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Effect of Nictitans Gland and Third Eyelid Excisions on Ocular Surface Integrity, pH, and Tear Production in Dogs

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Abstract

Prolapsed nictitans gland is a common affection in dogs. Several treatment options have been suggested such as removal of the prolapsed nictitans gland, excision of the third eyelid, or repositioning of the gland. Keratoconjunctivitis sicca (KCS) was reported to occur after excision of the prolapsed gland or the third eyelid, while recurrence is commonly associated with repositioning surgery. Therefore, the aim of this study was to investigate the effect of excisions of the nictitans gland or third eyelid on ocular surface integrity, pH, and tear production. Nictitans gland or third eyelid were excised surgically from healthy eyes in dogs. Corneal integrity, pH, and tear amount were examined for 7 months. Histological examination was performed after 7 months of surgery. Fluorescein staining was negative at the different time points in both groups. No significant change was observed in pH after excision of the third eyelid. In contrast, pH was significantly decreased after 2 weeks of removal of the nictitans gland. Tear amount was significantly declined 2 and 3 weeks after excision of the third eyelid and 3 weeks after excision of nictitans gland. The histological examination of different regions including, eyelids, cornea and conjunctiva in both groups compared to control revealed that all parts were completely normal. However, closer to the site of the third eyelid operation severe desquamation of epithelium and infiltration of inflammatory cells were observed. In conclusion, neither excisions of the third eyelid nor the nictitans gland induce the development of KCS or affect the ocular surface integrity.

KEYWORDS
Dog eye, Nictitans gland, Schirmer tear test, Third eyelid, Tear pH.

INTRODUCTION

The third eyelid also known as nictitans membrane is considered a conjunctival fold in the ventromedial fornix of many mammalian eyes (White and Brennan, 2018). In canine, it is supported by a T-shaped cartilage and contains the nictitans gland, which is responsible for production of 30% of the aqueous portion of tears (Gelatt *et al*., 1975; Saito *et al*., 2001; Davidson and Kuonen, 2004). The third eyelid contributes to formation of the precorneal film (tear film). The tear film consisted of three layers: the first layer is an oily layer produced and secreted by the multilobular meibomian glands (tarsal) in the margins of the eyelids and by the small Zeis glands. The second layer is an aqueous layer that is produced mainly by the lacrimal gland, the accessory (Krause's and Wolfring's) glands, and the nictitans gland. The third layer is a mucous layer produced mainly by the goblet cells of the conjunctiva and third eyelid glands (Jordan *et al*., 1990; Reece and Rowe, 2017). Prolapse of nictitans gland which is also known as third eyelid gland protrusion or cherry eye is one of the most common problems affecting the canine nictitans, and it may be unilateral or bilateral (Morgan *et al*., 1993; Aquino, 2008; Gómez, 2012). Cherry eye is more frequently occur in young dogs (up to 2 years) in breeds like Beagle, German Shepherd, Lhasa Apso, Labrador, Doberman, Bulldog, Chihuahua, Cocker Spaniel, Pekingese, Neopolitan Mastiff, poodle, Basset Hound, and brachycephalic dogs (Morgan *et al*., 1993; Mazzucchelli *et al*., 2012; Multari *et al*., 2016; White and Brennan, 2018). The pathogenesis of the prolapse is attributed to the weakness at the connective tissue attachment between the nictating gland and periorbital tissue, leading to pushing the gland from the normal ventral location to dorsally, then inflammation and hypertrophy occur, and appear as a pink colored mass over the free border of the third eyelid at the medial canthus (Edelmann *et al*., 2013). This abnormal enlargement prevents the gland from returning to its nor-

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mal location. Additionally, lymphoid hyperplasia of the nictitans gland occurred due to environmental allergens in young dogs may result in prolapse of the gland (Maggs, 2013).

There are different options that have been described for the treatment of the cherry eye. Trials with topical corticosteroid application with massage of the prolapsed gland have been performed; however, some veterinarians discourage the use of steroids (Rankin, 2013). Hence, several techniques have been established for repositioning the prolapsed nictitans gland, most commonly by several pocketing techniques or anchoring to the periosteum of the ventral orbit (Sapienza *et al*., 2014; Multari *et al*., 2016; White *et al*., 2018). Recurrence of the prolapse is usually seen in a high percentage after few weeks of replacement of the gland. Surgical failure is higher in hypertrophied or inflamed glands (Mazzucchelli *et al*., 2012; Multari *et al*., 2016; Michel *et al*., 2020). Additionally, repair is thought to be less successful in some giant and brachycephalic breeds such as Neapolitan Mastiffs and English Bulldogs (Martin, 2005).

Nictitating gland excision was the first described option for treatment of cherry eye; however, the removal is not recommended now to avoid the potential incidence of dry eye (keratoconjunctivitis sicca; KCS) that may occur due to decreased amount of tears (Barnett, 1978; Multari *et al*., 2016). Excision of the gland may decrease Schirmer tear test (STT) values (Saito *et al*., 2001), but the reported data explained that the reduction is highly variable. In the same context, third eyelid removal for treatment of prolapsed nictitans gland was previously described with several disadvantages including induction of KCS (Barnett, 1978). Removal of the third eyelid gland is only recommended when there is a malignant tumor due to its functions to promote tear secretion through its gland, to disperse tear over the corneal surface via its movement, to protect the cornea, and to assist with tear drainage (Bromberg, 1980; Saito *et al*., 2001). Therefore, it was necessary to investigate the difference between the total excision of the third eyelid and third eyelid gland removal on the different ocular parameters.

Thus, the present study aimed to evaluate the effect of removal of nictitans gland or the whole third eyelid as easy surgical techniques on the pH, tear production, and integrity of the cornea as examined by fluorescein dye and by histological examination.

