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Original Article

Protective Effect of Flax Seed on Brain Teratogenicity Induced by Lamotrigine in Rat Fetuses

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Abstract

The objective of this study was to assess the effects of the hydroalcoholic extract of *flax seed* on the teratogenic activity of lamotrigine in the brain of fetuses of rats who had received the drug. In this experimental study, 40 female rats were assigned randomly into four groups and after mating and confirming the vaginal plug, the control animals (group 1) were kept with no intervention, and the other three experimental groups were intraperitoneally injected with respective lamotrigine (75 mg/kg), and 100 and 200 mg/kg of *flax seed* hydroalcoholic extract. The drug was administered during the organogenesis period. Rats were sacrificed at the 20th day of gestation (one day before term) and fetuses were macroscopically examined, weighed and crown-rump length measured. Fetal brain specimens were processed for H&E and for histological study, using the ImageJ software. Results showed that fetuses of the experimental groups that received lamotrigine had reduced body weight, prefrontal cortical and hippocampal thickness, and pyramidal neurons in the hippocampus; Nevertheless, these factors were improved by high-dose administration of *flax seed* in the experimental group 3 and 4. Our research concludes that lamotrigine negatively influences the development of brain in rats and *flax seed* has a protective impact on these complications.

Keywords

lamotrigine, teratogenesis, flax seed

INTRODUCTION

According to the World Health Organization approximately 70 million people live with epilepsy worldwide[1] the majority of whom (>80%) are living in low- and middle-income countries.[2] This disease brings special issues for women, particularly in pregnancy. It is estimated that around 15% to 30% of women may have an increase in seizure frequency, most often in the first or third trimester.[3]

Lamotrigine is an anti-epileptic drug and a neuromodulator, which has been used in the treatment of epilepsy and mood disorders. This drug also is considered to be effective against partial tonic-clonic and secondarily generalized seizures.[4]

Herbal medicine is an area of complementary and alternative medicines that is readily amenable to empirical research.[5] *Flax (Linum usitatissimum)*, is a member of the genus Linum in the family Linaceae.[6] It is an edible oilseed/grain and is one of the oldest arable crops. In addition to being the richest plant source of a-linolenic acid (22% of whole flaxseed), lignans (range: 0.2–13.3 mg/g flaxseed), the *Flax* is also considered as an essential source of dietary

fiber (28% by weight), 25% of which is in the soluble form.[7-9] Various components of this herb have been shown to have antioxidant, anti-inflammatory, antiplatelet, hypoglycemia, and blood pressure-lowering properties.[10,11]

Although some pharmacologic and teratogenic effects of lamotrigine have been already studied, the teratogenic effects of this drug and the impacts of *flax seed* consumption during the pregnancy on the fetal brain development have not been scientifically clarified. Thus, this study was designed to evaluate the protective effects of *flax seed* hydroalcoholic extract on the teratogenic activity of lamotrigine in the developing rat brain.

MATERIALS AND METHODS

All experiments involving the animals were conducted according to a protocol approved by the Ethics Committee of the Shiraz University of Medical Science (SUMS), Iran.

Extraction method

The Flax seed was collected from Fars suburbs and after being authenticated by a botanist in the Research Center of Jahad-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. 500 ml of ethanol (70%) was added to 500 g of the plant powder in an appropriate container, and filtered after 72 hours. The solvent was removed at 35°C using a rotary apparatus. The Flax seed extract was incubated for two days at 40°C until it dried and then kept in refrigerator until the usage time.[12] Before using the extract we measured the flavonoid[13], total phenolic[14,15] and antioxidant activity[14] of the flaxseed.

Animal groups

Forty female Sprague-Dawley rats (180–220 g) were prepared and kept on a 12h light-dark cycle at controlled humidity and room temperature (20–23°C), and free access to food and water. After a ten-day habituation to the environment, two female and one male rat were placed in a cage for two days.[16] The vaginal plug was considered as the sign of zero-day of pregnancy (G0). Since this method was not certain, an eosin smear 3% was prepared from the vaginal discharges of the female rat and the existence of sperm was regarded as fertility.[13]

Afterward, the rats were randomly divided into four groups as follows:

The animals in the first group (control) were kept with no intervention, and the second (experimental 1), third (experimental 2) and fourth (experimental 3) groups were intraperitoneally (IP) injected with respectively lamotrigine (75 mg/kg), 100, and 200 mg/kg of *flaxseed* extract on days 9, 10 and 11 of pregnancy (corresponding to the fetal organogenesis stage).

