Histological Assessment of the Effect of Lacosamide on the Kidney Tissue of Pregnant Albino Rats

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Objective: Lacosamide is an antiseizure agent and most commonly prescribed drugs for epilepsy. The aim of this work was evaluating the effect of Lacosamide (at its therapeutic range) on the kidney of pregnant albino rats.

Methods: Forty pregnant rats were divided into two groups, group I received 1.5 ml/day distilled water in two divided doses throughout pregnancy. Rats in the group II received 1.5 ml/day distilled water (containing 36 mg Lacosamide) two divided doses. At the end of the experiment, blood samples were collected, and the sera were separated and used for biochemical analysis. The Kidneys were excised and examined by light and electron microscopic.

Results: Treatment with Lacosamide induced undesirable histopathological changes in the kidney. These changes were in the form of fusion and effacement of secondary foot processes, thickening and disruption of the glomerular basement membrane, widening of the Bowman’s spaces, cytoplasmic vacuolation, and swollen mitochondria with loss of their cristae. Such changes were confirmed by alteration of some biochemical parameters of kidney functions.

Significance: Authors concluded that Lacosamide induced adverse effects on the kidney of pregnant albino rats. Further investigations are needed to identify the mechanism of Lacosamide toxicity.

INTRODUCTION

Epilepsy is a common disorder affecting approximately more than 1.000.000 women in fertile period [1]. The prevalence of epilepsy was estimated to be 0.4 % in pregnant women [2]. Pregnant women with epilepsy are advised to maintain Antiepileptic Drugs (AED) [3]. Pharmacological treatment options of epilepsy comprise conventional AED (valproate) and second-generation agents (Lacosamide) [4]. Some undesired effects associated with conventional AED, such as Stevens–Johnson syndrome and memory deterioration [5]. The effectiveness of the second generation drugs, seems to be similar to that of the conventional AED. However, the second-generation drugs, offer a lower risk of interactions with other medications, simpler titration and improved tolerability [4]. Lacosamide was developed as an analogue of piracetam, a drug used to improve cognitive function [6], it is recognized for adjunct therapy of focal onset in children and adults [7].

Peak plasma concentrations of the drug in 1.3 hours. Two-thirds of the drug is excreted in the urine [8]. Studies reported adverse effects associated with Lacosamide therapy e.g. vomiting, irritability, headaches, inflammation of the nose and throat, and sleepiness [9]. Data on the adverse effects of Lacosamide during pregnancy is still limited [10]. So, this work aimed to assess the effect of Lacosamide on kidney of pregnant albino rats.
Materials and Methods

Lacosamide was available in the form of tablets 500 mg each. The chosen dose for adult humans was 2000 mg/day. The equivalent dose for adult rat weighting about 200 gm is 36 mg/day [11].

The forty pregnant rats were divided equally into two groups:

a) **Group I (control):** received 1.5 ml/day distilled water in two divided doses throughout pregnancy.

b) **Group II (treated):** received 1.5 ml/day distilled water (containing 36 mg Lacosamide) in two divided doses throughout pregnancy.

At the end of the experiment,

1. Blood samples were collected [12]. The sera were separated then preserved at −20°C for biochemical analysis. Creatinine level in mg/dl was determined [13]. Blood urea nitrogen measurement in mg/dl [14].
2. Rats were sacrificed then kidneys were excised and used for:
   A. Light microscopy: Sections were stained with
      1. Hematoxylin and Eosin to study the general histological features of selected kidney tissue.
   B. Immunohistochemistry, anti-bovine type IV (collagen rabbit serum was used as first antibody, and biotinylated anti-rabbit IgG goat serum was used as secondary antibody)