MATERIALS AND METHODS

Animals and Experimental Design

The experiment was carried out at the department of surgery, anesthesiology and radiology, veterinary teaching hospital, faculty of veterinary medicine, Assiut University, Assiut, Egypt. Animal handling and experimentation were approved by the Institutional Animal Care and Use Committee of Research Facilities, Faculty of Veterinary Medicine, Assiut University, Egypt according to the OIE standards and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Ten adult mongrel dogs of both genders (5 males and 5 non-lactating and non-pregnant females) were selected and housed in separate standard cages. The range of the bodyweight of the dogs was 15-20 kg with age of 12-18 months. All dogs had ad libitum access to food and water and were kept for 2 weeks to adapt. The dogs were divided into 2 groups: Group I, included 5 animals that underwent total removal of the third eyelid in the left eye and the right one kept as a control. Group II, included 5 animals that underwent an excision of the nictitans gland only in the left eye and the right eye kept as a control.

Surgical techniques

Dogs were fasted for 12 h, then examined clinically to record the physiological parameters including rectal temperature, heart rate, and respiratory rate. General anesthesia was induced using Xylazine HCl 2% (Xyla-ject 2%, Adwia pharmaceuticals, Egypt) and Ketamine HCl 10% (Alfasan Co, Netherlands) with doses of 1mg/kg and 10 mg/kg, respectively. In the lateral recumbency position, the operated area was prepared aseptically using an appropriate aseptic technique by washing the eyes with cleansing normal saline. In group I, after induction of general anesthesia, the animal was placed in a lateral recumbency position on the right side and let the operated eye facing upward on the operation table. The operated eye was opened using a wound dilator, the third eyelid was exteriorized, and then the third eyelid was crushed with artery forceps which applied at its base to prevent any hemorrhage. Finally, excision of the third eyelid was performed using a scalpel. The artery forceps was removed after few minutes followed by pressure using sterile gauze for hemostasis. For animals in group II, an incision in the bulbar surface was performed followed by dissection till reaching the nictitating gland and removed. Pressure using sterile gauze for few minutes was done, and then antibiotic ointment was applied to prevent any infection.

All the animals were kept for 7 months after surgery. The following evaluation methods were applied and monitored every one week for the first 2 months, then every 2 weeks for the next 2 months, then every 4 weeks for the last 3 months (the total examination period was 7 months).

Clinical examination

Animals' eyes were examined by the naked eye for any signs of inflammation such as redness or swelling.

Fluorescein staining

A drop of fluorescein stain was placed on the cornea. The dye produced a green fluorescence and stain the areas of ulceration. Images were taken using a camera.

pH measurement

The pH was measured by a pH paper, then images for the papers were taken to be analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Schirmer tear test (STT)

To measure the STT, Schirmer tear testing paper (OpStrip, Ophtechnic, India) was used. The down portion of the test paper was inserted in the anterior medial one-third of the conjunctival sac. The test paper was removed after keeping it for 1 minute and the wet portion was measured.

Histopathological examinations

At the end of the evaluation period (7 months after surgery), 3 randomly selected animals from each group were selected and euthanized using thiopental sodium (Anapental, Sigma tec, Egypt) in a dose of 85 mg/kg after sedation by Xylazine (1mg/kg) according to AVMA guidelines (AVMA Panel on euthanasia,

2020). The dog cadavers were further used for teaching of anatomy for veterinary medical students at the faculty. For histological evaluation, small specimens from the third eyelid, nictitating gland, upper and lower eyelids, cornea, and conjunctiva of the control groups were fixed in 10% buffered formalin solution in dibasic anhydrous sodium phosphate and monobasic acid phosphate (pH 7.0) for 48 hours. In group I underwent total removal of the third eyelid, the specimens were collected from the upper and lower eyelids, cornea, and conjunctiva. In group II underwent an excision of the nictitans gland only, the specimens were collected from the third eyelid, the upper and lower eyelids, cornea, and conjunctiva.

The collected samples were dehydrated through an ascending graded series of ethanol at 70%, 80%, 90%, and 100% for 90 minutes at each concentration. The samples were cleared by immersing in methyl benzoate then embedded in paraffin wax (Sigma Aldrich, USA) according to the standard methodology described before (Hussein and Abdel-Maksoud, 2020). Step serial transverse and meridian sections were cut at 4-5 µm thickness using a microtome (Richert Leica RM 2125, Germany) and mounted on glass slides.

Ocular sections were deparaffinized with xylene and rehydrated through a decreasing gradient of ethanol followed by washing in distilled water. Then, the sections were stained with hematoxylin/eosin (H&E), Crossman's trichrome, and Alcian blue/periodic acid Schiff (AB/PAS). H&E staining was used to identify and describe the general structures of the tissues. Crossman's trichrome stain was used to detect collagen and muscle fibers. The histochemical analysis was performed to demonstrate the presence of neutral mucopolysaccharides using PAS staining while the sulfated and carboxylated acid mucopolysaccharides were demonstrated by using the Alcian blue staining (Horobin, 2019). All obtained slides were examined by Leitz Dialux 20 Microscope and images were taken by a canon digital camera (Canon Powershot A95) for the histological and histochemical description.

Statistical analyses

Results of pH and Schirmer tear test are presented as means ± standard deviations. Statistical analysis of the data by one-way ANOVA followed by Tukey's HSD post hoc test was performed using SPSS software (Version 21) (IBM Corp., Armonk, NY). P values < 0.05 were considered significant.

RESULTS

Clinical examination

In the third eyelid removal group, redness of the bulbar conjunctiva (conjunctivitis) with cardinal signs of inflammation in the cornea was observed in all animals in the first week and continued till the second week. Additionally, prolapse of the third eyelid of the contralateral eye (Control) was observed in one dog at the 5th week and continued till the 7th week. In four animals, the prolapse of the control eye started earlier on week 3 and continued till the 5th week in one dog, the 6th week in one dog, the 7th week in one dog, and for 13 weeks in one dog.

In the nictitans gland removal group, redness of the bulbar conjunctiva (conjunctivitis) with inflammation in the cornea was observed in 3 dogs in the first week and disappeared in the second week. Prolapse of the third eyelid of the control eye was observed in the third week in two dogs, whereas it disappeared in one of them after a week and continued till the 7th week in the other dog.

