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Original Article

Anxiolytic Effect of *Echium amoenum* L. in Mice

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Abstract

Putative activity of hydroalcoholic and aqueous infusion extracts of *Echium amoenum* L. was investigated in mice using the rotarod model of motor coordination and the elevated plus maze model of anxiety. The extracts were administered intraperitonealy (i.p.) once, one hour before performing the tests. Preliminary phytochemical study of the plant, with standard procedures, showed that it contains saponins, flavonoids, unsaturated terpenoids and sterols. There was no evidence of tanins, alkaloids and cyanogenic glycosides. The hydroalcoholic extract of *Echium amoenum* in the dose range employed (125, 250 and 500 mg/kg) had no significant effect on motor coordination while the aqueous extract (62.5, 125, 250 and 500 mg/kg) disrupted motor coordination significantly. Intraperitoneal injection of aqueous extract (5, 10, 20, 30, 62.5, 80 and 125 mg/kg) showed a significant dose-dependent increase in time spent in open arm (OAT) with no significant change in open arm entries (OAE), closed arm entries (CAE) and total arm entries (TAE). The anxiolytic effect was most evident in 125 mg/kg group. It is almost evident that the extract produces its anxiolytic effect in the doses in which no change in motor activity is observable. Comparison of the dose response curve with the anxiolytic dose response of diazepam (0.25, 0.5, 1.0 and 2.0 mg/kg) in the same setting showed that the maximal efficacy of the extract is significantly lower than diazepam. Because of different maximal efficacies we were not able to calculate Extract/diazepam potency ratio but it does not seem to be more than 1/100. It is concluded that single administration of aqueous extract of *Echium amoenum* L. produces a significant but mild to moderate anxiolytic effect.

Keywords: Anxiolytic; Echium; Elevated plus-maze; Rotarod; Mice.

Introduction

Echium amoenum L. (Boraginaceae) is one of the important medicinal herbs in traditional Iranian medicine. Since it has long been used in traditional Iranian medicinal formulary, it represents an interesting source to search for various pharmacological activities. The phytochemical studies on *Echium amoenum* revealed the presence of many chemicals such as flavonoids, saponins and unsaturated terpenoids and sterols (1). It has been demonstrated that flavonoids possess mild sedative and anxiolytic effects. The naturally occuring flavonoids and their synthetic derivatives have been reported to selectively bind to the central benzodiazepine receptors, and to exert anxiolytic and other benzodiazepine-like effects in animals (2). In the present study we have investigated the anxiolytic activities of hydroalcoholic and aqueous infusion extracts of *Echium amoenum* by the elevated plus-maze (EPM) model of anxiety in mice. Furthermore, the activities of this extract on motor coordination were investigated using rotarod model.

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Experimental

Preparation of plant materials

The plants were collected in Chalus district in Iran. The flowers (10 g dry weight) were subjected to a hydroalcoholic extraction (900 ml) by shaking for 24 h at room temperature. The obtained hydroalcoholic extract was filtered and then diluted with saline to obtain concentrations of 125, 250 and 500 mg/10 ml.

Animals

Male NMRI albino mice (20-30 g; Pastur institute, Tehran, Iran) were used. Animals were housed in groups of eight to ten under a standard 12h light/dark cycle (lights on 07:00 hours) in a room maintained at 22 ± 2°C with free access to food and water. Each subject was only tested once. All the experiments were performed in spring from 8 a.m. to 3 p.m.

Apparatus and procedure

Rotarod

Loss of coordinated motor movement is one of the pharmacological effects of anxiolytic drugs (3). The effect of the plant extract on coordinated motor movement was assessed using rotarod test (3). Mice were trained to stay in rotarod apparatus (3 cm in diameter, 8 rpm) for 120 seconds and at least two times for each animal. Twenty four hours later the animals were injected with vehicle (saline), hydroalcoholic extract of plant (125, 250 and 500 mg/kg) intraperitoneally and placed in apparatus 1 hour later. The latency (in seconds) to drop off the rotarod was recorded up to a limit of 100 seconds.

