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Teratogenic Effect of Levetiracetam Drug on the Development of the Kidney in Embryo Rat

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Abstract

The new antiepileptic drugs, levetiracetam (LEV), are used to treat tonic-clonic seizures and myoclonic seizures in adults and children. Thirty pregnant rats were enrolled, which were divided into two groups A control (10), B treated (20). Group (A) were given distilled water orally for 15 days of pregnancy period. The other group was subdivided into two subgroups B_1, B_2 (each with 10 rats), which were treated with LEV for 14 days for subgroup (B_1) and 15 days for the other subgroup (B_2). The drug (350 mg/kg/day) is administered orally. Based on our results there was damage in the kidney due to the toxicity of the drug. The histopathological effects are represented by damage of cortical glomeruli in which there were hemorrhage, shrinking, accumulation of glomerular cells, and enlargement of capsular space. Severe malformations in the kidney were observed when the drug is used constantly during the organogenesis stages of pregnancy.

Keywords: Embryo rat, kidney, levetiracetam drugs.

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1. Introduction

Epilepsy is chronic condition which is characterized by repeated provocation epileptic seizures [1]. Levetiracetam (LEV) is one of the newest antiepileptic drugs (AEDs), it is used as adjunctive therapy for myoclonic seizures of juvenile myoclonic epilepsy, and primary generalized tonic-clonic seizures, partial-onset seizures. LEV had pharmacokinetic advantage. Its plasma concentrations peak is approximately one hour after oral administration, which rapidly and completely absorbed after oral intake [2, 3]. The plasma half-life of oral LEV is 7±1 hours in adults [4]. It's eliminated that 66% of the administered drug is remained unchanged [5]. The renal clearance is 0.6 ml/min/kg and 0.96 ml/min/kg is the total body clearance. LEV metabolism is not dependent on the liver cytochrome P450 enzyme system [4,5]. The precise mode of action of LEV is unknown. However, LEV can bind to synaptic vesicle protein SV_2A which is thought to prevent nerve conduction across synapses [6,7]. The mechanism of secretion is glomerular filtration and follows partial tubular reabsorption [9]. LEV drug that given to the pregnant female appears a significant increase in clearance during pregnancy and a coloration fall of blood concentrations [8]. Its malformation risk is unknown; thus this study was conducted to assess the effect of the drug on the kidney of the albino rat embryo.

2. Material and Methods:

Animal Breeding: Healthy pregnant female Albino rats *(Rattus rattus*) strain weighted 250-300 g, and aged 10-12 weeks were used which was obtained from Al-Nahrain University / the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies Animal House. All animals were housed at 21±4 ºĊ. The rats were allowed adlibitum access to water and food throughout the gestation [9].

Experimental Design: thirty pregnant females were divided into two groups: (A) represents control group, (B) represents treated group.

Group (A): given distilled water orally.

Group (B): treated with LEV drug, which is subdivided into two sub-groups (**B_1&B_2**) each include 10 rats.

Treatment:

Syrup levetiracetam (100mg/ml) (UCB Pharma, Inc., Smyrna, Ga., USA) was used for the treatment. The 1st group was treated with the drug orally for 14 days of pregnancy period. The other subgroup was treated orally for 15 days of pregnancy period.

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Newborn Collection:

Pregnant Females were anesthetized by mixing solution (Xylazine10 mg / kg and Ketamine 10 mg / kg of body weight) Embryo extraction was made by abdominal incision at the median line from placental sacs by cervical dislocation of uterus, remove the outer membranes of embryos and then kidney was taken from the treated and the control group, then fixed in Bouins fluid for 12-24 hours and later washed several times with 70% ethyl alcohol and was kept until use [10,11]. Paraffin embedded sections were made by rotary microtome. Stained with periodic acid Schiff (PAS) and haematoxylin-Harris and eosin stain [12]. Photographs were taken by microscope imaging compound type MEIJI Canon camera and that in the developed embryos Laboratory in the Department of Biology/College of Education for Pure Science (Ibn Al-Haitham) University of Baghdad.

3. Results:

Histopathology of Kidney:

1. Day Fourteen of Gestation:

The kidney of embryo in control group showed differentiation of glomerulus, glomerular capsule and clear collecting tubules (Figure 1). In the treated kidney, there was a Hemorrhage of glomerular cells and collecting tubules, accumulation and shrinkage of glomerular cell, Death of cells and detachment of the damaged cells. Degeneration of collecting tubules cells was evident by loss of nuclei (Figure 2).

