Original Article

Structural Alterations in the Rat Cornea and Retina Induced by Topiramate, A Histological and Immunohistochemical Study

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ABSTRACT

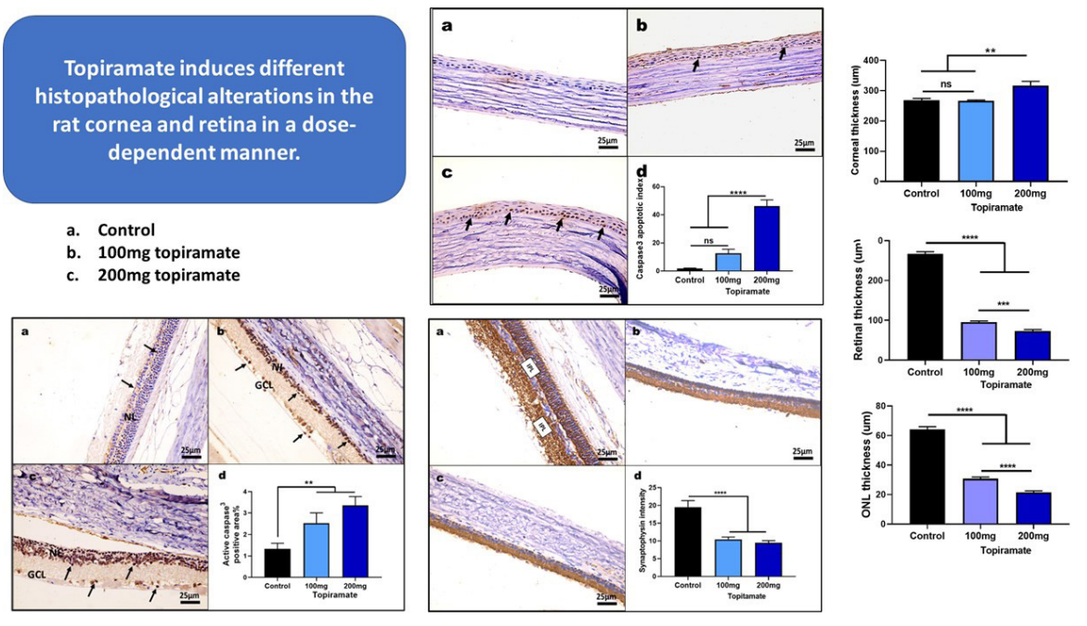
Introduction: The cornea is the chief refractive element of the eye while the retina is a portion of the nervous system containing the photoreceptor cells which process and transmit the light information to the brain. Topiramate is one of the antiepileptic drugs that is mainly used for the treatment of seizure disorder and migraine. However, its administration may be associated with eye pain and blurred vision.

Aim of the Work: This study is aimed to explore the impact of topiramate on the structure of rat cornea and retina using different histological, and immunohistochemical techniques.

Material and Methods: Thirty adult male albino rats were used in this study and were divided equally into three groups: the control group and two experimental groups that were administered topiramate at doses of 100mg, and 200mg/kg B.W. orally once daily for 28 days. After the experimental duration, the rats were sacrificed, and the eyeballs were dissected for staining with H&E, and Masson's trichrome in addition to immunolabelling with anti-caspase 3, and anti-synaptophysin, which is before a presynaptic protein that is closely linked to synaptic activity in the retina.

Results: The cornea showed apoptotic vacuolated epithelial cells, disorganization and wide separation of scleral fibers, focal loss of Descemet`s membrane and endothelium, with a significant increase in corneal thickness and caspase 3 apoptotic index in 200 mg topiramate group. The retina revealed a wide separation between the retinal layers and loss of demarcation between the inner and the outer nuclear layer with cellular vacuolation that showed pyknotic hyperchromatic nuclei. In addition to, disorganized scleral fibers, congested blood vessels, a significant reduction in retinal thickness and synaptophysin intensity, and a significant increase in caspase 3 area percentage.

Conclusion: The present findings indicate that topiramate induced adverse effects on the eye structure and it is recommended to be used strictly under medical supervision.



Graphical Abstract

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Key Words: Caspase3; cornea; retina; synaptophysin; topiramate.

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INTRODUCTION

Topiramate is one of the anticonvulsants and antiepileptic drugs that is mainly used to control seizures, prevention of migraine, and management of alcohol disorders and obesity[1,2,3].

Topiramate administration is also associated with a variety of adverse effects on the structure and function of different body organs such as the kidney and reproductive system[4,5]. It also induced fatigue, and dizziness with impairment of concentration and memory[6,7]. Various ocular undesirable effects and retinal dysfunction have been also reported with topiramate administration such as acute angle-closure glaucoma and acute onset myopia which consider the most ocular adverse impact of topiramate. Besides, ocular pain, headache, suprachoroidal effusions, scleritis, retinal hemorrhage, and visual field defects[8,9].

Topiramate is rapidly absorbed from the gastrointestinal tract with good bioavailability after each oral dose. It distributes to all the body tissues and organs with a half-life of about (20–30h) and it is mainly excreted unchanged through the kidney[10,11].

Topiramate produces its effects through a wide range of mechanisms of action. It blocks the calcium and the voltage-dependent sodium channels that can interfere with an ionic concentration in different body tissues. Topiramate also reduces both the carbonic anhydrase activity and the excitatory glutamate pathway. However, it enhances the inhibitory activity of gamma-aminobutyric acid (GABA), a significant inhibitory neurotransmitter in the CNS[12]. It was reported that prolonged topiramate administration induced irreversible inhibition of GABA transaminase with excessive accumulation of GABA, which is considered the main reason for neuronal activity fall, and morphological abnormalities with considerable loss of retinal function[8,13].

So, this experiment aimed to expose the impact of two different doses of topiramate on the structure of the cornea and the retina of the adult male rat.

MATERIAL AND METHODS

Chemicals

Topiramate was purchased in the form of film-coated tablets for oral use containing 100mg each (Sabaa, Egypt). Topiramate solution was prepared at a concentration of 40 mg/1ml by grinding 5 capsules (500 mg) and dissolving them in 12.5 ml of distilled water.

Experimental animals

Thirty adult male albino rats were used in this experiment with an average body weight of 200-250 grams each. The rats were preserved in the animal facility unit of the histology department, Faculty of Medicine, Tanta University. They were maintained for 1 week before starting the experiment without getting any treatment for acclimatization in well-ventilated cages with unrestricted access to diet and water. This experiment was done following the standard of the ethical committee of Tanta University (Approval number: 35226), which is in agreement with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Study design

The experimental animals were divided into three experimental groups with 10 rats in each:

Group I (control group): the rats were kept with no treatment through the experimental period.

Group II (100mg topiramate-treated group): the rats were administered 0.5ml of prepared topiramate solution (100 mg/kg body weight) orally, once, daily by a gastric tube for 28days[14].

Group III (200mg topiramate-treated group): the rats were administered 1ml of prepared topiramate solution (200 mg/kg body weight) orally, once, daily by a gastric tube for 28days[14].

Twenty-four hours after the last dose of topiramate treatment, the rats were anesthetized by an intraperitoneal injection of 50 mg/kg of sodium pentobarbital[15]. Next, the rats were sacrificed, and their eyeballs were enucleated and fixed immediately in 10% neutral-buffered formalin for 24 hours at 4ºC. Subsequently, the eyeballs were washed, dehydrated, cleared, and processed for paraffin wax embedding. The 5µm thickness of the eyeballs (5µm thickness) was deparaffinized with two changes of xylene, rehydrated with descending grades of alcohol (100%, 90%, 70%), and running tap water for staining with hematoxylin and eosin (H&E) and Masson trichrome stains to observe the structure of rat cornea and retina[16].

