B-4568
[1-8]

RESEARCH ARTICLE Indian Journal of Animal Research, Volume Issue : ()

Evaluation of Homologous Platelet Rich Plasma (PRP) Drop and Leucocyte Platelet Rich Fibrin (L-PRF) Membrane for Corneal Ulcer Healing in Dogs

Apoorva Mishra, Apra Shahi, Babita Das, Shobha Jawre,
Randhir Singh, Rajesh Vandre, Anju Nayak, Rakhi Vaish

10.18805/IJAR.B-4568

ABSTRACT

Background: To evaluate use of homologous platelet rich plasma (PRP) drop and leucocyte-platelet rich fibrin (L-PRF) clot/membrane as regenerative therapy for healing of corneal ulcer in dogs and its subsequent comparison with conventional therapy.

Methods: In this randomized study, 30 dogs were divided into three groups of 10 animals each. It was ensured each group had grade I, II and III ulcers. Group I received medicinal treatment with antimicrobial and non-steroidal anti-inflammatory eye drop. In Group II PRP drops and in Group III L-PRF membrane was used to evaluate corneal ulcer healing. Homologous platelet concentrates were prepared in laboratory and used for assessment of healing on various grades of corneal ulcer on basis on reduction in size and depth of ulcer, presence of stromal infiltration, scleral involvement, corneal opacity and vascularization.

Result: Post treatment, the size of corneal ulcer started to reduce gradually from day 7 onwards. Significant ($p \leq 0.05$) reduction was observed from day 10 in group I after instillation of drops Moxifloxacin and Flurbiprofen and in group II after instillation of PRP. It was measured as 5.35 ± 0.63 and 5.20 ± 0.98 mm at day 0, which reduced significantly ($p \leq 0.05$) to 3.90 ± 0.50 and 3.10 ± 0.98 mm at day 10 in group I and group II respectively. In group III there was significant reduction in size of ulcer from day 0 to day 7 measuring 7.80 ± 1.05 and 0.20 ± 0.11mm respectively. Thereafter, from day 10 onwards complete sealing of ulcer was observed in all the cases. Post treatment there was no significant reduction in corneal opacity from day 0 to day 30 in group I dogs and corneal opacity persisted specially in grade II and grade III ulcers. There was significant reduction ($p \leq 0.05$) in opacity from 2.90 ± 0.35 to 2.10 ± 0.38 and 3.00 ± 0.37 to 2.40 ± 0.34 in group II and group III dogs from day 0 to day 10 respectively. In group I, II and III there was significant ($p \leq 0.05$) reduction in vascularization from 1.50±0.30 to 1.20±0.13, 1.30±0.37 to 0.60±0.22 and 1.40±0.31 to 0.40±0.31 from day 0 to day 15 respectively. There was a significant reduction in vascularization on day 15 in group II and group III compared to group I animals.

Key words: Corneal opacity, Corneal ulcer/ ulcerative keratitis, Corneal vascularization, Leucocyte platelet rich fibrin, Platelet rich plasma, Regenerative therapy.

INTRODUCTION

A corneal ulcer is a result of break in the corneal epithelium that exposes the underlying corneal stroma. Conventional treatment adopted for corneal ulcer comprises of antimicrobial therapy, analgesics and anti-inflammatory agents. In humans, use of platelet concentrates was described to improve healing and to replace fibrin glue. Platelet-rich plasma (PRP) is dense platelet concentration in small volume of plasma obtained from centrifugation of whole blood with consequent presence of growth factors released by platelets. Various growth factors in PRP are involved in maintaining structure of cornea and corneal function, especially those related to transparency during repair process. Platelets adhere to injured corneal tissue, releasing numerous cytokines and growth factors that induce mitotic fibroblasts, resulting in production of collagen (Alio *et al*., 2012).

Leucocyte- platelet-rich fibrin (L-PRF) is a second-generation PRP, where autologousplatelets and leucocytes are present in a complex fibrin matrix to accelerate healing of soft and hard tissue and is used as a tissue-engineering scaffold.L-PRF is both a healing and interpositional biomaterial. It accelerates wound closure and mucosal healing due to fibrin and growth factor release (Hotwani and Sharma 2014).

Currently, human ophthalmology has described use of autologous solid platelet-rich plasma and other platelet

Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandary, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.

Corresponding Author: Apoorva Mishra, Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandary, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.
Email: mishra.ap07@gmail.com

How to cite this article: Mishra, A., Shahi, A., Das, B., Jawre, S., Singh, R., Vandre, R., Nayak, A. and Vaish, R. (2021). Evaluation of Homologous Platelet Rich Plasma (PRP) Drop and Leucocyte Platelet Rich Fibrin (L-PRF) Membrane for Corneal Ulcer Healing in Dogs. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4568.

Submitted: 09-06-2021 **Accepted:** 27-07-2021 **Online:** 31-08-2021

concentrates as an alternative for wound closure in perforated corneal ulcers. In humans and animals still there is lack of literature about use of regenerative therapy for corneal ulcer healing and hence present research work was designed.

MATERIALS AND METHODS

Animals

All animals having corneal ulcer, irrespective of age, sex, breed and grade of ulceration were randomly divided into three groups of 10 animal each (Table 1).

Therapeutic procedure

Initially in all animals, corneal ulcers were flushed with normal saline to remove debris and necrotic material.

