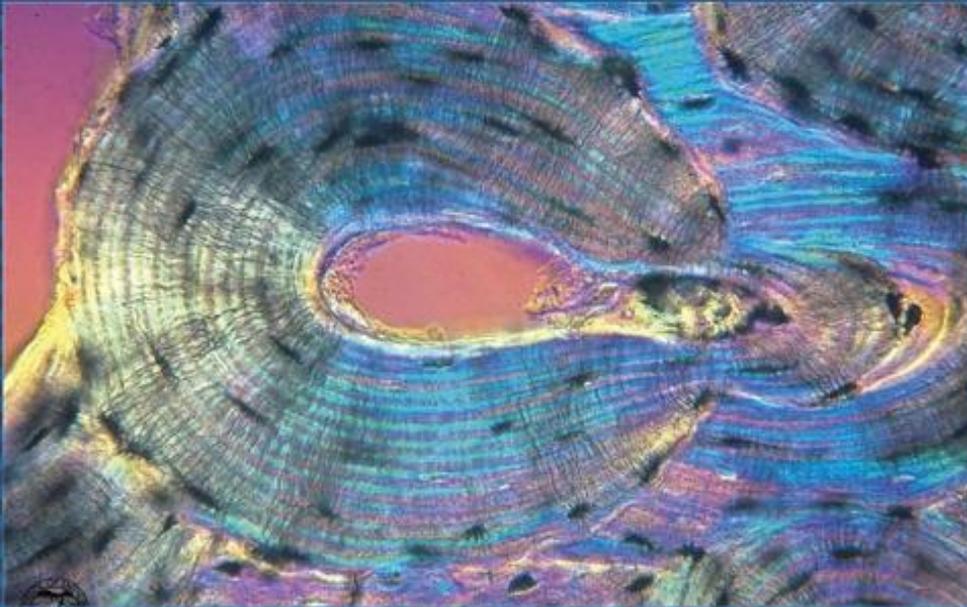




EGYPTIAN ACADEMIC JOURNAL OF  
**BIOLOGICAL SCIENCES**  
HISTOLOGY & HISTOCHEMISTRY

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ISSN  
2090-0775

[WWW.EAJBS.EG.NET](http://WWW.EAJBS.EG.NET)

Vol. 13 No. 1 (2021)



## Effect of Prenatal Topiramate Administration on Early Postnatal Expression of GFAP in Radial Glial Cells in Albino Rat Cerebellum and Spinal Cord

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### ARTICLE INFO

Article History

Received:3/2/2021

Accepted:17/3/2021

### Keywords:

Topiramate,  
cerebellum,  
spinal cord.

### ABSTRACT

**Background:** Topiramate is known as a modern drug approved for the treatment of patients with partial seizures. Radial glial cells are bipolar-shaped cells in the developing central nervous system of vertebrates. It can differentiate into other neurons and astrocytes. GFAP, Glial fibrillary acidic protein, is a glial intermediate filament protein that is known as the most comprehensive marker for astroglial cells including the radial glia

**Aim of the work:** There are few reports on topiramate administration during pregnancy. Therefore, this drug was chosen to investigate its effects, if any, especially the expression of GFAP in radial glia in the pups' cerebellum and spinal cord.

**Material and Methods:** Male and female rats were coupled in one cage for mating. On the next morning, mating was approved by the presence of sperms in the female vaginal swab, it was considered as Gestation Day zero:0. Twelve female pregnant rats were divided equally into two groups; control and topiramate treated. Topiramate drug was given to the treated group via orogastric tube from GD zero (0) till the time of delivery in a dose of 100mg/kg the control rats were administered tap water by orogastric tube. At the end of the experiment, the newly born pups (PN1) were weighed, anesthetized with Ketamine (60 mg/kg i.p.). The cerebellum and spinal cord were dissected and weighted. The specimens were fixed in Bouin's solution then processed for preparation of H& E and GFAP immunostained sections.

**Results:** In H& E-stained sections, the cerebellum in the control group was formed of subsequent layers, purkinje cells were arranged in one row, rounded or fusiform shape. GFAP positive Immunostained radial fibers (Bregman glia) were detected in granular and purkinje cell layers. The treated group showed that cells were disorganized and degenerated with focal areas of neuronal loss. Weak GFAP reaction was observed in Bregman glia in granular, purkinje and white matter cell layers. The lumbosacral segment of the spinal cord ventral horn showed large multipolar motor neurons with vesicular nucleus ("owl-eye" appearance), dendrites and axons were obvious. Glial cells with deeply stained nuclei were noticed. In the treated group, neurons appeared degenerated, vacuolated cytoplasm and pyknotic nuclei with focal areas of cellular loss. GFAP immunostained section in the control group showed an intense reaction in the radial fibers of the glia of the ventral funiculus, in contrast to the treated group which showed a faint reaction in both ventral funiculus and horn.

