

## **Effect of therapeutic dose of Topiramate on placenta albino rat**

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DOI: <https://doi.org/10.47372/uajnas.2019.n1.a18>

### **Abstract**

The aim of this study is to elucidate the effects of the therapeutic doses of topiramate on the placental structure of albino rats (one of the antiepileptic drugs). This study was conducted over a period of 6 months extending from October 1, 2011 to February 1, 2012.

. Thirty adult non pregnant female albino rats and 15 male ones of the same strains, weighing 150-200 grams, were purchased from the Animal House of the Faculty of Medicine, Assiut University, Egypt.

Thirty pregnant rats were used in this study and were classified in three groups: a) Control Group: consisted of 10 pregnant females with normal saline administration, b) Group one: consisted of 10 pregnant females with therapeutic dose of topiramate oral administration of 50mg /Kg body weight (Study Group I), and (c) Group two: consisted of 10 pregnant females with therapeutic dose of topiramate oral administration of 100mg /Kg body weight (Study Group II). The oral administration was done daily, from day one of pregnancy, for nineteen days of gestation.

Light microscopic examination, using Hematoxylin and Eosin (H&E) This study revealed that sections which were obtained from the study groups I & II showed multiple lesions that include decidual cells degeneration, hyaline deposition, particularly in decidual site, labyrinth zone and fetal blood vessels, Trophoblastic giant cells and spongiotrophoblasts had cytoplasmic vacuoles, presumably phagosomes, and pyknotic nucleus, the labyrinth layer of group I & II showed distortion of their plate-like architecture, deposition of more fibrinoid material and perivascular fibrosis and extensive areas of congestion and hemorrhagic in all placental layers. Therefore, topiramate induced dose independent structural changes in the placenta. So, it should be used with caution during pregnancy.

**Key words:** Topiramate, placenta, Histopathological changes.

### **Introduction:**

The anti-epileptic drug topiramate has been increasingly prescribed over the last decade not only to prevent seizures, but also to treat bipolar disorder and migraine headaches <sup>(9)</sup>.

Topiramate is a second-generation antiepileptic drug (AED) that is classified as 'Pregnancy Category-D drug' in terms of teratogenic risk <sup>(8)</sup>. It was approved by Food and Drug Administration (FDA) for the treatment of partial seizure in adult epileptic patients. This drug is also used in children with refractory partial seizures with or without secondary generalized tonic-clonic seizures <sup>(6)</sup>. Also, it was confirmed that topiramate has antidepressant activity in mice <sup>(1)</sup>.

Topiramate is as a Cytochrome P450 3A4 Inducer and Cytochrome P450 2C19 Inhibitor <sup>(16)</sup>. Topiramate, a derivative from naturally occurring d-fructose, shows a great effect in blocking the spread of seizures. Previous studies have demonstrated several possible mechanisms for its

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antiepilepsy activity, including enhancement of gamma-Aminobutyric acid (GABA) -evoked whole-cell currents in cortical neuron through a benzodiazepine-insensitive pathway<sup>(10,17)</sup>, inhibiting L-type calcium channels to control calcium influx and dendritic excitation<sup>(2)</sup>, inhibiting (RS)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate (AMPA) and kainate (KA) receptors in controlling calcium influx<sup>(20)</sup>. However, according to the FDA drug grading, topiramate has positive evidence of human fetal risk, but it has also potential benefits from use of the drug in pregnant women<sup>(15)</sup>.

Placenta is a very important channel for the exchange of materials between the maternal and fetal blood<sup>(7)</sup>. The relationship between the mother and fetus is very complex; this relation occurs through the placental tissue and control development of the fetus. So, the placenta was a target organ for drug or chemical-induced adverse effects. Drug-or chemical-induced placental functional depression and injury subsequently result in abnormal fetal growth or development leading to delay in growth of fetuses, fetal resorption and teratogenicity<sup>(5)</sup>.

Past studies have found that women taking topiramate during early pregnancy to prevent epileptic seizures had a two- to five-fold greater chance of giving birth to a baby with an oral cleft, but such studies did not focus on placental changes<sup>(9)</sup>. So, There is limited information on topiramate-induced changes in placenta.

The aim of this study is to evaluate the effects of the therapeutic doses of topiramate on the placental structure of albino rat fetuses.

