# Histopathological Changes in Developing Mice Brain Treated with Lamotrigine - An Experimental Teratological Study

**Shweta Singh[1], S.K. Pandey[2]**


## ABSTRACT

**Introduction:** The use of Lamotrigine, an antiepileptic drug, also used as a neuromodulator during pregnancy represents an inherent dilemma for clinicians and expectant mothers who must balance the risks associated with untreated disorders with the potential for occurrence of major congenital malformations. The risk for malformations with newer antiepileptic drugs, including lamotrigine, is less well characterized. The present study has been focused to find out the histopathological changes on the growing swiss albino mice brain after administering the drug during organogenesis (late) period of gestation.

**Material and methods:** In the present study the pregnant mice were divided into two experimental groups i.e. Gr1(treated with Normal Saline) and Gr2(treated on day 9 with LTG). LTG was administered at a dose of 150mg/kg body weight intraperitoneally, whereas the control group received normal saline of same volume by the same route. The pregnant mice were sacrificed with an overdose of ether on day 19$^{th}$ of gestation and the viable foetuses were collected. The foetuses were preserved in 10% formalin solution for 2-3 days. After fixation the brain was dissected out and processed for paraffin embedding followed by Haematoxylin and Eosin staining for histopathological study and finally the photomicrograpgy was done.

**Results:** The histopathological effects on the developing cerebral cortex revealed the destruction of the cortical layers , loss of normal architecture of sobcortical zone with oedematous changes in treated group.The ventricles were dilated with degeneration of their ependymal lining in treated group as compared to the control group.

**Conclusion:** Lamotrigine has shown various noxious effects on developing brain during organogenesis period of gestation. Therefore this antiepileptic drug should not be regarded as totally safe drug during pregnancy.


**Keywords:** Histopathological Changes, Developing Mice Brain, Lamotrigine

## INTRODUCTION

The use of drugs in pregnancy is a double edged sword. The drug itself may have harmful effects on the developing embryo and if left untreated the disease itself may be debilitating.[1] LTG crosses the placenta easily and rapidly, therefore, the maternal treatment leads to a considerable fetal exposure.[2] Not many studies have been undertaken on the microscopic effects of LTG on mice brain. There are few and inconclusive reports on the teratogenicity induced by lamotrigine. The present study has been undertaken to find out the microscopic changes on the growing brain of swiss albino mice embryo after administering the drug in late phase of gestation.(day-9)

## 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. Sixteen female Swiss albino mice of an average weight of 20-25 gm and about six weeks of age were used in this study. Animals were housed individually in plastic cages in animal house on a light dark cycle of 12:12 hours. The temperature was maintained at 20-25 degree centigrade with 65% relative humidity. Throughout the study animals were fed on pelleted diet obtained from local PashuAhar Kendra and tap water provided ad libitum.

The animals were divided into two groups Control Group containing six mice (Gr-1) and Treated Group containing ten mice (Gr-2). 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 pregnancy and was considered as day 0 of gestation. The pregnant mice were weighed and kept individually in separate cages. Treated mice belonging to Gr-2 received 0.4ml of stock solution intraperitoneally on day 9 whereas control mice (Gr-1) were injected with equal volume of normal saline intraperitoneally on the same day. Based on literature the dose was estimated to be 150mg per kg body weight.[3] The stock solution was made by dissolving 50mg strength of lamotrigine tablet (Lamez OD 50) in 5ml normal saline. The drug was procured from Intas Pharmaceuticals, Selaqui, Dehradun, India. The stock solution of LTG was prepared under sterile condition by pounding the tablet to powder and dissolving it in 5ml normal saline. Approximately 0.4 ml of stock solution contained 4mg drug. Treated mice received the drug intraperitoneally with the help of tuberculin syringe, whereas the control group received equal volume of normal saline intraperitoneally.

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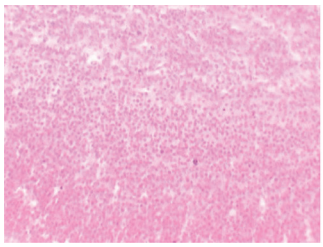
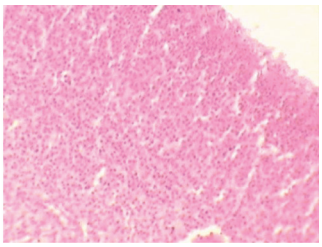
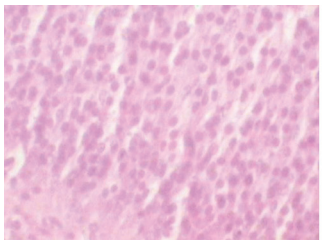


*Gr-1: Showing normal appearance of cerebral cortex.

