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Research Article

IN VITRO CHOLESTEROL BINDING AFFINITY OF TOTAL SAPONIN EXTRACTED FROM *GLYCYRRHIZA GLABRA*

ESKANDAR MOGHIMIPOUR[1], HOSSEIN KOOSHAPOUR[2], SAEED REZAEE[3], SAREH KHALILI[3], SOMAYEH HANDALI*[4]

[1]Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran., [2]Department of Pharmacognosy, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran., [3]Department of Pharmaceutics, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran., [4]Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Email: handali_s81@yahoo.com

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ABSTRACT

Objectives: Saponins are high molecular weight glycosides, consisting of a sugar moiety linked to a triterpene or steroid aglycone. Many saponins are good detergents and produce stable foam in water. Recently, it has been shown that oral administration of some saponins can prevent hypercholesterolemia, the phenomenon which is the result of complex formation with cholesterol. *Glycyrrhiza glabra* (Licorice) is a grassy plant with a height of 50-100 cm or more. The medicinal organ of the plant is constituted of the roots, containing 3-15% triterpene saponins that have many indications such as expectorant, anti-inflammatory, flavoring and foaming agent. The aim of the present study was to extract and characterize total saponin from licorice roots, and also evaluate the possible interaction between the saponin and cholesterol.

Methods: The collected roots of the plant were identified, dried, powdered and defatted with petroleum ether in a soxhlet apparatus. The air-dried powder was successively extracted with methanol, n-butanol and diethyl ether. Then foaming power of the extracted *Glycyrrhiza glabra* total saponin (GTS) was measured using the Ross-Miles foam column method and the index of emulsification (E_{24}) of the extracted saponin was also determined. The results were compared to data from *Quillaja saponaria* total saponin (QTS), and Tween 80 as a potent synthetic surfactant. Using a Du-Nouy tensiometer, critical micelle concentrations (CMCs) of the saponins were determined by measuring surface tension as a function of surfactant concentration. The effect of complex formation with cholesterol was determined by measuring the changes in surface tension and critical micelle concentrations due to addition of cholesterol in saponin solutions.

Results: The results showed that QTS had a relatively good ability to produce stable foam. In the case of reduction of surface tension and emulsification, the extracted total saponin had less power than QTS and Tween 80. The results also showed that the saponins are capable of forming complex with cholesterol.

Conclusion: It can be concluded that oral administration of total saponins of *G. glabra* and *Q. saponaria* may cause a reduction in cholesterol absorption through gastrointestinal system and finally a reduction in blood cholesterol. Also due to its excellent surface activity, it can be suggested that the total saponin from licorice roots is a suitable substitute for synthetic surfactants in food, drug and cosmetic industries.

Keywords: Saponin, *Glycyrrhiza glabra,* cholesterol, *Quillaja saponaria*

INTRODUCTION

Saponins are high molecular weight glycosides, consisting of a sugar moiety linked to a triterpene or steroid aglycone, with a general formula of many of them is C_nH_nO. Classical description of saponin is based on their surface activity, because many of saponins have cleansing properties, generate stable foam in water, bear hemolytic activity, are bitter and toxic for fishes [1]. Saponins are used to reduce surface tension in several cases such as extinguish of fire and as emulsifier [2]. These compounds have astringent flavor and act as powder to produce sneeze. The main part of saponin is glycoside that in pure form is colorless or white and is soluble in pure alcohol or diluted alcohol [3]. These compounds are usually extracted from higher plants, but today the use of marine sources is also considered for saponins production. Commercial saponins are prepared from *Yuka* and *Quillaja* [1, 3]. Characterization of complex formation between cholesterol and saponin was explained in 1909 by Windaus. Fundamental studies have shown the ability of different types of saponins to form complex with cholesterol. Saponins also form weak complexes with lecithin, ergosterol, amile alcohol, terpene, alcohols, phenols and thiophene. High molecular weight proteins and gallotannins also interfere with saponin [1]. In 1999, properties of micelle formation and cholesterol binding affinity of *Q. saponaria* were investigated. Based on their reports, the mechanism of linking saponins with cholesterol is important in understanding of bodily physiological processes such as the characteristics of membrane displacement, serum cholesterol, gallstones and gallbladder function. Also interaction of cholesterol and saponins, make it possible to predict the function of cholesterol in food products and pharmaceutical matrixes and cholesterol extraction processes [4]. Saponins exhibit surface activity characteristics due to the amphiphilic nature of their chemical structure [5].

