The Histological Changes of Albino Mice Embryos' Kidneys after Exposure In Utero to Topiramate

*Fatin Th. Abdul-Rhahman,[1] Luma I. Al-Allaf, *[2] Hatim A. Al-Nuaimy[3]*

Abstract

Background: The kidneys can be damaged by a large number of therapeutic agents. Mechanisms of injury are varied and all renal structures may be affected.

Objectives: To discuss the effect of topiramate (one of the new antiepileptic drugs) on renal histology by studying the pathological features of the kidneys of embryos of albino mice, Balbls species (Swiss origin, *Mus musculus*) after intrauterine exposure to that drug and to determine the frequencies of these pathological findings.

Setting: Laboratory of Postgraduate Studies at the Department of Anatomy, Histology and Embryology in Mosul College of Medicine, Mosul University, Mosul, Northern Iraq.

Methods: This study was conducted over a period of 2 months extending from July 1, 2010 to September 1, 2010. Male and female albino mice, Balbls species (Swiss origin, *Mus musculus*) were chosen from 10-12 weeks of age, their mean weight was about 28±2 gms and they were healthy. These animals were put in good environmental conditions and underwent acclimatization in a 12 hour light-dark cycle. The animals were put in plastic cages with food and water ad libitum.

Animals with age of 10-14 weeks and weight of 25 gms from both sexes were chosen for mating and the insurance of mating was made by noticing the vaginal plug .The pregnant females were isolated with the recording of the date of mating as day zero. Each pregnant female was housed in single cage with good environmental conditions for the duration of pregnancy and lactation and after weaning the males were isolated from females in big plastic cages with food and water ad libitum. Seventy five pregnant mices were used in this study and they were classified in two groups: **Group One:** consisted of 30 pregnant females with distilled water administration (Control Group). **Group Two:** consisted of 45 pregnant females with topiramate oral administration of 8.4 mg /Kg body weight (Study Group).The oral administration was done daily from day one of pregnancy for eighteen days.

The pregnant females from both groups were dissected using Ether inhalations at day eighteen of pregnancy .The pregnant females were dissected and the embryos were obtained from their uteruses and they were immersed for a few seconds in Nacl solution 0.9% to get rid of superficial blood. The embryos were examined using a magnifying lens .The weight of all embryos were measured using a sensitive electronic measure.

Two slices of 3-4 mm thickness from both kidneys were then taken and run in staining protocols using alcoholic Bouin's fixative, and then transferred to the Laboratory of Postgraduate Studies at the

1. **Assistant Lecturer, Department of Anatomy, Histology, and Embryology, College of Medicine, University of Mosul, Mosul, Iraq.**
2. **Lecturer, Department of Anatomy, Histology, and Embryology, College of Medicine, University of Mosul, Mosul, Iraq.**
3. **Pathologist, Unit of Histopathological Examination, Laboratory of Al-Jumhori Teaching Hospital, Ninavah, Health Office, Mosul, Iraq.**

**** Correspondence should be addressed to:***
Luma I. Al- Allaf
E-mail: Lumaallaf1971@yahoo.com

Department of Anatomy, Histology and Embryology in Mosul College of Medicine for tissue processing after 24 hours.

Multiple renal sections from both groups were stained with hematoxylin and eosin stain (H&E).
All sections were examined by an Ernst Leitz Wetzlar microscope (Germany). Photomicrographs were taken using a digital camera (Sony Cyber-shot, resolution 10.1 Mega pixels).

Results: Light microscopic examination using hematoxylin and eosin (H&E) revealed that the renal sections which were obtained from 40 out of 45 embryos (88.8%) in the study group showed various histological findings compared to that of control group .These findings include glomerular hyalinization, glomerular atrophy, interstitial fibrosis, tubular atrophy, tubular dilatation with sloughing of their epithelium, and vascular dilatation . Glomerular hyalinization was seen in the sections of 24out of 40 embryos (60.0%) while glomerular atrophy was observed in 12embryos (30.0%) in the study group.
Sections which were obtained from 30out of 40 embryos (75.0%) showed interstitial fibrosis. On the other hand, tubular atrophy was seen in 22 embryos (55.0%) while tubular dilatation with sloughing of their epithelium was observed in 16 embryos (40.0%).

