Journal of Current Veterinary Research

*ISSN: 2636-4026*

Journal homepage: http://www.jcvr.journals.ekb.eg

*Surgery*

# Preparation and Clinical Use of Autologous Platelet Rich Plasma Eye Drops in Dogs

Eslam Reda[1]*, Tarek N. Misk[1], Alaa Ghaith [2], Shaaban M. Gadallah[1]

*(1) Department of Surgery, Faculty of Vet. Medicine, University of Sadat City, Egypt.*

*(2) Department of Ophthalmology, Faculty of Medicine, University of Alexandria, Egypt.*

**corresponding author: eslamredaedrees@gmail.com* *Received: 10/12/2020 Accepted: 25/12/2020*

## ABSTRACT

Several studies demonstrate the effectiveness of eye drops Platelet Rich Plasma (E-PRP) as a promoter of the healing of different ophthalmological disorders in human, due to the high concentration of growth factors as EGF, PDGF-BB and Fibronectin which represent the key to obtain the desired regenerative effects on the ocular surface. There is different protocol for PRP eye drops preparation for use in human corneal disorders. This study was to evaluate an easy and affordable technique for preparation of canine autologous E-PRP according to standard measure used before for human corneal disorders to be used in canine corneal ulcers in comparison with control group, all treatments were applied four times daily. Twelve healthy dogs were divided into two groups first group included eight dogs which received autologous E-PRP compared with second group which included four dogs and received saline eye drops as control. Complete blood count is performed to whole blood and E-PRP to validate last one content of platelets which must equal more 1.5 those in whole blood. E-PRP contain platelets 2.41-fold than those in whole blood with one step centrifugation technique. Autologous canine E-PRP mean healing time was 3.62±0.23 days while with control group mean healing time was 6.75±0.30 days. In conclusion, As the E-PRP is now widely documented in human ophthalmology, it had been proven as a great result in veterinary practice when prepare autologous E-PRP by single step centrifugation to be used for canine corneal wound and healing rate was better than control group by significant difference. which make it novel option for treatment for canine corneal ulcer.

**Keywords:** *E-PRP, corneal ulcer, corneal wound healing, dogs*

## INTRODUCTION

Platelet rich plasma (PRP) is a blood derived product that provides higher concentration of essential growth factors (GF) by concentrating platelets in a small volume of plasma. The GF, including platelet-derived GF (PDGF), transforming GF-β (TGF-β), vascular endothelial GF (VEGF), fibroblastic GF (FGF), insulin-like GF (IGF) and epidermal GF (EGF), have a major role in wound healing and enhance the physiological process at the site of the injury (Carr, *et al.*, 2016). Concentrated GF lead to accelerated endothelial and epithelial regeneration and stimulate angiogenesis, collagen synthesis, soft-tissue healing, and homeostasis (Jee, *et al.*, 2016). PDGF-BB-induced tissue repair mechanisms appear to involve fibroblast proliferation, collagen production and new vessel formation and may involve direct effects on mesenchymal cells expressing PDGFR-α (Rodríguez and Alió 2019).

Now-a-days, E-PRP is being used in treatment of more than 30 diseases including tissue engineering and research, cardio-vascular surgery, gastroenterology, maxillofacial,

orthopedics surgery, urology and ophthalmology (Singla and Jain, 2017). In human ophthalmology some authors reported that E-PRP can be used to treat various ocular surface diseases, such as severe dry eye syndrome, persistent epithelial defects, and neurotrophic keratitis (Lee, *et al.,* 2016) .

Several protocols to obtain E-PRP were used, trying to optimize centrifugation speed and duration. The final products have different platelets and GF concentrations, as there is no standard protocol to these procedures and making it difficult to compare results. (Piccin, *et al.,* 2017).

The Ideal E-PRP has a platelet enrichment index of 1.6–2.5 times above the baseline values in whole blood. PDGF-BB-induced tissue repair mechanisms appear to involve fibroblast proliferation, collagen production and new vessel formation and may involve direct effects on mesenchymal cells expressing PDGFR-α. The E-PRP is a 100% autologous compound since it does not contain any additives (Rodríguez and Alió 2019).