Tissue processing

The animals were sacrificed at G20 (one day prior to term) and then the fetuses removed, blotted dry, and the following morphological examination was carried out. These included litter/fetal quality (i.e. appearance, weight, crown-rump length).

Histological preparations from the hippocampus and forebrain of the offspring were prepared by inclusion in paraffin, sectioned at intervals of 2 mm with a 6-µm thickness, and stained with hematoxylin-eosin. Then, nine histological sections from each fetus were prepared for stereological analysis using a grid for drawing and point counts, maintaining a space of 56 microns per cut. The same methodology was applied to the control group.

To measure the thickness of prefrontal cortex and hippocampus, the image of the sections (low-power magnification, ×4) was taken using a digital camera attached to the microscope [Olympus A×70]) and analyzed using the ImageJ software.

And finally the number of neurons in the prefrontal cortex and in the areas of CA1, CA2, and CA3 of the left hippocampus were counted with Eyepiece Micrometer grid.

Statistical analysis

Data were presented as mean and standard deviation and were analyzed by analysis of variance (ANOVA) using SPSS Windows version 22 (IBM Corp., Armonk, N.Y., USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

Each 100 gr of *flax seed* powder yielded 11 gr hydroalcoholic dried extract. The calculated levels for flavonoid and phenolic compounds were 98.13 mg/gr rutin equivalent and 104.30 mg/gr gallic acid equivalent, respectively. The extract antioxidant activity was 52.01%.

As shown in **Fig. 1**, in the lamotrigine-treated group we found decreased body weight and crown-rump length compared with those of the experimental groups ($p<0.05$).

The mean number of pyramidal neurons in the CA1, CA2 and CA3 decreased in lamotrigine groups compared to control group animals ($p<0.05$) **(Fig. 2)**. However, the mean number of pyramidal neurons in the CA1, CA2, and CA3 hippocampal subregions in control group was different from those of experimental groups 3 and 4 ($p<0.05$) **(Fig. 2)**.

Furthermore, the mean prefrontal and hippocampus thickness in lamotrigine groups decreased in comparison to the control rats ($p<0.05$) **(Fig. 3)**. On the other hand, the mean thicknesses of the prefrontal cortex and hippocampal layers increased significantly in the experimental group 4 compared to the control group ($p<0.05$) **(Fig. 3)**.

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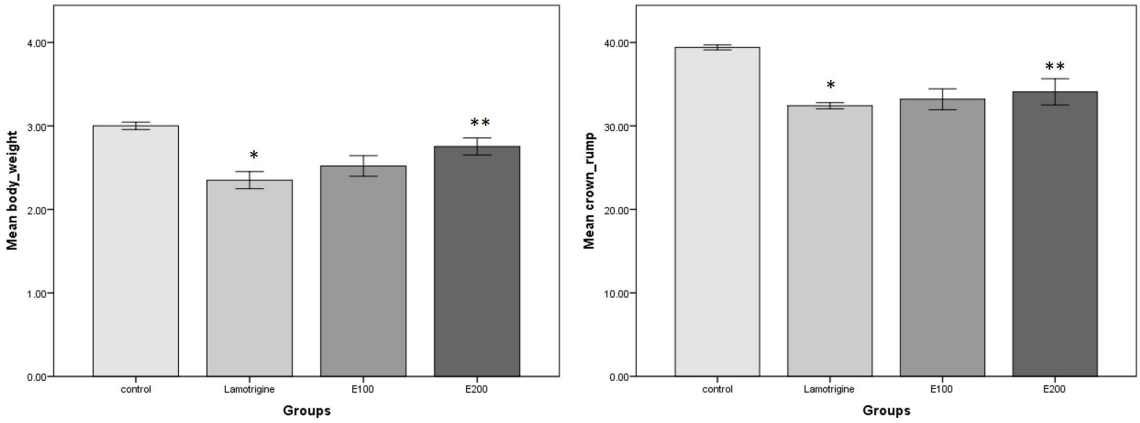

Figure 1. Fetal body weight and crown-rump in the studied groups. There were no significant differences between the experimental groups 3 and 4 and controls. The bars show mean values±SD. *p<0.05.

