



Research Article

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# The Antioxidant Effect of Echim Amoenum to Prevent Teratogenic Effects of Lamotrigine on the Skeletal System and Fetal Growth in Mice



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## Abstract

**Background and Aim:** Echim amoenum is a medicinal plant with abortive properties. This study was performed to determine the effect of lamotrigine and Echim amoenum hydroalcoholic extract on the development of congenital abnormalities in BALB/c mice.

**Materials and Methods:** In this experimental study, 120 female mature BALB/c mice were assigned to four groups and after mating and confirming the vaginal plug, the animals in the first group (G1) were kept with no intervention, and the second (G2), third (G3) and fourth (G4) groups were intra peritoneally (IP) injected with respective lamotrigine (25 mg/kg), and 100 and 200 mg/kg of Echim amoenum extract with Lamotrigine (for 7 days). On the 19<sup>th</sup> day, caesarean section was performed on the mice and embryos were examined for abnormalities. Their weight and height were measured. Data were analyzed by ANOVA.

**Results:** There were significant differences between G2 and G1 ( $p < 0.05$ ); the not significant difference was seen between G3 and G4.

**Conclusion:** lamotrigine have teratogenicity and should be used cautiously during pregnancy and Echim amoenum can improve it.

**Keywords:** Lamotrigine; Echim amoenum; Medicinal plant; Pregnancy; Teratogenicity

**Abbreviations:** AEDs: Antiepileptic drugs; RA: Rosmarinic Acid; GLA: Gamma-Linolenic Acid; ALA: Alpha-Linolenic Acid; IP: Intra Peritoneally

## Introduction

In spite of the fact that, the majority of children born to women with epilepsy are normal, they are at increased risk for malformations [1]. Antiepileptic drugs (AEDs) have the potential to affect fetal development throughout pregnancy and unfortunately, pregnant women should not stop their medication during this time. According to the Harmful effects of synthetic drugs and increasing contraindications to their usage, there is an increasing interest in the use of medicinal plants [2,3]. Medicinal plants have also the capacities to diminish drug-induced adverse effects [4]. Most women believe that herbal medicines are safe and consequently pregnant women may use these medicinal plants or their combinations during pregnancy [5].

Echim ammonium is belonged to the Boraginaceae family and is a biennial or perennial herb indigenous to the narrow

zone of the northern part of Caucasus and Iran. Echim genus (Boraginaceae) has 4 species in Iran [6] and only dried violet-blue petals of Echim amoenum have medicinal uses in Iran [6]. Echim ammonium that has been shown as a rich source of antioxidants, like flavonoids and rosmarinic acid (RA). This plant grows at Highlands at the altitude ranging from 60 to 2200 m and it has been advocated for a variety of effects such as demulcent, and analgesic, anti-inflammatory, common cold, sedative and anxiolytic [7]. The plant constituents have been isolated by different investigators; they include gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), delta6-fatty acyl desaturase, delta8-sphingolipid desaturase [6] pyrrolizidine alkaloids, mucilage, resin, potassium nitrate, and calcium salt combined with mineral acids.

Although some pharmacologic and toxic effects of lamotrigine have been investigated, however, the teratogenic effects of this drug and plant on the embryo during pregnancy have not been scientifically clarified. Therefore, in this study, the teratogenic effects of lamotrigine were studied in BALB/c mice.

### Materials

Female BALB/c mice between 8-12 week were purchased from the animal laboratory, Shiraz, Iran. lamotrigine was purchased from Sobhandarou Pharmaceutical Company and other chemicals from Merck Company (Germany). Echinium amoenum was collected in Fars (Iran).

### Experiments

In this experimental study, 120 female BALB/c mice between 8-12 week weighing 25-28 g were prepared and kept in proper health and light conditions (12/12 h light/darkness) without any food access limitation. After ten-day habituation to the environment, mouse, two female and one male mice were placed in a cage for two days [8]. The vaginal plug was considered as the sign of zero-day of pregnancy. As regards this method was not certain, an eosin smear 3% was prepared from the vaginal discharges of the female mice and the being of sperm was regarded as fertility. The other sign of fertility was female mice's weight increasing after fertility. That way the mice weight increases by 3-10% daily since the fourth day of pregnancy, the weight gain up to this level or more was regarded as the sign of fertility. Then, the mice were assigned to four groups. The animals in the first group (G1) were kept with no intervention, and the second (G2), third (G3) and fourth (G4) groups were intra peritoneally (IP) injected with respective Lamotrigine (25 mg/kg), and 100 and 200 mg/kg of Echinium amoenum extract with Lamotrigine (for 7 days) [9,10].