**Conclusion:** it could be concluded that daily regular administration of topiramate during a childbearing period in females may result in a neurotoxic effect in the cerebellum and spinal cord. So, the administration of this drug should be under strict medical observation.

## INTRODUCTION

Topiramate is known as a modern drug approved for the treatment of patients with partial seizures. It is prescribed to children with seizures. Moreover, it is safe to treat pregnant epileptic females with topiramate because it is less teratogenic than other old antiepileptic drugs (Palmieri and Canger, 2002).

Anomalies were reported in a newborn infant to a mother used topiramate during pregnancy; hirsutism and hypoplasia in the fifth nail. (Hoyme *et al.*, 1998). In addition, treatment with topiramate in pregnancy was documented to result in congenital ectrodactyly in rats and vertebral anomalies in rabbits (Kwarta *et al.*, 2006).

Radial glial cells are bipolar-shaped cells in the developing central nervous system of vertebrates (Rakic, 2009). It has the ability to differentiate into other neurons and astrocytes (Noctor *et al.*, 2001). The newly formed neurons travel along radial glial fibers for their final position (Rakic, 2009).

The cerebellar cortex is investigated in CNS neurogenesis because of its simple structure (Behesti and Marino, 2009). Bergmann glial cells, the radial glia in the cerebellum, can be observed early in development, and play a major role in the migration of granule and purkinje cells. They persist in the cerebellum in the postnatal period and are known as specialized astrocyte (Sild and Ruthazer, 2011). In mice, it is reported that there is a link between radial glial cells and the cerebellar cortex lamination from the embryonic till postnatal periods (Fricker-Gates, 2006).

During the development of the spinal cord in vertebrates, the radial glial cells are involved in gliogenesis (Fogarty *et al.*, 2005), growth of axons (Kadison *et al.*, 2006), and migration of neurons (Rakic, 2003). Lastly, radial glia change to astrocytes (Merkle *et al.*, 2004).

GFAP, Glial fibrillary acidic protein, is a glial intermediate filament protein that is known as the most comprehensive marker for astroglial cells including the radial glia (Zhang, 2001).

There are few reports on topiramate administration during pregnancy. Therefore, this drug was chosen to investigate its effects, if any, especially the expression of GFAP in radial glia in the pups' cerebellum and spinal cord.

## MATERIALS AND METHODS

### Experimental Animals:

Adult male and female rats were bought from the Faculty of Pharmacy Animal House, Mansoura University. Housing was in a properly-ventilated cage. The animals were supplied with food, water ad libitum daily. The use of animals was after the approval of Mansoura Faculty of Medicine ethical committee.

### Experimental Design:

Male and female rats were coupled in one cage for mating. On the next morning, mating was approved by the presence of sperms in the female vaginal swab, it was considered as Gestation Day zero:0.

Twelve female pregnant rats were divided equally into two groups; control and topiramate treated. Topiramate drug was crushed and dissolved in the drinking water, given to the treated group via orogastric tube from GD zero (0) till the time of delivery in a dose of 100mg/kg (Salah EL-Din

and Omar 2017). The control rats were administered tap water by orogastric tube.

#### **Histological Analysis:**

At the end of the experiment, the newly born pups (PN1) were weighed, anesthetized with Ketamine (60 mg/kg i.p.). The cerebellum and spinal cord were dissected and weighted. The specimens were fixed in Bouin's solution then processed for preparation of H& E and GFAP immunostained sections.

#### **GFAP Immunohistochemistry:**

After deparafination and hydration, sections were treated with GFAP antibody (mouse, 1:500). Sections were visualized by using 3,3diaminobenzidine. The sections were dehydrated, cleared and mounted with coverslip (Yossef *et al.*,2011). All chemicals were bought from Dako Company for chemicals.

#### **Quantitative Analysis:**

Sections were examined using Olympus R digital camera. The thickness of a molecular layer, number of purkinji cells, number of neurons in the spinal cord and the area percent of GFAP positive immunoreaction in cerebellum and spinal cord were calculated in five fixed non-overlapping fields. The measurement was done by Image J analysis software. Statistical analysis was carried out through SPSS program version 10, one-way ANOVA was used for analysis.  $P < 0.05$  was considered as significant.

