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Research Article

ADVERSE MACROSCOPIC CHANGES IN DEVELOPING MICE TREATED WITH LAMOTRIGINE ON EARLY AND LATE PHASE OF GESTATION

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A R T I C L E I N F O

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A B S T R A C T

Lamotrigine(LTG) is an anti epileptic drug(AED) and also used as a neuromodulator, in mood disorders. Recently, malformations have been reported in human foetuses, whose mothers were treated with LTG. However, it not been possible to establish a recognizable pattern of malformations in the human fetuses treated with LTG, therefore the lower animal has been used as experimental model for the present study. The objective of this study is to find out the macroscopic changes in the growing embryo of mice treated with LTG in both early and late phase of gestation.The pregnant mice were divided into two experimental group's i.e. early and late, each with two subgroups i.e. control group (treated with intra peritoneal injection of normal saline on Day 4 and Day 9 of gestation) and treated group (treated with intraperitoneal injection of LTG, 150mg/kg body weight on Day 4 and Day 9 of gestation. Fetuses were collected on day 19th of gestation and then observed for the various macroscopic abnormalities.The results of this study indicate that LTG administered intra peritoneally at high doses on day 9 of gestation induces intrauterine growth retardation (p value<.05), embryonic resorption (16.7%), external hemorrhage (39%), malformations of limb (34.9%). whereas these abnormalities were found in less number of foetuses treated in early phase (Day4) of gestation. As it is teratogenic in animals, so the pregnant mother should be treated cautiously and under appropriate guidelines.

INTRODUCTION

Lamotrigine (LTG) is an anti-epileptic drug (AED)[1] and also used as a neuromodulator, in mood disorders [2]. LTG crosses the placenta easily and rapidly, therefore maternal treatment leads to a considerable fetal exposure [3]. Recently, some malformations have been reported in human fetuses treated with LTG [4,5]. However, it has not been possible to establish a recognizable pattern of malformations in the human fetuses treated with LTG. The lower animal has been used as experimental model for the present study. Drugs that have been found to be teratogenic in man have caused similar effects in animals [6,7]. The objective of this study is to find out the macroscopic changes in the growing embryo of mice treated with LTG in early and late phase of gestation.

MATERIAL AND METHODS

The present study was conducted in the Teratology laboratory of the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi, after the approval of the Central Animal Ethical Committee.

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Thirty-two female Swiss albino mice of an average weight of 20-25 gm and about six weeks of age were used in this study. The animals were divided into four groups i.e. First Control Group (Gr-1), Early Treated Group (Gr-2), Second Control Group (Gr-3) and Late Treated Group (Gr-4).

Female mice were transferred in the evening to the cages containing male mice of the same stock in the ratio of 3:1. The presence of vaginal plug on the following morning indicated the Zero Day of pregnancy. Each pregnant mice were weighed and divided into treated and control groups. Based on literature the dose of LTG was estimated to be 150mg per kg [8,9,10]. The stock solution was made my dissolving 50mg strength of lamotrigine tablet in 5ml normal saline. Hence, approximately 0.4ml of stock solution contained 4mg drug. Treated mice of each group received the drug intraperitoneally with the help of tuberculin syringe, whereas the control group received equal volume of normal saline intraperinoneally (Fig-1).

The pregnant mice of each group were sacrificed with an overdose of ether anesthesia on day 19th of gestation. A midline laparotomy incision was made to open the abdomen and the uterine horns were exteriorized. A photograph of the uterine horns and its content was taken.

Fig 1 Photograph Showing The Method of Intraperitoneal Injection of Lamortigine In Mice

The sacs were inspected for sites of resorptions and the viable fetuses were collected. The fetuses were dried on a blotting paper and weighed with the placenta. The crown rump length (CRL) of each fetuses was recorded with the help of graph paper and then examined for external abnormalities if any. The photographs were taken and analysis was done using independent't 'test.

OBSERVATIONS AND RESULTS

The foetuses were collected from both early (Gr-1 and 2) and late (Gr-3and4) experimental groups after laparotomy of pregnant mice on Day 19th of gestation. The percentage of resorption in Gr-2 was 12.5%, whereas 16.67% in Gr-4(Table 1). The corresponding control groups did not show any resorption. The percentage of resorption was found to be more in Gr-4 as compared with Gr-2(Fig2, Table 1).

