Teratogenic Effect of Levetiracetam on Albino Mice Placentae

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ABSTRACT

Background: Levetiracetam is widely used as a newer antiepileptic drug in women of reproductive age, but data on its teratogenic effects, particularly on the placenta, are limited. This experimental study evaluated dose-related placental changes following prenatal exposure to levetiracetam in albino mice.

Methods: Forty F1-generation pregnant Swiss albino mice were randomly allocated to four groups (n=10 each). Group I received intraperitoneal distilled water (control). Groups II–IV received levetiracetam 600, 1200 and 1800 mg/kg body weight, respectively, from gestational day (GD) 5–11. All dams were sacrificed on GD19. Placentae were weighed, examined grossly and processed for histology (H&E). Trophoblastic, mucoid and fibrinoid degeneration and presence of dilated vessels were recorded. Data were analysed using one-way ANOVA and chi-square tests.

Results: Mean placental weight differed significantly among groups ($F=5.300$, $p<0.001$), being lower in Group II and higher in Groups III and IV compared with controls. Levetiracetam exposure produced marked, dose-related histopathological changes. Trophoblastic degeneration was present in 10% of control placentae versus 93.3%, 84% and 86.96% in Groups II–IV, respectively ($p<0.00001$). Mucoid degeneration occurred in 1.8% of controls vs 40%, 62% and 91.3% in Groups II–IV. Fibrinoid degeneration was found in 5.5% of controls vs 44.4%, 64% and 87% in Groups II–IV, while dilated vessels were seen in 1.8% of controls vs 2.2%, 20% and 93.5% in Groups II–IV (all $p<0.00001$).

Conclusion: Prenatal exposure to levetiracetam during organogenesis produced significant, dose-related placental degenerative changes in albino mice. These findings suggest potential teratogenic risk at the placental level and underline the need for cautious use and further mechanistic and clinical studies in pregnancy.

Keywords: *levetiracetam, pregnancy, placenta, teratogenicity, albino mice*

INTRODUCTION

Epilepsy is one of the most prevalent neurological diseases and its spread depends on age, race, social class, as well as geographic and national boundaries. Levetiracetam is currently approved as a novel antiepileptic drug (AED) which was synthesized for monotherapy or as adjunctive treatment of partial-onset seizures in adults and pediatric patients with epilepsy. [1, 2]. In India levetiracetam was approved in April, 2005. Studies have been undertaken to study the teratogenic effects of the drug but very little information is available. The present study was done in a medical college and research institutein North East India and planned to find information regarding the same

The North American antiepileptic epilepsy registry mentioned that the major congenital malformation rate for Levetiracetam is 2.03%. [3] Medor et al. in a study on pregnant women with epilepsy observed that the most frequently prescribed monotherapy drug were either Lamotrigine or Levetiracetam, probably owing to its high level of tolerability coupled with good efficacy. These drugs were found to be safer than older antiepileptic drugs. [4]

The teratogenic effects ofLevetiracetam are uncertain in pregnant women as the Standard and New Antiepileptics drug trial (SANAD) did not include Levetiracetam in its trial studies involving antiepileptic drugs on the human population. [5] Currently, insufficient clinical data exist with regard to reproductive safety in human subjects receiving the new antiepileptic drugs. [6]

With limited literature on the aspect of teratogenicity due to Levetiracetam, this study evaluates the same in fetuses of albino mice exposed in utero to the drug.

METHODS

The study was conducted in the Department of Anatomy with the Animal House of the institute (North Eastern Indira Gandhi Regional Institute of Health & Medical Sciences)after getting approval from the Animal Ethics Committee of the Institute (NEIGR/Pharma-AH/IEC/2014/01) (Proposal No. P-01/14) (Titled-“ Teratological Effect Of Levetiracetam on Pregnant Albino Mice: A Histomorphometric Study”) approved on 18/04/2014. For the study, adult albino mice with an average weight and age of about 20-30 gm and 80-100 days, respectively, were used.

