

Original Research Article

Teratogenic Effects of Lamotrigine on Thoraco-abdominal Organs of Developing Chick Embryos

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Abstract: There are number of newly developed antiepileptic drugs are currently in use, among them Lamotrigine (LTG) is more common. It is extensively used to control the generalized seizures and myoclonic seizures; it has not been possible to predict the teratogenic effects of LTG. The current study was designed to find out the teratogenic effects of Lamotrigine on heart and liver of developing chick embryo. Experimental eggs were injected with single injection of 2mg/egg therapeutic dose and 4mg/egg double the therapeutic dose. Histological examination of the liver revealed dilated central veins with degenerated hepatocytes with disturbed cellular architecture. Longitudinal sections of the heart showed thickened ventricular musculature with narrower lumen and haemorrhagic spots over atrial musculature.

Keywords: Lamotrigine, Antiepileptic, Liver, Chick Embryo

INTRODUCTION:

Lamotrigine (LTG) chemically 6-(2, 3-Dichloro phenyl) - 1, 2, 4-triazine 3, 5-diamine C₉H₇Cl₂N₅ is derived from pyrimethamine, which is chemically different from commonly available AEDS [1]. Lamotrigine is a known anticonvulsant drug used in the treatment of epilepsy and bipolar disorder. For epilepsy, it is used to treat focal seizures associated with Lennox-Gastaut syndrome, like many other anticonvulsant medications, lamotrigine also act as an effective mood stabiliser [2]. Lamotrigine acts as member of the sodium channel blocking class of antiepileptic drugs; it is a triazine derivate that inhibits voltage sensitive sodium channels, leading to stabilization of neuronal membranes. Lamotrigine is a weak inhibitor of dihydro folate reductase, but whether this effect is sufficient to contribute to a mechanism of action or increases risk to the fetus during pregnancy is not known. In clinical trials women were more likely than men to have side-effects. This is the opposite of most other anticonvulsants [3]. There are evidence showing interactions between lamotrigine and female hormones, which can be of particular concern for women on estrogen-containing hormonal contraceptives.

Almost all major antiepileptic drugs can cause hepatotoxicity including LTG. Liver is vulnerable to drug induced toxicity mainly because of its role as a primary organ of drug elimination and its subsequent exposure to potential toxins [4]. When clinicians prescribe LTG as a single therapy or polytherapy, must be alert to the possibility of serious hepatic reactions particularly in case of maximum dose with enzyme inducing agents, it may contribute to the overall risk of hepatic dysfunction. Chronic prenatal exposure to LTG during critical period of somatic and neural development may increase the risk for cleft lip and palate malformation in newborns, but the review studies have found that overall rates of congenital malformations in infants exposed to lamotrigine in utero are relatively low (1-4%)[5]. Some of the antiepileptic drugs are known teratogens like phenytoin, sodium valproate which can induce potential malformation with cardiac malformations too. The basic action of LTG is similar to that of other antiepileptic drugs. Therefore the present animal experiment was designed to investigate the LTG single dose injection in to developing chick embryo can induce any cardiac anomalies and hepatocellular damage in liver tissue.

MATERIALS & METHODS:

The fresh healthy fertile eggs of white leghorn chicken were obtained from government registered poultry form. The study was conducted in the department of anatomy, J N Medical College, Sawangi, Wardha. Prior approval was taken from the Institutional Ethical Committee, which has duly authorized by CPCSEA for animal experiments.

A total number of sixty eggs were incubated at $37 \pm 10C$ (60-80% humidity). The eggs with thin shell, cracked, unfertile, double yolks, decomposed, and excessively large or small eggs were excluded from the study. Eggs were candled on 3rd day of incubation in the order to discard the unfertilized eggs prior to exposure and outline was made to know the exact location of air cell. Eggs were divided into three groups 1, 2 and 3 each group having 20 eggs. 20 eggs of group 1 taken as control, remaining 40 eggs in group 2 and 3 are experimental eggs was injected with drug solution lamotrigine on 3rd day of incubation. The broad end of the egg was selected for the injection site. A hole was drilled in egg shell in the centre of the outlined area over the air cell with sterile 22-gauge needle. Group 1 control eggs were injected with 10 μ l of distilled water, experimental group 2 injected with therapeutic doses of lamotrigine 2mg/egg, experimental group 3 were treated with higher doses of lamotrigine 4mg/egg single injection with a tuberculin syringe.

