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Evaluation of Antidepressant Effects of Aerial Parts of *Echium vulgare* on Mice

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Abstract

Objective

In traditional medicine, *Echium* spp., including *E. vulgare* L., are utilized as exhilarant and mood stimulant. On the other hand, depression is a state of intense sadness, melancholia or despair that has advanced to the point of being disruptive to an individual's social functioning and/or activities of daily living. Therefore, finding effective and safe treatments is a hotly contested area in the present time. In this study, the antidepressant effects of aqueous and alcoholic extracts of *Echium vulgare* L. aerial parts were investigated on mice.

Materials and Methods

Boiling and percolation were used for aqueous and alcoholic extractions, respectively. Toxicity and anti-depressant studies were performed in male BALB/C mice. Three doses of 0.05, 0.2 and 0.35 g/kg for aqueous extracts and five doses of 0.01, 0.04, 0.07, 0.3 and 0.5 g/kg for alcoholic extracts were selected in the forced swimming test employing 8 mice in each group. Open field activity test was used to differentiate antidepression and locomotion effects. ANOVA and Tukey-Kramer tests were used for statistical analysis.

Results

The LD_{50} values of aqueous and ethanolic extracts were 1.22 g/kg and 1.21 g/kg, respectively. Aqueous and alcoholic extracts showed significant antidepressant effects starting at 0.05 g/kg and 0.07 g/kg, respectively. Open field test showed no significant changes in the activities of animals which received the ethanolic extract, but the aqueous extract decreased locomotor activities at higher doses.

Conclusion

The results showed that the aqueous extract at low doses and ethanolic extract at high doses have significant antidepressant effects. The effects of extracts were similar to imipramine and they may affect neurotransmitters, norepinephrine and serotonin. This herb might be considered as a useful drug in the management of depression.

Keywords: Antidepressant, Echium vulgare L, Forced swimming test, Imipramine, Open field test

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Introduction

The Boraginaceae family comprises of shrub plants that grow mainly in mild and tropical regions. This family is divided into 200 genus and 2000 species (1). These plants possess medicinal properties and most of them are mucilaginous and contain potassium nitrate. Flowers, stems, roots and leaves from these plants are used for medicinal purposes (2). *E. vulgare and E. amoenum* are both used under the name of "Gav Zaban" in Iranian traditional medicine. They are utilized as exhilarant, mood stimulant, diuretic and to fight common cold (1). Some nitrate and mucilage are found in leaves and aerial parts of *E. vulgare*. Other compounds are Allantoin, Phenyl propanoids such as Cinnamic acid and Rosmarinic acid, 4 carbon acids such as Citric, Fumaric, Malic and Succinic acids, Flavonoids and Flavonols, carbohydrates, lignans, lipids, steroids and Quinoids (3). Pharmacological studies have revealed hypotensive and anti tumor effects of *E. vulgare* aerial parts, leaves and stems (3).

Clinical depression is a state of intense sadness, melancholia or despair that has advanced to the point of being disruptive to an individual's social functioning or activities of daily living. It affects about 7–18% of the population on at least one occasion in their lives, before the age of 40. Approximately, two third of depressed patients experience suicide thoughts and 10-15% of them attempt suicide. The monoamine theory suggests that the main cause of depression is Serotonin, Norepinephrine and/or Dopamine deprivation in the central nervous system (CNS). Drugs that increase the level of these neurotransmitters in the CNS show anti-depressant activity (4). Recent theories suggest that monoamines act only as regulator to other more important brain neurobiological systems (5).

"Gav Zaban" herbs are used widely in traditional Iranian medicine. Its antidepressant function has been mentioned in traditional medicine. However, since this effect has not been scientifically studied, it has been pursued in the present work.

Materials and Methods

Animal

BALB/c male mice, weight 25-30 g were obtained from Razi Serum Organization and maintained at 21±2 °C, 12h/12h light cycle in the animal room of Mashhad School of Pharmacy. Food pellet and tap water were available ad libitum and each mouse was used once for experimentation.

Plant

Aerial parts of *Echium vulgare* L. were collected from Arsabaran forests at 1300-1800 m height (midway of Kelaleh to Abbas Abad villages in Eastern Azerbaijan Province). The plant was air-dried in the shadow (20-25 °C) and sent to Mashhad School of Pharmacy. It was identified by the School's Herbarium and a sample was deposited under the code number 040-0002-05.

