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Anticonvulsant Effect of Methanolic Extract of *Echium amoenum* Fisch and C.A. Mey. Against Seizure Induced by Picrotoxin in Mice

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Abstract: The effect of methanolic extract of *Echium amoenum* Fisch and C.A. Mey. against Picrotoxin induced seizure in mice. The extract with doses of 3.125, 6.25, 12.5 and 25 mg kg^{-1} were injected intraperitoneally to mice, 20 min before picrotoxin 10 mg kg^{-1}. The latency of seizure, death time and percentage of mortality were measured in animals. The latency of seizure was increased in groups that pretreated with different doses of extract and this effect was only significant at the dose of 6.25 mg kg^{-1} ($p<0.05$). Meanwhile, this dose delayed the death time and decreased the percentage of mortality significantly ($p<0.01$), as well. The results of this study revealed the anticonvulsant effect of methanol extract of *E. amoenum* (FM) and introduced this plant as a good candidate for further studies in the other models of convulsion.

Key words: *Echium amoenum* Fisch and C.A. Mey, anticonvulsant, picrotoxin

INTRODUCTION

Echium amoenum Fisch and C.A. Mey (FM) (Boraginaceae) is a biennial or perennial herb indigenous to the narrow zone of northern part of Iran and Caucasus, where it grows at highlands at the altitude ranging from 60-2200 m (Rechinger, 1967; Ghassemi *et al*., 2003) . This borage is different from the borage grown in Europe, *Borago officialis* L. There are four species of Echium in Iran (Mozaffarian, 1996).

There has been an increase in interest in *Echium* spp., including E. amoenum (FM), because of their medicinal and nutritional properties. The phytochemical studies on *E.* amoenum revealed the presence of many chemicals such as anthocyanidine (13%), flavonoid aglycons (0.15%) and trace amount of alkaloid (Anonymous, 2000; Zargari, 1996). These plants are rich of the fatty acid, especially in the seed oil. It contains significant amount of gamma linoleic acid (an essential fatty acid) which is also an important intermediate in the production of number of important compounds in the body (Anonymous, 2000; Zargari, 1996; Wretensjo *et al*., 1990). *E. amoenum* (FM) is one of the most important medicinal plants in Iranian traditional medicine (Zargari, 1996; Hooper, 1937). The flowers of this plant have been used as demulcent, anti-inflammatory and analgesic, anxiolytic and sedative in folk medicine of Iran (Hooper, 1937; Amin, 1991). Anxiolytic effect of the flower of this plant has been shown in two separate experimental studies on mice (Rabbani *et al*., 2004; Shafaghi *et al*., 2002).

In western traditional medicine, the flowers and the leaves of borage are used medicinally as antifebrile, anti-depressive, for the treatment of stress and of circulatory heart diseases, for pulmonary complaints, as a poultice for inflammatory swellings (Kast, 2001; Kapoor and Klimaszewski, 1999), as a diuretic (due to potassium nitrate), as a laxative, emollient and demulcent (due to the mucilage) and recently as a possible protective factor against cancer (Gonzalez *et al*., 1993). However this plant was used in Iranian traditional medicine for variety of CNS disorder (Zargari, 1996; Hooper, 1937; Amin, 1991). The analgesic effect of this plant was shown in previous research (Heidari *et al*., 2005b). There is any paper about anticonvulsant effect of this Iranian plant in the literatures, therefore the present research was carried out to investigative the effect of methanolic extract of *E. amoenum* (FM) on generalized seizure induced by picrotoxin, a widely used as a model for

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chemically-induced convulsion in mice (Meckenzie *et al*., 2002; Swinyard, 1969; Heidari *et al*., 2005a,b). This study is an attempt to establish a scientific response for the use of this plant as antiepileptic in Iranian traditional medicine.

MATERIALS AND METHODS

Animals: Male albino mice weighing 20-25 g were employed. The animals were obtained from The Neuroscience Research Center of Kerman University of Medical Sciences. They were housed in a room temperature 22±2 at 12/12 h light/dark cycle. They had free access to food and water except during the time of experiments. Animals were acclimatized to the laboratory for at least 1 h before testing and were used for once experiment only. The experiments were carried out between 9.00 and 14.00 h. The animals were distributed into groups of 10 as controls and test groups. This study was down in laboratory of Pharmacology of Faculty of Pharmacy in Kerman University of Medical Sciences in Iran at 2003. According to international rules considering animal experiments (Zimmermann, 1983), all efforts were made to minimize animal suffering and to reduce the number of animal used.

