



Management of Corneal Ulcers using Autologous Serum, Platelet Rich Plasma and Processed Human Amniotic Membrane in Dogs

A.R. Mecvan¹, J.J. Parmar¹, R.N. Bhatia¹, P.V. Parikh¹, N.R. Amin¹

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ABSTRACT

Background: In dogs the corneal ulcers are one of the major ophthalmic emergencies and can cure with routine medications, but in delay presentation will become challenge for veterinarians. In such cases advanced therapeutic management such as serum, platelet rich plasma and corneal grafts are needed for favorable outcome.

Methods: The present study was conducted on 24 dogs (29 eyes) with corneal ulcer categorized as a superficial (Group-I, n=15), deep corneal ulcers (Group-II, n=8) and descemetocoele (Group-III, n=6) which were treated with autologous serum, Platelet Rich Plasma and Processed decellularized human amniotic membrane grafting, respectively. All the affected eyes were given routine topical medications with eye drops Moxifloxacin, Flurbiprofen *q.i.d* till healing, 1% Atropine sulphate *b.i.d* for 5 days and Oral Doxycycline was given @ 5 mg/kg *b.i.d* for 10 days.

Result: The group-I (n=11, 15 eyes) treated with autologous serum eight to ten times-a-day as per severity with routine medication. Two dogs with entropion which was corrected first. The eyes gained vision within 10th-15th days. Developed complications managed medicinally till complete recovery. The group-II (n=7, 8 eyes) treated with platelet rich plasma drops five to seven times-a-day with routine medication. 75% eyes recovered up to 45th day. Developed complication were managed medicinally till complete recovery. The group-III (n=5, 6 eyes) treated with Decellularized Processed Human Amniotic Membrane Graft. 4 eyes gained complete transparency on 28th day of therapy while in 2 eyes developed complication were managed medicinally.

Key words: Amniotic membrane, Autologous serum, Corneal ulcer, Descemetocoele, PRP.

INTRODUCTION

Vision is a most valuable gift from the nature to living beings. The eye is a portal to the rest of the world. At its best, it's the only organ of vision and a biological camera (Dutta, 2006). Affections of the eye is a common in all animal species. If not treated promptly, vision may be impaired. The corneal affections are most common in dogs. Among them corneal ulcers are extremely painful, induce opacification, tearing, pawing, blepharospasm and pigmentation, which lead to a reduction in corneal transparency and ultimately vision loss. The treatment includes topical medications with autologous serum, anti-inflammatory, mydriatics, cyclosporine, hypertonic saline and surgical management with third eyelid flap, keratotomy, conjunctival pedicle grafting, application of tissue adhesives, soft contact lens, corneal transplantation and keratoplasty are all options for treating keratopathies (Gilger *et al.*, 2007). As the corneal ulcer is one of the major ophthalmic emergencies and delay presentations need advance therapeutic modalities for favorable outcome. The amniotic membrane promotes axonal regeneration by acting as a substrate for epithelial development. It aids epithelialization by increasing epithelial cell motility, improving basal epithelial cell adhesion, encouraging epithelial cell differentiation and avoiding apoptosis (Barros *et al.*, 2005). The application of platelet rich plasma in veterinary patients was well documented for wound

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management (Parmar *et al.*, 2022) but literature available regarding ophthalmic application of PRP and processed human amniotic grafts is very sparse. Thus, this paper presents a management of corneal ulcers using autologous serum, platelet rich plasma and processed human amniotic membrane in dogs.

MATERIALS AND METHODS

The study was undertaken on 24 dogs (29 eyes) presented with corneal ulcer with or without any vision abnormalities at the Department of Veterinary Surgery and Radiology,

College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat from July, 2021 to June, 2022. All these cases were categorized as a superficial (Group-I, n=15), deep corneal ulcers (Group-II, n=8) and descemetocoele (Group-III, n=6). The eyes of dogs from Group-I and II were treated with autologous serum eight to ten times a day and Platelet Rich Plasma five to seven times a day, respectively while the cases from Group-III were treated surgically with processed decellularized human amniotic membrane grafting.

