

Evaluation of the anxiolytic effect of *Echium amoenum* petals extract, during chronic treatment in rat

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Abstract

Echium amoenum (Boraginaceae), a very popular medicinal plant in Iran traditional medicine, is used as a tonic, tranquillizer, diaphoretic, cough and sore throat remedy, and antipneumonia. Preliminary phytochemical studies of the plant showed that it contains saponins, flavonoids, unsaturated terpenoids and sterols. In this study, an aqueous extract from petals of this plant was used 125 mg/kg as compared to diazepam 1 mg/kg, i.p., during two different treatment courses, 15 and 30 days. The aqueous extract was prepared by Soxhlet apparatus with distilled water. A sample size of 36 rats in 6 groups was selected for this experiment. Three groups were treated by saline, diazepam and extract daily for 15 days and the other groups for 30 days. Anxiolytic effect of extract was investigated in rat using the elevated plus-maze model of anxiety. After finishing these courses and 30 min after the last injections, the test was performed. The results revealed that in 30-day treatment course, time spent in open arms was significantly higher than that of 15-day treatment in both diazepam and extract groups and this effect was the highest in the diazepam group. So, the results of the study indicated a significant durationdependent increase in time spent in open arms of plus-maze.

Keywords: *Echium amoenum*; Aqueous extract; Anxiolytic; Elevated plus-maze

INTRODUCTION

Echium amoenum Fisch & C.A. Mey (Boraginaceae) is one of the important medicinal plants in Iranian traditional medicine (1,2). Petals of *E. amoenum* have been advocated for its demulcent, anxiolytic, sedative, anti-inflammatory and analgesic effects, especially for common cold, in folk medicine of Iran (1-4). The phytochemical studies on *E. amoenum* revealed that petals of this plant have anthocyanidine, flavonoid aglycons, traces of alkaloids (5,6), volatile oils (0.05%) (7) and rosmarinic acid (8). A review of literature revealed that *E. amoenum* has antioxidant activity in humans (9). This plant stimulates lymphocyte proliferation and inhibits humoral antibody synthesis (10).

Antibacterial effect of *E. amoenum* on *Staphylococcus aureus* was reported by Abolhassani (2004) (11). Iranian borago (*E. amoenum*) exerts analgesic and anticonvulsant effect in mice that was reported by Heidari et al. (12,13). Volatile constituents of *E. amoenum*, was extracted by Ghassemi et al. (7). These compounds include octadecane, heptadecane, viridiflorol, alpha cadinen etc. (7). Toxic pyrolizidine alkaloids of *E. amoenum* were separated by Mehrabani et al. (14). It has been demonstrated that flavonoids possess mild sedative and anxiolytic effects (15). The naturally occurring flavonoids and their synthetic derivatives have been reported to bind selectively to the central benzodiazepine receptors and to exert anxiolytic and other benzodiazepine like effects in animals (15).

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In the present study, we have investigated the anxiolytic activity of the aqueous extract, 125 mg/kg from petals of *E. amoenum*, comparing with a single dose of diazepam (1 mg/kg) during 15 and 30 day treatment courses, by the elevated plus-maze model of anxiety in rats.

MATERIALS AND METHODS

Plant and extract

An aqueous extract (pH=6) of dried flowers was used in this study. *E. amoenum* flowers were collected from Salmas district, Iran. Flowers of this plant were separated and dried in room temperature (22-24 °C). The plant materials were powdered and exhaustively extracted with distilled water in a Soxhlet apparatus under reduced pressure. After evaporation of the solvent in rotary evaporator and then in oven at 40 °C, the residue was diluted with saline to obtain 125 mg/kg dose.

Animals

Male Wistar Albino rats (180-200 g, Urmia University) were used. Animals were housed in groups of six, under a standard 12 h light/dark cycle in room maintained at 22 ± 4 °C with free access to food and water. A sample of 36 rats in 6 groups was selected for this experiment. Three groups were treated with saline, diazepam 1 mg/kg and extract 125 mg/kg daily for 15 days i.p. and 3 groups were treated as stated before but for 30 days. All the behavioral tests were performed from 9 am to 4 pm.