The two doses of Echinium amoenum and lamotrigine were injected daily since the zero-day of pregnancy till the seventh day. On the 19<sup>th</sup> day of pregnancy, the caesarean operation was done under anesthesia with chloroform. The caesarean cut was in shape of inverse Y. After the caesarean section, fallopian tubes were opened and embryos were removed and after that placed in normal saline. Embryos were heights were measured by a caliper from crown to rump and their weighed by a digital sensitive were measure it. The embryos were assessed in terms of observable abnormalities. Then, after using Alizarin dye and Alcian Blue, the skeletal abnormalities were investigated [11].

### Extraction Method

The seed of Echinium amoenum was collected from fars suburbs and after being authenticated by a botanist in the Research Center of Jihad-e-Keshavarzi, a herbarium sample was prepared and deposited in the Herbarium Unit of Medical Plants Research Center of Shiraz University of Medical Sciences, Iran. The collected plants were dried at a normal temperature and

the extraction was done by Percolation Method. To 500 gm of the plant powder in an appropriate container, 500 ml of ethanol 70% was added, and after 72 hours it was filtered. The solvent was removed at 35°C using a rotary apparatus. The Echinium amoenum extract was incubated for two days at 40°C to dry. Then, it was kept in refrigerator until the usage time [12].

### Standardization of The Extract

To standardize, the level of phenolic compounds and flavonoids, as well as the antioxidant capacity of the extract, were measured as follows:

#### Measurement of Flavonoid Compounds Level

Aluminum chloride and Rutin colorimetric method was run to test the total flavonoids [13]. At First, standard solutions that include the rutin in methanol 60% at concentrations of 25, 50, 100, 250, and 500 ppm were prepared. After that, 1 ml of these solutions was transferred into test tubes and blended with 1 ml of chloride aluminum 2%. Then, 6 ml of potassium acetate 5% was introduced and the optical density was read after 40 minutes at 415 nm wavelength. The concentration levels of the standard solutions were measured in three intervals. To assay the totally level of flavonoid in the extracts, 0.01-0.02 g of the extracts was dissolved with methanol 60%, reaching 10 ml. Then, the overall level of flavonoid was measured by chloride aluminum colorimetry. However, instead of the standard solution, 1ml of the extract was added. The total flavonoid level was calculated in mg/gr extract, equivalent to rutin.

#### Measurement of Total Phenolic Compounds

Total phenolic compounds were measured equivalent to gallic acid by Folin-Ciocalteu colorimetry [14]. The standard solutions were prepared at concentrations of 12.5, 25, 50, 62.5, 100, and 125 ppm of gallic acid in methanol 60%. After that, 0.1 ml of each sample was transferred into a test tube and 0.5 ml Folin-Ciocalteu 10% was introduced as a reactive agent. The solutions were left for 8 minutes at room temperature and 0.4 ml of sodium carbonate 7.5% was added. The tubes were held for 30 minutes at the laboratory temperature and then measured in three intervals by a spectrophotometer at 765 nm wavelength. To measure the total phenol in the extracts, 0.01-0.02 g of the extracts was solved with 60% methanol, reaching 10 ml and the total level of phenol was measured by Folin-Ciocalteu method. However, instead of the standard solution, 0.1 ml of extract solution was added. At the end, the total phenol level was derived from the read optical density in mg/gr extract in gallic acid equivalent.

#### Measurement of Antioxidant Activity

To assay, the antioxidant activity of the extract  $\beta$ -carotene model was used [14]. In a suitable container, 0.2 ml Tween 40, 500  $\mu$ L chloroform, 5 ml  $\beta$ -carotene (0.2 mg) and 20 ml linoleic acid (20 mg) were blended and incubated at 50°C for 10 minutes to remove the solvent. The solution was diluted using distilled water

and mixed with 4 ml of aliquots. The control solution, consisting of 0.2 ml ethanol and 0.2 ml of the extracted sample with 0.05 ml turmeric extract 0.15 ml ethanol, was prepared. The optical density in the Group 1(G1) was recorded at t=0 and t=90 at 470 nm wavelength, similar to the standard group. The specimens were incubated in a bain-marie at 500C. The antioxidant activity of the samples was assay based on the samples ability to prevent washing of β-carotene. The antioxidant activity of them was measured by the formula below [15]:

i.  $AA = 100 [14]$  Where,  $A_0$ : optical density at t = 0,  $A_t$ : optical density of the sample at t = 90,  $A_{0c}$  and  $A_{tc}$ : Optical density values in the control samples at t = 0, and t = 90, respectively.

**Statistical Analysis**

Data were presented as frequency, relative frequency, mean, standard deviation and were analyzed using ANOVA, posthoc Turkey, using SPSS 22 software.

