

Histopathological study of developmental toxicity of Carbamazepine in the mice

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ABSTRACT: Developmental toxicity is a structural or functional alteration, which interferes with normal growth, differentiation, and development. Carbamazepine is one of the most widely used antiepileptic drugs among women of childbearing age. Investigation of carbamazepine developmental toxicity for vital organs. The healthy pregnant females were divided into three groups. The (control) group received distilled water/day orally. Second and third group received 52.5, 65 mg/day of carbamazepine respectively by gastric gavage. Fetuses were delivered by hysterectomy on the 18th day of gestation. Each fetus was assessed for histopathological changes of liver, kidney and heart. Liver mice showed congestion, inflammation with areas of hydropic degeneration in second group and marked complete distortion of the hepatic cells and large areas of degeneration in third group. Kidney mice showed atrophy of glomeruli, degeneration of renal tubules in second group and complete distortion of glomeruli and renal tubules in third group. Cardiac mice showed loss of normal architecture, degeneration of the myocardial fibers in second group and marked widespread fragmentation and degeneration of myocardial fibers in third group. Histopathological changes of liver, kidney and heart due to Carbamazepine developmental toxicity represents as congenital anomalies of organs depending on overdose degree of carbamazepine.

Keywords: Carbamazepine, development, toxicity.

INTRODUCTION

Developmental toxicity is a structural or functional alteration, reversible or irreversible, which interferes with normal growth, differentiation and development. It is caused by environmental insult such as drugs (Rogers and Kavlock 2001). Carbamazepine is a tricyclic anticonvulsant drug and one of the most widely used antiepileptic drugs in the clinic. It is highly effective for cryptogenic, symptomatic partial seizures, generalized tonic-clonic seizures, trigeminal neuralgia and mood disorders. It is a neutral, lipid soluble compound that can easily pass the blood brain barrier and other membranes in the body (Hiremath et al., 2005). Carbamazepine is one of the most commonly used antiepileptic drugs in Europe among women of childbearing age with epilepsy. They need to make decisions about the safest drug to use well before pregnancy (Morrow et al., 2006).

Few drugs are proven teratogens. Older anticonvulsants are known teratogens such as carbamazepine. It is a developmental toxicant and exposure to it has a potential negative effect on the developing baby. It can cause harmful effects during any stage of development and leads to fetal death during the first two weeks post-conception. Teratogens induce major morphological changes during embryonic period because it is the stage of rapid cellular division and differentiation (organogenesis), this effect has been laid to genetic factors, associated with the pathology itself (Macara, 2000). The fetal phase is the period when growth and functional maturation of organs and systems already formed occurs. Teratogen exposure in this period will affect fetal growth, the size of a specific organ, or the function of the organ, rather than cause gross structural anomalies. The term fetal toxicity is commonly used to describe such an effect (Briggs, et al., 1998).

Carbamazepine is one of antiepileptic drugs which induces hypersensitivity syndrome. Due to broad spectrum side effects of carbamazepine, differentiation from hypersensitivity syndrome may be difficult, but it has been linked to this syndrome affecting the liver, lungs and kidneys in severe cases (Schlienger and Shear, 1998).

The aim of this study to investigate developmental toxicity of carbamazepine for microstructure of vital organs such as heart, liver and kidney.

MATERIALS AND METHODS

The healthy pregnant females 6- 8 weeks of age, weighing (25-35 g) were subjected for the study. The mating and pregnancy success was approved by appearance of vaginal plug which was considered a day zero of pregnancy. They were caged separately and allocated randomly into equal three groups (each n=20). The first was the control group; each pregnant mouse received 1ml of distilled water/day orally throughout gestational period. The second and third group received 52.5, 65 mg/day of carbamazepine respectively, dissolved in distilled water by gastric gavage throughout gestational period (Bauer et al., 2002 and Williams, et al., 2001).

Carbamazepine drug was in the tablet form and obtained from Novartis Farma S.P.A., Torre Annunziata, Italy for Novartis Pharma AG Basle, Switzerland. One tablet contains 400 mg of active ingredient, dispersed in 10 ml distilled water. All animals were killed by cervical dislocation on the 18th day of gestation and fetuses were delivered by hysterectomy. Each fetus was euthanized by decapitation. Chest and abdominal viscera were exposed by mid line incision. Heart, kidney and liver were removed. The fetal tissues were fixed in 10% buffered formalin for 72 hours, later they processed in automatic tissue processor and embedded in paraffin. Labeled blocks kept for fifteen minutes in freezer before cutting. Five micron thin sections obtained using a rotary microtome (Leica RM 2125), floated in warm water at 45°C and transferred to precleaned albumenized glass slides. Haematoxylin and eosin (H & E) used for general histology according to [9]. The sections studied under a light microscope (Leica DM 1000).

