Histological study on conjunctival and corneal reactions in rabbits induced by chronic topical application of latanoprost and travoprost

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Introduction
Chronic topical glaucoma therapy was reported to cause deleterious changes to the ocular surface epithelial layer.
Aim of the work
The aim of this study was to compare the histological changes in the cornea after chronic exposure to latanoprost preserved with 0.02% benzalkonium chloride (BAK) eye drops, travoprost preserved with sofZia eye drops and preservative-free artificial tears.
Materials and methods
Fifteen white rabbits were randomized into three groups (five animals each). They received once-daily topical application of one of the three treatments for 30 days. The first group (the control group) received preservative-free artificial tears (Refresh Plus). The second group received travoprost preserved with SofZia (Travatan Z). The third group received latanoprost preserved with 0.02% BAK (Xalatan). Enucleation was performed at the end of the experiment. Corneal samples were processed for light and transmission electron microscopic studies and conjunctival samples for light microscopic study. The mean epithelial height of the corneal epithelium, the mean number of goblet cells in the conjuctival epithelium and the mean area% of PAS-positive goblet cells were measured using an image analyzer. These results were statistically analysed using analysis of variance and the *t*-test.
Results
Latanoprost eye drops preserved in BAK produced toxic changes in the form of degeneration and desquamation of the superficial epithelial cells in the cornea, separation of Descemet's membrane and degeneration of endothelial cells, in addition to decreased number of goblet cells in the conjunctiva. Travoprost eye drops preserved in sofZia were safer and produced slight changes on the rabbit's ocular surface compared with latanoprost eye drops preserved in BAK.
Conclusion
It was concluded that antiglaucomatous drugs preserved with sofZia produced less corneal and conjunctival changes than those preserved with BAK.

Keywords:
benzalkonium, cornea, latanoprost, sofZia, travoprost

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Introduction

Glaucoma, in its various forms, is a blinding disease that affects millions of people worldwide [1].

The traditional treatment for glaucoma has involved lowering the intraocular pressure through the use of topical ocular hypotensive drops [2].

Although this treatment was shown to be effective at slowing down the progression of glaucoma, it comes at the cost of possible deleterious changes to the ocular surface over time [3].

In general, eye drops for the treatment of ocular hypertension and glaucoma are well tolerated, and short-term clinical trials have reported low incidences of patient withdrawal because of intolerance or adverse effects related to treatment. However, long-term treatment may be required for the rest of the patient's life [4].

Histopathological studies confirmed that antiglaucoma eye medications could exert toxic effects on the surface of the cornea. They induced chronic inflammation mostly due to the chloride of benzalkonium, the preserving agent in a majority of eye drops [5].

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The side effects of benzalkonium chloride (BAK) seemed to be dose and time dependent, increasing with larger amounts used over longer periods. Many patients using topical glaucoma medications frequently require long-term treatment with medication, thus increasing the probability of BAK-related side effects [6].

Alternative preservatives to BAK have emerged and seemed to be effective at preventing fungal and bacterial colonization of medication bottles. These preservatives seem to induce less structural damage and inflammatory effects on the ocular surface. Travoprost preserved with sofZia was the first prostaglandin analogue to be preserved with a non-BAK system [7].

BAK is the most commonly used preservative in topical multidose glaucoma medications. It is a quaternary ammonium compound that breaks down bacterial cell membranes, eventually leading to cell death. Travoprost preserved with sofZia, however, is the first prostaglandin analogue not preserved with BAK. SofZia is an oxidizing complex containing borate, zinc and sorbitol [8].

This study aimed to compare corneal and conjunctival changes due to the effect of latanoprost preserved with 0.02% BAK, travoprost preserved with sofZia and preservative-free artificial tears on the ocular surface of rabbits treated with a clinical regimen of once-daily dosing of one-drop instillation over a 30-day period using light and electron microscopes and morphometric measurements.

Materials and methods

The original research was approved by the review board of Histology Department, Faculty of Medicine, Cairo University, Cairo, Egypt.

Materials

Animals

In this study, 15 male white rabbits (weighing between 1.5 and 2 kg) were used. They were housed in the animal house of Kasr-El-Aini Faculty of Medicine, Cairo University, Cairo, Egypt. The animals received a standard diet for rabbits and were allowed free access to water. They were divided into three groups comprising five rabbits each.

Treatment protocol

The first group (the control group) received preservative-free artificial tears (Refresh Plus; Allergan, Irvine, California, USA). The second group received travoprost preserved with SofZia (Travatan Z; Alcon, Fort Worth, Texas, USA). The third group received latanoprost preserved with 0.02% BAK (Xalatan; Pfizer, New York, USA). For 30 days, all animals received the group-specific medication as a single drop in each eye; the eyelids were manually closed for 30 s to ensure retention of the drop. This study was approved by the ethics committee on animal research in the animal house of Kasr-El-Aini Faculty of Medicine, Cairo University, Cairo, Egypt, following international ethics and regulations for animal research in laboratory applications [9].

Methods

Histological study

On day 30 of the study, after instillation of the final drop, each rabbit was anaesthetized with 25 mg/kg ketamine hydrochloride followed by anaesthesia with pentobarbital (150 mg/kg) injected intravenously into the ear vein. Following anaesthesia, enucleation was performed.

Corneal samples from the left eye were cut into small cubes (~1 mm) and immediately fixed in 2% gluteraldehyde. Semithin sections were prepared and stained with toluidine blue and examined through a light microscope; ultrathin sections were prepared and stained with uranyl acetate and lead citrate and examined through an electron microscope. These procedures were performed at the regional centre for mycosis and its applications, Al-Azhar University [10].

Corneal and palpebral conjunctival samples from the right eye were fixed in neutral buffered formalin and processed for paraffin sections that were stained with H&E. Conjunctival sections were also stained with PAS stain and examined through a light microscope for structural changes.