Caspase-3 and Synaptophysin immunohistochemistry

For immunohistochemical staining, the eyeball sections were deparaffinized in xylene and rehydrated in descending grades of alcohol and distilled water. Then the sections were placed in preheated 10 mM sodium citrate buffer (pH 6.0) for 30 min in a hot water bath at 85 ºC for antigen retrieval. Next, the sections were left to slowly cool at room temperature for another 30 min. The endogenous peroxidase was inactivated by incubating the sections in 0.3% H_2O_2 in methanol for 30 min followed by proper rinsing in PBS. The sections were then incubated with 5% goat serum in phosphate buffer saline/triton-X100 for 30min to block the nonspecific background staining. Next, the sections were incubated with primary antibodies (Rabbit Polyclonal anti-Caspase-3, cell signaling, 9662 and Rabbit Polyclonal anti- Synaptophysin antibody, Abcam, ab14692) overnight at 4ºC. After proper rinsing in PBS, the sections were incubated with secondary antibody (Biotinylated Goat anti-Rabbit IgG (ab64256) solution for 30 min at RT followed by enzyme-labeled streptavidin and proper rinsing in the buffer. The sections were then incubated with DAB as a chromogen for 10min, counterstained with hematoxylin, dehydrated, and

mounted with DPX. The stained sections were examined under the light microscope and their photomicrographs were imaged and analyzed. Negative control was done by the similar preceding steps without adding the primary antibody which showed the absence of immunostaining. The positive immunoreactivity for caspase-3 appeared as brown nuclear staining in the immunoreactive cells, while synaptophysin appeared as brown staining in the synapses of outer& inner plexiform layers[17,18].

Morphometric Study

Ten different fields at the magnification of X400 from each cornea and retina were analyzed using Image J analysis software for the following parameters:

1. The total thickness of the cornea[19].
2. The total thickness of the retina[20].
3. The thickness of the outer nuclear layer of the retina[20].
4. The area percentage of caspase-3 immunostaining in the corneal epithelial layer and the retina[21,22].
5. The intensity of synaptophysin immunostaining in the retina[20].

Statistical analysis

The morphometric data were analyzed using GraphPad Prism 8 software. One-way analysis of variance (ANOVA) followed by Tukey's test was used to compare all studied groups. The value was expressed as mean ± standard error of the mean (Mean ± SEM). Differences were regarded as statistically significant if the probability value was ($p< 0.05$)[23].

RESULTS

The cornea

Investigation of H&E stained sections of the control group displayed the normal histological structure of the cornea that includes five layers; the non-keratinized stratified squamous epithelium, Bowman's layer, the stroma with regularly arranged parallel collagen fibers, and scattered spindle-shaped stromal cells, the thick homogenous non-cellular layer of Descemet`s membrane and the endothelium with a single layer of flat cells and flat nuclei (Figure 1a). On the other hand, the (100mg) topiramate treated group showed irregular corrugated corneal epithelium with vacuolated epithelial cells (Figure 1b). In addition to disorganization and separation of stroma's collagen fibers (Figure 1c). Although the (200mg) topiramate-treated group showed corneal epithelium with dark nuclei and vacuolated cytoplasm, some desquamated epithelial cells, focal area of complete loss of epithelium (Figures 2 a,b). The stroma showed an apparent increase in stromal thickness. Besides disorganized widely separated collagen fibers and area of eosinophilic homogenization with some inflammatory cellular infiltration. There was also focal loss of Descemet`s membrane and endothelium (Figures 2 b,c).

Masson's trichrome staining of corneal sections showed the control cornea with blue staining regularly arranged stromal collagen fibers (Figure 3a). On the other hand, the (100 mg) topiramate treated group displayed focal areas of wide separation between the stromal collagen fibers (Figure 3b). However, the corneal sections of the (200mg) topiramate treated group presented with widely separated stromal collagen fibers (Figure 3c).

Quantitative analysis of the corneal thickness of the experimental groups revealed a significant increase in the corneal thickness of the (200mg) topiramate-treated group (317.7±13.40) compared to the control group (268.8± 5.996) and the (100mg) topiramate-treated group (266.6± 2.806) (Histogram 1).

Caspase-3 immunohistochemical staining of the cornea showed a negative caspase-3 reaction in the control group (Figure 4a). A low number of apoptotic cells was present in the corneal epithelium of the (100mg) topiramate-treated group. However, the (200mg) topiramate treated group showed an increase in caspase-3 immunostained cells in the corneal epithelium, especially in the basal columnar cells (Figures 4 b,c). These findings were confirmed by quantitative analysis of the caspase-3 apoptotic index in the corneal epithelial layer of experimental groups, which showed significant growth in the apoptotic index in the (200mg) topiramate treated group (46.22±4.461) compared to the control (1.750± 0.4787 and the (100mg) of topiramate treated groups (12.66± 2.926). Moreover, there was a small insignificant increase in the caspase3 apoptotic index in the 100mg topiramate treated group compared to the control group (Figure 4d).

The retina

Examination of H&E stained sections of the control group showed the normal histological layers of the retina; retinal pigment epithelium, photoreceptor layer of rods and cones, outer limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer, and an inner limiting membrane. In addition to the overlying sclera and choroid (Figure 5).

On the other hand, topiramate-treated groups presented with a variety of structural alterations in the retina. The (100mg) topiramate treated group showed a wide separation between the retinal layers, especially between the outer nuclear layer and the inner nuclear layer as well as between the retinal pigment epithelium and the photoreceptor layer of rods and cones with some cellular vacuolation that showed pyknotic hyperchromatic nuclei (Figures 6 a,b). This group also presented with disorganization of scleral fibers, extravasation of blood cells in the choroid, and the detachment of the inner limiting membrane in some areas (Figure 6c). Dilated and congested blood vessels were also seen in this group with a reduction in total retinal thickness (Figure 6d).

As well, the (200mg) topiramate-treated group revealed disorganization with total loss of normal architecture of the retina in the form of focal separation between the retinal pigment epithelium and the photoreceptor layer of rods and cones, loss of demarcation between outer and inner nuclear layers, with multiple cellular vacuolations and apoptotic nuclei. There was disorganization and destruction in the scleral fibers with congested blood vessels (Figure 7 a,b,c).

Morphometric analysis of the retina also revealed a significant decrease (****P<0.0001) in the total thickness of the retina in the topiramate treated groups compared to the control group (266.4±5.789) with a significant reduction (***P<0.001) in the 200mg topiramate treated group (73.39±3.529) compared to that in the 100mg treated group (95.74±2.733) (Histogram 2a). Furthermore, there was a significant decrease (****P<0.0001) in the thickness of the outer nuclear layer in the topiramate treated groups compared to the control group (64.16±1.875), with a significant decrease (****P<0.0001) in the 200mg topiramate treated group (21.55± 0.9197) compared to the 100mg treated group (30.75± 1.180) (Histogram 2b).

Caspase-3 immunohistochemical staining of the retina showed a few positive immunolabelling cells in the nuclear layers of the control retina (Figure 8a). While there was an increase in the number of apoptotic cells in the nuclear cell layer and the ganglion cell layer in (100mg) and (200mg) topiramate-treated groups compared to the control group (Figures 8 b,c). Quantitative analysis of caspase-3 positive area percentage in the retina of the experimental groups revealed a significant increase (**P<0.05) in the 100mg (2.526± 0.4814) and the 200mg (3.358± 0.4121) topiramate treated groups compared to the control group (1.336± 0.2586), with an insignificant increase in the (200mg) compared to the (100mg) topiramate treated groups (Figure 8d).

The retina was immunostained with synaptophysin, a presynaptic vesicular protein to investigate the effect of topiramate on synaptic integrity and plasticity. The control group revealed a strong positive brown reaction to synaptophysin in both inner and outer plexiform layers of the retina (Figure 9a). On the other hand, the (100mg) & (200mg) topiramate-treated groups showed weak positive immune reaction for synaptophysin, in the outer and inner plexiform layers (Figures 9 b,C). Quantitative analysis of the mean grey value of synaptophysin in the retina of the experimental groups revealed a significant reduction (****P<0.0001) in the synaptophysin intensity in the 100mg (10.46±0.6298) and the 200mg (9.508±0.5642) topiramate treated groups in comparison to the control group (19.54±1.807), with no significant difference between both topiramate-treated groups (Figure 9d).