### **RESULTS**

#### **Histological Results:**

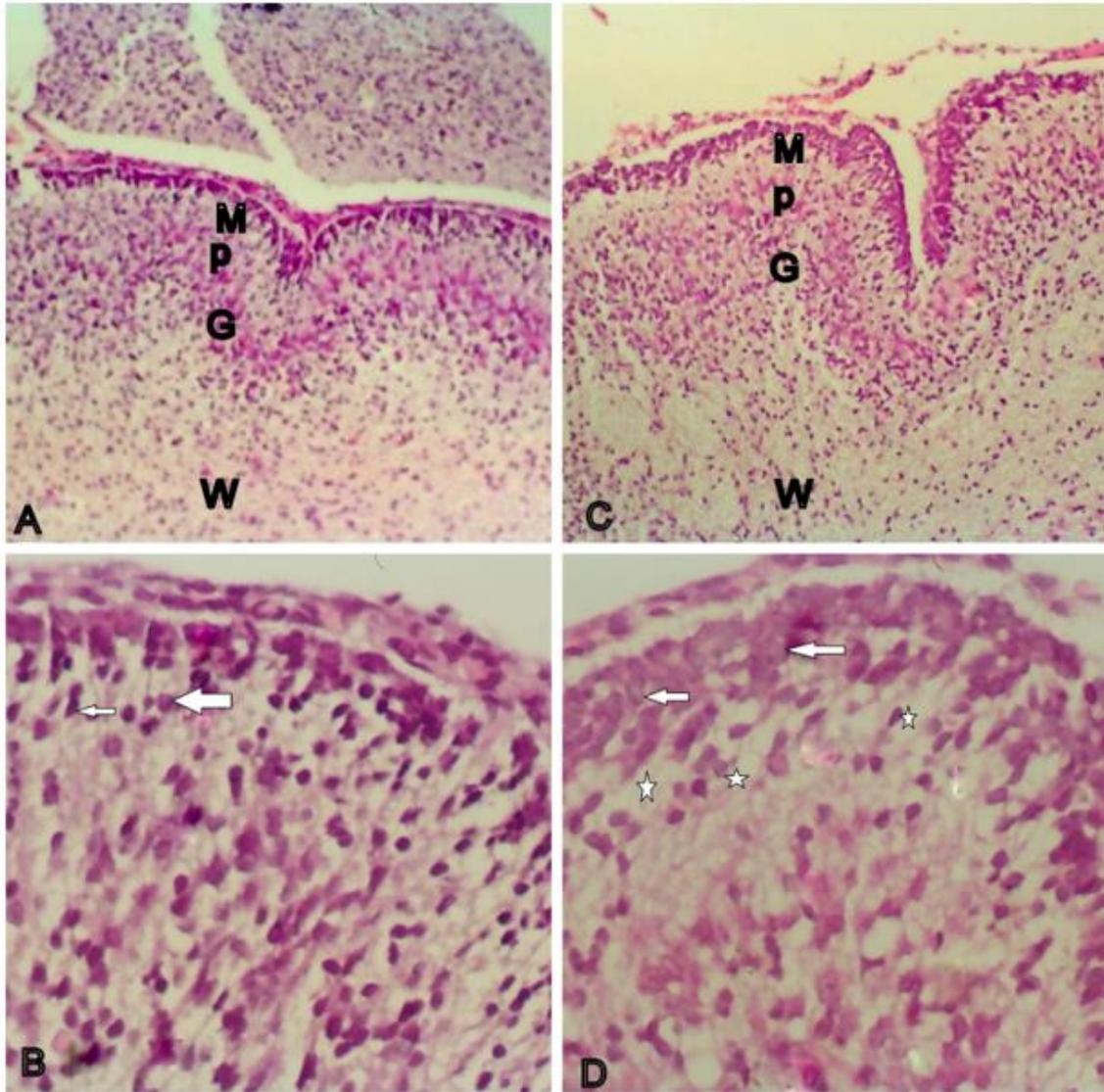
##### **1-The Cerebellum:**

In H& E-stained sections, the cerebellum in the control group

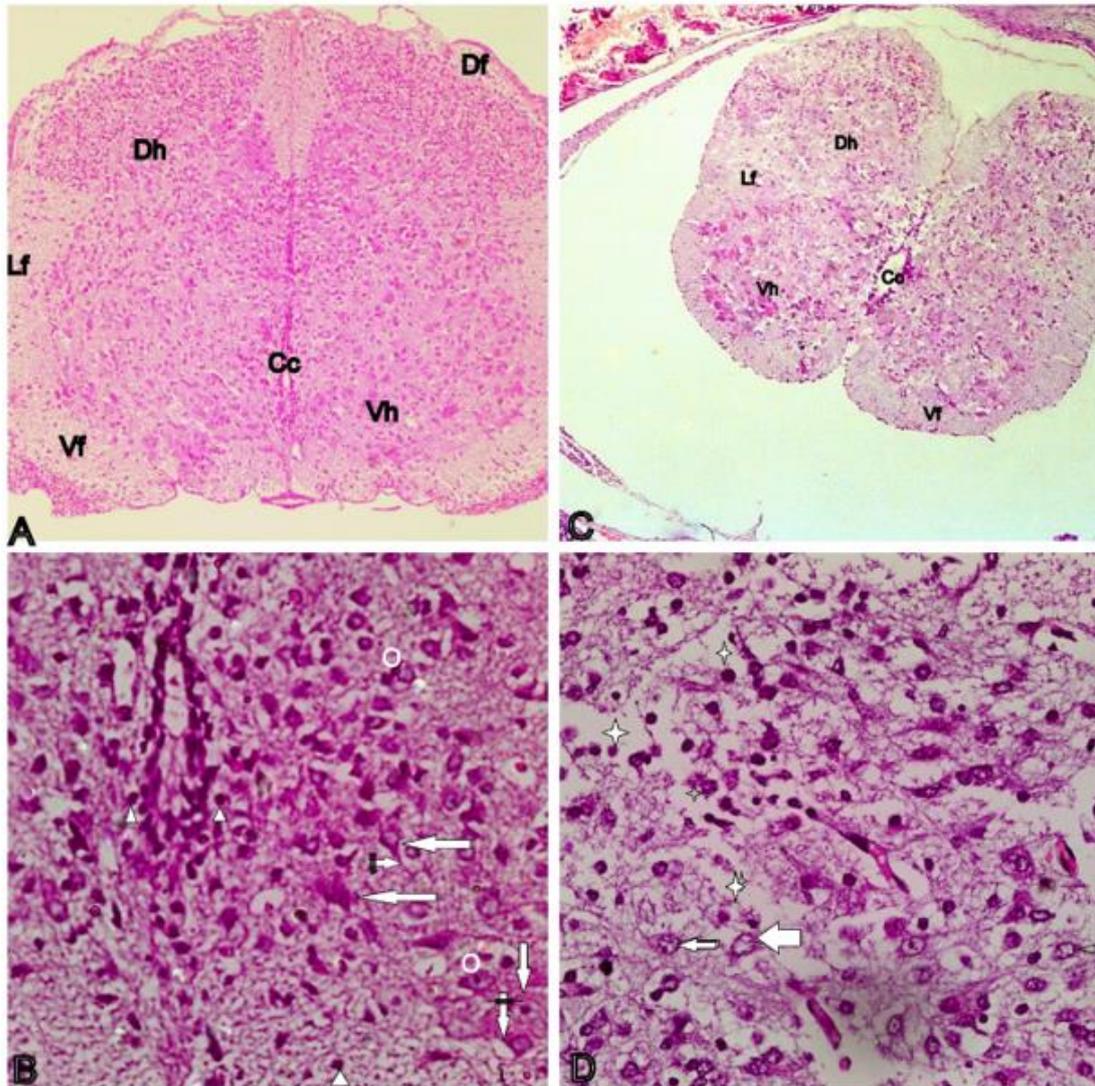
was formed of subsequent layers; molecular, purkinje, granule, and white matter. Purkinje cells were arranged in one row, rounded or fusiform shape (Fig.1: A, B). In immunohistochemistry-stained sections, GFAP positive Immunostained radial fibers (Bregman glia) were detected in granular and purkinji cell layers (Fig.3C). The treated group showed that cells were disorganized and degenerated with focal areas of neuronal loss (Fig.1: C, D). Weak GFAP reaction was observed in Bregman glia in granular, purkinje, and white matter cell layers (Fig.3D).