Ethical considerations

The most appropriate animal species was chosen for this research. Promotion of a high standard of care and animal well-being at all times was done. Appropriate sample size was calculated by using the fewest number of animals to obtain statistically valid results. Painful procedures were performed with ether inhalation to avoid distress and pain. Our standards of animal care and administration met those required by applicable international laws and regulations.

RESULTS

Histopathological findings

Liver

Light microscope examination of mice liver in the first day of delivery of first group (control group), showed normal structure of hepatic lobular pattern (Fig.1 A&B). But mice liver in the first day of delivery of second group, showed congestion, dilatation of central vein and blood sinusoids with areas of hydropic degeneration. Hepatocytes showed inflammatory cell infiltration with prominent fragmented pyknotic nuclei and mild vacuolated cytoplasm (Fig.2 A&B). Liver section of third group mice showed marked complete distortion of the hepatic cell cords, vacuolated hepatocytes with loss of intercellular demarcation in the hepatic lobules and large areas of degeneration. There were marked blood sinusoids dilatation and massive fragmented pyknotic nucleus in comparison to the second group (Fig.3 A&B).

Kidney

Light microscope examination of mice kidney in the first day of delivery of first group (control group), showed normal renal structure of both cortex and medulla (Fig. 4 A&B). But mice kidney in the first day of delivery of second group, showed enlarged vascular glomeruli, tight filling the glomerular capsular space, atrophied other vascular glomeruli and degeneration of epithelial lining of most tubules (Fig. 5 A&B). Kidney section of third group mice showed marked complete distortion of glomeruli, tight filling the glomerular capsular space and complete distortion of lining epithelium cells of the tubules (Figs. 6 A&B).

Heart

Light microscope examination of mice heart in the first day of delivery of first group (control group), showed normal histological appearance of myocardium (Fig. 7). But mice heart in the first day of delivery of second group, showed loss of normal architecture with fragmentation and degeneration of the myocardial fibers, (Fig. 8). Cardiac section of third group mice showed loss of normal architecture with marked widespread fragmentation and degeneration of myocardial fibers (Fig. 9).

DISCUSSION

Most studies of carbamazepine concentrated on its neurodevelopmental toxicity and morphological changes in the human and animal. Data of these studies was limited for effect of carbamazepine on microstructure of vital organs such as liver, kidney and heart although there are many observed congenital anomalies related to these organs. So our research attempts to study effect of carbamazepine use during pregnancy on histological structures of vital organs. The current study indicated to congestion, inflammation and areas of hydropic degeneration of mice liver in the second group and this consistent with Davion et al., 1984, who confirmed development of carbamazepine hepatitis due to immune-allergic mechanism with increasing dose of carbamazepine. Mice liver of third group showed marked complete distortion of hepatic cell cords, with loss of intercellular demarcation in the hepatic lobules and large areas of degeneration and this consistent with Rodríguez et al., (1989). Bjornsson, (2008) Explained that hepatotoxicity of carbamazepine due to hypersensitivity syndrome or immunological response to a metabolic generated drug-protein complex and liver involvement ranges from mild to severe degree depending on the dose .

The result of this study referred to atrophy of renal glomeruli, degeneration of epithelial lining of renal tubules of second group, marked destruction of glomeruli and epithelial cells of renal tubules of third group. These results are consistent with Okada, (2005), who confirmed that atrophy of glomeruli and degeneration of renal tubules leads to retarded renal growth.

According to, Tatsunori (2010), concentrations of carbamazepine and its metabolites are much higher in the brain, lung, liver, and kidney than its concentration in the blood. This suggests that the drug is retained in organ tissues and then this fact explains toxic effect of carbamazepine in liver and kidney which represents as histopathological changes.