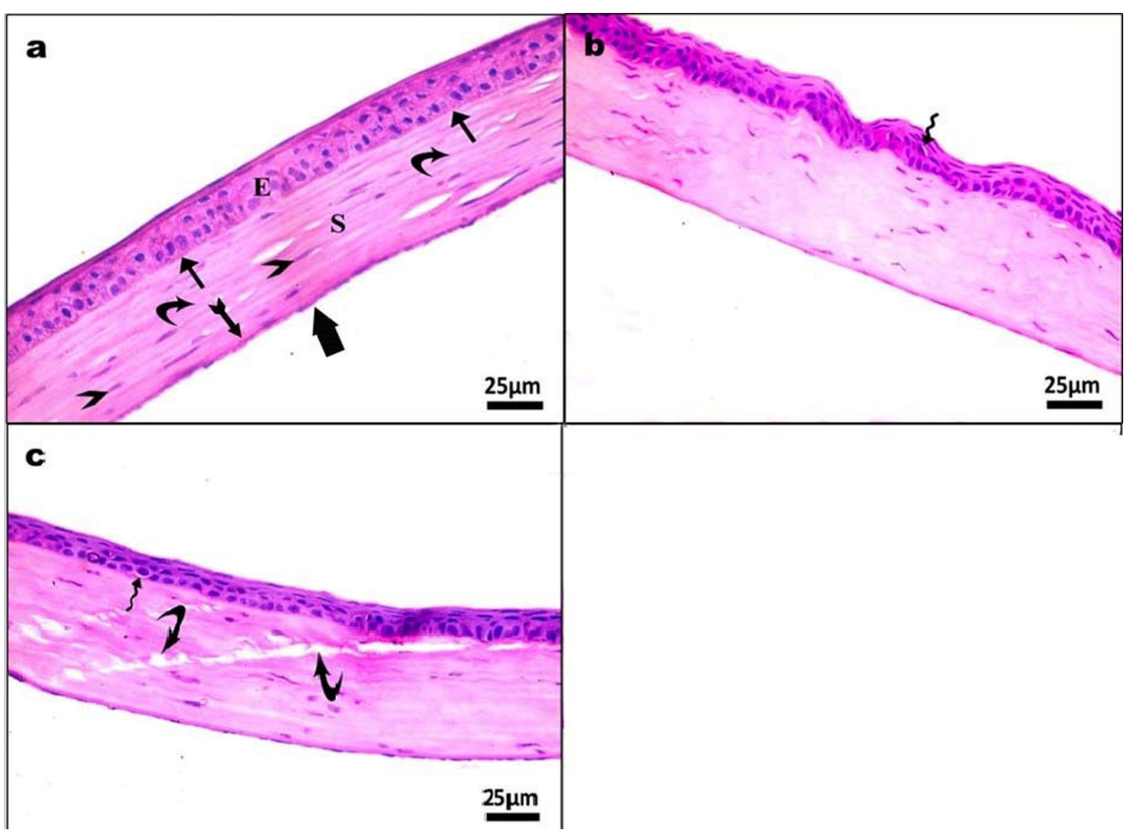


Fig. 1: Photomicrographs of the cornea showing [a] the five layers of control cornea; non keratinized ŝtratified squamous epithelium (E), Bowman's layer (arrow), ŝtroma (S) with regularly arranged parallel collagen fibers (curved arrow), and spindle-shaped ŝtromal cells (arrowhead), Descemet`s membrane (bifid arrow) and the endothelium (thick arrow). The (100mg) topiramate treated group showed [b] irregular corrugated corneal epithelium and vacuolated cytoplasm (zigzag arrow). [c] epithelial cells with vacuolated cytoplasm (zigzag arrow) with disconnection of ŝtroma's collagen fibers (curved arrow). (H&E. X400).

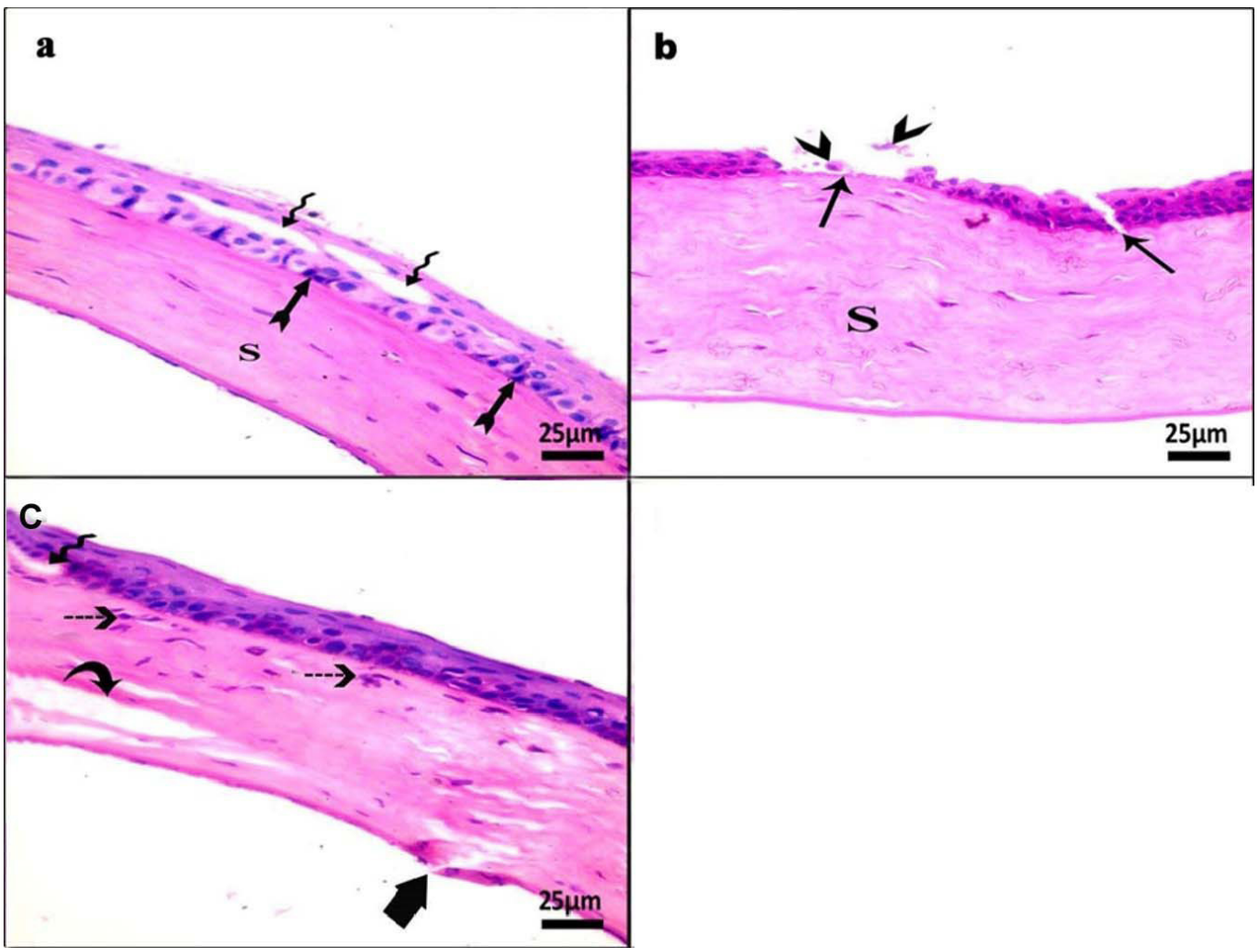


Fig. 2: Photomicrographs of the cornea from the (200mg) topiramate-treated group showing [a] ballooned vacuolated superficial epithelial cells (zigzag arrow), dark small nuclei (bifid arrow) with, eosinophilic homogenization of the stroma (S). [b] desquamated epithelial cells (arrowhead), focal area of epithelial loss (arrow), and stromal thickening (S). [c] vacuolated epithelial cell (zigzag arrow), stroma with disorganized widely separated collagen fibers (curved arrow), inflammatory cellular infiltration (dashed arrow) and focal loss of Descemet`s membrane and endothelium (thick arrow) (H&E. X 400).