Fluorescence staining

All animals in groups I and II were negative to the fluorescence staining test.

pH values

As shown in Fig. 1A, there was no significant difference in pH

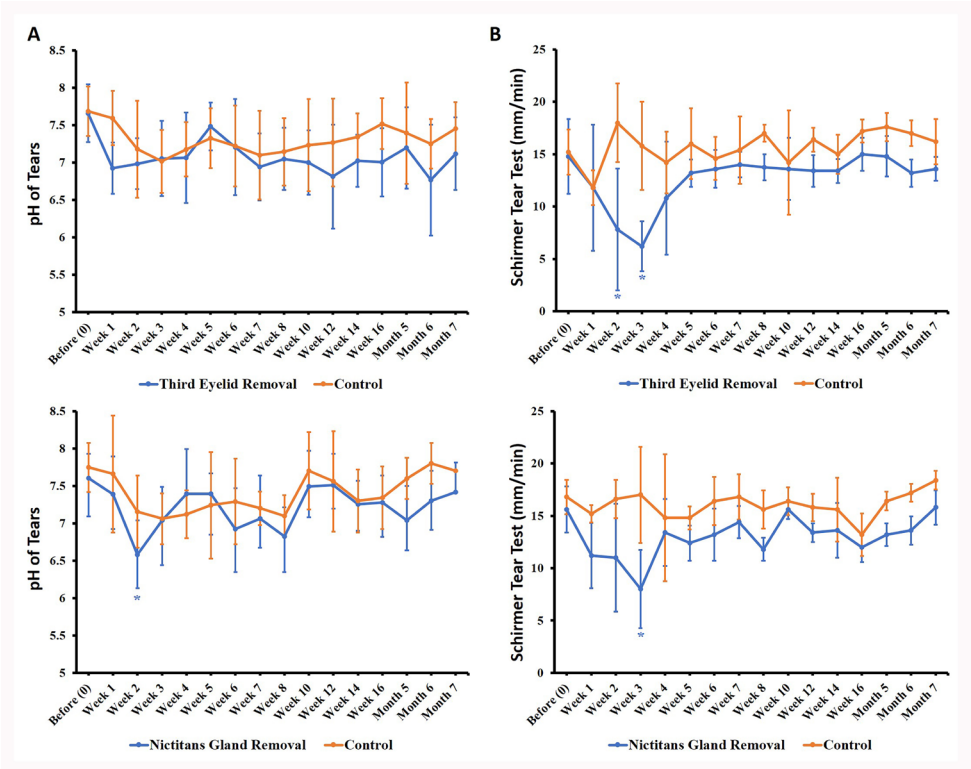

Fig. 1. Mean values of tears' pH (A) and Schirmer tear test (STT) (B) after excision of the third eyelid (upper graph) or the nictitans gland excision (bottom graph).

within the control eyes throughout the study compared to the baseline value. The pH value was decreased after one week of removal of the third eyelid to 6.92±0.34 compared to 7.65±0.38 (baseline). The pH started to increase again with no significant differences between the treated group for the different time points compared to the baseline. However, in the nictitans gland removal group, the pH was significantly ($p < 0.05$) reduced at the 2nd week (6.58±0.46) after removal of the nictitans gland then started to increase with no significant difference when compared to the baseline value (7.62±0.29).

Schirmer Tear Test

As shown in Fig. 1B, a significant reduction in the tear amount was observed at the 2nd and 3rd weeks of the third eyelid removal compared to the baseline value ($p < 0.05$). The STT was significantly decreased after 3 weeks of nictitans gland removal ($p < 0.05$), then starting from week 4, the amount of tears continued to increase and returned to normal baseline values.

Gross and microscopic observations

Anatomy of the third eyelid

As demonstrated in Fig. 2, the third eyelid (palpebra tertia) is well developed in dogs. It is considered as a semilunar fold of the conjunctiva, which emerges as a fold from the ventromedial angle of the conjunctiva. It is normally hidden within the orbit; however, its free border appears at the medial canthus and is covered partly by the lacrimal caruncle. The free border is darkly pigmented and directed dorsolateral. The movement of the third eyelid is adequate to sweep the entire surface of the cornea. The third eyelid is fixed in position by rich fasciae which are associated with the body of the third eyelid. Additionally, a dense connective tissue fascia, like a ligament inserts at both sides of the third eyelid. The third eyelid is supported by a thin hyaline cartilage plate having a T-shape, where the (T) column supports the body and the delicate (T) crossbar stiffens the free border of the fold. There is a gland surrounding the base of the cartilage. This is the superficial gland of the third eyelid. The deeper harder gland is absent in dogs (Fig. 2A-F).

Histology of nictitating membrane and gland

Histologically, the conjunctival fold consisted of conjunctival epithelium with an underlying stroma of fibrous connective tissue containing dense glandular and lymphoid tissue. The nictitating membrane is structurally supported with a flat T-shaped hyaline cartilage (Fig. 3A). The conjunctival epithelium differed in its structure in the bulbar (lymphoid region) and palpebral surfaces (non-lymphoid region). Accordingly, in the lymphoid regions, the epithelium associated with aggregated and solitary lymphoid follicles was simple cuboidal to squamous epithelium without goblet cells. The lymphoid follicles were observed close to the epithelial basal lamina. The non-lymphoid conjunctiva was covered with stratified columnar epithelium and many goblet cells were scattered among them. The goblet cells showed positive reactivity to PAS/ AB (purple color) (Fig. 3B and C). The parenchyma of the nictitating gland is composed of multilobular, tubulo-acinar structure. The nictitating gland is a mixed seromucous gland, therefore both mucous and serous secretory end-pieces were observed. However, the mucous secretory end-pieces were predominant. The seromucoid gland showed acidic muco-polysaccharides and neutral muco-polysaccharides when the sections stained with combined PAS/ AB (Fig. 3 D). The mucous secretory end-pieces were lined by tall columnar cells with pale acidophilic cytoplasm and basal oval nuclei and had a wide lumen. The boundaries of these cells were distinct. The serous secretory end-pieces were lined by pyramidal-shaped cells with deeply acidophilic cytoplasm and rounded basally located nuclei. It had a narrow lumen and the boundaries of these cells were hardly distinguished (Fig. 3E and F). The secretory end-pieces open directly into intercalated ducts lined with low cuboidal epithelium (Fig. 3E). All the intralobular ducts were lined with simple cuboidal epithelium (Fig. 4F). The interlobular ducts were lined with low cuboidal or flat squamous cells (Fig. 4F). These ducts open directly into the bulbar surface of the conjunctiva.