Procurement and Acclimatization of Animals

A total of 24 adult male and female Swiss albino mice were procured from the animal house. They were reared in a polypropylene cage (39 x 24 x 15 cm) in the animal house under the standard laboratory conditions (25° ± 2°C, 12-hour light-dark cycle, 60 % relative humidity) for 2 weeks for proper acclimatization. Dry rice bran was used as bedding material which was changed regularly to avoid any unhygienic conditions. They were fed on a pelleted diet and tap water ad libitum.

Maintaining of Mice Colony

To maintain the inbred mice colony, one adult male and two adult female mice were kept in a polypropylene cage as one set for natural mating. Several sets of mating mice were maintained. First-generation (F1) mice were reared from the initial 20 adult mice (F0). For experiment purposes, 40 healthy female mice and 10 male mice were selected from the F1 generation and they were reared. Then those 40 F1 generation female mice were categorized into four groups. Thereafter to study the effect of Levetiracetam, the 40 F1 generation female mice were mated with randomly selected F1 generation male mice. This process was done in the evening every day. On the next morning at 8 am, female mice were taken out from the mating cages and their vaginas were checked. The presence of the vaginal plug indicated pregnancy and was designated as day zero of gestation. In case of doubt, the plug was examined microscopically for the presence of sperms. Each sperm-positive mouse was housed individually in separate cages with similar laboratory conditions with access to water and food and all records of weight gain of pregnant female mice were kept till the day of sacrifice on the 19th day.

Drugs used

Levetiracetam was the drug used. It was purchased from the local market in the trade name of “Levi Pil” (in the form of injectable), manufactured by Sun pharmaceuticals industries Ltd. (Acme Plaza, Mumbai, India).

Procedure

Forty pregnant mice (F1) were taken and divided into four groups. Each group contained ten mice.

Group I: Control (given an equivalent amount of distilled water)

Group II: Treated with Levetiracetam (600mg/kg body weight)

Group III: Treated with Levetiracetam (1200mg/kg body weight)

Group IV: Treated with Levetiracetam (1800mg/kg body weight)

Drug administration

The treated mice were exposed to drugs or vehicles (distilled water) from gestation days through the intraperitoneal route. For the present study, Levetiracetam (in 10 ml solution) was administered to the female pregnant mice in the treated groups (Group II, III, and IV) with a concentration of 600,1200, and 1800mg/kg body weight respectively from 5th to 11th days of gestation through the intraperitoneal route. For the control group (Group I) 10ml of distilled water was injected intraperitoneally for the same period.

Sacrifice of the pregnant mice and collection of fetuses

Both treated and control mice of each group were sacrificed by cervical dislocation on the 19th day of gestation.

Immediately after cervical dislocation, uterotomy was performed, where the uterine horns were exteriorized after opening the abdomen by midline incision. The live fetuses were collected

along with the placenta after examining the uterine horns for sites of resorption and dead fetuses. All findings were recorded. The collected fetuses and placenta were blotted dry and weighed individually andwere examined for external malformations. After gross examination, the fetuses and placenta were preserved in a 10% neutral formalin solution for further examinations.

Histological study

After fixation, fetuses and their placentas from all 4 groups were taken for histological study. All the placentae were washed in tap water to remove the traces of formalin and then processed for paraffin wax embedding and then stained with hematoxylin and eosin (H & E) stain and were examined under a light microscope.

Statistical analysis

All data were entered into an excel sheet and mean and standard deviation (S.D.) were calculated. One-way ANOVA test and Chi-Square test were done using software SPSS version 16 (Statistical Package for Social Sciences) and Social Sciences Statistics online software. A p-value < 0.05 was considered as significant and a p-value < 0.001 was considered highly significant.