Plant Material: Flowers of *E. amoenum* (FM) (Mozaffarian, 1996; Anonymous, 2000) were collected from a farm at 80 km north of Ghazvin province in June 2002. Voucher specimens (No. 1001) were authenticated and then deposited in Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

Extract Preparation: The dried flowers of *E. amoenum* (FM) (50 g) were powdered and extracted with 80% aqueous methanol by percolation (72 h) method (Anonymous, 2000). The extract was filtered; residue was concentrated by rotary evaporator apparatus and then dried at room temperature, corresponding to a 20% yield. The residue was dissolved in normal saline for final suitable concentrations to give the desired experimental concentration (3.125, 6.25, 12.5 and 25 mg/10 mL).

Convulsion test and data recording: Picrotoxin with convulsive and fatal dose of 10 mg kg^{-1} was injected intraperitoneally to induction of generalized seizure (Meckenzie *et al*., 2002; Swinyard 1969, Avallone *et al*., 2000, Bum *et al*., 2004). The animals received different experimental doses of *E. amoenum* extract, 3.125, 6.25, 12.5 and 25 mg kg^{-1}, 20 min before Picrotoxin 10 mg kg^{-1} administration (Avallone *et al*., 2000; Bum *et al*., 2004). The onset time of seizure, duration of seizure, time of death and percentage of mortality were measured in the test and control groups (Meckenzie *et al*., 2002; Avallone *et al*., 2000; Bum *et al*., 2004). Mice were observed for 90 min after picrotoxin injection. Normal saline (10 mL kg^{-1}, i.p.) and Phenobarbital (40 mg kg^{-1}, i.p.) groups were considered as control and positive control, respectively (Meckenzie *et al*., 2002; Avallone *et al*., 2000; Bum *et al*., 2004).

Statistic analysis: Results are presented as Mean±SEM and statistical significance between groups were analyzed by ANOVA followed by Newman - Keuls test. Fishe exact test was used for comparison of percentage of mortality. $p< 0.05$ were considered significant (Avallone *et al*., 2000; Bum *et al*., 2004).

RESULTS

Effect of *E. amoenum* extract on the onset time of seizure induced by picrotoxin: As shown in Fig. 1A, pretreatment of animals with different doses of extract delayed the onset of seizure. Comparing with saline group, this effect was only significant ($p<0.05$) at the dose of 6.25 mg kg^{-1}.

Effect on duration of seizure: The extract decreased the severity of seizure and prolonged the duration of seizure induced by picrotoxin that was statistically significant ($p<0.01$) with dose of 6.25 mg kg^{-1} (Fig. 1B).

Effect on death time: Pretreatment of animals with different doses of *E. amoenum* extract decreased the severity of seizure and delayed the death time that was significant ($p<0.01$) with dose of 6.25 mg kg^{-1} (Fig. 1C).

Effect on percentage of mortality: Figure 1D shows that only the dose of 6.25 mg kg^{-1} of the extract decreased percentage of mortality significantly ($p<0.01$). The higher doses could not decrease percentage of mortality.

DISCUSSION

Picrotoxin as a $GABA_A$ antagonist has widely used as a model of chemically-induced convulsion and produces a generalized clonic-tonic convulsion that leads to death in most cases (Meckenzie *et al*., 2002; Swinyard 1969, Avallone *et al*., 2000; Bum *et al*., 2004). The results of the present study showed the anticonvulsant effect of the methanol extract of the flowers of *Echium amoenum* Fisch and C.A. Mey (F.M) in mice. In the first stage of this experiment we used the dose of 25 mg kg^{-1} , but due to toxic effect of this dose in animals , the dose of extract was decreased by 50% in the following stage of