Microscopic overview of the conjunctiva and cornea in control eyes

The bulbar conjunctiva (mucous membrane) covers the anterior surface of the globe (except cornea) and the palpebral conjunctiva lines the internal surfaces of the eyelids. The forniceal conjunctiva connects between bulbar and palpebral conjunctiva.

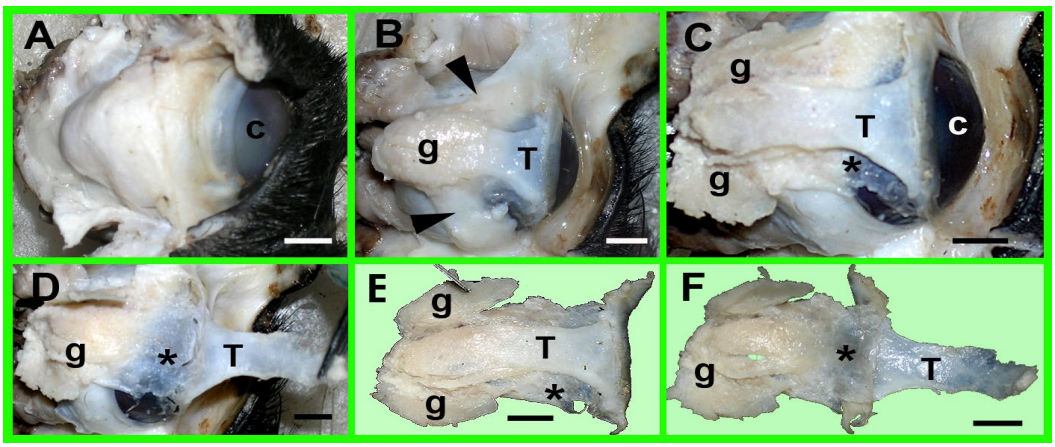

Fig. 2. Anatomy of the third eyelid in dog. A) The medial side of the eye after dissecting the medial canthus and removal of the superficial fascia and fat. The margin of the third eyelid appears just next to the cornea (c). B) The (T) shaped cartilage of the third eyelid appears, after dissecting the fascia. The gland (g) of the third eyelid wraps the basal half of the cartilage. The third eyelid is fixed in position by 2 ligaments (arrowhead), one from each side. C) The appearance of the whole cartilage after dissecting out the glandular tissue from its palpebral side. Note the bulbar mucous membrane (asterisk) of the third eyelid appears over the cornea. D) The appearance of the bulbar mucous membrane (asterisk) and the whole gland after reflecting the cartilage. E) The third eyelid has been dissected out the eye. Note the pigmented margin of the third eyelid. F) The appearance of the whole gland after reflecting the cartilage. The scale bar equals 0.5 cm.

The upper and lower eyelids consist of a fibrous tarsal plate and skeletal muscle fibers, bounded by the skin on the outer surface (Fig. 4A and B). In addition, there are a number of adnexal specializations, such as eyelashes (cilia) and glands. The tarsal or meibomian glands are modified sebaceous glands with long, multilobed acini completely occupied by lipid droplets that resemble a large bunch of grapes (Fig. 4B). Near the base of eyelashes, the glands of Moll which are apocrine sweat glands were observed. The glands of Zeis are unilobar sebaceous glands that open into the follicles (Fig. 4C). At the marginal area, the palpebral conjunctiva is lined by pigmented, non-keratinized stratified squamous epithelium that lies over a connective tissue substantia propria. Afterward, the palpebral conjunctiva changes abruptly into stratified columnar epithelium with goblet cells that increased markedly toward the fornix (Fig. 4D-G). Goblet cells were large, rounded cells and filled with glycoprotein (mucin) granules. Since the nature of the glycoprotein components in goblet cells differed in each cell therefore, it could be demarcated using AB/

