

Anxiolytic Effect of *Echium amoenum* During Different Treatment Courses

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Abstract: *Echium amoenum* (Boraginaceae) is a very popular medicinal plant which is used as a tonic, tranquillizer, diaphoretic, cough remedy, sore throat and pneumonia in Iran's traditional medicine. Preliminary phytochemical study of the plant, with standard procedures, showed that it contains saponins, flavonoids, unsaturated terpenoids and sterols. There was no evidence of tannins, alkaloids and cyanogenic glycosides. In this study an aqueous extract from petals of this botanical was used in 125 mg kg⁻¹ concentration as compared with diazepam 1 mg kg⁻¹ intraperitoneally (i.p.), during 2 different treatment courses, 15 and 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 last injections, the test performed. Results revealed that in 30 days treatment course, time spent in open arms was significantly higher than 15 days treatment in all groups and this effect is lower than diazepam. So we showed a significant time-dependent increase in time spent in open arm.

Key words: *Echium amoenum*, aqueous extract, anxiolytic, elevated plus maze

INTRODUCTION

Echium amoenum (Boraginaceae) is one of the important medicinal plants in Iranian traditional medicine (Hooper, 1937; Zargari, 1996). Petals of *E. amoenum* have been advocated for variety of effects such as demulcent, anti-inflammatory and analgesic, especially for common cold, anxiolytic and sedative in folk medicine of Iran (Amin, 1991; Hooper, 1937; Shafaghi *et al.*, 2002; Zargari, 1996). The phytochemical studies on *E. amoenum* revealed the presence of many chemicals such as flavonoids, saponins and unsaturated terpenoids and sterols (Salehzadeh, 1990). It has been demonstrated that flavonoids possess mild sedative and anxiolytic effects. The naturally occurring 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 (Medina *et al.*, 1997). In the present study we have investigated the anxiolytic activity of aqueous extract in 125 mg kg⁻¹ concentration from petals of *Echium amoenum* as compared with single dose of diazepam (1 mg kg⁻¹) during 15 and 30 days treatment courses, by the elevated plus maze model of anxiety in Rat. Bijan Shafaghi *et al.* (2002) used aqueous extract of *Echium amoenum* in dose range 62.5, 125, 250 and 500 mg kg⁻¹ and revealed that anxiolytic effect of extract was most evident in 125 mg kg⁻¹ group. About diazepam, they tested in dose range (0.25, 0.5, 1, 2 mg kg⁻¹) and showed that 1 and 2 mg kg⁻¹ increased open arm entries and time spending in open arm, respectively.

On the basis of the above facts, we used these doses to understanding the Anxiolytic effect of extract during 15 and 30 days treatment courses.

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 naturally on laboratory benches at 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⁻¹ concentration.

Animals: Total 36 Male Wistar Albino Rats (180-200 g; Faculty of Science; 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. All the experiments were performed from 9 a.m. to 4 p.m.

Elevated plus maze apparatus: In the elevated plus maze test, Rats were placed at 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 increase the percentage of time spent and/or entries into the open arms (Andreatini and

Bacellar, 1999). The elevated plus maze consisted of 2 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 for 15 days and eighteen Rats for 30 days. All of them were tested on apparatus once. After finishing each course and 30 min after last injection, the test performed.

Data analysis: All statistical calculations were done with SAS System (2000) version 7 for Windows. SAS Institute, Cary

RESULTS AND DISCUSSION

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

The classic anxiolytic benzodiazepine, diazepam, at 1 mg kg⁻¹ was used as positive control. Data analysis showed that diazepam significantly increased time spent in open arms in 30 days treatment and between both of treatment courses. But in 15 days treatment it wasn't significant. Benzodiazepines (BZDs) bind to a specific site on the GABA receptors. The presence of benzodiazepine on this site potentiates the effect of the GABA and increases the permeability to chloride ions and hyperpolarizes membrane of neuron and due to anxiolytic effect.

Table 1: Comparison of diazepam 1 mg kg⁻¹ with *E. amoenum* 125 mg kg⁻¹ on time spent in open arm in elevated plus maze.

Days	Treatment groups		
	Control	Diazepam	<i>E. amoenum</i>
15	83.09±23.9bc	63.75±24.7c	104.3±24.6abc
30	121.4±33.4abc	207.6±34.3a	194.3±20.2ab

Same words in p<0.05 in Tukey MRI test aren't significant

Table 2: Comparison of diazepam 1 mg kg⁻¹ with *E. amoenum* 125 mg kg⁻¹ on time spent in closed arm in elevated plus maze.

Days	Treatment groups		
	Control	Diazepam	<i>E. amoenum</i>
15	168.4±40a	146.6±44.9a	127.1±37.3a
30	132.9±26.1a	70.7±35.2a	63.5±14.8a

Same words in p<0.05 in Tukey MRI test aren't significant

Table 3: Comparison of diazepam 1mg kg⁻¹ with *E. amoenum* 125 mg kg⁻¹ on open arm entry in elevated plus maze.