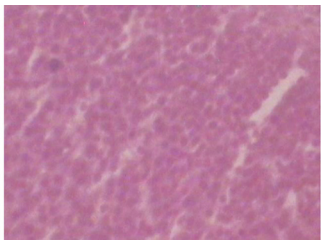


*Gr-2: Showing loss of the normal architecture of layers of cerebral cortex with edematous spaces in intercellular region

**Figure-1:** Microscopic structure of growing brain (cortex). 400X H&E

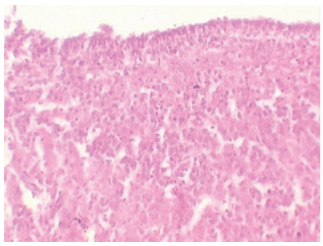


ǂ Gr-1: Showing normal architecture of cerebral cortex

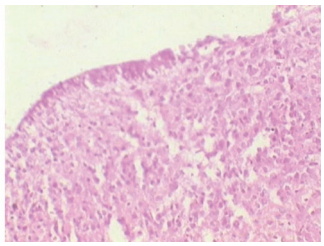


ǂ Gr-2: Showing pyknotic changes and destruction of neuronal cells.

**Figure-2:** Microscopic structure of growing brain (cortex) 1000X H&E

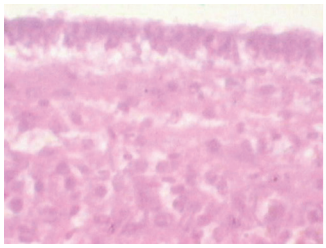


ǂ Gr-1: Showing normal appearance of control ventricle

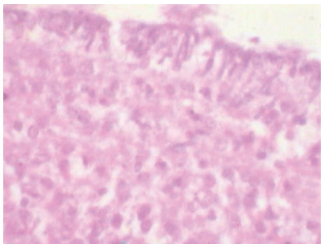


ǂ Gr-2: Showing destruction of the ependymal lining of the ventricle along with degenerative changes and edematous appearance in periventricular area.

**Figure-3:** Microscopic structure of growing brain (ventricle). 400X H&E



ɠ Gr-1: Showing normal appearance



ɠ Gr-2: Showing destruction of the ependymal lining of the ventricle with pyknotic changes in the cells of periventricular area.

**Figure-4:** Microscopic structure of growing brain (ventricle) 1000X H&E

The pregnant mice 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. The fetuses were preserved in 10% formalin solution in a jar for fixation. After fixation the brain was dissected out and processed for preparation of paraffin block and for histological examination. Haematoxylin and Eosin stained sections were examined under light microscope. The photomicrographs of the treated group were compared with control group. The photomicrographs for histological study were taken with the help of digital microscope (MOTIC 2000.1.13).

Observations and Results

The microscopic findings in the cerebral cortex of the treated group showed loss of normal architecture of layers of cerebral cortex with edematous spaces in the intercellular region (Fig-1). In oil immersion, the cerebral cortex of treated group showed pyknotic changes and destruction of neuronal cells, when compared with their control group (Fig-2). The ventricle showed destruction of the ependymal lining and edematous appearance in periventricular area (Fig-3). In oil immersion, the destruction of ependymal lining of the ventricle was well appreciated with pyknotic changes when compared with the control group. ( Fig.4)

## 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.[2] Late gestation period is particularly susceptible for induction of neural tube defects and a host of other malformations in mouse.[3] At this stage the mouse embryo develops primitive streak, notochord, early somites, gut, neural tube, neural crest, two branchial arches, heart, etc.[4] In Reproductive toxicity studies, the test substance is commonly administered throughout the period of organogenesis. This procedure may have the advantage of covering the entire susceptible period, but the disadvantage is that continuous dosing might activate maternal adaptive mechanisms, mask certain embryonic responses that are developmental stage specific and produce 'misleading results' .Rodents differ significantly from humans in their metabolism and ability to handle drugs and for this reason teratological studies on anti epileptic drug (AED) most commonly employ doses several times higher than recommended human doses.[5]

In previous studies similar to the present study, increased size of lateral ventricles and increased sub cortical density, volume density were observed in histological preparations from the cortex, subcortex, ependyma, and lateral ventricles.[6] The ventricles of the experimental pups were reported to be larger than that of the control pups and the plexiform layers in the experimental rat pups were relatively less clearly defined.[7]

In the present study the microscopic feature in the cerebral cortex of the treated mice showed loss of normal architecture of layers of cerebral cortex with edematous spaces in the

intercellular region, pyknotic changes and destruction of neuronal cells in the periventricular area.

Although brain growth varies among mammals, comparisons of brain development between species are possible.[8,9] Brain growth in all species follows a sigmoid trajectory when its weight is plotted against age. The period of rapid growth over a short period of time is known as brain growth spurt. The timing of brain growth spurt is different in relation to birth in different species and has to be taken into account when extrapolating results obtained in one species to another.[10] Neurotoxic effects of AEDs were systematically studied in infant rodents . It was determined that most AEDs cause apoptotic neurodegeneration in the developing rat brain at doses and plasma concentrations relevant for anticonvulsant treatment.[11] Lamotrigine at low doses was devoid of neurotoxic effects in infant rats. The results in the present study are in agreement with most of the findings of previous workers

## CONCLUSION

This study was undertaken to find out microscopic changes on the developing mice brain, in detail. A single dose of the drug was administered intraperitoneally in late phase of gestation to pregnant Swiss albino mice .The microscopic feature in the brain showed disintegration of the normal cytoarchitecture of various cerebral cellular layers, the ventricle was dialated with destruction of ependymal lining cells. Therefore, LTG should not be regarded totally safe drug during pregnancy until its safety is established in a large scale randomised study with longterm follow-up.

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