All the dogs covered under this study underwent detailed history and recording of clinical signs and diagnostic procedures. History pertaining to the primary complaint (cause, onset, initial clinical signs, progression, duration); earlier treatment, if any (previous diagnosis, treatment and its response) and any non-ophthalmic/ systemic disease conditions were recorded.

For confirmative diagnosis neuro-ophthalmic examinations viz., Menace response, Pupillary Light Reflex and Palpebral reflex (blink reflex) were carried out and grades as absent or present; absent, sluggish or present; absent, incomplete or present, respectively. Dazzle reflex was conducted by directing a direct beam of light at the ocular fundus and observing the positive blink reflex. Other special diagnostic procedures and gross eye examinations were carried out to confirm the lesion present on the cornea viz., Schirmer tear test was performed using sterile diagnostic strips (5 mm × 40 mm). The value was evaluated as: ≥15 mm/min (Normal production), 11-14 mm/min (Early or Subclinical or Mild KCS), 6-10 mm/min (Moderate KCS), <5 mm/min (Severe KCS), >25 mm/min (Epiphora). Slit lamp biomicroscope was used for detailed magnified illuminated examination of the adnexa (eyelids, lacrimal apparatus, orbit and para-orbital areas) and anterior segment (anterior part of the globe including the lens). The Fluorescein sodium ophthalmic strip was moistened with sterile distilled water and then placed in the palpebral fissure *cul-de-sac*. The visualization of fluorescein stain was enhanced by a cobalt blue light. It was graded as: Not retained -Normal cornea/no corneal defect, Retained-Corneal defect, Green spot - Corneal epithelial defect, Black spot-Descemetocoele. In selected cases tonometer was used to measure intraocular pressure. After instilling topical anaesthetic 0.5 percent proparacaine, very light pressure was applied on the globe. The normal IOP in dogs is somewhere between 15-18 mmHg.

All the eyes affected with corneal ulcer from all the groups were given routine topical medications with eye drops Moxifloxacin, Flurbiprofen *q.i.d* till healing, 1% Atropine sulphate *b.i.d* for 5 days and Oral Doxycycline was given @ 5 mg/kg *b.i.d* for 10 days.

The dogs of group-I were treated with autologous serum eight to ten times-a-day as per severity along with routine medication. All the cases with deep stromal corneal ulcer were treated therapeutically using Platelet rich plasma five to seven times a day as per severity along with routine

medication. 5 ml of whole blood was collected aseptically from cephalic vein in K2-EDTA collection vial and was centrifuged at 3000 RPM for 10 minutes. About 1/3rd of plasma supernatant after centrifugation was discarded and the remaining was utilized for therapeutic purpose. To avoid self-mutilation, application of Elizabethan collar was advised up to corneal healing. Dogs with respective healing times and outcomes were noted. All the cases with descemetocoele corneal ulcer were treated surgically using Decellularised Processed Human Amniotic Membrane graft following standard surgical protocols. The graft was anchored under operating microscope. Medical therapy was instituted prior to surgical procedure from the day of presentation. 0.5% Moxifloxacin eye drops *q.i.d.*, 0.03% Flurbiprophen eye drops *q.i.d.* and Atropine eye drops *b.i.d.* were instilled in all cases. All dogs received topical 0.5% Povidone iodine diluted with 0.9% normal saline in 1:2 ratios for ocular antisepsis 2-3 times prior to surgery. All the dogs were premedicated by using combination of Xylazine @ 1 mg/kg + Atropine @ 0.02 mg/kg Intramuscularly. A single injection of Ceftriaxone and Tazobactam @ 25 mg/kg body weight and Meloxicam @ 0.3 mg/kg given to eliminate ocular infection and pain along with Ringer lactate solution @10 ml/kg/hour, intravenously. Induction was done using a mixture of Ketamine (50 mg/ml) @10 mg/kg and Midazolam (10 mg/ml) @ 0.5 mg/kg in the ratio 2:1. Topical proparacaine was used to desensitize cornea. The anaesthesia was maintained following the same combination. The dogs were placed in lateral recumbency with the affected eye placed dorsally. Eye was draped with a light coloured drape with an operating window of about 5 cm diameter in the centre. Sterile drapes covered the rest of the dog's body to avoid contamination. Using dry sterile cotton buds, abnormal loose epithelium and debris from the stromal surface were removed. With a lance blade, ulcer bed and surrounding 1-2 mm area was scarified and bed was cleaned for acceptance of the graft. Decellularised, processed human amniotic membrane (Amnio-care TM) was cut with scissors to obtain a diameter greater than the corneal defect. The membrane was placed over the prepared ulcer bed, with the stromal side of the membrane facing the ulcer bed and secured in place as an onlay graft using 8-0 polyglactin 910 suture material (Fig 1). Temporary tarsorrhaphy (Fig 2) was done and kept in place for three days. Sufficient space was left at medial canthus for instillation of eye drops. The dogs covered under this study were advised Elizabethan collar for the first two weeks to prevent self-mutilation. The owners of the dogs were also advised to daily clean surroundings of the eye, especially the nasal fold with lukewarm water. They were also advised to give Doxycycline @ 5 mg/kg body weight *b.i.d.* orally for 10 days along with eye drops Moxifloxacin *q.i.d.*, Flurbiprofen *q.i.d.*, Atropine *b.i.d.* and Hydroxypropyl methylcellulose *b.i.d.* topically daily up to complete recovery. The complications of the therapeutic protocol were recorded and treated accordingly.

