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The Efficacy of Propolis and Gentamycin Treatments on a Corneal Ulcer in Pigeons (Comparative Study)

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Abstract

The aim of the study: Our study aimed to compare the efficacy of recurrent topical applications of watery soluble propolis and commercial gentamicin eye drop for the treatment of non-infected, chemical corneal ulcers. **Methods**: Twenty adult pigeons were used in this study. A round 5mm diameter circular filter paper disk immersed in 1 N NaOH solution for 5 sec has been used to create a corneal ulcer. The immersed filter disk has been placed upon the surface of the central corneal, centered on the pupil, and gently held in place wit for 30 sec. The corneal ulcer induced unilaterally in every one of the pigeons. The twenty pigeons were divided into two separated groups. Ten birds were treated with watery soluble propolis (propolis treated group) and the other ten birds were treated with Gentamicin eye drops solution 0.3% w/v (gentamicin treated group). **Results:** Clinical examinations of ulcerated corneas of the group which treated with gentamicin showed a decrease in inflammatory markers and opacity was better than the group treated with Propolis, but after 20 days, clinical signs of corneal ulcer disappeared in both groups. At the end of the 10th-day propolis treated corneal tissues histologically showed desquamation of corneal epithelium, edematous stroma with inflammatory cells, and congestion of blood capillaries. The antibiotic-treated group showed the intact epithelium and stroma of the cornea, After 20 days of inducing ulcers, ulcerative corneal tissue treated with propolis showed recovered whole epithelium, but the corneal endothelial cells within the tissue were observed to swell, fragment, and shed. However, the antibiotic treated group showed a magnificent histological improvement in corneal healing. **Conclusion:** both gentamicin and propolis promote corneal ulcer healing at variable rates, the study suggests that gentamicin have a more superior effect on the healing epithelium structure, and can be used as a preferred treatment for corneal ulcers.

Key words: corneal ulcer, gentamicin eye drop, pegion, propolis

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Introduction

Propolis can be specified as one of the resinous hive products acquired via worker bees from different plant parts (Pillai *et al.* 2010). As a honey-bee product, propolis was of high importance as alternative medicine and as a food. Its constituents were exerting pharmacological (anti-microbial, anti-cancer, and anti-inflammatory) effects (Inokuchi *et al.* 2006).

It is indicated that the bee glue (propolis) has anti-bacterial activities against a lot of typically faced cocci as well as gram positive rods, such as human tubercle bacillus, yet just limited activities against the gram negative bacilli. Propolis extracts were non-toxic in the experimental animals. In addition, aqueous solutions between (0.5 and 1%) were administered to humans as aerosols for effectively treating chronic and acute respiratory diseases; also, it was utilized as eye-drops (Shibata, *et al*. 2016).

The precise action mechanisms of propolis, royal jelly, and honey on the above-mentioned activities and diseases were not totally explained, and more researches are needed for explaining their particular contribution. Its use is on the basis of anti-fungal, anti-bacterial, anti-acne, anti-viral anti-oxidant, anti-inflammatory effects, microcirculation, epithelial in addition to topical anesthetic effects (Parashar and Agrawal, 2018).

The use of parenteral antibiotic eye drop formulations with non-marketed compositions or concentrations, commonly called fortified antibiotic eye drops, is a common practice in Ophthalmology in the hospital setting (Ferreiro *et al.* 2016).

Antibiotics are among the most common drugs used by ophthalmologists. They are used for the prophylaxis of infection after ocular surgery or surface trauma. In such circumstances, the corneal epithelial integrity has been disrupted, and a corneal epithelial defect is present in most of the cases. Any drug that disturbs healing of the epithelium should be administered with special caution (Lin and Boehnke 2000).

