONIUM GLYCYRRHIZINATE: A COMPREHENSIVE REVIEW OF ITS TRADITIONAL USE, PHYTOCHEMISTRY, PHARMACOLOGY & SAFETY.

Tinku Gupta, Mohd. Mujeeb

Phytomedicine Lab, Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi, India.

*Corresponding Author's E mail: vigneshrm21@gmail.com

Received 22 Nov. 2017; Revised 24 Nov. 2017; Accepted 5 Dec. 2017, Available online 20 Jan. 2018

ABSTRACT

Glycyrrhizic acid (GA) is a triterpene glycoside found in the roots of Licorice plants (*Glycyrrhiza glabra*). GA is the most important active ingredient in the Licorice root, and possesses a wide range of pharmacological and biological activities. GA coupled with glycyrrhetic acid and 18-beta-glycyrrhetic acid was developed in China or Japan as an anti-inflammatory, antiviral, and anti-allergic drug for liver disease. The application of natural compounds in the treatment of refractory diseases is a new trend in modern clinical medicine. Because of their satisfactory efficacy in clinic and low toxicity, more natural products are being used as alternative treatments for many diseases. This review summarizes the current biological activities of GA and its medical applications in different diseases. The pharmacological actions of GA include inhibition of cardiac apoptosis and necrosis; anti-inflammatory and immune regulatory actions; antiviral effects; and antitumor effects.

Keywords: GA, Licorice, Ammonium Glycyrrhizinate.

INTRODUCTION

Glycyrrhiza glabra is derived from the ancient Greek term glykos, meaning sweet, and rhiza, meaning root. It is known as Mulathi in north India and sweet wood in Mediterranean or part of Asia. It consists of dried, unpeeled, stolon and root which is a tall perennial herb, up to 2 m high found cultivated in Europe, Persia, Afghanistan and to a little extent is available in India like Punjab & Himalaya tract. Traditionally, its use involves a decoction of dried plant roots and stems.¹

It has been known from thousands of years. *Glycyrrhiza* plays an important role in various systems of medicine i.e. (Hindu Medicine) and is one of the principle drugs of the 'Susruta'. It continues to be used as a pharmacological agent as well as confectionery industry throughout India in the East and West. Last 50 years studies have yielded information which has prompted new interest in the pharmacological and physiological effects of this plant.

This research tells that the chemical structure of one of the principle agents in the root of the Licorice plant is a glycoside of a triterpene called glycyrrhetic acid and also the plant consists of glycyrrhizin, glycyrrhizic acid, glycyrrhetic acid, asparagine, sugars, resin and starch as main constituents.

The recognized components of Gancao include saponins (mainly glycyrrhizin (GA), 3.63–13.06%), flavonoids (1.5%), coumarin, alkaloids, polysaccharides, sitosterol, and amino acids. Besides from this, other components of Gancao GA and glycyrrhetic acid. In China and Japan GA used as a hepatoprotective drug due to the formation of glycyrrhetic acid in various metabolic processes of the human body. Therefore, pharmacologically both (GA & glycyrrhetic acid) shows the same effect. A triterpene glycoside glycyrrhizin obtained from Liquorice root (*Glycyrrhiza glabra*) and consists of one molecule of 18-glycyrrhetic acid and two molecules of glucuronic acid (18-glycyrrhetic acid-3-O--D-glucuronopyranosyl-()-beta-D-glucuronide. Glycyrrhizin is considered to be the main active constituent of Gancao as established by studies with experimental animal models and clinical studies.

AMMONIUM GLYCYRRHIZINATE

Structure

Glycyrrhizinic acid can be considered as the di-glucuronic acid conjugate of glycyrrhetic acid. This aglycone part of the molecule may occur in 2 forms, (18 alpha & 18 beta). Although the 18-alpha form has been found in amounts up to 13% of the total glycyrrhetic acid present, it is not clear whether this form occurs naturally or whether it is not formed during processing due to isomerization.²