This study revealed that sections which were obtained from 26out of 40 embryos (65.0%) in the study group showed multiple lesions include glomerular hyalinization/atrophy, interstitial fibrosis, and tubular atrophy/dilatation with sloughing of the tubular epithelium. On the other hand, glomerular hyalinization and interstitial fibrosis with intact tubules were noticed in 8cases (20.0%). Interstitial fibrosis, tubular atrophy/dilatation with sloughing of the tubular epithelium with intact glomeruli was seen in 4embryos (10.0%).

Conclusions: This study showed that the tubulointerstitial compartment is most frequently involved due to the effect of topiramate, but glomerular lesions were seen in a significant proportion of cases. When treating women during pregnancy, the risks of increased seizure frequency versus the risks of AED use must be weighed carefully.

Keywords: Epilepsy, New antiepileptic drugs, Topiramate, Kidney, Nephrotoxicity, Pathology**.**

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Introduction

The last ten years of the 20th century are called in neuroscience the "decade of the brain". This period has brought many advances to the treatment of neurological disabilities, including epilepsy. [1]

Epilepsy affects more than 2 million people in the world. [2] Despite many recent surgical advances, medications remain the mainstay of treatment. Approximately 70% of patients with epilepsy will become seizure-free using a single antiepileptic drug. [3] For the remaining 30%, recurrent seizures as well as intolerable adverse effects can have significant impact on the quality of life.

Multiple reviews of somatic and behavioral development in children of mothers with epilepsy are available. [4-24] The risk of anatomical deficits appear to be due to antiepileptic drug (AED) exposure since children of mothers without epilepsy, who are exposed to AEDs, have the same risk as children of women with epilepsy. [25] A recent large retrospective study found that 31% of AED exposed children had major malformation (14%) or developmental delay (19%). [17]

Although the risks of birth defects and neurodevelopmental deficits are increased in these children, consensus guidelines have not been able to delineate which of the AEDs have the greatest potential for teratogenesis based on the available data. [18] Further, there is a lack of consensus concerning the relative risks across different AEDs of impaired psychomotor development from in utero exposure.

Treatment choices in women with epilepsy are difficult due to conflicting risks. AEDs pose a risk of teratogenesis, but the seizures can also pose grave risks. Further, children of women who experienced greater than 5 convulsions had impaired cognitive outcomes.[26] There is a subset of women with epilepsy who can stop AEDs prior to pregnancy because they have very mild epilepsy, but the large majority of women with epilepsy cannot stop AEDs due to the greater risk posed by seizures. The present evidence supports a differential risk across AEDs with the greatest risk to the child's outcome posed by valproate. However, the magnitude of the risk is unknown. Further, whether differential risk exists for other AEDs is unknown.

A number of new antiepileptic drugs (AEDs) have been licensed for the treatment of seizures and topiramate is one of them.[1,27]

Topiramate (TPM) has generally been considered to be a highly effective new AED.[28] TPM has multiple mechanisms of action, including inhibition of sodium and calcium currents, blockade of the glutamate receptors and facilitation of GABA effects at the GABA-A receptor.[29] Cognitive side-effects and weight loss were reported by a high percentage of the patients taking TPM. [30] Another clinically relevant adverse effect of TPM is nephrolithiasis, with a reported incidence of 1.5%.[31] Despite the risk of the above mentioned side-effects, TPM offers high efficacy in the treatment of almost all seizures types.

Departing from the usual account of drug-induced renal disease according to the specific type of drug, this discussion can be divided into 3 main areas - glomerular injury, vascular injury and tubulointerstitial changes. In doing so, more emphasis will be placed on morphological findings although functional toxicity (with little or no structural abnormalities detected by routine techniques) plays an equally important role in certain classes of drugs. [32, 33]

The kidneys are primarily involved in filtering and concentrating various substances and chemical agents. These substances may reach high concentrations in the kidney and become toxic. Depending on the segment of the nephron targeted, different morphological changes are detected in the renal biopsy, which remains the gold standard for documenting kidney disease. [33]

Although we understand a great deal about its ultrastructure, filtration function and molecular phenotype, how certain drugs affect the kidneys remains obscure. [32- 34]

The aim of this study is to discuss the effect of topiramate on renal histology by studying the pathological features of the kidneys of *Mus musculus* embryos after intrauterine exposure to that drug and to determine the frequencies of these pathological findings.

Materials and Methods

This study was conducted over a period of 2 months extending from July 1, 2010 to September 1, 2010.

