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Surgery

Effect of Platelet Rich Plasma on Corneal Alkali Burn Ulcer in Rabbits

Mahmoud A Hassan[1*], Fatma Abozakaib Ali[2], Nani Nasreldin[3], Marwa M Elzeftawy[4], Mahmoud T. Nassef [5], Mohamed W. El-Sherif [1]

(1) Department of Surgery, Anesthesiology & Radiology, Faculty of Veterinary Medicine, New Valley University.

(2) Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Sohag University.

(3) Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, New Valley University.

(4) Department of Biochemistry, Faculty of Veterinary Medicine, New Valley University.

(5) Department of Surgery, Anesthesiology & Radiology, Faculty of Veterinary Medicine, Assiut University.

**corresponding author:* Mahmoud.atiya@vet.nvu.edu.eg *Received: 1/2/2021 Accepted: 10/3/2021*

ABSTRACT

Objective of this study to assess the efficacy of platelet rich plasma (PRP) in the treatment of extensive corneal ulcers in albino rabbits with two different administration techniques. A total of 24 rabbits were used in this study. After collecting intracardiac blood samples, platelet-rich plasma was obtained by double centrifugation. Alkali burns were inflicted on the central corneas of all rabbits using a sterile swab soaked in 1 M NaOH for 60s. Only Left eye in each rabbit was used. Animals were divided into 4 groups. GroupI (n=6) normal control group received PRP drop twice daily with 8-h intervals for 7 successive days. whereas group II (n = 6) did not receive any treatment and served as the control untreated group. The third group (n = 6) received only one-shot of subconjunctival PRP. The fourth group (n=6) received PRP drop twice daily with 8-h intervals for 7 successive days. Corneal histopathology was investigated for epithelial regeneration, presence of inflammation, and structural integrity of fibroblasts. Data were expressed as mean ± standard deviations. Data from experimental groups were statically analyzed using one-way ANOVA with Turkey's post hoc multiple comparisons tests using the GraphPad Prism software version 5 (San Diego, CA, USA) and $P<0.05$ was used to define statistically significant differences between the groups. Group III had a significantly high rate ($P<0.05$) of corneal epithelial healing as compared with the control group. Group IV had moderate restoration of corneal epithelium defects. Group III had a significantly better healing process after the fifth day as compared with group IV. Histopathological investigations revealed a steady fibroblast migration, quicker epithelial regeneration, and less inflammation in group III as compared with the other treated groups. A single dose of subconjunctival PRP injection had an effective alleviating effect on alkali-induced corneal burn rather than PRP drops

Keywords: Alkali-burn, cornea, PRP, Subconjunctival, ulcer.

INTRODUCTION

Corneal ulcer represents a great challenge in clinical ophthalmology due to the opacity of the cornea resulted by the healed corneal scar tissue (Suzuki *et al.*, 2003).

Corneal ulcer induced by an alkali-burn is an epithelial defect, with loss of stroma, stromal

inflammation, or a combination. Alkali are lipophilic agents that penetrate the eye more rapidly through denaturation of proteins and saponification of membrane lipids, thus they are even more harmful than acids ((Marquez-de-Aracena *et al.,* 2007; Del Cid & Escoriaza, 2009; Singh at al. 2013; Acosta *et al.,* 2014).and enter the eye.

Structural mechanism of corneal wound healing requires several materials such as growth factors, vitamins and glucose for the physiologic maintenance of ocular surface epithelial homeostasis. The lack of these materials can promote damage to the ocular surface leading to corneal defects (Kim *et al.*, 2012).

Blood is considered the easiest and most efficacious source to obtain such material. Until recently, various blood derivatives were used for this purpose, such as fetal calf serum, umbilical cord serum and autologous/allogenic serum(Khaksar *et al.,* 2013). Platelets are great suppliers of these growth factors and are known for their ability to heal epithelial and internal wounds. Platelets adhere to the damaged endothelium and start a healing reaction that includes the release of numerous cytokines and growth factors (Alio *et al.,* 2007). Platelet-rich plasma (PRP) has become a common treatment in the field of ophthalmology, plastic surgery, trauma and skin burns reconstructive surgery (Cervelli *et al.,* 2009; Girgin *et al.,* 2016).

