

## Clinical evaluation of topical tacrolimus ointment usage in different stages of keratoconjunctivitis sicca in dogs

Christina JOHN , Aswathy GOPINATHAN\* , Kiranjeet SINGH ,

Pallvi SHARMA, Chelladurai SOWBHARENYA , Sherin B. SARANGOM 

Division of Surgery, Indian Council of Agricultural Research-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India

Received: 01.01.2018 • Accepted/Published Online: 29.03.2017 • Final Version: 09.08.2018

**Abstract:** This study was conducted to investigate the effect of topical tacrolimus (0.1%) ointment in stimulating tear production in dogs with keratoconjunctivitis sicca (KCS). Thirty-six dogs (58 eyes) diagnosed with KCS were included in this study. KCS was grouped as early (E, n = 21 eyes), late (L, n = 21 eyes), and reflex tear due to corneal ulceration (R, n = 16 eyes) based on Schirmer tear test (STT) readings. The dogs in each group were randomly allotted treatment with either tacrolimus 0.1% ointment applied once daily (ET, n = 11 eyes; LT, n = 10 eyes; RT, n = 6 eyes) or cyclosporine (CsA) 0.05% eye drops instilled five times daily (EC, n = 10 eyes; LC, n = 11 eyes; RC, n = 10 eyes) for 2 months. The efficacy of the treatments was evaluated and comparison was made based on complete ophthalmic examination and a scoring system for various parameters like menace reflex, palpebral reflex, pupillary light reflex, conjunctival hyperemia, ocular discharge, corneal clarity, corneal ulceration, corneal vessel length, corneal vessel density, corneal pigmentation area, corneal pigmentation density, STT readings, fluorescein dye test, and Rose Bengal dye test. The effect of treatment was evaluated on day 15, 30, and 60 after treatments. Good quality digital photographs from a fixed distance were taken at each interval to aid subjective evaluation. Data were analyzed using one-way and repeated-measures ANOVA followed by Tukey's HSD test, Wilcoxon signed rank test, and Kruskal-Wallis test followed by Dunn's test to identify significant interactions. The level of significance was set to  $P < 0.05$ . Improvement in menace reflex and pupillary light reflex was observed in both treatment groups. Conjunctival hyperemia and ocular discharge decreased significantly after treatment with both of the drugs. There was no significant difference in improvement in tear production between treatment groups but tacrolimus 0.1% ointment significantly arrested the progression of pigments. The study concluded that both tacrolimus 0.1% ointment and CsA 0.05% eye drops improve tear production in KCS-affected dogs. Topical tacrolimus 0.1% ointment effectively arrests the progression of pigmentation compared to CsA 0.05% eye drops in KCS-affected dogs.

**Key words:** Tacrolimus, cyclosporine, lacrimostimulant, keratoconjunctivitis sicca, dogs

### 1. Introduction

Keratoconjunctivitis sicca (KCS), or dry eye, is a local immune-mediated disease that leads to the deficiency of precorneal tear film, which is crucial for preserving ocular surface health (1,2). The unrefreshed tear film, having altered composition like elevated tear osmolarity and the presence of proinflammatory mediators and proteases, no longer supports the normal functioning of the ocular surface (3). The deficiency in the aqueous component of the tear film predisposes the ocular surface to infection (4) and subsequent complications such as ulceration, vascularization, fibrosis, and pigmentation (1). Though there are several causes of KCS such as drug-induced toxicity, neurogenic or endocrine factors, hypoplasia, or adenitis of the lacrimal glands, a local immune-mediated destruction of the lacrimal glands was established in

histological studies (5). The risk factors for KCS are aging, breed predisposition in brachycephalic dogs, hormonal changes, and systemic diseases (1,3,6-8).

Stimulation of natural tear production was the mainstay of treatment for KCS to reduce associated clinical signs and thereby prevent loss of vision (9). The calcineurin inhibitors, cyclosporine A (CsA) and tacrolimus, are the lacrimostimulants widely used in dogs with KCS. CsA is the most commonly used lacrimostimulant for dogs with KCS either in the form of ointment (0.2%) or as an oil-based suspension (1%-2%). It has unique noncytotoxic effects on T cells at therapeutic concentrations and acts by inhibiting T-cell proliferation, allows regeneration of lacrimal glands and acini, and supports the secretory function (10). CsA has antiinflammatory properties on ocular tissues, prevents lacrimal gland apoptosis, and has a proliferative effect on

\* Correspondence: [aswathykiran77@gmail.com](mailto:aswathykiran77@gmail.com)

conjunctival goblet cells stimulating mucin production. It also has potent immunosuppressive properties, reflecting its ability to block the transcription of cytokine genes in activated T cells (9–14). Tacrolimus (formerly FK506) is a macrolide antibiotic from *Streptomyces tsukubaensis* that shows similarities to the immunomodulatory action of CsA (10,15). CsA therapy has been found to be less effective in bringing back tear production to normal levels and is an irritant to ocular tissues. It also fails to prevent the progression of corneal pigmentation in eyes severely affected with KCS (9). Topical administration of a 0.02% aqueous suspension of tacrolimus was found to enhance tear production in dogs with KCS (9). There are only a few studies on the effects of commercially available tacrolimus 0.1% ointment topically in eyes of dogs affected with KCS. This study was hence conducted with the aim of evaluating the efficacy of topical tacrolimus 0.1% ointment applied once a day and CsA 0.05% eye drops instilled five times a day in dogs with early and late stages of KCS, and also for KCS-associated corneal ulceration presented with reflex tearing.