Elevated plus-maze

The elevated plus-maze, a modification of that used by Lister (1987), consisted of two open arms (30 x 5 x 0.5 cm) and two closed arms (30 x 5 x 15 cm) with an open roof, arranged so that two pairs of identical arms were opposite to each other. Arms emerged from a central platform (5 x 5 cm), and the entire apparatus was raised to a height of 50 cm above floor level. The maze was constructed from black Plexiglass. Mice were administered the test compound and placed individually in the center of the maze, facing one of the open arms. The number of entries into both the open or enclosed arms and the amount of time spent in the open arms was recorded. Each test lasted for 5 min and each mouse was tested only once. The apparatus was cleaned between each test (4, 5). The test compounds were administered i.p. 30 min before the test in a volume of 10 ml/kg body weight. All tests were conducted between 08:00 and 14:00.

Data analysis

Statistical analysis of quantal data of rotarod test was performed with a probit-log(dose) model (Probit procedure), if the fit was successful fiducial confidence interval for ED50 of falling from rotarod was reported. Statistical analysis of graded responses of plus-maze was done with an analysis of covariance (ANCOVA) using the Generalized Linear Model (GLM procedure) with response as the independent, dose as the covariate and replicates as a nested random factor. All statistical calculations were done with SPSS for Windows (1999) statistical software package (6). In all statistical tests $p<0.05$ was considered significant.

Results and Discussion

Effects of EA extracts on motor coordination

The effect of EA aqueous extract on the motor coordination of mice was evaluated by the rotarod test. Statistical analysis of the latency to fall from the rotarod revealed that i.p. administration of EA at 500 mg/kg ($p<0.01$) and 250, 1000 mg/kg ($p<0.05$) produced significant motor incoordination compared to

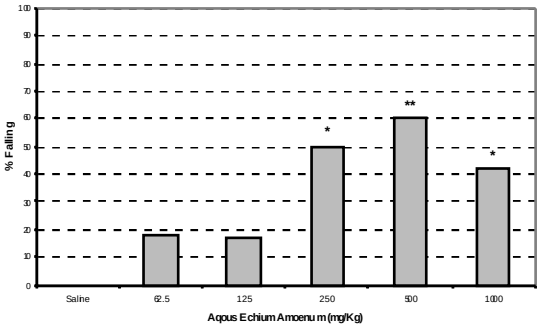

Figure 1. Effects of i.p. administration of EA aqueous extract on %falling from rotarod apparatus. Behavior was monitored 1 hour after drug administration. Data are the means ± SEM of at least 10 animals.

** $p<0.01$ Dunnett test.

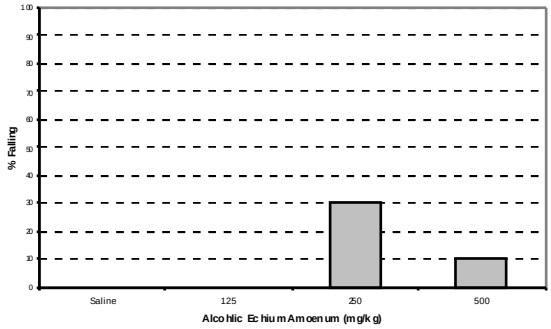

Figure 2. Effects of i.p. administration of EA alcoholic extract on %falling from rotarod apparatus. Behavior was monitored 1 hour after drug administration. Data are the means ± SEM of at least 10 animals.

** $p<0.01$ Dunnett test.

vehicle control (Figure 1). Although different doses of hydroalcoholic extract had no significant effect on it (Figure 2).

Effects of diazepam on mice anxiety behavior in EPM apparatus

The classic anxiolytic benzodiazepine, diazepam, at 0.25, 0.5, 1 and 2 mg/kg was used as positive control. Statistical analysis of plus-maze data revealed that diazepam significantly increased the time spent in open arms (OAT) (Figure 3). These results confirm the suitability of the method used in the present study. Although various doses of diazepam had no effect on the number of open or closed arm entries (Figures 4-6).