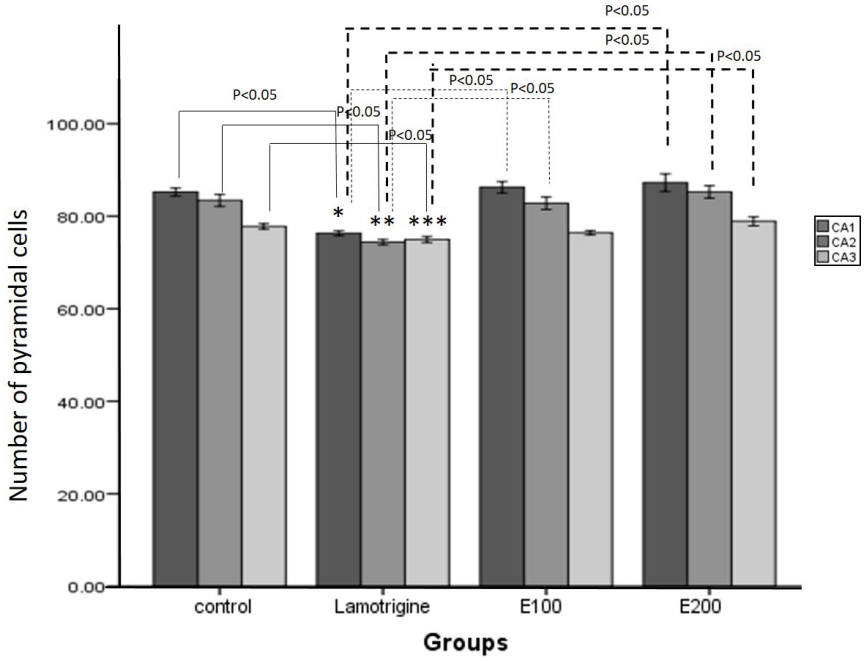

Figure 2. Total pyramidal neuron number of CA1, CA2, and CA3 subdivisions of the left hippocampus in different groups. The bars show mean values±SD. *p<0.05 compared to the lamotrigine groups.

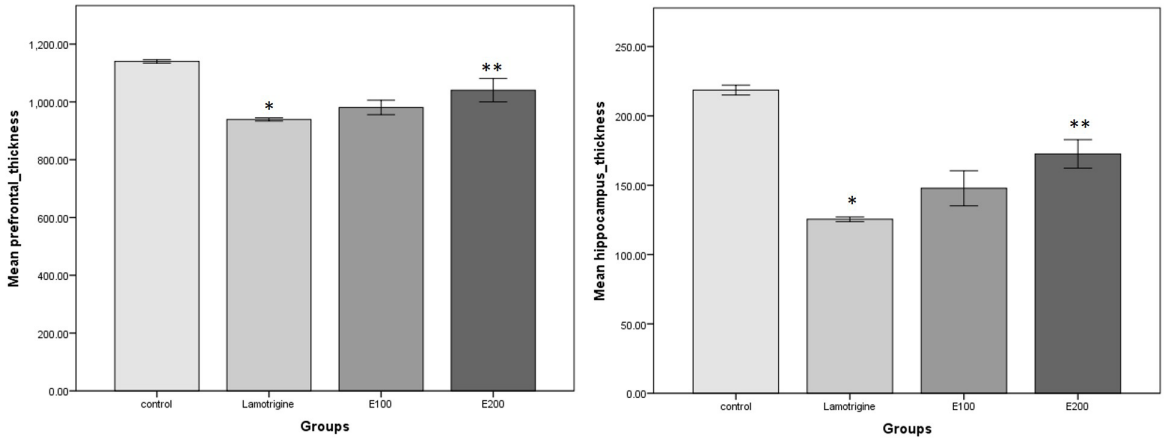

Figure 3. Mean thickness of prefrontal cortex and hippocampal layers in the study groups. The bars show mean values±SD. * p<0.05 compared to the lamotrigine groups.