Medicinal treatment

Local instillation of eye drops Moxifloxacin (0.5%) one to two drops four times a day and eye drop Flurbiprofen (0.03%) two drops four times a day for 15-20 days in group I animals. If needed, tear substitute eye drop containing hydroxypropyl methylcellulose (0.1%) two drops four times a day for 15-20 days.

Preparation of homologous platelet rich plasma drop

20-25 ml blood was collected into vials containing acid citrate dextrose 3.2% as anticoagulant. The blood was centrifuged at relative centrifuge force (RCF) of 200 gravities (g) for 10 minutes and upper layer composed of plasma platelets and WBCs (buffy coat) was collected and transferred to an empty siliconised glass tube to be homogenized. Homogenized sample was again centrifuged at RCF of 400g for initial 5 minutes and subsequently at RCF of 600g for another 5 minutes. The supernatant 2/3rd was discarded as platelet poor plasma (PPP) and remaining 1/3rd called as platelet rich plasma (PRP) was used as eye drop (Fig1). PRP was stored in sterilized eppendorf tubes at 4^{o}C (Kececi *et al*., 2014). 1-2 drops of prepared PRP was instilled 4 times/day for 15- 20 days in affected eye of group II animals.

Preparation and surgical placement of homologous leucocyte- platelet rich fibrin clot

10-15ml of blood was collected from healthy donor by venipuncture in sterile manner. Blood obtained was placed into a sterile tube without any anticoagulant and centrifuged at 20^{o}C and 2700-3000 rpm for 10 minutes. L-PRF was formed as a clot above red corpuscles base (Choukroun *et al*., 2000). It was pressed manually to form a membrane (Fig 2). This L-PRF membrane was immediately placed over the corneal ulcer and affixed with conjunctiva.

The surgical procedure was performed under adequate general anaesthesia in all animals of Group III. General anaesthesia was induced using, Inj. Atropine sulphate @ 0.04 mg/kg b.wt intramuscularly, after 5 minutes Inj. Diazepam hydrochloride @ 0.5 mg/kg b.wt intravenously and after 5 minutes Inj. Propofol @ 4 mg/kg b.wt intravenously. Maintenance of anaesthesia was done by using Isoflurane at 1.28% MAC, post endotracheal intubation.Topical anaesthesia of eye was achieved by applying a gauze soaked in Lignocaine hydrochloride (4%) for 5 minutes. Eyes were rinsed with aqueous normal saline solution. Eyelids were opened with an eyelid speculum and epithelium was debrided with a sponge. L-PRF membrane was placed to cover whole cornea and was affixed with conjunctiva using 5-0 polyglactin 910 suture material in animals of group III. Temporary tarsorrhapy was done using 2-0 silk in quill pattern. Sutures were removed after 7 days (Fig 3) (Lewin, 2000).

Parameters of the study

Ophthalmic examination

Pre-operative ophthalmic examination was performed on 0 day before starting the treatment which included: Fluorescein dye test on day 0 preoperatively to check location of ulcer and measure size and depth of corneal ulcer. Direct ophthalmoscopic examination was performed with help of direct ophthalmoscope to check fundus and intraocular pathology of eye. Ultrasonographic examination was done by using 7MHz linear transducerforany intraocular pathology.

Grading of corneal ulcer

Grading of corneal ulcer was done as per score card given by Harrison (1975) with some modification (Table 2). Grading was done before start of medication on and cases were allocated randomly in group I, II and III.

Grading of corneal opacity

Grading of extent of injury and prognosis on basis of opacity of cornea was done as per the score card of Panda *et al*. (1995), (Table 3). Grading was done before start of medication on 0 day followed by day 7, 10, 15, 20 and 30 post treatment.

Grading of corneal vascularization

Grading of corneal vascularization was done as per arbitrary score card (Table 4). Grading was done before start of medication on 0 day followed by day 7, 10, 15, 20and 30 post-treatment.