Morphometric study

Using an image analyzer computer system (Leica Qwin 500), the following parameters were measured:

(1) The mean epithelial height of the cornea, which was measured in 10 nonoverlapping high-power fields for each specimen at magnification ×400 using the interactive measuring menu.
(2) The number of goblet cells in the conjunctiva (PAS-stained sections), which were counted in 10 high-power fields for each specimen at magnification ×400 using the interactive measuring menu.
(3) The mean area% of PAS-stained goblet cells in the conjunctiva, which was measured in 10 high-power fields for each specimen at magnification ×400 in relation to the area of the standard measuring frame, which was 7381.11 μm^2.

Statistical analyses of the obtained results were performed using analysis of variance and the *t*-test.

Results

Light microscopic results

The cornea of controls

The cornea of rabbits in the control group consisted of a nonkeratinized stratified squamous epithelium formed of a single layer of columnar basal cells with oval nuclei resting on a clear basement membrane, intermediate two or three layers of polyhedral cells interdigitating with each other with rounded nuclei and two or three layers of superficial flattened squamous cells with flattened nuclei (Figs 1a, b and 3). The stroma consisted of parallel collagen bundles with spindle-shaped corneal fibroblasts (keratocytes) between the lamellae of the collagen bundles (Figs 2a, b and 3). Descemet's membrane was seen clearly as a homogeneous acellular membrane. The corneal endothelium appeared as a single flat cell

layer with flattened nuclei on the posterior surface of Descemet's membrane (Fig. 2a and b).

The cornea of the second group (travoprost preserved with sofZia)

The cornea of the second group showed the epithelium with slight desquamation and degeneration of superficial cells (Figs 4a, b and 6), whereas the stroma appeared to be formed of regularly arranged bundles of collagen fibers with spindle-shaped keratocytes between these bundles (Figs 4a, b, 5a, b and 6). There were no changes in Descemet's membrane or the endothelium when compared with the control group (Fig. 4a and b). Substatia propria exhibited separation of collagen fibers (Figs 4a and 5a).

The cornea of the third group (latanoprost preserved with benzalkonium chloride)

The cornea of the third group showed marked desquamation and separation of the epithelium (Fig. 7a and b). There was leucocytic cellular infiltration under the epithelium at the limbus (Fig. 8a and b). Some of the endothelial cells were separated from Descemet's membrane in some areas (Fig. 7a). Descemet's membrane showed separation in some parts from the stroma leaving wide spaces in between. The endothelium showed darkly stained small endothelial cell nuclei (Fig. 9a and b). Semithin sections showed desquamated epithelium and keratocytes with dark nuclei (Fig. 10).

The conjunctiva

The conjunctiva of the control group was composed of stratified columnar epithelium with numerous interspersed goblet cells in the surface layer. Beneath the conjunctival epithelium, a loose vascular connective tissue was detected (Fig. 11a and b), whereas the conjunctiva of the second group showed fewer goblet cells compared with the control group (Fig. 11c and d). The conjunctival epithelium of the third group became thinner and disrupted with desquamation of superficial conjunctival epithelial cells with fewer scattered goblet cells in between (Fig. 11e and f).

Transmission electron microscopic results

Changes in the different layers of the cornea of the rabbit's eye were evaluated using an electron microscope.

The control group

In the control group the superficial epithelial cells of the cornea appeared flattened with flattened nuclei. Intermediate large polyhedral cells with rounded nuclei had characteristic wing-like shape. Interdigitations were found between adjacent wing cells (Fig. 12). The basal cells were columnar, resting on a basement membrane. The stroma consisted of regularly arranged collagen fibers, and keratocytes were scattered between them (Fig. 13). Endothelial cells were seen beneath Descemet's membrane having flattened nuclei and many organelles such as mitochondria and rER (Fig. 13). Keratocytes were spindle shaped with elongated nuclei and scanty cytoplasm. Collagen fibers appeared to be formed of individual fibrils (Fig. 14).

The second group (travoprost with sofZia)

Areas of degeneration and desquamation were noticed in the superficial layer of the epithelium. The underlying wing cells appeared normal. Interdigitations were found between these cells. The basal epithelial cells showed many vacuoles (Fig. 15).

The third group (latanoprost with benzalkonium chloride)

The superficial epithelial cells appeared degenerated and vacuolated with dark nuclei. The winged cells showed many cytoplasmic vacuoles surrounding the nucleus. Many cytoplasmic vacuoles were noticed in the basal cell layer of the epithelium (Fig. 16). Stromal keratocytes had dark shrunken nuclei (Fig. 17a). Endothelial cells showed many vacuoles (Fig. 17b).

Morphometric results

Mean epithelial height of the cornea

In the latanoprost group, there was a highly significant decrease ($P \leq 0.001$) in mean epithelial height of the cornea when compared with the control group, whereas there was no remarkable difference between the travoprost and control groups (Table 1 and Chart A).

Mean number of goblet cells in the conjunctiva/HPF

In the latanoprost and travoprost groups, there was a significant decrease ($P \leq 0.001$) in the mean number of goblet cells in the conjunctiva/HPF (High Power Feild) when compared with the control group. The decrease was more obvious in the latanoprost group (Table 1 and Chart B).

Area% of PAS-stained goblet cells in the conjunctiva

In the latanoprost and travoprost groups, there was a significant decrease ($P \leq 0.001$) in the mean area% of PAS-stained goblet cells in the conjunctiva when compared with the control group; this was markedly reduced in the latanoprost group (Table 1 and Chart C).

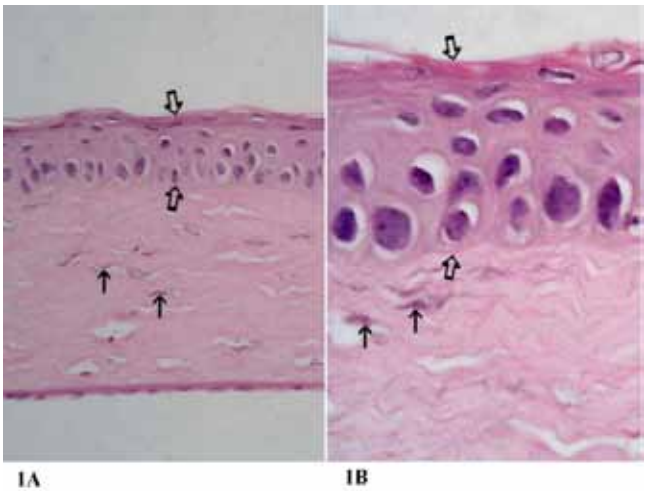

Figure 1. Photomicrograph of a section of a rabbit's cornea from the control group, which received preservative-free artificial tears eye drops: showing the stratified squamous nonkeratinized corneal epithelium (thick arrows) formed of a few cell layers. The basal epithelial cells are columnar with oval nuclei, intermediate layers of polyhedral cells and upper layers of flattened cells. The stroma are formed of parallel arranged collagen bundles with spindle-shaped keratocytes in between (thin arrows).
H&E, (a) × 400; (b) × 1000.