Our results indicated to degeneration of myocardial muscle fibers in second group and third group depending on dose of carbamazepine. These results explain the morphological changes of cardiac muscle due to carbamazepine use which represent as cardiac congenital anomalies such as ventricular septal defect, atrial septal defect and underdeveloped left heart according to Roguin (1995). Sipes (2011), reported that drug capacity disrupts embryogenesis and causes developmental defects depends on many factors including chemical properties, dose and time of exposure, genetic susceptibility, bioavailability, biotransformation and chemical interactions with biological systems. Embryonic susceptibility to drugs insult is determined by the sensitivity and specificity of drugs or their metabolites which interact with pathways during particular developmental stages. The potential for adverse outcomes is dependent on the complex cellular and molecular process governing morphogenesis, growth and differentiation and the higher response of developing embryo to local or systemic perturbations.

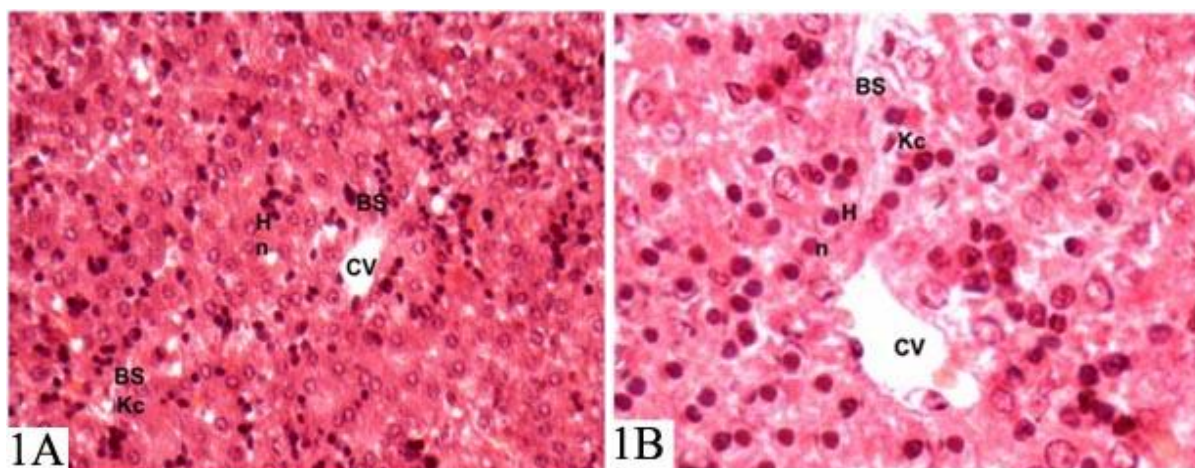


Figure 1.(A&B). A photo micrograph of liver tissue of mice in the first day of delivery of the first (control) group shows cell cords radiating from the central vein (CV), The hepatocytes (H) with defined cell lining and rounded nucleus (n) separated by blood sinusoids (BS) which containing Kupffer cells (Kc). 1A (H&E X 400) and 1B (H&E X 1000).

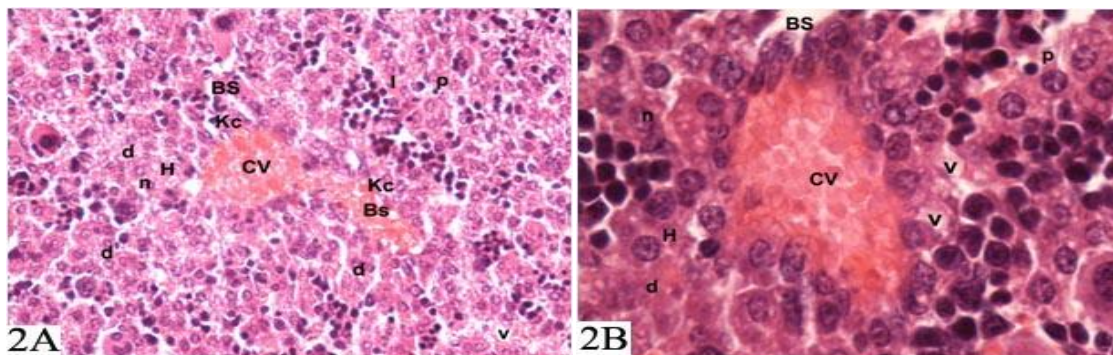


Figure 2. (A&B). A photo micrograph of liver tissue of mice in the first day of delivery of second group shows congestion, dilatation of the central vein (CV) and blood sinusoids (BS) containing many Kupffer cells (Kc). The hepatocytes (H) shows prominent fragmented nuclei (n) vacuoles (V) and pyknotic nuclei (P) with marked inflammatory cell infiltration (I) and areas of hydropic degeneration (d). 2A (H&E X 400) and 2B (H&E X 1000).