Extraction

Extraction by boiling was used for the aqueous extract. The dried aerial parts (400 g) were powdered and boiled in 2 liters of water for 15 minutes. After filtration, extract-containing plates were heated at 40 °C until the solvent was evaporated. The leftover extract, brown in color, was kept in the fridge until its administration. Normal saline was used as solvent. Extraction percentage was 3% (12 g from above 400 g dried plant).

Maceration was used to obtain alcoholic extract. First, ether de petrol was used to get ride of the fat contents of the plant. Then, ethanol 95% was used to macerate the dried plant for 48 hours. After filtration, the solvent was evaporated at 30 °C. The leftover extract as green thin layers was kept in the fridge until its administration. Normal saline with few drops of Tween 80 was used as a solvent. Extraction percentage was 0.5%.

Acute Toxicity Study

To determine LD_{50} and maximum tolerated dose, doses of 0.5 g to 3 g were injected intraperitoneally (IP) into mice. Five mice were used for each dose group and mortality was recorded after 24 hours.

Depression Test

This test was performed in two sessions. In pretest session (24 hours before main session), each mouse was forced to swim in a cylindrical-shaped container (diameter 10 cm, height 25 cm, water height 11 cm, temperature 25±1 °C) (6). This preliminary test is stress inducing and mice gradually lose their movement behavior (7). After 15 minutes, animals were removed and blow-dried. Then, 23.5 hours later the relevant sample was administered IP. The main test was performed 30 minutes later. In this test, each mouse was left in the same container for 6 minutes and the following behaviors were recorded during the last 4 minutes:

1. Immobility: floating in water without swimming.
2. Swimming: active movement of extremities and circling in the container.
3. Climbing: active movement of forelimbs on the container wall (7, 8).

Sample Administration

The following samples were administered IP 30 minutes before the test in groups of 8 mice:

- Negative control: normal saline 10ml/kg for aqueous extract and normal saline with Tween 80 for alcoholic extract.
- Positive control: Fluoxetine 10 or 20mg/kg or Imipramine 15 or 30 mg/kg.
- Aqueous extract: 0.05, 0.2, 0.35 g/kg of *E. vulgare* aqueous extract.
- Alcoholic extract: 0.01, 0.04, 0.07, 0.3, 0.5 g/kg of *E. vulgare* ethanolic extract.

Open-Field Test

The open field test was designed to measure behavioral responses such as locomotor activity, hyperactivity, and exploratory behaviors. This test performed, as mice mobility affects their ability to swim or climb. In this test, effects of administration on mobility of animals were evaluated. Open-filed apparatus was made as reported (9). Each mouse was placed in the center of the open field, and its behavior observed for 5 min. The parameters evaluated were the total number of squares crossed, the number of outer squares (those adjacent to the walls) crossed, and the number of inner squares crossed; the three measures referred to as total (TL), peripheral (PL), and central locomotion (CL), respectively. The numbers of leanings (one or two paws in contact with the wall), rearings (the mouse standing on its two hind paws without touching the walls), groomings (face cleaning, paw licking, fur licking, head scraping, and rubbing), and defecations were also recorded (9). At the end of each test, the whole area was cleaned with a wet sponge and a dry paper towel.

Statistical Analysis

PCS software was used for LD_{50} calculation. Analysis of Variance (ANOVA) and Tukey-Kramer were used to analyze difference among groups (Instat Software).

Results

Acute toxicity

LD_{50} for the aqueous and alcoholic extracts were 1.22 g/kg and 1.21 g/kg, respectively. Maximum tolerated dose was 0.5 g/kg for the aqueous and 0.71 g/kg for the alcoholic extracts.

Anti-depressant effect of aqueous extract

Data resulting from comparing the effect of IP injection on immobility revealed that all three doses show significant effect ($p< 0.001$) (Figure 1). Climbing was also affected by the extract administration and showed significant difference, in comparison with the control one ($p<0.001$) (Figure 2). Two doses of extracts have significant effects on the swimming time, compared to the normal saline solution ($p<0.001$) (Figure 3).

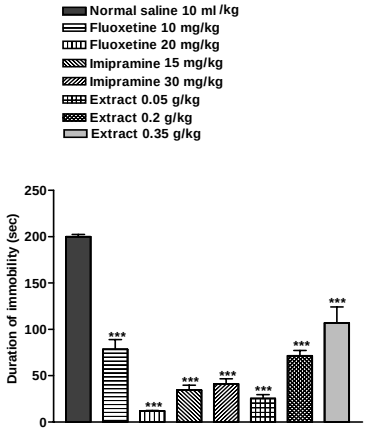


Figure 1. Effect of IP injection of the aqueous extract on immobility. Each bar represents mean response from 8 mice ± SEM (*** indicates p< 0.001).