Platelet-rich plasma is a non-toxic and non-immunogenic blood component obtained by centrifuging whole blood to get a cellular constitute of platelet-enriched plasma. PRP contains several growth factors, including insulin-like growth factors 1 and 2, transforming growth factor, hepatocyte growth factor, fibroblast growth factor, and vascular endothelial growth factor(Everts *et al.,* 2020). Besides, it includes bioactive factors or non- growth factors; serotonin, histamine, dopamine, calcium, adenosine, fibronectin, fibrin, and vitronectin. These factors have been suggested to accelerate epithelial and endothelial regeneration, provoke angiogenesis and cell differentiation, enhance the hemostatic response, increase collagen synthesis, and assist cell migration, thus stimulate soft tissue healing (Çirci *et al.*,2016; Girgin *et al.*,2016). The aim of the present study was to evaluate the effect of autologous PRP on the healing of alkali-burn induced corneal ulcer through topical and subconjunctival injection in a rabbit model.

MATERIALS AND METHODS

The present experimental study design was approved by the animal handling and Fac Vet Med ethics committee Ref. 4/2020, New valley University. The current study was done at the Department of Surgery, Faculty of Veterinary Medicine, New Valley University.

Animals

A total of 24 adult male New Zealand albino rabbits, with mean body weight 2 kg (±200 gm) were used in the experimental study. Animals were housed in batteries (rabbit house) under standard conditions in a room with controlled temperature (25±5 °C) and relative humidity (60-70%) with a 12-hour light/dark and free access to dry food pellets and tape water. Rabbits were kept for a week to adapt the laboratory conditions before starting the experiment. All animal experiments were conducted in accordance with the guide of the National Institutes of Health (NIH 1985) for the care and use of laboratory animals.

Prior to study all rabbits underwent a complete visual and ophthalmoscopic examination (indirect ophthalmoscope, keeler standard ophthalmoscope) and sodium fluorescein 0.5% (FUL-GLO, Akorn) exam to exclude rabbits with ophthalmic conditions.

Experimental design

All rabbits except *group(I)* were subjected to corneal ulcer induction using alkali burn technique as described by (Khaksar *et al.,* 2013). Blood samples were collected for autologous PRP preparation.

Preparation of PRP

Intracardiac Blood Sampling

Animals received anesthesia using intramuscular injection (21gauge) of ketamine (25mg/kg) and xylazine (1mg/kg), then 8.5 ml of blood was aspirated by intracardiac puncture **(Gimeno *et al.,,* 2006)** which is the best method to avoid blood coagulation resulting from other sites of blood collection with a 10-ml syringe (PRP tube) containing 1.5 anticoagulant (acid citrate dextrose gel), then samples underwent double centrifugation **(Figure 1).**

Centrifugation technique

First centrifugation, at 3000 rpm at 15 mins. the 6-ml plasma fraction and white cells were aspirated by a Pasteur pipette into another tube without anticoagulant and underwent second centrifugation at 4000 rpm at 20 mins to obtain a 2-level plasma, the upper level consisting of platelet poor plasma (PPP), the lower level consisting of platelet rich plasma (PRP) (Khaksar *et al.,* 2013; Ariede *et al.,* 2015) with slight modification.

The PPP was first aspirated to avoid mixing up with the PRP. The PRP was then gently aspirated with another pipette and placed in a sterile tube.

Autologous PRP samples were transferred into 5 ml presterilized eye droppers and stored it in the refrigerator at 4ºC according to (Wu *et al.*, 2015). Each dropper was labeled with the same ID number of the corresponding rabbit.

Corneal ulcer animal model

Animals were anaesthetized with xylazine (xylaject, Adwia , Egypt) 2% (1mg/kg IM) and ketamine (rotexmedica,Germany) (25 mg/kg IM). The corneal alkali burn was made by using a sterile swab soaked in 1M NaOH **(*Alpha chemicals*)** on the central cornea for 1 min (Figure 2). Burning was induced in one eye of each rabbit in all groups. the ocular surface was rinsed with 2 ml of physiological saline for 2 mins. Fluorescein dye test was used to confirm and delimit the burn lesion induced **(Figure 2).**

After creation of animal model. Animals were randomly allocated into 4 groups (6 animals per group *n=6*), as follow:

Group I (Control normal group) received only one drop of the pre-prepared (autologous PRP) twice daily with 8-h intervals for 7 successive days.

