

The Effects of *Glycyrrhiza Glabra* L. Total Extract on Liver Mitochondrial Respiratory Function

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Glycyrrhiza glabra L. and its major constituent, glycyrrhizin, are known to possess a plethora of pharmacological properties including anti-inflammatory, anti-viral, antitumoral and hepatoprotective. In this paper we report that Glycyrrhizin induced an improvement of respiratory function in isolated liver mitochondria by increasing both basal and ADP-stimulated respiration, whereas the total extract of *Glycyrrhiza glabra* L. elicited an increase of basal respiration and an inhibitory effect on the active respiration.

Keywords: *Glycyrrhiza glabra*, mitochondria, respiratory function

Glycyrrhiza glabra L. also known as “liquorice” or “sweet wood” is an undershrub belonging to the Fabaceae family, widely distributed in subtropical and warm temperate regions including Mediterranean countries, South Europe, Asia Minor, Egypt, Turkistan, Iran [1]. The parts of the plant which have been reported to possess pharmacological effects are rhizomes and roots. They have a rich content consisting of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances that are responsible for a host of therapeutic effects [1]. Indeed, *Glycyrrhiza glabra* L. is known to elicit several beneficial effects namely expectorant, antimicrobial, hypolipidemic, anti-atherosclerotic, antiviral, hypotensive, hepato-protective, spasmolytic, anti-diuretic, anti-mutagenic, and anti-inflammatory ones [1]. Glycyrrhizin (GL) also known as the glycyrrhizic acid, the major constituent of *Glycyrrhiza glabra* L. root, was used in Asia as remedy for chronic hepatitis [1, 2]. GL is a triterpenic compound with various therapeutic activities mainly anti-inflammatory, anti-viral, anti-tumor and hepatoprotective [2, 3]. Recent studies demonstrated that glycyrrhizic acid inhibits hepatocyte apoptosis by down-regulating the expression of caspase-3 and inhibiting the release of cytochrome C from mitochondria into the cytoplasm [2].

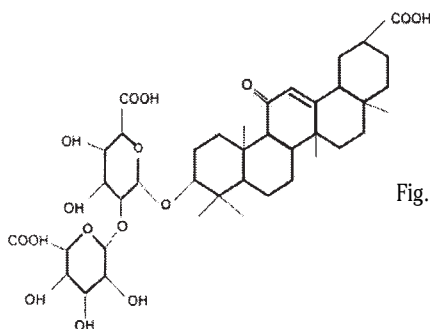


Fig. 1. Chemical structure of glycyrrhizin

Due to its chemical structure (fig. 1) consisting of one molecule of glycyrrhetinic acid and two molecules of glucuronic acid, GL also possesses hormone-like effects at adrenal cortex level [4, 5]. Moreover, this compound is an inhibitor of CCl₄-induced hepatocyte apoptosis via p53-mediated mitochondrial pathway and can delay the progression of liver fibrosis determined by CCl₄ in rats [6].

The aim of the present study was to investigate the effects of glycyrrhizin (GL) versus total extract of *Glycyrrhiza glabra* L. (Gg) on hepatic mitochondria respiratory function.

Experimental part

Materials and methods

All experimental procedures used in this study were conducted in accordance with the Directive 2010/63/EU on the protection of animals used for scientific purposes. The experimental protocol was approved by the Committee for Ethics Research of the University for Medicine and Pharmacy of Timișoara, Romania.

Animals were fed ad libitum and housed under standard conditions (constant temperature and humidity of 22.5 ± 2° C and 55 ± 5%, 12-h light/dark cycle). Twenty-four hours prior to the experiment solid food was withdrawn with no limitation in water supply.

All chemicals used for mitochondria isolation, incubation and oxygen consumption studies such as: mannitol, sucrose, HEPES, Trisma Base, BSA (bovine serum albumin - fatty acid free), EGTA, MgCl₂ · 6H₂O, KH₂PO₄, L-glutamic acid (sodium salt), L-malic acid, ADP (potassium salt), cytochrome c, oligomycin, and FCCP (carbonylcyanide p-trifluoromethoxyphenylhydrazone) were analytical grade or of highest purity commercially available. Most reagents were from Sigma Aldrich, Germany. Glycyrrhizin was purchased from Extrasynthese, France and the methanolic total extract of *Glycyrrhiza glabra* was obtained from Plafar, Romania.

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Isolation of rat liver mitochondria

Female Sprague-Dawley rats weighing 250-300 g were anesthetized with xylazine (5 mg/kg) and ketamine (20 mg/kg) intraperitoneally. Livers were quickly removed and rinsed in ice-cold 0.9% KCl solution. The tissue was minced with scissors to small pieces and then manually homogenized in isolation buffer which contains 210 mM mannitol, 70 mM sucrose, 10 mM HEPES (pH = 7.4), improved with 125 mg bovine serum albumin (BSA) 5 mg/mL and 0.25 mL EGTA 1 mM [7]. Liver mitochondria were isolated by means of differential centrifugation (Hettich Rotina 38R centrifuge) according to a previously described protocol [7]. In brief, the protocol consisted of 3 centrifugations at 4°C and different rotation speed (750 x g, 7000 x g and 7000 x g). After the final centrifugation the pure mitochondrial pellet was obtained and kept on ice throughout the experiment. Protein concentration was determined by the biuret method [8] after mitochondria solubilization with 1% deoxycolate, using BSA as standard. The final mitochondrial protein concentration in the Clark electrode chamber for all experiments was 1 mg/mL [7, 9].