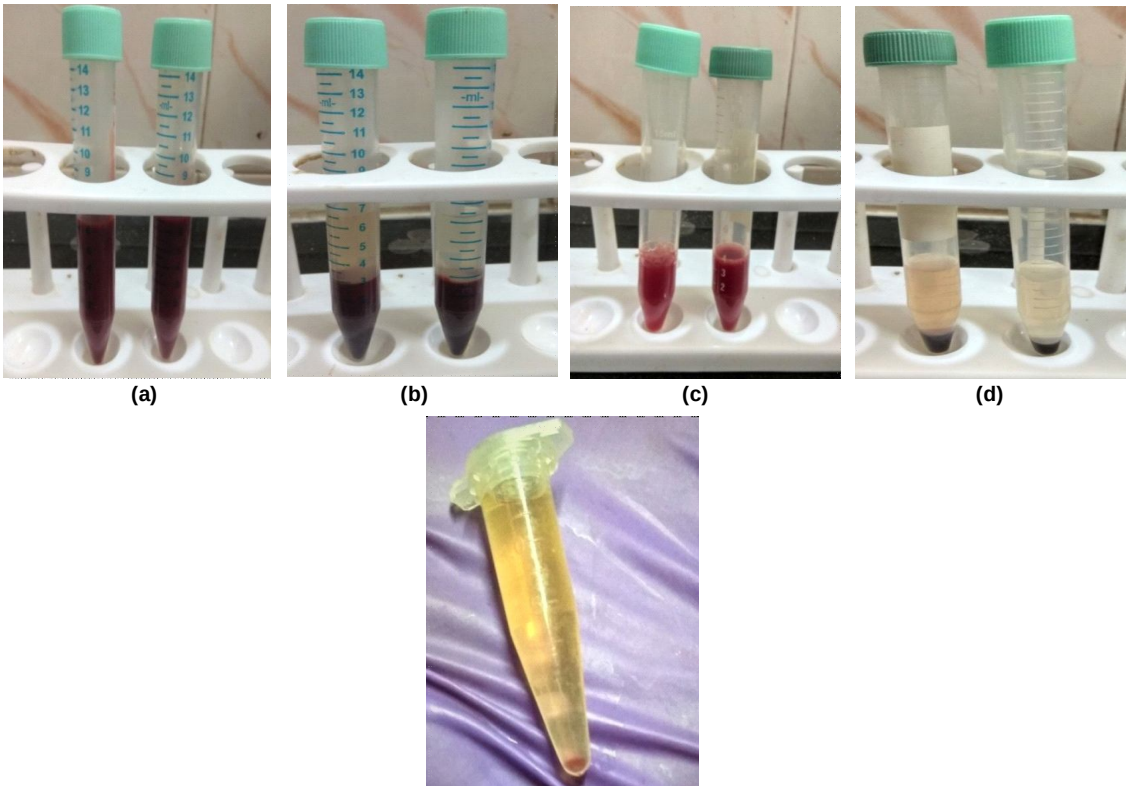
RESULTS AND DISCUSSION

Ophthalmic examination

All thirty dogs showed mild to moderate lacrimation, 83.33% (n=25) dogs showed corneal opacity and 80% (n=24) cases had corneal vascularization of various grades. Ocular surface pain was clinically presented by blepharospasm,

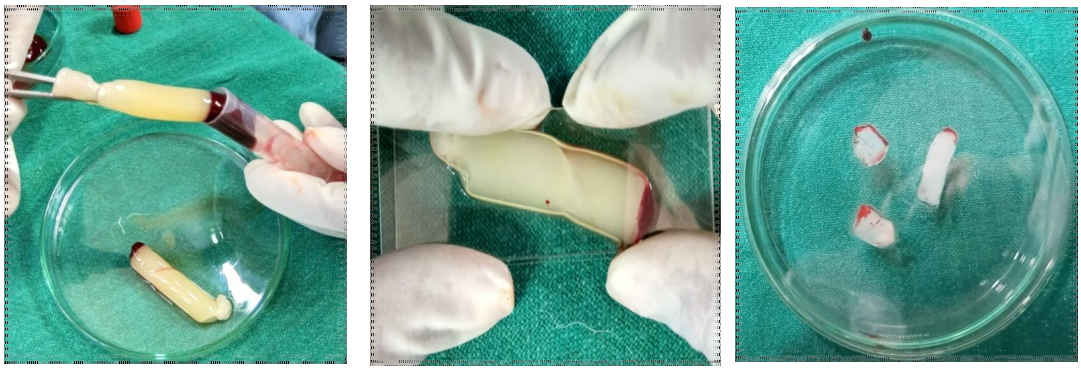
Table 1: Treatment protocol.

Group	No. of animals	Treatment protocol
I	10	Medicinal treatment - Eye drop Moxifloxacin and Flurbiprofen @ 1-2 drops 4 times/day+Tear substitute
II	10	Homologous platelet rich plasma drop (PRP) @ 1-2 drops 4 times/day
III	10	Homologous leucocyte - platelet rich fibrin clot (L-PRF) affixed on the cornea



(a) **(b)** **(c)** **(d)** **(e)**

Fig 1: Preparation of PRP (a) Blood with anticoagulant ACD (b) Blood components after first spin (c) Homogenization (d) Blood components after second spin (e) Platelet Rich Plasma.



(a) **(b)** **(c)**

Fig 2: Preparation of PRF (a) Leukocyte-platelet rich fibrin clot (b) Manual compression of L-PRF clot (c) Different sizes of L-PRF membranes after compression.

Table 2: Grading of corneal ulcer.

Features	Grading of corneal ulcer		
	Mild	Moderate	Severe
Size	2 mm - 4 mm	4-7mm	>7mm
Depth of ulcer	<20%	20-50%	>50%
Stromal infiltrate	Dense and superficial	Dense upto mid-stroma	Dense anddeep stromal
Scleral involvement	NIL	Occasionally present	Present

Table 3: Grading of corneal opacity.

Corneal opacity	Grade	Score
No corneal opacity	I	1
Corneal haziness but visible iris detail	II	2
Significant corneal haziness obscuring iris detail	III	3
Complete corneal opacity with no view of iris or pupil	IV	4

Table 4: Grading of corneal vascularization.

Corneal vascularization	Grade	Score
No vascularization	0	0
Fine tree like capillaries advancing from corneoscleral junction towards ulcer	I	1
Prominent blood vessels advancing from corneoscleral junction towards ulcer along with congestion around ulcer	II	2
Blood vessels at corneoscleral junction along with prominent zone of vascularization around ulcer	III	3

Table 5: Pre and post treatment size of corneal ulcer (mm).