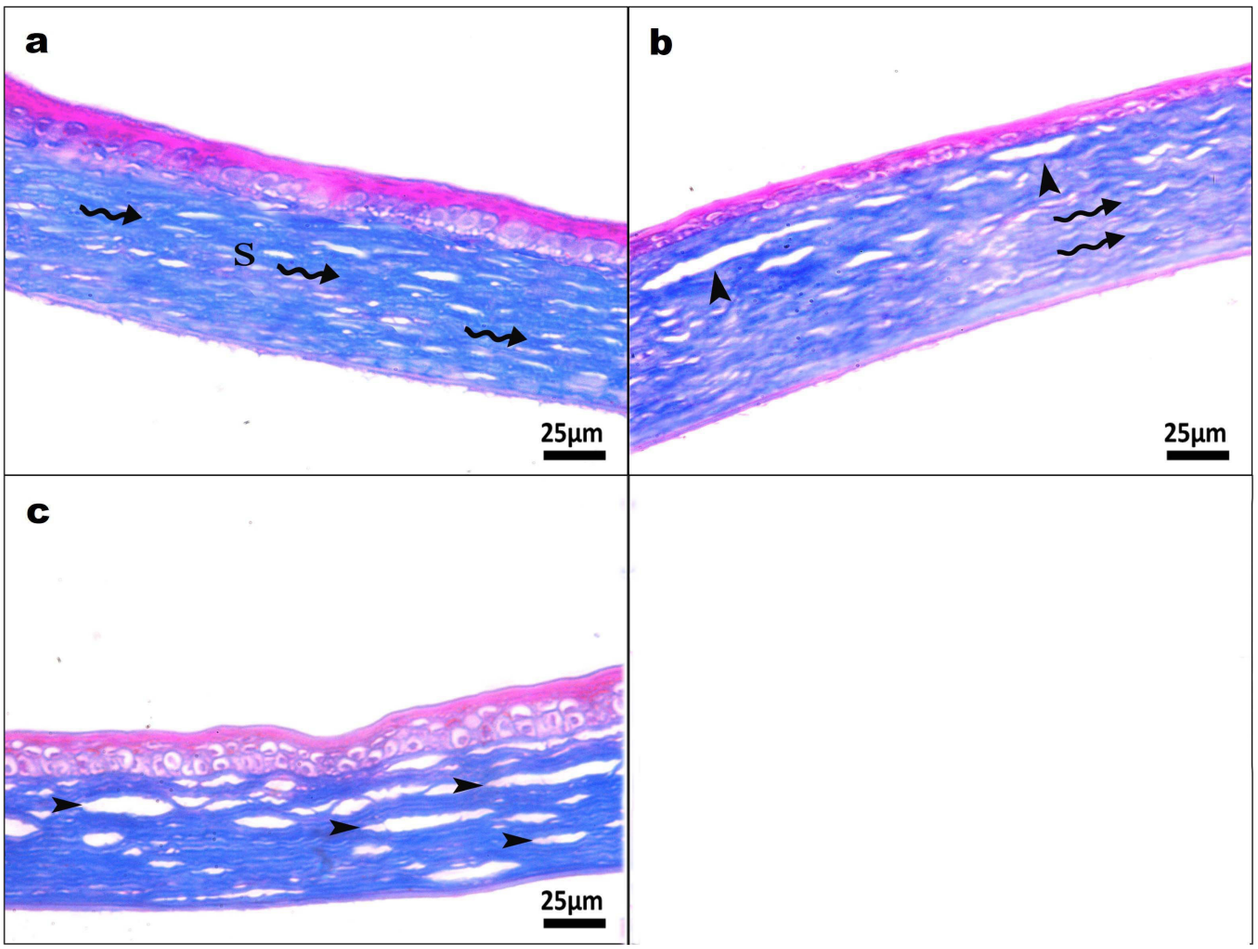


Fig. 3: Photomicrographs of the cornea from [a] the control group showing regular arranged stromal (S) fibers (zigzag arrow). [b] the (100mg) topiramate treated group showed regularly arranged stromal collagen fibers (zigzag arrow) with focal areas of wide separation in between the collagen fibers (arrowhead). [c] the (200mg) topiramate treated group showed widely separated stromal collagen fibers (arrowhead). (Masson's trichrome stain, X400).

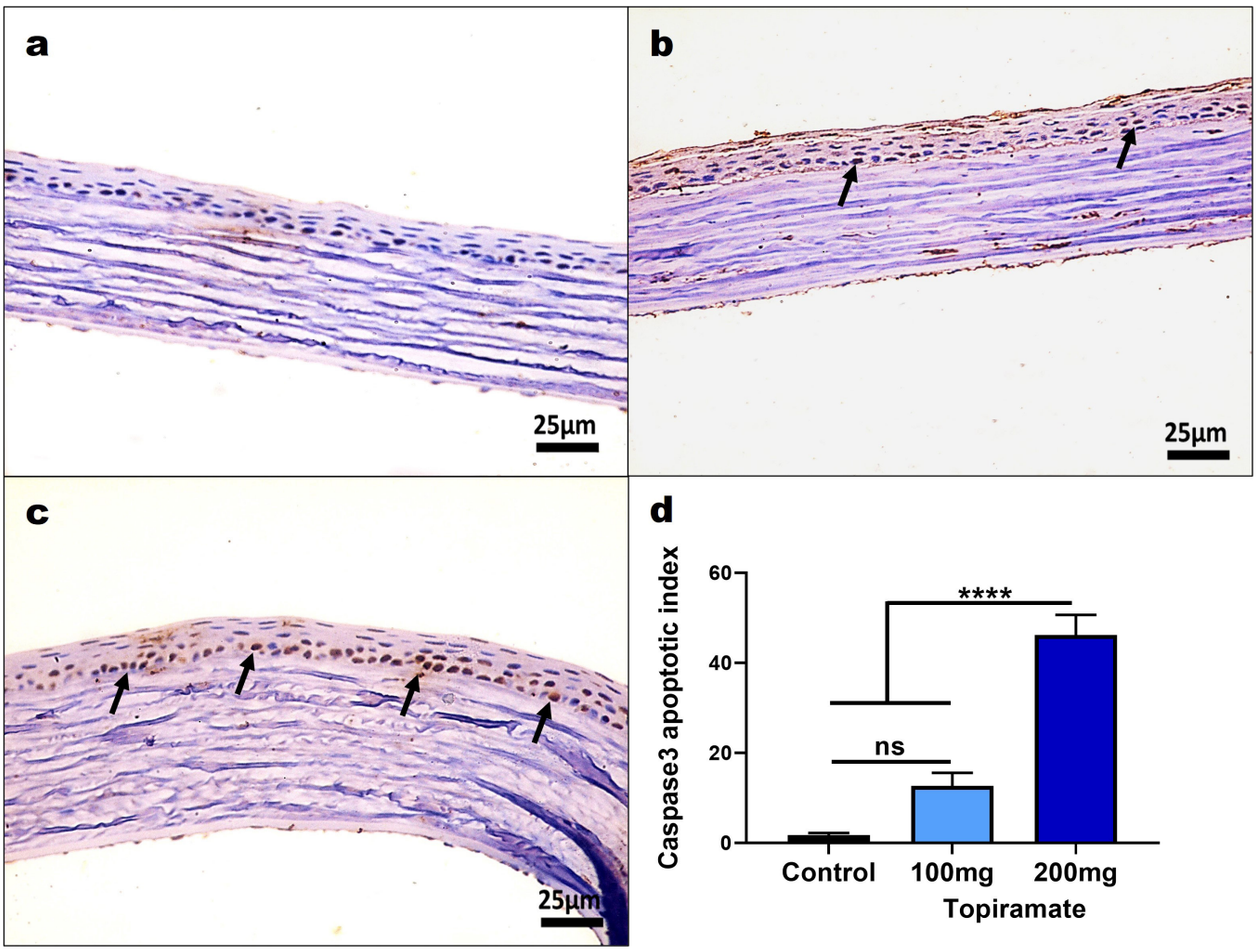


Fig. 4: Photomicrographs of caspase 3 immunohistochemical staining of the cornea showing [a] control group with negative caspase reaction. [b] 100mg topiramate-treated group with a low number of apoptotic cells (brown) in corneal epithelium. [c] 200mg topiramate treated group with a high number of apoptotic cells (arrow). [d] quantitative analysis showing a significant increase in the apoptotic index in the 200mg topiramate treated group compared to other groups, with insignificant differences between the 100mg topiramate treated group and control group. Data are presented as mean ±SEM, ns: nonsignificant, ****P<0.0001.