The embryos were collected on 19th day of incubation by breaking the egg shell and were observed for gross malformations of heart and liver. The viscera collected were fixed in 10% buffered formalin for further processing for histological observations after staining with H & E.

RESULTS:

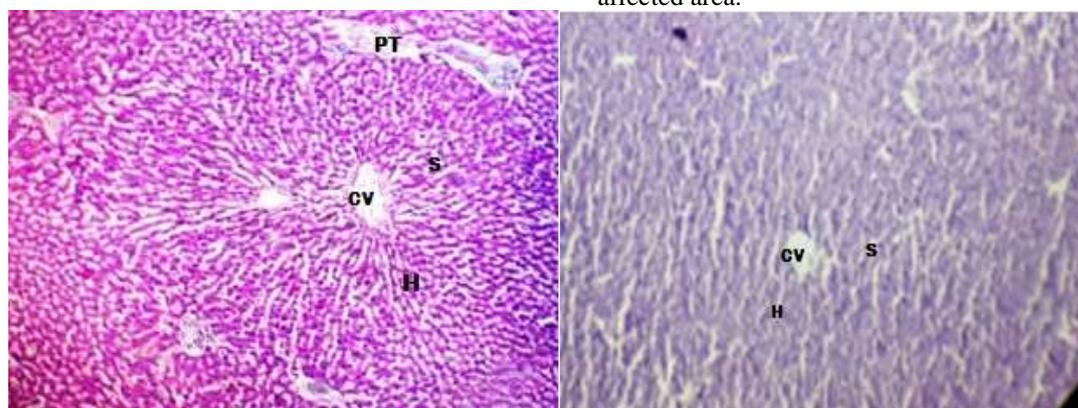


Fig 1: Histological Sections of Liver of Group 1 & Group 2 chick embryo showing normal architecture with Central vein (CV), Small Sinusoids (S), Hepatocytes (H).

On gross examination of higher dose LTG injected (group 3) chick embryos only 60% embryos were found to be viable, LTG injected group 2 embryos shows 70% viability, whereas in control only 10% embryos were dead. No visceroptosis seen in control and treated group 2 chick embryos. LTG treated group 3 viable embryos, total visceral defects were 42.8% out of which, visceroptosis 14%, thin anterior abdominal wall 21%, ectopia cardis 7% were found.

The liver parenchyma in control (group 1) and lower doses (group 2) of LTG groups were showed no abnormalities. On histological observation of liver sections of control eggs reveals normal hepatic architecture with central vein, radiating hepatic cords and haemopoiesis (Fig 1). On light microscopic examination of higher dose LTG treated liver displayed granular degeneration of hepatic cells with distorted cytoarchitectural pattern. Central vein was markedly dilated with discontinuous lining epithelium. The cells closer to dilated central vein were more degenerated than the peripheral cells. Enlarged hepatocytes with pyknosis of nuclei and scattered coagulative necrosis seen in some of the sections (Fig 2.A & 2.B).

On morphological observation of heart, the architecture of heart in control (Fig 3.A) and group 2 (Fig 3.B) embryos display conventional microscopic features expect some muscular wall thickness and differing intensity of haemorrhagic spots in group 2. The haemorrhagic spots (Fig 4.B) were observed over both the atria while dissecting the heart of group3 embryos. Higher doses LTG treated hearts were elongated with thickened ventricular musculature (Fig 4.A) as compared to that of group 2 embryos. Some of the heart specimens show haemorrhagic spots on thickened ventricular wall resulting into myocyte necrosis (Fig 4.C) subsequently degeneration of the affected area.

