ORIGINAL ARTICLE

# *Glycyrrhiza glabra* and *Glycyrrhiza echinata* — Sources of Low Hemotoxic Saponins

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**Introduction:** *Glycyrrhiza glabra* L. (licorice) is one of the most used medicinal plats as an expectorant, anti-inflammatory, anti-ulcer, sweetener, antioxidant, antibacterial, antiviral, antimycotic or anti-tumor agent. In our country *G. echinata* (Russian licorice) is the dominant species of the *Glycyrrhiza* genus. Several substitution cases were reported between *G. glabra* and *G. echinata* in the last years. The **aim** of this study was to evaluate the surface-active properties, related to the potential toxicity of Russian licorice, using the medicinal species *G. glabra* as standard. **Material and method:** Tests were performed according to the methods described by valid Pharmacopoeias. The foam index was identified (FI), followed by the evaluation of haemolysis capacity. A spectrophotometric method, more sensitive than the haemolytic index (HI) was used to determine the 50% haemolytic dose ($HD_{50}$) of the two vegetal products, ammoniacal glycyrrhizate and *G. echinata* saponins.
**Results:** *Glycyrrhiza echinata* (FI 400; $HD_{50}$ = 9153±210 µg/ml), *G. glabra* (FI 250, $HD_{50}$ = 12382±172 µg/ml) and the tested saponins (ammoniacal glycyrrhizin HD 50 = 63.25±1.4 µg/ml and *G. echinata* saponins $HD_{50}$ = 42.5±1.2 µg/ml), had low haemolytic capacity.
**Conclusions:** The surface-active properties of the tested vegetal products and substances do not create an impediment when using small amounts of the product, creating the perspective of new research on the use of saponins isolated from *G. glabra* and *G. echinata* as possible ingredients in parenteral formulation.

**Keywords:** *Glycyrrhiza glabra*, *Glycyrrhiza echinata*, saponins, haemolysis, $HD_{50}$

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## Introduction

*Glycyrrhiza glabra* L. (licorice) is one of the most used medicinal plants worldwide, being documented in the second century before Christ in the Chinese literature [1]. It is one of the top five herbal drugs employed in ayurvedic medicine [2]. Licorice was used by Scythians, Greeks and Romans as an expectorant, anti-inflammatory, anti-ulcer, and sweetener [3,4]. New pharmacological actions have been recently reported: hepatoprotective, antispasmodic, antioxidant, antibacterial, antiviral, antimycotic, anti-tumor, estrogen, inhibitor of serotonin reuptake, depigmenting [5].

Licorice has limited spreading area in the Romanian spontaneous flora, being documented only in Vrancea, Galați and Botoşani counties. *Glycyrrhiza echinata* (Russian licorice) is the dominant *Glycyrrhiza* genus species in our country [6]. In the last years, several substitution cases were reported between the medicinal licorice and Russian licorice roots [7].

The roots and rhizomes of licorice are known as the medicinal drug *Liquiritiae radix*. It contains mainly saponins, glycyrrhizic acid being the representative triterpene saponin. *Glycyrrhiza echinata* roots also contain saponins, but they lack glycyrrhizic acid [7–9].

The foam index and haemolytic capacity are important characteristics of saponin drugs, related to the potential toxicity of the vegetal product [10].

The aim of this study was to identify and compare the surface-active potential of *Liquiritiae radix* and the roots of *Glycyrrhiza echinata*, and to correlate these values to eventual new therapeutic uses of *Glycyrrhiza echinata*.

## Material and method

Plant material: Licorice roots and Russian licorice roots were obtained from cultures of the Faculty of Pharmacy, Timişoara. The roots were dried at room temperature and pulverized. Saponins were extracted using the method perfected by Cucu and Grecu [11]. Voucher specimens of the roots (identification code CD-*G.glabra*-011, CD-*G.echinata*-009) were deposited at the aforementioned institution.

Reagents: Ammoniacal glycyrrhizin was purchased from Extrasythese (France).

Foam index (FI) was determined according to the method indicated as quality control of plant material by the World Health Organization [12].

Hemolytic index (HI) was determined according to the method recommended by the Romanian Pharmacopoeia X$^{th}$ edition [13], using bovine blood provided by the Faculty of Veterinary Medicine of Timişoara.

50% haemolytic dose – $HD_{50}$ (µg/ml) – equal volumes of 0.5 % red blood cells and different dilutions of saponins or of the two extractive solutions (obtained according to the Romanian Pharmacopoeia X$^{th}$ edition, Haemolytic Index Method) were incubated at 37°C for 30 minutes, then centrifuged 1 minute at 10,000 rotations per minute, using a Sigma 1-15 centrifuge (B.Braun Biotech International). The free hemoglobin in the supernatants was measured spectrophotometrically at 530 nm [14–16] (Spekol 1300 spectrophotometer – Analytik Jena AG). Saline and distilled water were included as minimal and maximal

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Table I. Foam indices and the haemolytic indices of *Glycyrrhiza glabra* and *G. echinata*

| Species | Foam index | Haemolytic index |
|---|---|---|
| *Glycyrrhiza glabra* | 250 | 166 |
| *Glycyrrhiza echinata* | 400 | 200 |

haemolytic controls. The haemolytic percent developed by the saline control was subtracted from all groups. The experiment included triplicates for each concentration.