Gentamycin is well-known use in ophthalmology, as eye drop is stable solution. The commercially available concentration is 3 mg/ ml which is the ideal concentration recommended for the treatment of eyelid and conjunctiva infections. Gentamycin belong to the aminoglycoside group, the mechanism of action is by means of binding to the bacterial ribosome, inhibiting protein synthesis. In general it is bacteriostatic, but in higher doses, it may has bactericidal property (Moeller *et al*. 1999).

The transparent cornea is considered as the eye's outer layer at the front. The cornea is coated, and dryness is prevented via the harderian gland. The cornea shape can be changed via Crampton's muscles; therefore, providing the birds with greater accommodation ranged than

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what is possible for the mammals. In addition, the ciliary muscle alters the corneal curvature for corneal accommodation and moves the ciliary body anteriorly as part of the lenticular accommodative mechanism (Shehan, 2012).

This transparency is associated with the remarkable regularity of the protein content of the corneal stroma and the relative paucity of cells packed with organelles that would otherwise diffract light. The protein is, of course, collagen fibrils, each of them the same diameter, spaced equally apart by a similar distance. The regularity of the collagen fibrils that provide the cornea with its structural integrity renders them 'invisible' to the light rays passing through them. 'Invisible' as long as their regularity is maintained (Williams, 2014).

Essentially, the cornea includes 5 layers, which are Bowman's layer, epithelium, Descemet's membrane, stroma, and endothelium. It is specified as one of the body's few avascular tissues. There are no blood vessels in a healthy and normal cornea (Mayakkanna, et al. 2018). Furthermore, the anterior ciliary artery that is derived from the ophthalmic artery creates an arcade at the limbus (Sharma and Vajpayee, 2008).

The cornea has a protective epithelium, a dense collagenous stroma lined by an endothelium giving a tough resilient structure. It is transparent (a biological glass, we might say at least until trauma or infection strikes) (Williams, 2014). In the eye, the significant cornea functions are to protect the eye structures, contribute to the eyes' refractive power, to focus the light rays on the retina with the least scatter and optical degradation (Sridhar, 2018).The cornea, in addition to the tear film and conjunctiva, is the main components related to the ocular defense system against microbial infections. Whereas the corneal epithelium is acting as a mechanical barrier, the chemical and cellular components regarding conjunctiva as well as pre corneal tear film acting as biologic protective systems (Sharma and Vajpayee, 2008).

Comparable to that of humans, the avian corneal epithelium has a true Bowman's layer. It was assumed that removing such a layer following blunt, abrasive trauma might be affecting the corneal epithelialization that cannot be seen in the mammal species (Seruca, et al. 2012).

Sterile corneal ulceration might happen because of systemic dermatologic or connective tissue disease and thermal or chemical injuries (Sharma and Vajpayee, 2008). In birds, ocular diseases are infrequently studied. An increase in keeping extremely high-priced caged birds makes it a requirement for veterinarians to handle the ornithoophthalmology' problems. This, there is a need for finding species-orientated, differentiated solutions in investigations and adequate ocular diseases' treatments in birds (Korbel, 1991). In this study, the main goal is to compare the early healing effects related to watery soluble propolis and gentamycin on the corneal ulcer in pigeon eye.

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Materials and Methods

Twenty adult pigeons have been utilized in the present research. The birds have been clinically healthy and kept in cages at the animal house, Veterinary Medicine College, Univ. of Basrah. Water and food have been freely provided throughout the period of the adaptation**.** The experimentation has been performed on one eye of every one of the animals.

Method for Inducing Ulcer

A round 5mm diameter circular filter paper disk that has been produced with the use of the standard paper bunch, this disk has been immersed in 1 *N* NaOH solution for 5 sec; filter paper has been utilized due to the fact that it is molded easily to cornea in the case where it is wet. The eye-lid was secured manually in open position. The immersed filter disk has been placed upon the surface of the central corneal, centered on pupil and gently held in place wit for 30 sec. The corneal ulcer induced unilateral in every one of the pigeons (figure1 a, b, and c).