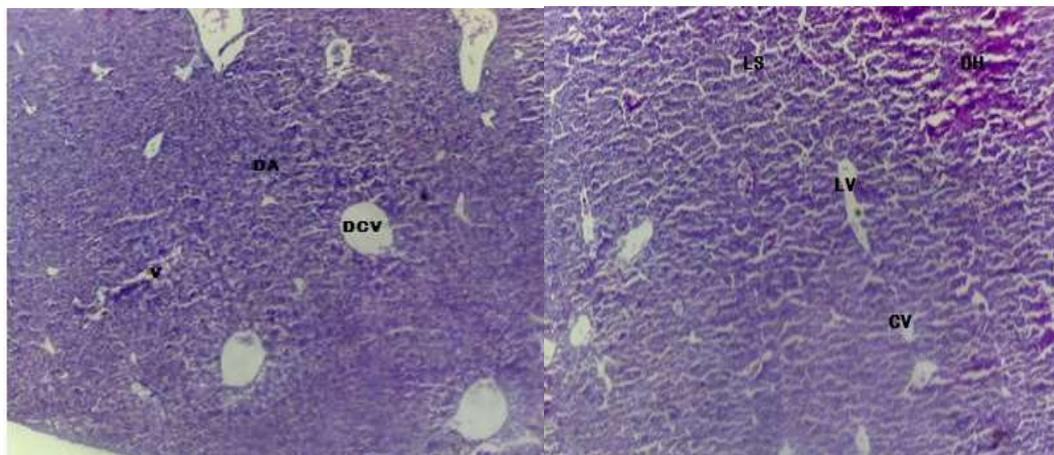


Fig 2: Histological Section of Liver of Group - 3 (higher dose of LTG treated) chick embryos Showing Dilated central vein (DCV), Large Vacuoles (LV), enlarged sinusoids (LS) with Degenerated hepatocytes (DH).

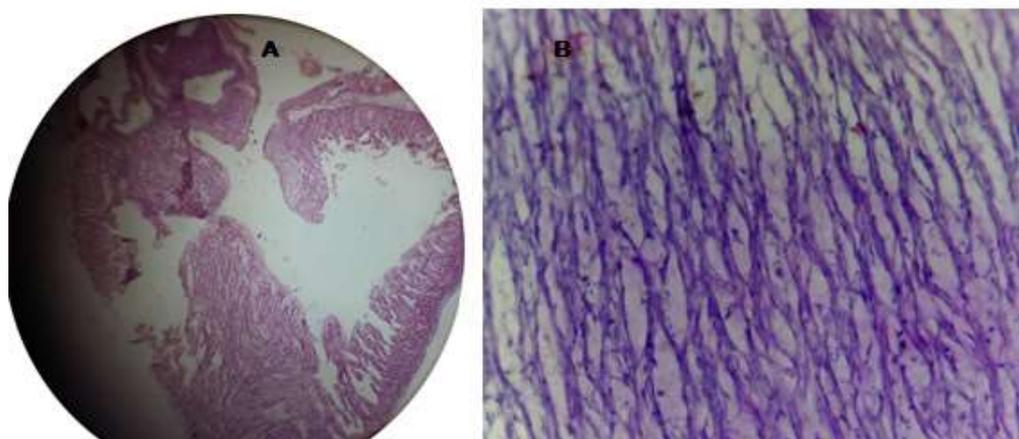


Fig 3: Paraffin sections of Heart A. Control heart H&E 100X, B. Treated heart (group 2) H &E 400X showing normal architecture of heart, cardiac muscle fibre with branching, intercalated discs and central nucleus.