Group \ Days	0	7	10	15	20	30
I (Mean ± SE)	$5.35^{a}\pm0.63$	$4.55^{Aab}\pm0.59$	$3.90^{Abc}\pm0.50$	$2.65^{Acd}\pm0.36$	$1.70^{Ade}\pm0.27$	$0.65^{e}\pm0.26$
II (Mean ± SE)	$5.20^{a}\pm0.98$	$3.95^{Aab}\pm0.89$	$3.10^{Aabc}\pm0.98$	$2.05^{Abc}\pm0.94$	$1.45^{ABbc}\pm0.85$	$0.50^{c}\pm0.40$
III (Mean ± SE)	$7.80^{a}\pm1.05$	$0.20^{Bb}\pm0.11$	$0.02^{Bb}\pm0.02$	$0.00^{Bb}\pm0.00$	$0.00^{Bb}\pm0.00$	$0.00^{b}\pm0.00$

Mean value within same group (a,b,c,d,e) and between the groups (A,B) with different superscript varied significantly ($p<0.05$) at different time intervals

photophobia, epiphora and enopthalmos. Damaged corneal epithelium exposed underlying layer of collagen or hydrophilic stroma which retained fluoroscein stain. It gave fluorescent green stain or apple green stain around border of the ulcerated cornea. In grade III ulcers with descemetocele, the Descemet's membrane did not show fluorescein staining because it is hydrophobic, but it was surrounded by fluorescein positive stromal defect. Four cases of descemetocele and two cases of perforated descemetocele causing iris prolapse were recorded.

Pre and post treatment evaluation of corneal ulcer for clinical healing

Pre and post treatment size of corneal ulcer (mm)

Post treatment size of corneal ulcer reduced gradually from day 7 onwards (Table 5). Significant ($p \leq 0.05$) reduction was observed from day 10 in group I and II whereas complete recovery was not observed in grade III ulcer even at day 30 and two cases of descemetocele out of 10 showed severe pigmentation, scar and blindness. In group III there was significant reduction in size of ulcer from day 0 to7 and from day 10 onwards complete sealing was observed in all cases. However, prominent scar was visualized on day 10 which started to faint gradually upto day 30 (Fig 6).

In group I and II depth of ulcer reduced gradually from day 7 onwards. In group I, grade I ulcer showed complete sealing whereas grade II and III ulcers showed incomplete sealing on day 30 (Fig 4). In group II depth of corneal ulcer reduced from day 20 onwards and corneal transparency was observed at day 30 (Fig 5). However, in grade III ulcers with descemetocele, incomplete sealing was observed. In group III complete sealing was observed in 6 out of 10 cases on day 7 while on day 15 all cases depicted complete sealing of corneal ulcers and perforations without any leakage.

Pandey (2016) evaluated use of tarrsorraphy and third eyelid flap with medicinal treatment of Moxifloxacin and Flurbiprofen and reported that on 30th post-operative day corneal ulcer healed completely with scar on 45th day. In dogs with third eyelid flap covering complete healing without scar formation was observed in 33.33% cases and scar was present in 66.66% cases. Similar findings were reported by Kim (2009) and Singh *et al.* (2015).

Alio *et al.* (2007) reported that autologous platelet-rich plasma promoted healing of dormant corneal ulcers in eyes threatened by corneal perforation and was accompanied by reduction in pain and inflammation. Alio *et al.* (2013) illustrated use of autologous fibrin membrane combined with solid platelet rich plasma as effective surgical alternative for closure of corneal perforations. In all cases corneal perforation was sealed on 7th day postoperatively with no evidence of leakage even when moderate pressure was applied to the globe.

PRP has been proved effective in treating corneal ulcer because of presence of corneal receptors for numerous growth and mitogenic factors present in the platelets *viz.* epidermal growth factors, platelet derived growth factors (PDGF), insulin like growth factors (IGF) and transforming growth factors (TGF). These promote migration, mitosis and differentiation of corneal cells as well as extracellular matrix production.

In case of L-PRF, fibrin strands of membrane binds to stromal collagen fibers of cornea, thus contributing to sealing

Table 6: Pre and post reduction in corneal opacity score.

Group \ Days	0	7	10	15	20	30
I (Mean ± SE)	2.20±0.36	2.20±0.36	2.20±0.36	2.00±0.26	1.70±0.30	1.30±0.15
II (Mean ± SE)	2.90^{a}±0.35	2.50^{a}±0.31	2.10^{ab}±0.38	1.50^{b}±0.27	1.40^{b}±2.67	1.40^{b}±2.67
III (Mean ± SE)	3.00^{a}±0.37	3.00^{a}±0.39	2.40^{ab}±0.34	1.70^{bc}±0.21	1.10^{c}±0.10	1.00^{c}±0.00

Mean value within same group (a,b,c) with different superscript varied significantly ($p<0.05$) at different time intervals.

Table 7: Pre and post reduction in corneal vascularization score.

Group \ Days	0	7	10	15	20	30
I (Mean ± SE)	1.50^{a} ± 0.30	1.60^{a} ± 0.27	1.30^{a} ± 0.15	1.20^{Aab} ± 0.13	0.50^{b} ± 0.27	0.50^{Ab} ± 0.27
II (Mean ± SE)	1.30^{a} ± 0.37	1.40^{a} ± 0.40	1.20^{a} ± 0.36	0.60^{Bab} ± 0.22	0.30^{b} ± 0.21	0.00^{Bb} ± 0.00
III (Mean ± SE)	1.40^{a} ± 0.31	1.20^{a} ± 0.29	0.80^{ab} ± 0.13	0.40^{Bbc} ± 0.31	0.00^{c} ± 0.00	0.00^{Bc} ± 0.00

Mean value within same group (a,b,c) and between the groups (A,B) with different superscript varied significantly ($p<0.05$) at different time intervals.

the defect. Fibrin membrane/patch gradually disappear over wound in 7-8 days constituting a physiologic and biologically active solution for corneal perforation.