##### **2-The Spinal Cord:**

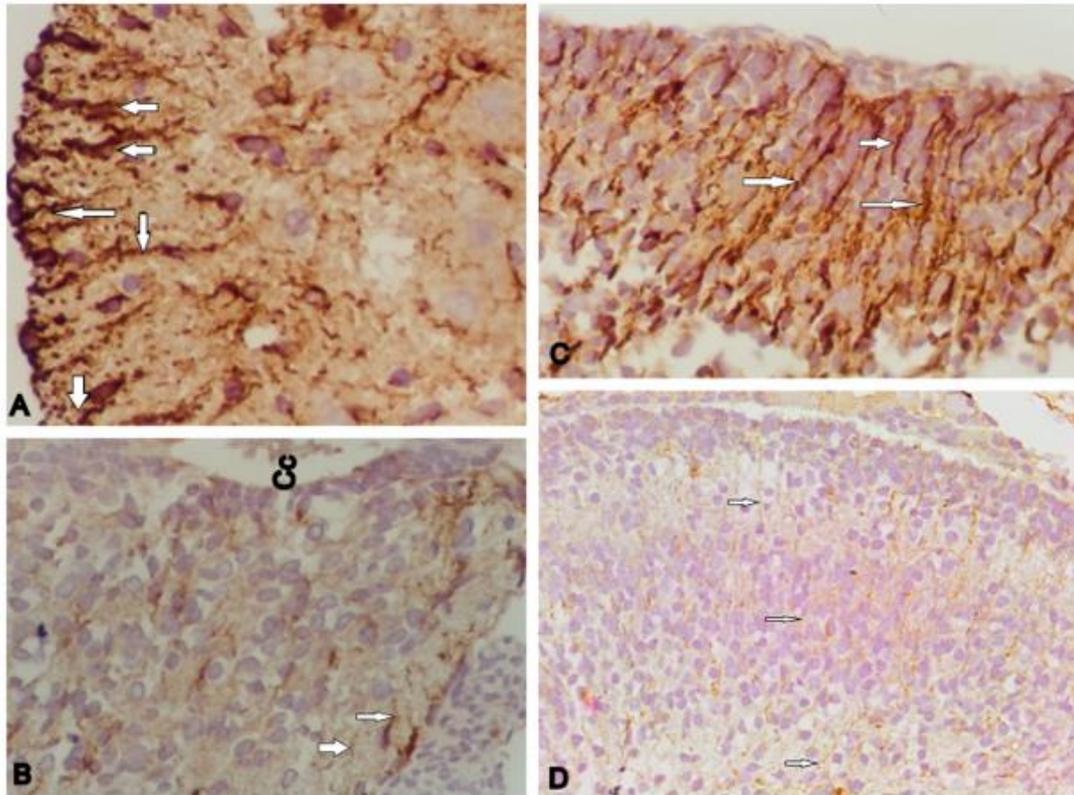
The lumbosacral segment of the spinal cord in the control group consisted of; ventral horn (Vh), dorsal horn (Dh), Ventral funiculus (Vf), dorsal funiculus (Df), Lateral funiculus (Lf) and the central canal (Cc). The ventral horn showed large multipolar motor neurons with vesicular nucleus ("owl-eye" appearance), dendrites, and axons were obvious. Glial cells with deeply stained nuclei were noticed (Fig.2: A, B). In the treated group, neurons appeared degenerated, vacuolated cytoplasm and pyknotic nuclei with focal areas of cellular loss (Fig.2:C, D). GFAP immunostained section in the control group showed an intense reaction in the radial fibers of the glia of the ventral funiculus and to a lesser extent in the ventral horn (Fig.3: A). while the treated group showed a faint reaction in both ventral funiculus and horn (Fig.3: B).



**Fig.1:** A, B. photomicrograph in the control cerebellum formed of subsequent layers; molecular (M), purkinje (p), granule(G) and white matter (W). The Purkinje cells were arranged in one row, rounded or fusiform shape (arrows). C, D. The treated group shows that cells were disorganized and degenerated (arrows) with focal areas of neuronal loss ( \* )(H&E stain,A,CX100; B,D X400)



**Fig.2:**A. The lumbo-sacral spinal cord in control group consists of ; ventral horn (Vh),dorsal horn (Dh), Ventral funiculus (Vf), dorsal funiculus (Df) , Lateral funiculus (Lf) and central canal (Cc).**B.** The ventral horn shows large multipolar motor neurons with vesicular nucleus (arrows), dendrites, and axons are obvious (crossed arrows), cells with 'owl-eye appearance' (o). Glial cells with deeply stained nuclei were noticed (arrow head). **C.** The treated group, lumbo-sacral spinal cord consists of ; ventral horn (Vh),dorsal horn (Dh), Ventral funiculus (Vf), dorsal funiculus (Df), Lateral funiculus (Lf) and central canal (Cc). **D.** neurons appears degenerated, vacuolated cytoplasm and pyknotic nuclei (arrows) with focal areas of cellular loss (\*) (H&E stain,A,CX100; B,D X400) .



**Fig.3:** A. The lumbosacral spinal cord in control spinal cord shows intense GFAP reaction in the radial fibers of the glia of the ventral funiculus and to lesser extent in the ventral horn (arrows). B. GFAP immunostained section in treated spinal cord shows faint reaction in both ventral funiculus and horn (arrows). C. GFAP positive Immunostained radial fibers (Bregman glia) is detected in granular and Purkinje cell layers of control cerebellum (arrows). D. Weak GFAP reaction is observed in Bregman glia in granule, Purkinje and white matter cell layers of treated cerebellum (arrows). (GFAP immunostain A&C X100; B, DX400).

### Morphometric Results:

#### 1- Body and Cerebellar Weights of The Rats:

Topiramate treated rats exhibited a significant change in their body and cerebellar weights in comparison to the control rats (Graph 1).

#### 2- Thickness of Molecular Layer:

Significant thinner layer was detected in the treated group. (Histogram.1).

#### 3- Number of Purkinje Neurons in Cerebellum:

Significant decrease in the number of neurons in the treated group (Histogram.2).