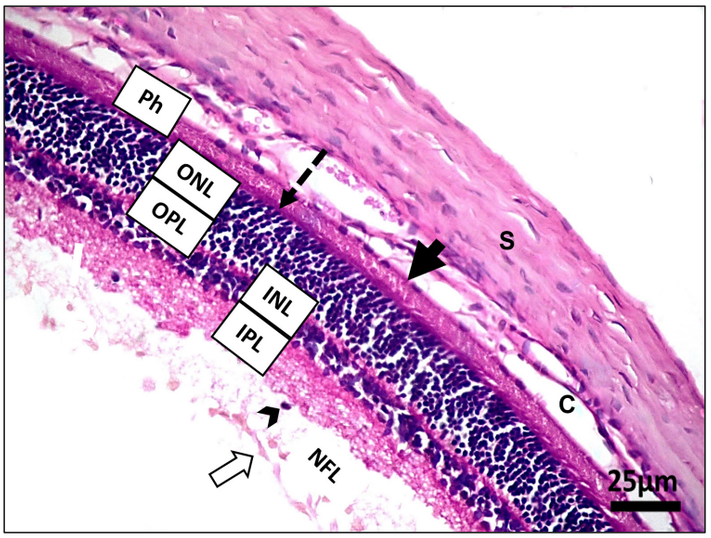


Fig. 5: Photomicrographs of the retina from the control group showing the ten layers of rat retina; retinal pigment epithelium (arrow), photoreceptor layer of rods and cones (PH), outer limiting membrane (dashed arrow), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (arrowhead), nerve fiber layer (NFL) and inner limiting membrane (thick white arrow) with the overlying sclera (s) and choroid (c). (H&E. X400).

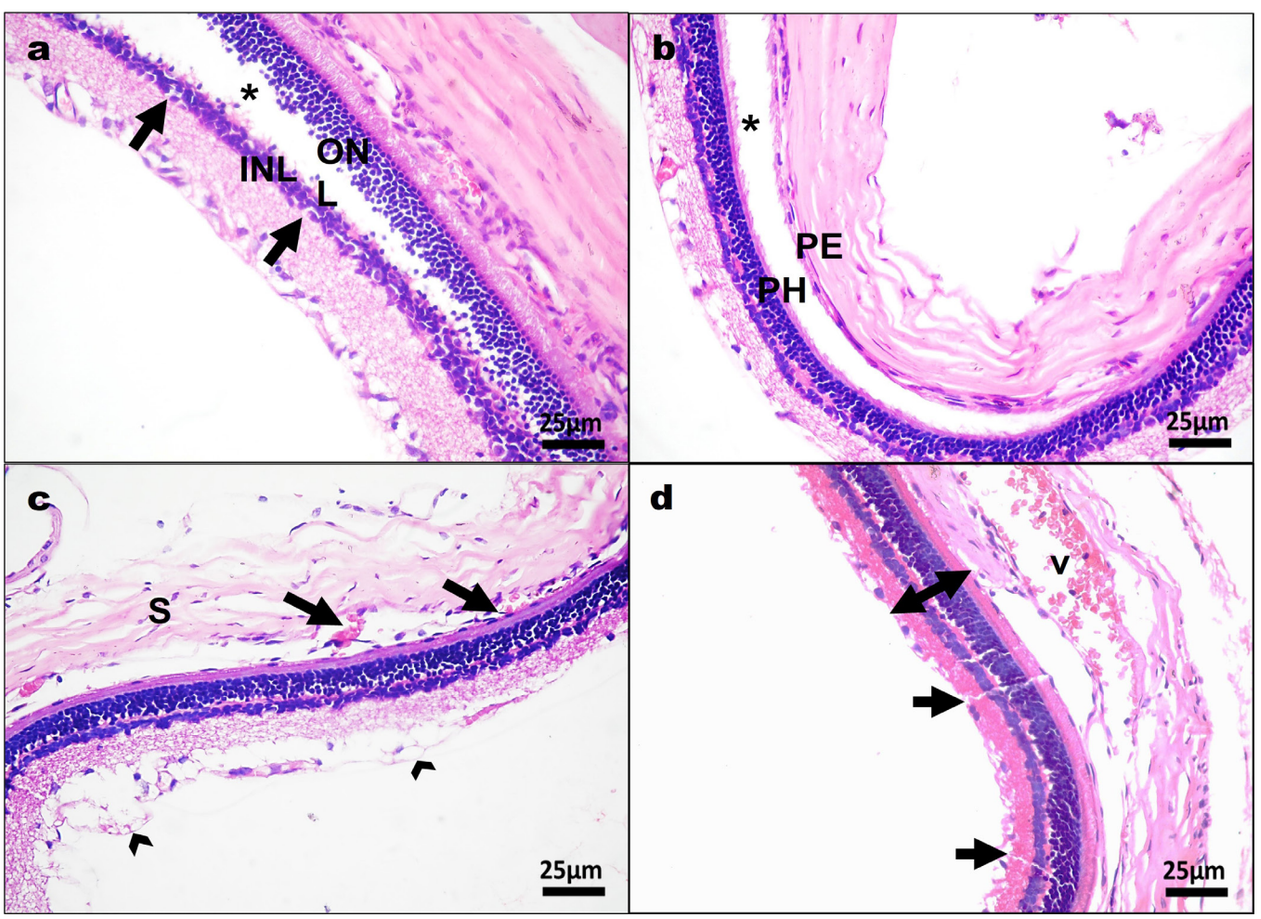


Fig. 6: Photomicrographs of the retina from 100mg topiramate treated group showing [a] a wide separation (star) between outer (ONL) and inner (INL) nuclear layers, some cellular vacuolation with pyknotic hyperchromatic nuclei (arrow). [b] a wide separation (star) between retinal pigment epithelium (PE) and photoreceptor layer of rods and cones (PH). [c] disorganization of scleral fibers (S), extravasation of blood cells (arrow) in the choroid with a detachment of inner limiting membrane (arrowhead). [d] splits in the retina (arrow), reduction in total retinal thickness (double-headed arrow) with congestion and dilatation in scleral's blood vessels (V). (H&E. X400).