Animals

Male and female albino mice, Balbls species (Swiss origin, *Mus musculus*) were chosen with 10-12 weeks of age, their mean weight was about 28±2 gms in healthy condition. These animals were obtained from the animal houses of both the College of Medicine and the College of Veterinary Medicine in Mosul University, Mosul, Northern Iraq. These animals were keep in good environmental conditions and underwent acclimatization in a 12-hour light-dark cycle. The animals were put in plastic cages (30×13×13 cm) from North Kent Plastic Cages, England with food and water ad libitum. [35]

Animals with age of 10-14 weeks and weight of 25 gms from both sexes were chosen for mating and the insurance of mating was made by noticing the vaginal plug.[36] The pregnant females were isolated with the recording of the date of mating as day zero.[37] Each pregnant female was housed in single cage with good environmental conditions for the duration of pregnancy, lactation and after weaning .The males were isolated from females in big plastic cages (41×25×13 cm) from North Kent Plastic Cages , England with food and water ad libitum. [35]

Drug Exposure

Seventy five pregnant mice were used in this study and they were classified in two groups: **Group One:** consisted of 30 pregnant females with distilled water administration (Control Group). **Group Two:** consisted of 45 pregnant females with topiramate oral administration of 8.4 mg/Kg body weight (Study Group). The oral administration was done daily from day one of pregnancy for eighteen days using white tablets of topiramate (Medutics Industries, Syria). The dosage was prepared by dilution of the drug with 100 ml distilled water and it was given using special syringes which were used for these purposes.

Animal Dissections

The pregnant females from both groups were dissected using ether inhalations [35] on day eighteen of pregnancy. [38, 39]

The pregnant females were dissected by longitudinal thoracoabdominal incision and the embryos were obtained from their uteruses and immersed in a Nacl solution 0.9% for a few seconds in order to get rid of superficial blood. The embryos were then examined using a magnifying lens. The weights of all the embryos were measured using an electronic sensitive measure (Type Shimadzu Aw5320, Japan).

Two slices of 3-4 mm thickness from both kidneys of each embryo were taken using the microtome and run in staining protocols using alcoholic Bouin's fixative, a popular method for medical renal biopsies. They were then transferred to the Laboratory of Postgraduate Studies at the Department of Anatomy, Histology and Embryology in Mosul College of Medicine for tissue processing after 24 hours. Multiple renal sections from both groups were stained with hematoxylin and eosin stain (H&E).

All sections were examined in a Ernst Leitz Wetzlar microscope (Germany). Photomicrographs were taken using a digital camera (Sony; Cyber-shot, resolution 10.1 Mega pixels).

Results

Light microscopic examination using hematoxylin and eosin (H&E) revealed that the renal sections which were obtained from 40 (88.8%) out of 45 embryos in the study group showed various histological findings compared to that of the control group .These findings include glomerular hyalinization, glomerular atrophy, interstitial fibrosis, tubular atrophy, tubular dilatation with sloughing of their epithelium, and vascular dilatation .

This study revealed that sections which were obtained from 26 out of 40 embryos (65.0%) in the study group showed multiple lesions that include glomerular hyalinization/atrophy, interstitial fibrosis, and tubular atrophy/dilatation with sloughing of the tubular epithelium (Figures 1, 2).

Glomerular hyalinization was seen in the sections of 24 out of 40 topiramate exposed embryos (60.0%) while glomerular atrophy was observed in 12 embryos (30.0%) in the study group**.**

Sections which were obtained from 30 out of 40 embryos (75.0%) in the study group showed interstitial fibrosis**.**

On the other hand, tubular atrophy was seen in 22 embryos (55.0%) while tubular dilatation with sloughing of their epithelium was observed in 16 embryos (40.0%)**.** Vascular dilatation was observed in 2 embryos and it was accompanied with of glomerular hyalinization, interstitial

fibrosis, and tubular atrophy. On the other hand, glomerular hyalinization and interstitial fibrosis with intact tubules were noticed in 8 cases (20.0%). Interstitial fibrosis, tubular atrophy/dilatation with sloughing of the tubular epithelium with intact glomeruli was seen in 4 embryos (10.0%). Most of the renal sections in the study group showed the presence of intratubular amorphous substances in form of eosinophilic plagues.