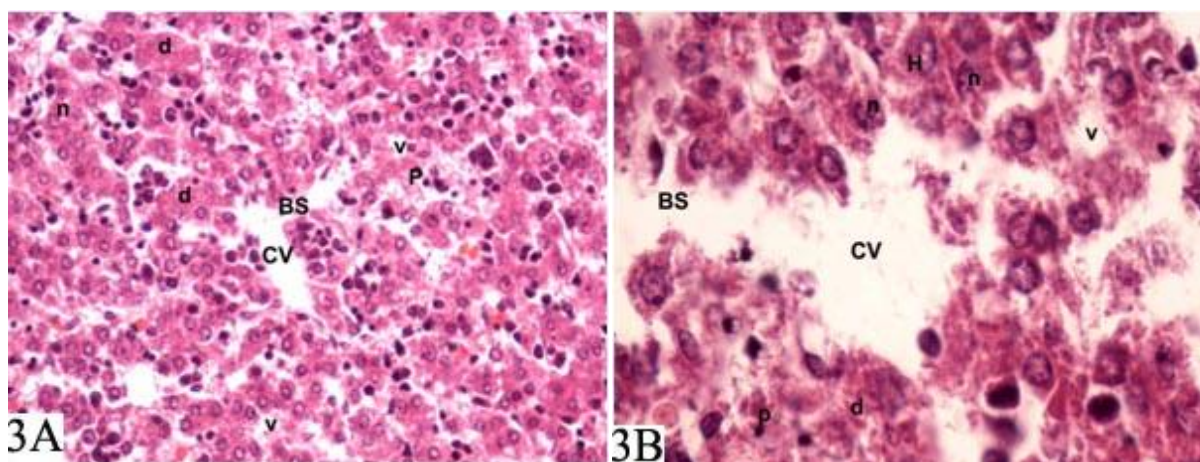


Figure 3. (A&B). A photomicrograph of liver tissue of mice in the first day of delivery of the third group shows widening central vein (CV) and blood sinusoids (BS), severe distortion and shrinkage of hepatocytes (H) with illdefined cell lining, small fragmented nucleus (n) and pyknotic nuclei (P), increase of number, size of vacuoles (v) and degenerated areas (d) . 3A (H&E X 400) and 3B (H&E X 1000).

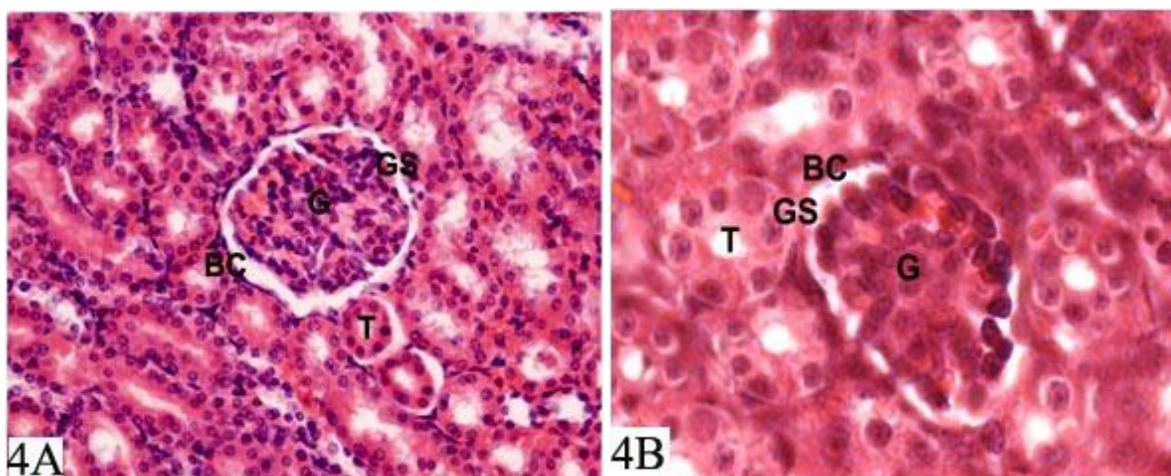


Figure 4. (A&B). A photomicrography of kidney tissue of mice in the first day of delivery of the first (control) group shows normal glomeruli (G), normal glomerular capsular space (GS), with flat epithelium lining the Bowman's capsule (BC) and normal cells in the lining epithelium of the tubules (T) . 4A (H&E X 400) and 4B (H&E X 1000).