## 2. Materials and methods

### 2.1. Grouping of cases and ophthalmic evaluation findings

Due permission to conduct the present clinical experiment was obtained from the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of the Environment, Forest, and Climate Change, Government of India (No. F.25/33/2016-CPCSEA, dated 16/02/2017). This study was conducted in 58 eyes of 36 client-owned dogs with KCS referred to the ophthalmology units of the clinics. Among a total of 67 dogs diagnosed with KCS, 31 dogs had KCS due to nonimmune-mediated causes (iatrogenic, endocrine, drug-induced, or dry eye in young dogs with viral keratitis) and were excluded from the study. A detailed history was recorded from the owners and complete ophthalmic examination was carried out for all the dogs. The ophthalmological examination included the Schirmer tear test (STT), indirect ophthalmoscopy, fluorescein dye test (FDT), Rose Bengal dye (RBD) test, and complete evaluation of the cornea, anterior and posterior chamber of the eye, and adnexa. Physiological parameters like heart rate (HR), respiratory rate (RR), and rectal temperature (RT) were recorded. Blood samples were collected and parameters like hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), and differential leukocyte count (DLC) were evaluated using standard procedures. Blood glucose estimations were performed using a ROMOCHECK glucometer (Romsons Scientific & Surgical Pvt. Ltd., Nunhai, Agra, Uttar Pradesh, India). Serum samples were subjected to  $T_4$ ,  $T_3$ , TSH, and

cortisol estimation using commercially available ELISA kits (BlueGene Biotech, Putuo, Shanghai, China). Blood smears were also evaluated for detecting the presence of any hemoprotozoan parasites.

The STT was done using Schirmer tear strips (OpStrip, Ophtech Unlimted, Gurgaon, Haryana, India) before the application of any topical anesthetics. The strips were placed on the lower fornix of each eye after folding the strip of filter paper (35 mm × 5 mm) at the notch and putting the folded portion between the cornea and lower lid. The paper was left in position for 1 min and the amount of tear production (mm/min) was measured (16,17). The STT readings were scored from 1 to 4 in the increasing order of severity as 1: normal,  $\geq 15$  mm/min; 2: mild or subclinical KCS, 11–14 mm/min; 3: moderate to mild KCS, 6–10 mm/min; and 4: severe KCS,  $\leq 5$  mm/min (18). The dye-impregnated strips were used for performing FDT (OptiGlo, Ophtech Unlimted) and the RBD test (Optitech Eye Care, Tarun Enterprises, Allahabad, Uttar Pradesh, India). These strips were moistened with sterile normal saline and a drop was placed on the fornix. The eyes were thoroughly washed with normal saline and were examined for epithelial loss. The degree of loss of epithelium was scored from 0 to 2 depending on the severity as 0: no dye retention, 1:  $< 50\%$  retention of dye, and 2:  $> 50\%$  retention of dye. The RBD retention intensity was scored from 0 to 3 in two exposed conjunctival zones (medial and lateral) and the cornea as 0: no spots, 1: few separated patches, 2: many separated patches, and 3: confluent patches. The sum of the scores from each zone was calculated (maximum of 9) and any value greater than 3 was considered positive for KCS (19).

Depending on the severity of KCS, dogs were grouped into early (E), late (L), and reflex tear (R) groups. The dogs with mild and moderate STT readings were placed in group E ( $n = 21$  eyes). Those dogs with severe STT scores and mucoïd discharge were placed in group L ( $n = 21$  eyes). The dogs presented with corneal ulceration of nontraumatic origin along with signs of KCS in the ipsilateral eye were placed in group R ( $n = 16$  eyes). Thereafter, the 58 eyes of 36 dogs selected for the study were randomly divided into 6 treatment groups as EC and ET (early KCS), LC and LT (late KCS), and RC and RT (reflex KCS). The groups EC, LC, and RC were subjected to treatment with tacrolimus 0.1% ointment (Talimus, Ajanta Pharma Limited, Mumbai, India), applied once a day for 2 months, and groups ET, LT, and RT were subjected to treatment with CsA 0.05% eye drops (Cyclomune, Sun Pharmaceutical Industries Ltd., Halol, Gujarat, India) instilled five times a day for 60 days (Table 1). In addition to medical management, tarsorrhaphy was performed in the RC and RT groups. Alpha-tocopherol capsules (Evion, Merck Limited, Mumbai, India) and omega-3 essential

**Table 1.** Grouping of patients and protocols used for the treatment of KCS.

Groups	Treatment groups	Medical/surgical management	Treatment protocol	Number of eyes
Group E	EC	Medical	Cyclosporine (0.05%)	10
	ET	Medical	Tacrolimus (0.1%)	11
Group L	LC	Medical	Cyclosporine (0.05%)	11
	LT	Medical	Tacrolimus (0.1%)	10
Group R	RC	Medical	Cyclosporine (0.05%)	10
	RT	Medical	Tacrolimus (0.1%)	6

fatty acid syrup (FILO-VET, VC Pharmaceuticals, Ernakulam, Kerala, India) were also supplemented along with treatment in all the groups.

## 2.2. Posttreatment evaluation

The effect of treatment was assessed by analysis of digital photographs taken on the 0th (day of presentation), 15th, 30th, and 60th posttreatment days and by scoring various parameters (20) as mentioned hereafter. Conjunctival hyperemia was scored from 0 to 2 as 0: no conjunctival hyperemia, 1: mild hyperemia, and 2: moderate hyperemia. Ocular discharge was scored from 0 to 2 as 0: no ocular discharge, 1: slight ocular discharge at the medial canthus, and 2: discharge across the cornea or on eyelid margins. The area of corneal pigmentation was demarcated as within the area of a clock by dividing the cornea into 3 circular parts and was scored from 0 to 4 as 0: no corneal pigmentation, 1: pigment over less than 25% of the cornea, 2: pigment over 25%–50% of the cornea, 3: pigment over 50%–75% of the cornea, and 4: pigment over >75% of the cornea. The corneal pigment density was scored from 0 to 3 as 0: no pigment, 1: iris easily visualized through the pigment, 2: iris partially visualized through the pigment, and 3: iris not visible through the pigment. The corneal vessel length was scored from 0 to 3 as 0: no corneal vessels, 1: limbal corneal vessels, 2: vessels halfway towards the corneal axis, and 3: vessels extending to the axial cornea. The corneal vessel density was scored from 0 to 4 as 0: no vessels, 1: vessels extending to the cornea from <25% of the circumference of the limbus, 2: vessels extending to the cornea from 25%–50% of the circumference of the limbus, 3: vessels extending to the cornea from 50%–75% of the circumference of the limbus, and 4: vessels extending to the cornea from >75% of the circumference of the limbus. The clarity of the cornea was assessed based on visual examination and was scored from 1 to 4 as 1: clear, 2: hazy, 3: moderate opacity, and 4: complete opacity; the depth of corneal ulceration was scored from 0 to 3 as 0: no ulcer, 1: shallow ulcer, 2: moderate ulcer, and 3: deep ulcer (21).