2. Day Fifteen of Gestation:

As in the day fifteen, the kidney of embryo in control group showed differentiation of glomeruli which are covered by Bowman capsule that consist of two layers (Inner visceral layer and outer parietal layer). In addition to that there was clear differentiation to collecting tubules (Figure 3). In treated group, enlargement of capsular space, glomerular cell shrinkage, cell death and detachment of cells from basement membrane, and damage of the Bowman capsule were observed (Figure 4).

Discussion:

The embryonic stage is critical stage. Exposure to drugs during this period can cause toxic effects to the fetus, such as changes in size or function of certain organs, developmental and behavioral abnormalities, or growth restriction [13].

Our results showed that in the treated kidney, there was a Hemorrhage of glomerular cells and collecting tubules in comparison with the control group. The drug might be possibly responsible for this event. This result is in agreement with [14] who mentioned that blood accumulation with damaged cells (loss of their nuclei) of collecting tubules may be caused by the high concentration of the drugs in the biologically active organs in the body.

The current study showed that there were glomerular cells accumulations, cells are characterized by abnormal aggregation of cells, loss their nuclei, glomerulus shrinking and

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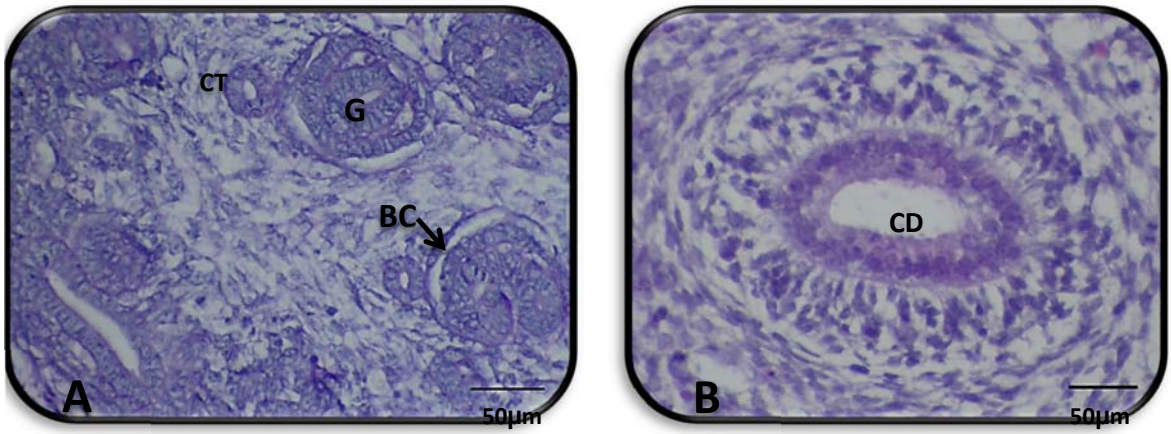
atrophy in comparison with the control groups. This result is in agreement with [15] who reported that cell accumulation disorder may be due to increase in the number of abnormal cells or reduce the number of normal cells. [16] Mentioned that glomerular cell accumulation might be occur due to the destruction of nucleus, or may be due to the glomerular cell loss and extracellular matrix accumulation.

There was death of cell of collecting tubules which is characterized by decreasing size of cell or swelling and loss of their nucleus. This result is compatible to what was obtained by [17], who reported that cell death, cell shrinkage, cytoplasm eosinophilia, presence of a shrunken and small nucleus with chromatin condition due to the toxicity of drug.

There was enlargement of the space that was present between the glomerulus and Bowman's capsule. This result is compatible to what was observed by [18], who reported that the appearance of abnormal enlarged capsular space may result from disappearance of glomerular cells or shrinkage. [19] Reported that enlargement of capsular space might be due to induction of cytoplasmic microtubules because of the toxicity of the drugs.

Damage of collecting tubules which were evident by loss of their nucleus, detachment of epithelial cells from basement membrane when compared with the control group. This result is compatible to what was obtained by [20], who reported that damaged cell might be caused by the cell death which may occur by toxicants.