Effects of EA aqueous extract on the EPM

EA doses below 250 mg/kg were chosen to clarify anxiety effects in EPM model. MANOVA -revealed that EA aqeuous extract at 125 mg/kg significantly increased the time spent in open arms [$F(7,152)=3.465$, $P=0.002$], (Figure 7).

Benzodiazepines (BDZs) are the most widely prescribed class of psychoactive drugs in current therapeutic use, despite the important unwanted side-effects that they produce such as sedation, muscle relaxation, ataxia, amnesia, ethanol and barbiturate potentiation and tolerance. Searching for safer BDZ-receptor (BDZ-R) ligands it has been demonstrated the existence of a new family of ligands which have a flavonoid structure, first isolated from plants used as tranquilizers in folkloric medicine, some natural flavonoids have been shown to possess a selective and relatively mild affinity for benzodiazepine receptors. Some of those compounds, such as 6,3-dinitroflavone were found to have a very potent anxiolytic effect (7). These compounds exhibit a high affinity for the benzodiazepine receptors. Due to their selective pharmacological profile and low intrinsic efficacy at the benzodiazepine receptors, flavonoid derivatives, auch as those described, could represent an improved therapeutic tool in the treatment of anxiety.

The elevated plus-maze is currently one of the most widely used model of animal anxiety, having been employed by many research laboratories in the past 6 years and has been extensively validated for use with both rats and mice (4,5). Its validity in our study was supported by the observation that diazepam, a classic anxiolytic, significantly increased the time spent in the open arms.

The behavior observed using the EPM in the present study confirmed the anxiolytic activity of diazepam as reported previously (6). Using this test the EA increased the percentage of time spent in the open arms.

The EA extract, similarly to diazepam, increased the time spent in the open arms. These results are suggestive that EA has an anxiolytic-like effect in the plus-maze test.

Statistic analysis of plus-maze data (MANOVA) revealed that among four parameters in anxiety assessment, proportion of time spent in the open arms was significantly chaned [$F_{OAT}(7,152)=3.465$, $P=0.002$] and for other parameters there wasn't any significant result (Figures 8-10). Post hoc tests (Dunnett 2 sided test) revealed that 125 mg/kg of EA produced a statistically significant effect compared to vehicle control ($P=0.005$). Moreover, using multivariate analysis of covariance (MANCOVA) revealed that there is a significant dose response relationship [$F(1,152)=22.636$, $p<0.0001$] particulary in 125 mg/kg dose. These results are suggestive that EA has an anxiolytic-like effect in the plus-maze test. The behavioral profile induced by Echium extract is similar to that induced by diazepam. Our data represents that aqueous extract of plant has a significant but not very potent anxiolytic effect.

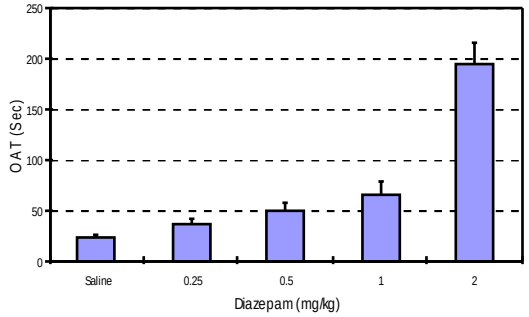

Figure 3. Effects of increasing doses of diazepam on percent time spent in the open arm of the elevated plus maze.

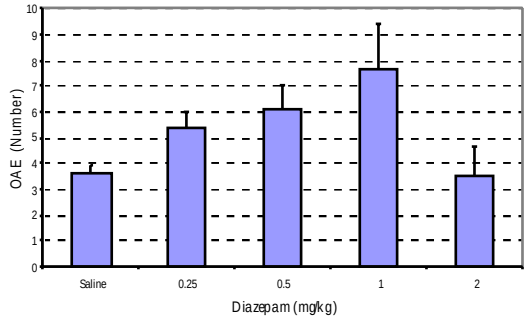

Figure 4. Effects of increasing doses of diazepam on the open arm entry of the elevated plus maze.