### **Material and methods:**

This study was conducted over a period of 6 months, from October 1, 2011 to February 1, 2012.

Thirty adult non pregnant female albino rats and 15 male ones of the same strain weighting 150-200 grams in healthy condition, were purchased from the Animal House of the Faculty of Medicine, Assiut University; the female rats were separated and housed for one week to be sure that they were non pregnant. All over the period of experiment, the animals were housed in capacious cages with natural ventilation at room temperature (37°C). They received tap water and food (rodent pellets) ad libitum.

One fertile male rat was placed into each cage with two females overnight, and the insurance of mating was made by noticing the vaginal plug (Vaginal redness and slight swelling)<sup>(12)</sup>; on the following morning and regarded as Day 0 (zero) of gestation (GDO)<sup>(4)</sup>, then the males were returned to their separate cages.

Topiramate was dissolved in distilled water and was administered by intra-gastric route from day 6 through day 19 of gestation<sup>(4,18)</sup>.

### **Drug Exposure:**

Thirty pregnant rats were used in this study and were classified into:

**Control Group**, Consisted of 10 pregnant females with normal saline administration.

**Study Group 1**, consisted of 10 pregnant females with therapeutic dose of Toriramate oral administration of 50 mg/kg daily, and

**Study Group II**, consisted of 10 pregnant females with therapeutic dose of Toriramate oral administration of 100 mg/kg daily. The dosing regimen was based on human therapeutic dose (HED).<sup>(15)</sup>

**Animal Dissections:**

The pregnant females from all groups were dissected, using cervical dislocation on day twenty of pregnancy.

The pregnant females were dissected by longitudinal thoracoabdominal incision and the Placenta of the control and the treated groups were firstly fixed for histological investigation by light microscopy using; 10% formalin solution for 24 h. Washing was done in running water to remove excess fixative, then dehydrated using serial ascending series of ethyl alcohol. Specimens were cleared in xylene and embedded in paraffin wax at 56 degrees in hot air oven. Paraffin wax tissue blocks were prepared for sectioning at 5  $\mu$  thicknesses using Reichert microtome. The obtained tissue sections were mounted on glass slides, deparaffinized, stained with Haematoxylin and counterstained by Eosin for the light microscope<sup>(3)</sup>. Photomicrographs were taken using a digital camera (Sony; Cyber-shot, resolution 14.0 Mega pixels). Tissue processing was done in the Laboratory of Aden University. Three placentae were chosen randomly from each mother with a total of thirty placentae in each group.

**Statistical analysis:**

Results were expressed as frequency. All statistical analysis were done using Statistical Package for the Social Sciences (SPSS) (version 20), the Fisher exact test was performed to test the significance level between the treated groups and negative control group. Values of  $P < 0.05$  were considered statistically significant

**Results:**

Placenta is an organ with circulation from both the mother and the fetus. Rat placenta has two zones: 1) junction zone, which is composed of outer giant cells that separated exteriorly located maternal deciduas, basalis and inner trophospongium which consist of highly packed basophilic spongioblast cells 2) labyrinth zone, which is composed of communicated network of the maternal lacuna and embryonic capillaries<sup>(14)</sup>. The materno-embryonic nutritional and gaseous exchange is believed to take place in the labyrinth<sup>(14)</sup>.

Gross placental examination in both study groups (I & II) showed neither significant reduction in weight nor gross anomalies as compared to the control group.

Histological examination and the structural changes in the placentas of groups I & II were seen in the different layers 1) deciduas basalis site 2) basal zone and 3) labyrinth.

This study revealed that sections which were obtained from study groups I & II showed multiple lesions that include decidual cells degeneration (Table 1), pyknosis of nuclei of giant cells with and perinuclear halo in some of them and basophilic cells with vacuoles were noticed (Figures 4) ( $p=0.000001$ ) and hyaline deposition (table 2) in decidual site, labyrinth zone and fetal blood vessels were mostly hyalinized (Figure 2B & 3); this was primarily due to increased amount of fibrinoid material ( $p=0.000001$ ). Trophoblastic giant cells and spongiotrophoblasts had cytoplasmic vacuoles, presumably phagosomes, and pyknotic nucleus (Figures 1A, 2B and 4B). Vacuolization and hemorrhagic cysts are seen (Figures 1A&B, 3). An increased deposition of the fibrinoid material and abundant fetal mesenchyme, compared to scanty fetal mesenchyme in the control was seen (Figures 1B and 3). In the decidual layer of group I, a marked degeneration of the cells (vacuolization) was seen (Figures 1A,2B,3 and 4B) (Table 1). Some