Results

Histopathological Results

a) **Light microscopy (H&E):** Group I (control group): showed Malpighian corpuscles (formed of glomerulus surrounded by Bowman’s capsule) and tubules (Proximal Convoluted Tubule (PCT), Distal Convoluted Tubule (DCT) and collecting tubules). The PCT and DCT were lined by cuboidal cells, however, the DCT exhibited a greater number of lining cells, and wide Lumina with no brush border (Figure 1a). Group II: some shrunken glomeruli with wide capsular spaces. Some of the glomerular tuft nuclei were irregular and deeply stained. Some of the epithelial cells lining the renal tubules showed cytoplasmic vacuolization, intraluminal exfoliation as well as small darkly stained irregular nuclei. The brush border of the some PCT became disrupted (Figure 1b).

b) **Light microscopy (immunohistochemistry):** Group I (control group): no brownish coloration in the mesangial matrix indicating negative reaction for collagen IV (Figure 2a). Group II: strong positive reaction for collagen IV (Figure 2b).
Figure 2: Photomicrographs of renal cortex transverse sections (Collagen IV immunostaining X1000).

a) 2a: group I: showing no brownish coloration in the mesangial matrix indicating negative reaction for collagen IV.

b) 2b: group II: showing strong positive reaction for collagen IV (arrow).

Electron Microscopy:

a) Group I (control group): showed renal corpuscles formed tuft of capillaries lined with fenestrated endothelium and wrapped by podocytes (had large irregular euchromatic nuclei, primary processes and secondary processes with filtration slits). The glomerular basement membrane appeared with its trilaminar structure. The epithelial cells lining the PCT showed many mitochondria lodged between the basal infoldings and a noticeable number of mitochondria of different sizes and shapes were situated on the upper portion of the cells. In addition to mitochondria, endoplasmic reticulum, ribosomes, Golgi complex and lysosomes appeared clearly. At apex of the epithelial cells, tall and closely packed projections called microvilli appeared clearly. The nuclei of these epithelial cells were large, euchromatic with well demarcated nuclear membrane. The nuclei contained clumps of heterochromatin and nucleoli. The epithelial cells lining the DCT were resting on a regular basement membrane that exhibited basal infoldings ran perpendicular to it (Figures 3a & 3b).

b) Group II: showed many histopathological changes. Thickening of the glomerular basement membrane and loss of its trilaminar structure, some of the secondary foot processes were effaced or fused the epithelial cells lining some PCT had small nuclei with irregular ill demarcated nuclear envelope. Loss of cristae or swollen of their mitochondria were longitudinally arranged with. The epithelial cells lining some DCT showed small nuclei with increased heterochromatin. Their cytoplasm exhibited rarefaction, loss of basal infoldings, disorganized mitochondria with loss of their cristae (Figures 3c & 3d).

Biochemical Results: Statistical analysis of the mean levels of Urea and creatinine in groups I and II revealed significant increase in group II as compared with group I (P< 0.05) (Table 1).

Table 1: Means and standard deviations of Urea and creatinine of pregnant rats in groups’ I – II.

| Measurements | Pregnant rats at one day after delivery | T test |
|--------------|---------------------------------------|--------|
|              | Group I (n=20) | Group II (n = 20) | t       | P-value |
| Urea (mg/dl) | Mean ± SD Range 37.39 ± 1.066 | 49.65 ± 1.480 | 2.119 | 0       |
| Creatinine (mg/dl) | Mean ± SD Range 0.663 ± 0.021 | 0.781 ± 0.008 | 2.178 | 0       |
Figure 3: Electron micrograph of the ultrathin renal cortex section (TEM ×10,000 magnification).

a) 3a: group I: showing Glomerular Basement Membrane (GBM) formed of a central lamina densa and lamina rara on either side, separating between Capillary Space (Cs) and Urinary Space (Us). Foot processes of podocytes (arrow) are separated from one another by a regular narrow space called Filtration Slit (Fs).

b) 3b: group I: showing the lining epithelium of proximal convoluted tubules. The Nucleus (N) is rounded, regular contour and euchromatic. Mitochondria (M) are basally oriented and lie within basal infoldings. Apical surface has numerous Microvilli (Mv) forming the brush border.