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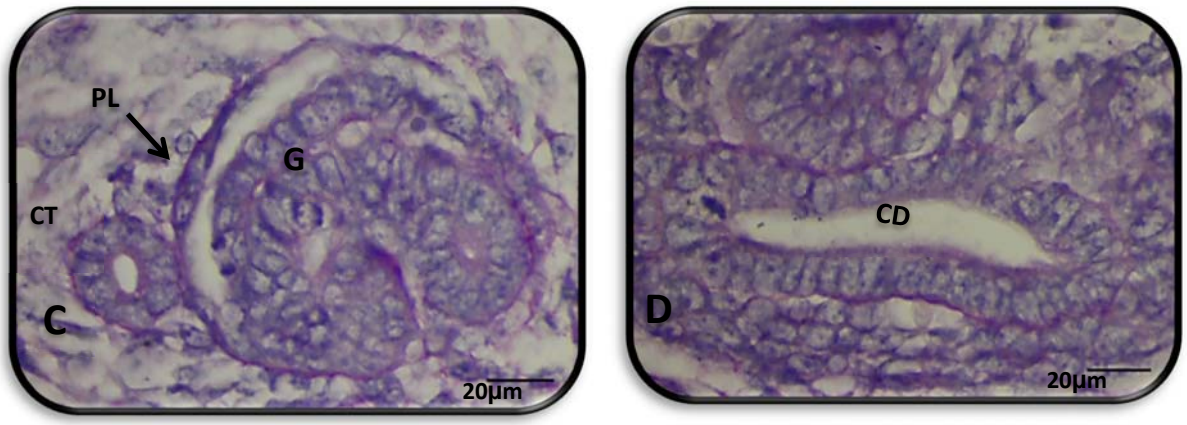
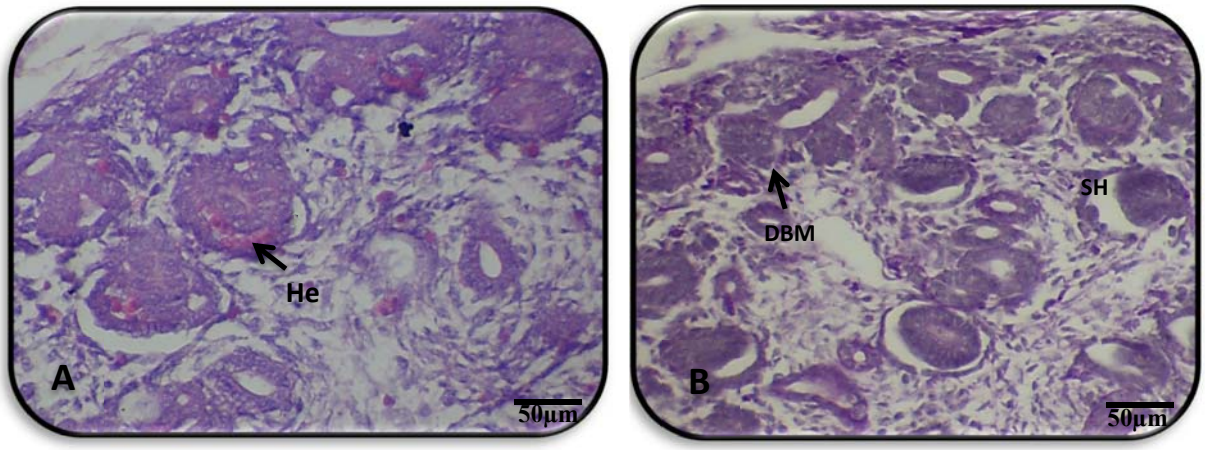


Figure (1): Cross section in kidney of embryo at (14th) day of gestation in (control group) showed: (BC) Bowman's capsule, (CD) Collecting duct, (CT) Collecting tubules, (G) Glomerulus, (PL) Parietal layer. (A) :(H&E), scale bare: 50µm, 40X. (B): (PAS), Scale bare 50µm, 40X. (C)&(D) : (PAS)، Scale bare: 20µm , 100X.