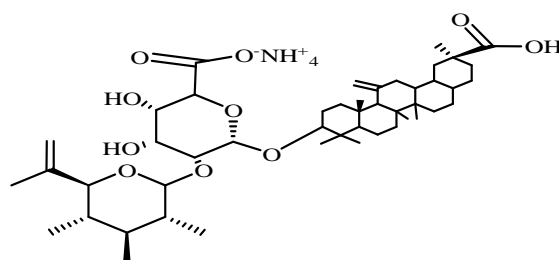


Fig1. Chemical structure of ammonium salt of Glycyrrhizinic acid

Chemical Properties

Substance Name	Ammonium Glycyrrhizin-ate
Chemical name	(3-beta, 20-beta)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2- O-beta-D-glucopyranuronosyl-alpha-D-glucopyranosiduronic acid, monoammonium salt
Synonyms	Glycyrrhizinic acid, ammonium salt
Molecular formula	C ₄₂ H ₆₁ O ₁₆ NH ₄ (anhydrous)
Molecular weight	839.96 D (anhydrous)

Method of extraction

Sonication

For glycyrrhizin, the procedures were adopted from an earlier report. Briefly, 0.6g of sample were extracted with 20 ml of methanol/water mixture (70:30 v/v) at room temperature for 10 min and centrifuge at 2000 rpm for 10 min. The procedures were repeated three times. The extracts were combined; excess solvent was evaporated with the rotary evaporator and filtered.

Super critical fluid extraction (SFC-CO₂)

SC-CO₂ technology has been used to plant extract widely. SC-CO₂ extraction was a very suitable method for Liquorice. Several reports have described. Briefly, sample was accurately weighed, then extracted by the following methods. The pulverized sample was packed into a sample cartridge. Different concentration of ethanol (75%, 80%, 85%, 90% and 95%) was as co-solvent. The extraction temperature was set at 40, 45, 50 & 55 respectively. Liquid carbon dioxide at high pressure (20, 25, 30, 35 MPa) was then allowed to flow into the sample cartridge. When the pressure reached the aim pressure opened the vent valve of the extractor and then the two separators. The crude extraction was obtained by reduced pressure evaporation.

Microwave-assisted extraction

This method is very suitable for fast extraction. The extraction time only need 4-5 min. briefly, 10g sample is (50) mesh with the mixture of 100ml solvent which ethanol concentrations of 50-60% (v/v), ammonia concentration of 1-2% (v/v). The suspensions were irradiated under microwaves in pre-setting procedures (15s power on, 15s power off for three times to the desired temperature (about 85-90) and then 3s power on for heating and 15s power off for cooling), but not allowed to super-boil. The extraction was filtered and excess solvent was evaporated with the rotator evaporator.^{3,4}

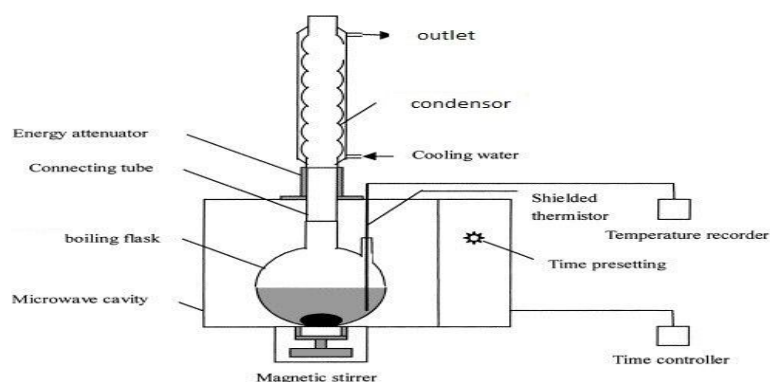


Fig. 2. Microwave-assisted extraction

High performance liquid chromatographic analysis

The apparatus was equipped with a binary gradient pump, auto sampler, column oven and diode array detector was used. The elution consists of mobile phase of methanol, water and acetic acid and CH₃OH/3%HOAc was 33/67. Detection was at 254nm. Room temperature and flow rate was set at 1.0ml/min. For all the experiments, 20µl of standards and sample extract were injected. The column used for separation was DOS (4.6mm i.d.*25cm).

Chemical constituents

A number of chemical constituents have been isolated from liquorice, which are water-soluble. They consist of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances. Glycyrrhizin a triterpenoid compound used for the sweet taste of Liquorice root. This compound represents a mixture of potassium-calcium-magnesium salts of glycyrrhizic acid. Among the natural saponins, it composed of a hydrophilic part, glucuronic acid, and a hydrophobic fragment, glycyrrhetic acid.