Licorice is dried powder of rhizomes and roots of the plant *G. glabra*, which is yellow in color and sweet taste. The main part of licorice root contains 2 to 4% glucose, 2 to 4% sucrose, 25 to 30% amidone, 2 to 4% asparzhin, albominoid materials, resin and oil, but the main component is Glycyrrhizic acid or Glycyrrhizin which is a triterpene saponin [6, 7, 8, 9]. The extract of root is laxative and useful medicine in urinary disease, bronchial and gastric troubles [10].

The aim of the study was to extract total saponin of the plant, investigate its characteristics and determine the probable affinity to form complex with cholesterol.

MATERIAL AND METHODS

QTS was purchased from Sigma, Swiss. Cholesterol and Tween 80 were obtained from Merck, Germany. All of the solvents were of the analytical grade.

Plant Materials

The roots of *G. glabra* were collected from Ahvaz (Iran), and indentified in department of Pharmacognosy, Faculty of Pharmacy,

Ahvaz Jundishapur University of Medical Sciences. The roots of the plant were ground into powder and stored at room temperature (25ºC).

Extraction of total saponin

The powdered roots of *G. glabra* was defatted in a soxhelet apparatus with petroleum ether (boiling range 40-60 ºC) for removing lipids and phenolic compounds. The air-dried powder was extracted with methanol for 48 h. The solvent was removed under vacuum by rotary evaporator (Heidolph, Germany) and the resulting brown residue was suspended in water, then centrifuged at 2500 rpm for 45 min, and the supernatant was separated and extracted with water saturated n-butanol. Butanol phase concentrated in rotary evaporator at 80ºC and the dry residue was dissolved in the least methanol quantity (30 ml), and then precipitated by addition of diethyl ether. Finally, total saponin of the plant (GTS) was freeze-dried (Operon, Korea) and stored at room temperature [11, 12, 13].

Foaming Ability

Different concentrations of GTS (0.25- 8 mg/ml), QTS (0.2- 10 mg/ml) and Tween 80 (0.216 - 10.8 mg/ml) in double-distilled water were prepared. 5 ml of each concentration was added to three tubes and tubes were vortexed for 5 seconds and after one minute the foam height was measured. The results of average foam height were plotted as a function of surfactant concentration.

Determination of Emulsification Index (E_{24})

3 ml of aqueous solution of different concentrations (0.04- 3 mg/ml) of GTS, QTS and Tween 80 were added to the three tubes contaning 3 ml of liquid paraffin was added to each tube. Then they were vertexed at high rate for 2 min to form emulsion. The samples were stored at 25°C for 24 h and then the emulsified layer thickness was measured. The emulsification indexs (E_{24}) were plotted as a function of the concentration.

Surface Tension and Critical Concentration Micelle Formation (CMC) Studies

Using the stock solution, different dilutions of GTS (0.003- 4 mg/ml) QTS (0.007- 4 mg/ml) and Tween 80 (0.001- 5.4 mg/ml) in volumetric flask were prepared and vortexed for 5 seconds and then were kept at room temperature for 12 h. Then surface tensions of the solutions were measured using Du-Nouy Ring Tensiometer at 25˚C. The results of mean values of surface tensions were plotted as a function of saponin concentration. Concentration range in which did not observe significant change of surface tension was identified as critical concentration of micelle formation (CMC) [14].

Determination of Surface Tension of Saturated Saponin Solutions of Cholesterol

Different concentrations of saponin solutions saturated with cholesterol were prepared and vortexed for 5 seconds. Then the solutions were kept at room temperature for 12 h and surface tensions of the solutions were measured as mentioned previously.