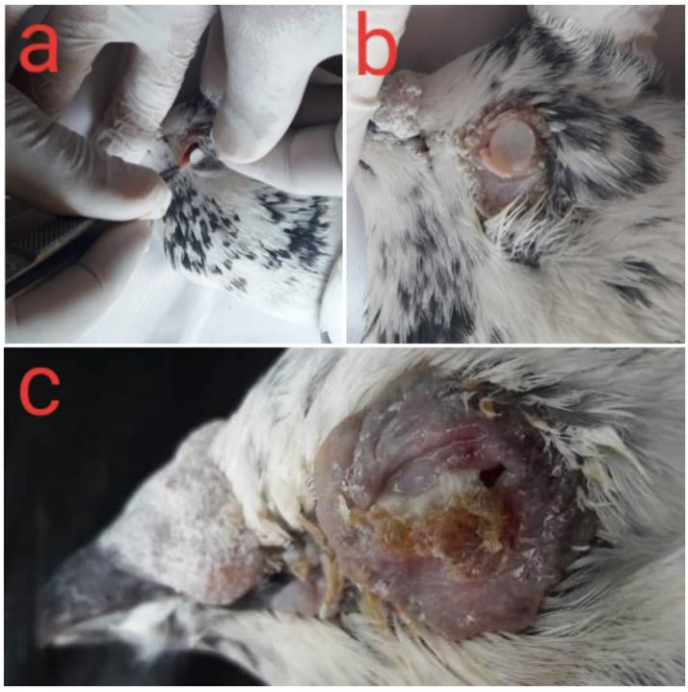

Figure (1): a., b. filter paper disc saturated with 1 *N* NaOH solution was put on the corneal surface of the pigeon. c. Corneal ulceration was obvious with lacrimation and eyelids adhesion.

The twenty pigeons were divided into two separated groups. Ten birds were treated with watery soluble propolis twice a day (propolis treated group) and the other ten birds were treated with Gentamicin eye drops solution 0.3% w/v also twice a day (gentamicin treated group).

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Clinical evaluation

External and detailed ophthalmic inspections of both eyes have been performed. The eyes have been inspected for the presence of corneal ulcer, eyelids adhesion, lacrimation, infection, or pus.

Histopathological inspection

On 10^{th}, 20^{th} days following the induced corneal ulcer that sacrifices the birds, the entire eye has been excised from the head with curved surgical scissor. Eyes were immediately placed in 10% formalin. The samples were sent to a private laboratory, routine processing of the tissue, the sections of the tissues have been stained by the Hematoxylin–Eosin stains (H&E).

Results:

Clinical Results

Clinical findings of the corneal ulcer in pigeons treated with propolis and gentamycin within different 2^{nd}, 5^{th} ,10^{th}, 20^{th} days are included:

In both groups at the 2^{nd} day of inducing corneal ulcers, there was edema, eyelids adhesion, signs of conjunctivitis, and obvious corneal ulcer (figure 1. C). After 5 days, there was lacrimation, opacity, but no eyelid adhesion. The gentamycin treated group showed a slight improvement in reducing these clinical signs (lacrimation and opacity).

In the 10^{th} day after inducing corneal ulcer, propolis treated group showed a reduction in the inflammatory signs, but tearing and slight opacities were still exist. However, in the gentamycin treated group, clinical results showed a remarkable improvement in the healing process.

On the 20^{th} day, both groups did not show differences, as the eye was close to normal and there was no obvious difference with the other normal eye.

Histopathological results

At the end of the 10^{th} day after ulceration, propolis treated corneal tissues showed desquamation of corneal epithelium, edematous stroma with inflammatory cells, and congestion of blood capillaries (figure 2, 3). After 20 days, ulcerative corneal tissue treated with propolis showed attenuated mononuclear cells and recovered whole epithelium, but also, the corneal endothelial cells within the tissue were observed to swell, fragment, and shed (figure 4, 5). However, in antibiotic treated group, after 10 days, the histaopathological examination showed the intact epithelium and stroma of the cornea with the keratocytes (figure

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6, 7), but after 20 days the antibiotic treated group showed improvement in corneal healing (figure 8).