DISCUSSION

Lamotrigine is a popular anti-epileptic drug for women who are either pregnant or consider getting pregnant. A commonly held view is that lamotrigine is a safe drug for an expectant mother and has a low rate of teratogenicity.[17,18] But the evidence of lamotrigine teratogenicity remains uncertain.[19,20]

The anti-epileptic drug can produce both anatomical (i.e. MCM) or behavioural (i.e. cognitive) teratogenicity. Several theories have been posited for the mechanisms of teratogenesis in the women with epilepsy on anti-epileptic drugs. The currently proposed mechanisms including folate deficiency, ischemia, neuronal suppression, reactive intermediates (e.g. free radicals or epoxides), and anti-epileptic drug-induced neuronal apoptosis were defined.[19]

In this study, as shown in **Fig. 1**, we found decreased body weight and crown-rump length in the lamotrigine-treated group compared to the flaxseed-treated animals. Morrow et al. reported that prenatal exposure lamotrigine produced fewer major congenital malformations than observed with valproic acid.[21] The major congenital malformation rate for lamotrigine exposures was 3.2% (95% CI: 2.1–4.9). However, the study was insufficiently sensitive to exclude a substantially increased risk of major congenital malformation (risk ratio [RR]: 0.92; 95% CI: 0.41–2.05).[22] Also, Mobini et al. in 2019 showed that lamotrigine can be considered a risk factor for malformations.[23] The same authors found that lamotrigine doses were significantly higher in the cases of major congenital malformation than that observed in the controls.[21]

In this research, we observed that the mean number of pyramidal neurons in the lamotrigine-exposed groups were significantly reduced in comparison with the controls ($p<0.05$) **(Fig. 2)**. These results are consistent with previous studies on lamotrigine.[24-27]

On the other hands, in experimental groups 3 and 4, there was a significant increase in the mean number of neurons ($p<0.05$) **(Fig. 2)**. This indicates that high doses of *flax seed* reduced the teratogenic effects of the lamotrigine on the development of nervous system.[28]

The results of the mean number of pyramidal neurons and cortical thicknesses can be attributed to the large amounts of fatty acids from omega-3 family in *flaxseed*, which has 57% of omega-3 fatty acids in their composition.[29]

This study demonstrated that the mean thickness of prefrontal cortex and hippocampus decreased in lamotrigine groups compared to the control group ($P<0.05$). In contrast, when compared to control group, the mean thickness of prefrontal cortex and hippocampus increased significantly in the experimental group 4 **(Fig. 3)**.

Oxygen free radicals attack objects on the polyunsaturated components of membranes and may cause a serious organizational dysfunction within cells and tissues. It has been suggested that the administration of omega-3 polyunsaturated fatty acids may have an ameliorating effect on such damage by two possible ways. First, omega-3 polyunsaturated fatty acids may increase the level of catalase within the peroxisome and in the cytoplasm resulting in enhanced defense against free oxygen radicals. Second, omega-3 polyunsaturated fatty acids, which have been supplemented, may be replaced with polyunsaturated fatty acids components of the membranes that had been attacked by oxygen free radicals.[30,31]

CONCLUSION

The results of the present study clearly revealed that lamotrigine consumption during pregnancy has teratogenic effects on the fetal developing rat brain and *flax seed* also has positive influences against these teratogenic impacts. Further research must be carried out to corroborate these findings and establish their applicability to humans.

Conflict of Interest

The authors declare they have no conflict of interest.

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Защитное действие льняного семени на тератогенность мозга, вызванную ламотриджином у эмбрионов крысы

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Резюме

Целью данного исследования было оценить влияние водно-спиртового экстракта льняного семени на тератогенную активность ламотриджина в мозге эмбрионов крыс, получавших лекарственные препараты. В этом экспериментальном исследовании 40 самок крыс были случайным образом распределены на четыре группы, и после спаривания и подтверждения наличия вагинальной пробки контрольные животные (группа 1) были выращены без вмешательства, а остальным трём экспериментальным группам внутрибрюшинно вводили ламотриджин (75 мг / кг).) и 100 и 200 мг / кг водно-спиртового экстракта льняного семени. Препарат вводили в период органогенеза. Крыс умерщвляли на 20-й день беременности (за день до срока), и плод исследовали, взвешивали и измеряли их длину под микроскопом. Образцы мозга эмбриона обрабатывали для окрашивания гематоксилином и эозином и для гистологического исследования с использованием программного обеспечения ImageJ. Результаты показали, что у эмбрионов экспериментальных групп, получавших ламотриджин, была снижена масса тела, толщина префронтальной коры и гиппокампа, а также пирамидные нейроны в гиппокампе. Тем не менее, эти факторы были улучшены путём приёма высоких доз льняного семени в экспериментальных группах 3 и 4. Наше исследование пришло к выводу, что ламотриджин отрицательно влияет на развитие мозга крыс, а льняное семя оказывает защитное воздействие на эти осложнения.

Ключевые слова

ламотриджин, тератогенез, льняное семя