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cells contained more fibrous materials, particularly at the periphery of the decidua (Table 2). Expensive areas of congestion (Figures 2A) and hemorrhagic and deposition of acidophilic hyalinized areas were found (Table 3). Similar findings were observed in decidual layer of group II.

The trophoblastic layer of group I showed an increase in the number and size of cell clusters. Some cells showed signs of degeneration, other showed signs of death, markedly dark cytoplasm and small dark (pyknotic) peripheral nuclei. Basophilic cells (spongiotrophoblasts) showed vacuoles in their cytoplasm and degenerate nuclei (Figures 1B&2B). Glycogen cells became more numerous, cytolysis, degenerated, prominent, and occupying most of it's thickness ((Figures 4A),(Table 1). Hyaline material deposited within the cell clusters (table 2). Examination of group II revealed similar structural changes as group I, with extensive degeneration and increase in the apoptotic cells.

The labyrinth layer of groups I & II showed distortion of their plate-like architecture. Deposition of more fibrinoid material and perivascular fibrosis and abundant fetal mesenchyme were found (Table 2). Fetal blood vessels were mostly hyalinized and showed an increased cellular debris and congestions (Table 2 &3). Excess glycogen cell clusters in the walls of the labyrinth were found.

**Table 1:** Comparison of histopathological effect of Topiramate in placenta (Maternal deciduae).

Placental findings	Degeneration of decidua cells (Vacuolization)				Total	
	Present		Absent			
	NO	%	NO	%	NO	%
Group I (control) (30)	0	0.0	30	100.0	30	100.0
Group II (30)	29	96.7	1	3.3	30	100.0
Group III (30)	30	100.0	0	0.0	30	100.0
Total	59	65.6	31	34.4	90	100.0

**Table 2:** Comparison of histopathological effect of Topiramate in placenta (hyaline deposition).

Placental findings	Hyaline deposition				Total	
	Present		Absent			
	NO	%	NO	%	NO	%
Group I (control) (30)	1	3.3	29	96.7	30	100.0
Group II (30)	30	100.0	0	0.00	30	100.0
Group III (30)	30	100.0	0	100.0	30	100.0
Total	61	67.8	29	32.2	90	100.0

**Table 3:** Comparison of histopathological effect of Topiramate in placenta (congestion & hemorrhage).

Placental findings	Congestion & Hemorrhage				Total	
	Present		Absent			
	NO	%	NO	%	NO	%
Group I (control) (30)	2	6.7	28	93.3	30	100.0
Group II (30)	30	100.0	0	0.0	30	100.0
Group III (30)	30	100.0	0	0.0	30	100.0
Total	22	73.3	8	26.7	90	100.0

