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Chinese Journal of Natural Medicines 2013, **11**(4): 0354–0361
Chinese Journal of Natural Medicines

Hepatoprotective effects of *Astragalus kahiricus* root extract against ethanol-induced liver apoptosis in rats

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Available online 20 July 2013

[ABSTRACT] The hepatoprotective activity of the ethanol extract of *Astragalus kahiricus* (Fabaceae) roots against ethanol-induced liver apoptosis was evaluated and it showed very promising hepatoprotective actions through different mechanisms. The extract counteracted the ethanol-induced liver enzymes leakage and glutathione depletion. In addition, it demonstrated anti-apoptotic effects against caspase-3 activation and DNA fragmentation that were confirmed by liver histopathological examination. Moreover, the phytochemical study of this extract led to the isolation of four cycloartane-type triterpenes identified as astrasieversianin II (**1**), astramembrannin II (**2**), astrasieversianin XIV (**3**), and cycloastragenol (**4**). The structures of these isolates were established by HRESI-MS and 1D and 2D NMR experiments. The antimicrobial, antimalarial, and cytotoxic activities of the isolates were further evaluated, but none of them showed any activity.

[KEY WORDS] Hepatoprotective; Apoptosis; Rats; Fabaceae; *Astragalus kahiricus*; Cycloartane triterpenes

[CLC Number] R965 **[Document code]** A **[Article ID]** 1672-3651(2013)04-0354-008

1 Introduction

Apoptosis, or physiologically programmed cell death, is recognized as an important pathologic feature in the development of most liver diseases [1]. In recent years, there has been renewed interest in plant medicines for the treatment of a wide variety of liver diseases [2-3]. Unfortunately, the conventional or synthetic drugs used are inadequate, and sometimes can have serious side effects. This is one of the reasons why many people all over the world are turning towards complementary and alternative medicine [4]. The genus *Astragalus* L. (Fabaceae) is the largest genus of flowering plants [5]. It comprises over 3 000 species [6-7]. In Egypt, the genus is represented by 35 species [8-9]. Several *Astragalus* spp. have previously shown hepatoprotective actions [10-12]. Previous phytochemical studies performed on some Egyptian *Astragalus* species have resulted in the isolation of a series of cycloartane-type saponins [13-17]. Radwan *et al*. initiated a study on the aerial parts of *Astragalus kahiricus* (AK) isolating four new saponins named, kahiricoside II, kahiricoside III, kahiricoside IV, and kahiricoside V [18].

In this study, the hepatoprotective effect of the alcoholic root extract of *A. kahiricus* against acute ethanol-induced liver apoptosis in rats was evaluated. Another, three saponins namely astraversianin II (**1**), astramembrianin II (**2**) and astraversianin XIV (**3**), and the aglycone cycloastragenol (**4**) were isolated. Also, the antimicrobial, antimalarial, antileishmanial and cytotoxic activities of the isolated compounds were investigated.

2 Results and Discussion

2.1 *Serum AST and ALT*

[Received on] 12-Feb.-2012

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These authors have no conflict of interest to declare.

Alcohol-induced liver injury was estimated by measuring plasma transaminase activities. In the ethanol group, serum ALT and AST activities were significantly increased and reached 153% and 130%, respectively, as compared to control group. The two indices in ethanol/low AK, ethanol/high AK and ethanol/silymarin group were significantly decreased when compared with the ethanol alone group. Moreover, their levels were returned to normal levels (Figs. 1A and 1B)