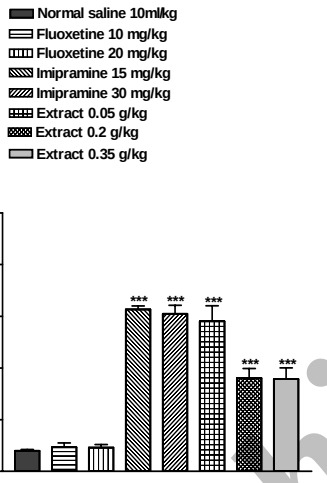


Figure 2. Effect of IP injection of the aqueous extract on climbing. Each bar represents mean response from 8 mice ± SEM (*** indicates p< 0.001).

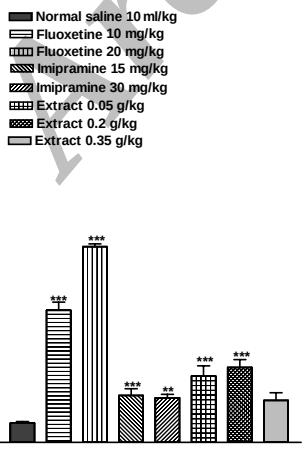
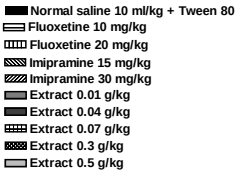


Figure 3. Effect of IP injection of the aqueous extract on swimming. Each bar represents mean response from 8 mice ± SEM (*** indicates p< 0.001).



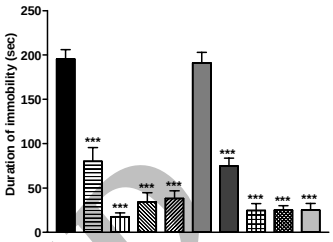
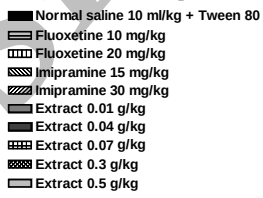


Figure 4. Effect of IP injection of the alcoholic extract on immobility. Each bar represents mean response from 8 mice ± SEM (*** indicates p< 0.001)



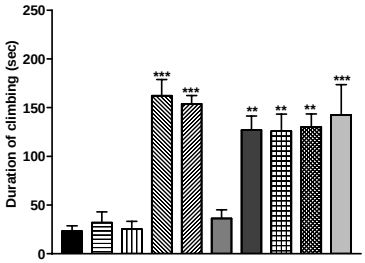
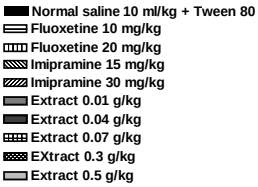


Figure 5. Effect of IP injection of the alcoholic extract on climbing. Each bar represents mean response from 8 mice ± SEM (** and *** indicate p< 0.001 and p< 0.001, respectively).



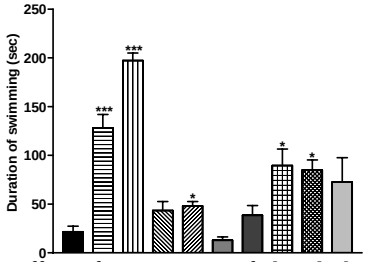


Figure 6. Effect of IP injection of the alcoholic extract on swimming. Each bar represents mean response from 8 mice ± SEM (* and *** indicate p< 0.05 and p< 0.001, respectively)

Anti-depressant effect of alcoholic extract

The lowest dose of the alcoholic extract had no significant effect on immobility. However, by increasing the dose, immobility time was significantly reduced ($p<0.001$). At 0.04 g/kg, immobility time reduced by 62% and this was reduced further up to 87% with the higher doses (Figure 4). Climbing was not significantly affected at the lowest dose. However, starting from 0.04 g/kg a significant effect was revealed ($p<0.001$). This effect remained almost at the same level, by increasing the dose (Figure 5). Swimming time increased with 2 doses (0.07 and 0.3 g/kg) (Figure 6).

Table 1. Effects of aqueous and alcoholic extracts in open-field test. Results are shown as Mean ± SEM (n=6). Aqueous extract and alcoholic extracts are compared to normal saline and normal saline + Tween 80, respectively. *, ** and *** indicate $p<0.05$, $p<0.01$ and $p<0.001$, respectively. TL: Total Locomotion , PL: Peripheral Locomotion, CL: Central Locomotion.