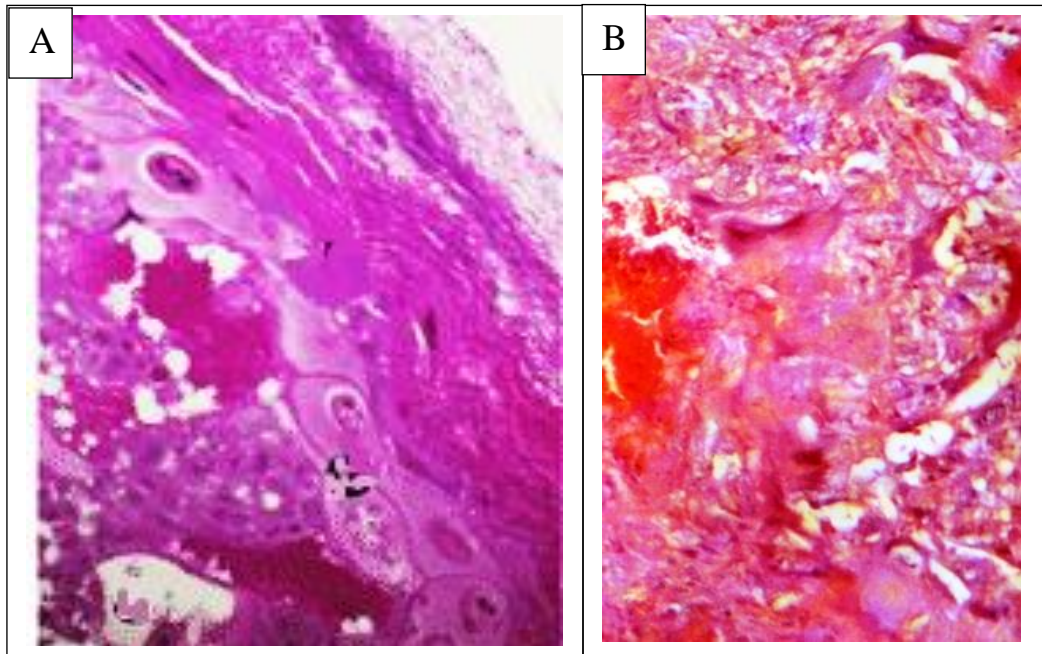


Fig. 1: Section of treated placenta stained with Haematoxylin and Eosin stain showing (A) Hemorrhagic areas & cysts and degenerate trophoblastic cells, and (B) Decidual necrosis and wide areas of hemorrhage.

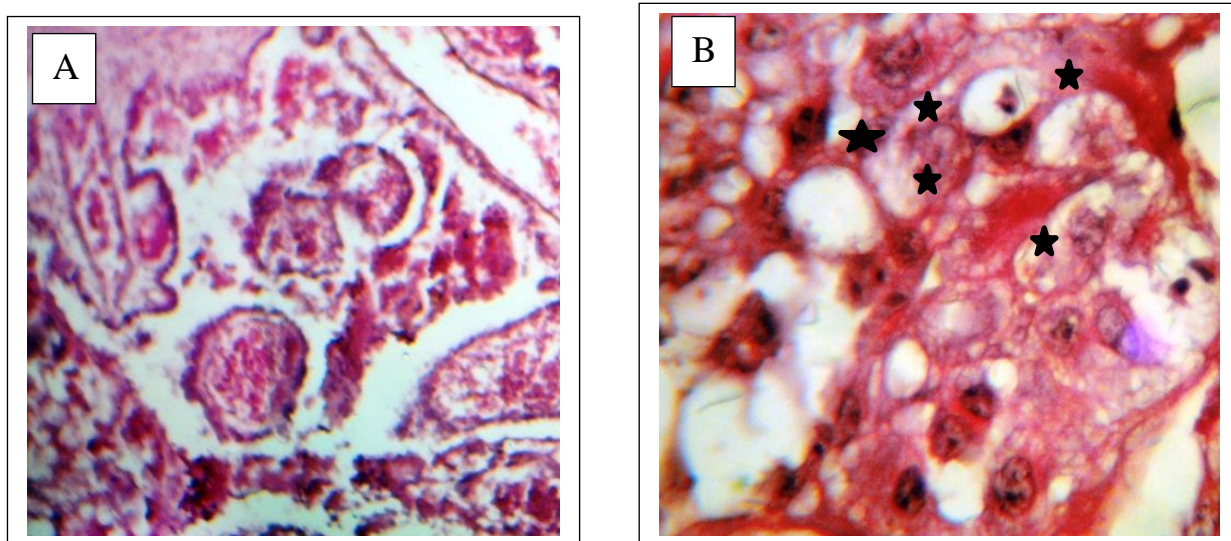


Fig. 2: Section of treated placenta stained with Haematoxylin and Eosin stain showing (A) Congestion of fetal blood vessels and hyaline deposition of its wall (thickening of blood vessel



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wall) and hemorrhage, and (B) Vacuolated and degenerated large cells, areas of hyaline deposition(stars).