Figure 2: Ten days after ulceration, propolis-treated corneal tissue showing complete desquamation of corneal epithelium (black arrow) and edematous stroma with inflammatory cells (white arrow), there were congestion of blood capillaries (yellow arrow) H&E stain 100X.

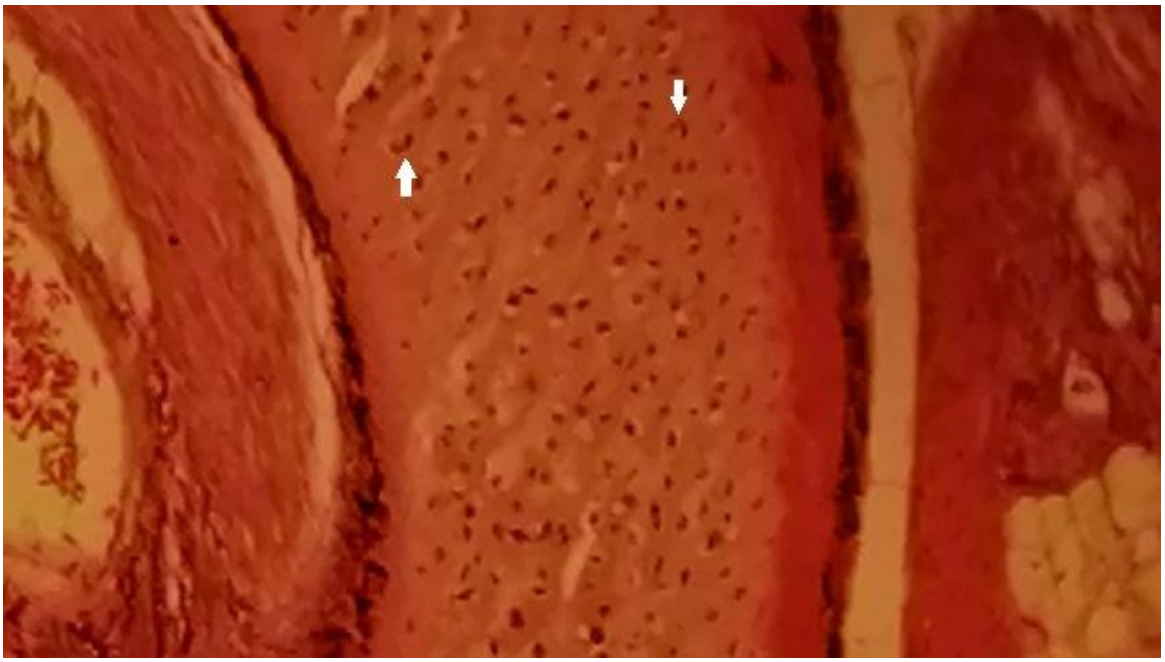

Figure 3: After 10 days, ulcerative corneal tissue treated with propolis observed the infiltration of inflammatory cells (white arrows) H&E stain 100X.