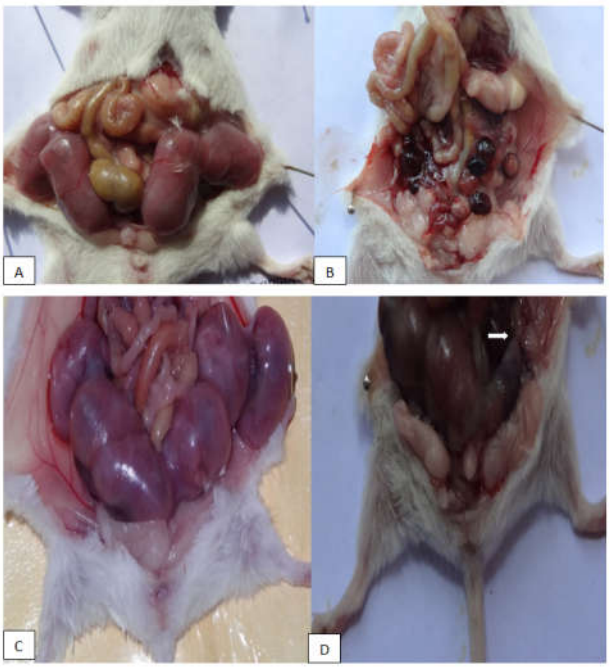

Fig 2 Photographs of Ventral View of First & Second Experimental Mice on Laprotomy

The mean weight of fetuses of Gr-2 was 1.11809 gm and the mean crown rump length (CRL) 22.095mm while the mean weight of the corresponding control was 1.16250gm and the CRL 22.572mm respectively (Table2)

Table 1 Showing Pregnancy Outcomes of Both Experimental Groups

	Group-1	Group-2	Group-3	Group-4
Total number of mother mice	6	10	6	10
Implantation	36	64	41	66
Resorption	0	8	0	11
Live fetuses	36	56	41	55

Table 2 Showing Weight and Crown Rump Length of First Experimental Group

	Group	N	M	S. D	S. E
Weight	1	36	1.16250	.114875	.019146
	2	56	1.11809	.154240	.020611
CRL	1	36	22.572	1.3860	.2310
	2	56	22.095	1.5901	.2125

N=number of fetuses
M=mean
SD=standard deviation
SE=standard error of mean

The mean weight of Gr-4 fetuses was 1.02113 gm as compared to 1.14834 gm in its corresponding control. The mean CRL of the foetuses of this group was 21.835 mm as compared to 22.388 mm of its corresponding control (Gr-3). A significant decrease in weight ($p < .004$) and CRL ($p < .010$) was observed only in late treated group

On gross examination of foetuses of early treated group revealed, external haemmorhagic spot at periauricular area in two cases and, periorbital area in one case. The other gross abnormalities like shortening of limb was seen in one case and ligated mark proximal to the ankle in one case in the same group. No such abnormality was observed in corresponding control group. (Fig-3, 4).

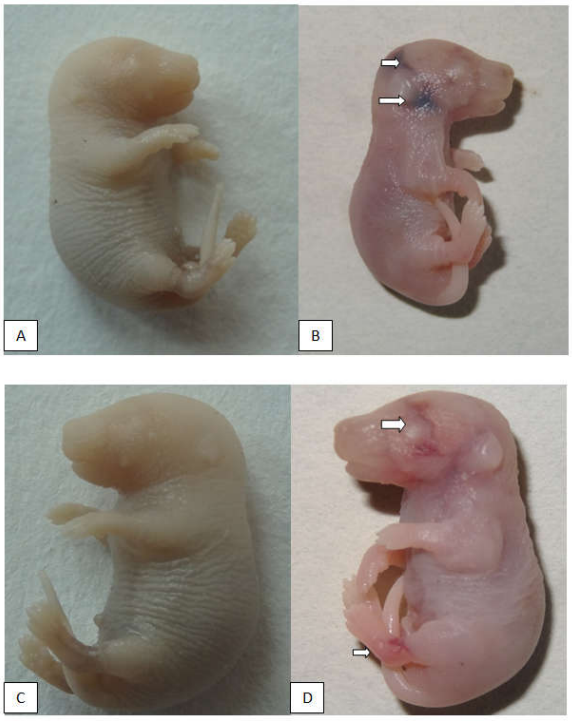

Fig 3 Photographs of Lateral View of First Experimental Group

A and C: Showing normal appearance of the control fetus. **B:** Showing preauricular and preoccipetal haemorrhagic area with shortening of forelimbs. **D:** Showing periorbital haemorr-hagic area, and ligated mark proximal to ankle. (→)

The foetuses of late treated group, showed growth retardation, generalized oedematous appearance and preauricular haemorrhage, haemorrhagic patch was observed on the lower left abdomoinal wall with the shrinkage of placenta in one case, while one fetus of this group also showed haemmorhagic spot on the left lower jaw (Fig-4,5).