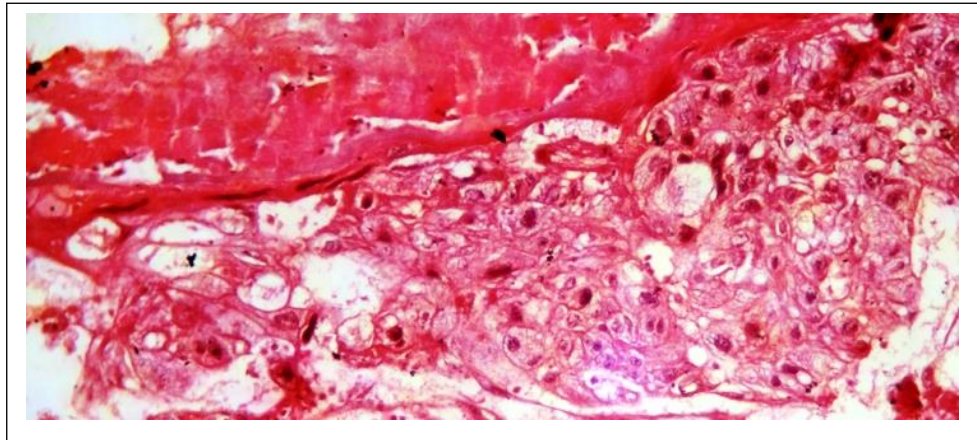


Fig. 3: Section of treated placenta stained with Haematoxylin and Eosin stain showing necrotic areas, cytolysis of glycogen cells, areas of hemorrhage and hyaline deposition.

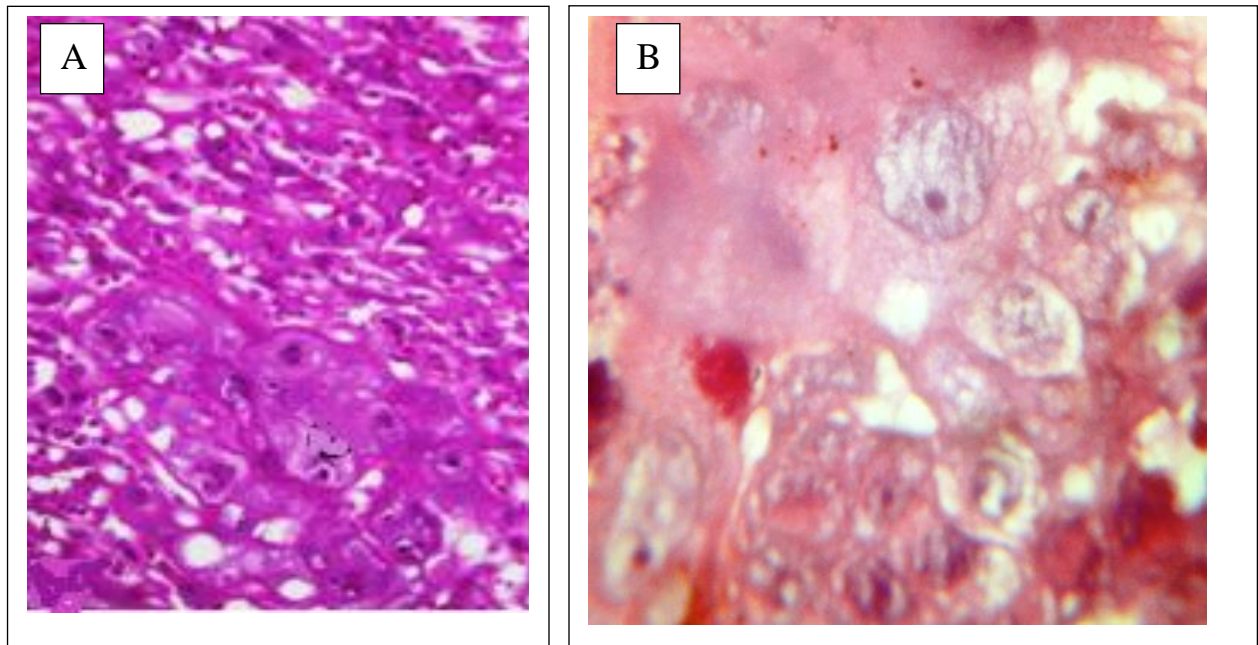


Fig. 4: Section of treated placenta stained with Haematoxylin and Eosin stain showing (A) Necrotic areas and cytolysis of glycogen cells, and (B) Alteration in nucleus and cytoplasmic vacuoles of giant cells with pyknotic nucleus

