Egyptian J. Desert Res., 57, No.2, 325 -331 (2007)

FLAVONOID CONTENT AND BIOLOGICAL EFFECT OF THE DESERT MEDICINAL PLANT *ASTRAGALUS BOETICUS* L.

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An ethyl acetate extract containing flavonoids was obtained from *Astragalus boeticus* L. Seven flavonoids were isolated and identified as rutin, hyperoside, isoquercitrin, narcissin, quercetin, kaempferol and isorhamnetin for the first time. The extract was found to be practically non-toxic (acute oral toxicity >5g kg^{-1} in mice). It was investigated for antihypoxic activity in two experimental models: hypoxia-haemic and circulatory. The antihypoxic activity was especially pronounced in the model of circulatory hypoxia.

Our studies indicate that the ethyl acetate extract from *Astragalus boeticus* has a low acute oral toxicity and a remarkable antihypoxic effect, especially in a model of circulatory hypoxia. It is therefore very promising for further pharmacological and biochemical experiments, which will be focused on evaluating the mechanism of antihypoxic activity.

Keywords: *Astragalus boeticus* L., Leguminoseae, biological activity, flavonoids.

Astragalus boeticus L.(Leguminoseae) is one of the largest and most widely distributed genera of about 2000 species, mainly in the North Temperate regions and Tropical African mountains (Evans, 1998 and Bolus, 1999). The genus *Astragalus* has 37 species indigenous to Egypt. *Astragalus boeticus* L. is annual herb growing in Egypt (Hutchinson ,1973 and Tacholm, 1974). Some *Astragalus* species are used as feed by livestock and wild animals particularly in Asia, others are used as foods, cosmetics, and as a vegetable (Rizk, 1986). A number of *Astragalus* species are used in Chinese traditional medicine as antihypertensive, antidiabetic, diuretic and tonic (Konoshima, 1996; Hikino *et al*., 1976). They also have hepatoprotective, antioxidative, immunostimulant and antiviral properties, whereas others have shown toxic activity (Hartwell, 1970) and have been used for the treatment of leukemia and uterine cancer. In many cases the cytotoxic principals can pass to humans through milk and meat (Rios and Waterman, 1997).

The constituents of *Astragalus boeticus* include polysaccharides, saponins, flavonoids, amino acids and trace elements .

Mostly flavones and flavonol glycosides and their aglycones displaying different biological activities have been previously isolated from *Astragalus* species. The most interesting properties of flavonoids are their antioxidative, vasodilator and antimicrobial traits (Krasteva *et al*., 2000).

The study was planned as there are no reports on the chemical constituents of this plant to date. The aim of the present study was to obtain a dry ethyl acetate extract from above-ground parts of *Astragalus boeticus* which contained flavonoids and to test it for acute oral toxicity and brain antihypoxic activity.

MATERIALS AND METHODS

I-Plant Material

Astragalus boeticus L. were collected from the area adjacent to Al-Arish road (65 Km west) during the vegetative growth seasons of the year 2006. Samples were air-dried in shade, ground to fine powder and stored for subsequent investigations . The plant was identified by the late Prof. V. Tackholm, Faculty of Science, Cairo University.

II- Extraction and Phytochemical Study

The UV- spectra were recorded on a SPECORD UV-VIS spectrometer with diagnostic shift reagents (Mabry *et al*., 1970).

Air-dried plant material (1Kg) was powdered and extracted with (5L) 90% ethanol. After the removal of ethanol in vacuo, the aqueous residue was consecutively treated with chloroform and ethyl acetate. The ethyl acetate extract was evaporated to dryness to give a solid residue (14g), which was suspended in 5%DMSO (dimethyl sulfoxide) in water for pharmacological experiments .

The ethyl acetate extract was chromatographed on a cellulose column, using a 0-95% ethanol linear gradient. Seventy fractions, 60ml each, were collected and analyzed by thin layer chromatography (TLC) on silica gel. Identical fractions were put together: 1-20, 21-40, 41-52, 53-70. Fractions 1-20 were rechromatographed on a Sephadex LH-20 using methanol as eluent. Further purification was achieved by column chromatography on a polyamide, eluting with 0-90% ethanol gradient, followed by preparative paper chromatography(PPC) or TLC. Final purification was carried out on a Sephadex LH-20 with methanol as eluent. Five flavonol glycosides and three flavonol aglycones were isolated and identified by chromatographic, chemical and spectroscopic methods.