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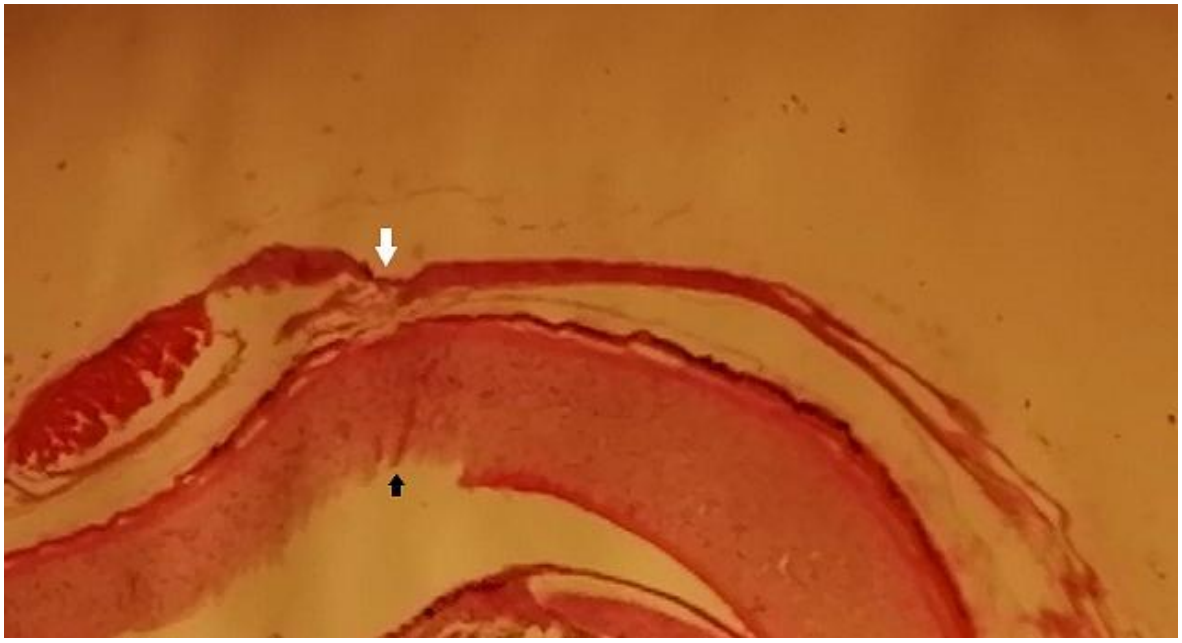

Figure 4: Ulcerative corneal tissue treated with propolis (at the end of the 20th day) showed attenuated mononuclear cells and recovered whole epithelium (white arrow), but also, the corneal endothelial cells within the tissue were observed to swell, fragment, and shed (black arrow). H&E, 40X.

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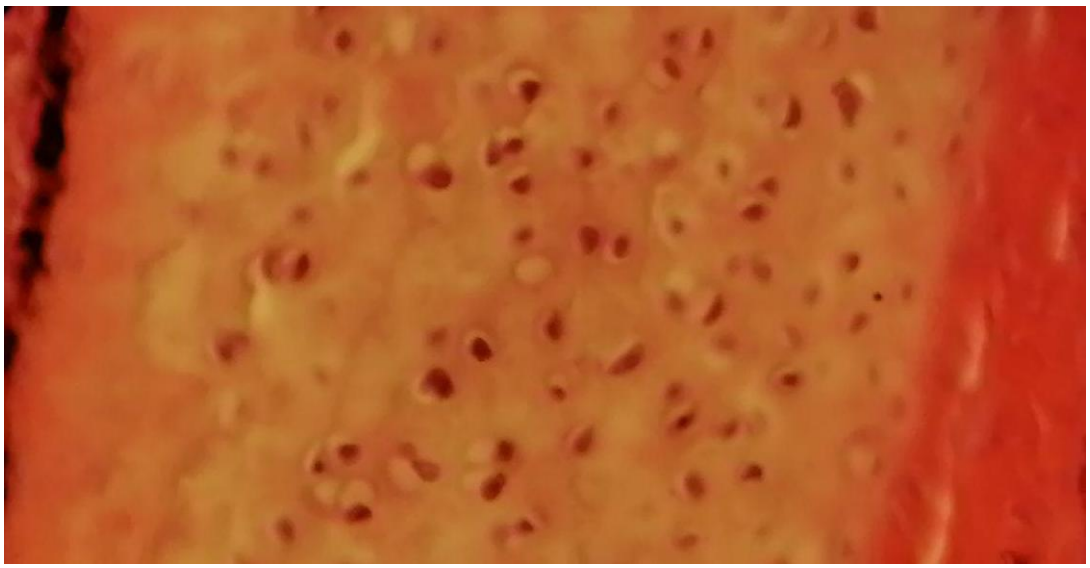

Figure 5: Ulcerative corneal tissue treated with propolis (after 20 days) shows normal stromal and infiltration of a few inflammatory cells. H&E, 400X.