Group II (Control untreated group) the animals of this group were modeled, and the corneal burn was left untreated. Few drops of normal saline solution were used as placebo drug.

Group III (PRP subconjunctival treated group) in this group, each rabbit received a single dose subconjunctival autologous PRP next day after induction of corneal ulcer. A 0.5 ml autologous PRP was injected into the superior bulbar conjunctiva using a 1ml insulin syringe and 27-gauge half-inch needle (Tanidir *et al.,* 2010).

Group IV (PRP topical treated group) the next day after induction of ulcer model. The rabbits of this group were administered one drop of the pre-prepared (autologous PRP) twice daily with 8-h intervals for 7 successive days according to regimen described by (Acosta *et al.*, 2014).

Samples for scheduled evaluation and assessment

Whole blood sample

1ml of whole blood on EDTA was separated for baseline whole blood analysis.

Platelet count sample

From prepared autologous PRP, 200µL samples were taken to count average platelets in sample.

*Values represent mean ± SE. SPSS version 23 (independent-sample t-test). Significance: ***p<0.001 comparing to control rabbits. PRP= Platelets rich plasma, SE=Standard error.*

Tissue samples

An animal from each group was euthanized at 5th and 10th day of the study, while others were euthanized at the day 18 by the end of the experiment. Each animal's cornea was removed by the same surgeon 1 mm close to limbus, rinsed in normal saline solution, then fixed in phosphate-buffered 10% formaldehyde in labeled glass containers and sent for histopathological evaluation.

Evaluation and assessment processes

Naked eye and indirect ophthalmoscopy

Rabbits were evaluated through naked eye examination, ophthalmoscopic examination and fluorescein dye test daily for 18 days. Corneal opacity, duration of blepharospasm, corneal vascularization, duration of ocular discharge, condition of the conjunctiva (edema and hyperemia) and the presence of any pathological discharge and subjective symptoms (pain, discomfort, lacrimation, or photophobia) were recorded and documented.

The severity of corneal opacity was graded 0-3 according to the scale designed by (Kozák *et al.*, 2002) as follow; Grade (0) represents a completely clear cornea, Grade (1) represents faint corneal haze, Grade (2) represents blurred iris detail, Grade (3) represents pupil not visible.

Corneal vascularization was graded 0-3 according to the scale designed by (Kozák *et al.*, 2002) as follow; Grade (0) represents no vascularization, Grade (1) superficial focal vascularization, Grade (2) superficial diffuse vascularization, Grade (3) superficial deep vascularization.

Fluorescence dye test and corneal ulcer measurements

A piece of blotting paper containing the fluorescein dye was touched to the surface of eye. The light (blue light) was applied directly on eye, so any defect problems found on the cornea would be stained by the fluorescein dye and take a green color under the blue light, by this technique we can limited and determine the damage and detect the main cause of the cornea problem by measuring the size of lesions with detect the shape of it and location by effect of the staining (Jassim *et al.*, 2020).The ulcerated cornea was outlined with florescence dye and measured with caliber on days 0,3,6,10,18.

Histopathological examination of corneal tissue samples

Tissue samples were processed, paraffin embedded samples were sections at 5 µm thickness , Each sample yielded ten slides with three specimens per slide and was stained with Harris hematoxylin and Eosin H&E (Carleton, Drury *et al.*, 1980, Bancroft, D *et al.*, 1996) and Masson's Trichrome stains (specific stain for collagen content) (Sereno, Vala *et al.*, 2015), and observed under light microscopy for histopathological evaluation. The histopathological observation was performed by Olympus® CX 41 RF light microscope (Olympus Corporation, Tokyo, Japan).

Morphometric study

The images were taken randomly from the Hx & E stained tissue slides and processed to the Image J program versus 1.48 software (NIH), where it was selected and the region of each lesion (thickness of corneal epithelium) were measured and calculated (Abràmoff *et al.*, 2004).