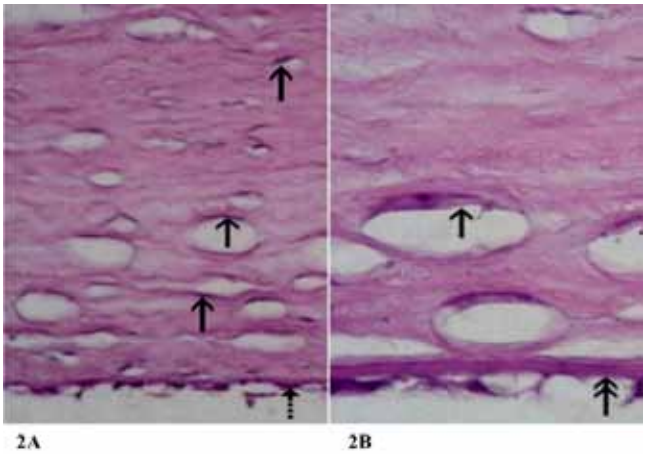

Figure 2. Photomicrograph of a section of a rabbit's cornea from the control group showing keratocytes in the stroma (thin arrows). The Descemet's membrane appears as a homogeneous acellular membrane (double arrow). The endothelium appears as a single layer of flattened cells with flattened nuclei (dotted arrows).
H&E, (a) × 400; (b) × 1000.

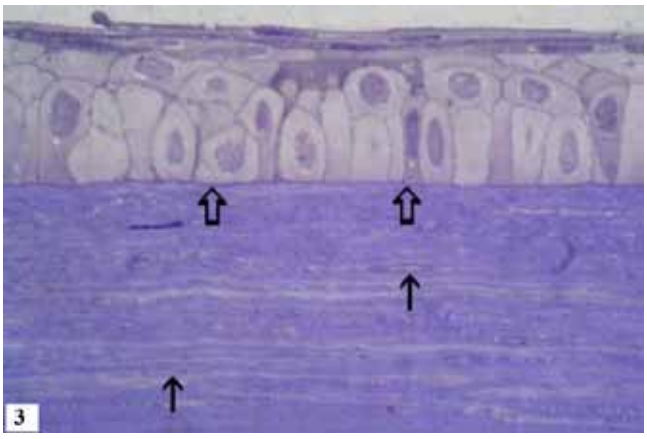

Figure 3. Photomicrograph of a semithin section of a rabbit's cornea from the control group showing the corneal epithelium resting on a clear basement membrane (thick arrows). The stroma show regular collagen bundles with normal spindle-shaped keratocytes in between (thin arrows).
Toluidine stain, × 1000.

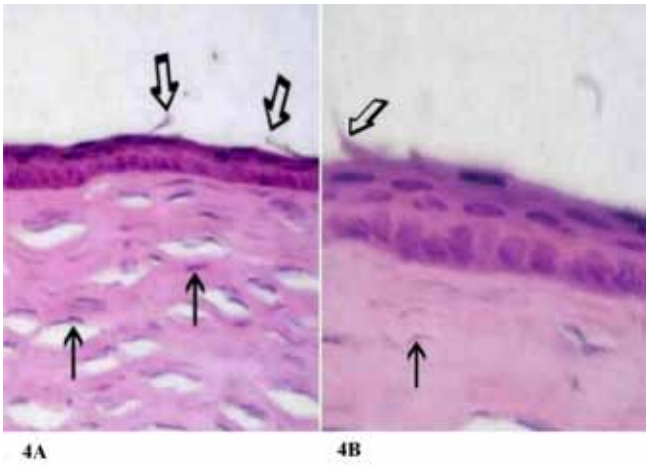

Figure 4. Photomicrograph of a section of a rabbit's cornea that received travoprost eye drops with SofZia (group II) showing the stratified squamous nonkeratinized corneal epithelium. The superficial epithelial cells show slight desquamation (thick arrows). The stroma are formed of parallel arranged collagen bundles with spindle-shaped keratocytes in between (thin arrows).
H&E, (a) × 400; (b) × 1000.

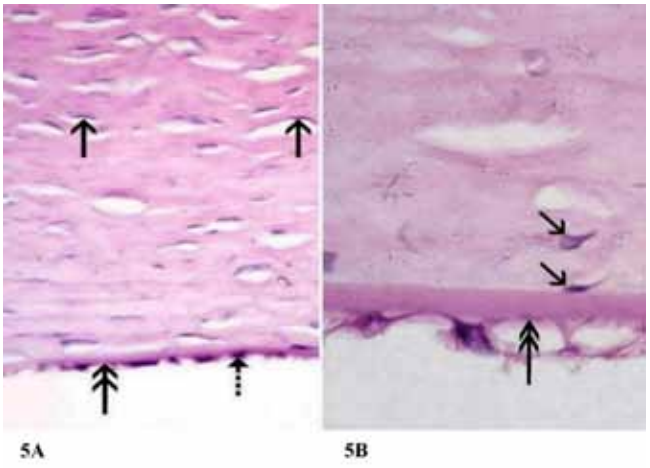

Figure 5. Photomicrograph of a section of a rabbit's cornea (group II) showing stroma (thin arrows). The Descemet's membrane appears as a homogeneous acellular membrane (double arrows). The endothelium appears as a single layer of flattened cells with flattened nuclei (dotted arrows).
H&E, (a) × 400; (b) × 1000.