E-PRP for ophthalmological use is carried out through a one-step centrifugation process. (Kim, *et al.,* 2012, Lee, *et al.,* 2016, Rodríguez and Alió 2019). All previous data for preparation of E-PRP carried out from blood human to different ophthalmological disorders, but another Author prepared E-PRP and used it for corneal ulcers in dogs and cats using double centrifugation technique and got a valued result about its use in corneal healing (Di Pietro, 2017)

The aim of this study was to evaluate the one step centrifugation technique used before in human ophthalmology and validate it hematologically and clinically on induced canine corneal ulcer to be used in veterinary practice.

## MATERIALS AND METHODS

This study was carried out at Faculty of Veterinary Medicine Hospital and its Laboratory at Sadat City University after getting approval from Animal Rights Association at Egypt.

***Autologous platelets rich plasma Eye drops preparation***

PRP preparation was carried out under strict sterile conditions using sterile and disposable materials at the laboratory of Faculty of Veterinary Medicine Sadat City University.

Blood sample collection from jugular vein. 10 ml in a tube containing 3.2% sodium citrate as anticoagulant to put it immediately at centrifuge once only (10 min at 1600 r/min) using Centrifugation unit is (L600) with rotor, After the centrifugation, each tube had three distinct layers. The lower, red layer with the vast majority of the red cells, and the upper yellow representing the poor platelets plasma PPP. Between both layers, there was platelets rich plasma above thin white layer consisting of white cells called buffy coat. E-PRP was aspired by syringe around 4 ml above the buffy coat and transferred to new sterilized amber glass bottles with eye drop applicators, as we obtain around 1/3 amount of blood as an E-PRP eye drops. (Rodriguez, *et al.,* 2020)

Preservation at -20 C for one month as it found that freezing of E-PRP increase platelets concentration (Rodríguez and Alió 2019) and when use it must kept at -4 C during one week only as the E-PRP preparation kept during use only for one week when refrigerated at -4C . The final preparation placed at bottles wrapped in aluminum foil for protection from ultraviolet light to avoid degradation of vitamin A. The bottles being used were maintained under refrigerated conditions at - 4 _C. (Kim, *et al.,* 2012). There were no dilutions of the PRP. The substance so created was termed E-PRP (Alio, *et al.,* 2015)

***Hematological evaluation*** of the E-PRP through platelets count in the whole blood and another time after E-PRP preparation using the digital hematological counter Mythic 18 (Orphée, Montpellier, France) at SADAT CITY university laboratory The Ideal E-PRP has a platelet enrichment index of 1.6–2.5 times above the baseline values in whole blood. (Rodríguez and Alió 2019).

***Clinical evaluation*** by Appling autologous E-PRP on induced corneal wound healing in dogs four times daily.

***Animals***

The present study was carried out on 12 apparently healthy dogs: (9 males and 3 females) mixed breed (mongrel) dogs, aging 1.52±0.31 years and weighing 15.7±2.13 kg. Group (I) included 8 dogs received E-PRP compared with group (II) which included 4 dogs and received normal saline as control group. The dogs were anaesthetized with a combination of 1 mg/kg

xylazine HCl (Xylaject®) Amoun pharmaceutical CO. Egypt as tranquilizer followed by 10 mg/kg ketamine HCl (ketamine hydrochloride inj. USP ® by Rotexmedica – Trittau Germany) as general anaesthia IV through cephalic vein then add benoxinate hydrochloride 4mg eye drops (Benox®) as local anaesthia. The dogs were placed in lateral recumbency. A pediatric eyelid speculum (lid retractor) was placed for exposure of the cornea.

A 6-mm calibrated corneal trephine was placed in the center of the cornea. The trephine depth was 240 µg which include the anterior third of the corneal stroma then a crescent bevel-up blade which was used to curette the trephined button from the center of the cornea (Aldavood, *et al.*, 2003) . Then apply fluorescein stain 10% immediately after removing the incised button to stain the corneal wound as shown in figure (1).