#### **Discussion:**

The placenta not only provides a link between the circulation of two distinct individuals (maternal and fetal), but also acts as a barrier to protect the fetus from xenobiotics in the maternal blood<sup>(14)</sup>. In this study, the administration of topiramate to pregnant rats resulted in degenerative

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changes affecting all layers of the placenta. This finding is in agreement with Omer et al. Emany and Omer (2017) who reported that rats treated with topiramate showed severe degeneration of placental structure and function and caused teratogenic defects<sup>(5,13)</sup>. This indicates that topiramate induced different degrees of impairment; thus, it may lead to reduced uteroplacental blood flow which appears to be associated with an increased risk of poor outcome. Our study showed that topiramate causes degeneration and necrosis at the maternal deciduas which may occur due to deficiency in the maternal vascular circulation. These results are similar to the deficient vascular development described in the homozygous gene knockout models in which deletion of the entire adrenomedullin (Adm) gene is responsible for potent, and endogenous vasodilator peptide in the germ line produces embryos with little vascularization in the yolk sac and thin umbilical cords and the few fully formed vessels were revealed to be “leaky” on angiography<sup>(13)</sup>.

Congestion and hemorrhages as well as fibrosis, deposition of hyaline material, vacuolization of cells and degeneration of cells all are structural damage of the placenta which interrupt the uteroplacental blood flow. These findings are in agreement with other studies which suggest that topiramate might cause cell degeneration and death; thus giant cells act as biological elimination of degenerated trophoblasts<sup>(11)</sup>. It has been proved that topiramate inhibits placental carnitine transport has resulted in increased fibrinoid deposition and thickening of placental barrier, thus giving us the reason for pathogenesis of teratogenic changes in fetuses<sup>(13,14)</sup>.

#### **Conclusion:**

Our results suggested that topiramate induced dose independent structural changes in the placenta; therefore it should be used with caution during pregnancy.

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## تأثير الجرعة العلاجية من عقار التوبراميت على مشيمة الفئران البيضاء

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DOI: <https://doi.org/10.47372/uajnas.2019.n1.a18>

### الملخص

تهدف الدراسة إلى توضيح التغيير النسيجي الذي يحدثه التوبراميت (أحد أدوية الصرع) على طبقات البلاسنتا لاجنة الفئران البيضاء بعد تعرضهم له داخل الرحم.

استغرقت الدراسة ستة أشهر خلال الفترة من 1 أكتوبر 2011م حتى 1 فبراير 2012م. وأجريت على 30 فأرة بالغة غير حامل و 15 ذكر بالغ من نفس السلالة لغرض التزاوج (معدل أوزانهم 150 - 200 جم) قسمت الفأرات الحوامل إلى ثلاث مجاميع: المجموعة الضابطة أعطي لها محلول ملحي، ومجموعة الدراسة الأولى أعطي لها الجرعة العلاجية من التوبراميت (50 مجم/ كج) والثانية أعطي لها الجرعة العلاجية الأخرى من العقار (100 مجم/ كج).

❖ أعطيت الجرعة يوميا عبر الفم بدء من يوم ثبوت الحمل وحتى اليوم التاسع عشر من الحمل. تم الفحص النسيجي للشرائح تحت المجهر الضوئي، باستعمال صبغة الهيماتكسولين و الايوسين. أظهرت العينات للمجموعة الأولى والثانية من الجرعة العلاجية لعقار التبروميت تغييرات في كل طبقات المشيمة ولم تختلف تلك التغييرات في مجموعة 2 عنها في المجموعة 3، وشملت تحلل خلايا المشيمة في الجدار الساقط، وترسب مادة الفيبيرين لاسيما على الجدار الساقط وفي طبقة المتاهة وحول أوعية دم الجنين. وأظهرت طبقة المتاهة في جدار المشيمة تحطم شبه كامل وتحلل لخلاياها المختلفة. و ظهر الاحتقان والنزف في كل طبقات المشيمة.

لهذا فإن عقار التوبراميت أظهر تغييرات في تركيب المشيمة ووجب اخذ هذا العقار بحذر اثناء فترة الحمل.

**الكلمات المفتاحية:** توبراميت، مشيمة، التغييرات النسيجية المرضية.