Elevated plus-maze apparatus

In the elevated plus-maze test, rats were placed in the center of the plus-maze facing one of the open arms. During a 5-min test period, the following data were recorded: number of entries and time spent in the open and closed arms. Anxiolytic compounds selectively increased the

percentage of time spent and/or entries into the open arms (16). The elevated plus-maze consisted of two open arms and two closed arms with open roof, arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 75 cm above floor level. Eighteen rats received solutions i.p. for 15 days and eighteen rats for 30 days. All animals were tested on apparatus once. After finishing each course and 30 min after the last injection, the test was performed.

Data analysis

The data were analyzed using Analysis of Variance, completely randomized block design (ANOVA2). Means were separated by using the Tukey Multiple Range Test, SAS System version 7 for Windows. In all statistical tests $P < 0.05$ was considered to be significant.

RESULTS

The results of data analysis showed that time spent in open arms decreased in diazepam group and increased significantly ($P < 0.05$) in extract group in 15 day course. In 30-day course, this time increased significantly in both of treatment groups compared to their own control groups (Fig. 1). Time spent in closed arms decreased in diazepam and extract groups in both of treatment courses (Fig. 2). Our data showed that number of open arm entry was same in 15-day groups but increased in 30-day course in both of treatment groups (Fig. 3). Observation of reduction in number of closed arm entry in each course is shown in Fig. 4.

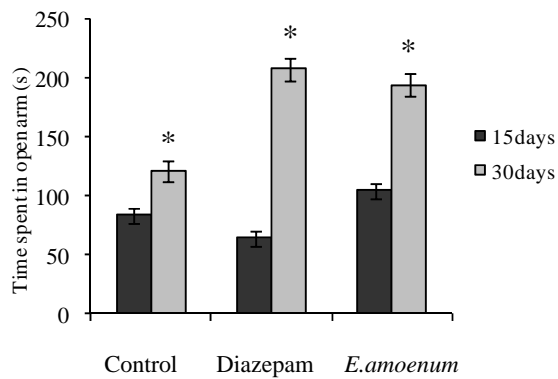


Fig. 1. Effect of i.p. administration of *E. amoenum* aqueous extract, 125 mg/kg and diazepam, 1 mg/kg on time spent in open arms in the elevated plus-maze during 15- and 30-day. Each column indicates the mean \pm SEM in 6 rats. * $P < 0.05$ compared to corresponding control groups.

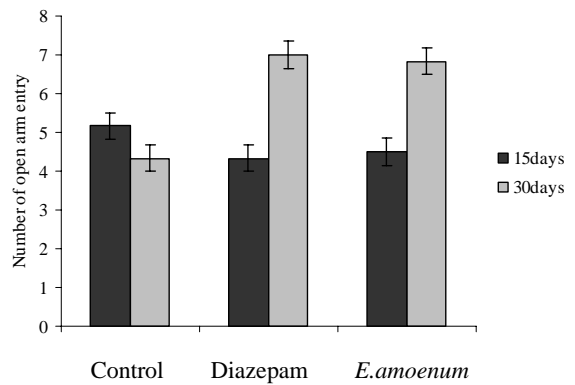


Fig. 3. Effect of i.p. administration of *E. amoenum* aqueous extract in 125 mg/kg compared with diazepam 1 mg/kg during 15- and 30- day treatment on the number of open arm entry in rat. Each column indicates the mean \pm SEM in 6 rats.

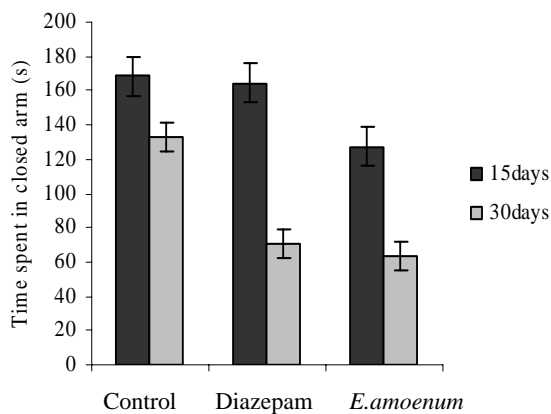


Fig. 2. Effect of i.p. administration of *E. amoenum* aqueous extract, 125 mg/kg compared to diazepam 1 mg/kg during 15- and 30-day treatment on time spent in closed arms in rat. Each column indicates the mean \pm SEM in 6 rats.