The yellow color of Liquorice is due to the presence of flavonoid i.e. chalcone (liquiritin, isoliquiritin). The isoflavones glabridin and his-paglabridins have significant antioxidant activity and estrogen-like activity.⁵⁻⁸

Mechanism of action

The number of beneficial mechanism are shows by the *Glycyrrhiza glabra* isolated components (Glycyrrhizin and glycyrrhizic acid).

- Growth Inhibition and cytopathology of numerous RNA and DNA viruses.⁹
- Glycyrrhizin reduce hepatic metabolism of aldosterone and suppress 5-(beta)-reductase which is responsible for the well-documented pseudoaldosterone syndrome.¹⁰
- Inhibit the activity of phospholipase A2, an enzyme critical to numerous inflammatory processes.¹¹
- Inhibits cyclooxygenase activity and prostaglandin formulation as well as indirectly inhibiting platelet aggregation.¹²
- Glycyrrhizin and glabridin inhibits the generation of reactive oxygen species (ROS) by neutrophils at the site of inflammation.^{13,14}
- In vitro studies give idea about Liquorice isoflavones, hispaglabridin A & B, inhibit (Fe.sup.3)-induced mitochondrial lipid per-oxidation in rat liver cells.¹⁵

- Glycyrrhizin lowers lipid peroxide values in animal models of liver injury caused by ischemia reperfusion.¹⁵

Pharmacological Activity

Traditionally, the plant is recommended for gastric and duodenal ulcers and dyspepsia as an anti-inflammatory agent during allergic reactions. Locally, it is used as a laxative, emmenagogue, contraceptive, galactagogue, anti-asthmatic drug and antiviral agent. The various studies done by plant scientists and experimental pharmacologists on its bioactivities revealed that the plant may be a source of new drugs and therapeutic agents for the treatment of a variety of diseases and ailments could be manufactured.¹⁶

The ammonium salt of glycyrrhizic acid is characterized by an anti-inflammatory activity, anti-ulcer, anti-allergic, antidote, antioxidant, antiviral, anti-tumor, anticonvulsant activity.¹⁷ Glycyrrhizic acid has been used to treat chronic hepatitis, inhibiting the penetration of the hepatitis A virus into hepatocytes. Glycyrrhizic acid and its salt and esters are cosmetic ingredients that function as flavouring agents or skin –conditioning agents.² Powdered extract of the drug with water was used in the treatment of sore throat cough bronchial catarrh or demulcent action.¹⁸

Apart from that, salt of glycyrrhizinate can be used in Anticoagulant, Anticancer, Antimicrobial, Antiviral, Antioxidant and Anti-inflammatory, Antidiabetic, immunomodulation (especially in swine flu) & many other diseases also.

Side Effects and Cautions¹⁹

- In large amounts, glycyrrhizin salt can cause high blood pressure, water retention, & low potassium levels, which could lead to heart problems.
- Combined administration of glycyrrhizin salt with diuretics (water pills), corticosteroids, or other medicines that decrease the body's potassium levels.
- Pregnant women should avoid large amounts of Liquorice as food, as some research suggests it could increase the risk of preterm labor.

Toxicity study

Orally intake of large dose (>50 g crude drug per day) for a prolonged period leads to hypocalcaemia, hypernatraemia, oedema, hypertension and cardiac disorder. Hypokalemia is the greatest threat when liquorice preparations high in glycyrrhizin are prescribed for prolonged periods. Liquorice causes fluid retention. Patients should be placed on a high potassium and low sodium diet. Special precautions should be taken with elderly patients with hypertension or cardiac, renal or hepatic disease.²⁰

Conclusion:

Glycyrrhiza glabra is the most important ancient medicinal plant, according to traditional pharmacopeia. Its pharmacological activities have been proved like antitussive, antidiabetic, hepato-protective, antimicrobial, antiulcer, antiviral, anticancer etc. so, it indicates that it shows wide biological activity.