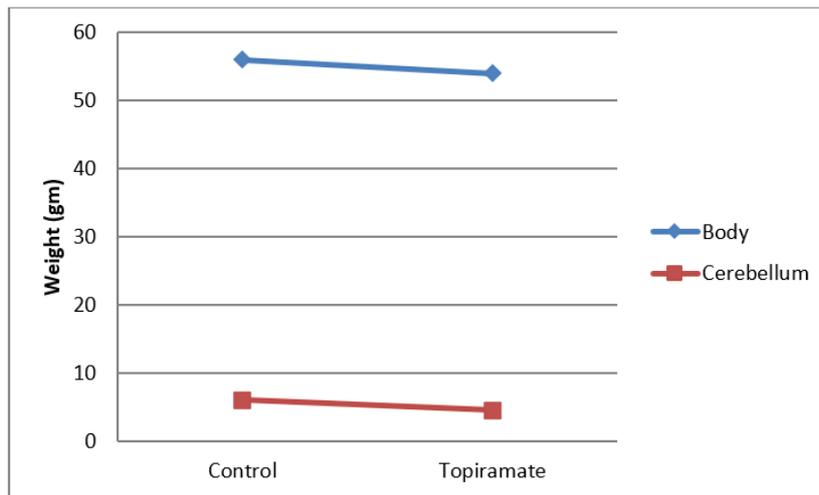
#### 4- Number of neurons in the spinal cord:

Significant decrease in the number of neurons in the treated group (Histogram.3).

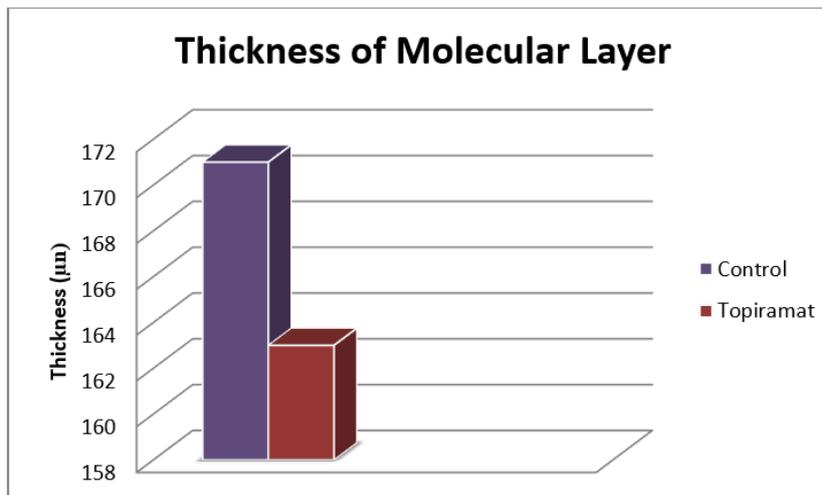
#### 5- Area % of GFAP Positive Fibers in Cerebellum and Spinal Cord:

Significant reduction in the treated group (Histogram.4).

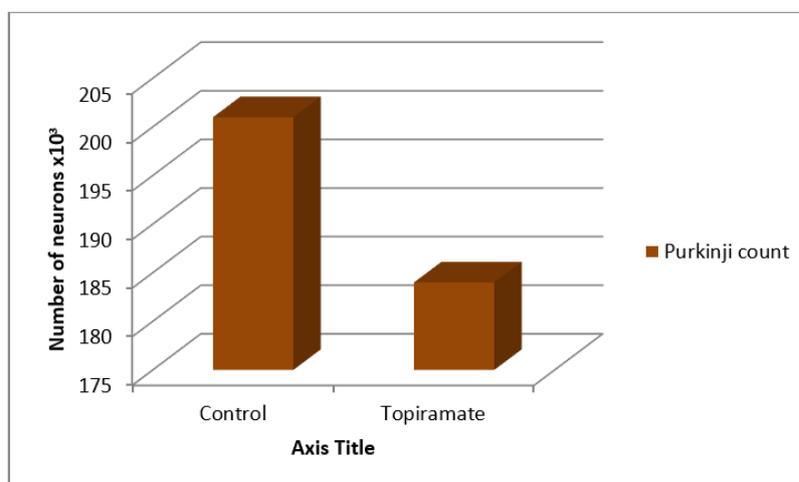
## Effect of topiramate on GFAP in radial glia in cerebellum and spinal cord<sup>37</sup>