Ten different non-overlapping randomly selected fields from each slide at a magnification of 400 were quantified for: area Mean % of corneal epithelial thickness in H&E-stained sections.

Statistical Analysis

Data were expressed as means ± standard deviations. Data from experimental groups were statistically analyzed using one-way ANOVA with Tukey's post hoc multiple comparisons tests using the GraphPad Prism software version 5 (San Diego, CA, USA). $P < 0.05$ was used to define statistically significant differences between the groups (Ali, M Abdel-Maksoud *et al.*, 2021).

Figure 1. Blood collection, PRP preparation, and preservation.

Figure 2. Induction of corneal ulcer animal model.

RESULTS

Platelet count in both whole blood & Platelet rich plasma:

The result showed that there is high increase of platelet count in (PRP) in compared with whole blood in control rabbits.

Table. 1. Blood variables (mean ± S.E)، for control (*n* = 5) and PRP (*n* = 5) groups of rabbits.

	Groups	
	whole blood	PRP
Platelet's count /µl	392.667 $\times 10^3$ ± 18.39	1158.333$\times 10^3$ ± 18.39***

Finding of indirect ophthalmoscopic examination

Freshly burned corneas of all groups become cloudy immediately after burning and subsequently turned opaque within 24 h. All rabbits showed blepharospasm due to pain on the first 3days following ulcer formation and the eyes were semi-closed. Corneal edema was most prominent in the first week after surgery in all groups after inducing ulcers.

Corneal opacity and degree of vascularization are recorded in table 2.

Table. 2. the corneal opacity evaluation and corneal vascularization degree.

Corneal opacity Score							Corneal Vascularization Score				
	Animal No.		Day 0	Day 6	Day 12	Day 18		Day 0	Day 6	Day 12	Day 18
	C1	Group *(II)* Control untreated	3	3	2	2		3	2	2	2
	C2		3	3	1.5	1		3	2	2	2
	C3		3	3	2	--		3	2	2	2
	C4		3	3	2	2		3	2	--	--
	C5		3	3	2	2		3	2	2	--
	Animal No.		Day 0	Day 6	Day 12	Day 18		Day 0	Day 6	Day 12	Day 18
	1	Group (*IV*) PRP drops treated	3	2	2	1		3	2	1	1
	2		3	3	1	0		3	3	1	1
	3		3	3	2	2		3	3	1	1
	4		3	2	--	--		2	2		--
	5		3	2	1	--		2	2	1	--
	Animal No.		Day 0	Day 6	Day 12	Day 18		Day 0	Day 6	Day 12	Day 18
	1	Group (*III*) PRP subconjunctival injection treated	3	1	--	--		2	1	--	--
	2		3	1	0	0		2	1	0	0
	3		3	1	0	--		2	1	0	--
	4		3	2	0	0		3	2	0	0
	5		3	2	1	0		3	1	0	0

Finding of Florescence dye test and corneal ulcer measurement progress

the result showed that in table 3.

Table.3. The corneal ulcer diameter progress in *mm*.

Animal No.		Day 0	Day 3	Day 6	Day 10	Day 15	Day 18
C1		0.6×0.6	0.6×.06	--	--	--	--
C2	Group *(II)*	0.5×0.5	0.5×0.5	0.5×0.5	0.5×0.5	0.4×0.4	0.4×0.4
C3	Control untreated	0.6×0.6	0.6×0.6	0.5×0.5	0.5×0.5	0.4×0.4	0.4×0.4
C4		0.4×0.4	0.4×0.4	0.4×0.4	--	--	--
C5		0.4×0.4	0.4×0.4	0.4×0.4	0.4×0.4	0.4×0.4	--
Animal No.		Day 0	Day 3	Day 6	Day 10	Day 15	Day 18
1		0.6×0.6	0.3×0.3	0.2×0.2	0.2×0.2	0.2×0.2	--
2	Group (*IV*)	0.5×0.5	0.5×0.5	0.4×0.4	0.2×0.2	0.2×0.2	0.2×0.2
3	PRP drops treated	0.4×0.4	0.3×0.3	0.2×0.2	0.2×0.2	0.2×0.2	0.2×0.2
4		0.4×0.4	0.3×0.3	--	--	--	--
5		0.3×0.3	0.2×0.2	0.2×0.2	--	--	--
Animal No.		Day 0	Day 3	Day 6	Day 10	Day 15	Day 18
1	Group (*III*)	0.3×0.3	0.3×0.3	--	--	--	--
2	PRP	0.3×0.3	0.3×0.3	0.2×0.2	0.2×0.2	0.2×0.2	0.2×0.2
3	subconjunctival	0.5×0.5	0.4×0.4	0.4×0.4	--	--	--
4	injection treated	0.4×0.4	0.3×0.3	0.2×.02	0.2×0.2	0.2×0.2	--
5		0.3×0.3	0.3×0.3	0.2×0.2	0.2×0.2	0.2×0.2	0.2×0.2