Pre and post treatment reduction in corneal opacity score

Post treatment there was no significant reduction in corneal opacity score from day 0 to 30 in group I and corneal opacity persisted specially in grade II and III ulcers (Table 6). There was significant reduction ($p \leq 0.05$) in opacity score in group II and III from day 0 to 10. In animals of group II there was no significant difference ($p \geq 0.05$) from day 10 to day 30 but transparent cornea was observed at day 30 except in cases of staphyloma or perforated corneal ulcers (Fig 5). In group III dogs there was significant reduction in opacity score from day 15 to 20. Corneal transparency was achieved fully even in grade III ulcers, except at areas of descemetocele and corneal perforations where a zone of cloudy white scar was observed (Fig 6).

These findings are in accordance with Alio *et al.* (2013), Merlini *et al.* (2014) and Simona *et al.* (2017). Corneal opacity develop due to absorption of fluid from tear film by stromal layer of cornea. After placement of L-PRF membrane, the abraded stroma was sealed completely which prevented further absorption of fluid. Gradually, due to presence of growth factors in L-PRF, existing oedema reduced leading to transparent cornea.

Post treatment reduction in corneal vascularization

In group I, II and III there was significant ($p \leq 0.05$) reduction in vascularization score from day 0 to 15 (Table 7). Significant reduction in vascularization on day 15 in group II and group III compared to group I animals. In group I complete resolution of corneal vascularity was not observed unlike group II and group III. Usual avascularity of cornea was restored on day 30 and 20 in group II and group III animals.

Eaton *et al.* (2017) discussed a significant direct positive correlation between vascularization scores and time to reepithelialization. Reduction in corneal vascularization with the healing of ulcer was also found by Dulaurent (2014) and Ion *et al.* (2015).

Corneal vascularization and fibrosis typically increase during healing of ulcers. These are undesirable consequences of healing because they leave a persistent haziness and reduce clarity of vision. Platelet concentrates are anti-inflammatory in nature and provide growth factors to promote rapid healing which may be the reason of earlier and complete resolution of corneal vascularity.

Clinical healing of corneal ulcer

Complete clinical healing in terms of sealing of corneal ulcer, maximum reduction of corneal opacity and vascularization varied significantly between all three groups (Table 8). It was significantly delayed in group I in comparison to group II and group III. Earlier healing was observed in group III. Grade III ulcers with descemetocele and staphyloma showed formation of anterior synechia and consecutive blindness in group I and II, while vision was retained and clinical healing was seen in group III. Although, a white spot at area of sealing of corneal ulcer was present upto day 30. On day 40, it became faint but remained present.

Kim (2009) and Singh *et al.* (2015) observed similar findings. Merlini *et al.* (2014) reported complete healing of corneal ulcers in 5-10 days postoperatively when treated with PRP drops and covered with third eyelid. Alio *et al.* (2012) described that platelet activation occurs when the PRP eye drops are instilled. Similar findings are reported by Kim *et al.* (2012) and Simona *et al.* (2017). In agreement with this, Alio *et al.* (2013) reported sealing of all cases

Table 8: Clinical healing of corneal ulcer in days.

Group	Days
I (Mean ± SE)	41.00^{A} ± 4.00
II (Mean ± SE)	28.50^{B} ± 5.53
III (Mean ± SE)	8.40^{C} ± 0.83

Mean value between the groups (A,B,C) with different superscript varied significantly ($p<0.05$).

of perforated ulcer on 7th postoperative day using autologous fibrin membrane combined with solid platelet rich plasma.

Earlier healing of superficial corneal ulcer is because only anterior epithelium is injured it heals rapidly as compared to the deep ulcers where, stromal layers, which has no power of regeneration were also injured and eroded. Moxifloxacin helps to combat infection and Flurbiprofen reduces inflammation but both these drugs do not contain any active principles to augment healing process of