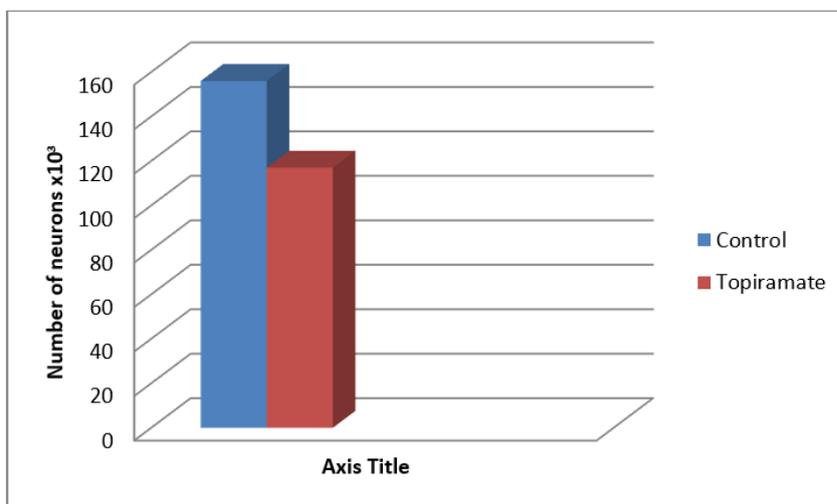
**Graph. 1:** Topiramate treated group exhibited an insignificant change in their body and cerebellar weights in comparison to the control group.



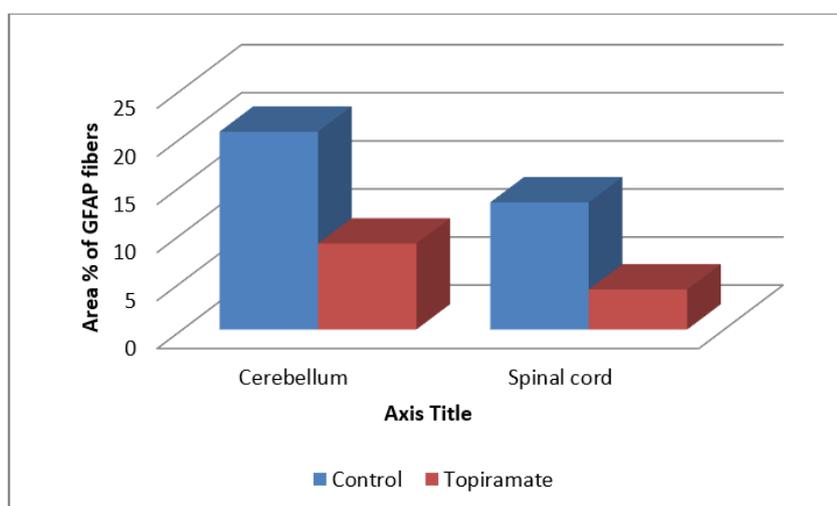
**Histogram.1:** Significant thinner molecular layer (measured in µm) was detected in the treated group.



**Histogram.2:** Number of purkinje neurons in cerebellum showed a significant decrease in the number of neurons in the treated group.



**Histogram.3.** A number of neurons in the spinal cord showed a significant decrease in the number of neurons in the treated group.



**Histogram.4:** Area % of GFAP positive fibers in cerebellum and spinal cord showed a significant reduction in the treated group.

## DISCUSSION

Topiramate is commonly used in females in the childbearing period. It was approved as a prophylaxis for migraine in 2004 in the United States (Kurth and Hernandez-Diaz,2010). Also, it could be also used in females with eating disorders (Tata and Kockler,2006), sleep disorders (Aurora *et al.*,2010), and other psychological disorders (Berlin *et al.*,2011).

In this study, the newly born pups showed a non-significant change in the body or cerebellar weights among treated and control groups. This comes in agreement

with Singh and Mishra (2005) who reported that topiramate has no effect on the body weight of newborn pups, while Ogura *et al.* (2002) reported that other antiepileptic drugs like phenytoin induced reduction in brain weight in neonatal mice brain and Allam *et al.* (1987) who reported a reduction in the size of the chick cerebellum treated with phenytoin.

The control cerebellum in this work was formed of subsequent layers; molecular, purkinje, granule and white matter. Purkinje cells were arranged in one row, rounded or fusiform shape. In immunohistochemistry-stained

sections, GFAP positive Immunostained radial fibers (Bregman glia) were detected in granular and purkinje cell layers. While the treated cerebellum showed that cells were disorganized and degenerated with focal areas of neuronal loss. According to Hoogland and Kuhn (2010), Bregman glia in the cerebellum, which are a subtype of radial glia, are parallel in a direction in the rodent cerebellum mainly in rostrocaudal axis. Also, in zebrafish, Bergmann glia, was observed molecular layer of the anterior cerebellar lobes (Than-Trong and Bally-Cuif, 2015).

In parallel to our results that molecular cell layer was thinner in treated cerebellum than that in the control cerebellum and the cells were disorganized and degenerated with focal areas of neuronal loss, it was reported that molecular layer in the treated cerebellum with phenytoin was thinner than that of the control group in mice, also, phenytoin administration in high or low doses in the neonatal mice resulted in pyknotic cells in the granular cell layer (Ohmori *et al.*, 1999).