RESULTS AND DISCUSSION

This clinical study was conducted to assess the effectiveness of medicinal and surgical care of corneal ulcers, as well as the management of complications.

The dogs (n=24) covered under this study were also subjected for detailed history and clinical examinations of corneal ulcer. The causative factors noted in the cases of corneal ulcers in 29 eyes are presented in Table 1. The

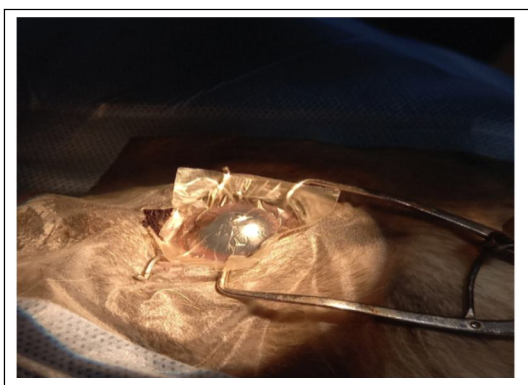


Fig 1: Placement of the graft over the whole cornea.



Fig 2: Temporary tarsorrhaphy.

Table 1: Causative factors in the cases of corneal ulcers.

Causative factor	No. of eyes	Per cent
Traumatic injury	19	65.52
Keratoconjunctivitis sicca	7	24.15
Entropion	2	6.89
Chemical injury	1	3.44
Total	29	100.00

traumatic injuries 65.52 % to the cornea were found to be the highly prominent factor for the corneal ulcer followed by keratoconjunctivitis sicca 24.15%, entropion 6.89% and chemical injury 3.44%. The results obtained in this study are in accordance with earlier studies of Mandell (2000). However, Kim *et al.* (2009) reported that keratoconjunctivitis sicca (KCS) as the most common cause of ulcerative keratitis followed by logophthalmos, bacterial infection, nasal fold trichiasis and trauma. The inherent lower sensitivity of cornea in brachycephalic dogs breeds negatively affect the function of cornea protective mechanisms, which leads to traumatic injury to the cornea and allow the ulcers in the initial stages to go unnoticed by owners (Kecova *et al.*, 2004).

The affected dogs were presented from three days to 15 days of onset of symptoms of corneal ulcers. The dogs presented with corneal ulcer were also examined for the signs grossly and also using slit lamp biomicroscope, which revealed conjunctival hyperemia in all the affected eyes with varying degree of corneal opacity along with corneal oedema and neovascularization. The dogs with superficial ulcer on cornea exhibited epiphora, episcleral congestion, stippled appearance of cornea, whereas superficial vascularization was also evident in chronic cases. In deep ulceration common clinical signs were epiphora, blepharospasm due to pain, corneal oedema, excavation of cornea, purulent exudate due to secondary bacterial infection and mucopurulent discharge commonly observed only in Pug breed. The dogs with desmetocele exhibited almost similar signs to the deep ulcer. Similar clinical signs were observed by Wilkie and Whittaker (1997).