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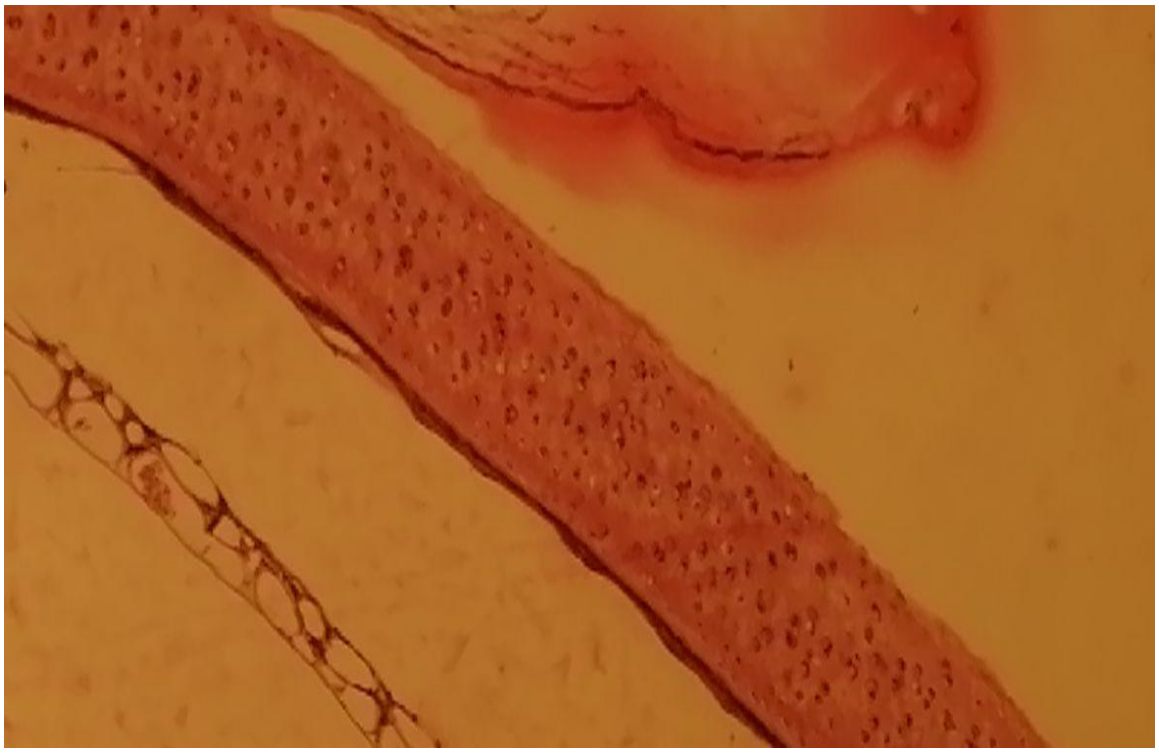

Figure 6: After 10 days of ulceration, antibiotic-treated group that shows the intact epithelium and stroma of the cornea with the keratocytes. H&E, 100X.