	TL	PL	CL	Leaning	Rearing	Grooming	Defecation
Normal saline 10 ml/kg	130.16 ± 9.82	114/66 ± 8.78	15.5± 2.14	9.5± 1.33	0.5± 0.22	0.33± 0.21	0.33± 0.21
Normal saline 10 ml/kg + Tween 80	132.33 ± 5.41	117.68± 5.78	14.66± 1.98	9.33± 1.28	0.5± 0.22	0.33± 0.21	0.33± 0.21
Fluoxetine 10 mg/kg	107.83± 19.45	73.68± 17.64	24.16± 5.78	6.16± 1.45	0 ± 0	0.66±0.33	0.83± 0.31
Fluoxetine 20 mg/kg	154.83± 10.84	125.66± 9.05	29.16± 3.75	7.33± 1.5	0.16± 0.17	1 ± 0.26	0.5± 0.34
Imipramine 15 mg/kg	119.16± 6.55	97±8.62	22.16± 2.67	6.33± 0.84	0.16± 0.17	0.16± 0.17	0.5± 0.34
Imipramine 30 mg/kg	94.83*± 11.25	85.66± 10.42	9.16± 1.95	6.83± 1.34	0 ± 0*	0.5± 0.22	0 ± 0
Aqueous extract 0.05 g/kg	111.33± 13.92	98± 11.62	13.33± 2.39	9.16± 1.17	0 ± 0*	0.33±0.21	0.5± 0.34
Aqueous extract 0.2 g/kg	51.33**± 19.88	39.16**± 15.54	12.17± 5.05	2.66***± 1.08	0 ± 0*	0.33± 0.21	0 ± 0
Aqueous extract 0.35 g/kg	49.83**± 10.64	42.17**± 11.24	7.66± 2.43	0.33***± 0.21	0 ± 0*	0 ± 0	0 ± 0
Alcoholic extract 0.07 g/kg	147.33± 14.79	123.17± 11.13	24.16± 4.44	10.83± 1.49	0.16± 0.17	1.16± 0.4	0 ± 0
Alcoholic extract 0.3 g/kg	103.66± 7.72	94.33± 8.79	9.33± 2.20	6± 0.85	0.33± 0.21	0 ± 0	0 ± 0
Alcoholic extract 0.5 g/kg	102.5± 8.29	90.83± 8.39	11.67± 1.94	6.5± 1.17	0.16± 0.17	0.66± 0.33	0 ± 0

Open-field test

Results are as shown in Table 1. Groups of 6 mice were used for each treatment.

A. TL

a. From the positive controls, only imipramine 30 mg/kg had a significant difference compared to the control group ($p<0.05$). Thus, total locomotion of animals in this group is reduced by drug treatment.

b. Two doses (0.2 and 0.35 g/kg) of the aqueous extracts affected total locomotion significantly ($p<0.01$). The lowest dose (0.05 g/kg) has no effect on total locomotion ($p>0.05$).

c. None of the alcoholic extracts had significant effect on total locomotion.

B. PL

a. Imipramine and Fluoxetine had no significant effect.

b. Two doses (0.2 and 0.35 g/kg) of the aqueous extracts affected the the peripheral locomotion significantly ($p<0.01$). The lowest dose (0.05 g/kg) had no effect on this locomotion.

c. None of the alcoholic extracts had significant effect on the peripheral locomotion.

C. CL

None of the treatments had significant effect on the central locomotion.

D. Leaning

a. Imipramine and Fluoxetine had no significant effect.

b. Two doses (0.2 and 0.35 g/kg) of the aqueous extracts affected leaning significantly ($p<0.01$). The lowest dose (0.05 g/kg) had no effect compared to the control.

c. None of the alcoholic extracts had significant effect on leaning.

E. Rearing

a. Imipramine and Fluoxetine had no significant effect.

b. All three aqueous extracts affected rearing significantly ($p<0.05$) and showed reduction in animal rearing.

c. None of the alcoholic extracts had significant effect on rearing.

F. Grooming

None of the treatments had significant effect on grooming.

G. Defecation

None of the treatments had significant effect on defecation.

Discussion

In this study, antidepressant effects of *E. vulgare* have been studied. For this purpose, modified forced swimming test was employed that in addition to immobility, it studies swimming and climbing. In this test, animal behaviors are divided into three groups: immobility, climbing and swimming. Immobility is the symbol of depression. Antidepressants that selectively inhibit norepinephrine uptake reduce immobility and selectively increase climbing without affecting swimming. Serotonin reuptake inhibitors also reduce immobility but increase swimming instead of climbing (7). To determine the appropriate doses, acute toxicity tests were done, then the main test was performed. The obtained results show that both aqueous and alcoholic extracts have significant antidepressant effects.