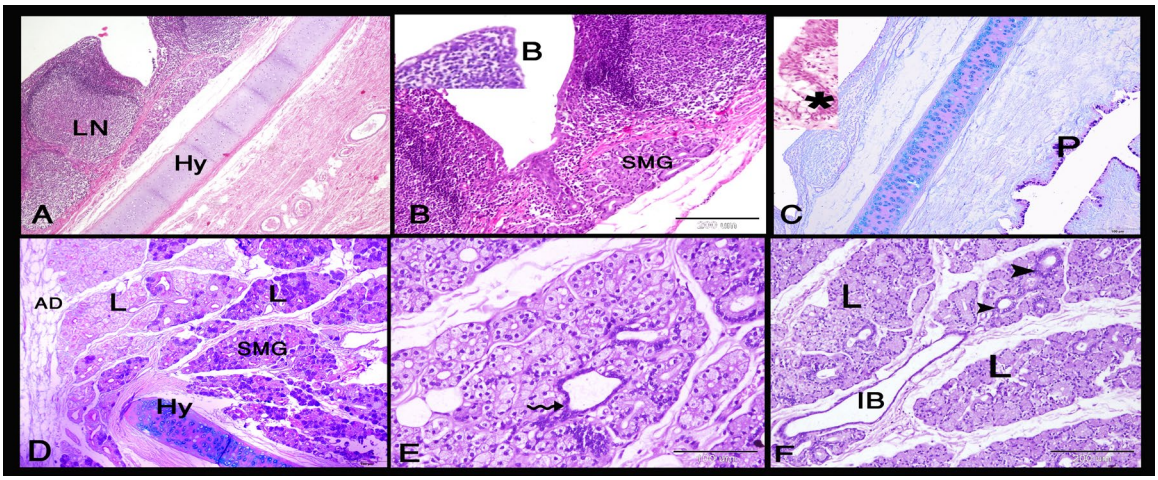

Fig. 3. Histology of the nictitans membrane, gland and duct. A) Paraffin section stained with H&E and showing the conjunctival fold which consisted of conjunctival epithelium with an underlying stroma of fibrous connective tissue containing dense glandular (G) and lymphoid tissues (LN). The nictitating membrane is structurally supported with hyaline cartilage (Hy). B, C) Sections stained with H&E and PAS/AB, respectively showing the conjunctival epithelium in the bulbar (B) region associated with aggregated and solitary lymphoid follicles was lined by simple cuboidal to squamous epithelium (inset). The palpebral (P) conjunctiva was covered by stratified columnar epithelium and many goblet cells were scattered among them (inset). The goblet (asterisk) cells showed positive reactivity to PAS/ AB (purple color). D) Section stained with PAS/AB showing the parenchyma of the nictitating gland that is composed of multilobular (L), tubulo-acinar structure showing the mixed secretion of the gland. E, F) Stained sections with H&E showing the intercalated ducts (wavy arrow), the intralobular ducts were lined with simple cuboidal epithelium (arrowheads). The interlobular ducts (IB) were lined with low cuboidal or flat squamous cells. Abbreviations: SMG (seromucoid gland) and AD (adipose tissue).

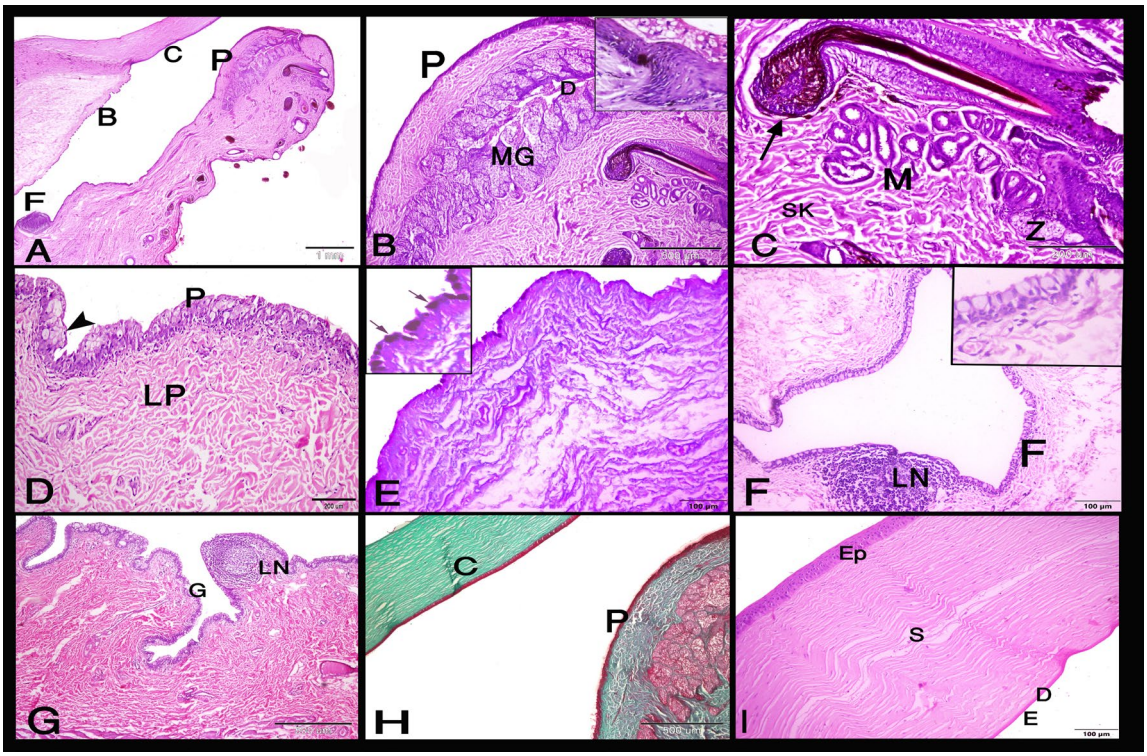

Fig. 4. Conjunctiva and cornea in the control group. A) Paraffin section stained with H&E showing the conjunctiva divided into bulbar (B), forniceal (F), and palpebral (P) regions. B, C) The eyelids consist of a fibrous tarsal plate with meibomian gland (MG) and its duct (D) and skeletal muscle fibers (SK), bounded by the skin on the outer surface (inset). In addition, there are a number of adnexal specializations, such as cilia (Ci) and glands, the glands of Moll (M), and the glands of Zeis (Z). D-G) The palpebral conjunctiva lined by stratified columnar epithelium with goblet cells that increased markedly toward the fornix (F). E) PAS/AB-stained section showing the goblet cell in the palpebral conjunctiva. Numerous lymphatic nodules (LN) are found throughout the conjunctiva. H, I) The cornea consisted of four layers: epithelium (Ep), stroma (S), Descemet's membrane (D), and endothelium (E).

PAS staining. The acidic glycoprotein was stained blue by AB, and the neutral glycoprotein was stained pink by PAS. However, in dogs, most of goblet cells contained a mixture of blue- and pink-stained glycoprotein, appearing as purple staining (Fig. 4E). Numerous lymphatic nodules or conjunctiva-associated lymphoid tissue (CALT) are found throughout the conjunctiva (Fig. 4G). The dog cornea consisted of four layers: epithelium, stroma, Descemet's membrane, and endothelium. The epithelium was stratified squamous epithelium. The corneal stroma was the thickest layer as it forms about 90% of the corneal thickness and it consisted of several bundles of collagen fibers. The cytoplasmic processes of the keratocytes (fibroblasts) run between the collagen bundles. Descemet's membrane was located at the innermost part of the stroma, and underneath a single layer of squamous endothelial cells was observed (Fig. 4H and I).

