

HEPATOTOXICITY EFFECTS OF AQUEOUS EXTRACT OF *ECHIUM AMOENUM* IN RATS

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Summary

Hepatotoxicity effects of *Borago officinalis* has been accepted due to pyrrolizidine alkaloid constituents. This study was performed to evaluate the hepatotoxicity effect of *Echium amoenum*, indigenous to Iran, in rats. Rats were divided into 8 groups (n=7/group). They were treated for 1 or 2 weeks with saline or *E. amoenum* at doses of 100, 200 or 400 mg/kg/day. Rat liver functions were assessed by serum biochemistry of alanine aminotransferase (ALT), aspartate aminotransferases (AST) and alkaline phosphatase (ALP). Liver sections were stained with H&E for histopathological assessment. Serum levels of ALT and ALP were reduced significantly in both 1 and 2 weeks treated groups (p<0.05) compared to controls. ALT reductions were observed only in 2 weeks treated groups. All treated groups had a normal architecture of liver tissue. No sign of evidence of characteristic hepatotoxicity was found in rats treated with *E. amoenum* extract.

Key words: Borage, *Echium amoenum*, Hepatotoxicity, Liver function tests

The interest in phytomedicine by the professional and lay public is increasing steadily. Several recent surveys from Europe and the US have demonstrated a sharp rise in the use of botanical drugs within a few years (1-2). However, our knowledge of the potentials and risks of botanical drugs is still limited and efforts to elucidate them should be intensified.

Iranian borage (*Echium amoenum*) is a large hairy annual herb that is a member of Boraginaceae family. It grows in most of Europe, in the Mediterranean region, and also in northern parts of Iran (3). *Echium* genus has 4 species in Iran (4,5) and only *E. amoenum* has medicinal uses (4,6). It has long been used as atonic, tranquillizer, diaphoretic and as a remedy for cough, sore throat and pneumonia. It's known in traditional medicine of Iran as Gol-e-Gavzaban (7).

Anxiolytic effect of the flower of this plant has been shown in two separate experimental studies in mice. In Western medicine, the flowers and the leaves of borage have been similarly used as antifebrile, antidepressant, anxiolytic, ameliorant of heart and pulmonary disturbances, poultice for inflammatory swellings, diuretic, laxative, emollient and demulcent, and recently as a possible protective factor against cancer (8).

It has been shown as a rich source of antioxidants, like rosmarinic acid (RA) and flavonoids (8). Different investigators have isolated the plant constituents; they include gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), delta6-fatty acyl desaturase, delta8-sphingolipid desaturase (9), pyrrolizidine alkaloids, mucilage, resin, potassium nitrate and calcium salt combined with mineral acids (3).

Hepatotoxicity of plants containing pyrrolizidine alkaloids is well established. Acute toxicity is reproducible in animals and seems to be related to biotransformation of pyrrolizidine alkaloids by cytochrome P450s into pyrrole derivatives, which then act as alkylating agents (10).

The present study was designed to determine the hepatotoxicity effect of aqueous extract of *E. amoenum*, in rats.

Methods

Animals

Adult male 3-month-old Wistar rats (200-250g) were obtained from Razi institute (Karaj, Iran) and housed in polypropylene cages (4-5/cage). All animals received food and water ad libitum. Rats were kept in standard environmental conditions (temperature $23\pm 2^{\circ}\text{C}$ and a 12h light/dark cycle). All animal experiments were carried out in accordance with the regulations of the Ethics Committee of the Qazvin University of Medical Sciences. Two investigations have been conducted in rats. The first one assessed the acute toxicity and a second, chronic (1 and 2 weeks) hepatotoxicity study of aqueous extract of Iranian borage.