Animals with corneal ulcer show clinical signs of pain, epiphora, pawing, photophobia, blepharospasm, conjunctival hyperaemia, chemosis, tearing, rubbing of eyelids and corneal opacity. The signs of keratitis or corneal ulcers may arise from ciliary muscle spasm secondary to reflex anterior uveitis and direct stimulation of sensory corneal nerves. Neovascularisation denotes the complicated ulcers, in which healing was delayed by ocular factor *i.e.* eyelid defects or non-ocular factors such as self-mutilation, inappropriate medical therapy (Kern, 1990). Vessels invade into cornea in response to various pathologic processes in vascular method of corneal stromal healing. In majority of cases discolouration of the cornea and purulent discharge were the symptoms noticed by the owner (Mandell, 2000).

All the dogs (n=24) with corneal ulcer covered under this study were subjected to various neuro-ophthalmic examinations. The findings are presented in Table 2. Out

Table 2: Neuro-ophthalmic examinations of eyes with corneal ulcers in dogs.

Type of Response	Menace response	Pupillary light reflex	Palpebral reflex	Dazzle reflex
Positive	16	22	29	22
Sluggish	4	00	00	00
Negative	9	7	-	7
Total	29	29	29	29

of 29 eyes affected with corneal ulcer, 55.17% eyes (n=16) were positive for menace reflex, 31.03% eyes (n=9) were negative for menace reflex and 13.79% eyes (n=4) were sluggish. Pupillary light reflex was found to be positive in 75.86% eyes (n=22) with comparatively small, superficial ulcers along with almost negligible corneal opacity; and negative in 24.14% eyes (n=7). Dazzle reflex was found to be positive in 75.86% (n=22) eyes and negative in 24.14% (n=7) eyes. The palpebral reflex was found positive 100% (n=29) of the affected eyes. Pupillary light reflex and Dazzle reflex could not be ascertained in the eyes with extensive corneal opacity and oedema. The findings of present study concurred with Felchle and Urbanz, (2001).

The eyes (n=29) from dogs (n=24) affected with corneal ulcers were subjected for the special diagnostic procedures on the day of presentation. The mean($\pm$ SE) (mm/min) value of Schirmer's tear test was 15.79 ± 4.1 mm/min which was within the normal range (15 to 25 mm/min) (Gelatt and MacKay, 1998). The STT values were recorded lower in 7 eyes from Pug breed (n=5) and equally in Pomeranian and Shih Tzu breeds (n=1, each) of dog. STT was performed before applying any topical medication and inducing sedation or anaesthesia as suggested by Munro (2001) and Morreale (2003). These findings were found to be in accordance with Kim *et al.* (2009).

The ulcerated cornea (n=23) were found to be positive (79.31%) as they stained greenish with fluorescein dye while the cases (20.69%) of descemetocoele (n=6) were negative. In descemetocoele, only margins of the corneal ulcers were stained while protruded descemet's membrane does not stain and thus it was considered as a negative. Only the ends of abrasive cornea stained greenish in cases of descemetocoeles because exposed descemet's membrane in the centre does not stain. Fluorescein stain aids in the diagnosis of corneal ulceration, the dye not only stains the eroded area of cornea, but also migrates under the loose flaps of the epithelium and stains the surrounding anterior stroma and this makes the ulcer appear larger than the actual size (Whitley and Gilger, 1999; Moore, 2003). The positive fluorescein dye test is suggestive of complete loss of epithelium. Negative Fluorescein dye test was considered as an indication of healthy cornea or complete corneal healing (Bromberg, 2002).

The mean($\pm$ SE) (mm Hg) value of Intraocular pressure was measured on the day of presentation in 23 eyes was 15.22 ± 0.42 mm Hg which was within the normal range (10 to 20 mm Hg) Gelatt and MacKay (1998). IOP was not recorded in five eyes with descemetocoele and a case with iris prolapse, because it may lead corneal perforation as suggested by Gouille (2012). Similar findings were also observed by (Broadwater *et al.* 2008). IOP was not recorded in five eyes with descemetocoele and a case with iris prolapse, because it may lead corneal perforation as suggested by Gouille (2012). In the present study, IOP was measured in all the dogs using Tonopen-Vet by applanation tonometry. The applanation tonometry could be used to measure



Fig 3: Pre-treatment with autologous serum.