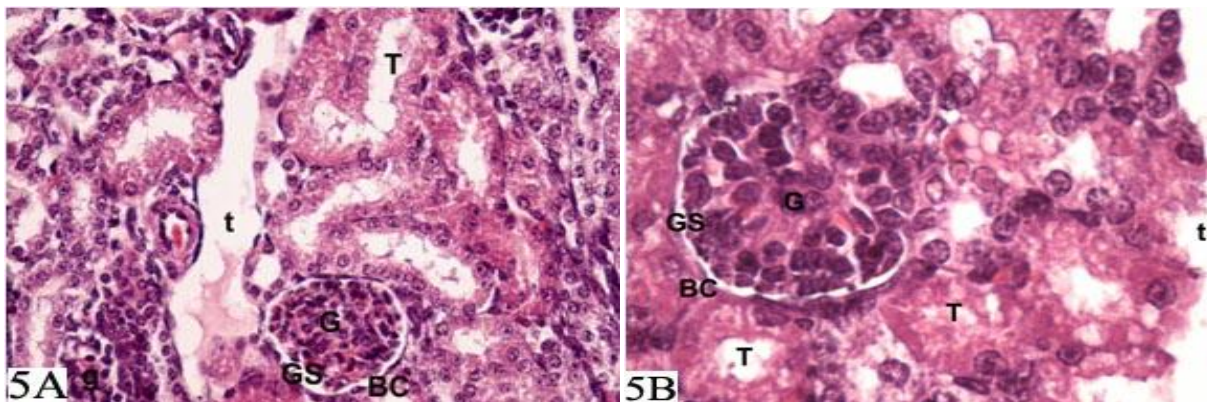


Figure 5. (A&B). A photomicrography of kidney tissue of mice in the first day of delivery of second group shows some enlarged vascular glomeruli (G), decrease of glomular spaces (GS), with flat epithelium lining of Bowman's capsule (BC), atrophied other glumeruli (g), degeneration of some epithelial lining of tubule (T) and complete destruction of epithelial cells of other tubules (t). 5A (H&E X 400) and 5B (H&E X 1000).

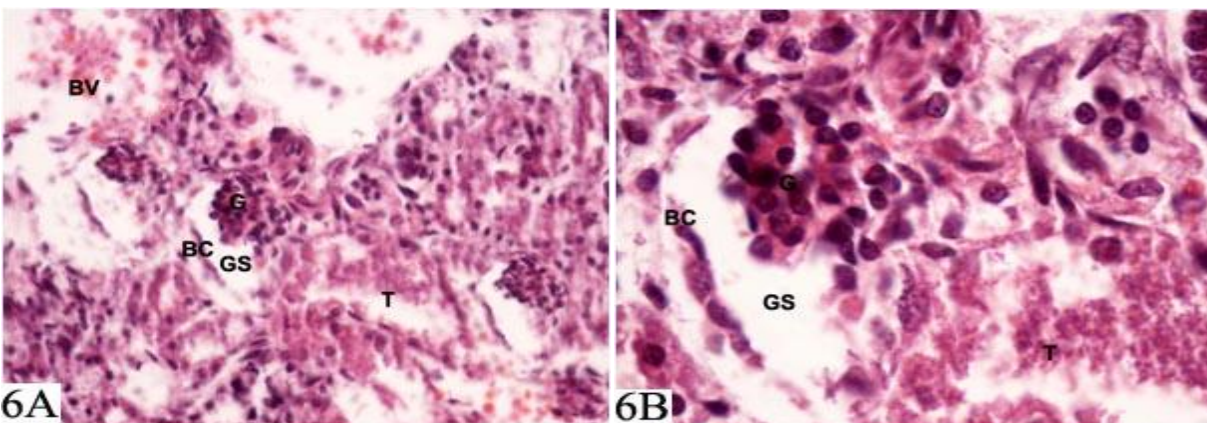


Figure 6. (A&B). A photomicrography of kidney tissue of mice in the first day of delivery of third group, shows small and shrunked vascular glomeruli (G), widening of the glomerular space (GS), with destruction of epithelial lining cells of the Bowman's capsule (BC) and epithelial lining cells of the tubules (T) with destruction of blood vessels (BV). 6A (H&E X 400) and 6B (H&E X 1000).