RESULTS

On histological examination, Levetiracetam-treated placentae showed marked degenerative changes as compared to the control group. The basal zone of the treated placentae showed trophoblastic cell degeneration and mucoid degeneration in all the treated groups II, III, and IV. (Fig 1) Fibrinoid degeneration in the basal zone of the placenta was identified in group II and group III. (Fig 1) Dilated blood vessels were noted in the basal zone of the placenta of group IV. (Fig 1) In the labyrinth zone, all the treated groups (Group II, III and IV), showed trophoblastic cell degeneration, mucoid degeneration, and fibrinoid degeneration. (Fig 2)

FIGURES AND IMAGES

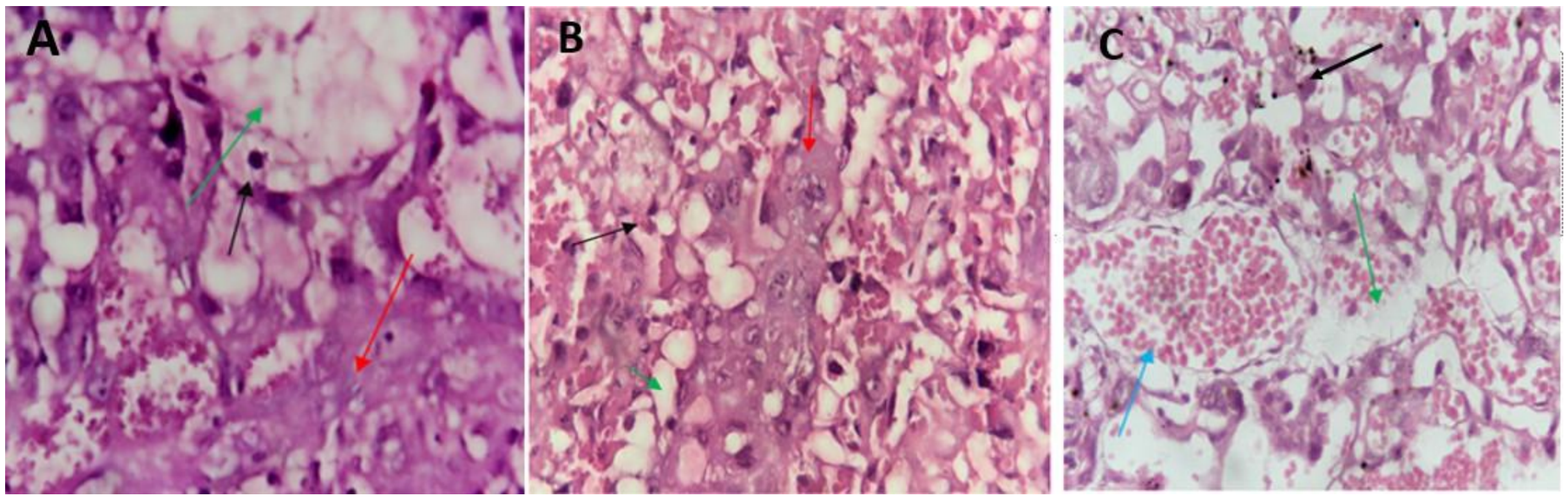

Fig 1: The basal zone of the treated placentae showed trophoblastic cell degeneration (Black arrows) and mucoid degeneration (Green arrows) in all the treated groups of II (A), III (B) and IV (C). Fibrinoid degeneration (Red arrows) is seen in group II (A) and III (B). Dilated blood vessels (Blue arrow) seen in group IV (C) (H & E, 400x)