The calculated LD_{50} implies that both extracts could be categorized as "relatively toxic" materials. The presence of toxic pyrrolizidine alkaloids have been reported in this plant (3). More than 300 pyrrolizidine alkaloids have been reported in more than 6000 plants of Compositae, Boraginaceae and Leguminoseae families. The *Echium* genus is one of the important plants that shows toxic effects on the liver system. These alkaloids cause renal and/or hepatic toxicities in humans.

In evaluating antidepressant effects by the swimming test, when mice are forced to swim in a limited space, they quickly abandon swimming and stand still. Many antidepressant drugs reduce this despair behavior of mice which shows that this immobility behavior might be a measure of lowered mood in the mice (10). This test is faster and cheaper in mice than rats and thus is a suitable screening test for antidepressants (11). Drugs that potentiate central dopaminergic and alpha adrenergic systems reduce immobility behavior of mice in this test (12). However, a new method of evaluating animal behaviors has been recently introduced that in addition to immobility, measures swimming and climbing behaviors. Drugs that are selectively inhibit norepinephrine reuptake reduce immobility and increase climbing without significantly affecting swimming results. Although selective serotonin reuptake inhibitors (SSRIs) reduce immobility, they increase swimming time without influencing climbing. This new method can help in identifying the mechanism of action of antidepressants (8).

In this study, the aqueous extract in all three doses causes a significant reduction in immobility, compared to the control. However, increasing the dose increases the immobility time. The effect of the low dose of this extract is comparable to imipramine (15 and 30 mg/kg). Central depression is the reason behind its less effectiveness at higher doses. This has been shown in previous studies (3) and the resulted data from the open field study also confirms this observation (Table 1). The alcoholic extract shows a positive effect on the immobility reduction at the last four doses. This effect reaches a plateau at the last three doses and is comparable to imipramine (15 and 30 mg/kg).

It is well known that *E. vulgare* contains several flavonoid compounds (3). In one

study on *Ginkgo biloba*, it was shown that the flavonoids-containing leaves extract inhibits mono-amino oxidase enzyme (MAO) (13). In another study on *Hypericum perforatum*, it was found that a flavonoid extract of this plant shows a significant antidepressant effect on the swimming test (14). Moreover, flavonoid compounds from this plant have similar chemical structures to known MAO inhibitors. Indeed, the flavonoids quercetin, luteolin and kaempefrol from *H. perforatum* show significant *in vitro* MAO inhibitory effects (15). Interestingly, quercetin and kaempefrol are present in *E. vulgare* extracts (3). Concerning the mechanism of action of *E. vulgare* extracts, these extracts affected both climbing and swimming behaviors and no selective action was observed. This is similar to imipramine effects. Therefore, it might be concluded that the extracts have no selective effect on serotonin or epinephrine and a non specific effect of MAO inhibition could explain their effects.

To differentiate antidepressant effects from effects on central locomotion, the open-field test has been used. The open-field test is thought to provide indices of motor activity, emotional reactivity, and exploration (9). Seven factors were measured (Table 1). Peripheral locomotion and leaning relate to motor activity in a novel environment (16). Rearing is assumed to be under the influence of several processes, including motor abilities (17). These three factors are labeled "motor reactivity axis". CL/TL ratio relates to approach/avoidance responses of the unprotected part of the open field and together with rearing are supposed to reflect opposing tendencies: exploration and emotivity (17).

However, they are independent of grooming and defecation. High scores for grooming and defecation are considered to reflect a higher level of emotivity, and these two variables are more sensitive to anxiolytic drugs than those related to exploration (9).

Observing table 1 shows that the motor reactivity factors (PL, leaning and rearing) and TL have been affected by the extracts. Only the high dose of the aqueous extract reduced these factors, indicating a lower motor reactivity and suggesting a central depression. In this study, immobility caused by 30 mg/kg imipramine was higher than 15 mg/kg imipramine.

As it is clear from table 1, TL was lower in the 30 mg/kg imipramine group. Drowsiness was evident in this group that could explain this observation.

Conclusion

Results from this study indicate that low doses of the aqueous extract of the aerial parts of *E. vulgare* and high doses of the alcoholic extract have a clear antidepressant activity that is comparable to imipramine. Moreover, this effect, similar to imipramine, might be related to effects on both norepinephrine and serotonin and/or inhibition effect on MAO that eventually leads to higher availability of norepinephrine and serotonin in synapses. It is suggested that more studies in this regard should be pursued to obtain more knowledge about the role of *E. vulgare* in depression.

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