The epithelial defect area in all cases was assessed daily by using digital photographs captured with a digital camera every 24 hours until epithelial healing was complete. All eye drops administered to animals 4 times daily (Freire, Andollo *et al.*, 2014) . Each dog was placed in a separate clean cage with neck collar to control infection during treatment period.

***Statistical Analysis***

The frequency, mean, and standard deviation of the duration of corneal epithelial healing after topical application of E-PRP were calculated and compared control group using ANOVA, were performed using SPSS statistical software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, version 26.0. Armonk, NY: IBM Corp) and a *P* value less than 0.05 was considered statistically significant.

Fig. (1): Cornea, Dog. fluorescein stain after trephine machine incision in cornea with depth 240 µ and width 6mm.

**RESULTS**

Hematological evaluation was performed to whole blood and E-PRP to ensure the ability of this animal to provide an E-PRP preparation matching with Ideal E-PRP enrichment index 1.6–2.5 times above the baseline values in whole blood

The results were average platelets concentration in whole blood was 150.5*1000, SE 8.48 while average platelets in group (I) after preparation is 374.9*1000 SE 61.05 which represent 2.41, SE 0.25 times than those in whole blood.

Table (1) showing platelets concentration in whole blood and in E-PRP for each dog with average range 2.4% increase in E-PRP than those of whole blood.

| | whole blood PLT | PRP PLT | % |
|---|---|---|---|
| dog 1 | 149 | 284 | 1.9 |
| dog 2 | 146 | 261 | 1.8 |
| dog 3 | 203 | 778 | 3.8 |
| dog 4 | 165 | 369 | 2.2 |
| dog 5 | 142 | 345 | 2.4 |
| dog 6 | 133 | 279 | 2.1 |
| dog 7 | 128 | 421 | 3.2 |
| dog 8 | 138 | 262 | 1.9 |
| AVERAGE | 150.5 | 374.875 | 2.4125 |
| SE (standard error) | 8.48 | 61.05 | 0.25 |

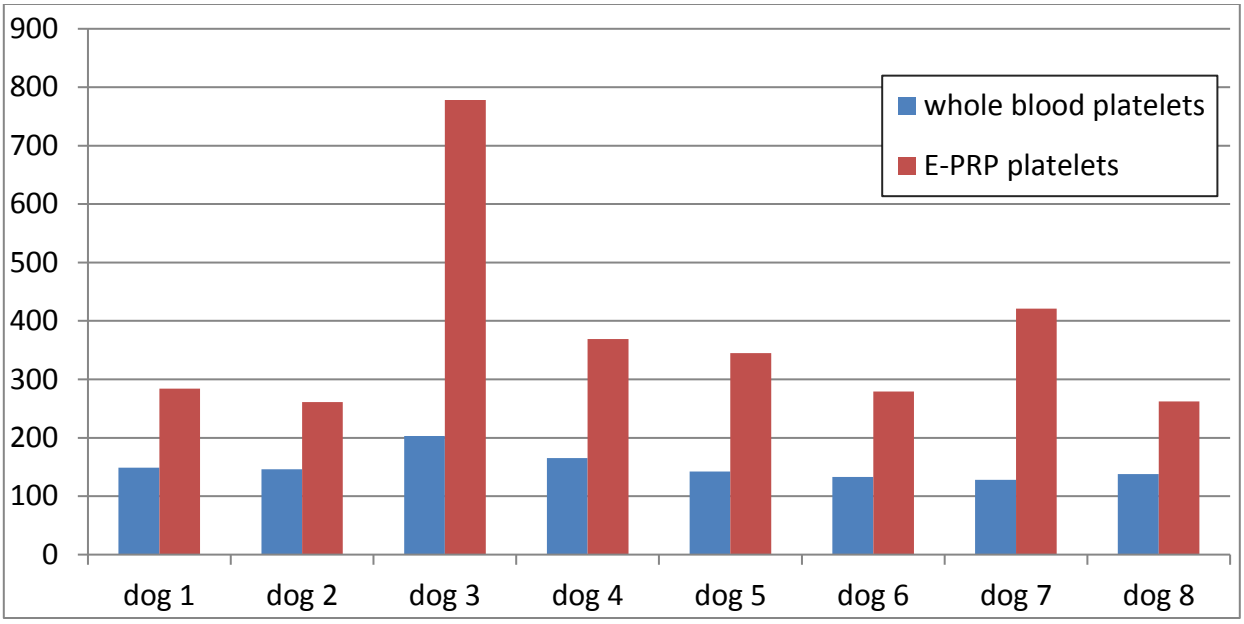


**Fig. (2)**: Showing hematological analysis of platelets count for each dog in whole blood and E-PRP preparation using single step centrifugation.