## 2.3. Statistical analysis

Data were analyzed statistically using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Summary statistics (mean

$\pm$  SE and median  $\pm$  SE) were calculated for all variables. Single measurements of continuous variables between groups E, L, and R were compared using one-way analysis of variance (ANOVA). Continuous data of the parametric variable (STT) within and between groups EC, ET, LC, LT, RC, and RT at different time intervals were analyzed using repeated-measures ANOVA. For both one-way ANOVA and repeated-measures ANOVA, the post hoc Tukey's HSD test was employed. An independent samples t-test was used to compare the parametric data at different time intervals between EC and ET, LC and LT, and RC and RT. Nonnormally distributed data or nonparametric variables (scores) on different days were compared using the Kruskal–Wallis test. Dunn's test was used for analyzing pairwise differences in groups if there was a tied rank for the Kruskal–Wallis test (22). The Wilcoxon signed rank test was used to evaluate nonparametric data at different time intervals within a group. The level of significance was set to  $P < 0.05$ .

## 3. Results

The average age of dogs with KCS in the study was 3 years and most of the animals were in the age group of 1 to 5 years. Nearly 61% of the affected animals were female. Breeds like Chinese pugs, Indian Spitz, German shepherd, and Mastiff were found to be commonly affected. The mean  $\pm$  SE values of physiological, hematological, and serum biochemical parameters did not show any significant variation ( $P > 0.05$ ) between groups E, L, and R except for eosinophil count (Table 2) and the mean values of each parameter remained within normal limits. Eosinophil values were significantly higher ( $P < 0.05$ ) in group E compared to group L.

On the day of presentation, the menace reflex was significantly higher ( $P < 0.05$ ) in group E compared to groups L and R, whereas the amount of ocular discharge was significantly higher ( $P < 0.05$ ) in groups L and R compared to group E before the initiation of either of the treatments (Table 3). Corneal clarity was significantly higher ( $P < 0.05$ ) in group R (opaque), followed by groups L and E, respectively (Table 3). The score for corneal ulceration was

**Table 2.** Mean  $\pm$  SE values of physiological, hematological, and biochemical parameters of dogs in groups E, L, and R on day 0.

Parameters	Group E (n = 16)	Group L (n = 12)	Group R (n = 8)
RT ( $^{\circ}$ C)	39.42 $\pm$ 0.07	39.32 $\pm$ 0.10	39.3 $\pm$ 0.06
RR (breaths/min)	33.76 $\pm$ 1.12	34.00 $\pm$ 1.11	36.31 $\pm$ 0.67
HR (beats/min)	112.5 $\pm$ 1.64	105.4 $\pm$ 2.71	109.1 $\pm$ 2.55
Hb (g/dL)	12.58 $\pm$ 0.31	12.07 $\pm$ 0.39	12.81 $\pm$ 0.30
PCV (%)	38.24 $\pm$ 0.82	37.48 $\pm$ 1.39	39.0 $\pm$ 1.10
RBC ( $\times 10^6/\mu$ L)	4.579 $\pm$ 0.08	4.420 $\pm$ 0.10	4.69 $\pm$ 0.12
WBC ( $\times 10^9/L$ )	16746 $\pm$ 792.7	20169 $\pm$ 1395	19344 $\pm$ 856.7
Glucose (mg/dL)	93.5 $\pm$ 1.62	99.43 $\pm$ 2.47	96.69 $\pm$ 2.19
Neutrophils (%)	73.86 $\pm$ 1.38	75.52 $\pm$ 1.56	74.19 $\pm$ 1.65
Lymphocytes (%)	13.90 $\pm$ 1.22	13.00 $\pm$ 0.98	11.69 $\pm$ 0.77
Monocytes (%)	2.905 $\pm$ 0.33	3.524 $\pm$ 0.35	3.50 $\pm$ 0.38
Eosinophils (%)	1.333 $\pm$ 0.28 <sup>a</sup>	0.571 $\pm$ 0.14 <sup>b</sup>	0.93 $\pm$ 0.23 <sup>ab</sup>
Basophils (%)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.06 $\pm$ 0.06
TSH ( $\mu$ g/L)	0.29 $\pm$ 0.01	0.31 $\pm$ 0.01	0.31 $\pm$ 0.03
T <sub>4</sub> (nmol/mL)	4.12 $\pm$ 1.06	2.74 $\pm$ 0.49	6.39 $\pm$ 2.31
T <sub>3</sub> (nmol/mL)	0.40 $\pm$ 0.09	0.35 $\pm$ 0.05	0.60 $\pm$ 0.11
Cortisol (nmol/L)	28.08 $\pm$ 1.57	31.46 $\pm$ 0.98	28.95 $\pm$ 1.72

<sup>a,b</sup>Differ significantly ( $P < 0.05$ ) between groups.

**Table 3.** Mean  $\pm$  SE and median  $\pm$  SE values of scores of ophthalmic parametric and nonparametric variables of dogs in groups E, L, and R on day 0.