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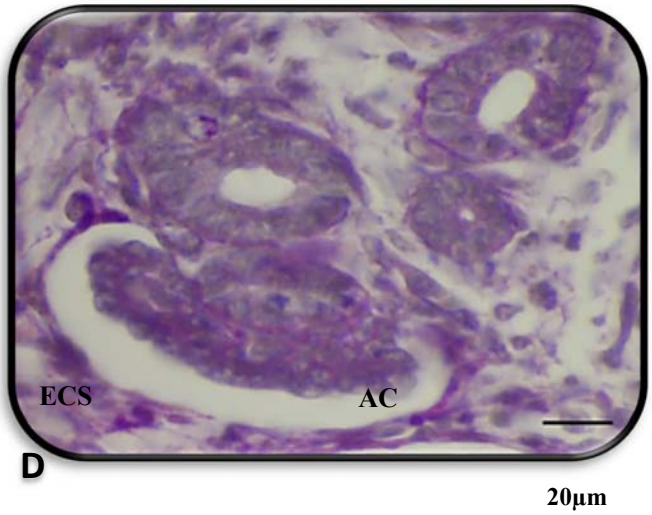
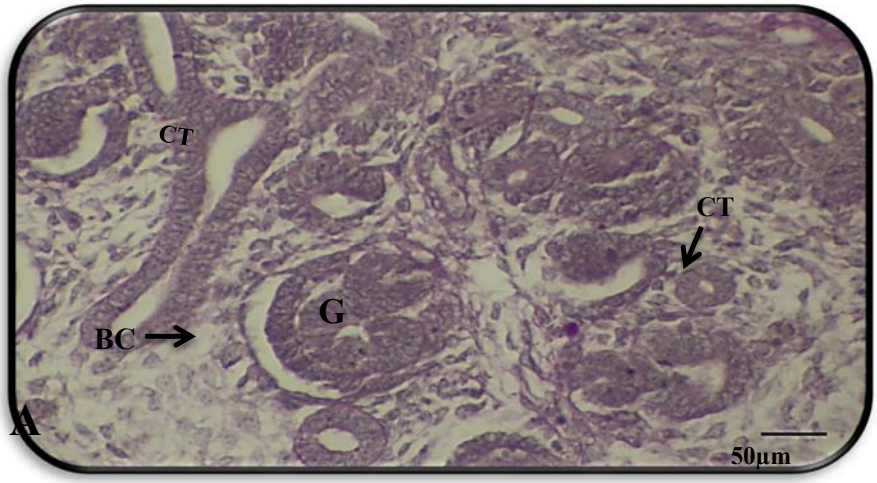


Figure (2): Cross section in kidney of embryo at (14th) day of gestation in (treated group) showed: (AC) Accumulation, (DBM) Damaged of basement membrane, (He) Hemorrhage, (ECS) Enlargement capsule space, (SH) Shrinkage glomerular. (A): (PAS)، scale bare: 100µm,10X .(B): (H&E), scale bare: 50µm, 40X . C: (PAS), scale bare: 50µm, 40X. D: (PAS) , Scale bare: 20µm, 100X.



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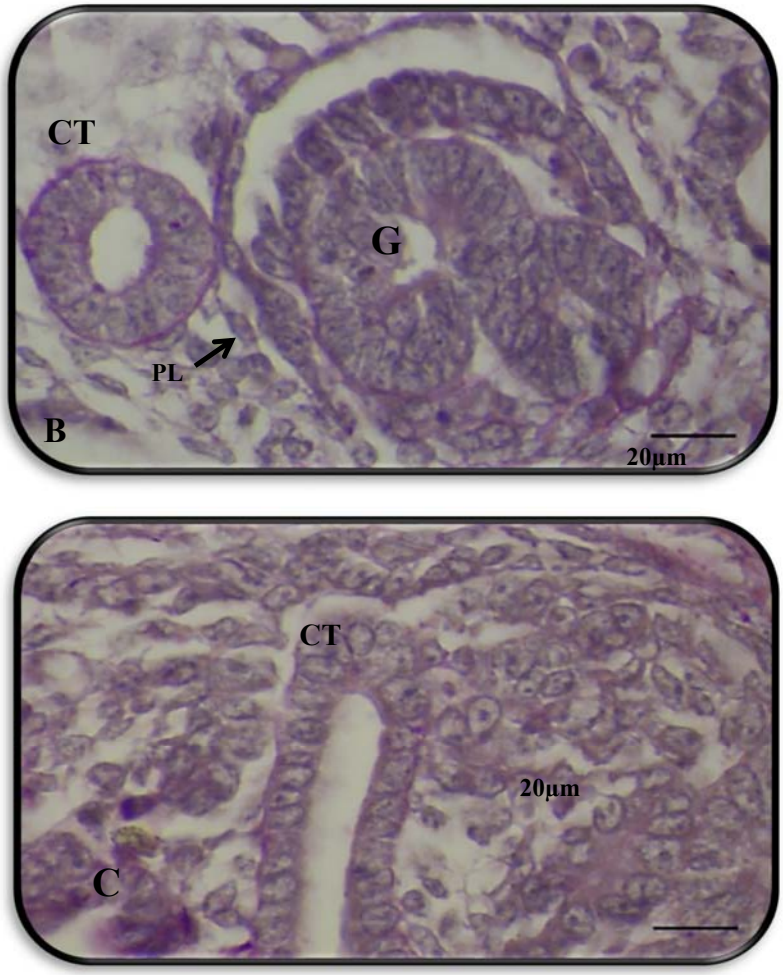

Figure (3): Cross section in kidney of embryo at (15th) day of gestation in (control group) showed: (BC) Bowman's capsule, (CT) Collecting tubules, (G) Glomerulus, (PL) Parietal layer. A: (PAS), scale bare: 50µm 40X .(B)&(C): (PAS), Scale bare: 20µm, 100X.