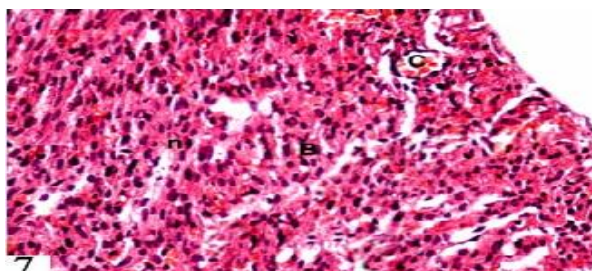


Figure 7. A photomicrography of mice myocardium in the control group shows branching and anastomosis cardiac muscle fibers (B) with acidophilic sarcoplasm and central elongated vesicular nuclei (n) with capillaries in between (C). (H&E X 400).

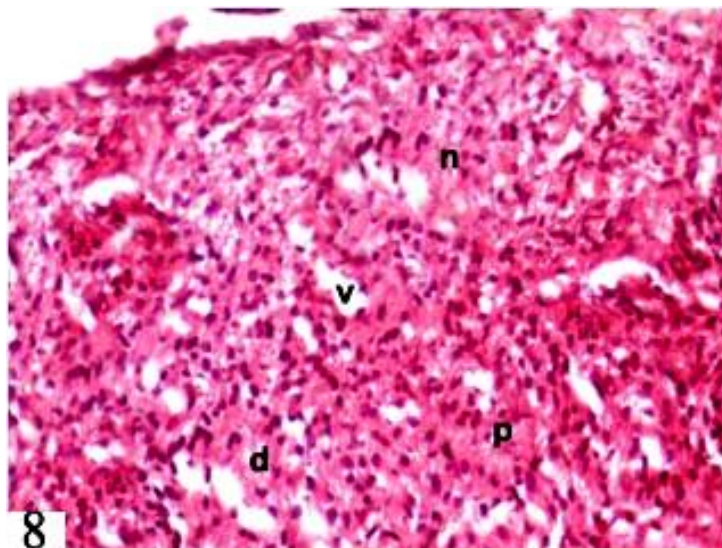


Figure 8. A photomicrography of mice myocardium in the second group shows loss of normal architecture, fragmentation and degeneration of the myocardial fibers (p), large size of vacuoles (v) with acidophilic sarcoplasm, degenerated area (d) with degenerated nuclei (n). (H&E X 400).

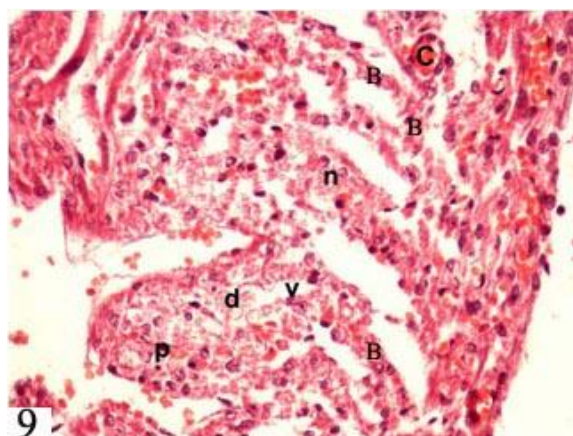


Figure 9. A photomicrography of mice myocardium in the third group shows loss of normal architecture and widespread fragmentation and degeneration of the myocardial fibers (B), huge number and size of vacuoles (v) with acidophilic sarcoplasm, degenerated nuclei (n) and pycknotic nuclei (p) and increase of number and size of degenerated areas (d). (H&E X 400).

CONCLUSION

Histopathological changes of liver, kidney and heart due to developmental toxicity of Carbamazepine represents as morphological changes and congenital anomalies of these organs depending on overdose degree of carbamazepine.

Recommendations

Carbamazepine use during pregnancy should be restricted as much as possible. It is recommended that further researches in human should be performed to verify our results.

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