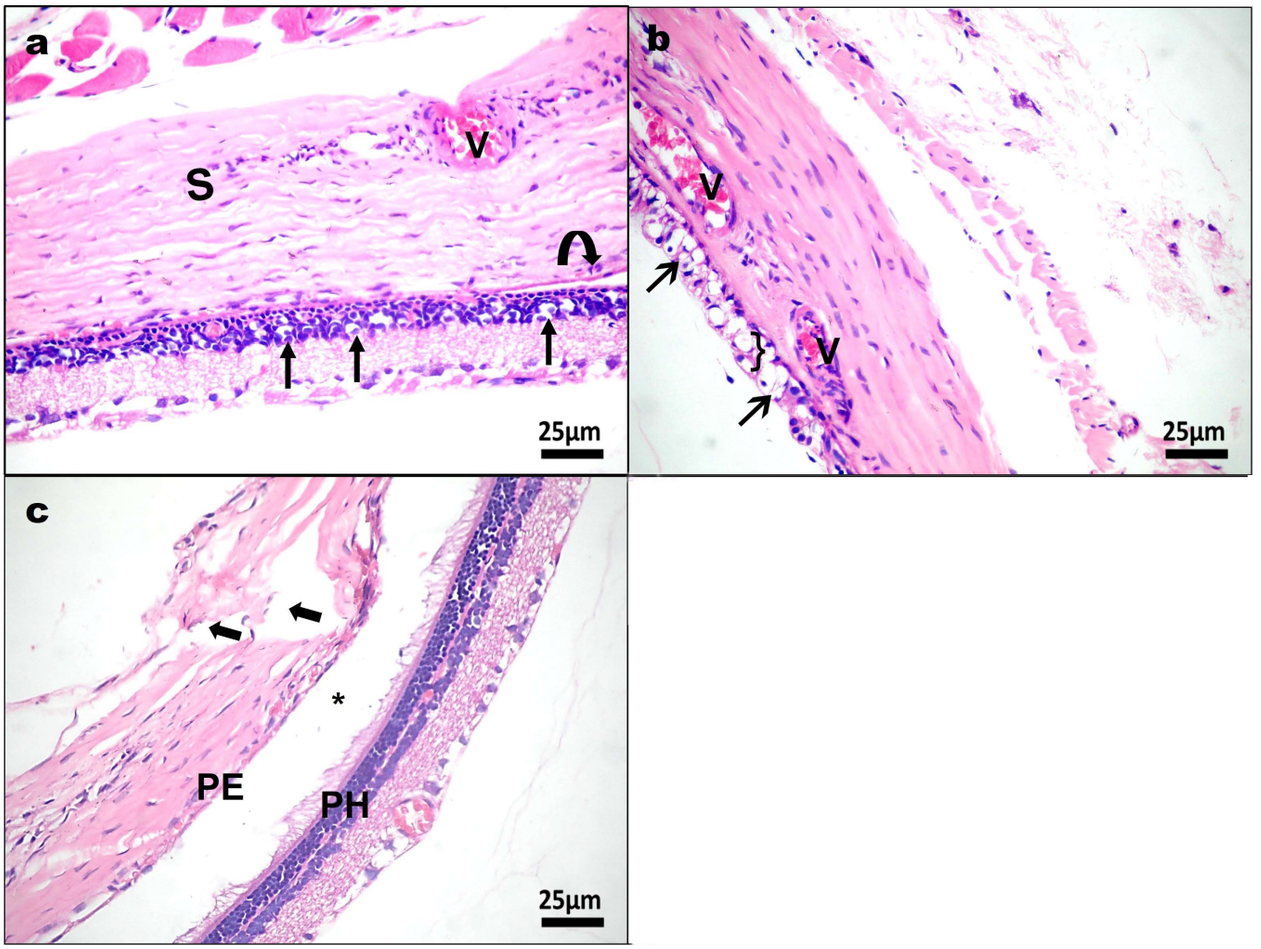


Fig. 7. Photomicrographs of the retinal from 200 mg topiramate treated group showing [a] focal separation between the retinal pigment epithelium and the photoreceptor layer of rods and cones (curved arrow), disorganized and loss of demarcation between outer and inner nuclear layers with multiple cellular vacuolations (arrow) and congested blood vessel (V) in overlying sclera (S). [b] disorganization with total loss of normal architecture of retina ({), excessive cellular vacuolation with apoptotic nuclei (arrow), and congested blood vessels (v). [c] a wide separation (star) between the retinal pigment epithelium (PE) and photoreceptor layer of rods and cones (Ph). Disorganization and destruction (arrow) in scleral fibers are noticed. (H&E. X 400).

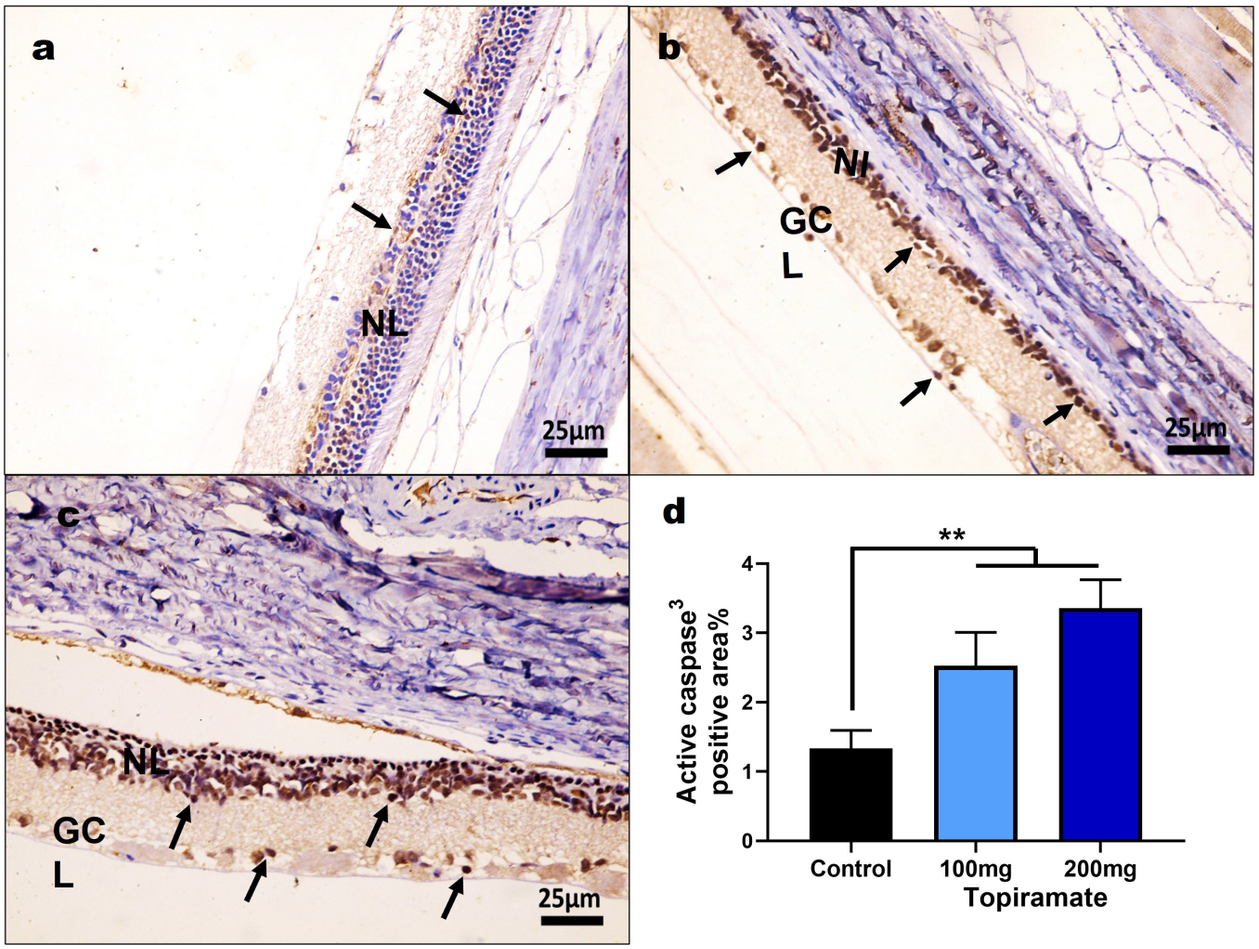


Fig. 8: Photomicrographs of caspase-3 immunohistochemical staining of the retina showing [a] the control retina with a few positive immunolabelling cells (arrow) in the nuclear layers [NL]. An increase in the number of apoptotic cells (arrow) in the nuclear cell layer (NL) and the ganglion cell layer (GCL) in [b] 100mg and [c] 200mg topiramate-treated groups. [d] quantitative analysis revealed a significant increase in the caspase-3 positive area percentage in both topiramate treated groups in comparison to the control group, with an insignificant increase in the 200mg compared to100mg topiramate treated groups. Data are presented as mean ±SEM, $^{**}P<0.05$.