Finding of histopathological investigation

<u>Group I (Control normal group)</u>

Corneal sections from the central part of the cornea of control normal group revealed the characteristic histological structure of cornea, where it was composed of three distinct layers, the outer epithelium, the inner endothelium, and the intermediate stroma. The corneal epithelium was a non-keratinized stratified squamous epithelium consisting of a single basal layer of columnar cells and 2-3 layers of intermediate polygonal (wing) cells covered with 1-2 layers of superficial flattened squamous cells. The epithelium was supported by Bowman's membrane. The stroma was composed of parallel lamellae of regular dense acidophilic collagenous fibers forming the main bulk of the cornea. Keratocytes were flattened cells scattered in the ground substance between the stromal lamellae. The inner endothelium of the cornea was composed of single layer of flattened cells supported by a thick homogenous Descemet's membrane **(Figure. 3).**

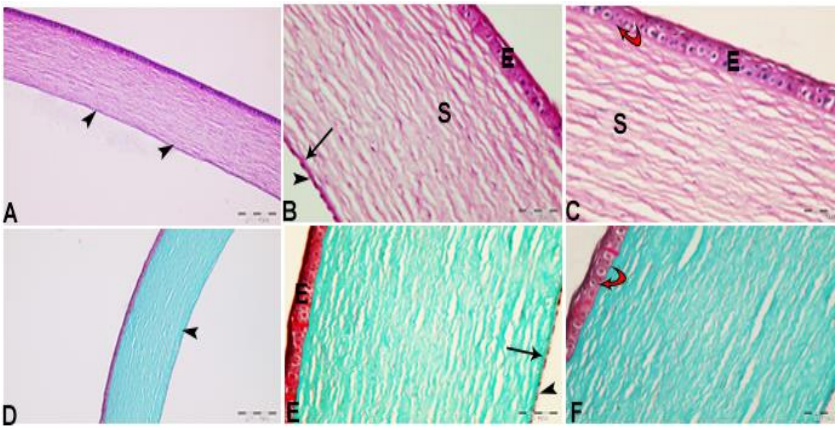

Fig. 3: Photomicrograph of sections from the central part of the normal cornea: showing epithelium (E), intact substantia propria (S) that forms the main bulk of the cornea and the endothelium. Regular continuous Bowman's layer (red arrow). Descemet's membrane (black arrow) and endothelium (black arrow heads) are intact. (A-C, H&E stain, D-F, Masson's trichrome, the bar size was indicated under each picture).

Group II (Control untreated group)

Corneal sections from the central part of the cornea of control group showed necrosis and detachment of corneal epithelium corneal epithelium with focal discontinuity and denudation, while other parts revealed focal disorganization and stratification. Moreover, the corneal epithelium showed vacuolated cytoplasm and some hyperchromatic, pyknotic and karyolytic nuclei. The Bowman's membrane appeared irregular with focal disruption in some areas. The underlying stroma showed extensive separation of collagen lamellae with wide spaces in between. keratocytes were large and irregularly dispersed in between the lamellae. The Descemet's membrane was apparently thinned out and disrupted in some areas. Mononuclear cellular infiltration and invasion with small blood vessels could be observed in between the irregularly dispersed collagen fibers of the stroma **(Figure.4)**