Plant material and preparation of the extract

Flowers of *E. amoenum* (obtained from Alamout) a village in Qazvin provinces, Iran) in June 2006. Identity of the plant was authenticated by Qazvin Agriculture and National Resources Research Center, Iran. A voucher specimen (986) was deposited in the Institute's Herbarium. An aqueous extract of the *E. amoenum* was prepared. In brief 100 g of the dried flowers placed in a container with 1000 ml of water, and boiled for 15 min. the preparation was left standing to cool, filtered and dried in oven in $40-45^{\circ}\text{C}$. the extract was diluted with saline.

Acute toxicity and lethality test (LD₅₀)

The LD₅₀ of the extract was determined by administering aqueous extract diluted in normal saline to rats using the intraperitoneal route. Animals in groups of seven received one of 0.75, 1.5, 2, 2.5 and 3 g/kg of aqueous extract. And observed for 24 h for number of deaths. The LD₅₀ was calculated by Litchfield and Wilcoxon methods (PHARM/PCS version 4).

Chronic hepatotoxicity test

Rats were divided into 4 groups (n=7), a saline treated control group and three experimental groups. Three doses (100, 200 and 400 mg/kg i.p.) of aqueous extract diluted with saline were administered in separate groups of animals.

Serum analyses

After one or two weeks of treatment, the animals were anesthetized and blood samples were collected by cardiac puncture. To prepare the serum samples, blood samples from rats were immediately centrifuged at 3000g for 10 min. The supernatant serum were separated from the pellet and immediately used for biochemical analyses.

The following biochemical parameters were assayed: alanine aminotransferase (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP) using biochemical analysis kits (Parsazmun Co., Tehran, Iran).

Liver histopathological assessment

Liver sections were fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene, and embedded in paraffin. Sections (4-5 μ m thick) were prepared and then stained with hematoxylin and eosin (H-E) dye for microscopic observation, including cell necrosis, fatty change, ballooning, fibrosis and inflammation.

Statistical analysis

The dose of the mean lethal dose (LD₅₀) of aqueous extract of *E. amoenum* and its associated 95% confidence limits was calculated by Litchfield and Wilcoxon methods (PHARM/PCS version 4). The data were expressed as mean values \pm S.E.M and tested with one-way ANOVA followed by the multiple comparison test of Tukey-Kramer. A value of $p < 0.005$ was considered to be statistically significant.

Results

An LD₅₀ value of *E. amoenum* extract was 2.03 g/kg (%95 CL: 2.54, 1.62).

There were no signs of clinical toxicity in animals during either the 1 or 2 weeks treatment.

E. amoenum extract administration did not produce significant ($p > 0.05$) changes in liver weight compared to the controls (Table 1).

Table 1: Effect of *E. amoenum* extract on the liver weight

	Control	100mg/kg	200mg/kg	400mg/kg
1 week treatment	11.09 \pm 0.24	10.42 \pm 0.43	10.92 \pm 0.25	9.99 \pm 0.24
2 weeks treatment	10.84 \pm 0.25	11.07 \pm 0.33	12.12 \pm 0.26	11.26 \pm 0.48

Data are reported as mean (g) \pm SEM in groups.

The serum levels for ALT and ALP were significantly lower ($p < 0.05$) than controls at both 1 and 2 weeks treatment (Fig. 1-2). AST showed a significant reduction ($p < 0.05$) only after 2 weeks *E. amoenum* treatment (Fig. 3).

The histological observations basically supported the result obtained from enzyme assays. The histological architecture of liver sections of rats treated with *E. amoenum* showed a normal lobular pattern and hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein.