Days	Treatment groups		
	Control	Diazepam	<i>E. amoenum</i>
15	5.16±1.5a	4.33±1.3a	4.5±0.72a
30	4.3±0.49a	7.0±1.57a	6.83±1.14a

Same words in p<0.05 in Tukey MRI test aren't significant

Table 4: Comparison of diazepam 1mg kg⁻¹ with *E. amoenum* 125 mg kg⁻¹ on closed arm entry in elevated plus maze

Days	Treatment groups		
	Control	Diazepam	<i>E. amoenum</i>
15	4.33±0.91a	3.83±1.22a	3.16±0.79a
30	4.5±0.76a	3.66±0.91a	4.33±0.84a

Same words in p<0.05 in Tukey MRI test aren't significant

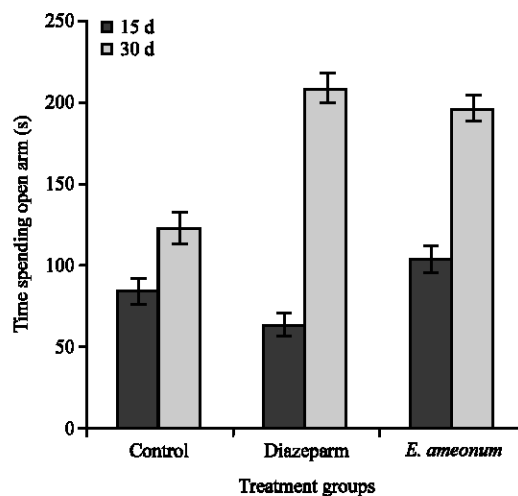


Fig. 1: Effect of diazepam 1 mg kg⁻¹ and *E. amoenum* 125 mg kg⁻¹ on time spent in open arms during 15 and 30 days treatment. **p<0.05 Tukey test.

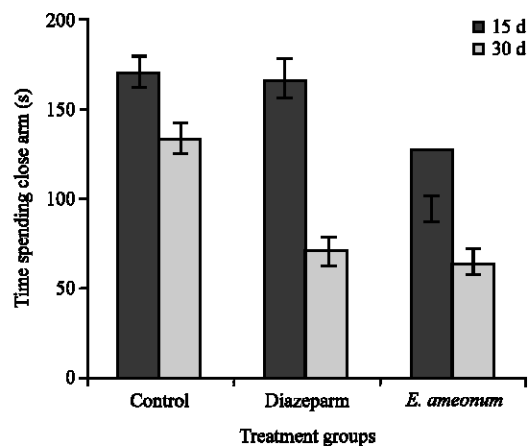


Fig. 2: Effect of diazepam 1 mg kg⁻¹ and *E. amoenum* 125 mg kg⁻¹ on time spent in closed arms during 15 and 30 days treatment.

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

Table 1-4 Searching for safer BZD-receptor 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 (Shafaghi *et al.*, 2002). Some of those compounds, such as 6,3-dinitroflavone were found to have a very potent anxiolytic effect (Wolfman *et al.*, 1996).

The *E. amoenum* extract, similarly to diazepam, increased the time spent in the open arms. Data analysis showed that *E. amoenum* significantly increased time

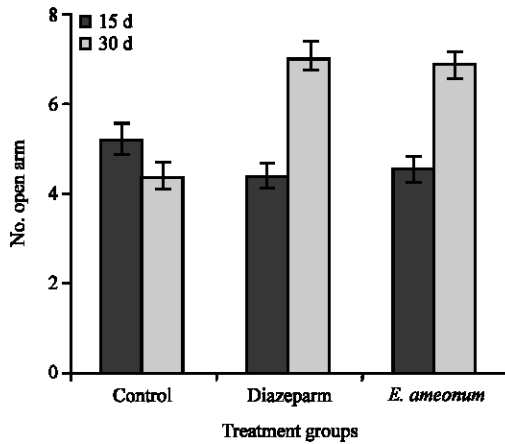


Fig. 3: Effect of diazepam 1 mg kg⁻¹ and *E. amoenum* 125 mg kg⁻¹ on open arm entry during 15 and 30 days treatment.

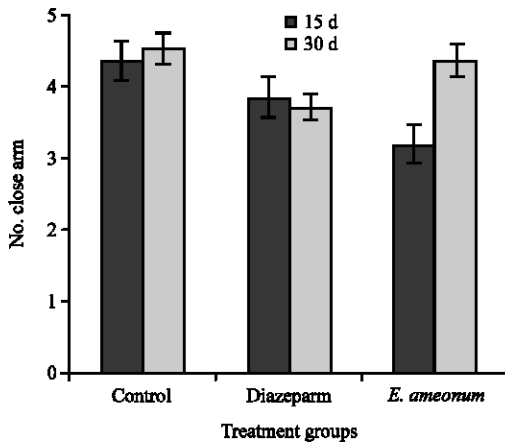


Fig. 4: Effect of diazepam 1 mg kg⁻¹ and *E. amoenum* 125 mg kg⁻¹ on closed arm entry in 15 and 30 days treatment.

spent in the open arms during 15 and 30 days treatment. These results are suggestive that *E. amoenum* has an anxiolytic effect in the plus maze test shows in Fig. 1-4.

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