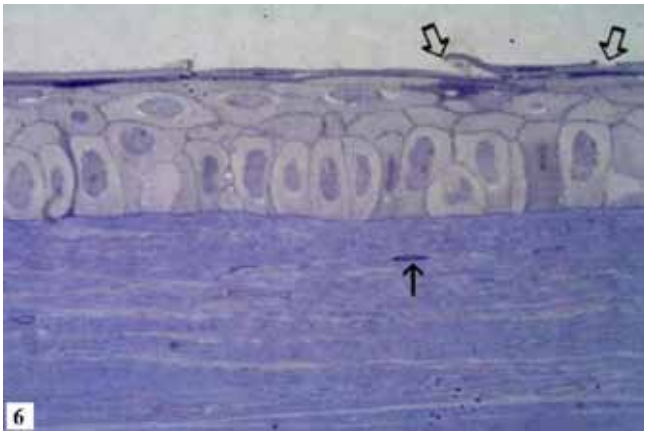

Figure 6. Photomicrograph of a semithin section of a rabbit's cornea (group II) showing slight desquamation of the superficial epithelial cells (thick arrows). The stroma show regular collagen fibers with normal keratocytes (thin arrow) in between.
Toluidine blue stain, × 1000.

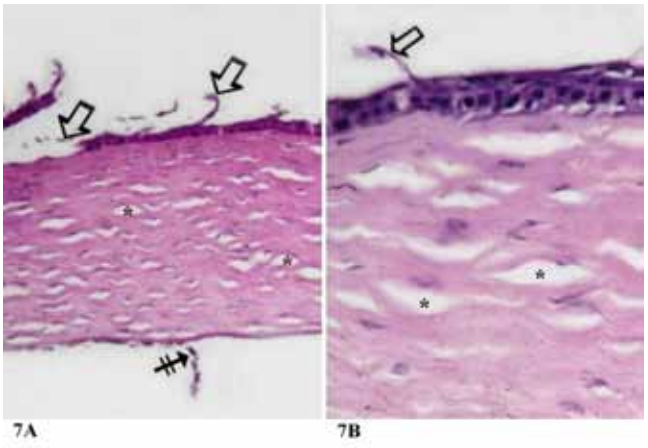

Figure 7. Photomicrographs of a section of a rabbit's cornea that received latanoprost eye drops preserved with benzalkonium chloride (group III) showing corneal epithelial damage in the form of desquamation and separation of epithelial cells (thick arrows). Some of the endothelial cells (crossed arrow) are separated from Descemet's membrane. Note wide separation of collagen fibers in substatia propria (star).
H&E, (a) × 200; (b) × 400.

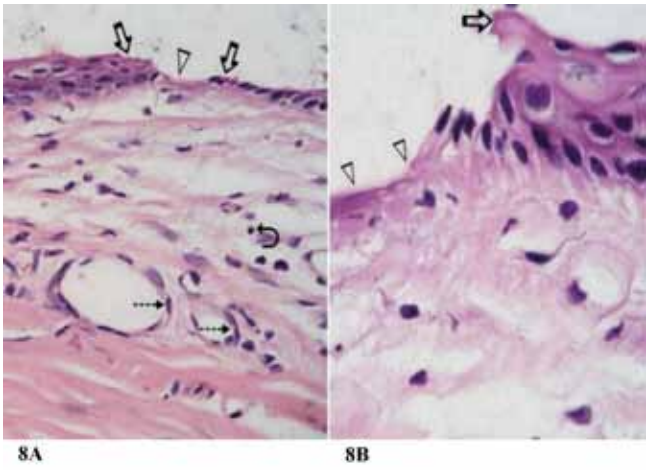

Figure 8. Photomicrograph of a section at the limbus and adjacent sclera (group III) showing desquamation of the epithelium (thick arrows) leaving a bare area devoid of an epithelial covering (arrow heads). There was leucocytic infiltration under the epithelium (curved arrow). Note the limbal blood vessels (dotted arrows).
H&E, × 1000.

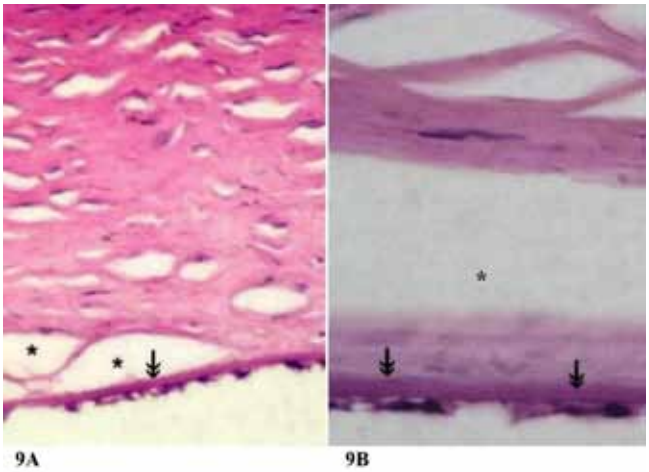

Figure 9. Photomicrograph of a section of a rabbit's cornea (group III) showing the stroma that are formed of collagen bundles with spindle-shaped keratocytes in between. Collagen bundles are widely separated. The Descemet's membrane (double arrows) appeared separated from the stroma leaving a wide space in between (stars).
H&E, (a) × 400; (b) × 1000.

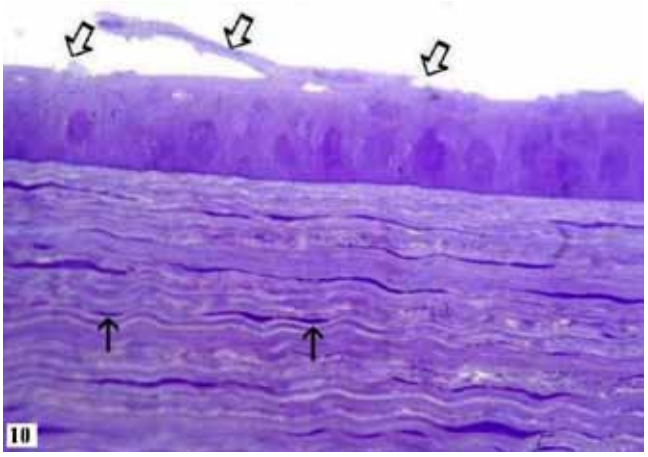

Figure 10. Photomicrograph of a semithin section of a rabbit's cornea (group III) showing desquamation and separation of the epithelium (thick arrows). The keratocyte nuclei appear dark (thin arrows).
Toluidine blue stain, × 1000.