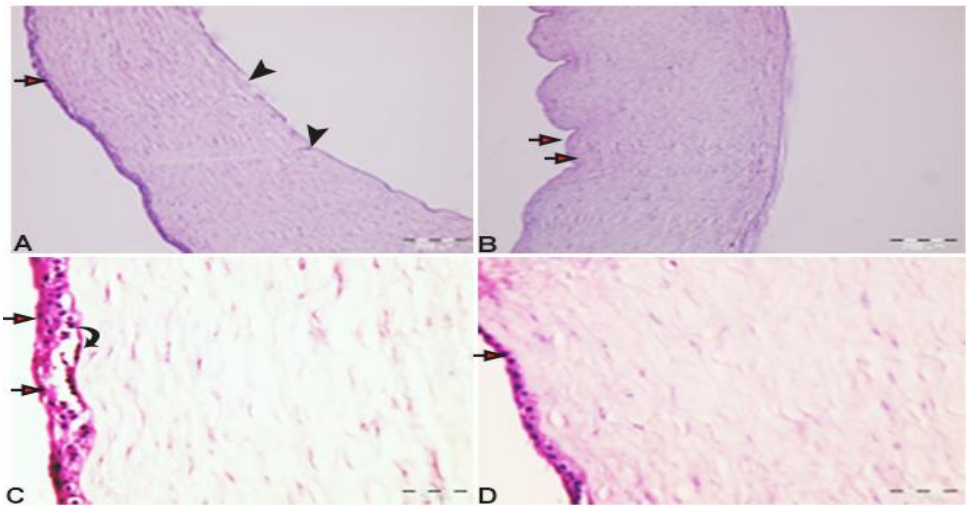

Fig. 4: Photomicrograph of sections from the central part of the cornea of **control group**: showing (A-D): necrosis and detachment of corneal epithelium (red arrowhead), Descemet's membrane separated from the stroma (black arrows). (B): complete loss of part of epithelium (corneal ulcer) (red arrow heads). (C): irregular Bowman's layer (black arrow). (H&E stain, the bar size was indicated under each picture).

Corneal sections from the central part of the cornea of control group showed necrosis and complete loss of part of the epithelium (corneal ulcer), abnormal architecture of the corneal tissue with reduced corneal thickness was noticed. Additionally, epithelial vacuolations were seen and some epithelial cells exhibited dark nuclei. The substantia propria appeared wide with thin collagen bundles in the upper half and compacted bundles in lower half and the Descemet's membrane was separated from the corneal stroma and the endothelium **(Figure.5)**