Statistic analysis: t-test was performed. Data were analyzed using Excel 2003 software and expressed as the mean±standard errors.

## Results

Foam indices and haemolytic indices ware measured for the extractive solutions of the two species (Table I).

The measurement of the haemolytic index was a preliminary step, followed by the measurement of the 50% haemolytic doses (Table II) of the vegetal products.

For the saponins isolated from *G. echinata* $HD_{50}$ = 42.5±1.2 μg/m and for ammoniacal glycyrrhizate $HD_{50}$ = 63.25±1.4 μg/ml.

Table II. Haemolytic activities of *Glycyrrhiza glabra* and *G. echinata* extracts on bovine erythrocytes

| 2 % extract (μl) | Haemolysis percentage | |
|---|---|---|
| | *Glycyrrhiza glabra* | *Glycyrrhiza echinata* |
| 300 | 13.53±0.18 | 14,67±0.18 |
| 500 | 22.23±0.09 | 24.04±0.34 |
| 700 | 29.86±0.15 | 36.27±0.19 |
| 900 | 37.80±0.14 | 48.96±1.12 |
| 1000 | 40.23±0.27 | 53.23±0.90 |
| Water | 100.00±2.1 | |
| Saline | 0±0.15 | |
| $HD_{50}$ (μg/ml) | 12382±172 | 9153±210 |

All values represent the mean ± SE (n=3 tests). p<0.01 significant differences compared to saline, the minimal haemolysis control.

## Discussions

Surface-active properties of saponins were known and exploited since antiquity, many plants being used as detergents. In modern times, saponins are widely used in pharmaceutical formulations, food, drink and cosmetic industries [17].

*Glycyrrhiza echinata* (FI 400) presents higher foaming capacity than *G. glabra* (FI 250), as expected, Russian licorice being often used to obtain fire extinguishing foam mixtures and as mild detergent for fine fabrics [8].

The haemolytic index of the two Glycyrrhiza species shows the same variation trend as the foam index, with higher value for *G. echinata* (HI 200) than *G. glabra* (HI 166). However the two values are close and comparable, suggesting that Russian licorice saponins have low haemotoxicity.

In order to confirm and validate the promising preliminary result, a quantitative spectrophotometric method was used. We tested the aqueous extracts because on the market the vegetal product is widely encountered. *Glycyrrhiza echinata* does not contain glycyrrhizic acid, the representative saponin of *G. glabra* [7]. The haemolytic activity of saponins extracted from *G. echinata* was compared to that of ammoniacal glycyrrhizin, one of the most studied and used chemical compound of licorice.

Glycyrrhiza glabra shows the lowest haemolytic capacity (HI 166), correlated with the highest vegetal product concentration that haemolyses 50% of the erythrocytes in the tested suspension. *G. echinata* also possesses reduced haemolytic capacity.

Studies on Glycyrrhiza uralensis saponins, species that has a similar chemical profile (5% glycyrrhizic acid) as *G. glabra*, reported low haemolytic activity. Further more, G. uralensis saponins proved to have adjuvant potentials on the cellular and humoral immune responses of ICR mice against ovalbumin, also being less membrane harmful than saponins isolated from Platycodon grandiflorum or Quillja saponaria [15]. Quillja saponaria saponins ($HD_{50}$ = 19.91 μg/ml) are considered safe and are recommended for vaccine formulations; additional to their surface-active properties, they stimulate the initiation of the immune response [18].

The chemical structure of saponins is directly related to their haemolytic activity, steroidal saponins having superior haemolytic activity than triterpene saponins. An oleanolic saponin mixture showed higher haemolytic activity than a dialyzed reference saponin mixture from Merck® (HI 30,000) [16]. Monodesmosides were less haemolytic than bisdesmosides [10,19]. *G. glabra* saponins and the ammoniacal glycyrrhizate belong to the low haemolytic categories: triterpene saponins, monodesmosides. The haemolytic capacity of *G. echinata* saponins is situated in the same range as that of *G. glabra*, suggesting also the presence of the triterpene aglycone.

## Conclusions

The present study compared the haemolytic and foam generating abilities of Glycyrrhiza glabra and *Glycyrrhiza echinata*.

*G. echinata* showed superior surface-active properties than *G. glabra*. The ammoniacal glycyrrhizin is one of the low haemolytic risk saponins due to the high HD50. Saponins extracted from *G. echinata* showed a moderate haemolytic profile.

Both vegetal products and the tested saponins had low haemolytic capacity, a parameter that does not create an impediment when used in adequate quantities.

The results obtained by the present study set the perspective of new research on the use of saponins isolated from *G. glabra* and *G. echinata* as possible ingredients in parenteral formulations.

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