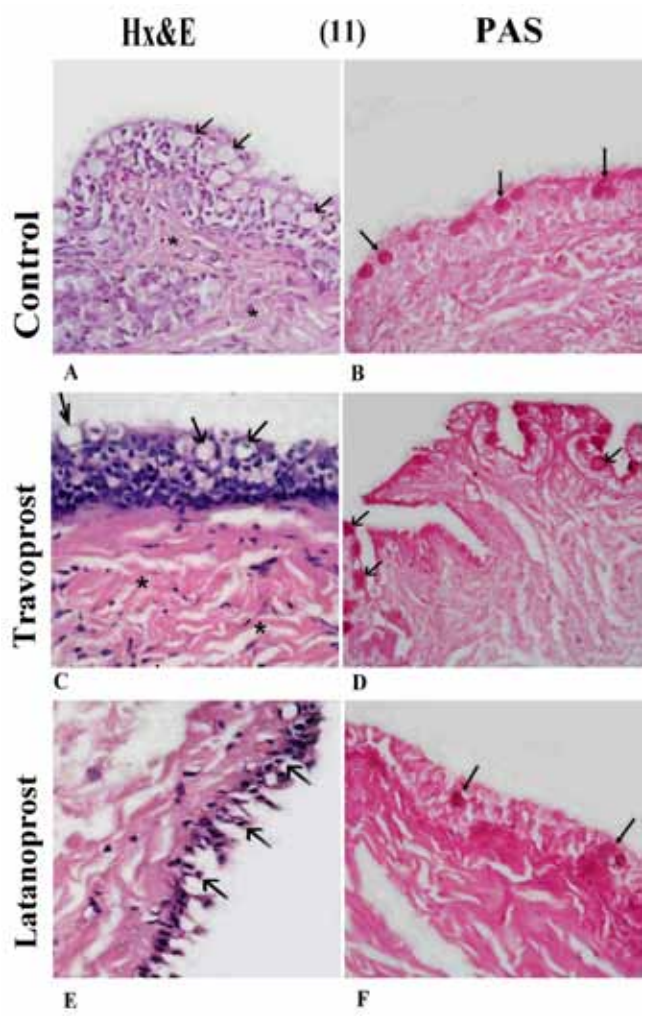

Figure 11. Photomicrographs of sections of rabbit conjunctiva: (a, b) controls that received preservative-free artificial tears eye drops showing the stratified columner epithelium with numerous interspersed goblet cells in the surface layer (arrows). Note the underlying connective tissue (stars); (c, d) rabbits that received travoprost eye drops with SofZia showing no remarkable difference from the control group; (e, f) rabbits that received latanoprost eye drops preserved with benzalkonium chloride showing thinning and disruption of the conjunctival epithelium with fewer goblet cells (arrows).
H&E, (a, c, e) × 400; PAS, (b, d, f) × 400.

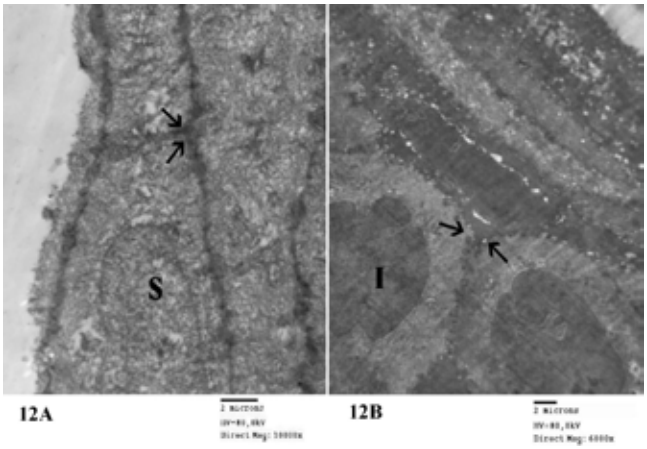

Figure 12. TEM photomicrograph of a rabbit's cornea that received preservative-free artificial tears eye drops showing superficial flattened cell (S) with flattened nuclei. Intermediate wing cells (I) are polyhedral with rounded nuclei. Interdigitations (arrows) are seen between superficial and intermediate cells (arrows).
TEM, (a) × 10 000; (b) × 6000.

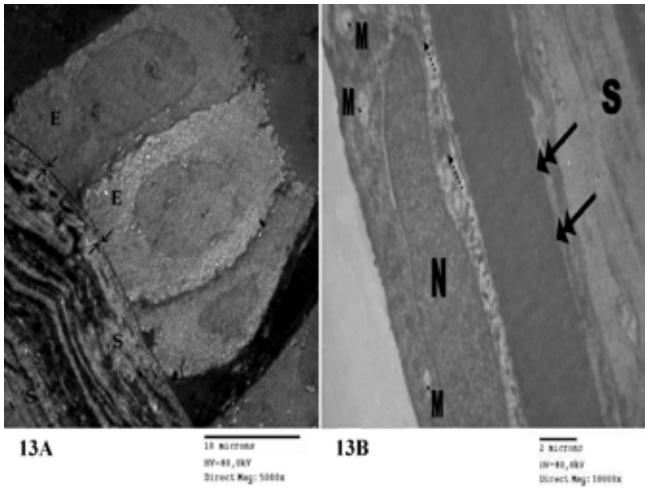

Figure 13. TEM photomicrograph of a rabbit's cornea that received preservative-free artificial tears eye drops showing the basal cell layer of the corneal epithelium (E) resting on the basement membrane (thin arrows). The collagen fibers of the stroma (S) are regularly arranged. Descemet's membrane appears as a homogeneous layer (double arrows) on which endothelial cells are resting. These endothelial cells have flattened nuclei (N) and the cytoplasm has many organelles such as rER (dotted arrows) and mitochondria (M).