c) 3c: group II: showing thick Glomerular Basement Membrane (GBM) which separates between Capillary Space (Cs) and Urinary Space (Us). Foot processes of podocytes (arrow) are separated from one another by a regular narrow space called Filtration Slit (Fs). Effacement of foot processes is obvious (double arrow).

d) 3d: group II: showing lining epithelium of proximal convoluted tubules. Nucleus (N) is rounded. The basal cytoplasm is rich in Mitochondria (M) which show pleomorphism. Apical surface has numerous microvilli (Mv). Cytoplasmic vacuolation are seen (arrowhead).

Discussion

The current research revealed that administration of Lacosamide to pregnant albino rats induced degenerative changes in their kidney tissue. The changes were in the form of thickening and disruption of the glomerular basement membrane, fusion and effacement of secondary foot processes, pyknosis of some of the glomerular tuft nuclei and widening of the Bowman’s spaces. Lacosamide treatment also led to disorganized mitochondria in the cells lining the renal tubules, cytoplasmic vacuolization and intraluminal exfoliation. In addition, Lacosamide treatment led
to an increased heterochromatin contents of some nuclei of the cells lining the renal tubules, such pathological changes were confirmed by parallel significant elevation of the levels of urea and creatinine. These findings were in line with Leblanc and Plaisance who reported occurrence of acute kidney injury and potentially life-threatening nature of Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) two months after starting Lacosamide to a 75-year-old man with glioblastoma, With leukocytosis, eosinophilia and thrombocytopenia [15].

Also, the results of the present study were in line with some researchers who also reported occurrence of kidney injury after the initiation of Lacosamide treatment for new-onset seizures in a 23-year-old fertile female patient. Based on the time course of the patient’s elevation in the level of serum creatinine and the exclusion of other causes, the authors suggested that Lacosamide treatment was contributed to the acute kidney injury [16]. On the other hand, the results of the current study were antithesis to other researchers who didn’t detect major side effects any patients after use of intravenous Lacosamide in patients with renal impairment [17]. The results of the present study also were contrary to another study, the authors presented two pediatric cases of Lacosamide overdose for one month and didn’t observe severe side effects in the patients. So, they suggested that accidental treatment with an overdose of Lacosamide in children for long period was not accompanied with serious side effects [18].

In the current study, the degenerative changes that were observed in the kidney might be due to disturbance in the oxidant/antioxidant ratio. Such suggestion was supported by researchers [19-23] who explained that genotoxic potential of Lacosamide by decreased free radical neutralization and/or an increased production of free radicals.Other researcher reported that, relationship between serum lacosamide concentrations and clinical efficacy is not well understood; thus, therapeutic drug monitoring is not routinely recommended [24-27]. Yet, we demonstrated that measuring serum lacosamide concentrations in the critically ill population during continuous renal replacement therapy may be useful to individualize dosing programs [28-34]. Further pharmacokinetic studies of lacosamide may be necessary to generate widespread dosing recommendations [5]. Mahmoud SH reported that Continuous Renal Replacement Therapy (CRRT) is used for managing acute kidney injury in critically ill patients [35-37]. Removal of Antiepileptic Drugs (AEDs) by CRRT could be significant and may complicate patients’ intensive care unit stay [11]. Other researcher reported that additional studies are necessary before specific dosing recommendations can be made for Lacosamide in critically ill patients receiving Renal Replacement Therapy (RRT) after evaluating current literature for dosing recommendations for the use of antiepileptic medications in patients receiving RRT [2].

Conclusion
Authors concluded that Lacosamide induced dangerous effects on the kidney of pregnant albino rats. Continuous assessment of the kidney functions during Lacosamide therapy is advised. In addition, further investigations are recommended to clarify the mechanism of Lacosamide toxicity [38].

Declaration of Conflicts of Interest: No conflicts of interest.

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