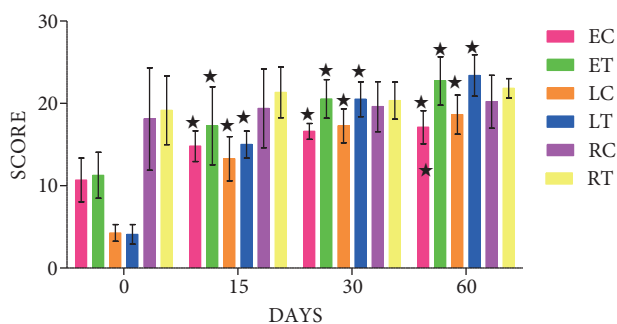
Parameter	E (n = 16)	L (n = 12)	R (n = 8)
Menace reflex	1.81 $\pm$ 0.09 <sup>a</sup>	0.71 $\pm$ 0.20 <sup>b</sup>	0.13 $\pm$ 0.09 <sup>b</sup>
Palpebral reflex	1.91 $\pm$ 0.07	1.67 $\pm$ 0.11	1.88 $\pm$ 0.09
Conjunctival hyperemia	0.81 $\pm$ 0.19	1.05 $\pm$ 0.16	1.06 $\pm$ 0.11
Ocular discharge	1.14 $\pm$ 0.10 <sup>a</sup>	1.71 $\pm$ 0.10 <sup>b</sup>	1.63 $\pm$ 0.13 <sup>b</sup>
Corneal clarity	1.61 $\pm$ 0.18 <sup>a</sup>	2.66 $\pm$ 0.27 <sup>b</sup>	3.62 $\pm$ 0.17 <sup>c</sup>
Corneal ulceration	0.14 $\pm$ 0.08 <sup>a</sup>	0.57 $\pm$ 0.16 <sup>a</sup>	2.56 $\pm$ 0.16 <sup>b</sup>
Corneal vessel length	0.14 $\pm$ 0.1 <sup>a</sup>	0.38 $\pm$ 0.16 <sup>ab</sup>	0.94 $\pm$ 0.23 <sup>b</sup>
Corneal vessel density	0.14 $\pm$ 0.1 <sup>a</sup>	0.38 $\pm$ 0.15 <sup>a</sup>	1.31 $\pm$ 0.27 <sup>b</sup>
Corneal pigmentation area	1.14 $\pm$ 0.24	2.33 $\pm$ 0.38	1.25 $\pm$ 0.27
Corneal pigmentation density	1.33 $\pm$ 0.25	1.81 $\pm$ 0.27	1.81 $\pm$ 0.28
STT (mm/min)	11 $\pm$ 0.58 <sup>a</sup>	4.19 $\pm$ 0.23 <sup>b</sup>	18.5 $\pm$ 1.35 <sup>c</sup>
FDT	0.14 $\pm$ 0.14 <sup>a</sup>	1.29 $\pm$ 0.10 <sup>a</sup>	1 $\pm$ 0.00 <sup>b</sup>
RBD test	3.14 $\pm$ 0.31 <sup>a</sup>	4.48 $\pm$ 0.21 <sup>b</sup>	4.5 $\pm$ 0.22 <sup>b</sup>

<sup>a,b,c</sup>Differ significantly ( $P < 0.05$ ) between groups.

significantly higher ( $P < 0.05$ ) in group R compared to the other two groups. The degree of corneal vessel length and degree of corneal vessel density were also significantly higher ( $P < 0.05$ ) in group R compared to group E (Table 3). Fluorescein dye retention was significantly higher ( $P < 0.05$ ) in group R, whereas the RBD test showed significantly higher ( $P < 0.05$ ) retention in groups L and R compared to group E. Since the dogs in the study were grouped on the basis of STT values, significantly higher ( $P < 0.05$ ) mean  $\pm$  SE values of the STT were recorded in group R, followed by groups E and L, respectively (Table 3).

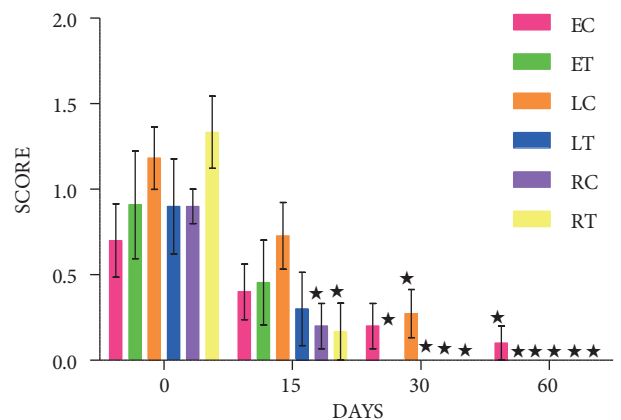
The post treatment mean  $\pm$  SE STT scores showed significant improvement ( $P < 0.05$ ) in tear production from day 15 in groups E and L following treatment with either 0.1% tacrolimus or 0.05% CsA (Figure 1). Group R also showed marked improvement in tear production. There was no significant difference ( $P < 0.05$ ) in the efficacy of CsA 0.05% eye drops and tacrolimus 0.1% ointment as lacrimostimulants when applied 5 times daily and as a single application daily, respectively. In group R with KCS-associated corneal ulceration, pretreatment STT scores showed near normal tear production. Although topical application of either of the lacrimostimulants enhanced tear production in this group, the rise in tear production was statistically insignificant.

The basic ocular reflexes associated with vision like menace reflex and pupillary light reflex improved significantly ( $P < 0.05$ ) with treatment in all the groups. Conjunctival hyperemia also improved significantly ( $P < 0.05$ ) in all groups with either of the treatments (Figure 2). There was no significant difference between two treatments in any of the treatment groups. In groups E and L, significant improvement ( $P < 0.05$ ) was evident from day 30 onwards, whereas in group R, significant improvement ( $P < 0.05$ ) was observed by day 15. Scores for ocular discharge also showed significant improvement ( $P < 0.05$ ) from day 30 in

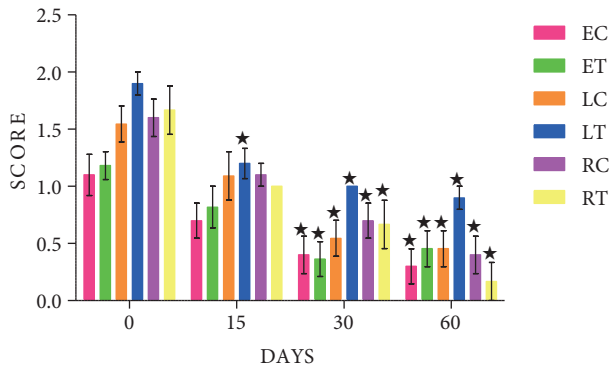


**Figure 1.** Mean  $\pm$  SE values of the score for STT readings; \*: differ significantly ( $P < 0.05$ ) compared to day 0.