Oxygen consumption studies

Oxygen consumption studies were performed using a Clark-type electrode (Hansatech Instruments Ltd.) at 37°C in a specific incubation buffer containing 100mM KCl, 2 mM KH₂PO₄, 10 mM HEPES and 1 mM MgCl₂ (pH 7.4). Respiratory rates were expressed as nmolO₂/min/mg mitochondrial protein. Data were recorded with the Oxygraph Plus software and the results were expressed as the mean ± S.D.

The sequence of additions used was as follows: basal respiration of mitochondria (State II - Std II) was determined in the presence of complex I dependent substrates (glutamate + malate) after the addition of test compounds glycyrrhizin - GL or total extract of *Glycyrrhiza glabra* - Gg vs. DMSO (as control), the oxidative phosphorylation (State III - Std III) was measured after ADP was added in the electrode chamber. In State III, ADP is rephosphorylated, and it reflects the maximal capacity of mitochondria to synthesize ATP, which positively depends on the degree of coupling. The respiratory control

ratio (RCR) was calculated as the ratio of State 3 and State 2 oxygen consumption rates.

The intactness of the outer mitochondrial membrane was checked by the subsequent addition of exogenous cytochrome *c*. A stimulation of respiration by exogenous cytochrome *c* indicates the loss of cytochrome *c* from mitochondria due to damage the outer membrane during the isolation procedure [7].

Statistical analysis

Data are presented as mean and standard deviation and were analyzed using one-way ANOVA and Tukey's and Bonferroni's post hoc analysis. All p values were considered significant at the < 0.05 level.

Results and discussions

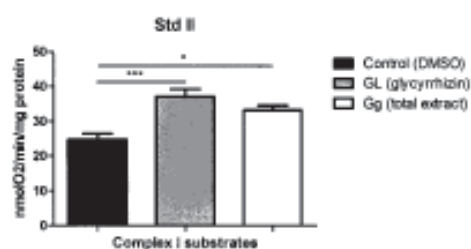
In the present study we assessed the effects of two concentrations (1 μL and 10 μL) of glycyrrhizin, a compound extracted from *Glycyrrhiza glabra* L. and the total extract of *Glycyrrhiza glabra* L. on liver mitochondrial respiration in the presence of complex I dependent substrates (glutamate and malate). Dimethylsulfoxide (DMSO), the solvent of the two tested compounds, served as control.

Our results indicated that addition of 1 μL of GL induced significant changes in all three respiratory parameters. Basal respiration was significantly higher in the presence of both compounds, but more important in the case of GL, as compared to control (fig. 2A). Interestingly, we found an important increase of the active respiration (Std III) in the presence of GL but not in the presence of Gg (fig. 2B). Since RCR is directly proportional with Std III, the decrease of Std III in presence of Gg led to a decrease of RCR as compared to control (fig. 2C).

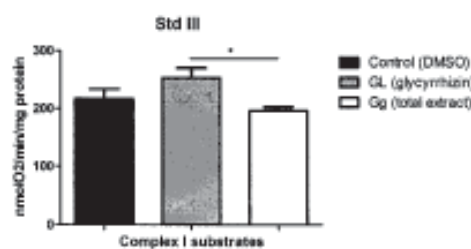
In the presence of the 10 μL of GL, basal respiration (Std II) was still increased, albeit in lesser extent as compared to the previous case (fig. 3A). However, the higher concentration (10 μL) of phytochemicals showed opposite changes on active respiration of isolated mitochondria. A strong inhibition of Std III in the presence of 10 μL of Gg was found (fig. 3B) together with an important decline of RCR (fig. 3C).

Glycyrrhiza glabra L. is a well-known traditional medicinal herb with various pharmacological properties.

A.



B.



C.

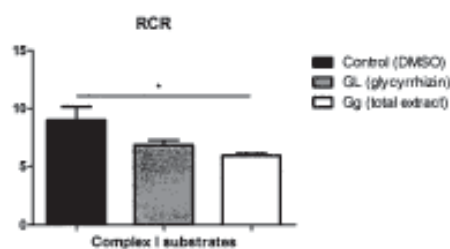


Fig. 2. Quantification of respiratory parameters in presence of complex I dependent substrates (glutamate+malate) and after addition of 1 μL of test compounds: A. A significant increase of basal respiration was observed after the addition of both glycyrrhizin (GL) and total extract of *Glycyrrhiza glabra* L. (Gg) as compared to the control (DMSO); B. A higher Std III was induced after glycyrrhizin addition; C. RCR calculated after addition of Gg was significantly decreased due to the decrease of Std III