Microscopic overview of the conjunctiva and cornea in the group-I operated eyes

In group I, the histological sections of the upper and lower eyelids, cornea, and conjunctiva we examined. We demonstrated that the palpebral conjunctiva lining the inner aspect of the eyelids, at the different regions including the marginal, forniceal, and bulbar, with its adnexal structures was completely normal in comparison with the control one (Fig. 5A and B). The palpebral conjunctiva was varied in their lining epithelium from stratified squamous epithelium to stratified columnar epithelium with interspersed goblet cells (Fig. 5C-H). In addition, the corneal epithelium and stroma were completely normal (Fig. 5I). No signs of inflammation or degeneration were observed in the lining epithelium of the cornea and conjunctiva.

Microscopic overview of the conjunctiva and cornea in the group- II operated eyes

In group II, the specimens were collected from the third eyelid, the upper and lower eyelids, cornea, and conjunctiva. The palpebral conjunctiva of the superior eyelid at different regions and the cornea were normal without any lesions (Fig. 6 A-F). In the lower eyelids, the lining epithelium and underlying connective tissue in the inner aspect of the palpebral conjunctiva were also normal (Fig. 7 A-D). However, closer to the site of the third eyelid operation, we observed some abnormalities including severe desquamation of epithelium, and the lamina propria was infiltrated with mononuclear inflammatory cells (Fig. 7E). Additionally, the epithelium in the bulbar side showed vacuolar degeneration with slight infiltration with mononuclear inflammatory cells (Fig. 7F and G). The lymphoid follicles showed severe depletion and exhaustion along with severe congestion of blood vessels. A few seromucoid glands close to the lymphoid follicles were observed (Fig. 7H and I).

DISCUSSION

Prolapsed nictitans gland is a common, important ocular condition in dogs. Various surgical techniques such as excising or replacing the prolapsed gland have been described, but effective intervention is still depending on the personal surgeon preference (Gómez, 2012; Mazzucchelli *et al*., 2012). A big debate in literature was revealed about the need of replacing or anchoring the nictitans gland over the removal of the nictitans gland or the third eyelid. Therefore, investigating the potential of total excision of the third eyelid or the nictitans gland as suitable options for treatment of prolapsed gland of the third eyelid is required.
The tear film aids in the preservation of the integrity of the ex-

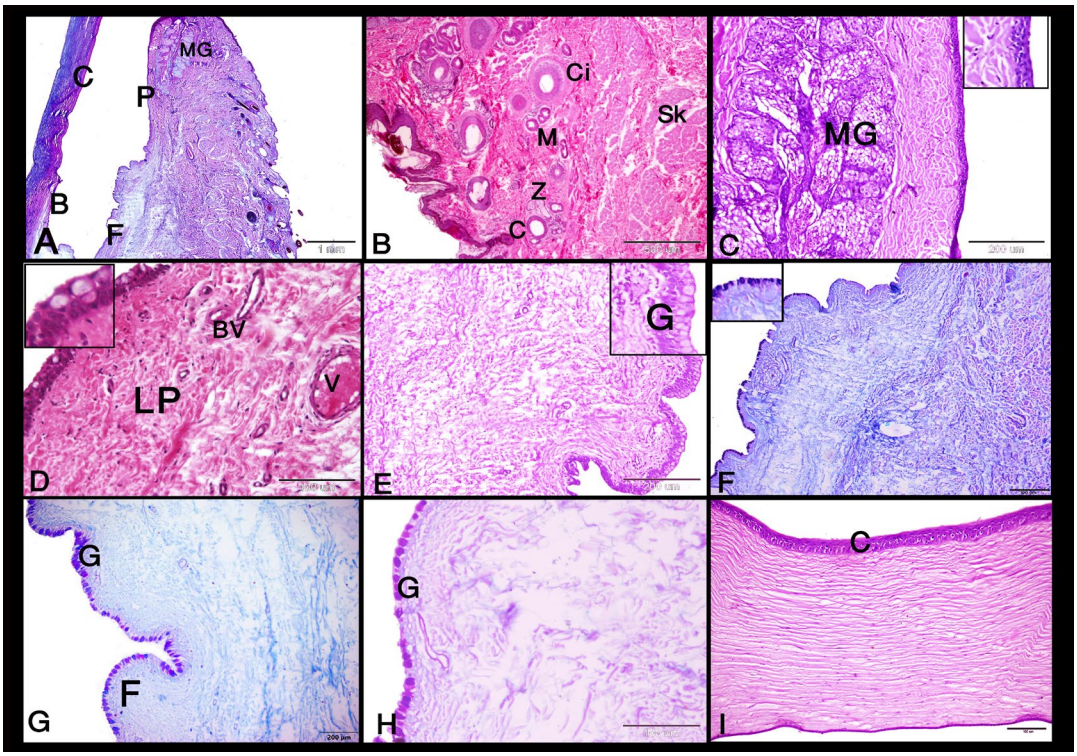

Fig. 5. Upper and lower eyelids, cornea, and conjunctiva in group I. A-C) Paraffin section stained with PAS/AB and H&E showing the palpebral conjunctiva (P) lining the inner aspect of the eyelids, at the different regions including the marginal, forniceal (F), and bulbar (B), with its adnexal structures was completely normal in comparison with the control one. C-E) The palpebral conjunctiva was varied in their lining epithelium from stratified squamous epithelium (C, inset) to stratified columnar epithelium with interspersed goblet cells (D, E, and Inset). F-H) PAS/AB showing the purple color of goblet cell in the palpebral and forniceal (F) conjunctiva. I) The corneal (C) epithelium and stroma were completely normal. Abbreviations: MG; meibomian gland, LP; lamina propria, BV; blood vessel, Ci; cilia, SK; skeletal muscle, Z; Zeis, M; Moll gland, V; vein, and G; goblet cells.