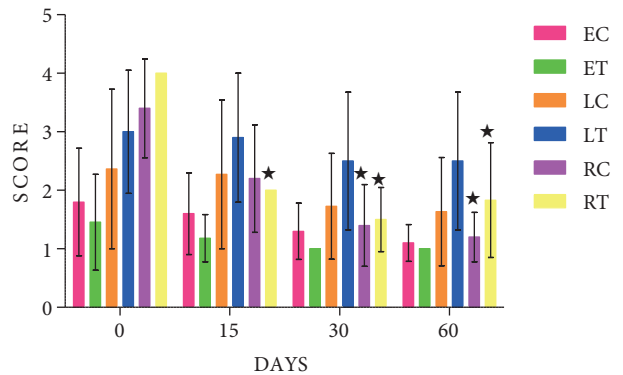
all groups irrespective of treatments (Figure 3). In group L, treatment with 0.1% tacrolimus significantly reduced ( $P < 0.05$ ) mucoid ocular discharge from day 15 (Figure 3). The corneal clarity significantly increased ( $P < 0.05$ ) in group R from day 15 onwards with 0.1% tacrolimus treatment (Figure 4). Integrity of corneal epithelium was found to increase significantly ( $P < 0.05$ ) in group R compared to the other two groups from day 15. The healing of corneal ulcers did not differ significantly ( $P < 0.05$ ) with either of the treatment protocols in groups E and R, but the loss in corneal epithelium was significantly less ( $P < 0.05$ ) in group LT compared to group LC on day 15. Posttreatment parameters like corneal vessel length, corneal vessel density, and corneal pigmentation area did not show a significant variation ( $P > 0.05$ ) from pretreatment values at any interval of time between any groups, except in group R. Group RC showed a significant increase ( $P < 0.05$ ) in corneal vessel length on day 15. There was a significant decrease ( $P < 0.05$ ) in corneal vessel density in groups RC and RT by day 60. In groups LC and LT, vascularity was less visible because of corneal pigmentation. The corneal pigmentation density significantly increased ( $P < 0.05$ ) in groups EC and RC from day 30 and day 15, respectively (Figure 5). No significant increase ( $P < 0.05$ ) in corneal pigmentation density was observed between any other treatment groups. It was observed that the progression of pigmentation was less among 0.1% tacrolimus groups compared to 0.05% CsA groups. Fluorescence dye retention was significantly less ( $P < 0.05$ ) in groups RC and RT from day 30 onwards. There was significant reduction ( $P < 0.05$ ) in RBD uptake in all the treatment groups from day 15 and a significant decrease ( $P < 0.05$ ) in RBD retention in group ET compared to group EC at day 30 (Figure 6). Photographs of eyes of KCS-affected dogs representing each treatment group at different intervals of time were also recorded to depict the effect of treatment (Figure 7).



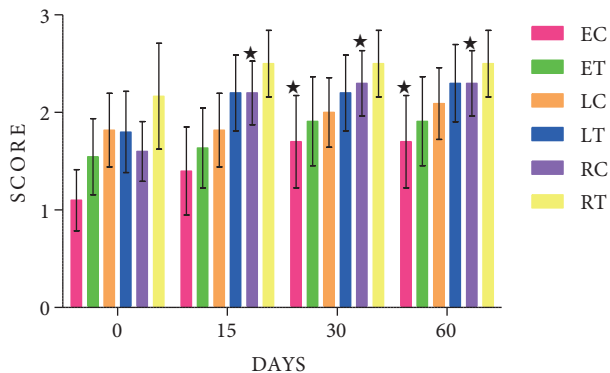
**Figure 2.** Mean  $\pm$  SE values of the score for conjunctival hyperemia; \*: differ significantly ( $P < 0.05$ ) compared to day 0.



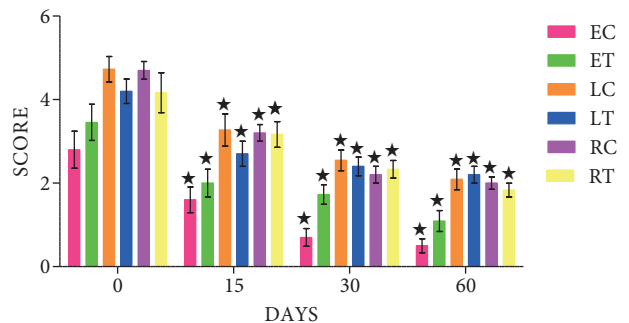
**Figure 3.** Mean ± SE values of the score for ocular discharge; \*: differ significantly (P < 0.05) compared to day 0.



**Figure 4.** Mean ± SE values of the score for corneal clarity; \*: differ significantly (P < 0.05) compared to day 0.



**Figures 5.** Mean ± SE values of the score for density of corneal pigmentation; \*: differ significantly (P < 0.05) compared to day 0.



**Figure 6.** Mean ± SE values of the score for retention in RBD test; \*: differ significantly (P < 0.05) compared to day 0.

**4. Discussion**

The results of this study support the use of both CsA 0.05% eye drops, instilled five times daily, and tacrolimus 0.1% ointment, applied once daily, for improving tear production in dogs affected with KCS and to reduce the deteriorating clinical signs associated with KCS. There was a marked increase in tear production in all dogs treated with either of the two drugs within 2 weeks. For arresting the progression of corneal pigmentation associated with KCS, tacrolimus 0.1% ointment was more effective than CsA 0.05% eye drops. Tacrolimus 0.1% ointment showed better response in stimulating the tear production and arresting the progression of corneal pigmentation even in dogs with low STT values. Although the concentration of tacrolimus used in our study differs from earlier investigations, the results corroborated the findings of a previous study (9).