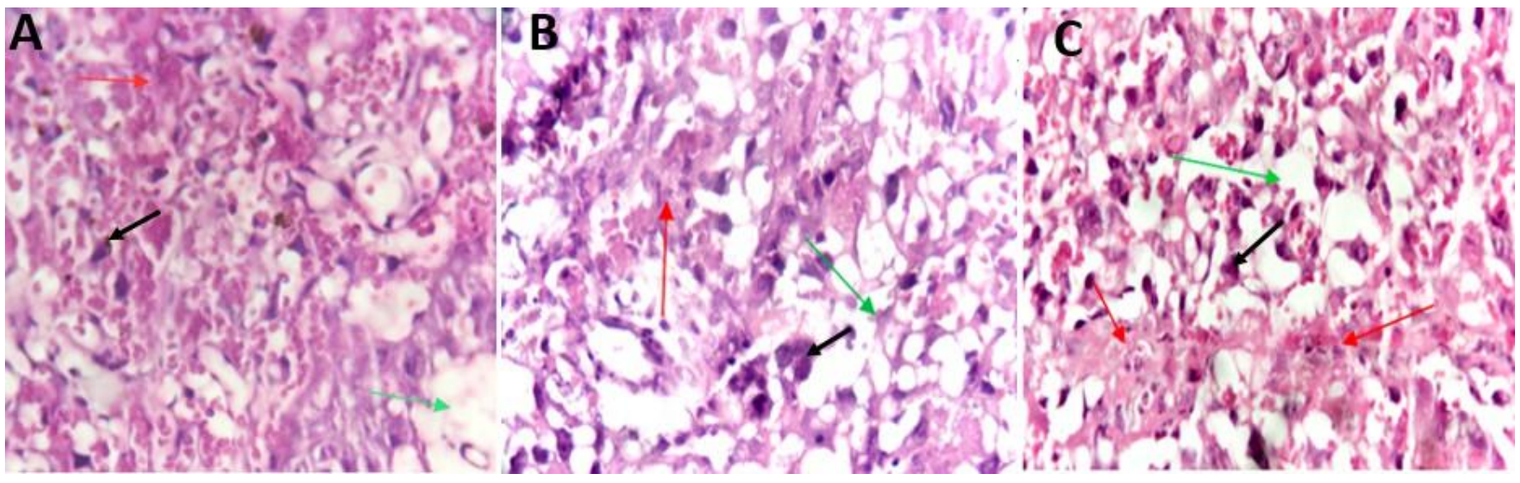

Fig 2: In the labyrinth zone, all the treated groups II (A), III (B) and IV (C) showed trophoblastic cell degeneration (Black arrows), mucoid degeneration (Green arrows), and fibrinoid degeneration (Red arrows) (H & E, 400x)

TABLES

Table 1&2: Trophoblastic degeneration and Mucoid degeneration of the treated placentae

Groups	Trophoblastic degeneration Present	Trophoblastic degeneration Absent	p-value
Group I (n=55)	5 (10%)	50 (90%)	< 0.00001
Group II (n=45)	42 (93.3%)	3 (6.7%)	
Group III (n=50)	42 (84%)	8 (16%)	
Group IV (n=46)	40 (86.96%)	6 (13.04%)	

Table 1&2: Trophoblastic degeneration and Mucoid degeneration of the treated placentae (1)

Groups	Mucoid degeneration Present	Mucoid degeneration Absent	p-value
Group I (n=55)	1 (1.8%)	54 (98.2%)	< 0.00001
Group II (n=45)	18 (40%)	27 (60%)	
Group III (n=50)	31 (62%)	19 (38%)	
Group IV (n=46)	42 (91.3%)	4 (8.7%)	

Table 1&2: Trophoblastic degeneration and Mucoid degeneration of the treated placentae (2)

Table 3&4: Mucoid degeneration and of the treated placentae and Dilated vessels in the treated placentae

Groups	Fibrinoid degeneration Present	Fibrinoid degeneration Absent	p-value
Group I (n=55)	3 (5.5%)	52 (94.5%)	< 0.00001
Group II (n=45)	20 (44.4%)	25 (55.6%)	
Group III (n=50)	32 (64%)	18 (36%)	
Group IV (n=46)	40 (87%)	6 (13%)	

Table 3&4: Mucoid degeneration and of the treated placentae and Dilated vessels in the treated placentae (1)