TEM, (a) × 5000; (b) × 10 000.

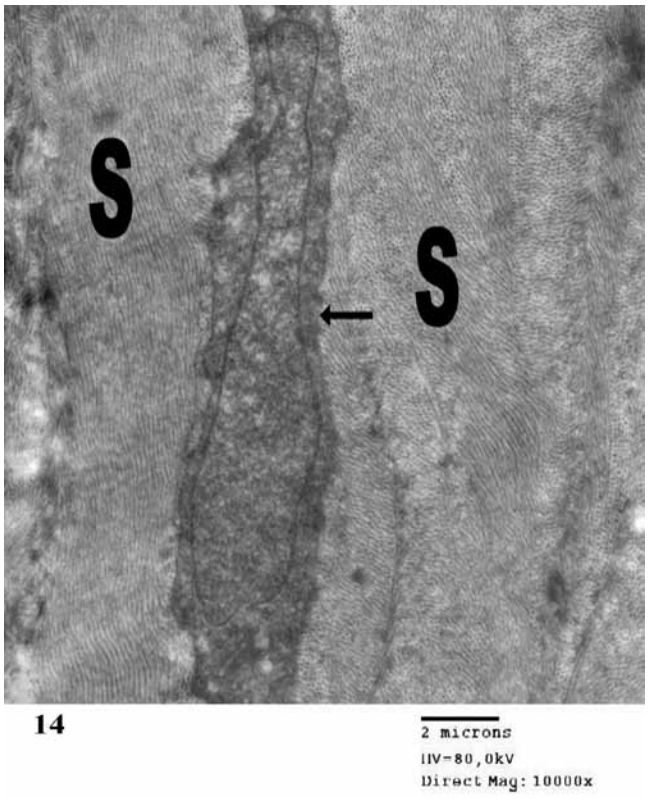

Figure 14. TEM photomicrograph of a rabbit's cornea that received preservative-free artificial tears eye drops showing a spindle-shaped keratocyte (arrow). It has an elongated large nucleus and scanty cytoplasm. Stromal collagen lamellae (S) are seen formed of individual fibrils.

TEM, × 10 000.

Figure 17. TEM photomicrograph of a rabbit's cornea that received latanoprost eye drops preserved with benzalkonium chloride showing keratocytes with dark nuclei (thin arrows) between stromal lamellae (S). Beneath the Descemet's membrane (D) is seen an endothelial cell showing many vacuoles (thick arrows) and a small shrunken nucleus (N).

TEM, (a) × 4000; (b) × 10 000.

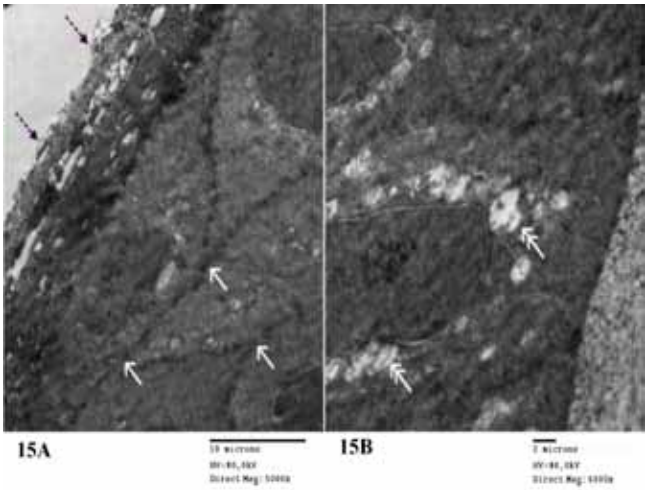

Figure 15. TEM photomicrograph of a rabbit's cornea that received travoprost eye drops with SofZia showing desquamation of the superficial layer of the epithelium (dotted arrows) with interdigitations (thin arrows) between superficial and intermediate cells. There were many vacuoles in the cytoplasm of the basal cell layer of the corneal epithelium (double arrows).

TEM, (a) × 5000; (b) × 6000.

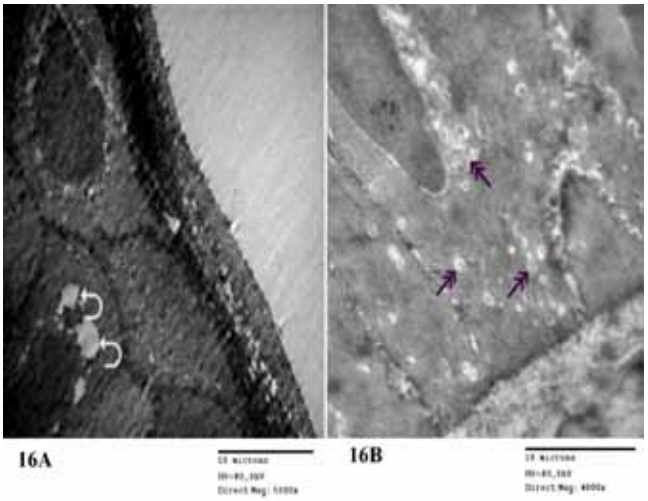

Figure 16. TEM photomicrograph of a rabbit's cornea that received latanoprost eye drops preserved with benzalkonium chloride showing many cytoplasmic vacuoles around the nucleus of wing cells (curved arrows) and many vacuoles in the cytoplasm of the basal cell layer of the corneal epithelium (double arrows).

TEM, (a) × 5000; (b) × 4000.