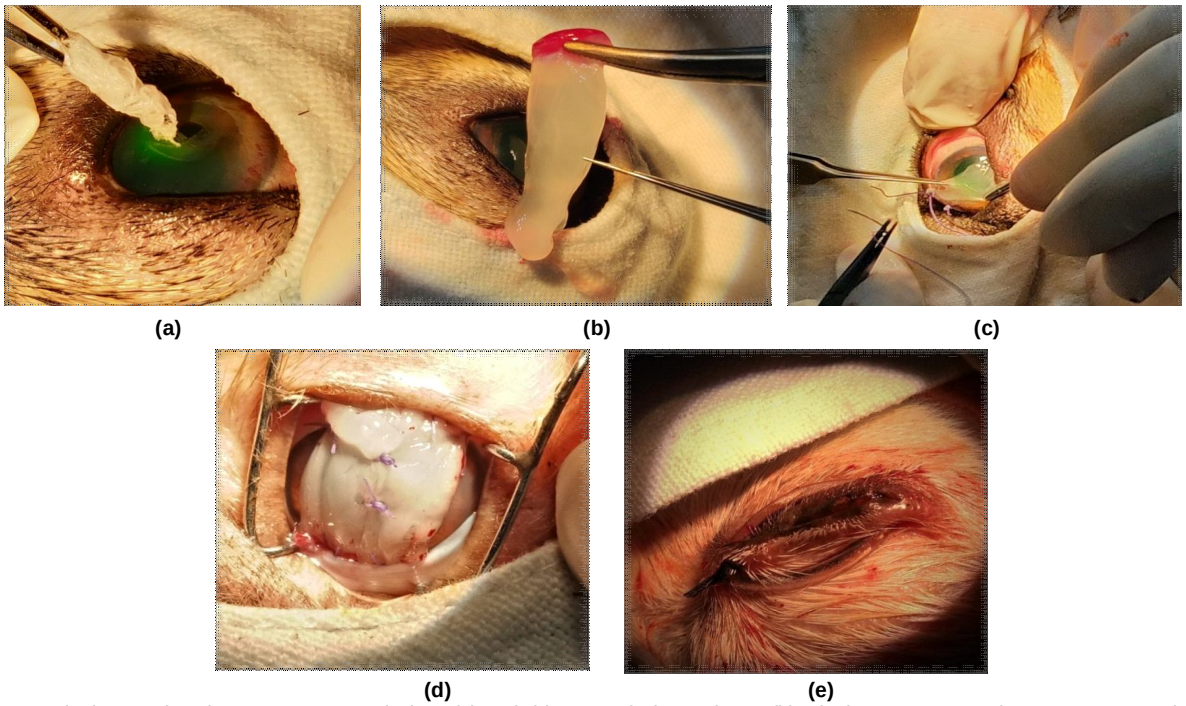


Fig 3: Surgical procedure in group III corneal ulcer (a) Debridement of ulcer edges (b) Placing L-PRF membrane over corneal ulcer (c) Suturing L-PRF membrane over conjunctiva (d) L-PRF membrane completely covering the cornea (e) Temporary tarsorrhaphy by placing quill suture.

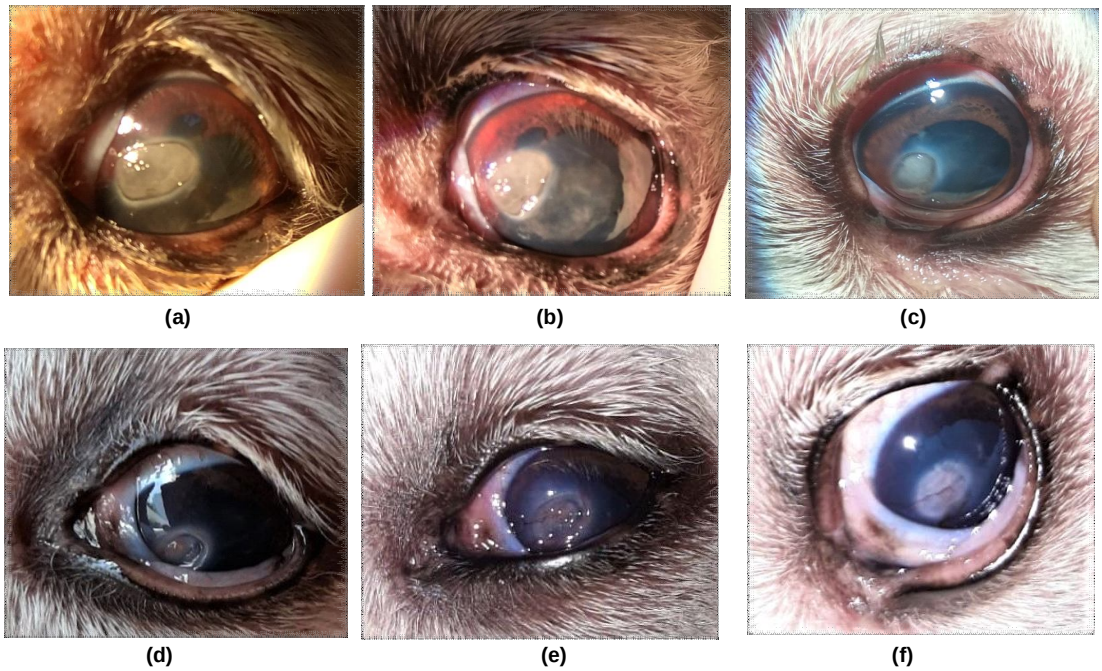


Fig 4: Grade II corneal ulcer treated with Moxifloxacin and Flurbiprofen (Group I) (a) Day 0 (b) Day 7 (c) Day 10 (d) Day 15 (e) Day 20 (f) Day 30.