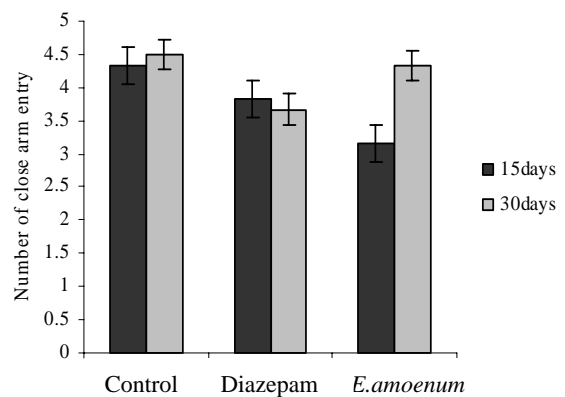


Fig. 4. Effect of i.p. administration of *E. amoenum* aqueous extract 125 mg/kg compared to diazepam 1 mg/kg during 15- and 30-day treatment on number of closed arm entry in rat. Each column indicates the mean \pm SEM in 6 rats.

DISCUSSION

Effects of diazepam on rat anxiety behavior in plus-maze apparatus

Diazepam, the classic anxiolytic benzodiazepine, at 1 mg/kg was used as positive control. It has been shown that diazepam at 0.03-3 mg/kg, i.p. has anxiolytic activity in rat and mice, and

increases open arm exploration (17). Shafaghi et al. used diazepam at doses of 0.25, 0.5, 1, 2 mg/kg, and the results showed that diazepam at 1 and 2 mg/kg increased open arm entries and time spending in open arms, respectively (4). Data analysis of the present study showed that diazepam (1 mg/kg), significantly increased time spent in open arms in 30-

day treatment and between both of treatment courses. However, in 15-day treatment, this time was reduced. Statistical analysis of plus-maze data of diazepam revealed that among the four parameters in anxiety assessment, proportion of time spent in open arms was significantly changed, and for other parameters there were no significant results (Fig. 1-4). Benzodiazepines (BZDs) bind to a specific site on GABA receptors. The presence of BZDs on this site potentiates the effect of GABA, increases the permeability to chloride ions, hyperpolarizes membrane of neuron and therefore, results in anxiolytic effect (18). Literature review indicates that behavioral anxiety in mice is related to decreased number of GABA and benzodiazepine receptors in cerebral cortex (19). Therefore, diazepam as an anxiolytic drug increases the number of GABA and benzodiazepine receptors in cerebral cortex. The difference between the results of 15 and 30 days treatments with diazepam may probably be the result of time interval needed for these changes.

Effect of E. amoenum extract on rat anxiety behavior in plus-maze apparatus

Searching for safer BZD-receptor ligands has led to discovery of a new family of these ligands with a flavonoid structure, first isolated from plants and used as tranquilizers in folk medicine. Some natural flavonoids have been shown to be selective and possess relatively mild affinity for benzodiazepine receptors (4). Some of those compounds, such as 6,3'-dinitroflavone were found to have a very potent anxiolytic effect (20). Data analysis of the present study showed that the extract of *E. amoenum* (125 mg/kg) significantly increased the time spent in the open arms in 15- and 30- day treatments, and reduced the time spent in closed arms and the number of closed arm entries. The results suggests that *E. amoenum* has an

anxiolytic effect by using the plus-maze test. Shafaghi et al. used aqueous extract of *E. amoenum* at doses of 62.5, 125, 250, and 500 mg/kg, and the results showed that anxiolytic effect of the extract was most evident in 125 mg/kg group (4). Anxiolytic effect of ethanolic extract of *E. amoenum* flowers at 50 mg/kg was revealed by Rabbani et al. in 2004 (21).

Considerably, Soxhlet is an aggressive procedure because of temperature shock, and may reduce the extract effects, so it is recommended to use safer methods such as percolation.

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