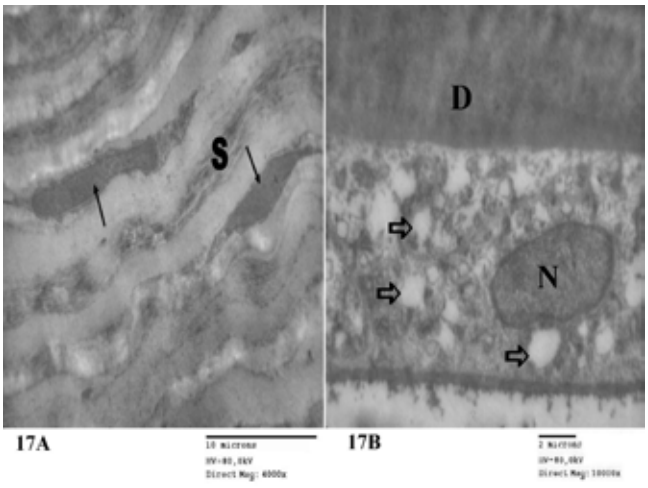

Table 1. Results of statistical analysis of cornea and conjunctiva

Mean±SD	Control (group I)	Travoprost (group II)	Latanoprost (group III)
Corneal epithelial height	18.57 ± 1.96	18.68 ± 5.2.26	10.81 ± 0.78*,#
Conjunctival goblet cell number/HPF	6.25 ± 0.29	3.9 ± 0.16*	1.6 ± 0.26*,#
Conjunctival goblet cell area%	18.88 ± 1.91	15.2 ± 1.83	3.43 ± 0.6*,#

*Significant at $P<0.001$ compared to group I (control).
#Significant at $P<0.001$ compared to group II (travoprost).

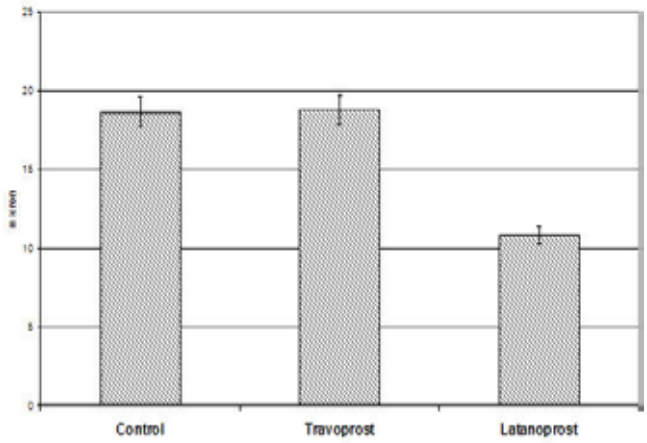

Chart A. Mean epithelial height of the cornea.

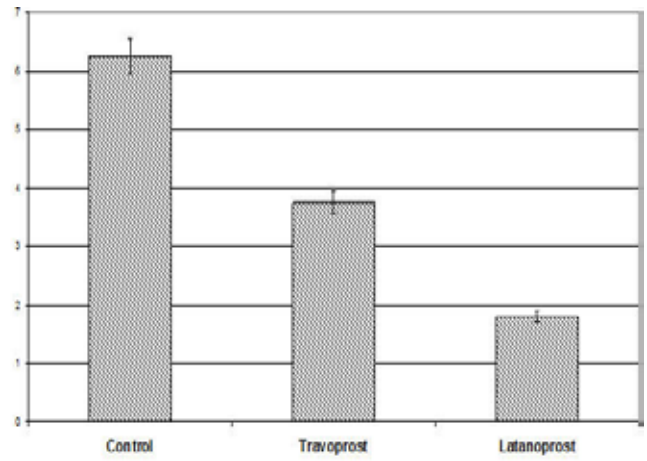

Chart B. Mean number of goblet cells in the conjunctiva/HPF.

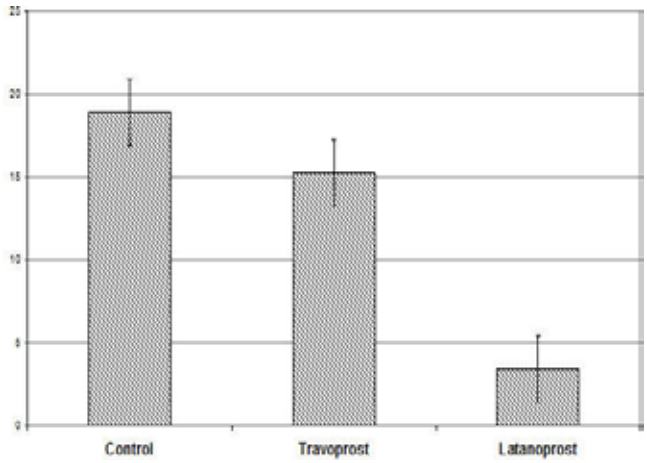

Chart C. Mean area% of goblet cells in the conjunctival epithelium.

Discussion

Glaucoma is the second most common cause of blindness in the world today [1]. Medication is the first-line therapy for patients with open-angle glaucoma or ocular hypertension. The majority of patients with these conditions receive topical medical treatment in the form of eye drops [11].

In the present study, using a light microscope, corneal sections treated with travoprost with sofZia were seen to show slight epithelial degeneration and desquamation. There was no remarkable change in other corneal layers. Electron microscopic examination also showed slight degeneration and vacuolation in the epithelium. These findings were in accordance with other studies that used transmission electron microscope to study corneal tissue treated with travoprost with sofZia. They observed that the corneal epithelium showed a normal number of surface microvilli with slight blunting of microvilli tips with no other deleterious effects [12].

In addition, other studies utilized confocal microscopy to evaluate corneal epithelial cell morphology and cell size in New Zealand white rabbits after exposure to travoprost with sofZia and latanoprost with BAK. They found that travoprost with sofZia did not cause significant superficial corneal epithelial loss, whereas latanoprost with 0.02% BAK exposure led to more superficial cell loss in this *in-vivo* model [7].

In the present study, through a light microscope, corneal sections treated with latanoprost with BAK were seen to have severe damage in the form of degeneration, desquamation and separation of the epithelium. There was also lymphocytic infiltration under the epithelium at the limbus. Descemet's membrane showed separation in some parts from the stroma leaving wide spaces in between.