The efficacy of both the drugs was evaluated in dogs that had not received any previous treatment for KCS. The half-life of CsA, when administered topically, is

not available in the literature, but it is believed to reach therapeutic concentrations in corneal tissue quickly (23). Investigations have shown a significant degree of penetration of CsA into inflamed tissue (24). Previous studies have shown CsA at 0.1% to be effective in the treatment of KCS in canines (25). On the other hand, tacrolimus is 10 to 100 times more potent than CsA in vitro (26) and hence a single application was advised. Today 0.1% and 0.03% tacrolimus preparations are widely used in veterinary ophthalmic therapy (9,20,27). Studies in experimental animal models have already demonstrated extremely low blood concentrations of tacrolimus after topical administration (15) and hence systemic adverse effects are seldom anticipated, providing short-term safety in clinical use. Topical broad-spectrum antibiotics are often used for the management of KCS since secondary bacterial infection is considered as a major sequel of KCS (28). Despite the use of topical antibiotics, both 0.1% tacrolimus and 0.05% CsA effectively reduced the mucoid discharge that developed with defective tearing.

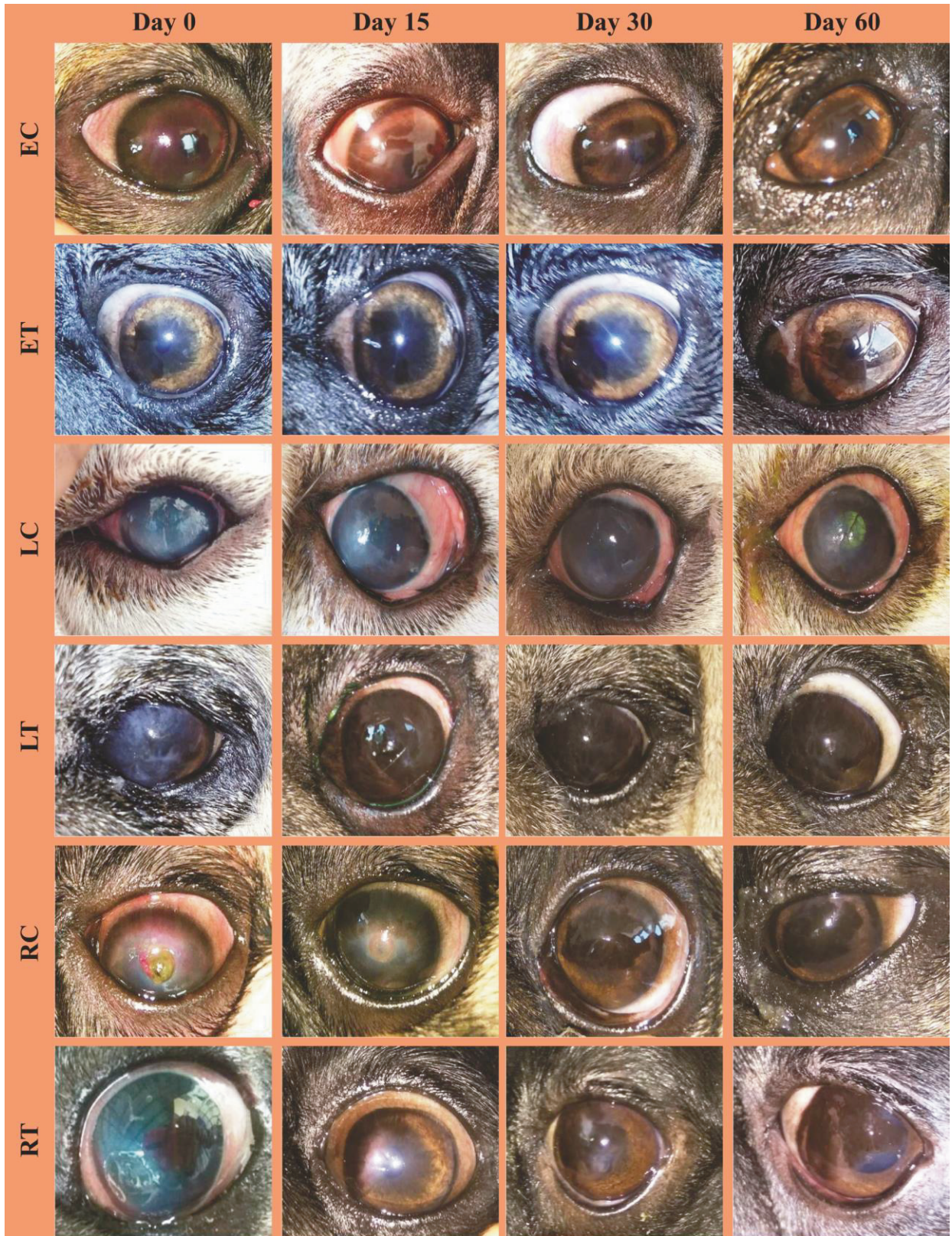


Figure 7. Follow-up photographs of dogs representing each group (EC, ET, LC, LT, RC, and RT) on days 0, 15, 30, and 60.

The STT is the commonly employed diagnostic test to detect KCS. The mean normal value of the STT is 20 mm/min in dogs and 17 mm/min in cats (16). The normal range of STT readings is 15–25 mm/min. Values of 0–9 mm/min were considered to demonstrate KCS while values of 10–14 mm/min were considered as borderline tear deficiency (29). For a comprehensive investigation of the effects of the drugs used in our study, we classified KCS into different stages based on STT readings as recorded on the first day of presentation. Mild to moderately low STT values between 6 mm/min and 14 mm/min were considered as early KCS and a severely low STT value of lower than 5 mm/min was considered as late KCS. Such a classification was not identified in literature. Posttreatment STT scores at different intervals improved with both CsA and tacrolimus at their respective concentrations. A quantitative difference in tear production was not observed between 2% CsA and 0.03% tacrolimus up to 12 weeks in a previous study (20). Interestingly, the concentration of the drugs used for evaluation in the above study was proportionately similar to that used in our study.

