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Phytochemical Evaluation and Effect of Saponins' Mixture Isolated from *Astragalus monspessulanus* on HepG2 Cell Line

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Authors' contributions

This work was carried out in collaboration between all authors. Author VB obtained from butanol extract saponin fraction. Author MKB design the study. Authors RS and VT wrote the protocol and performed the statistical analysis. Author IK wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Short Communication

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ABSTRACT

Aims: Purified saponin fraction was obtained from butanol extract of the aerial parts of *Astragalus monspessulanus* var. *monspessulanus.* The fraction was analyzed by HPLC and six saponins were determined. The saponin mixture was investigated for possible cytotoxicity on HepG2 cell line.
Study Design: HPLC, *in vitro* study, biochemical analysis - lactate dehydrogenase leakage in medium
Place and Duration of Study: Laboratory of Drug metabolism and drug toxicity, between September and November 2013.
Methodology: The saponin mixture was studied on HepG2 cell line in 3 different concentrations –1, 2, 4 mg/ml for 24, 48 and 72 hours. The release of lactate dehydrogenase in the medium was measured spectrophotometrically.
Results: It was found that the saponins have statistically significant cytotoxicity only in the

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highest concentration 4 mg/ml on 48 and 72 hours. At this concentration the sample increased the level of LDH (lactate dehydrogenase) with 37 % on 48 h and with 52 % on 72 h.
Conclusion: Purified saponin fraction, isolated from *Astragalus monspessulanus* was found to be cytotoxic at the highest concentration 4 mg/ml on 48 and 72 hours in HepG2 cell line.

Keywords: Astragalus monspessulanus; saponins; LDH leakage; HepG; cytotoxicity.

1. INTRODUCTION

Astragalus species are well known with their use in traditional medicine [1]. Many of them have been documented to contain saponins. The *Astragalus* species are known to have numerous and varied biological activities, exerting immune modulating, antimicrobial, antiviral, tumor-inhibiting, anti-inflammatory, hepatoprotective, cardio-vascular, etc. [2,3,4].

However, the lack of information on one of their relatives, *Astragalus monspessulanus var. monspessulanus* L., makes this species a target for further investigation.

Isolated liver cells are used as a suitable model for assessing new perspective compounds. The perspective new compounds from synthetic and natural origin with predictable hepatic metabolism must be examined for cytototoxicity.

The present study investigates the effects of purified saponin fraction (PSF) containing six saponins on HepG2 cell line.

2. MATERIALS AND METHODS

2.1 General Experimental Procedures

Thin layer chromatography (TLC) study was carried out on precoated silica gel plates (Kieselgel G, F254, 60, Merck) with solvent systems n-BuOH-AcOH-H_2O (4:1:1), EtOAc-HCOOH-AcOH-H_2O (32:3:2:6). The spots were visualized by heating at 110ºC for 10 min after spraying with anisaldehyde/H_2SO_4 reagent. Column chromatography was carried out with Diaion HP20 (Supelco, USA) and Kieselgel 60 (0.040-0.063 mm, Merck). HPLC analysis was accomplished with Waters binary HPLC pump, Model 1525 and Waters UV/visible detector, Model 2479 over prepacked column Phenomenex® (C18 100A 250 x 4.60 mm 5 micron). Isocratic elution was carried out with water-trifluoracetic acid 0.03% - acetonitrile (55:45) at a constant flow rateof 1ml min^{-1}. The chromatogram was monitored at 204 nm.

2.2 Materials

The overground parts of the *Astragalus monspessulanusvar. monspessulanus* was collected from Rodopi Mountain, Bulgaria in May 2010. The plant was identified by Dr D. Pavlova from Faculty of Biology, Sofia University, Bulgaria where the voucher specimen has been deposited (N SO 107533).

Human hepatocellular carcinoma cell line (HepG2) (DSMZ-No. ACC-180) was obtained from DSMZ (Leibniz Institute German Collection of Microorganisms and Cell Cultures). RPMI medium (Gibco BRL, USA), Fetal bovine serum (Gibco BRL, USA). Penicillin-streptomycin (10,000 U/ml penicillin and 10mg/ml streptomycin) (Gibco BRL, USA).

2.3 Obtaining and Chemical Characteristics of Purified Saponin Fraction (PSF)

Air-dried powdered plant material (280g) was subjected on exhaustive extraction with 80% MeOH. After partial evaporation the aqueous solution was successively extracted with CH_2Cl_2, EtOAc and n-BuOH. The n-BuOH extract was chromatographed over Diaion column with the system H_2O–MeOH (0-100%) to give 18 main fractions (A-R). Fraction N was subjected to Low-pressure liquid column chromatography over Silica gel eluting with CH_2Cl_2-MeOH-H_2O (18:11:1) to give 28 fractions. After TLC analysis the fractions showing similar content were combined. Subfraction 10-13 which contained mostly triterpene saponins was studied by HPLC Fig. 1.