Statistical Methods

To compare the results of foam height, emulsification indices and surface tension in different samples, univariate ANOVA and general linear model were utilized. In the presence of any differences, Tukey test was utilized to analyze the difference. Non-parametric ANOVA was also used to compare the results of CMC determination.

RESULTS AND DISCUSSION

The extracted total saponin of *G.glabra* was 7% of the primary total weights of the plant material. The results of foam measurement are listed in Table 1. As shown in the table, by enhancement in the concentration of GTS, QTS and Tween 80, the foam height was increased to the maximum value. QTS showed a maximum foam height of 9.3±1 cm. Also, there was a significant different between the two total saponin if compared with control solution (P<0.001). Tween 80 could not form stable foam. Due to ability of saponins in producing, it may be suggested that these compounds are good candidates for being used in shampoos instead of alkanolamides. A shampoo should produce stable foam. Alkanolamides are often used to prepare stable foam, but because of producing nitrosamines, they are potentially carcinogenic compounds. So, GTS and QTS can be substituted the alkanolamides in formulation of a Shampoo [15].

The results of emulsion stability showed that there was a significant correlation between the saponin concentration and formation of emulsions (Fig 1). A comparative analysis between the total saponins and Tween 80 which was utilized as positive control showed that the onset of emulsification ability for the saponins was significantly slower than the synthetic surfactant (P<0.001). Tween 80 is non-ionic surfactant which is used for several purposes such as for preparation of oil in water emulsions in pharmaceutical products, cosmetics, and industrial detergents [16]. Also, emulsion stability of QTS was higher than GTS (P<0.001).

The results of the measurement of surface tension of GTS, QTS solutions and Tween 80 aqueous solution are shown in Fig (2). It is apparent from Fig (2) that by the increasing in the concentration of saponins, the surface tension of the solutions was reduced. Surface activity of GTS is significantly slower than QTS and Tween 80 (P<0.001). In spite of an apparent difference, there was no statistical significant difference between CMC of total saponins and Tween 80. The CMC value of GTS, QTS and Tween 80 was 0.53, 0.42 and 0.34 mg/ml, respectively. CMC is one of the most important physical parameters of surfactants that influence on the properties of a surfactant such as solubilization power, viscosity, osmotic pressure, density and polarity. Industrial application of a surfactant is always based on the value of its CMC [17].

The results of the measurement of surface tension of different concentrations of GTS and QTS aqueous solutions saturated with cholesterol are shown in Fig (3) and (4). According to these figures, enhancing of the concentration of GTS and QTS decreased their surface tension. The presence of cholesterol in the saponins solutions significantly decreased their surface tension. (P<0.001). Based on the results, it can be deduced that saponins could form complex with cholesterol.

Hypercholesterolemia is a major cause of cardiovascular disease such as atherosclerosis and coronary heart disease. Many patients prefer nondrug therapies for many reasons including adverse effects of anti-hyperlipidemic agents, contraindications and allergic reactions to drugs. Therefore, it is necessary to develop new safe and effective cholesterol-lowering agents from natural sources [18]. It has been reported that saponins can form insoluble complexes with cholesterol and reduce blood cholesterol levels in humans [19]. Several studies have shown that administration of ginseng saponins may decrease serum cholesterol, especially LDL cholesterol, and increase HDL cholesterol [20]. Jung *et al*. in 2007 reported that saponin extracted from *Pleurospermum kamtschaticum* was effective in reducing hypercholesterolemia and hyperlipidemia [21]. It has been shown that micellar solutions of saponins extracted from the *Q. saponaria* are efficiently able to solubilize very large hydrophobic molecules such as cholesterol, phytosterols and phenanthrene [22]. Based our results, oral administration of GTS and QTS can be used to reduce blood cholesterol. However, further investigation is necessary to determine their chemical structure and animal studies are needed in order to evaluate their *in vivo* efficacy and toxicity.