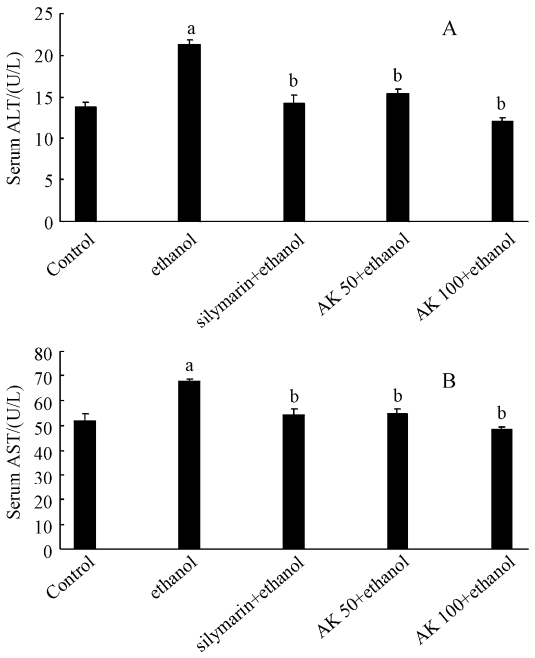

Fig. 1 A & B Effect of AK extract on liver function tests in rats subjected to acute intoxication with ethanol. Mean ± SEM, *n* = 8. Statistical analysis was done using one way ANOVA, followed by Tukey's post-hoc test for multiple comparisons at *P* < 0.05
a: *P* < 0.05 *vs* control, b: *P* < 0.05 *vs* ethanol

2.2 *Liver reduced glutathione (GSH) and lipid peroxides level*

Experiments were carried out to evaluate whether the enhanced hepatotoxicity found in the ethanol group was associated with elevated oxidative stress. The ethanol group showed a marked and significant decrease by 50% in the liver content of GSH as compared to the control group. Ethanol/low AK and ethanol/high AK groups significantly counteracted GSH depletion induced by ethanol intoxication in a dose-dependent manner. However, the level of liver GSH in these two groups was still significantly lower than the control value. On the contrary, the ethanol/silymarin group did not show any improvement in liver GSH level as compared to the control and ethanol intoxicated groups (Figs. 2A and 2B). MDA, as one index of lipid peroxidation, was significantly higher in the ethanol intoxicated group than the control group reaching 233%. Unfortunately, the ethanol/low AK and ethanol/high AK groups significantly elevated the liver lipid peroxides level as compared to the ethanol alone group. Moreover, there was no difference in the level of MDA between ethanol/silymarin group and ethanol alone group (Figs. 2A and 2B).

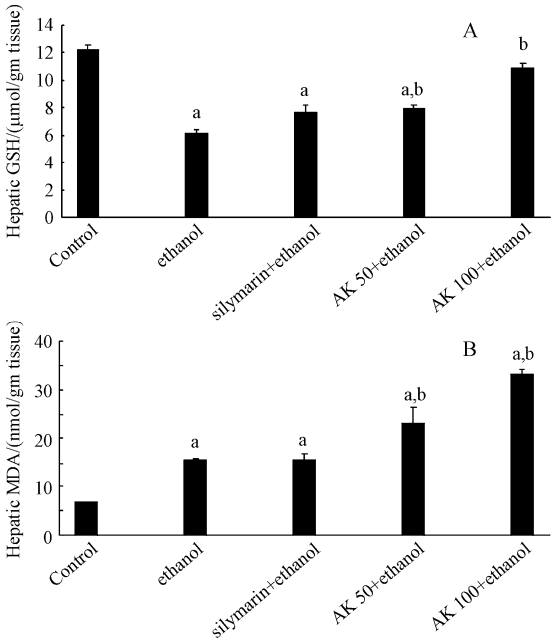

Fig. 2 A & B Effect of AK extract on liver GSH and MDA in rats subjected to acute intoxication with ethanol. mean ± SEM, *n* = 8. Statistical analysis was done using one way ANOVA, followed by Tukey's post-hoc test for multiple comparisons at *P* < 0.05
a: *P* < 0.05 *vs* control, b: *P* < 0.05 *vs* ethanol

2.3 *DNA fragmentation assay and liver caspase 3 activity*

Ethanol-induced hepatic apoptosis was examined by a DNA fragmentation assay. The ethanol group induced a significant increase by 2.5 fold in DNA fragmentation as compared to the control group. The two extract groups significantly lowered the fragmentation of DNA compared with the ethanol group. However, the level of fragmentation in the low dose group was slightly, but not significantly, lower than from the high dose of the extract. Similarly, the ethanol/silymarin group resulted in a significant decrease in DNA fragmentation as compared to the ethanol intoxicated group, but did not bring the increase in DNA fragmentation back to the normal value. Ethanol-induced apoptosis involves the activation of cysteine-dependent aspartate-specific proteases or caspases; therefore, the activity of caspase-3 was determined after acute ethanol administration. The activity of the enzyme was significantly elevated in ethanol group by two-fold compared with the control group. The two extract groups significantly lowered the liver caspase compared