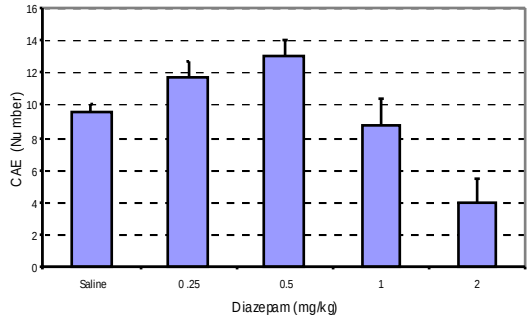

Figure 5. Effects of increasing doses of diazepam on the close arm entry of the elevated plus maze

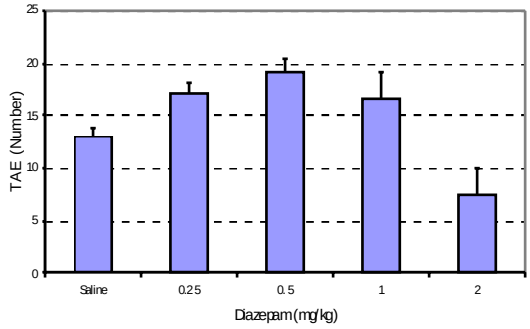

Figure 6. Effects of increasing doses of diazepam on the total arms entries of the elevated plus maze.

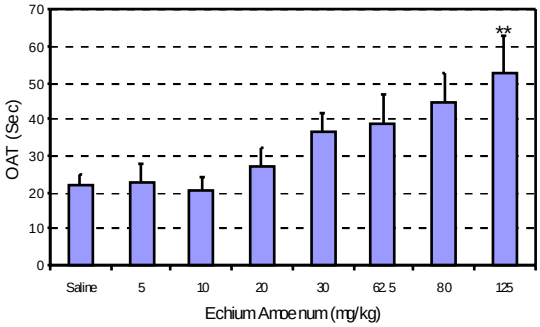

Figure 7. Effects of i.p. administration of EA aqueous extract on percent time spent in the open arms of the elevated plus maze..
** $p<0.01$ Dunnett test.

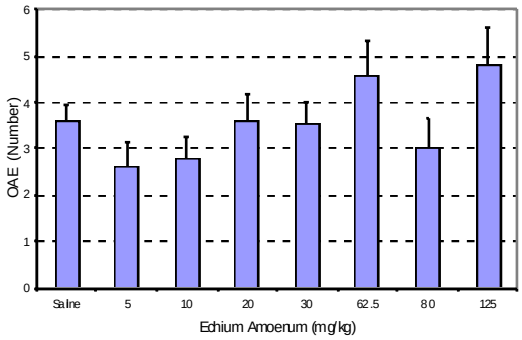

Figure 8. Effects of i.p. administration of EA aqueous extract on the open arm entry of the elevated plus maze.

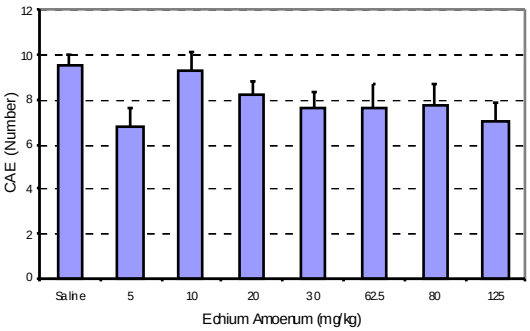

Figure 9. Effects of i.p. administration of EA aqueous extract on the close arm entry of the elevated plus maze.

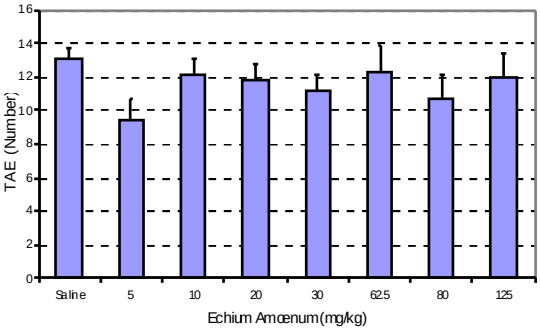

Figure 10. Effects of i.p. administration of EA aqueous extract on the total entries of the open and close arms of the elevated plus maze.

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