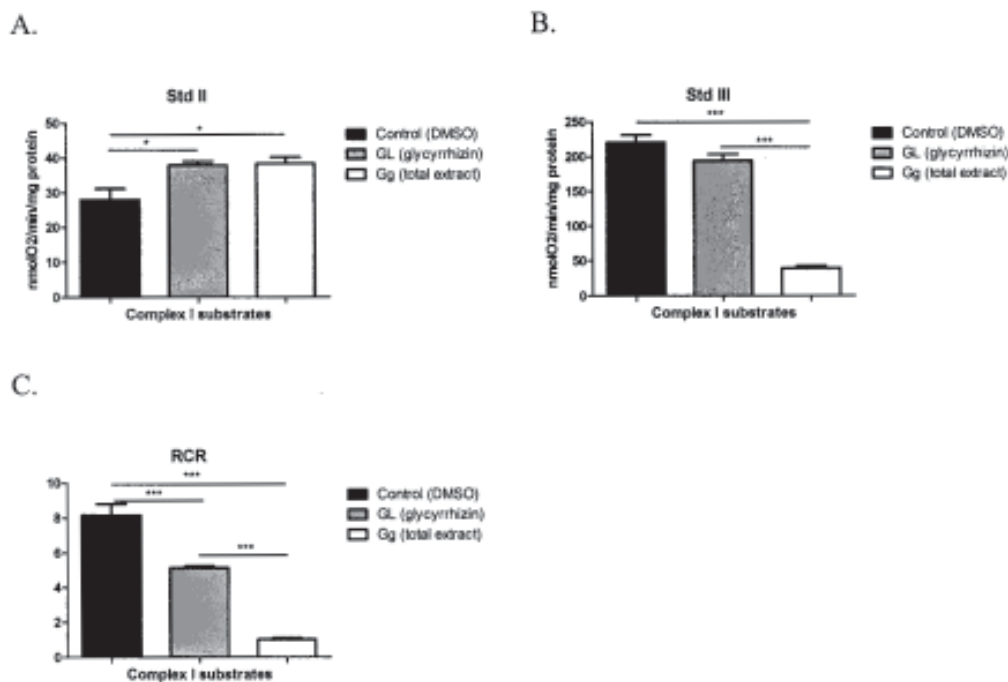


Fig. 3. Quantification of respiratory parameters in the presence of glutamate+malate and after addition of 10 μ L of test compounds: A. Addition of GL and Gg determined a clear improvement of Std II as compared to control (DMSO); B. A significant decline of Std III was observed when Gg was added; C. RCR was significantly decreased after GL and Gg additions as a consequence of Std III decline.

The protective effects of glycyrrhizin in the settings of liver and metabolic diseases represent an active field of research in the past years. In this line, it was shown that the ethanolic extract of *Glycyrrhiza glabra* possessed antidyslipidaemic activity [10]. A recent study demonstrated that the rhizomes of *Glycyrrhiza glabra* prevented the hepatorenal damage induced by acetaminophen [11]. Also, administration of the compound had a protective effect against acute liver injury induced by CCl₄ [12] and by endotoxin [13] in mice. More recently, glycyrrhizin was proven to be able to improve lipoprotein lipase expression and insulin sensitivity in obese rats [14].

The protective effects of the phytochemicals are related to their impact on mitochondria function. In this line, a recent study demonstrated the inhibitory effect of glycyrrhizin on CCl₄-induced hepatocyte apoptosis via p-53 mediated mitochondrial pathway [6]. The authors reported an increased expression of anti-apoptotic proteins, a decreased expression of pro-apoptotic proteins, and the inhibition of the cytochrome c release [6].

Our results indicated that both GL or Gg elicited an increased basal and active respiration when administered in the small dose. The effect of glycyrrhizin on hepatic mitochondria respiratory function was a protective by inducing and improvement of both basal and active respiration, which is in accordance with the data from literature [6, 13].

However, the beneficial effect on active respiration was reversed, especially in the case of *Glycyrrhiza glabra* (total extract) when the dose was ten times higher. Since the degree of inhibition of oxidative phosphorylation was not similar for GL in the same dose, we can speculate that inhibitory effect of the total extract is the consequence of the higher doses of the other constituents of *Glycyrrhiza glabra*.

Conclusions

Our results showed that both glycyrrhizin and the total extract of *Glycyrrhiza glabra* L. dose-dependently modify the respiratory function of liver mitochondria isolated from healthy animals in the presence of complex I dependent substrates. Glycyrrhizin (1 mL) was particularly effective in increasing mitochondrial respiration. Whether the beneficial effect of the small dose of glycyrrhizin can be recapitulated in animals with experimental models of liver disease remains to be proven.

Acknowledgements. Research supported by the HURO/1101/086/2.2.1 cross-border project. Grant Contract Proiect bilateral Romania-Ungaria 665/2013.

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Manuscript received: 28.11.2013