The content of flavonoids in the ethyl acetate extract, calculated as hyperoside, was determined by spectrophotometry at 320nm (European

Pharmacopoeia, 2002). A Hewlett Packard 8452A array diode spectrometer was used for the measurements.

III- Biologically Study

1- Animals

Animals used in the experiments were male mice, strain H, mass 22-25g, kept under standard conditions in animal house (water and food, 12h dark and light cycle). The following groups of animals were used in the study: control groups and 3 experimental groups for each model, haemic and circulatory hypoxia. Each group comprised 6 animals. Controls were treated with vehicle (5% DMSO in water) in the same volume as the treated animals (0.1mL per 10g b.w.). No effects of the vehicle were observed.

2-Acute toxicity

Acute oral toxicity (LD_{50}) was estimated by the up-and-down procedure. No behavioral changes or mortality were observed during the period of 14 days.

3-Haemic and circulatory hypoxia

Thirty minutes after oral administration of ethyl acetate extract 250, 125 and 62.5 mg kg^{-1}, namely (1/20,1/40 and 1/80 of LD_{50}) , $NaNO_2$ (360mg kg^{-1}) was applied i.p. to each mouse and the antihypoxic activity was estimated as the latent time of evidence of hypoxia in minutes according to the method of (Roshtina and Ostrovskaya,1981) .

Thirty minutes after oral administration of the ethyl acetate extract at 125, 62.5, 31.3mg kg^{-1}, namely (1/40, 1/80 and 1/160 of LD_{50}), to each mouse and the antihypoxic activity was estimated in minutes as the latent time of evidence of hypoxia.

4- Statistical analysis

The LD_{50} data were assessed by the AOT 425 statistical program. Anti-hypoxic activity was expressed relative to the control and was compared by Student's paired t-test.

RESULTS AND DISCUSSION

Seven known flavonoids were isolated for the first time from the above – ground parts of *Astragalus boeticus* L. The compounds were identified as rutin (1), hyperoside (2), isoquercitin (3), narcissin (4), quercitin (5), kaempferol (6) and isorhamnetin (7) by chromatographic, chemical and spectral methods.

The Rf-values of glycosides 1-4 (solvent system A) and aglycones 5-7 (solvent system B) are presented in table(1).

UV spectra of isolated flavonoids revealed different profiles, recorded alone or after the addition of some diagnostic shift reagents (Table 1). The spectral data showed flavonol structure with an *o*-dihydroxy group on B-ring

for compounds 1,2,3,5, free hydroxyl groups on C-5 and C-7 for all compounds and a substituted hydroxy groups on C-3′ on B-ring for 4 and 7.

Total concentration of flavonoids in the extract, calculated as hyperoside, was 3.8% .

The extract is practically non toxic (LD_{50} > 5g kg^{-1}) in mice. In contrast, the literature data about LD_{50} of pure quercetin (97-98%) show relatively high toxicity (p.o.LD_{50} 160 mg kg^{-1} in mice) (Merck Index,1983). As mentioned above the total flavonoid content in our extract is only 3.8 %, which can explain the difference in LD_{50} values. A statistically significant antihypoxic activity of the extract was established in the experimental model of haemic and circulatory hypoxia in mice . The effect was found to be dose-dependent (Table 2) in a range of 250-62.5mg kg^{-1}(1/20-1/80 parts of LD_{50}) for haemic and 125-31.75mg kg^{-1} (1/40-1/160 parts of LD_{50}) for circulatory hypoxia. There are literature data that administration of sodium fluoride (substance that induces circulatory hypoxia) increases the blood histamine content and decrease the oxygen carrying capacity (Sumina and Shugaev, 1978). The study of Karcher *et al.* (1984) shows that a preliminary acute treatment of mice with *Ginkgo biloba* extract, which also contains quercetin, kaempferol and other flavonoids, has a significant protective effect on other forms of hypoxia such as hypobaric hypoxia.