These results were in accordance with those of other studies, which found that prolonged use of topical ocular medications preserved with BAK may exacerbate sequelae associated with ocular surface disease and had adverse effects on the conjunctiva and cornea. These effects included the induction of subclinical inflammation, reduction of corneal epithelial barrier function, destabilization of the tear film, cataract formation and an overall higher incidence of patient complaints of dryness and irritation [13,14]

A corneal *in-vivo* confocal microscope was used to evaluate the corneal epithelial cell layers in rabbits after exposure to latanoprost with (BAK) 0.02%. The solutions were applied at 5 min intervals 15 times and it was found

that the superficial epithelium of the cornea showed partial desquamation of epithelial cells, irregular cell shape, loss of cell borders, swollen cells and inflammatory cell infiltration in the limbus area [15].

Multiple daily applications of BAK lead to serious damage to the ocular surface and even cause corneal ulcer vascularization and scarring, similar to what occurs with a chemical burn. However, in this study less remarkable adverse effects were observed, which may be due to less aggressive applications as we used a single daily instillation of one drop per day for a month [16].

In the present study, electron microscopic examination showed degeneration and vacuolation of the epithelial cells. Similar experiments assessed corneal damage with SEM. They found that in latanoprost-treated corneas there was cell membrane wrinkling, increase in the number of epithelial holes, cell peeling and decreasing epithelial cell layers [14].

Some keratocytes were dark and shrunken and there was aggravated separation of collagen fibers in the stroma. In addition, Descemet's membrane was separated in some parts from the stroma leaving wide spaces in between. These observations are consistent with those of other studies, which observed that, in the normal cornea, BAK resulted in intracellular and extracellular oedema and contraction of keratocytes [17]. In contrast, we explained the separation of collagen bundles as an effect of formalin fixation as it was not noticed in gluteraldehyde-fixed semithin sections stained with toludine blue in all groups.

The electron microscopic examination showed that the endothelial cell cytoplasm had many vacuoles and similar other results were explained as being vacuoles of swollen mitochondria [17].

These adverse effects of medications may be due to preservatives that induced the reduction of corneal cell proliferation and viability, which impaired corneal healing and disrupted epithelial barrier functions, which were more prominent with the use of BAK as a fixative than with sofZia [16].

This may also explain lymphocytic infiltration under the epithelium as an attempt at healing and protection against infection, which became easier with epithelial barrier disruption.

One of the mechanisms of action of BAK is that it kills organisms through action on cell walls causing disruption of cytoplasmic membranes with leakage of cellular contents including hydrolytic enzymes. This effect may explain the severe adverse reactions affecting corneal tissues [18].

As regards the conjunctiva, examination of the conjunctival sections through the light microscope after administration of travoprost with sofZia showed that the conjunctiva is composed of stratified columnar epithelium rich in goblet cells with an underlying connective tissue layer. There were no remarkable differences from the eyes treated with preservative-free artificial tears.

On comparison with group III, which received latanoprost with BAK, severe damage in the form of conjunctival epithelial cell disruption with severe loss of conjunctival goblet cells was seen. These results were also supported by statistical analyses.

Goblet cells appeared to be one of the most sensitive cell types to toxins in the tear film. These cells were extremely metabolically active, which was evident from the abundant rough endoplasmic reticulum and prominent Golgi complexes. This reflected their high rate of absorption of chemicals from their extracellular milieu, raising the possibility of these cells being hypersensitive to the presence of toxic substances in their environments [19].

Loss of goblet cells leads to decreased mucin secretion, which then leads to instability of the tear film, decreased nutrition to the superficial conjunctival epithelial cells with resulting increased mechanical damage to conjunctival and corneal surface cells, and decreased ability of the tear film to be distributed evenly on the ocular surface [20].

Hence, we recommend the use of safer drops with minor side effects and look forward to new medications avoiding these adverse reactions.

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Conflicts of interest

There is no conflict of interest to declare.

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الملخص العربى

دراسة هستولوجية عن تأثير الأستخدام المزمن لقطرات العين لاتانوبروست وترافوبروست علي الملتحمة و القرنية في الأرانب

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المقدمة: ان علاج مرض الجلوكوما المزمن يسسبب تغييرات ضارة علي الطبقة الطلائية لسطح العين.

هدف الدراسة: مقارنة التغييرات الهستولوجية في القرنية والملتحمة بعد الأستخدام المزمن لقطرة العين لاتانوبروست المحفوظة في مادة البنزالكونيوم كلورايد و قطرة ترافوبروست المحفوظة في مادة السوفزيا و قطرة الدموع الصطناعية الخالية من المواد الحافظة.

الأدوات و الطرق المستخدمة: اجري البحث علي خمسة عشر من الأرانب البيضاء. قسمت الأرانب الي ثلاث مجموعات (خمسة أرانب لكل مجموعة). تم اعطاء احدي القطرات لكل مجموعة مرة يوميا لمدة ثلاثون يوما. في نهاية التجربة تم انتزاع العين و تحضير عينات القرنية للدراسة بالمجهر الضوئي والالكتروني وتحضير عينات الملتحمة للدراسة بالمجهر الضوئي. استخدم جهاز تحليل الصورة بالكومبيوتر لقياس متوسط ارتفاع النسيج الطلائي للقرنية، وقياس متوسط عدد الخلايا الكأسية في الملتحمة. أيضا تم قياس متوسط نسبة مساحة التفاعل الأيجابي لصبغة شف للكشف عن وجود حمض البريوديك.

النتائج: اظهرت النتائج أن قطرة لاتانوبروست المحفوظة في مادة البنزالكونيوم كلورايد ادت الي تغييرات ضارة علي هيئة تحلل وانفصال بعض الخلايا السطحية للنسيخ الطلائي للقرنية، انفصال غشاء ديسمت، تحلل بعض الخلايا المبطنة لغشاء للقرنية بالاضافة الي نقص عدد الخلايا الكأسية في الملتحمة. أما قطرة ترافوبروست المحفوظة في مادة السوفزيا كانت أكثر أمانا و ادت الي تغييرات طفيفة في سطح العين مقارنة بقطرة لاتانوبروست المحفوظة في مادة البنزالكونيوم كلورايد.

الخلاصة: قد خلصت الدراسة الي أن الأدوية المضادة للجلوكوما المحفوظة في السوفزيا أقل ضررا علي القرنية والملتحمة من تلك المحفوظة في مادة البنزالكونيوم كلورايد.