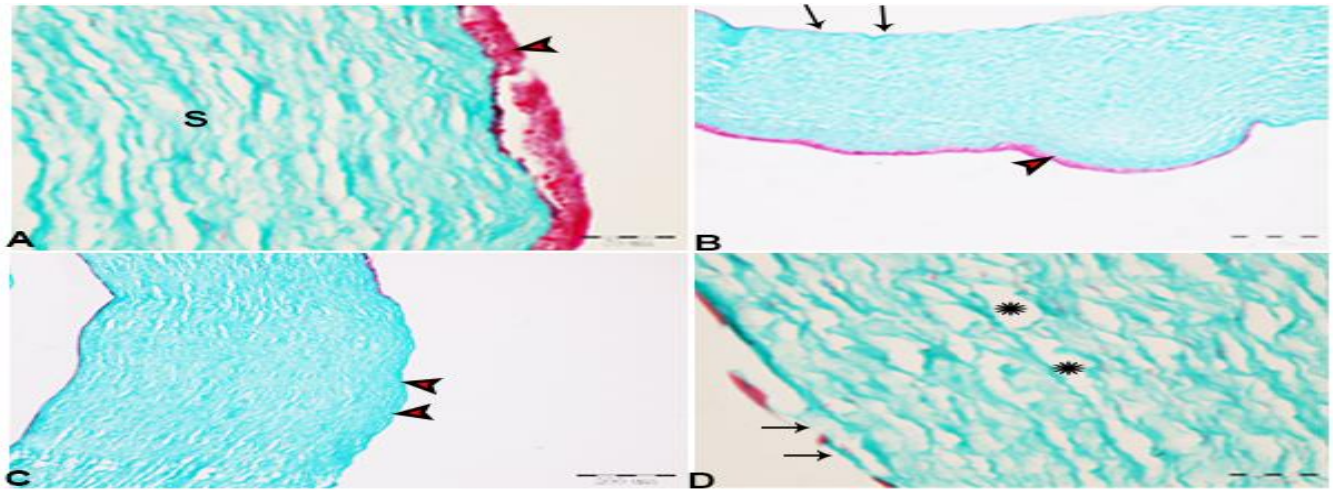

Fig. 5: Photomicrograph of sections from the central part of the cornea of **control group**: showing (A): necrosis and detachment of corneal epithelium (red arrowhead), (B): reduced corneal thickness with abnormal architecture (red arrowhead). (C): complete loss of part of epithelium (corneal ulcer) (red arrow heads). (D): widely separated collagen bundles of substantia propria (S) (Stars), Descemet's membrane separated from the stroma (black arrows). (Masson's trichrome, the bar size was indicated under each picture)

Group III (PRP subconjunctival treated)

Histopathological sections from the central part of the cornea from *left eye of group I* showing corneal epithelium restored its complete normal thickness. Normal substantia propria with regular arrangements of collagen bundles without any gabs. Normal intact Descemet's membrane and endothelial cells (**Figure. 6**)

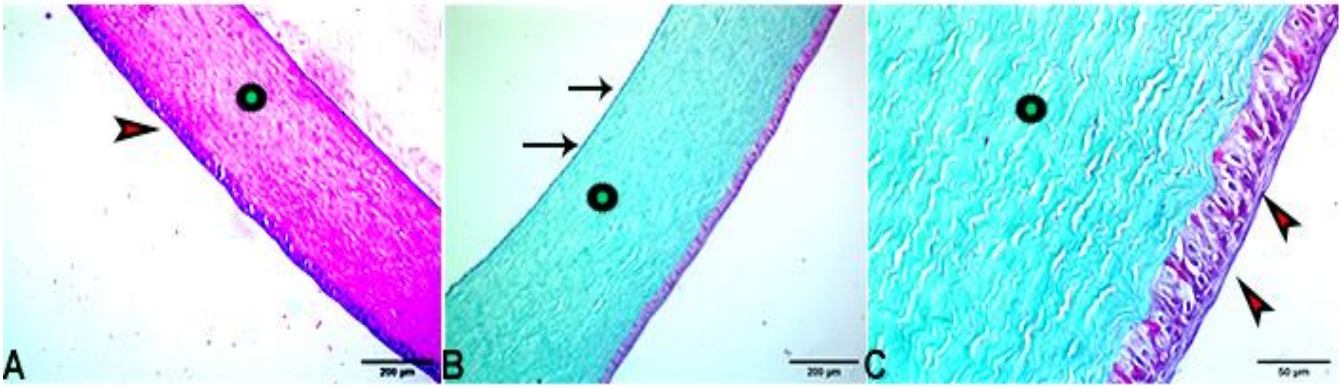

Fig.6: Photomicrograph of sections from the central part of the cornea from left eye of Group I treated with PRP by subconjunctival injection showing corneal epithelium restored its complete normal thickness (red arrow heads), normal substantia propria (star), Descemet's membrane and endothelial cells are in normal intact (black arrows) (A, H&E stain, B-C, Masson's trichrome stain the bar size was indicated under each picture).

Group IV (PRP drops treated)

Histopathological pictures of corneal sections from the central part of the cornea from *left eye of group II* showed corneal epithelium starts to restore its normal structure but still thin and shows hydropic degeneration. The substantia propria shows separated thin collagenous lamellae with a waving appearance. Degeneration of Descemet's membrane and endothelial cells **(Figure.7).**