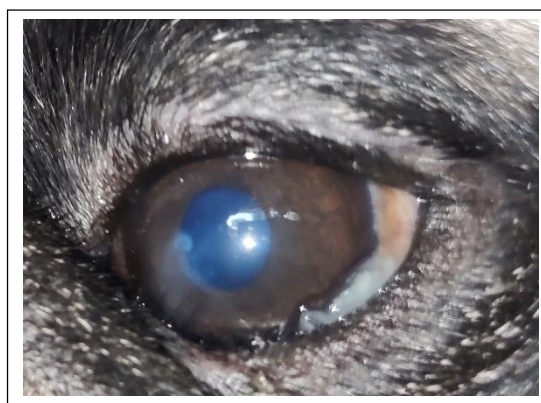


Fig 4: 15th day Post-treatment.

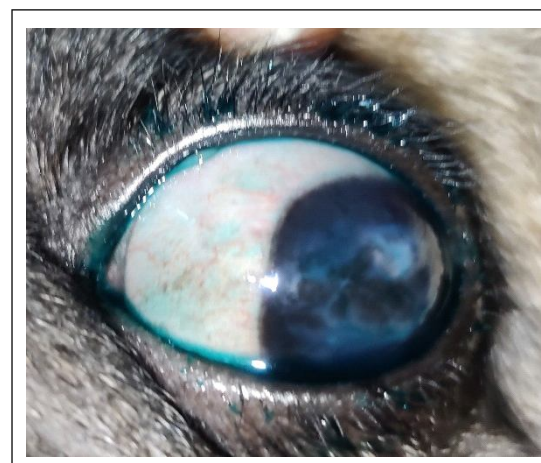


Fig 5: Corneal melanosis post treatment with autologous serum.

intraocular pressure in dogs because of its ease of use and superior accuracy (Gelatt and Brooks, 2011). Spiessen *et al.* (2015) stated that there could be a tendency to over/under estimation of the IOP by applanation or rebound tonometry in the presence of different corneal pathologies.

The affected eyes (n=29) from all the groups along with respective treatments were also treated with topical antibiotic Moxifloxacin and topical anti-inflammatory



Fig 6: Corneal scar post-treatment with autologous serum.

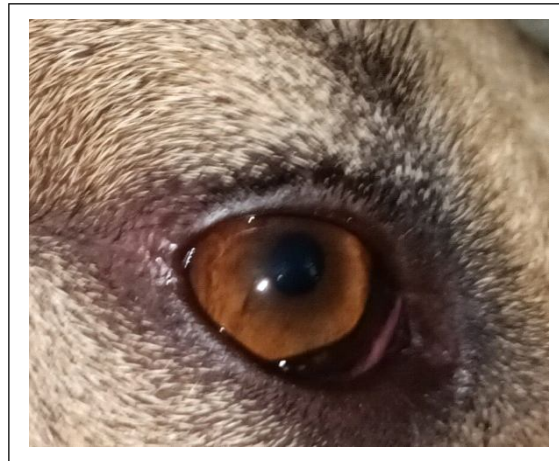


Fig 9: Complete transparency of cornea observed on 45th day.

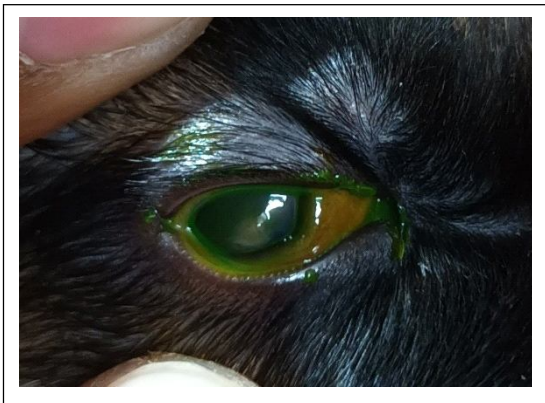


Fig 7: Pre-treatment with platelet rich plasma.