***Clinical Evaluation*** was performed daily by fluorescein stain which recorded daily to achieve the results which are explained in figure (2) which shows that the complete epithelial healing time is more rapid with E-PRP group than control group by significant difference.

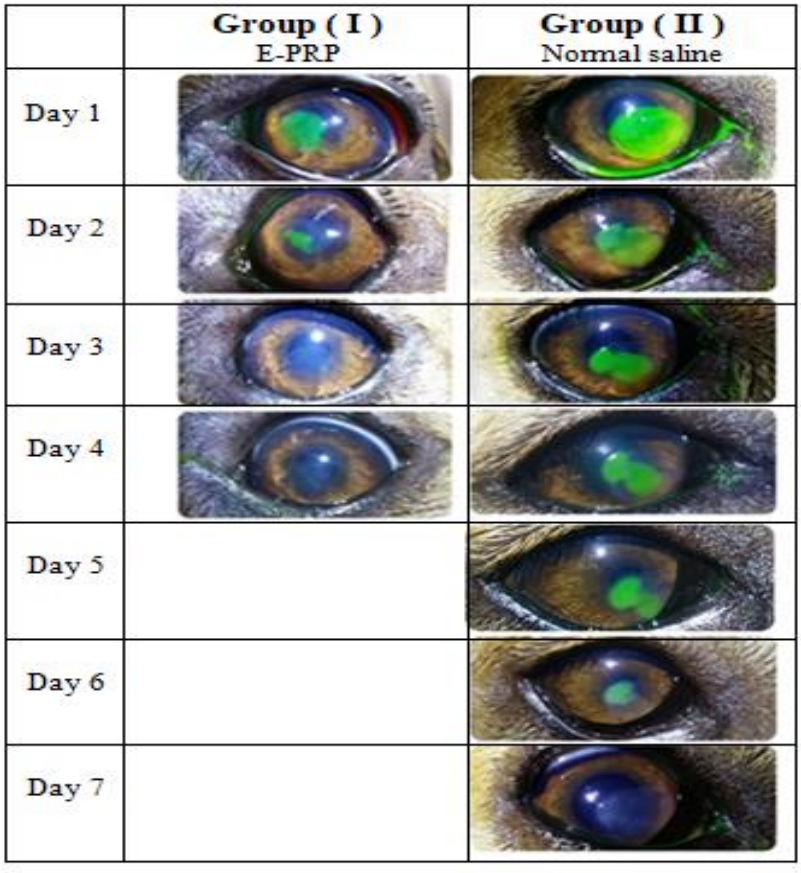


**Fig. (3):** Cornea, dog stained by fluorescein stain 10% to evaluate healing time daily progress in group (I) and (2) till complete healing.