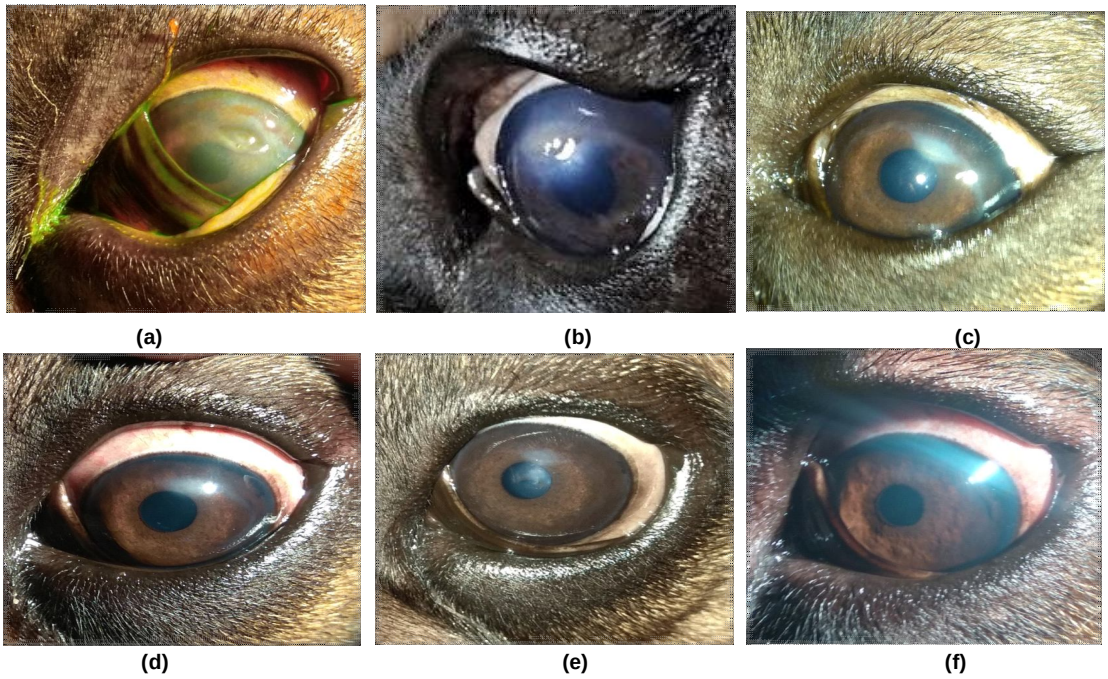


Fig 5: Grade II corneal ulcer treated with PRP (Group II) (a) Day 0 (b) Day 7 (c) Day 10(d) Day 15 (e) Day 20(f) Day 30.

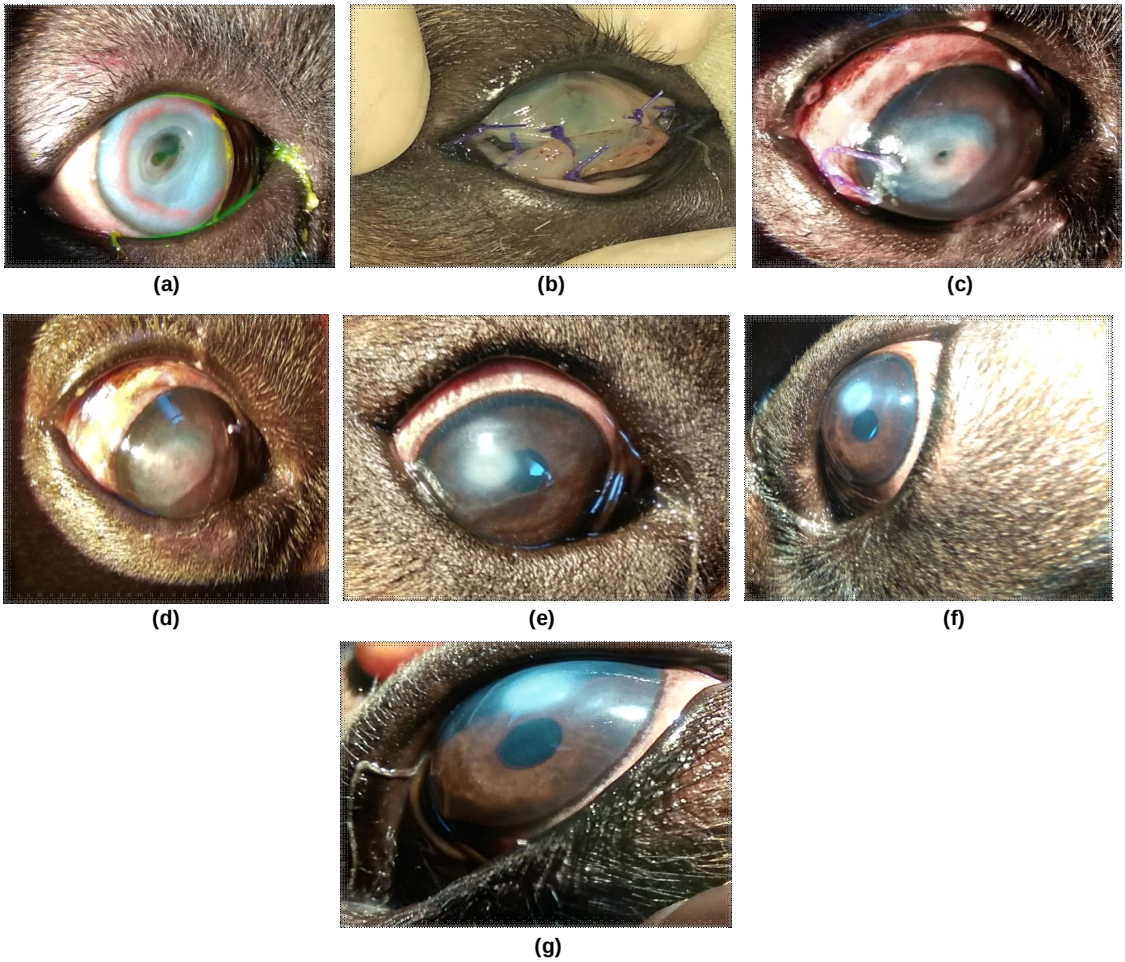


Fig 6: Grade III corneal ulcer treated with L-PRF membrane (Group III)(a) Day 0 (b) L-PRF membrane affixed on cornea (c) Day 7 (d) Day 10 (e) Day 15(f) Day 20 (g) Day 30.

damaged cornea. This lacuna is well fulfilled by homologous platelet concentrates PRP and L-PRF. These platelet concentrates, in a small volume of plasma, contain high concentration of essential growth factors and cell adhesion molecules which play a major role in wound healing an enhance physiological process at the site of injury.