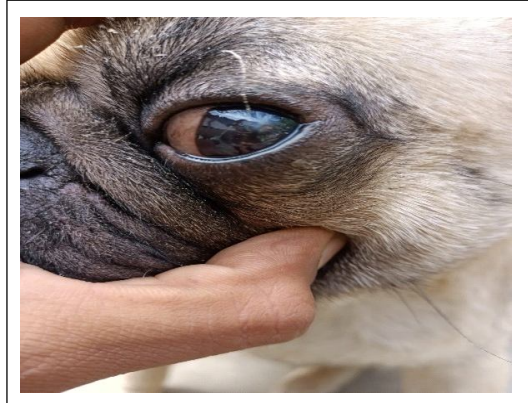


Fig 10: Corneal melanosis post-treatment with platelet rich plasma.

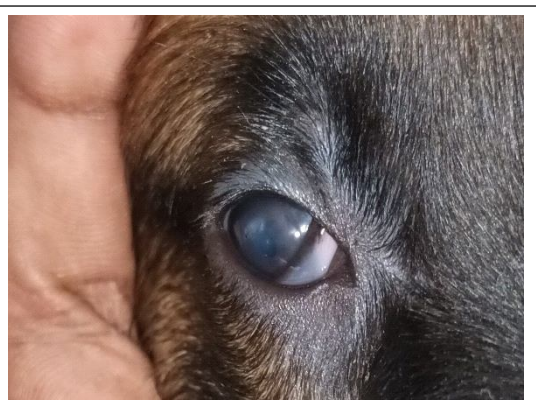


Fig 8: 20th day post-treatment.



Fig 11: Pre-operative desmetocele.

Flurbiprofen *q.i.d*, Topical 1% atropine sulphate *b.i.d* eye drops for 5 days and Oral Doxycycline was given @ 5 mg/kg *b.i.d* for 10 days. Townsend, (2007) reported that topical fluoroquinolones can penetrate intact corneal epithelium and it is effective in treating infected corneal ulcer. Topical 1% atropine sulphate *b.i.d* for 5 days (parasympatholytic) was

included to control ciliary muscle spasm and associated ocular discomfort and to prevent synechia formation as uveitis is commonly associated with corneal ulcerations as also suggested by Brooks (2005). Oral Doxycycline was given @ 5 mg/kg *b.i.d* for 10 days as it inhibits matrix metalloproteinases through primarily restriction of the gene

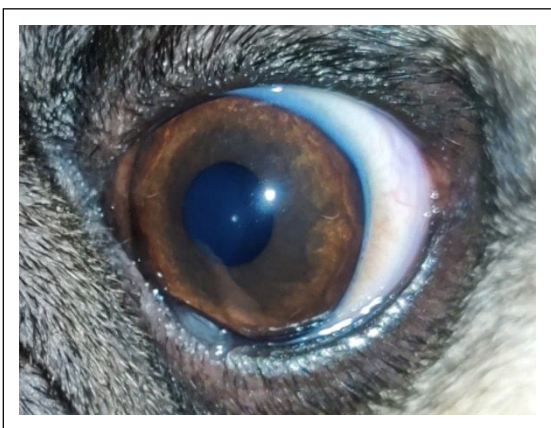


Fig 12: 21st day post-operative.



Fig 13: Corneal scar formation post grafting.

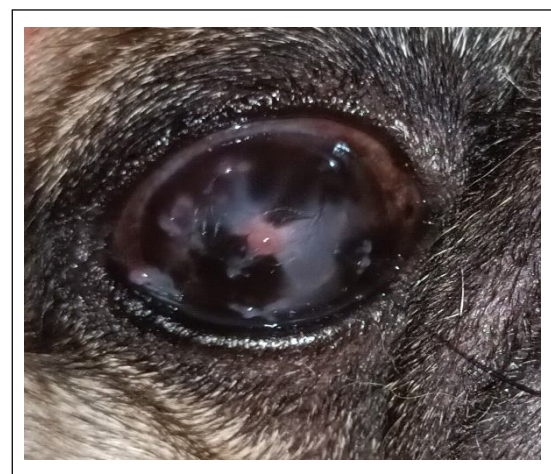


Fig 14: Corneal melanosis post grafting.

expression of neutrophil collagenase and epithelial gelatinase, suppression of alpha1-antitrypsin degradation and scavenging of reactive oxygen species.