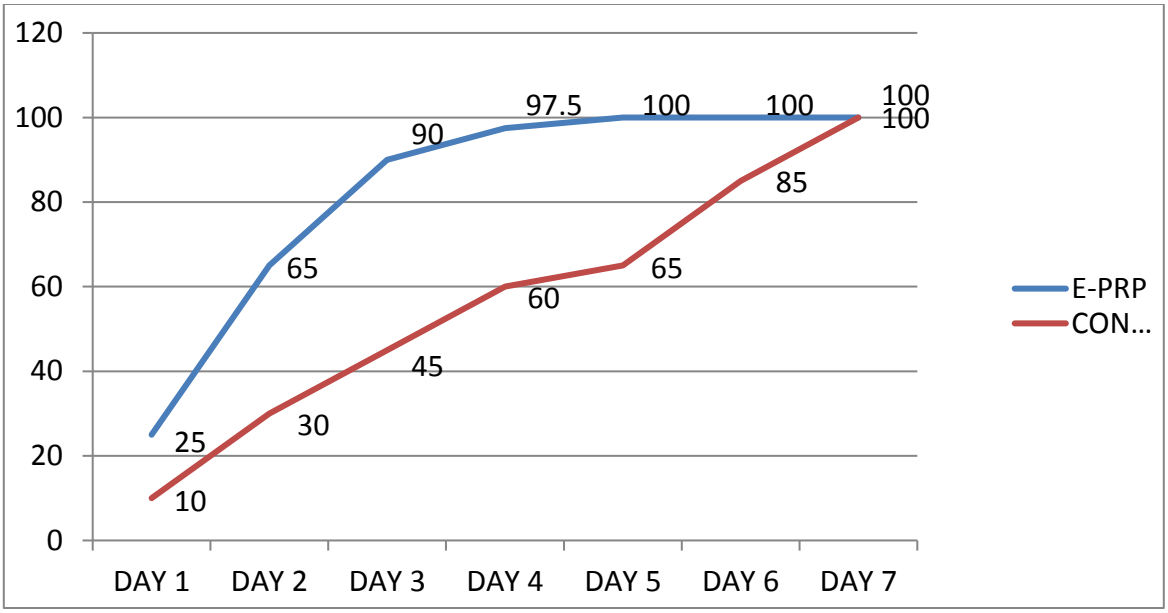


**Fig. (3)**: showing daily improvement in corneal wound healing for group (I) and (II) till complete healing.

So, the results showed that the group (I) mean healing time by day was 3.62±0.23 days while with group (II) mean healing time was 6.75±0.30 days which considered significant difference with *p. value* $P \leq 0.0001$ according to figure (3)

In group (I) daily photograph of corneal wound stained by fluorescein stain showed that the mean healing rate at first day was 25.2% with SE ±3.3 and at the second day was 65.4% with SE ±5.2 while at third day 90.3 % with SE±3.8 and finally at fourth day mean healing rate was 97.6 with SE±2.5.

While in group (II) mean healing, rate was 9.2% with SE±5.3, 30.1% with SE±5.2, 44.7% with SE±5.4, 59.5% with SE±6.3, 70.6% with SE±5.1, 86.3%with SE±4.7 and finally 100% at 1,2,3,4,5,6 and 7 days respectively.

## DISCUSSION

In human ophthalmology , the use of blood derivatives represents an alternative therapeutic approach that is gaining interest in regenerative medicine due to its potential to stimulate and accelerate tissue healing (Anitua, *et al.*, 2015) . PRP is known to be powerful, effective, and safe cure for various wound-healing processes. Multiple commercial PRP separation systems have been developed for both human and veterinary use (Carr, *et al.*, 2016). New challenge is to obtain a high-quality preparation, easy to prepare and stable, all properties that make it suitable for a routine clinical use, although most of the methods require expensive and sophisticated technical equipment. In this report we obtained effective, safe and inexpensive eye drops PRP termed as E-PRP to be used in veterinary clinical practice as therapeutic tool to enhance epithelial wound healing in ocular surface disease in dogs.

When Hematological evaluation was performed to all blood samples collected as whole blood and E-PRP , the result proved that the average platelets concentration in E-PRP was 2.4 times than those in whole blood which matching with E-PRP enrichment index 1.6–2.5 stated recently in human ophthalmology by Alio at 2019 (Rodríguez and Alió 2019) .

As the effectiveness of E-PRP depend on preparation method , this platelets concentration in E-PRP was a result of depending on one step centrifugation technique which is matching with authors who depend on this methods in human E-PRP preparation (Kim, *et al.*, 2012, Lee, Kim *et al.*, 2016, Rodríguez and Alió 2019). However this result didn`t match with authors who depend on double centrifugation technique (Di Pietro 2017, Alizadeh, Balagholi *et al.*, 2019). Which mean further studies is recommended for the most appropriate preparation methods.