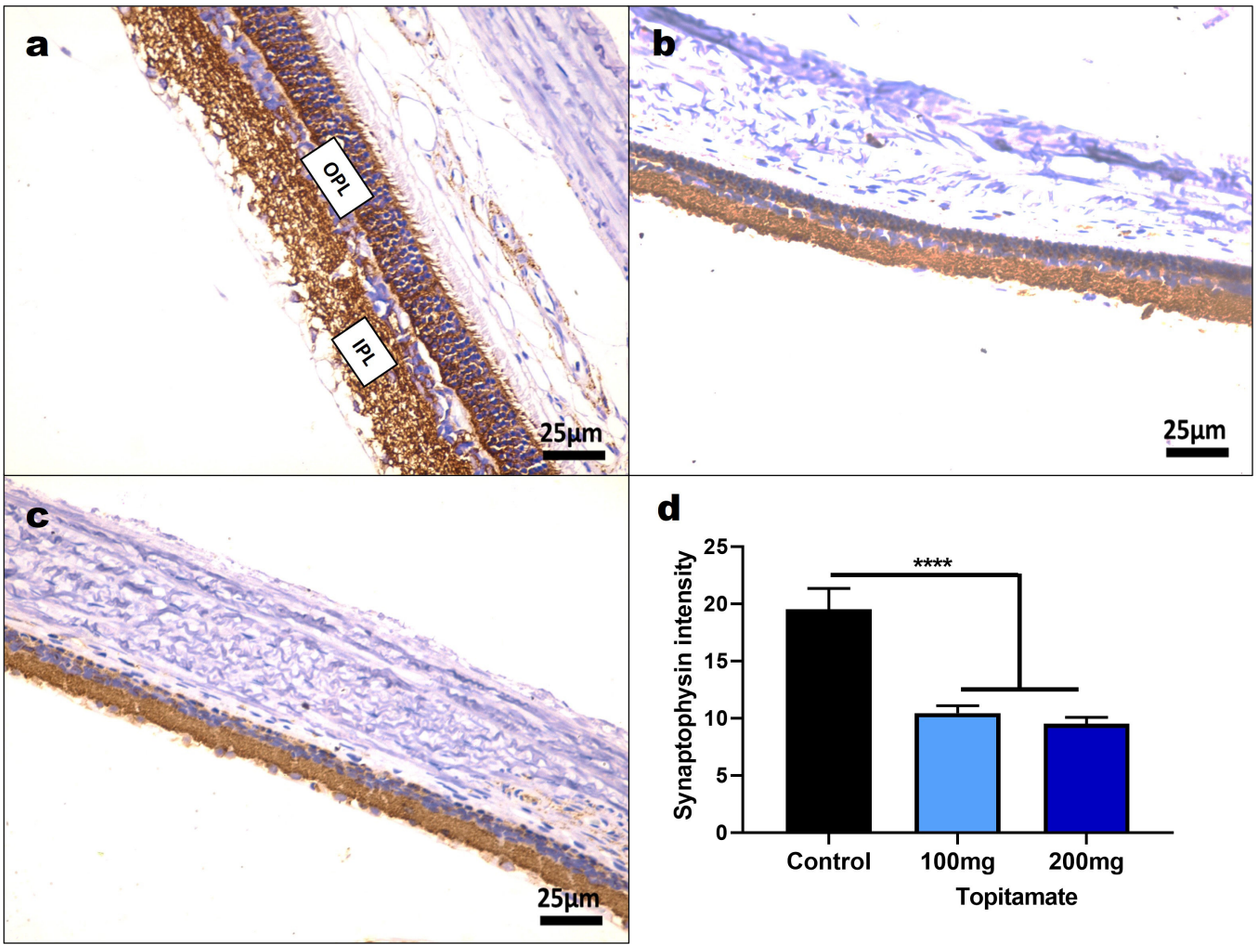
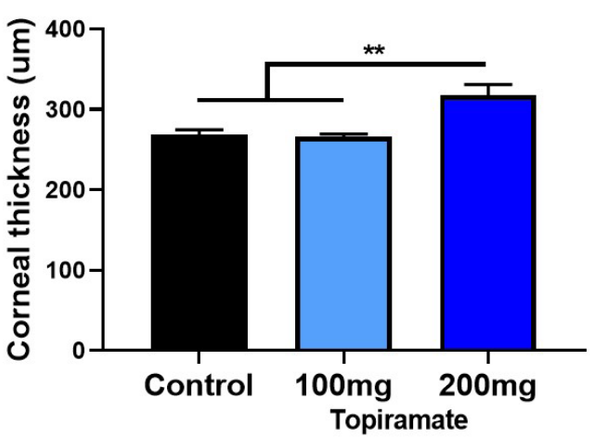
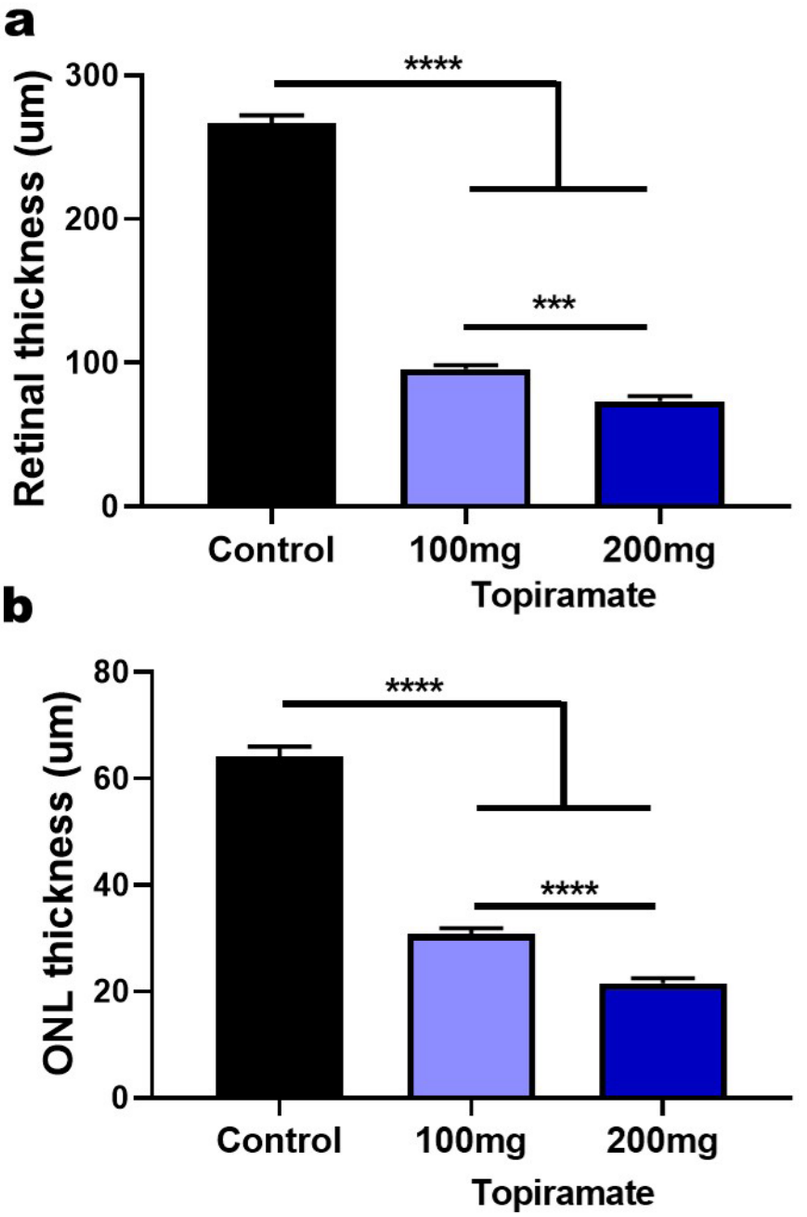


Fig. 9: photomicrographs of synaptophysin immunohistochemical staining (brown) of the retina showing [a] the control group with a strong positive immunoreaction in the inner (IPL) and outer (OPL) plexiform layer. A decrease in synaptophysin positive immune reaction in [b] 100mg and [c] 200mg topiramate-treated groups. [d] quantitative analysis revealed a significant reduction in synaptophysin intensity in the topiramate treated groups compared to the control group. Data are presented as mean ±SEM, ****P<0.0001.



Histogram1: quantitative analysis showing a significant increase in the corneal thickness of the 200 mg topiramate-treated group compared to the control and 100mg topiramate-treated groups. Data are represented as mean ±SEM. **P<0.001.



Histogram2: quantitative analysis showing a significant decrease in the thickness of [a] total retinal and [b] outer nuclear layer (ONL) in 100 mg and 200 mg of the topiramate treated groups compared to the control group. the Data are represented as mean ±SEM. ****P<0.0001.

DISCUSSION

Topiramate is one of the anti-epileptic drugs that causes irreversible severe ophthalmological lesions[24], such as acute myopia and acute angle-closure glaucoma which most probably developed within the normal therapeutic range and usually manifest within 10 days of drug initiation[9,25]. Moreover, the increase in the intraocular pressure, corneal edema, moderate conjunctival congestion, retinal striae, and even blindness were also seen in topiramate-treated patients in a dose and duration-dependent manner[26,27]. That is why the patients must be told about the possibility of acute ocular side effects even after using small doses of topiramate that must be administered under the care of an ophthalmologist[28]. Consequently, this work was designed to study the influence of topiramate with two different doses on both cornea and retina using different histological and immunohistochemical methods.

The histological analysis of rat cornea demonstrated alterations in all the corneal layers, focusing on the corneal epithelial layer, there are multiple cellular vacuolations with pyknotic hyperchromatic nuclei with a significant increase in the apoptotic index as established with caspase

immunostaining in the corneal epithelium layer. While the stoma showed disorganized widely separated collagen fibers with inflammatory cellular infiltration. In addition to, the focal loss of the Descemet`s membrane and the endothelial layer of the cornea with an increase in the corneal thickness.