Table (1). Chromatographic and UV spectral data of identified compounds.

Compd.	R_f value	UV (λ_{max} nm)
Rutin (1)	0.15	(MeOH)257,355;(+NaOMe)272,405;(+$AlCl_3$)275,422;(+$AlCl_3$/HCl)265,396;(+NaOAc)269,393; (+NaOAc/H_3BO)260,372
Hyperoside(2)	0.43	(MeOH)253,355;(+NaOMe)323,405;(+$AlCl_3$)272,420;(+$AlCl_3$/HCl)272,393;(+NaOAc)265,387
Isoquercitrin(3)	0.50	(MeOH)255,357;(+NaOMe)278,407;(+$AlCl_3$)278,432;(+$AlCl_3$/HCl)275,405;(+NaOAc)278,398; (+NaOAc/H_3BO)265,385
Narcissin(4)	0.23	(MeOH)252,347;(+NaOMe)277,405;(+$AlCl_3$)279,355,407;(+$AlCl_3$/HCl)279,345,400;(+NaOAc)278,325,382; (+NaOAc/H_3BO)253,347
Quercetin(5)	0.27	(MeOH)254,367;(+$AlCl_3$)٢٦٨,٤٣٧;(+$AlCl_3$/HCl)٢٦٣
Kaempferol(6)	0.52	(MeOH)٢٦٧,٣٦٥;(+$AlCl_3$)٢٦٨,٣٥٠,420;(+$AlCl_3$/HCl)267,348,418
Isorhamnetin(7)	0.59	(MeOH)253,368;(+$AlCl_3$)267,425;(+$AlCl_3$/HCl)260,418

Table (2). Antihypoxic activity of ethyl acetate extract of *Astragalus boeticus* L. on two models of brain hypoxia

Haemic hypoxia		Circulatory hypoxia	
Dose (mg kg^{-1}.part of LD_{50})	Activity (%)[a]	Dose (mg kg^{-1}.part of LD_{50})	Activity (%)[a]
Control	100 ± 6	Control	100 ± 5
Group 1(625mg kg^{-1}.1/80)	101 ±7[b]	Group 1(31.75mg kg^{-1}.1/160)	140 ± 9[b]
Group 2(125mg kg^{-1}.1/40)	127.8 ±13[b,c]	Group 2 (62.50mg kg^{-1}.1/80)	195 ± 11[b,c]
Group 3(250mg kg^{-1}.1/20)	208 ±19[b,c,d]	Group 3 (125mg kg^{-1}.1/40)	230 ± 18[b,c,d]

Data are expressed as mean ±SD(n = 6)
Statistically significant difference : [b] $p < 0.05$(compared to control); [c] $p < 0.05$ (compared to group 1) : [d] $p < 0.05$ (compared to group 2) .

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Received: 23/11/2006
Accepted: 11/04/2007

المحتوى الفلافونيدي والتأثير البيولوجي لنبات البهلق (*Astragalus boeticus*) كنبات طبي صحراوي

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تشير دراستنا في هذا البحث أن مستخلص الاثيل اسيتات المأخوذ من نبات البهلق (*Astragalus boeticus*) له تأثير سمية وتأثير معاد للنشاط المضاد لنقص الأكسجين في الدورة الدموية.

ومن المبشر بالخير لكل التجارب العقاقيرية والكيمياء الحيوية ان تركيزها على اية النشاط المضاد لنقص الأكسجين ، مستخلص الاثيل اسيتات يحتوي على مركبات فلافونيدية وعددها سبعة مركبات تم فصلها والتعرف عليها وهي الرتين – هيبيروسيد – الايزوكيرسيترين – نار سيزين – كيرسيتين – كامبيقيرول و ايزورامنتين . وجد أن مستخلص الاثيل اسيتات غير سام (الجرعة حادة السمية إذا أخذت عن طريق الفم في الفئران وهى تمثل > ٥جم / كجم).

المستخلص يكشف عن النشاط المضاد للنقص في وصول الأكسجين لأنسجة الجسم بنموذجين هما Circulatory وhypoxia-haemic النشاط المضاد لنقص الأكسجين واضح خصوصا في نموذج الدورة الدموية.