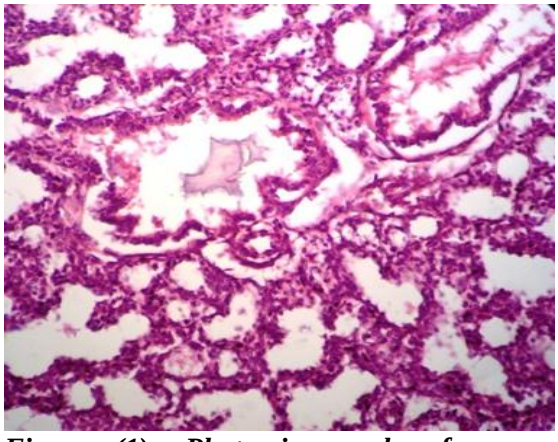

Figure (1): Photomicrograph of group 2 showing interstitial fibrosis, tubular dilatation and glomerular hyalinization and atrophy with intratubular amorphous aggregates. (H&E X600).

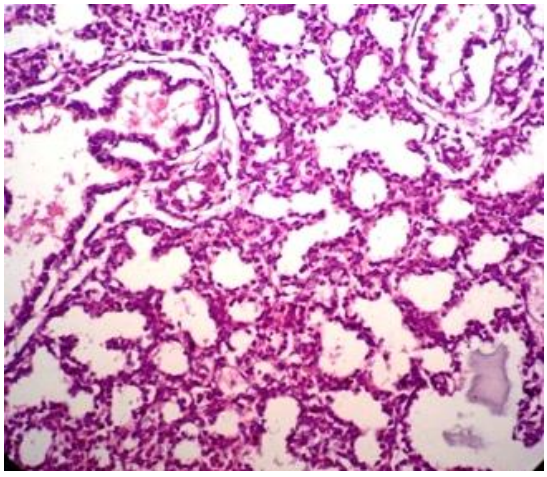

Figure (2): Photomicrograph of group 2 showing interstitial fibrosis, tubular dilatation and glomerular hyalinization and atrophy with intratubular amorphous aggregates. (H&E X600).

Table (1) showed the frequency of the different histological findings in the kidneys of the embryos of the study group.

Table (1): The frequencies of different histological findings in the kidneys of the embryos of the study group.

Histological Findings	*Frequency Number out of 40 (%)*
Glomerular Hyalinization	*24 (60.0%)*
Tubular Atrophy	*22 (55.0%)*
Vascular Lesions	*2 (5.0%)*
Glomerular Atrophy	*12 (30.0%)*
Tubular Dilatation	*16 (40.0%)*
Interstitial Fibrosis	*30 (75.0%)*

Discussion

Pregnancy in patients with seizure disorders can be complicated by a variety of maternal and fetal issues. Patients can experience higher rates of seizures because of the lower serum plasma levels of their antiepileptic drugs (AEDs). [40, 41]

During pregnancy, the volume of distribution and hepatic metabolism of AEDs are increased. This, along with decreased compliance with AEDs because of concerns about effects on the fetus, leads to an increase in seizure frequency, which is observed in as many as 17-33% of pregnancies. [42, 43]

This study revealed that the renal sections which were obtained from 26 out of 40 embryos (65.0%) in the study group showed multiple lesions that include glomerular hyalinization/atrophy, interstitial fibrosis, and tubular atrophy/dilatation with sloughing of the tubular epithelium. These findings are consistent with that of Alwin and Arthur in 2009, where they classified the drug-induced renal disease into 3 main areas- glomerular injury, vascular injury and tubulointerstitial changes. [44] Several studies of Detwiler et al in 1994, Valeri in 1996, Laurinavicius in 1999, Alwin and Arthur in 2009 reported that these lesions are frequently seen together as shown in this study. [44- 47]

This study revealed that the 38 out of 40 cases (95.0%) among sections obtained from embryos

that were exposed to topiramate in utero were tubular lesions including tubular atrophy/tubular dilatation with sloughing of their epithelium. These findings are coming in according with that of Alwin and Arthur in 2009 [44] who reported that the tubulointerstitial compartment is most frequently involved, but glomerular lesions are seen in a significant proportion of cases; they summarized the cytopathic effects of a number of drugs on the renal tubules and they reported that varying degrees of tubular degeneration and regeneration are commonly observed. [44]

As shown in this study sloughed epithelial cells, cell debris and proteinaceous material are a frequent finding of drug-induced renal injury. Some tubules become dilated and simplified, predisposing to rupture in more advanced cases .A study which was done by Wasserstein et al in 1995 and another by Depierreux et al in 1994 suggested that tubular atrophy may reflect chronicity and severity. [31, 48]