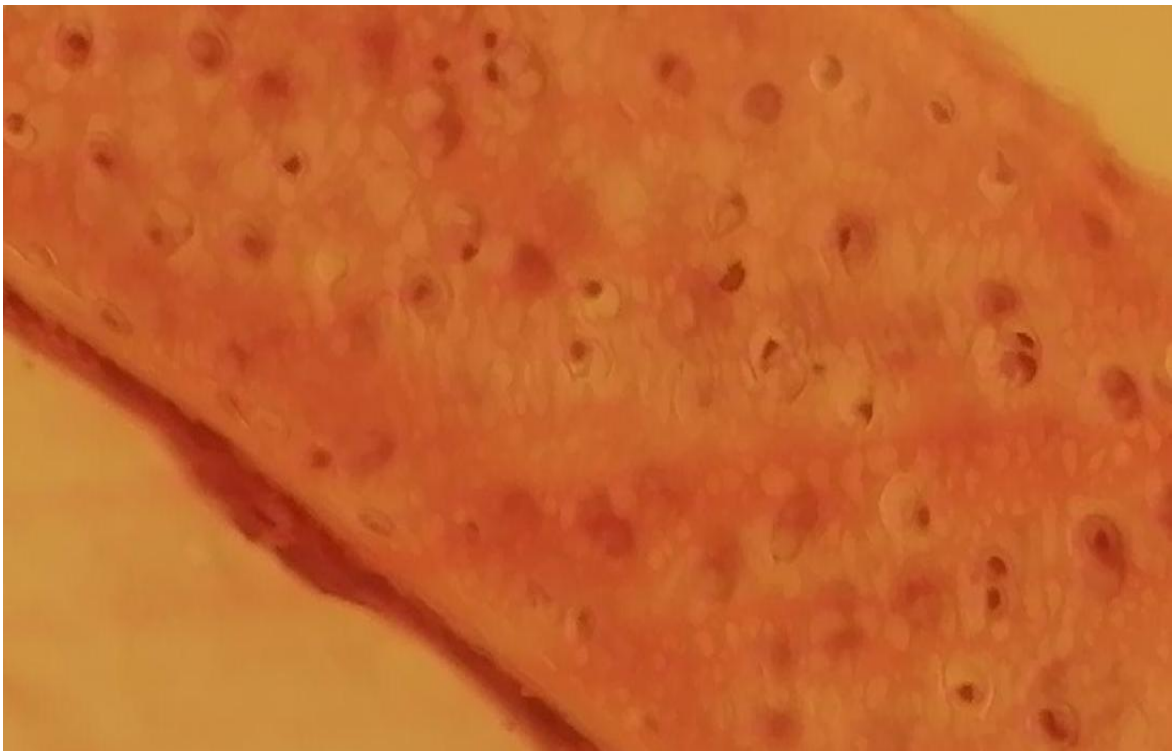

Figure 7: after 10 days, ulcerated antibiotic-treated group that shows the intact epithelium and stroma of the cornea with the keratocytes. H&E, 400X.

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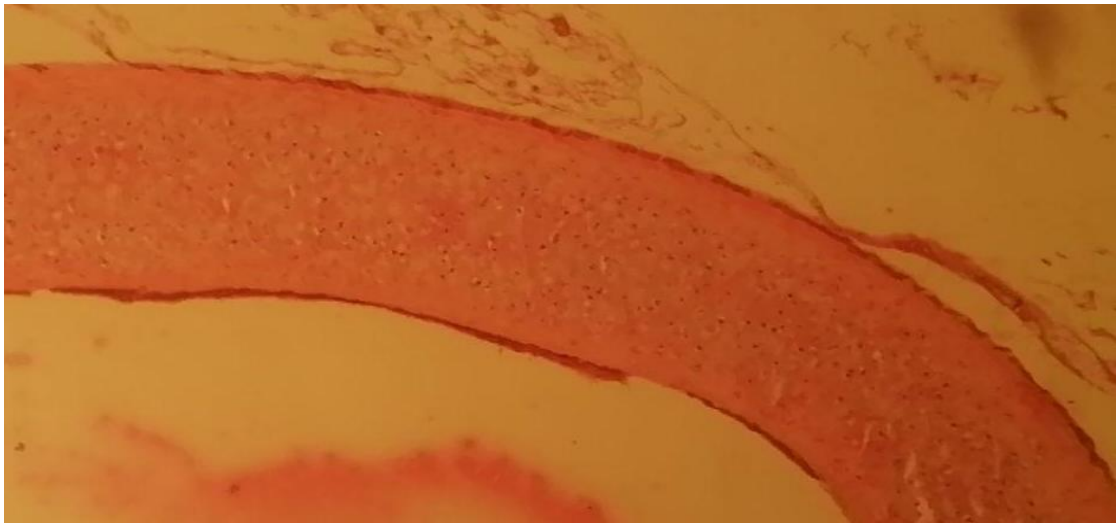

