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The Effects of Lamotrigine on Fetus Resorption and Histologic Changes in Cranium of Fetus of Albino Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Author NS conceptualizes the study, data analysis, drafting and finalizing of the results was done by author SFAR. Authors NAS and BBR critically reviewed the article. Finally reviewed and approved by author AK. Data collection and session organization was facilitated by author HS. All authors read and approved the final manuscript.

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ABSTRACT

Background: Lamotrigine is a member of antiepileptic drugs, it belongs to the sodium channel blocking agent's class and it is pregnancy category C drug. While its teratogenic effects are not hidden by the doctors but it is the preferred drug being prescribed in pregnancy. The current study aims to investigate the effects of fetus resorption and histologic changes in cranium of fetus of albino mice and to compare their weight changes due to lamotrigine therapy.
Methodology: It was an experimental animal study conducted in collaboration of anatomy and surgery department at animal house of University of Lahore in 2019. The duration of study was 25 days, twenty-four albino mice (12 males and 12 females) were placed in conventional cages in

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pairs for mating purpose. Female mice Group A, controls in which normal saline was administered intraperitoneally on 10th day of gestation and Group B, in which lamotrigine was given intraperitoneally on the 10th day at the dose of 10 mg/kg. Maternal health was monitored daily during the intervention. Body weight, food and water consumption, and changes in general health, behavior, activity and any sign of toxicity were checked daily. After 18th day the pregnant mice were sacrificed under euthanasia and fetuses were removed and histologic assessment was carried out.

Results: Weight of mice treated with lamotrigine decreased significantly (p-value=0.03) and fetus resorptions were also more (p-value=0.013) in Group B. Histologic assessment reveled that there were cleft of lip and palate in group B.

Conclusion: Lamotrigine increased the fetal resorption and decrease the weight and seemed to be responsible for inducing cleft of lip and palate at 10mg/kg dose in albino mice.

Keywords: Lamotrigine; fetus resorption; cranial abnormalities; albino mice.

1. INTRODUCTION

In Pakistan, USA, and Europe Lamotrigine is marketed by the name of Lamictal that is an anticonvulsant drug used for the therapy of bipolar disorder and epilepsy. Lamotrigine is a member of antiepileptic drugs and it belong to the sodium channel blocking agent's class [1-2], it is effective regime for the depressed phase of bipolar disorder, and it is inactivated by .Hepatic glucuronidation [3]. The precise root for the wider range of its action is unknown, however along with sodium channel blocking ability the proposed mode of action is its activity as T-type calcium channels can be related to the actions of the drug [4]. Stevens–Johnson syndrome, a life-threatening skin reaction, has also been warned by Lamotrigine use, other diseases include DRESS syndrome and toxic epidermal necrolysis [5]. Decrease in white blood cell count (leucopenia) is also associated with Lamotrigine use [6].

The risk in pregnant women due to Lamotrigine use is rated Pregnancy Category Risk C. If benefits outweigh potential risks, only then its use is recommended in pregnancy. FDA issued a warning in 2006 September regarding the use of Lamotrigine during the first trimester. According to them the use of the medication earlier led to an increase in the threat of cleft lip and palate deformity in new born children [7-8]. Along with this it also documented that taking lamotrigine in early pregnancy may result in fetus resorption i.e loss fetus [9]. Offspring (Average age = 4.2 years) who came in contact with lamotrigine in utero indicated no signs of adversarial effects, a prospective study's report [10].; Breastfeeding is not recommended during treatment by the manufacturer because Lamotrigine is found in breast milk as well [11].

There are various studies in which it is documented that fetus resorption is associated with intervention with teratogenic drugs and various genetic and environmental factors are thought to be responsible for childlessness in females [12]. According to Kenny et., al. Females suffer from epilepsy in child bearing age and in that scenario hindrances predisposed by antiepileptic drugs make their life more difficult [13]. The current study aims to investigate the effects of fetus resorption and histologic changes in cranium of fetus of balb c albino mice and to compare their weight changes due to lamotrigine therapy.

2. METHODOLOGY

It was an experimental animal study conducted in collaboration of anatomy and surgery department at animal house of University of Lahore in 2019. The duration of study was 25 days, twenty-four albino mice (12 males and 12 females) were purchased from veterinary university, and they were placed in conventional cages in pairs for mating purpose. Veginal plug of each mice were observed in early morning for conforming the mating on daily basis once the vaginal plug was observed the mice were separated from males and were divided into two groups i.e. Group A, controls in which normal saline was administered intraperitoneally on 10th day of gestation and Group B, in which lamotrigine was given intraperitoneally on the 10th day at the dose of 10mg/kg. Maternal health was monitored daily during the intervention. Body weight, food and water consumption, and changes in general health, behavior, activity and any sign of toxicity were checked daily. After 18th day the pregnant mice were sacrificed under euthanasia that was induced by using chloroform. After scarification fetuses were removed. The resorptions were identified after dissecting the uterine horn, rinsing

them and keeping them in phosphate-buffered saline for 10 minutes. The uterine horns were subsequently stained with a few drops of a solution of 10% ammonium sulfide for 10 minutes to identify the number of implantation sites which appear as dark rings (Qureshi et al., 2009). Prior to experiment animals were acclimatized for 4-5 days in the Animal House of Postgraduate Medical Institute, Lahore. The animals were kept conventionally in iron cages under an artificial light regime (6am-6pm = day, 6pm-6am = night), at optimum temperature (24±2°C) and relative humidity 55 ± 5% in hygienic conditions, provided with pellet food and water ad- libitum daily. The study was approved by ERC of University of Lahore. Statistical Package for Social Sciences (SPSS) version 18 was used for data analysis. All the quantitative data was collected on proforma. Any difference in the quantitative measurement was tested by student t-test to identify which group mean differed. The p-value less than 0.05 was considered statistically significant.