The response to menace reflex improved with increase in corneal clarity and decrease in inflammation. An improperly moistened cornea loses its shine and becomes opaque in KCS (27). The rise in tear production once the treatment was initiated might have contributed to the corneal lubrication and thereby restoration of corneal integrity. Corneal clarity increased consistently in dogs presented with corneal ulceration-induced reflex tearing also. Although a significant relationship was not observed in corneal healing time, the dogs that developed corneal ulceration due to KCS with reflex tearing under 0.05% CsA therapy showed faster healing compared to dogs under 0.1% tacrolimus treatment, possibly due to the low degree of corneal ulceration in the former group compared to that in the latter. Clinical improvement in pupillary light reflex was also observed during the healing phase of the corneal ulcer.

The drastic reduction in mucoid discharge in parallel to the rise in tear production was evident in all the dogs with either of the drugs during the course of treatment. Both CsA and tacrolimus also have antibacterial activity (30). In a previous study, dogs treated with 0.2% CsA ointment two times a day showed a reduction in lacrimal gland inflammation and regeneration of lacrimal acinar lobules (12). In our study, we used a lower concentration of CsA (0.05%) instilled regularly in a much shorter interval of time. CsA as 0.2% ointment or 1% to 2% drops is inherently capable of reducing the infiltration of T lymphocytes into the lacrimal gland acini and their proliferation, and it promotes apoptosis, thereby decreasing inflammation (9,31). Since tacrolimus is more potent than CsA, a single application could stimulate sufficient tear production and suppress infection and inflammation associated with KCS.

The CsA and tacrolimus at their respective concentrations and time intervals of application in our study could appreciably improve the conjunctival clarity and mucoid discharge in KCS-affected eyes. In a previous clinical study that compared 2% CsA with 0.03% tacrolimus in KCS-affected dogs, no significant difference was found in combined clinical scores for conjunctival hyperemia and ocular discharge (20). Interestingly, an early reduction in mucoid discharge was observed with 0.1% tacrolimus compared to 0.05% CsA in our study.

The corneal neovascularization remained dormant once treatment using either of the drugs was initiated. Although topical CsA and tacrolimus effectively inhibit T-lymphocyte proliferation and inflammatory prostaglandins responsible for the corneal neovascularization (30), it may take a longer time for a significant decrease in vascularity to be achieved in addition to the lower concentration of both the drugs used in our study. Corneal vascularization resolved when topical CsA ointment was used at 0.2% twice a day for chronic keratitis in a previous study (24). The initial increase in corneal vessel length in the reflex tear group with corneal ulceration could be attributed to healing in the corneal epithelium, which demands neovascularization.

Confining and reducing corneal pigmentation is the most challenging aspect while treating KCS. Corneal pigmentation progresses with the in-growth of pigmented conjunctival epithelial cells following persistent corneal epithelial injury and when the depth of injury exceeds the regenerative capabilities of the resident corneal epithelial cells (32). This is because the melanocytes at the limbus, which act as a final line of defense against oxidative damage in the cornea, initiate to play their role as scavengers of free radicals (33). The 0.05% CsA could not arrest the progression of corneal pigmentation in dogs with KCS and led to a slower progression of pigmentation over time, but the spread of corneal pigmentation was less in dogs treated with 0.1% tacrolimus. Rather, the concentration of CsA may not have been enough to contend with the reactive oxygen species to arrest the spread of pigmentation. Contradictory reports exist regarding the efficacy of 1% and 2% CsA in inhibiting the progression of pigmentation and resolving pigmentation completely (25,27,34). Unfortunately, in dogs with ulcerative keratitis and reflex tearing, there was an initial increase in the pigment deposition. The topical 0.1% tacrolimus more effectively controlled the progression of pigmentation in these dogs as compared to 0.05% CsA by day 60. However, both 0.05% CsA and 0.1% tacrolimus could not reduce or completely resolve the pigmentation that was already established on the cornea. Previous studies with 2% CsA and 0.03% tacrolimus also showed similar results (20).

Tacrolimus has been found to be effective in dogs unresponsive to cyclosporine therapy for stimulating tear production (20,23) and for arresting the progression



of corneal pigmentation (9). Although tear production improved with both 0.05% CsA and 0.1% tacrolimus, an evaluation of the effect of 0.1% tacrolimus, particularly in dogs, where the progression of pigmentation could not be arrested amply by 0.05% CsA, is lacking in our study.

In conclusion, both tacrolimus 0.1% ointment and CsA 0.05% eye drops improve tear production in KCS-affected dogs. Topical tacrolimus 0.1% ointment effectively arrests the progression of pigment epithelium compared to CsA 0.05% eye drops in KCS-affected dogs.