In two dogs with superficial corneal ulcer, the entropion was recorded as predisposing cause of ulcer, thus before initiation of topical medications, the surgical correction of entropion was done by Hotz- Celecucus technique. The sutures of these cases were removed on 12th post-operative day. The eyes treated with autologous serum, 73.33% eyes (n=11) regained vision within 10th days post-treatment day whereas 26.67% (n=4) regained vision on 15th post-treatment day (Fig 3 to Fig 4). During the treatment in a pug dog melanosis (Fig 5) was observed while in two pug dogs corneal scar was noted (Fig 6). The melanosis and corneal scar were managed using topical application of Cyclosporine (1%) eye drops *b.i.d.* and Prednisolone eye drops *b.i.d.* for five days., respectively. Similar results were also observed by Patil and Kelawala, (2015) and Patel (2020). Serum contain alpha-2 macroglobulin with anti-collagenase activity that helps in healing of ulcer (Brook, 2005).

Out of eight eyes diagnosed with deep corneal ulcer (Fig 7), 50.00 % eyes (n=4) showed complete healing with scar formation and corneal vascularisation on 10th post-treatment day. Corneal oedema and keratitis reduced subsequently by 20th day post therapy (Fig 8). On 30th post-treatment day two eyes showed complete healing and corneal transparency. On 45th day 75.00 % eyes (n=6) recovered completely with transparency of cornea (Fig 9), whereas in two eyes of a pug dog complete corneal melanosis (Fig 10) was observed. Almost similar results were also observed by Rechichi *et al.* (2020) and Ahmed *et al.* (2021) while melanosis was attributed to Pug breed as a sequel of corneal ulcer as stated by Patel (2020).

A total of 6 eyes (n=5 dogs), were diagnosed as a descemetocoele (Fig 11) and subjected for the corneal grafting and interrupted sutures using Polyglactin 910 No. 8-0, the tarsorrhaphy suture was removed on the 3rd post-operative day. The ophthalmic examinations revealed intact grafts along with mild granulation on the ulcer bed with oedema around the ulcer bed. In all the dogs no corneal neovascularization was noted. On day 7th in a Pomeranian dog graft dehiscence was observed. Thus, this case was managed only with topical medications while in other dogs marked reddish pink granulation was observed. There was absence of corneal oedema and no neovascularization was observed. On 14th post-operative day, light pink granulation was observed throughout ulcer bed indicated healing progress while in a pug dog there was reddish pink granulation observed. On the 21st post-operative day all 4 eyes were presented with complete healing (Fig 12) but in 2 eyes of a dog, bilateral corneal scar (Fig 13) was observed which might be due to irregular topical medication. On 28th post-operative day all 4 eyes presented with complete corneal healing indicated by corneal transparency while in a dog unilateral melanosis (Fig 14) was observed which was managed by topical medication with eye drops cyclosporine (1%) till complete regression. The findings of the present study supported by (Wichayacoop *et al.* 2005; Vongsakul *et al.* 2009; Costa *et al.*, 2019; Korittum *et al.*, 2019) and

(Nagashree *et al.*, 2020). Better results over the conventional methods of treatment of descemetocoele could be attributed by the low immunogenicity and presence of certain growth promoters of the graft, inhibiting metalloprotease and the zero chances of graft rejection (Hao *et al.* 2000; Riau *et al.*, 2010 and Gholipourmale kabadi *et al.*, 2015). Less corneal vascularization and scar formation could be attributed to the properties of the graft which was also reported by Tseng and Li (1999) and (Nakamura *et al.*, 2004).

CONCLUSION

The routine topical medications along with autologous serum in superficial ulcer, platelet rich plasma in deep ulcer and decellularised processed human amniotic membrane graft in descemetocoele helps to heal with minimum manageable complications.

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Conflict of interest

No any conflict of interest among the authors.

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