Figure 8: after 20 days, ulcerated antibiotic-treated group that shows the intact epithelium and stroma of the cornea H&E stain 100X.

Discussion

This study was performed to compare the efficacy of recurrent topical applications of watery soluble propolis and gentamicin eye drop for the treatment of chemical corneal ulcers.

On the fifth day after inducing the corneal ulcers, the clinical signs of the eyes in both groups that were almost identical due to the similarity of the lesions showed lacrimation, opacity, but no eyelid adhesion.

After the 10th day, propolis treated group showed a reduction in the inflammatory signs, but tearing and slight opacities were still exist. This indicates that propolis has an anti-inflammatory effect on tissues. These results came in agree with (Öztürk, *et al.* 2000) when they investigated the effect of propolis in the treatment of alkali-injured rabbit eyes and they found the effects of dexamethasone and propolis on healing of injured corneas were similar and significantly better than controls.

However, in the gentamycin treated group, clinical results showed notable improvement. Corneal ulcers can range from small epithelial erosions that will heal in less than a week to a descemetocoele that may rupture by the end of the day. Treatment of such varied lesions depends on a correct assessment of how deep the ulcer is and whether it is in the process of healing (Williams, 2014).

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After the 20th day, the two groups did not show apparent differences, as the eyes looked close to normal, meaning that both treatments contribute to protecting and improving the healing of the corneal ulcer. Nevertheless, the gentamicin eye drops did better at improving healing clinical signs.

At the day 10th, Histopathological sections of propolis treated corneal tissues showed desquamation of corneal epithelium, edematous stroma with inflammatory cells, and congestion of blood capillaries. but, the antibiotic-treated group showed the intact epithelium and stroma of the cornea with the keratocytes. That is meaning propolis showed a prolonged inflammatory stage or delayed corneal healing.

As we knew, that propolis has anti-bacterial and anti-inflammatory effects, the use of anti-inflammatory treatment is a two-edged sword; it may protect the cornea from host inflammatory response but at the risk of enhancing the infection.

In 1990, Carmichael et al. (during an 18-month period) were performing a randomized study of 40 selected patients affected with a bacterial corneal ulcer. Two groups were compared: one treated with antibiotics only and the other with antibiotics in addition to steroids. Complications were similar in the two groups. They did not notice any delay in the rate of healing of the ulcer with the use of a topical steroid.

According to the result After 20 days, both groups showed recovered whole epithelium, however, corneal ulcers treated with gentamicin revealed a well-developed improvement in healing. Whereas the eyes treated with propolis had a swell, fragment, and shed corneal endothelial cells.

The aminoglycoside gentamicin is a stable antibiotic with good penetration in the cornea when used in the correct dose (Moeller *et al.* 1999).

In 2000, Lin and Boehnke developed an in vitro epithelial wound-healing model to evaluate the toxicity of antibiotics and they did find 0.3% gentamicin sulfate did not retard epithelial healing, the 0.3% commercial preparation, a further lowering of the concentration may help to reduce the toxic effect.

Conclusion

both gentamicin and propolis promote corneal ulcer healing at variable rates. the study suggests that gentamicin have a more superior effect on the healing epithelium structure, and can be used as a preferred therapy for corneal ulcers**.**

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