The presence of amorphous aggregates inside the lumen of the tubules was shown in 10 embryos (25.0 %); these lesions were accompanied with tubular dilatation or atrophy or accompanied with glomerular atrophy. These findings may explain why some drugs predispose to crystal deposition and can result in urolithiasis and obstructive changes, [44] and are in accordance with that of Wasserstein et al in 1995 who reported that one of the clinically relevant adverse effect of TPM is nephrolithiasis, with a reported incidence of 1.5%. [31]

Several studies reported that these crystals are mostly dissolved with routine tissue processing, forming amorphous aggregates surrounded by giant cells. It has been reported that several drugs have all been associated with tubular crystallopathy, urolithiasis and interstitial nephritis. [49- 52] On the other hand, this study revealed that sections which were obtained from 30 embryos showed interstitial fibrosis. A study done by Alwin and Arthur in 2009 reported that when this lesion sets in, the disease is deemed chronic while Depierreux et al. in 1994 suggested that this lesion may reflect the severity. [48]

This study revealed that sections of 36 embryos showed glomerular injury; these findings are consistent with that of other studies which reported that several drugs including anticonvulsant drugs may lead to glomerulopathy and suggested that the common mechanism for glomerular injury is immune complex deposition along the sub-epithelial aspect of the Glomerular Basement Membrane (GBM). [53- 63]

Conclusion

The tubulointerstitial compartment is most frequently involved due to the effect of topiramate, but glomerular lesions are seen in a significant proportion of cases.

In addition, while some drugs (topiramate is one of them) primarily injure a specific renal parenchymal structure, effects are seen in other compartments because they are inter-connected by feedback mechanisms.[44] Physicians should be familiar with the wide range of medications harmful to the kidney and be aware of the lesions they bring about. New targets in epilepsy treatment, the search for antiepileptic agents with more selective activity and lower toxicity continues to be an area of intensive investigation. Moreover, it could be concluded that when treating epilepsy during pregnancy, the risks of increased seizure frequency versus the risks of AED use must be weighed carefully. [40]

Epilepsy should be treated during pregnancy by a team of providers, including a perinatologist, who can focus on balancing the risks of seizures versus the administration of AEDs. Further studies are needed to resolve this issue and determine the contribution of various risk factors.

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التغيرات النسيجية لكلى أجنة الفئران المهقاء بعد تعريضهم لجرع من عقار التوبريميت داخل الرحم

فاتن ذنون عبد الرحمن،[1] لمـى العلاف، [1] حاتم عبد المجيد النعيمي [2]

1– فرع التشريح والأنسجة والأجنة، كلية الطب، جامعة الموصل، الموصل، العراق؛ 2– وحدة الفحص النسيجي المختبرى، مستشفى الجمهوري التعليمي،دائرة صحة نينوى، الموصل، العراق

الملخص

الخلفية: يمكن أن تتعرض الكليتان لتلف ما نتيجة العديد من العوامل العلاجية. إن ميكانيكية حدوث الضرر متنوعة وكل التراكيب الكليوية من الممكن أن تتضرر.

هدف الدراسة: تهدف الدراسة إلى مناقشة تأثيرعقار التوبريميت (وهو يستعمل كعلاج للصرع) على النسيج الكلوى عن طريق دراسة الملامح المرضية لكلى اجنة الفئران المهقاء بعد تعرضها لهذا العقار وهى فى ارحام أمهاتها كما تهدف الدراسة الى تحديد تكرار تلك التغيرات المرضية.

مكان الدراسة: مختبر الدراسات العليا في كلية طب الموصل، جامعة الموصل غربى مدينة الموصل شمال العراق.

طرق الدراسة: اجريت الدراسة خلال الفترة المحصورة من الاول من شهرتموز سنة 2010 الى الاول من شهرايلول من نفس العام. تم اختيار مجموعة من ذكور واناث الفئران المهقاء السويسرية الاصل وبعمر عشرة الى اثنى عشر اسبوعا وفى صحة جيدة، كان معدل اوزانهم2 ± 28 غرام وتم وضع الحيوانات فى ظروف بيئية مناسبة وتمت اقلمتها فى اثنتى عشرة ساعة من دورة من الضوء– الظلام. تم وضع الحيوانات فى اقفاص بلاستيكية مع تزويدهم بالغذاء والماء. تم اختيار حيوانات باعمار عشرة الى اربعة عشر اسبوعا وبوزن خمس وعشرين غراما من كلا الجنسين للتزاوج وتم التاكد من حدوث التزاوج بملاحظة السدادة المهبلية. وتم عزل الاناث الحوامل مع تسجيل يوم التزاوج واعتباره يوم الصفر.كل انثى حامل وضعت فى قفص انفرادى وبظروف بيئية جيدة طول فترة الحمل والارضاع. تم عزل الذكور عن الاناث فى اقفاص بلاستيكية كبيرة مع تهيئة بالغذاء والماء. خمس وسبعون انثى اختيرت لهذه الدراسة وتم تصنيفهن الى مجموعتين كالتالى:

المجموعة الاولى: تتكون من ثلاثين انثى حامل باعطائها ماء مقطراً.