From the clinical evaluation of corneal wound healing , the significant difference between group ( I ) received E-PRP and group ( II ) received saline indicated the effectiveness of this method of preparation and this improvement in corneal wound healing may be due to higher concentration of platelets and therefore some growth factors as; There was a positive and significant correlation between PDGF-BB in the E-PRP and platelets (Rodriguez, *et al.*, 2020) so

the E-PRP prepared contain high level of this growth factors that presence is essential for corneal wound healing.

Another growth factors EGF, PDGF-BB and fibronectin in the E-PRP as PDGF-BB-induced tissue repair mechanisms appear to involve fibroblast proliferation, collagen production and new vessel formation, EGF which play an important role in accelerating the wound healing process, producing less scarring and stimulation of the granulation tissue (Rodríguez and Alió 2019), and Fibronectin is the first to reach to corneal wound, filling the bed of the lesion, which produces cell migration during the repair process of the corneal epithelium. (Ljubimov, *et al.*, 2015). Still there are need for further studies to evaluate the E-PRP as autologous preparation in veterinary medicine and subsequent concentrations of different growth factors responsible for this success.

## CONCLUSION

When autologous E-PRP prepared by single step centrifugation technique, the platelets concentration was more than two folds those in whole blood and it was effective preparation in healing of canine corneal wound compared with normal healing by significant difference. So Autologous E-PRP is considered effective, inexpensive and easy to be prepared by veterinarian for treatment of canine corneal wound healing. However, there is a clear need for more detailed prospective studies on other indications and other blood derivatives and formulation in order to complement our findings for other eye affections and to clarify mechanisms involved in autologous PRP treatment in different veterinary ophthalmological disorders.

## REFERENCES


Aldavood, S., *et al.*, (2003). "Effect of acetylcysteine on experimental corneal wounds in dogs." 35(6): 319.

Alio, J. L., *et al.*, (2015). "Eye platelet-rich plasma in the treatment of ocular surface disorders." Current opinion in ophthalmology 26(4): 325-332.

Alizadeh, S., *et al.*, (2019). "Autologous platelet-rich plasma eye drops accelerate re-epithelialization of post-keratoplasty persistent corneal epithelial defects." 14(2): 131.

Anitua, E., *et al.*, (2015). "Autologous serum and plasma rich in growth factors in ophthalmology: preclinical and clinical studies." 93(8): e605-e614.

Carr, B. J., *et al.*, (2016). "Canine platelet-rich plasma systems: a prospective analysis." Frontiers in veterinary science 2: 73.

Di Pietro, S. (2017). Department of Veterinary Science, University of Messina, Polo Universitario Annunziata, 98168 Messina, Italy: Arch Vet Sci Technol 2017 AVST: 104. DOI: 10.29011, AVST-104/100004.

Freire, V., *et al.*, (2014). "Corneal wound healing promoted by 3 blood derivatives: an in vitro and in vivo comparative study." Cornea 33(6): 614-620.

Jee, C.-H., *et al.*, (2016). "Effect of autologous platelet-rich plasma application on cutaneous wound healing in dogs." 17(1): 79-87.

Kim, K. M., *et al.*, (2012). "Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis." 56(6): 544-550.

Lee, J. H., *et al.*, (2016). "Autologous platelet-rich plasma eye drops in the treatment of recurrent corneal erosions." 30(2): 101-107.

Ljubimov, A. V., *et al.*, (2015). "Progress in corneal wound healing." 49: 17-45.

Piccin, A., *et al.*, (2017). "Platelet gel: a new therapeutic tool with great potential." 15(4): 333.

Rodríguez, A. E. and J. L. Alió (2019). Eye Platelet-Rich Plasma (E-PRP) for Corneal Regeneration. Corneal Regeneration, Springer: 317-345.

Rodriguez, A. E., *et al.*, (2020). "Quantification of Growth Factors and Fibronectin in Diverse Preparations of Platelet-Rich Plasma for the Treatment of Ocular Surface Disorders (E-PRP)." 9(6): 22-22.

Singla, A. and M. Jain (2017). "Biological Regeneration of Tissues-Latest Therapeutic Approach Using Platelet Rich Plasma." Blood Res Transfuse J 1(1): 1-3.