**Results**

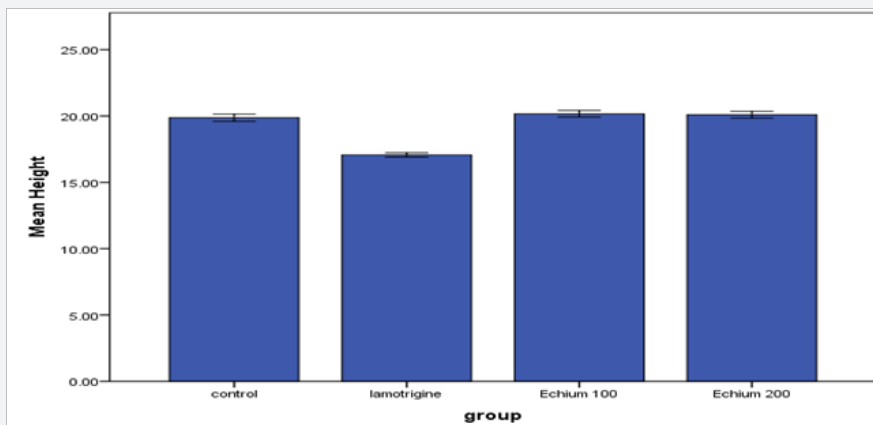
Each 100 gr of Echium amoenum powder yielded 8.5 gr hydro alcoholic dried extract. The calculated levels for flavonoid

and phenolic compounds were 85.37 mg/gr rutin equivalent and 117.91 mg/gr gallic acid equivalent, respectively. The extract antioxidant activity was 37.28%. The indices of embryos' height and weight were measured (Table 1) and (Figures 1 & 2). There were embryos in all selected mice; however, posthoc Tukey test showed that both doses of the extract caused a not significant decrease in embryos' height and weight in comparison with the G1 ( $p < 0.05$ ); however, there is a significant difference was observed in G2 compare with G1 regarding these parameters ( $p > 0.05$ ).

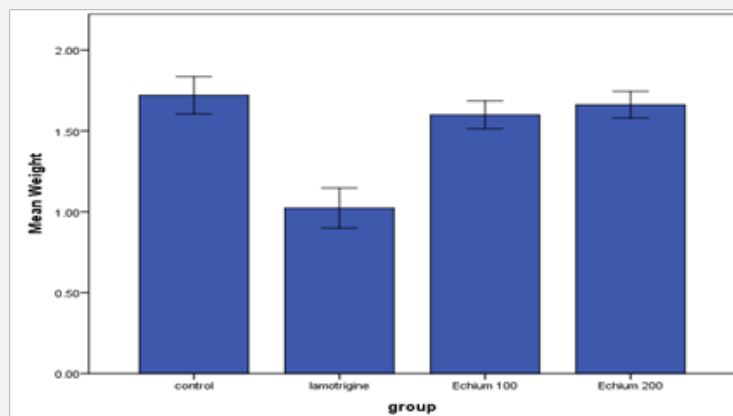
**Table 1:** The comparison of the mice embryos' height and weight mean (± standard deviation) in the groups under study.

Variable Group	Control	lamotrigine	Extract 100 mg/kg	Extract 200 mg/kg
Height (mm)	19.86±1.47	17.06±0.86*	20.16±1.36	20.10±1.39
Weight (gr)	1.72 ± 0.63	1.02 ± 0.67*	1.59 ± 0.47	1.66 ± 0.45

\*  $p < 0.05$  in comparison with control and lamotrigine groups.



**Figure 1:** The comparison of the mice embryos' height mean (± standard deviation) in the groups under study ( $p < 0.05$ ).



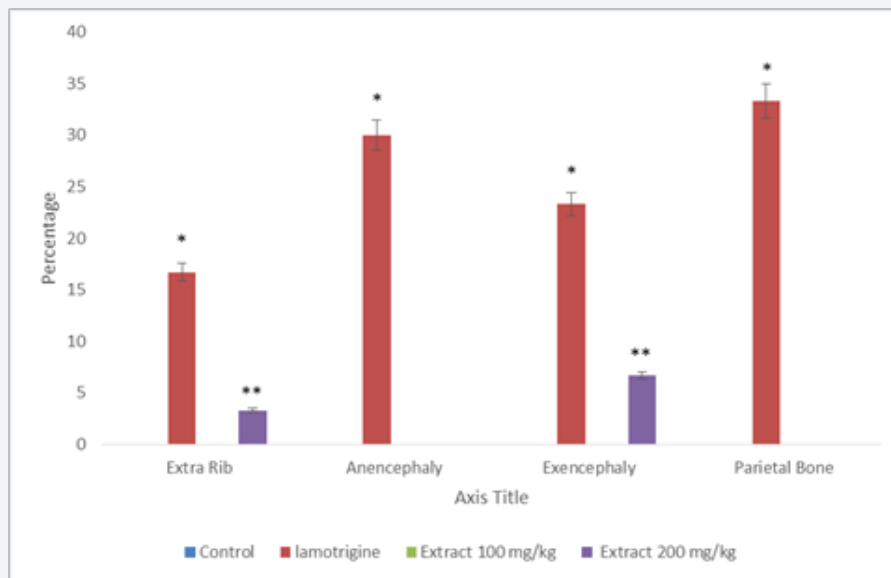
**Figure 2:** The comparison of the mice embryos' weight mean (± standard deviation) in the groups under study ( $p < 0.05$ ).