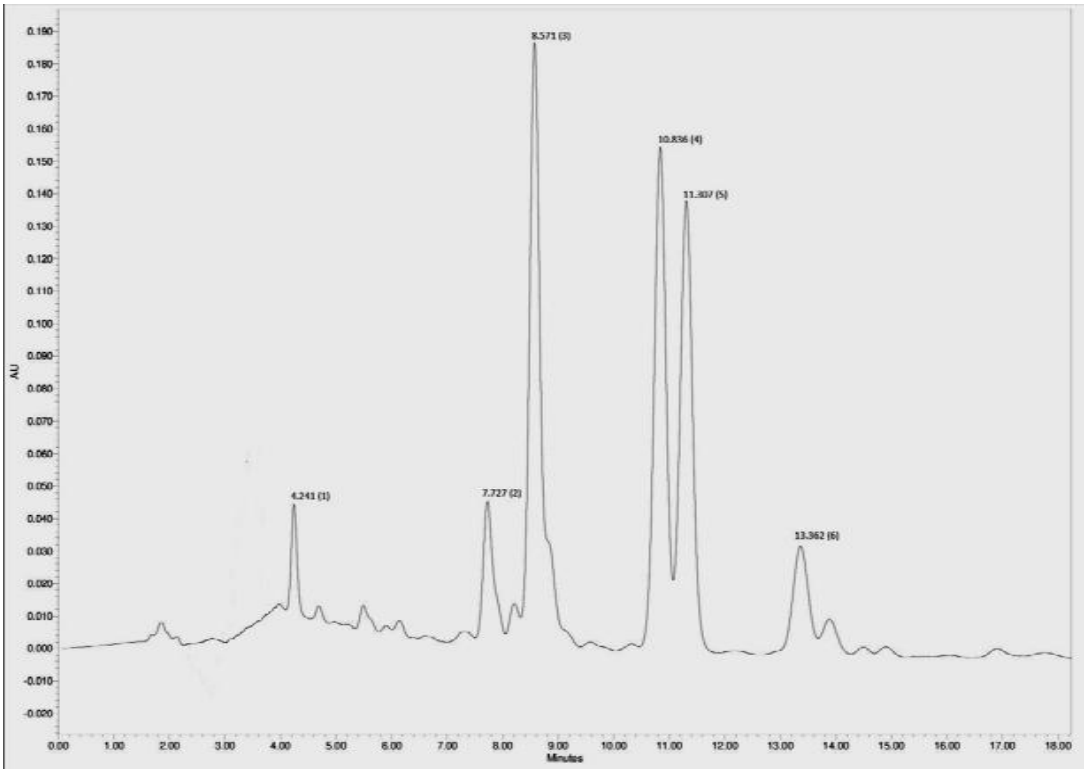

Fig. 1. HPLC profile of saponin fraction

Under optimized HPLC chromatographic conditions, six peaks of different triterpenesaponins were well separated in 18 minutes program Fig. 1. Three of the peaks referred to the main components in the saponin mixture with retention time t_R=8.57 min (**3**), t_R=10.84 min (**4**), t_R=11.31 min (**5**). Except them there are three more peaks which correspond to the minor saponins / t_R=4.24 min (**1**), t_R=7.73 min (**2**), t_R=13.36 min (**3**)/.

2.4 Cell Cultures

Human hepatocellular carcinoma cell line (HepG2) was cultured with RPMI medium supplemented with 10% fetal bovine serum and 2% penicillin-streptomycin (10 000 U/ml and 10mg/kg streptomycin). The cells were maintained at 37ºC in a humidified atmosphere of 5% CO_2/95% air and were treated with 1, 2 and 4 mg/ml saponins' mixture for 24, 48 and 72 hours.

Lactate dehydrogenase (LDH) release. Lactate dehydrogenase release was measured as described by Bergmeyer et al. [5].

2.5 Statistical Analysis

Statistical analysis was performed using statistical program 'MEDCALC'. Results are expressed as mean ± SEM for 6 experiments. The significance of the data was assessed using the nonparametric Mann–Whitney test. Values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ were considered statistically significant. Three parallel samples were used.

3. RESULTS AND DISCUSSION

Purified saponin fraction was obtained from the BuOH extract of *Astragalus monspessulanus* bycolumn chromatography over Diaion and Silica gel. It was analysed by HPLC and six saponins were determined Fig. 1.