## References

- Sanchez RE, Innocent G, Mould J, Billson FM. Canine keratoconjunctivitis sicca: disease trends in a review of 229 cases. *J Small Anim Pract* 2007; 48: 211-217.
- Dodi PL. Immune-mediated keratoconjunctivitis sicca in dogs: current perspectives on management. *Vet Med Res Reports* 2015; 6: 341-347.
- Bhavsar AS, Bhavsar SG, Jain SM. A review on recent advances in dry eye: pathogenesis and management. *Oman J Ophthalmol* 2011; 4: 50-56.
- Davidson HJ, Kuonen VJ. The tear film and ocular mucins. *Vet Ophthalmol* 2004; 7: 71-77.
- Kaswan RL, Martin CL, Chapman WL Jr. Keratoconjunctivitis sicca: histopathologic study of nictitating membrane and lacrimal glands from 28 dogs. *Am J Vet Res* 1984; 45: 112-118.
- Hartley C, Williams DL, Adams VJ. Effect of age, gender, weight, and time of day on tear production in normal dogs. *Vet Ophthalmol* 2006; 9: 53-57.
- Whitley RD, McLaughlin SA, Gilger BC, Lindley DM. The treatments for keratoconjunctivitis sicca. *Vet Med* 1991; 86: 1076-1093.
- Cullen CL, Ihle SL, Webb AA, McCarville C. Keratoconjunctival effects of diabetes mellitus in dogs. *Vet Ophthalmol* 2005; 8: 215-224.
- Berdoulay A, English RV, Nadelstein B. Effect of topical 0.02% tacrolimus aqueous suspension on tear production in dogs with keratoconjunctivitis sicca. *Vet Ophthalmol* 2005; 8: 225-232.
- Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506. *Immunol Today* 1992; 13: 136-142.
- BenEzra D, Hemo I, Maftzir G. In vivo angiogenic activity of interleukins. *Arch Ophthalmol* 1990; 108: 573-576.
- Bounous DI, Carmichael KP, Kaswan RL, Hirsh S, Stiles J. Effects of ophthalmic cyclosporine on lacrimal gland pathology and function in dogs with keratoconjunctivitis sicca. *Vet Comp Ophthalmol* 1995; 5: 5-12.
- Gao J, Schwab TA, Addeo JV, Ghosn CR, Stern ME. The role of apoptosis in the pathogenesis of canine keratoconjunctivitis sicca: the effect of topical Cyclosporin A therapy. *Cornea* 1998; 17: 654-663.
- Matsuda S, Koyasu S. Mechanisms of action of cyclosporine. *Immunopharmacology* 2000; 47: 119-125.
- Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, Goto T, Okuhara M, Kohsaka M, Aoki H et al. FK-506, a novel immunosuppressant isolated from a *Streptomyces*. II. Immunosuppressive effect of FK-506 in vitro. *J Antibiot* 1987; 40: 1256-1265.
- Bexfield N, Lee K. *BSAVA Guide to Procedures in Small Animal Practice*. 1st ed. Gloucester, UK: BSAVA; 2010.
- Sansom J, Barnett KC, Long RD. Keratoconjunctivitis sicca in the dog associated with the administration of salicylazosulphapyridine (sulphasalazine). *Vet Rec* 1985; 116: 391-393.
- Corr A. Canine dry eye. *Canine keratoconjunctivitis sicca (KCS)*. Metropolitan Vet Associates 2015; 4: 1-4.
- Van Bijsterveld OP. Diagnostic tests in the sicca syndrome. *Arch Ophthalmol* 1969; 82: 10-14.
- Hendrix DVH, Adkins EA, Ward DA, Stuffle J, Skorobohach B. An investigation comparing the efficacy of topical ocular application of tacrolimus and cyclosporine in dogs. *Vet Med Int* 2011; 5: 1-5.
- Sarangom SB, Venugopal SK, Martin KDJ, Narayanan MK, Mini M, Anoop S. Incidence and predisposing factors of keratopathies in Chinese Pugs. *Indian Vet J* 2014; 91: 41-43.
- Elliott AC, Hynan LS. A SAS® macro implementation of a multiple comparison post hoc test for a Kruskal-Wallis analysis. *Comput Methods Programs Biomed* 2011; 102: 75-80.
- Kaswan RL, Salisbury MA, Ward DA. Spontaneous canine keratoconjunctivitis sicca: a useful model for human keratoconjunctivitis sicca: treatment with cyclosporine eye drops. *Arch Ophthalmol* 1989; 107: 1210-1216.
- Gilger BC, Allen JB. Cyclosporine A in veterinary ophthalmology. *Vet Ophthalmol* 1998; 1: 181-187.
- Olivero DK, Davidson MG, English RV, Nasisse MP, Jamieson VE, Gerig TM. Clinical evaluation of 1% cyclosporine for topical treatment of keratoconjunctivitis sicca in dogs. *J Am Vet Med Assoc* 1991; 199: 1039-1042.

## Acknowledgments

The authors acknowledge the scholars and scientific and technical staff of Division of Surgery and Teaching Veterinary Clinical Complex and Referral Veterinary Polyclinic, ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India, for providing necessary support and help at various levels for the study. This investigation was funded by the ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly, Uttar Pradesh, India.

26. Tocci MJ, Matkovich DA, Collier KA, Kwok P, Dumont F, Lin S, Degudicibus S, Siekierka JJ, Chin J, Hutchinson NI. The immunosuppressant FK506 selectively inhibits expression of early T cell activation genes. *J Immunol* 1989; 143: 718-726.
27. Radziejewski K, Balicki I. Comparative clinical evaluation of tacrolimus and cyclosporine eye drops for the treatment of canine keratoconjunctivitis sicca. *Acta Vet Hung* 2016; 64: 313-329.
28. Pawde AM. Ocular affections in domestic animals: incidence, therapeutic and surgical management. *Indian J Vet Surg* 2006; 27: 35.
29. Kaswan RL, Bounous D, Hirsh SG. Diagnosis and management of keratoconjunctivitis sicca. *Vet Med* 1995; 90: 539-560.
30. Martin CL. Cornea and sclera. In: Martin CL, editor. *Ophthalmic Disease in Veterinary Medicine*. 1st ed. London, UK: Manson Publishing Ltd.; 2005. pp. 241-297.
31. Izci C, Celik I, Alkan F, Ogurtan Z, Ceylan C, Sur E, Ozkan Y. Histologic characteristics and local cellular immunity of the gland of the third eyelid after topical ophthalmic administration of 2% cyclosporine for treatment of dogs with keratoconjunctivitis sicca. *Am J Vet Res* 2002; 63: 688-694.
32. Slatter DH. Keratoconjunctivitis sicca in the dog produced by oral phenazopyridine hydrochloride. *J Small Anim Pract* 1973; 14: 749-772.
33. Sacca SC, Roszkowska AM, Izzotti A. Environmental light and endogenous antioxidants as the main determinants of non-cancer ocular diseases. *Mutat Res* 2013; 752: 153-171.
34. Morgan RV, Abrams KL. Topical administration of cyclosporine for treatment of keratoconjunctivitis sicca in dogs. *J Am Vet Med Assoc* 1991; 199: 1043-1046.