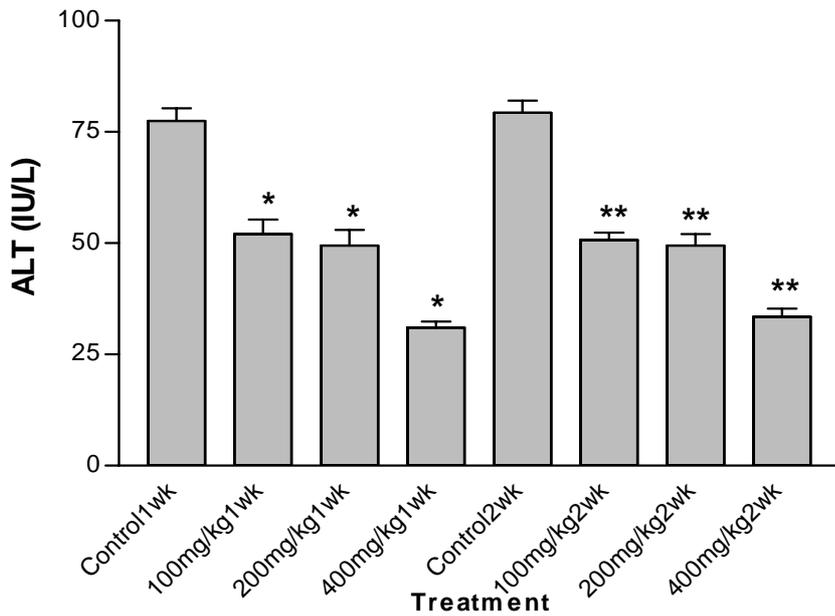


Fig 1. Effects of *E. amoenum* extract in rats on ALT in Low dose (100mg/kg), medium dose (200mg/kg) and high dose (400 mg/kg) experimental groups in contrast to controls for 1 week or 2 weeks (*p<0.05 vs. 1 week treatment; **p<0.05 vs. 2 weeks treatment)

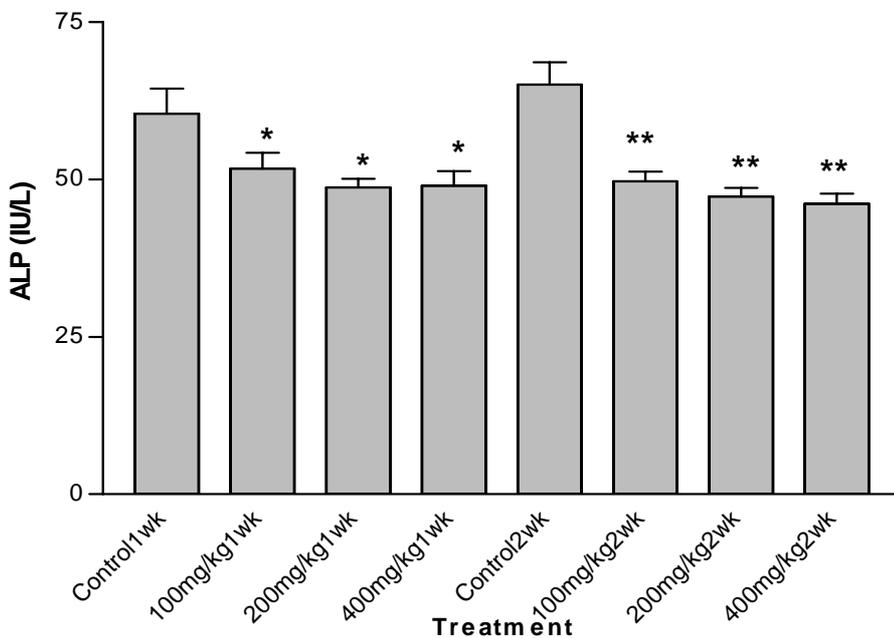


Fig 2. Effects of *E. amoenum* extract in rats on ALP in Low dose (100mg/kg), medium dose (200mg/kg) and high dose (400 mg/kg) experimental groups in contrast to controls for 1 week or 2 weeks (*p<0.05 vs. 1 week treatment; **p<0.05 vs. 2 weeks treatment).