The ballooning and vacuolation of the corneal epithelial cells with dark nuclei could be attributed to an alteration in the mitochondrial membrane with the release of much more cytochrome C, an apoptosis mediator[29]. Meanwhile, a previous study revealed that the apoptotic cells constantly shed into the tear film and this goes in line with the finding of desquamated cells or even focal areas of complete loss of corneal epithelium[30].

The destruction and disorganization of the sclera's collagen fibers with topiramate were observed previously by[31] during studying scleral changes in uveal effusion syndrome. He attributed that to the changes in the scleral permeability with an accumulation of GAG-like substances; primarily proteodermatan sulphate and proteochondroitin sulphate. These deposits obstruct trans-scleral water flow, resulting in scleral edema. A previous study[32] also attributed the eosinophilic homogenization of corneal stroma to inflammatory infiltration by neutrophils which produce matrix metalloproteinases and different proteases that break up collagen fibers and damage the normal corneal cytoarchitecture. Moreover, the spacing and disarrangement of the collagen bundles could be the results of the increase in the collagen fibers cross-linking aroused by glycation[21].

The histological findings and the morphometric results of the cornea of topiramate-treated groups also revealed an increase in the corneal thickness with topiramate administration. This was constant with the outcomes of Kerimoglu *et al*, 2009[33], who stated the increase in the central cornea in a patient after ten days only of topiramate administration that markedly decrease after three weeks of topiramate cessation. This corneal thickening may be attributed to the accumulation of fluids in the corneal stroma with consequent disorganization and separation of collagen fibers. The same finding was detected by another study which stated that the osmotic disturbance in topiramate-treated patients is the main cause of tissue edema and increased corneal thickness[34]. The harmful effects of topiramate on the cornea were also attributed to its effect on the function of aquaporins water channels that control the intraocular fluid development, and pressure[35]. As well as, the alteration of the endothelial layer disturbed the procedure of fluid movement out of the stroma, resulting in corneal edema with an increase in corneal thickness[36], which is considered one of the inflammatory markers in the cornea[37,38]. These theories were enforced by our findings of focal loss of the endothelium and increase in corneal thickness.

Generally, topiramate-induced corneal changes are hypothesized to be caused by oxidative stress. This was in agreement with Cejka et.al. 2015, who stated that oxidative stress with an increase in reactive oxygen species (ROS) was the principal cause of corneal changes in different pathological conditions like inflammation, dry eye disease, keratoconus, endothelial dystrophy, and bullous keratopathy[39].

This study also showed different structural alterations in the retina of topiramate-treated groups. There was cellular vacuolation with a reduction in the cell population of outer and inner nuclear layers and even focal areas of loss of all retinal layers which was confirmed by the significant decrease in thickness of both outer nuclear layer and whole retinal. These findings were in agreement with a previous study, which reported retinal damage and disruption in nuclear layers and ganglion cell layers with the anti-epileptic drugs such as vigabatrin[40].

The present work showed the appearance of splits in the retina and the separation of the retinal pigment epithelium from the photoreceptor layer of rods and cones. This was in agreement with Rosenberg et. al. 2017, who was the first to report the potential of retinal detachment in patients shortly after oral topiramate[41]. As they could be linked to topiramate-related membrane potential alterations and the disordered fluid flow in the retinal pigment epithelium which may affect the fluid balance in the subretinal space by influencing the retinal pigment epithelium pump's efficiency and/or the blood-retinal barrier's integrity.

Regarding the blood vessels, light microscopic examination revealed the presence of multiple congested blood vessels in the cornea, the retina, and the sclera. which may be attributed to the inflammatory process and cellular infiltration. This pathogenic process is divided into two phases; The 1st phase is the release of an angiogenic growth factor such as vascular endothelial growth factor VEGF[42]. while the 2nd phase is characterized by the activation of inflammatory cytokines such as TNF-a, interleukins, and MMPs, that are secreted by inflammatory cells. MMP-9s are proteolytic enzymes that disrupt extracellular matrix networks and enable the moving of endothelial cells to other locations, resulting in the development of new blood vessels[43]. It was also reported that topiramate induced vascular congestion in the placentas of female rats which belonged to topiramate's direct toxicity on the uterine blood vessels[44].

In the present study, immunohistochemical staining of the retina with anti-caspase-3 as apoptosis-related protein showed a significant increase in the caspase-3 area percentage in topiramate treated groups in comparison to the control group. The same findings were previously found by other scientists during studying age-related alterations in the retina and oxidative stress was supposed to be the possible mechanism through which topiramate could induce retinal degeneration[45]. On the other hand, immunostaining of the retina with anti-synaptophysin, a marker for synaptic activities showed a significant decrease in synaptophysin intensity in topiramate treated groups

compared to the control group. The same finding was recorded previously in the rat retina with aging where there was a decrease in the synaptic density and the thickness of the outer plexiform layer[20]. That could be linked to the decrease in the total retinal thickness and such thinning affects the density of synaptic layers[46].

CONCLUSION

Analysis of eye sections of the topiramate-treated groups revealed different histopathological alterations in the rat cornea and retina in a dose-dependent manner. Therefore, it could be recommended to use topiramate with care and under strict medical supervision with further histological, functional, and ophthalmological studies are recommended.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التغيرات الهيكلية في قرنية وشبكية الجرذان التي يسببها التوبيراميت دراسة نسيجية وهستوكيميائية مناعية

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مقدمة البحث: القرنية هي العنصر الانكساري الرئيسي للعين بينما الشبكية هي جزء من الجهاز العصبي يحتوي على الخلايا المستقبلة للضوء التي تعالج معلومات الضوء وتنقلها إلى الدماغ. ان التوبيراميت هو أحد الأدوية المضادة للصرع التي تستخدم بشكل أساسي في علاج اضطراب الصرع والصداع النصفي. ومع ذلك ، قد يصاحب تناوله ألم بالعين وعدم وضوح الرؤية.

الهدف من البحث: تهدف هذه الدراسة إلى معرفة تأثير مادة التوبيراميت على بنية قرنية وشبكية الجرذان باستخدام تقنيات نسيجية وهستوكيميائية مناعية مختلفة.

مواد و طرق البحث: تم استخدام ثلاثين ذكرًا بالغًا من الجرذان البيضاء في هذه الدراسة وتم تقسيمها بالتساوي إلى ثلاث مجموعات: المجموعة الضابطة ومجموعتين تجريبيتين تم إعطاؤهما توبيراميت بجرعات ١٠٠ مجم ، و ٢٠٠ مجم / كجم من وزن الجسم عن طريق الفم مرة واحدة يوميًا لمدة ٢٨ يومًا . بعد فترة التجربة ، تم التضحية بالجرذان ، وتم تشريح مقل العيون للصبغ بالهيماتوكسيلين والأيوسين ، وصبغة الماسون الثلاثية بالإضافة إلى التفاعل المناعي بمضاد كاسباس ٣ ، ومضاد السينابتوفيسين ، وهو بروتين موجود قبل التشابكات العصبية ومرتبط ارتباطًا وثيقًا بنشاط التشابكات العصبية بشبكية العين.

نتائج البحث: أظهرت القرنية تفريغ الخلايا الظهارية مع الموت المبرمج ، وعدم انتظام واتساع بين الألياف الصلبة العينية، وفقدان بؤري لغشاء ديسيميت والبطانة ، مع زيادة معنوية في سمك القرنية ومؤشر كاسباس ٣ في مجموعة ٢٠٠ مجم توبيراميت. كما كشفت شبكية العين عن فاصل واسع بين طبقات الشبكية وفقدان الفاصل بين طبقة الانوية الخارجية والداخلية مع فجوة خلوية وظهرت الانوية بلون غامق. وأظهرت الصلبة العينية عدم انتظام في أليافها مع احتقان الأوعية الدموية. ويشير التحليل الاحصائي أيضًا إلى انخفاض كبير في سمك الشبكية وزيادة لون السينابتوفيزين مع زيادة كبيرة في النسبة المئوية لمساحة كاسباس ٣.

الأستنتاج: تشير النتائج الحالية إلى أن التوبيراميت يسبب آثارًا ضائرة على بنية العين ويوصى باستخدامه بدقة تحت إشراف طبي.