Skeletal abnormalities of embryos in all groups are summarized in (Table 2) and (Figure 3). The rate of extra rib, anencephaly, exencephaly, and parietal bone abnormality of the mice embryos was zero in group 1(G1), but the rates of them in group2 (G2) was 5(16.7%), 9(30%), 7(23.3%), and 10(33.3%). On the other side, we examined the frequency of abnormalities in the four groups, those of G1 and G2 were compared and the difference was obtained significant ( $p<0.05$ ).also, G4 in extra

rib and Exencephaly. Based on the test, the abnormalities of the extra rib and Exencephaly were significantly higher in Group 4 compare than Gruop1 ( $p<0.05$ ). In addition, no case of extra rib, parietal bone abnormality, Anencephaly, and Exencephaly was observed in Gruop1 and Gruop3 (Table 2) and (Figure 2). The rate of abnormalities was higher in the lamotrigine and after that in the higher dose of the extract in comparison with the lower dose was seen.

**Table 2:** The frequency of observed abnormalities in mice's embryos\*.

Group abnormalities	Extra Rib		Anencephaly		Exencephaly		Parietal Bone	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
Control	0	0	0	0	0	0	0	0
lamotrigine	5	16.7	9	30	7	23.3	10	33.3
Extract 100 mg/kg	0	0	0	0	0	0	0	0
Extract 200 mg/kg	1	3.3	0	0	2	6.7	0	0



**Figure 3:** The comparison of the mice embryos' abnormalities in the groups under study ( $p<0.05$ ).

**Discussion**

This study tried to examine the teratogenicity of lamotrigine and antioxidant activity of Echium amoenum in BALB/c mice. In the present study, the lamotrigine drug caused skeletal abnormalities and height and weight loss in embryos. Padmanabhan et al, in 2003 reported that administering lamotrigine as a single dose of 50-200 mg/kg body weight can induce intrauterine growth retardation in mice, where as multiple doses of 25, 50, 75 mg/kg body weight cause a dose-dependent increase in embryonic resorption and craniofacial malformations [9]. Marchi et al. [16] in 2001 report edteratogenic effects, such as reduction in body weight and morphological changes in the brain, when lamotrigine was administered to rats at 4 times the median effective dose [16]. On the other hand both

doses of the extract caused a not significant decrease in embryos' height and weight in comparison with the G1 ( $p<0.001$ ). Studies have shown that Echium amoenum contains certain amounts of fatty acids such as linoleic acid [17] and antioxidant compounds such as a rosmarinic acid [18]. And maybe it has been caused the balance weight [19].

The comparison between the group 1(G1) and group 2 (G2) showed that there was a significant difference in external and skeletal abnormalities and the lamotrigine caused anencephaly, exencephaly, extra rib abnormalities, and lack of parietal bone in mice embryos ( $p<0.05$ ). Numerous study has argued that lamotrigine caused the teratogenic in man and have similar effects in animals, This view is supported by Prakash and et al. [20]. Previous research has shown a negative correlation

between the lack of absorption of folic acid and skeletal abnormalities. The teratogenicity of lamotrigine in animals has also been attributed to reduced absorption of folate [2].

on the other side, in this study, abnormalities of extra rib and Exencephaly were observed in group 4 (G4); however, their incidence was higher with a dose of 200 mg/kg. Therefore, it can be said that the incidence of these abnormalities depends on the dose. Apigenin is an estrogen flavonoid available in aromatic plants. It has a slow metabolism and the adsorption and desorption phases happen slowly and therefore accumulation of this flavonoid in the body is probable. Because it has been already shown that *Echium amoenum*, especially its hydro alcoholic extract, has high amounts of apigenin, the presence of this component in *Echium amoenum* hydro alcoholic extract could be one of the reasons for external and skeletal abnormalities.

## Conclusion

In view of the obtained results about external and skeletal abnormalities including extra rib and Exencephaly, it seems that *Echium amoenum* should be taken cautiously during pregnancy. This study showed that *Echium amoenum* plant was able to create abnormalities in mice, and its teratogenic effects were dose-dependent and of course, the teratogenic effects of this plant should be studied more precisely.

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