Table1:The value of formation of foam in different concentrations of GTS, QTS and Tween 80 (mg/ml) after 1 min (Mean±SD)

Concentration of GTS (mg/ml)	Foam height (cm)	Concentration of QTS (mg/ml)	Foam height (cm)	Concentration of Tween 80 (mg/ml)	Foam height (cm)
0.25	0.7±0.2	0.2	1.4±0.1	0.216	1.5±0.2
0.5	1.3±0.2	0.5	2.3±0.1	0.54	2.2±0.5
0.75	2±0.5	1	3±0.4	1.08	2.2±0.2

1	2.5±0.5	2	3±0.4	2.16	2.1±0.4
2	2.1±0.2	3	4±0.4	4.32	2.5±0.3
3	2.7±0.6	4	5.8±0.2	6.48	2.8±0.3
4	3.3±0.8	6	6.2±0.2	8.64	3.4±0.5
5	3.8±0.2	8	7.3±0.2	10.80	3.1±0.4
8	6.5±1.3	10	9.3±1	-	-

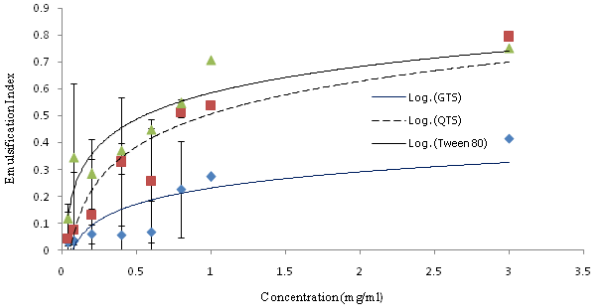

Figure 1: Formation of emulsions with different concentrations of GTS, QTS and Tween 80 (n=6)

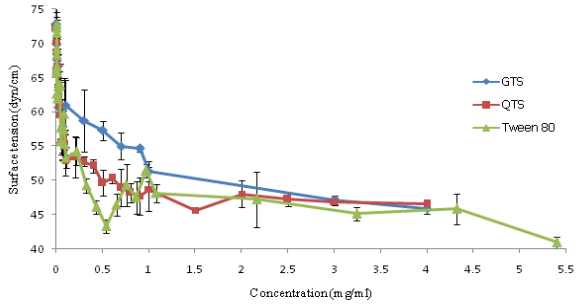

Figure 2: Changes in surface tension at different concentrations GTS, QTS and Tween 80

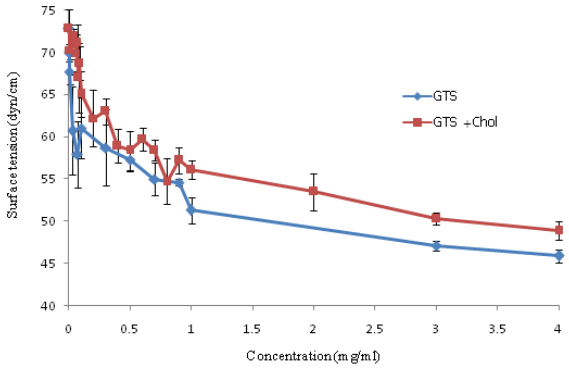

Figure 3: Changes in surface tension at different concentrations of GTS and GTS + cholesterol

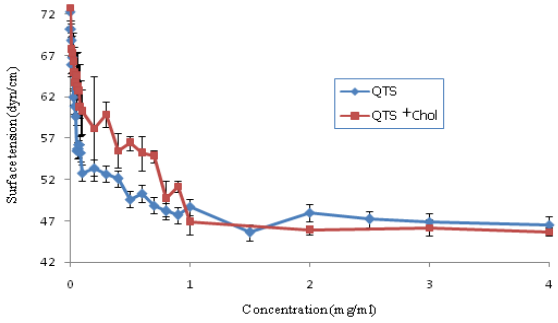

Figure 4: Changes in surface tension at different concentrations of QTS and QTS+ cholesterol

CONCLUSION

The results of the present study indicated that GTS is a potent agent to produce stable foam, stable emulsion and is able to lower water surface tension significantly. Also, it can be concluded that GTS and QTS are capable of forming complex with cholesterol and may be considered a cholesterol lowering agents.

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