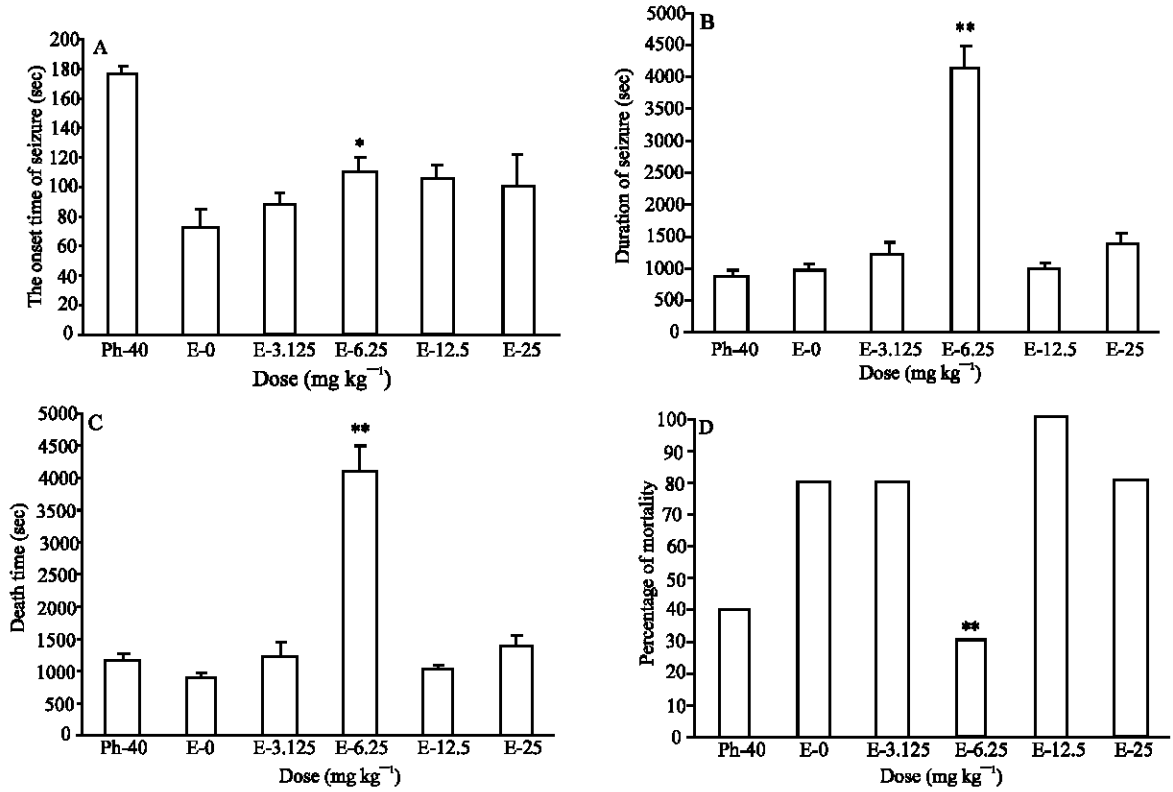

Fig. 1: The effect of the flower of *E. amoenum* (FM) extract on the (A) onset time of seizure, (B) duration time of seizure, (C) death time and (D) percentage of mortality in mice. Normal saline 10 mL kg^{-1}, phenobarbital 40 mg kg^{-1} or different doses of extract were injected intraperitoneally 20 minutes before picrotoxin 10 mg kg^{-1}. Each column indicates the Mean±SEM in 10 mice. *$p<0.05$ and **$p<0.01$; significant difference from saline control group

experiment. The most effective dose of the extract was 6.25 mg kg^{-1} in this study. This dose of extract significantly delayed the onset time of seizure, death time and percentage of mortality, in comparison to control group ($p<0.05$). This dose of extract also increased duration of seizure. With this dose of extract that decreased the severity of seizure, the animals have survived for longer period. This can be explanation for increased duration of seizure with this dose of extract. Higher doses of extract 12.5 and 25 mg kg^{-1} exerted less anticonvulsant effect than the 6.25 mg kg^{-1}. The higher doses of the extract may produce concentrations higher than therapeutic level and produced nonpharmacologic (Shargel and Yu, 1999) or toxic effects. These non-pharmacologic effects can be attributed to the presence of toxic component, Pyrrolizidine alkaloids in this plant (Boppre *et al*., 2005; Wretensjo *et al*., 1990). There is not any paper about anticonvulsant effect of this Iranian plant in the literature for comparison of these findings with others.

It is believed that plants of Boraginaceae family are rich of fatty acids, especially gamma linoleic acid, and flavonoids (Anonymous, 2000; Zargari, 1996; Wretensjo *et al*., 1990). There are some evidences about anticonvulsant effect of this fatty acid (Yehuda *et al*., 1994; Voskuyl *et al*., 1998) and some flavonoid compounds (Kavvadias *et al*., 2004; Du *et al*., 2002; Griebel *et al*., 1999). Salgueiro *et al*. (1997) showed anxiolytic effects of some natural and synthetic flavonoids in rats and found that these compounds exerted their effects through the central benzodiazepine receptors. Therefore, it seems that the antiseizure effect of *E. amoenum* Fisch and C.A. Mey (F.M) may be related in part to linoleic acid and/or flavonoid compounds present in the extract. Similar effect was observed with *Rosmarinus officinalis* L. Extract in our previous experiments (Heidari *et al*., 2005a). However determination of the role of each compounds in the anticonvulsant effect of the extract is a wide field for more investigations.

In conclusion, the findings reported in this study indicate that *Echium amoenum* Fisch and C.A. Mey may contains novel bioactive principles with anticonvulsant properties that is parallel with use of this plant in Iranian folk medicine. However more pharmacological and toxicological experiments are needed for use of this plant as an official herbal drug in clinical use.

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