3. RESULTS

Maternal weight gain during pregnancy ranged between 19.00-24.00, 13.00-15.00 in group A and B respectively. The mean maternal weight significantly increased in Group A compared to group B (p-value=0.03) as shown in Table 1. There was statistically significant difference in fetal resorptions between group A and B (p=0.013) more fetuses were seemed to be resorbed in group B (lamotrigine treated group). On histologic assessment of cranium, we observed gap in palate of animals of group B however, cranium of group A seemed to be normal under microscope. Histological findings are shown in Figs. 2 and 3.

Table 1. Showing the mean weight in groups A and B

Variable	Group A Mean ± SEM n=5	Group B Mean ± SEM n=5	p-value
Maternal Wt. Gain (gm)	21.80±0.86	14.2±0.37	0.03

Table 2. Showing number of fetal resorptions in Group A and B

Variable	Group A Mean ± SEM n=1	Group B Mean ± SEM n=16	P-value
Fetal resorptions	0.2±0.2	3.2±0.66	0.013

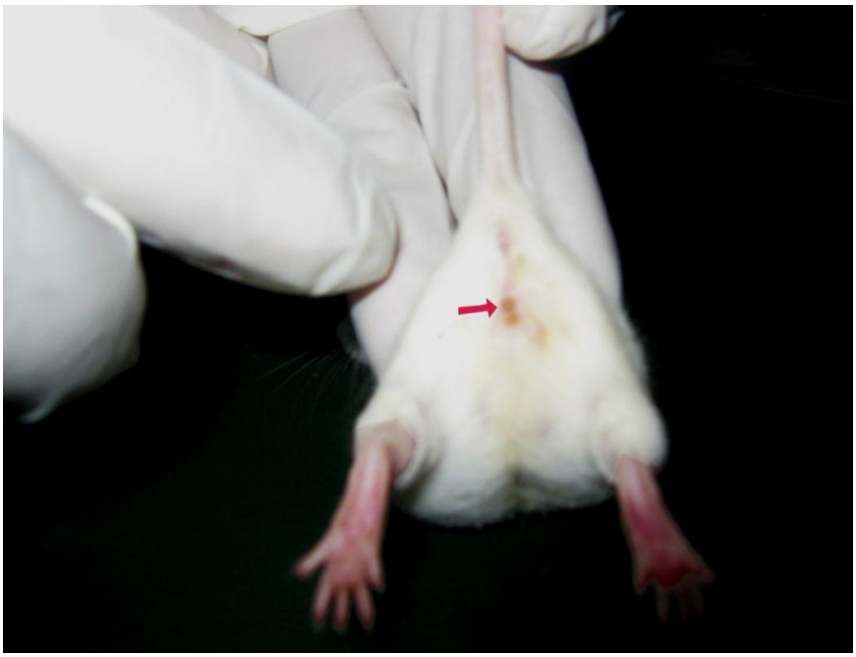

Fig. 1. Photomicrograph showing the vaginal plug

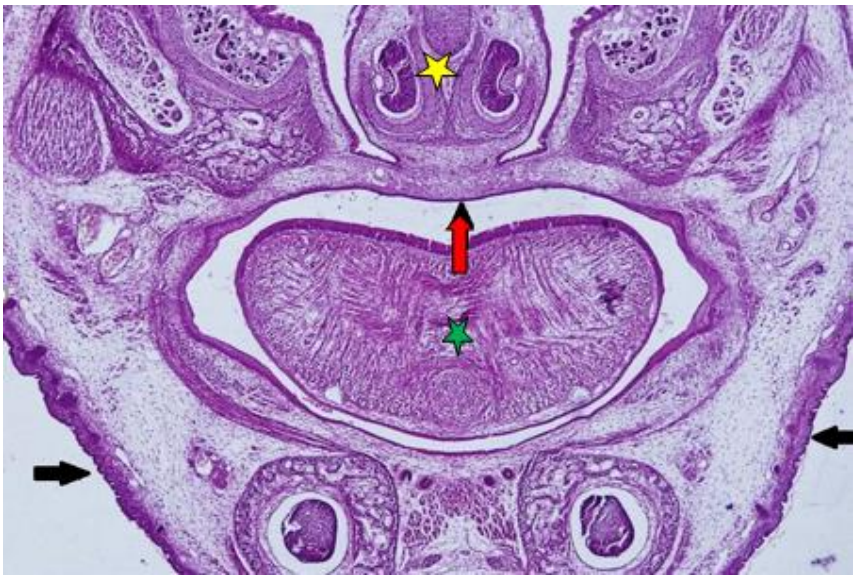

Fig. 2. Photomicrograph of histological section of palate and cranium in coronal plane showing the normal palate (Red arrow), and normal lips (Black arrows) in control group A. Yellow star (Nasal septum), Green star (Tongue).H&E stain X4