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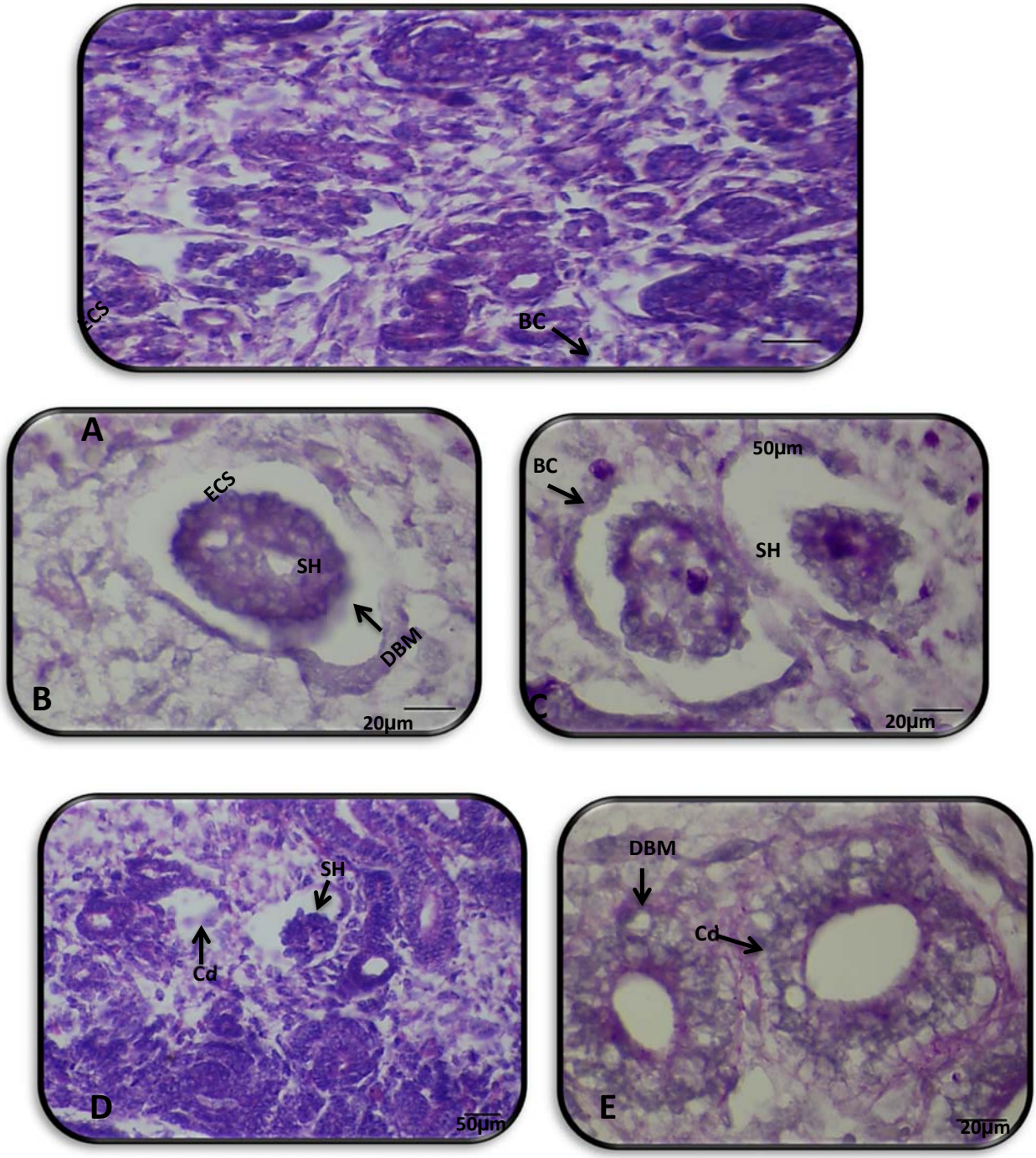

Figure (4): Cross section in kidney of embryo at (15th) day of gestation in (treated group) showed: (BC) Damage of the Bowman's capsule, (Cd) Cell death, (DBM) Damaged of basement membrane, (ECS) Enlargement capsule space, (SH) Shrinkage glomerular. (A): (H&E), scale bare: 50µm, 40X. (B) & (C): (PAS), scale bare: 20µm, 100X . D: (H&E), scale bare: 50µm, 40X. E: (PAS) Scale bare: 20µm, 100X.

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