Topiramate treated pups showed weak GFAP reaction in Bregman glia in granular, purkinje and white matter cell layers of the cerebellum. A similar result was reported in cisplatin administration, the rat cerebellum showed altered radial glia fibers (Pisu *et al.*, 2005). Also, GFAP positive reactions in the hippocampus of topiramate-treated rats were significantly less than in the control rats (Han *et al.*, 2008).

In this study, the lumbosacral spinal cord in the control group showed the ventral horn was formed of large multipolar motor neurons with vesicular nuclei. While, the treated group, some neurons appeared

degenerated, vacuolated cytoplasm and pyknotic nuclei. This finding may be explained by the fact that topiramate causes a deficiency in vitamin B12 and folic acids which are vital in nerve cell formation in all nervous system (Ray, 2011).

GFAP immunostained section in the control group showed an intense reaction in the ventral funiculus and to a lesser extent in the ventral horn. Similar localization of GFAP-positive radial cells was reported on the surface of the spinal cord in zebrafish (Than-Trong and Bally-Cuif, 2015). In contrast, the treated group showed a faint reaction in the spinal cord. Similar results were observed with lamotrigine, an anticonvulsant drug, it reduced the expression of GFAP in the spinal cord lumbosacral region of treated rats (Jun *et al.*, 2013).

Our findings in this study could be explained according to the following mechanisms; it is well known that topiramate reduces the activity of the voltage-dependent sodium channels and potentiates the inhibitory neurotransmitter GABA (Porter and Meldrum, 2004). At the same time, it was reported that GABA-A receptors are located in astrocytes and Bregmann glia in the cerebellum (Farber K, Kettenmann, 2005), and in the spinal cord (Bohlhalter *et al.*, 1996). While GABA-B receptors are expressed in glial cells in cerebellum, cerebral cortex, and spinal cord (Pastor *et al.*, 1995). Activation of GABA receptors in GFAP-expressing cells results in inhibition of proliferation of those cells (Liu *et al.*, 2005).

From this study, it could be concluded that daily regular administration of topiramate during the childbearing period in females may result in neurotoxic effect in the cerebellum and spinal cord. So, the administration of this drug

should be under strict medical observation.

### Conflicts of Interest

The author declares that she has no conflict of interest.

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### ARABIC SUMMARY

تأثير اعطاء عقار التوبرامات قبل الولادة على اباتة البروتين الغروي الليفى الحمضى في الخلايا الدبقية الشعاعية مبكرا بعد الولادة فى المخيخ والنخاع الشوكي للفأر الأبيض

هاجر عطا الله حشيش

قسم التشريخ والاجنة - كلية الطب- جامعة المنصورة

يستهدف هذا البحث دراسة تأثير التعرض لعقار التوبرامات فى فترة ما قبل الولادة على المخيخ والنخاع الشوكي للفئران. تم استخدام 12 من اناث الفئران البيضاء البالغة الحامل، وقسمت الى مجموعتين: 6 فئران فى كل مجموعة. استخدمت الاولى كمجموعة ضابطة، اعطيت ماء مقطر عن طريق الفم من بدايه الحمل وحتى وقت الولادة. اعطيت المجموعة الثانية عقار التوبرامات المذاب فى الماء عن طريق الفم (100مجم/كجم). بعد الولادة، تمت التضحية بالفئران الحديثه الولاده من كل مجموعة وجمعت عينات من المخ وتم تجهيزها لعمل قطاعات من شمع البارافين وصبغت بالهيماتوكسيلين والايوسين للدراسة الهستولوجية وصبغت أيضا والصبغة المناعية للبروتين الغروي الليفى الحمضى. وتم استخدام جهاز تحليل الصور لقياس حجم وعدد الخلايا فى المخيخ والنخاع الشوكي، كما تم خضوع هذه القياسات للعمليات الاحصائية اللازمة.

لم يسبب عقار التوبرامات تغيرا ملحوظا فى وزن الجسم او وزن المخيخ فى المجموعه المعالجه مقارنة بالمجموعة الضابطة. تسبب عقار التوبرامات فى تقليل عدد وحجم الخلايا فى كلا من المخيخ والنخاع الشوكي فى المجموعه المعالجه مقارنة بالمجموعة الضابطة. كما ادى عقار التوبرامات الى انخفاض ابانه البروتين الغروي الليفى الحمضى فى طبقات الخلايا فى كل من المخيخ والنخاع الشوكي للفئران المعالجه بالمقارنة مع الفئران فى المجموعة الضابطة.

ونستنتج من هذه الدراسة أن استخدام عقار التوبرامات اثناء الحمل بشكل يومي يمكن أن يؤدي الى تأثيرات عصبية سمية طويلة الأمد فى المخيخ والنخاع الشوكي مما قد يؤثر على الوظائف العصبية.