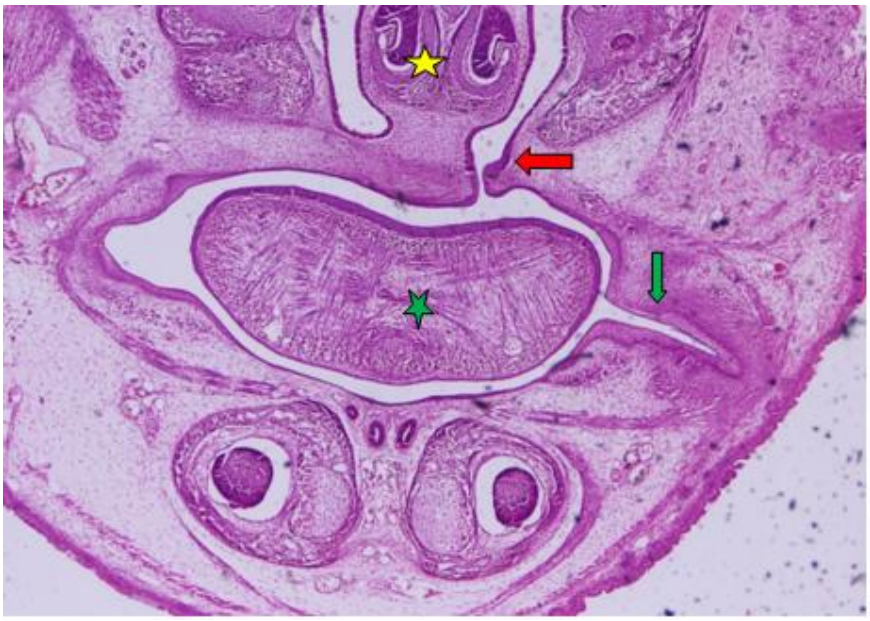

Fig. 3. Photomicrograph of histological section of palate and cranium in coronal plane showing the cleft palate (Red arrow), partial cleft lip (Green arrow), Yellow star (Nasal septum), Green star (Tongue) in group B. H & E. X4

4. DISCUSSION

In this study maternal weight in LTG treated group 'B' was reduced when compared with Control group 'A' and it was very highly significant, However Lamotrigine administration to mother has no effect on food intake, but it may be due to loss of fetuses owning to the treatment with Lamotrigine, the findings are consistent with the observation of [14], when LTG was given on day 7th and 8th at a dose of 25 mg/Kg and 50mg/kg body weight respectively. El-Sayyad et

al. also observed reduction in maternal body weight when lamotrigine 50 mg /Kg body weight every other day from 6th day of gestation until parturition, was given to albino rats of Wistar strain [15]. This indicates dose related toxic effects on maternal weight gain. In another study it was found that when compared to control weight reduction was observed in lamotrigine treated group. Fetal resorptions were statistically significant in Lamotrigine treated group 'B' when compare with control group 'A'. Similar finding of fetal resorptions were also reported by Afshar et al., [16-17] and statistically significant when Carbamazepine is given at a dose of 30 mg/kg/day on gestational day 6 to 15 in experimental group I, and 60 mg/kg/day on gestational day 6 to 15 in experimental group II. In this study a solitary (10 mg/kg body weight) high dose of lamotrigine was injected because large dose has long lasting intense and constant teratogenic effect compared to short intervals smaller doses which might result in repair of damaged tissue [18]. Comparison of fetuses which shows cleft palate and cleft lip between control 'A' and 'B' were significant, In this study fetuses of group B shows, two fetuses (09%) have unilateral cleft palate and partial cleft lip, two fetuses (09%) with bilateral cleft lip (Fig. 2. while one fetus (4.5%) showed contra-lateral cleft palate and cleft lip (Fig. 3). In group "B" bilateral Cleft lip and contra lateral cleft palate & lip was observed, while in other group's only unilateral cleft palate and lip was observed [19]. Whatever the site of clefting was, the focus of this study was to observe cleft palate and cleft lip induced by lamotrigine and its prevention by different doses of folic acids. Lamotrigine has adverse effects of decreasing folic acid level of mother and fetus, consequently resulting in reducing methylation of DNA and especially during organogenesis, the period of rapid cell growth and differentiation Rogawski, M [20-21].

5. CONCLUSION

Lamotrigine induced weight loss and increased the number fetal resorptions in group B. Histologic findings revealed that lamotrigine may cause cranial abnormalities at high doses, so when prescribed in pregnancies the dose must be assessed on risks versus benefits phenomena.

CONSENT

It is not applicable.

ETHICAL APPROVAL

ERC approval was taken from University of Lahore and ethics reference code 2405720PULPA was issued.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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