Reference

1. Kataria H, Singh G, Gupta A, Jalhan S and Jindal A. Pharmacological activities on glycyrrhizia glabra: A Review. Asian Journal of Pharmaceutical and Clinical Research. 2013; 6 (1):5-7.
2. Opinion of the scientific committee of food on glycyrrhizinic acid and its ammonium salt European commission health and consumer protection directorate-general SCF/CS/ADD/EDUL/225 final 10 April 2003.
3. Xuejun P, Huizhou L, Guanghe J and Youn S. Microwave-assisted extraction of glycyrrhizic acid from Liquorice root. Biochemical Engineering Journal Volume 5, Issue 3, July 2000, Pages 173–177.
4. Wei W, Jianzhong Y, Che Z, Yi L and Wenhua S. Supercritical Carbon Dioxide Extraction of Glycyrrhizin from Liquorice Root. 7th Italian Conference of Supercritical Fluids and Their Applications.
5. Obolentseva GV, Litvinenko VL and Ammosov AS. Pharmacological and therapeutic properties of Liquoricepreparations (A Review). Pharm Chem J. 1999; 33:24-31.
6. Yamamura Y, Kawakami J, Santa T et al. Pharmacokineticprofile of glycerrhizin in healthy volunteers by a new high performance liquid chromatographic method. J Pharm Science 1992; 81:1042-1046.

7. Vaya J, Belinky PA and Aviram M. Antioxidant constituents from Liquorice roots: isolation, structure elucidation and antioxidative capacity toward LDL oxidation. *Free Radic Bio Med* 1997;23: 302-313.
8. Tamir S, Eizenberg M and Somjen D et al. Estrogen like activity of glabrene and other constituents isolated from Liquorice root. *J Steroid Biochem Mol Biol* 2001;78:291-298. <http://europa.eu.int/comm/food/fs/sc/scf>
9. Crance JM, Biziagos E and Passagot J. Inhibition of hepatitis A virus replication in vitro by antiviral compounds. *J Med Virol* 1990. 31: 155-160.
10. Armanini D, Karbowiak I and Funder JW. Affinity of liquorice derivatives for Mineralocorticoid and Glucocorticoid receptors. *Clin Endocrinol (Oxf)* 1983. 19:609-612.
11. Okimasu E, Moromizato Y and Watanabe S. Inhibition of phospholipase A2 and platelet aggregation by glycyrrhizin an anti-inflammatory drug. *Acta Med Okayama* 1983. 37:385-391.
12. Ohuchi K and Tsurufuji A. A study of the anti-inflammatory mechanism of glycyrrhizin. *Mino Med Rev.* 1982; 27: 188-193.
13. Akamatsu H, Komura J, Asada Y and Niwa Y. Mechanism of anti-inflammatory action of glycyrrhizin: effect on neutrophil functions including reactive oxygen species generation. *Planta Med.* 1991; 57:119-121.
14. Wang ZY and Nixon DW. Liquorice and cancer. *Nutr cancer.* 2001; 39: 1-11.
15. Nagai T, Egashira T, Yamanaka Y and Kohno M. The protective effect of glycyrrhizin against injury of the liver caused by ischemia-reperfusion. *Arch environ contam toxicol.* 1991; 20:432-436.
16. Harwansh K, Patral C, Paretal K, Singh J and Biswas R. Pharmacological studies of *Glycyrrhizia glabra*: A review. *Pharmacology online.* 2011; 2: 1032-1038.
17. Kale S, Shete R, Kore K, Patil B, Bhatada R and Pattankude V. Anticonvulsant activity of glycyrrhizic acid in mice. *Journal of Pharma Research and Development International.* 2010; 10(2).
18. Iino S, Tango T, Matsushima T et al. Therapeutic effects of stronger neo-minophagen C at different doses on chronic hepatitis and liver cirrhosis. *Hepatol Res.* 2001; 19:31-40.
19. Isbrucker RA and Burdock GA. Risk and safety assessment on the consumption of Liquorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. *Regulatory Toxicology and Pharmacology.* 2006; 46: 167–192.

20. Marianecci C, Rinaldi F, Mastriota M, Pieretti M, Trapasso E, Paolino D and Carafa M. Anti-inflammatory activity of novel ammonium glycyrrhizinate/niosomes delivery system: Human and murine models. *Journal of controlled release*. 2012; 164: 17-25.