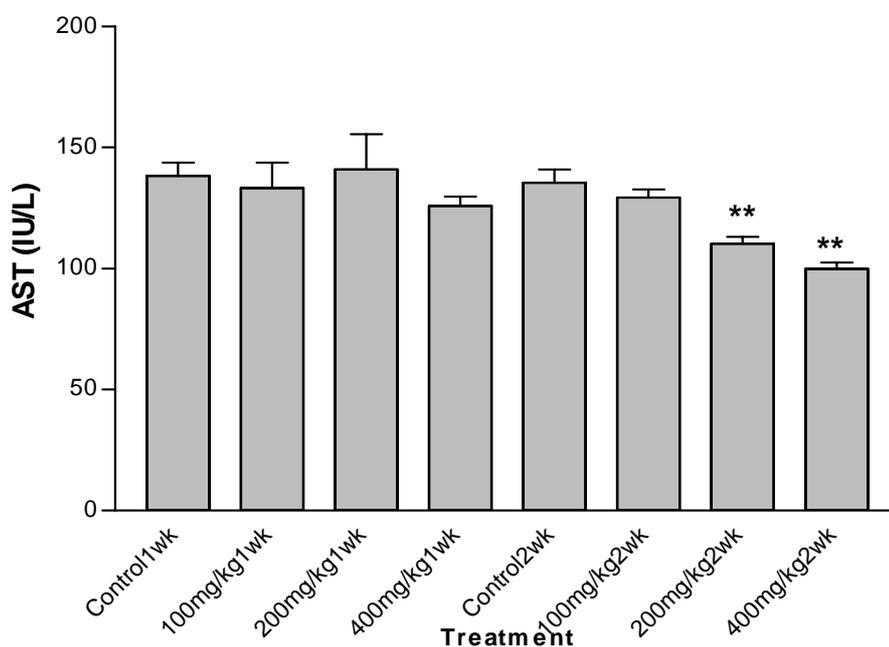


Fig 3. Effects of *E. amoenum* extract in rats on AST in Low dose (100mg/kg), medium dose (200mg/kg) and high dose (400 mg/kg) experimental groups in contrast to controls for 1 week or 2 weeks (** $p < 0.05$ vs. 2 weeks treatment).

Discussion

The enzyme assay data clearly indicate that there was no significant increase in any of the three major marker enzymes for hepatotoxicity. Actually there were reductions in the enzymes activities. On the other hand we observed a normal histopathological condition in liver tissue sections.

These observations would suggest that *E. amoenum* extract might have a hepatoprotective effect, rather than being hepatotoxic. Earlier studies have shown that the total alkaloid content of dried petals of *E. amoenum* is 0.01%. In these petals, structures of four pyrrolizidine alkaloids namely: echimidine I, echimidine isomer II, 7-angeloyl retronecine III and 7-tigloyl retronecine IV were identified (11). Hepatotoxicity of pyrrolizidines is well documented (10) but it is possible that the trace amount of pyrrolizidine alkaloids in this plant could not be adequate to producing hepatotoxicity.

On the other hand, in another study has been suggested that the major phenolic compound of *E. amoenum* extract is rosmarinic acid (RA) (4) and antioxidant activity of Iranian *E. amoenum* has been suggested due to its bioactive antioxidant components, especially rosmarinic acid and flavonoids (8).

Rosmarinic acid, an important constituent of this plant, is an ester of caffeic acid and 3,4-dihydroxyphenylacetic acids. It is commonly found in species of the Boraginaceae. There are a number of reports on the antioxidative activities of RA which all confirm that RA has strong antioxidant activity even higher than vitamin E. In this regard, the reported positive effects of RA include enhancement of superoxide and hydroxyl scavenging (12), inhibition of both low-density lipoprotein (13) and oil oxidation (14), suppression of arachidonate metabolism formation (15), inhibition of hemolysis (16). Free radicals, including the superoxide radical, hydroxyl radical, hydrogen peroxide, and lipid peroxide radicals have been implicated in liver diseases (17).

Therefore the possible hepatoprotective effect of *E. amoenum* may be due to preventing the process of lipid peroxidation and scavenging of free radicals. However the exact mechanism is still unknown. Further studies are warranted to isolate the active components and to clarify in detail, the pathway of the protective mechanism on chemically induced or viral-induced liver injuries.

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