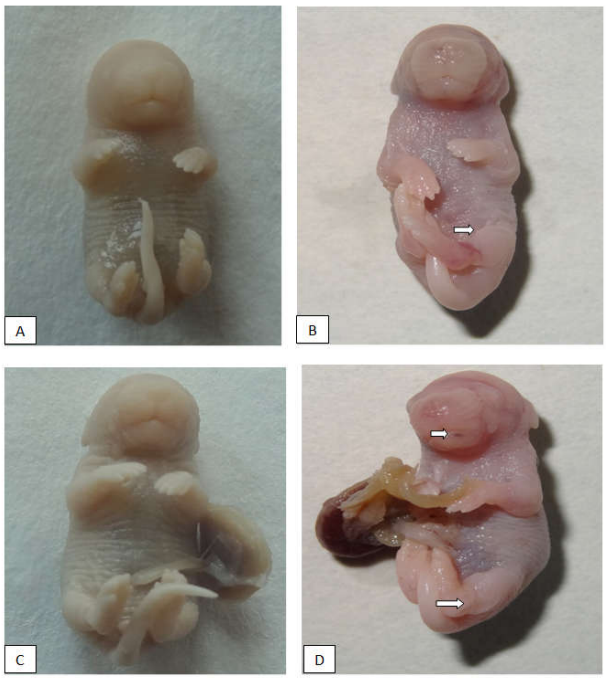

Fig 4 Photograph of Venral View of Frist & Second Experimental Group

A and C: Showing normal appearance of control fetus. **B:** Showing shortening of left forelimb and ligated mark in left hind limb. (→) **D**: Showing growth retardation with haemmorhagic spot on left lower jaw (→) and shortening of limbs

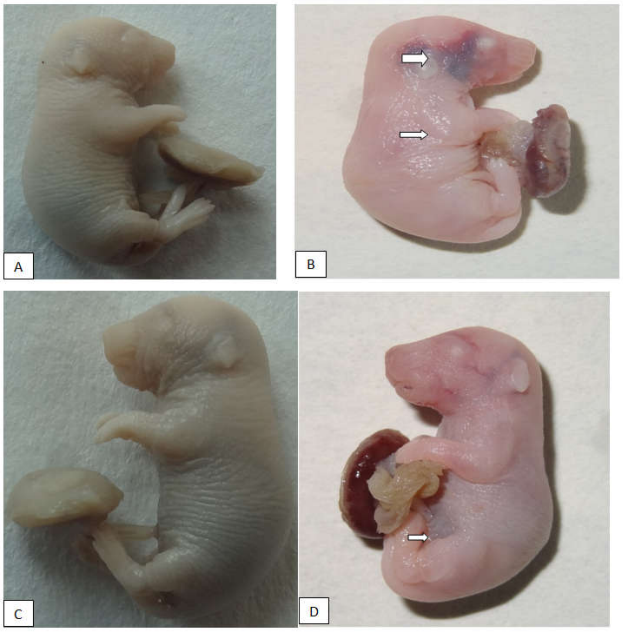

Fig 5 Photographs of Lateral View of Second Experimental Group

A and C: Showing normal appearance of control fetus. **B**: Showing periorbital and auricular haemorrhagic areas, shortening of limbs and shrinkage of placenta **D:** Showing growth retardation, generalized edematous appearance, preauricular haemorrhage and haemorrhagic patch on lower left abdominal wall (→).

Table 3 Showing Weight and Crown Rump Length of Second Experimental Group

	Group	N	M	S. D	S. E
Weight	3	41	1.14834	.263280	.041117
	4	55	1.02113	.152827	.020607
CRL	3	41	22.388	1.0068	.1572
	4	55	21.835	1.0350	.1396

DISCUSSION

LTG is a phenyl triazine derivative, initially developed as an antifolate agent. Lamotrigine crosses the placenta easily and rapidly, therefore, the maternal treatment leads to a considerable fetal exposure [3,11,12,13]

R. Padmanabhan, *et al* [2003] in their study found that the administration of LTG in multiple low doses resulted in a better maternal survival and increased incidence of embryonic resorption.In the present study resorption was observed in both the treated groups.

Fetuses of the high dose LTG group exhibited significant intrauterine growth retardation and the fetal body weight was also found to decrease proportionately with increasing doses of LTG. [8,14,15].

The present study showed significant growth retardation in the mice fetuses when LTG was given to the mother in late phase of gestation i.e.9th day of gestation. However the results were not stastically significant in the early treated group.

Sah N *et al* (2013) in their study, found exposed rat fetus with well defined, dark brown swelling over the parieto-occipital region and the cervicothoracic junction[14]. Hemorrhages of various sizes were found over the body; especially over the cervicothoracic junction by Mohanty C *et. al* (2011)[16] In the present study external haemorrhage was found in both the treated group in the preauricular, preorbital, preoccipetal area and also on the trunk.

Late gestation period is particularly succeptible for induction of neural tube defect and host of other malformations in mouse [17,18,19,20]. At this stage the mouse embryo develops primitive streak, notochord, early somites, gut, neural tube, neural crest, branchial arches, heart etc[21].R. Padmanabhan, *et al*(2003) observed, varying degrees of caudal regression, malrotation and meromelic anomalies of hindlimbs including classical mermaid syndrome. In the present study, malformations of the forelimbs and hindlimb in both the experimental groups were observed in the form of malrotation, shortening, haemmorhage and constriction rings.

CONCLUSION

The present study revealed LTG to be teratogenic in developing Fetus of Swiss Albino Mice. Resorption and growth retardation was observed in both the treated groups however, growth retardation was statistically significant only in late treated group.

In gross examination external haemorrhage and malformations of both the limbs were found in both the treated groups when compared to their corresponding controls.

Therefore LTG should not be regarded totally safe during pregnanc and should be used cautiously and under proper guidelines laid down by concerned authorities.

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