CONCLUSION

On the basis of present study, it is concluded that no adverse reaction was found during regenerative therapy of corneal ulcer using homologous L-PRF membrane and PRP drop. Homologous L– PRF membrane showed significantly earlier clinical healing followed by PRP drop and conventional treatment of Moxifloxacin and Flurbiprofen. Moreover, both platelet concentrates were found at par in early reduction of corneal odema, congestion and vascularization in comparison to conventional treatment. Leucocyte-Platelet rich fibrin (L-PRF) membrane is easy to prepare and can be exploited as a sealant or regenerative graft.

REFERENCES

Alio, J.L., Abad, M., Artola, A., Rodriguez-Prats, J.L., Pastor, S. and Ruiz-Coleccha, J. (2007). Use of autologous platelet-rich plasma in the treatment of dormant corneal ulcers. Opthalmology. 114: 1286-1293.

Alio, J.L., Montiel, F.A. and Rodriguez, A.E. (2012). The role of “eye platelet rich plasma” (e-prp) for wound healing in ophthalmology. Current Pharmaceutical Biotechnology. 13: 1257-1265.

Alio, J.L., Rodrigurez, A.E., Martinez, L.M. and Rio, A.L. (2013). Autologous fibrin membrane combined with solid platelet-rich plasma in the management of perforated corneal ulcers. Journal of American Medical Association Opthalmology. 131: 745-751.

Choukroun, J., Adda, F., Schoefûer, C. and Vervelle, A. (2000). PRF: An opportunity in period implantology. Implantodontie. 42: 55-62.

Dulaurent, T., Azoulay, T., Goulle, A.F., Dulaurent, A and Mentek, M. (2014). Use of bovine pericardium (tautopatch) graft for surgical repair of deep melting corneal ulcers in dogs and corneal sequestra in cats. Veterinary Opthalmology. 17: 91-99.

Eaton, J.S., Hollingsworth, S.R., Holmberg, B.J., Brown, M.H., Smith, P.J. and Maggs, D.J. (2017). Effects of topically applied heterologous serum on reepithelialisation rate of superficial chronic corneal epithelial defects in dogs. Journal of American Veterinary Medical Association. 250: 1014-1022.

Harrison, S.M. (1975). Grading corneal ulcers. Annals of Ophthalmology. 7(4): 537- 539.

Hotwani, K. and Sharma, K. (2014). Platelet rich fibrin - a novel acumen into regenerative endodontic therapy. Restorative Dentistry and Endodontics. 39: 1-6.

Ion, L., Ionascu, I. and Birtoiu, A. (2015). Melting keratitis in dogs and cats. Agriculture and Agricultural Science Procedia. 6: 342-349.

Kececi, Y., Ozsu, S. and Bilgir, O. (2014). A cost – effective method for obtaining standard platelet rich plasma. Wounds. 26: 232-238.

Kim, J.Y. (2009). A retrospective study of ulcerative keratitis in 32 Dogs. International Journal of Applied Research in Veterinary Medicine. 7: 27-31.

Kim, K.M., Shin, Y.T. and Kim, H.K. (2012). Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis. Japanese Journal of Opthalmology. 56: 544-550.

Lewin, G.A. (2000). Temporary lateral tarsorrhaphy for the treatment of lower lateral eyelid entropion in juvenile dogs. American Journal of Veterinary Surgery. 146: 439-440.

Merlini, N.B., Fonzar, J.F., Perches, C.S., Sereneo, M.G., Souza, V.L., Estanislaus, C.A., Rodas, N.R., Ranzani, J.J.T., Maia, L., Padovani, C.R. and Brandao, C.V.S. (2014). Use of platelet rich plasma on corneal ulcers in dogs. ArquivoBrasileiro de MedicinaVeterinaria e Zootecnia. 66: 1742-1750.

Panda, A., Khokar, S., Rao, V., Das, G.K. and Sharma, N. (1995). Therapeutic penetrating keratoplasty in non-healing corneal ulcer. Opthalpmic Surgery, Laser and Imaging Retina, 26: 325-329.

Pandey, P. (2016). Surgical management of corneal ulcer in dogs. M.V. Sc. thesis (Surgery and Radiology), Nanaji Deshmukh Veterinary Science University, Jabalpur.

Simona, P.D., Chiara, C., Francesca, A., Rosario, P., Giusi, V. and Elisabetta, G. (2017). Platelet rich plasma eye drops: preparation, storage and clinical use in dogs and cats, preliminary results. Archives on Veterinary Science and Technology. 104: 1

Singh, N.P., Jhirwal, S.K. and Gahlot, T.K. (2015). Management of corneal ulcer: Rsearch and reviews. Journal of Veterinary Sciences. 2: 4-5.