posed eyeball surface and conjunctiva that lines the eyelid and covers the eyeball tissues (Saito *et al*., 2001). The nictitating membrane contributes to the formation and secretion of the precorneal tear film which plays a protective role in clearing debris from the surface (Samuelson, 1999; Maggs, 2013). The seromucoid (mixed) gland of the nictitating membrane which is located at its base produces a portion of the mucous and aqueous secretion of the tear film. The glands empty their secretion in the excretory duct system which opens directly in the bulbar conjunctival surface (Giuliano *et al*., 2002). The lymph nodules in the third eyelid and conjunctiva (conjunctival associated lymphoid nodules) play an important role in the local immunological protection of cornea and conjunctiva against pathogens (Chodosh *et al*., 1998; Giuliano *et al*., 2002). Fluorescein is commonly used to detect any disorder in the precorneal tear film, corneal epithelial defects, and corneal ulcers (Zeev *et al*., 2014). In the current study, the fluorescein stain was negative at the different time point in both treatment groups which indicate the absence of the injuries or micro-injuries of the corneal surface and the full integrity of the cornea.

The pH value was decreased non-significantly after one week of the third eyelid excision and at the 2nd week after removal of the nictating gland. These results are contrary to Saito *et al* who reported a quick increase in pH after excision of the nictating gland (Saito *et al*., 2001).

Tear production is reported to originate from the lacrimal gland and the nictitans gland with percentages of 70% and 30%, respectively (Maggs, 2013). Schirmer tear test (STT) is commonly used as a semi-quantitative standard test for evaluating aqueous tear production (Petersen-Jones, 1997; Kim *et al*., 2019; Hussein *et al*., 2021). Helper *et al*. (1974) assessed the effect of lacrimal gland or nictitating gland removal on tear volume by measuring STT values. They reported that tear production declined by 10–37% (average 23%) after removal of the lacrimal gland whereas it declined by 29–57% (average 42%) after the excision of the nictitans gland (Helper *et al*., 1974). Gelatt *et al*. (1975) showed that the surgical removal of the nictitating gland resulted in a 9.2% reduction of Schirmer tear test values by 4 months. Both Helper *et al*. (1974) and Gelatt *et al*. (1975) studies reported that there was no KCS on clinical observation after removal of the third eyelid gland. However, Martin (2009) has recorded that removal of the nictitating membrane gland induced developing KCS by 42.8% and is more serious in many breeds that are susceptible to KCS. McLaughlin *et al*. (1988) evaluated the effects of removing the lacrimal gland and the third eyelid gland independently on tear production in cats. They reported that the tear amount decreased by 34.1% and 15.7% on STT after removal of the lacrimal gland and third eyelid gland, respectively. In the current study, the amount of tears decreased significantly by 59.45% and 58.10% after 2 and 3 weeks of third eyelid removal, respectively. Removal of the nictating gland resulted in a significant reduction of the tear production by 30% after 3 weeks.

Gupta *et al*. (2016) have reported the removal of the nictitans gland in 8 eyes suffering from the cherry eye in 5 dogs, with no significant decrease in tear production even after removal of the third eyelid gland. The authors added that dogs treated by removal of the nictitans gland showed excellent outcomes in terms of immediate postoperative cosmetic appearance. No postoperative complications were recorded during a three-year follow-up except in one Beagle that had bilateral cherry eyes. This dog showed the signs of KCS in one eye two years after excision of the nictitans gland with a Schirmer tear test reading of <5 mm/min. No signs of KCS or "dry eye" were recorded in any animal in both groups of surgical interference for 7 months of the study which agree with the study of Saito *et al*. (2001). It has been reported that the absence of KCS was attributed to the maintenance of the tear volume by increasing the reflex secretion leading to a compensatory increase in tear production by the lacrimal gland resulted in very minor defects to the corneal surface (Saito *et al*., 2001). The obtained observations have been supported by the microscopic examination of the eyelids, cornea, and conjunctiva in both groups compared to the control. The different parts were completely normal and free from any signs of degenerations or lesions. However, closer to the site of the third eyelid operation,