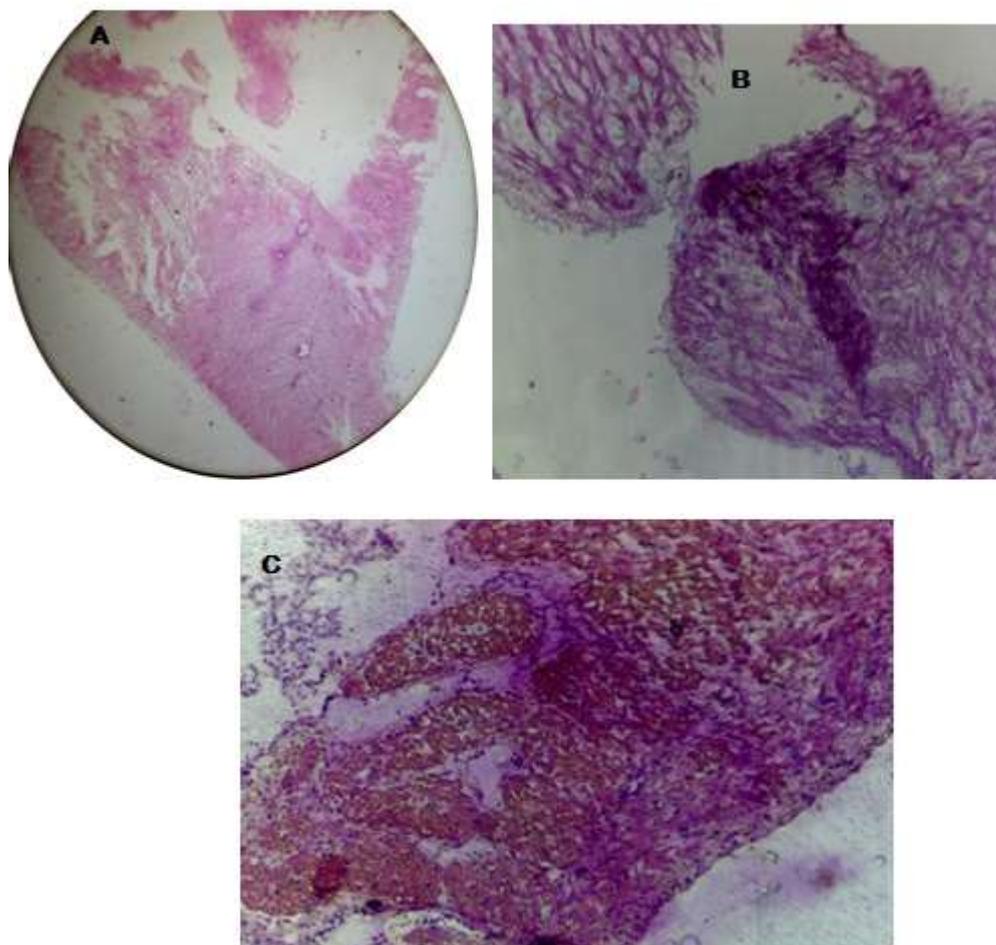


Fig 4: Paraffin sections of treated Heart (group 3) A. Thick ventricular musculature of hypertrophied heart H&E 100X, B. Haemorrhagic spots over atrial musculature H &E 400X, C. Degenerated cardiac myocytes H&E 100X.

DISCUSSION:

Pregnant women with epilepsy are greatest problem in all over the world. Selection of ideal antiepileptic drug is very crucial to control the seizures in pregnant women. Lamotrigine is a new antiepileptic drug developed to control convulsions, bipolar disorder and depression. Several studies are done to understand the teratogenic effects of older antiepileptic drugs, but paucity studies are available on lamotrigine. Like several other AED's, lamotrigine also may influence on organogenesis stage of development where organs undergo sequence of cell division, migration differentiation and cell death [6].

The embryonic differentiation in chick embryos begins almost simultaneously with incubation; the undifferentiated stage with all major organogenesis begins by 48 hr of incubation. Hence any drug injected

during first few hours of incubation will represents derangement of embryogenesis and organogenesis [7].

In the present study hepatic cell degeneration and hypertrophy of cardiac muscle have reported. Similar kind of results reported by M Singh [8] with single injection of Dilantin on chick embryos. Our study indicates that exposure of maximum therapeutic dose of LTG during organogenesis results hepatotoxic changes in liver and hypertrophic changes in hearts with myocyte degeneration to some extent. The exposure of therapeutic dose of LTG did not have any effect on body growth rate: on the other hand higher doses of LTG retarded the body growth, architectural change at tissue level. Hepatocyte degeneration with infiltration of inflammatory cells in portal area, dilated central vein, congestion, extensive vacuolation of the cytoplasm was reported by Tureci [9] in their experiment on chick embryo treated with leviteracetam and valproic acid.

Singh M [10], have been reported that 68% thoraco-abdominal malformations, out of which ectopiacardis 10%, visceroptosis 27%. Histological examination of liver sections revealed granular degeneration of hepatic cells with loss of cytoarchitectural pattern. The results are in conformity with current experimental study. In another study, cardiovascular defects in addition to absence of kidneys and skeletal deformities have been reported by Collins *et al.* Literature on LTG therapy it may be concluded that usage of lamotrigine as a safe and effective medication gives better responses at therapeutic dose but use in pregnant women to control seizures with increasing dose is may not recommended and the risk has to evaluated by the clinician before advising to the patient [11, 12].

It has been summarized that single injection of lamotrigine beyond the therapeutic dose during critical stage of embryogenesis produces hepatic damage with deleterious effects, increases myocyte damage with haemorrhagic spots on atrial musculature. But the malformations reported with the administration of lamotrigine are less and no major gross malformations observed.

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