One of the markers for cytotoxicity of biologically active compounds is the increased leakage of the enzyme lactate dehydrogenase (LDH) in the medium. Administered alone saponins' mixture (SM) increased the LDH release in medium. The effect is statistically significant, concentration and time-dependent, most prominent in 4 mg/ml at the 72 hour.

There is no statistically significant leakage of LDH in the medium at 24 hour in the 3-rd concentrations.

On 48 hour–only 4 mg/ml saponins' mixture had statistically significant cytotoxic effect, compared to the control – it increased LDH leakage in the medium with 37%.

On 72 hour, again 4 mg/ml saponins' mixture had statistically significant cytotoxic effect, compared to the control–it increased LDH leakage in the medium with 52% Fig. 2.

Our results correlate with other literature data about effects of saponins from *Astragalus* on HepG2 cell line. Ionkova et al. determined that hairy roots cultures from *A. membranaceus* can be used as means of reliable supply of cycloartane saponins to extend the research to human clinical studies [6]. An investigation in order to characterize the intracellular proteins regulated by astragaloside IV, the major active triterpenoid in *Radix Astragali* was carried out, with the conclusion that it possesses a chemoprotective effect against human hepatocellular carcinoma cell line HepG2 [7]. Apoptosis induction in the human hepatocellular HepG2 cell line was confirmed for saponins from *Astragalus* [8]. Based on the information available and according our results we suggested that these effects of the saponins' mixture, isolated from *Astragalus monspessulanus*, on hepatocellular carcinoma cell line HepG2 might be due to formation of metabolites, which had cytotoxic effects on this cell line. These cytotoxic effects of SM on HepG2, suggested that the saponins had

protective activity on this hepatocellular carcinoma cell line. This study gives preliminary results about the effect of saponin mixture.

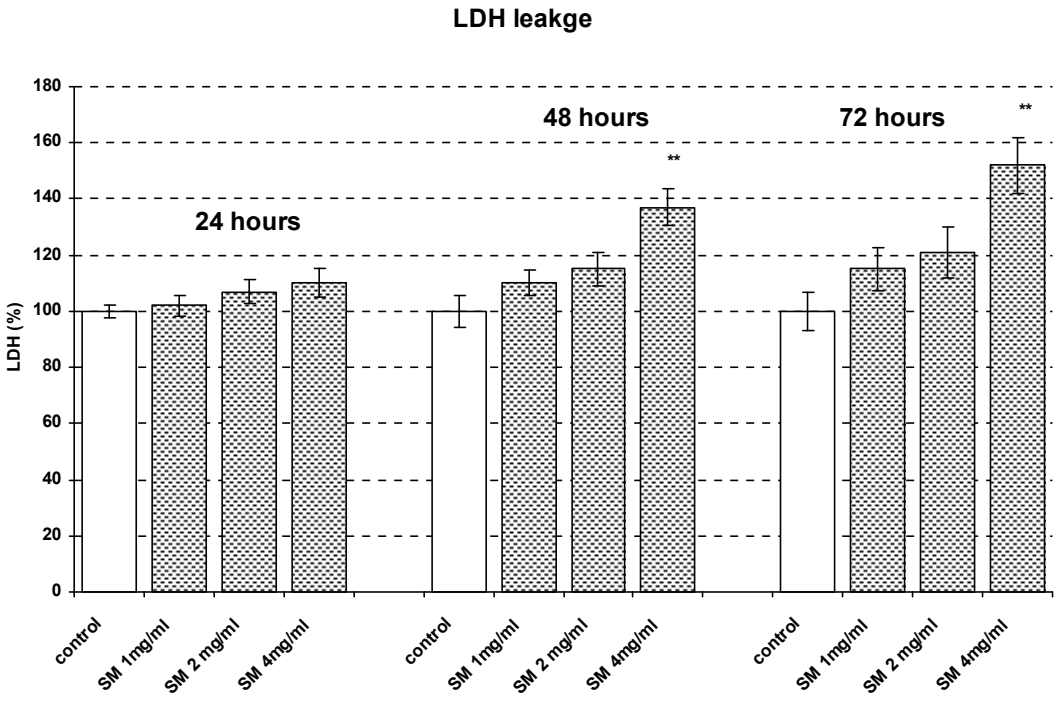

Fig. 2. Effect of saponins' mixture (SM) on LDH leakage in HepG2 cell line

4. CONCLUSION

In conclusion, the results of the present study indicate that administered alone, saponins' mixture, isolated from *Astragalus monspessulanus*, revealed cytotoxic effect on HepG2 cell line, by increasing the LDH leakage in the medium at the highest concentration 4 mg/ml on 48 and 72 hours.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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