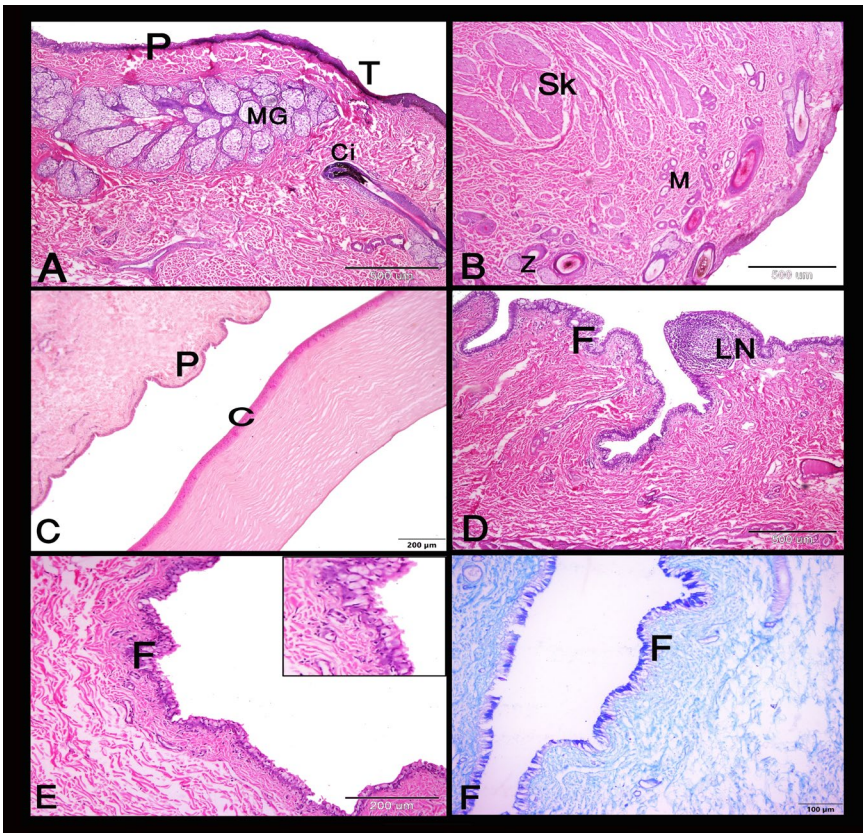

Fig. 6. Superior eyelids, cornea, and conjunctiva in group II. A-E) Paraffin sections stained with H&E showing the palpebral (P), forniceal (F) conjunctiva of the superior eyelid at different regions and the cornea (C) were completely normal without any lesions. F: PAS/AB-stained section showing the purple color of goblet cells in forniceal conjunctiva. Abbreviations: MG; meibomian gland, Ci; cilia, SK; skeletal muscle, Z; Zeis, M; Moll gland, T; tarsal plate, and LN; lymph nodule.

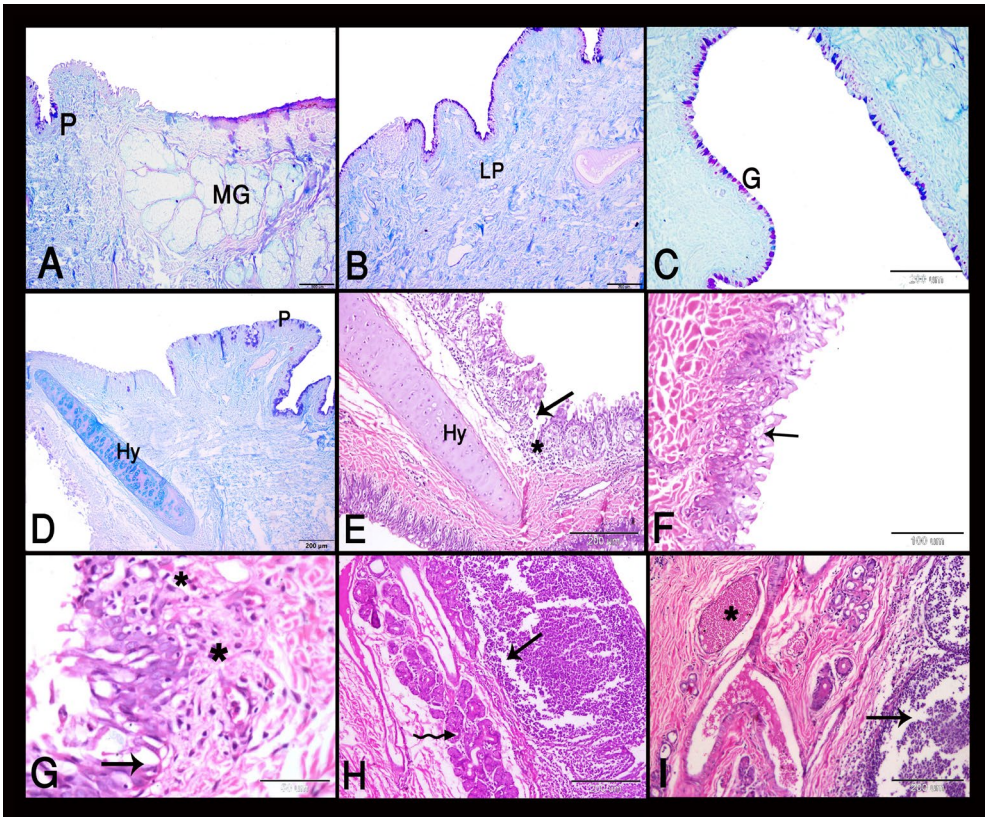

Fig. 7. A-C) showing the palpebral conjunctiva (P) of the lower eyelids in group II. The lining epithelium and underlying connective tissue (LP) was normal. C) PAS/AB staining showing the purple color of goblet cells (G) in forniceal conjunctiva. D) PAS/AB staining showing the hyaline cartilage of third eyelid (Hy) without the nictitans gland. E) showing the severe desquamation of epithelium (arrow) and the lamina propria was infiltrated with mononuclear inflammatory cells (asterisk). F, G) showing the vacuolar degeneration (arrow) in the epithelium of the bulbar side and the lamina propria was infiltrated with mononuclear inflammatory cells (asterisk). H, I) The lymphoid follicles showed severe depletion, exhaustion (arrow) and severe congestion of blood vessels (asterisk). A few seromucoid gland close to the lymphoid follicles were observed (wavy arrow).

some abnormalities including severe desquamation and vacuolation of epithelium, and the lamina propria was infiltrated with mononuclear inflammatory cells. In addition, the lymphoid follicles showed severe depletion, exhaustion and severe congestion of blood vessels. This could be attributed to the manipulation of the third eyelid during the excision of the gland.

According to results from this study, the authors recommend the removal of the third eyelid in case of any disorder affecting it without fear. Moreover, prolapsed nictitans gland could be treated by excision of the gland as it is technically simple without developing KCS. The authors suggest using artificial tears as postoperative care for 3 weeks after removal of the third eyelid or the nictitans gland to avoid the changes in the volume and pH of tears during this period. In the present study, the main limitation is the relatively low sample size, so further studies with a larger sample size are required. Additionally, the postoperative period of evaluation is only 7 months that may require to be elongated for 1-2 years.

CONCLUSION

Excisions of the third eyelid or the nictitans gland induce significant changes in the pH of tears after two weeks of nictating gland removal and recordable changes after a week of the third eyelid removal. Moreover, tear production is significantly decrease at 2 weeks and 3 weeks after the third eyelid removal and at week 3 of the nictating gland excision. Neither excisions of the third eyelid nor the nictitans gland induce the development of KCS or affecting the ocular surface integrity.

CONFLICT OF INTEREST

The authors have declared that no competing interest exist.

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