Groups	Dilated vessels Present	Dilated vessels Absent	p-value
Group I (n=55)	1 (1.8%)	54 (98.2%)	< 0.00001
Group II (n=45)	1 (2.2%)	44 (97.8%)	
Group III (n=50)	1 (20%)	49 (98%)	
Group IV (n=46)	43 (93.5%)	3 (6.5%)	

Table 3&4: Mucoid degeneration and of the treated placentae and Dilated vessels in the treated placentae (2)

DISCUSSION

During the organogenesis period of the developing embryo, any chemical-physical or environmental agent can be the reason for inducing teratogenic effects. The period of day 6 to day 15 in rodents (albino mice) is highly critical to any injury leading to congenital malformation or even death of embryo corresponding to the intensity of insult due to teratogen.

This study revealed that exposure to Levetiracetam during the period of organogenesis induces teratological changes in the developing albino mice. [7]

In the present study, we evaluated the gross and microscopic features of the placenta in albino mice. There was a significant increase (p-value <0.001) in the weight of the placenta in Group III and IV as compared to control Group I. In contrast, there was a significant decrease in placental weight of Group II as compared to control Group I.

On histological examination, Levetiracetam-treated placentae (Group II, III, and III) showed marked degenerative changes as compared to the control group. The basal zone of the treated placentae showed trophoblastic cell degeneration and mucoid degeneration in all the treated groups of II, III, and IV. Fibrinoid degeneration was observed in the basal zone of the placenta in group II and group III. Dilated blood vessels were noted in the basal zone of the placenta of group IV. In the labyrinth zone, all the treated groups (Group II, III, and IV) showed trophoblastic cell degeneration, mucoid degeneration, and fibrinoid degeneration.

There is no data available on placental changes caused due to Levetiracetam in mice but there is one study done by Huda Omer et al. in rats, where they had observed dose-dependent necrosis in maternal deciduae and fibrin deposits in placentae due to cell degeneration due to Levetiracetam. [8]

Kwieciñska P et al. as shown in their study that Levetiracetam can cross the placenta and its exposure decrease in beta –HCG secretions which is a marker of trophoblastic differentiation and increased in caspase 3 activity which is a marker for apoptosis and derangement of trophoblastic cells, resulting in fetal growth restrictions which may be a possible explanation for decrease weights for the placenta of Group II in which fetuses were exposed to 600 mg/kg Levetiracetam doses during the period of organogenesis. [9] But in placentae of Group III and IV placental weight is seen significantly increased with the presence of fibrinoid changes and mucoid degeneration alongside dilated blood vessels.

Svalheim S et al. while studying the effect of Levetiracetam on Wistar rats showed that Levetiracetam is affecting the functioning of the ovary and alerting hypo-thalamopituitary-gonadal axis. [10] Furthermore Levetiracetam is reported for altering reproductive balance as synaptic vesicle glycoprotein 2A (SV2A) receptors are not only present in the central nervous system but also found in most endocrine tissues which may be causing an increase in placental weight in Group III and IV due to dose-dependent, the altered hormonal status of pregnant female mice resulting in dilated vessels resulting in more blood perfusion in the placenta of Group II and IV resulting in an increase in weight of placenta of fetuses of Group II and IV. [11]

Levetiracetam is extremely water-soluble, so this allows rapid and complete absorption after oral administration. It is not metabolized by the liver and is hence free of non-linear elimination kinetics and free of major drug-drug interactions. It further lacks protein binding. [12,13] But in this study, in group IV (1800mg/kg body weight) the effect of Levetiracetam is limited compared to other groups as it may be possible that Levetiracetam may be following altered pharmacokinetics leading to second pass effect with higher doses which is yet to be confirmed.

CONCLUSION

This study was to evaluate placental changes of Levetiracetam-treated Albino mice and to study the histopathological changes of placentae. It concluded that prenatal exposure to Levetiracetam induces teratological effects. The weight of the placenta was significantly increased in the treated groups and histological examination revealed that the Levetiracetam-treated placentae showed marked degenerative changes

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