المجموعة الثانية: تتكون من خمس واربعين انثى حامل بتجريعها عقار التوبريميت عن طريق الفم وبجرعة 8.4 mg/Kg.تم التجريع يوميا من اليوم الاول من الحمل لمدة ثمانية عشر يوما. تم تشريح الاناث الحوامل واخذ الاجنة من الارحام وغمسها فى محلول Nacl 0.9% للتخلص من الدم السطحى لبضع ثوان. تم فحص الاجنة باستعمال عدسة مكبرة وتم قياس اوزان الاجنة باستخدام ميزان الكترونى حساس. تم اخذ شرائح نسيجية وبسمك 3-4mm من كلى الاجنة وتم تمريرها فى برنامج الصبغ المعتاد بعد استمال مثبت بوين وذلك فى مختبر الدراسات العليا في كلية طب الموصل لغرض اجراء المعاملة النسيجية بعد اربع وعشرين ساعة تم اخذ عدة شرائح من كلى اجنة المجموعتين كلتيهما وصبغها بالهيماتوكسيلين ايوسين وتم اخضاعها للفحص بالمجهر الضوئى الالمانى. تم اخذ الصور باستعمال كاميرا رقمية نوع سونى يابانية الصنع.

النتائج: ان الفحص المجهرى الضوئى باستعمال الهيماتوكسيلين ايوسين اظهر ان الشرائح الماخوذة من كلى اربعين (88.8%) جنيناً من ضمن خمس واربعين جنيناً عائداً للمجموعة الثانية قد اظهرت تغييرات نسيجية متنوعة اذا ما قورنت بالعائدة للمجموعة الاولى. شملت تلك التغييرات ضمور الكبيبة، المظهر الشفافى للكبيبة، التليف البينى، ضمور النبيبات الكليوية مع توسعها وتلف الخلايا الظهارية لها مع توسع الاوعية الدموية. لوحظ المظهر الشفافى الكبيبى فى اربع وعشرين (60.0%) حالة بينما لوحظ ضمور الكبيبة فى اثنتي عشرة (30.0%) حالة. اظهرت الشرائح الماخوذة من كلى ثلاثين(70.0%) جنين وجود التليف البينى. من جهة اخرى سجلت اثنتان وعشرون (55.0%) حالة من ضمور النبيبات الكليوية بينما لوحظ توسعها وسلخ الخلايا الظهارية لها فى ست عشرة (40.0%) حالة. فى هذه الدراسة كان وجود تغييرات نسيجية متعددة فى ست وعشرين (65.0%) حالة تشمل ضمور النبيبات الكليوية، توسع النبيبات الكليوية وسلخ الخلايا الظهارية لها، المظهر الشفافى الكبيبى، التليف البينى وضمور الكبيبة. بينما لوحظ من جهة اخرى وجود ضمور النبيبات الكليوية، توسع النبيبات الكليوية وسلخ الخلايا الظهارية لها مع التليف البينى وبوجود كبيبة طبيعية فى اربع (10.0%)حالات. وجد ان هتاك ضمور الكبيبة والمظهر الشفافى للكبيبة، التليف البينى دون وجود اى تغييرات فى النبيبات الكليوية النبيبات الكليوية فى ثمانى (20.0%)حالات.

الاستنتاجات: ان جزء الكلى البيني النبيبى كان أكثر أجزاء الكية تأثرا بعقار التوبريميت علما ان الكبيبة أيضا قد تاثرت وبصورة ملحوظة بنفس العقار. عند معالجة النسوة الحوامل بأدوية الصرع يجب اجراء موازنة دقيقة بين الخطر الناجم عن احتمال حدوث زيادة فى نوبات الصرع وبين الخطر الناجم عن ادوية الصرع.

الكلمات الدالة: الصرع، ادوية الصرع الجديدة، الكلية، سمية الكلى، علم الامراض.