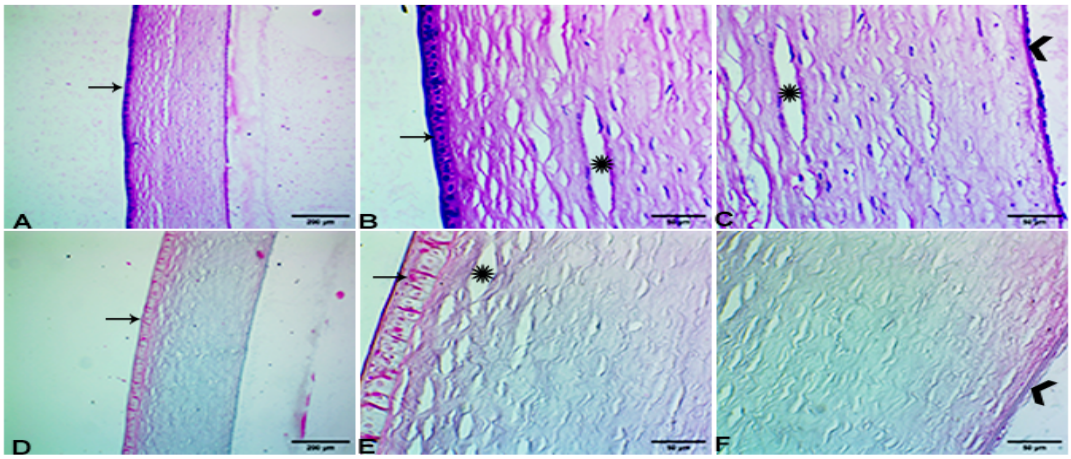

Fig. 7: Photomicrograph of sections from the central part of the cornea from left eye of Group II treated with PRP drops showing: corneal epithelium starts to restore its normal structure but still thin and shows hydropic degeneration (black arrows). The substantia propria shows separated thin collagenous lamellae with a waving appearance (stars). Descemet's membrane and endothelial cells are degenerated (black arrow heads). (A-C, H&E stain, D-F, Masson's trichrome stain the bar size was indicated under each picture).

DISCUSSION

Corneal ulcer (epithelial defects) is one of the greatest problems in the practice of the ophthalmology. It may result from mechanical trauma, immunological defect and infections, burns by chemical materials, neurogenic, basement membrane disorders and corneal epithelial **(Jassim *et al.*,, 2020).**

In our study, according to (Jassim *et al.*,, 2020; Zheng *et al.*,2019; Abdelwahab *et al.*, 2017;Khaksar *et al.*, 2013; Ye *et al.*,, 2006; Klang *et al.*, 1999), we used sodium hydroxide as alkaline chemical to induce corneal ulcer model in rabbits as the direct contact of NAOH may

cause severe irritation to the skin, mucous membranes and eye. However, some authors used alcohol for ulcer induction (Acosta *et al.,* (2014). Others used trephine machine in a rabbit model (Tanidir *et al.,* (2010).

Researchers have related the burning effect of NaOH to the anion (hydroxyl) group that causes saponification of the fat and lipids, resulting in tissue softening, followed by increasing the penetration ability of the cation chemicals (Mashige,2015). While other researchers (He et al,2006; Lee et al,2013) attributed the deleterious effect of alkali on cornea to its strong inflammatory reaction which is characterized by cell infiltration and production of proteolytic enzymes, cytokines oxidative derivatives that could cause severe loss of the extracellular matrix.

PRP refers to the concentration of many platelets in a small volume of plasma, at least 1.0×10^6 cells/μL, resulting in the 3 to 5-fold enrichment of growth factors (Yun *et al.,* 2016). Platelets play pivotal roles in both hemostasis and wound healing, because of the presence of granules that release growth factors. These growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-ß (TGF-ß), vascular endothelial growth factor, basic fibroblastic growth factor, and epidermal growth factor (Pelletier *et al.*,2013) could further stimulate other cells to migrate into the injured area and facilitate the healing process. PRP is a well-known angiogenic, anti-inflammatory, and anticatabolic agent (EI-Sharkawy *et al.*,2007; Bendinelli *et al.*,2010).

It should be noted that the blood sample in our investigation had to be collected intracardiacally as (Gimeno *et al.*,, 2006) because we were not able to avoid blood coagulation by using different puncture sites, such as the ear or femoral vein. However, some authors collect blood from ear vein as (Acosta *et al.,* 2014) and from jugular vein as (Yang *et al.*,, 2018).

In the present study, the animals showed an average whole blood platelet count of 392.667 platelets per microliter, which is within the normal range for the animal model used (Efeoglu, *et al.*,2004). Normally, platelet counts of New Zealand White rabbits vary from 250,000 to 750,000, and hematocrit values are 36% to 48% (Demirdöven, et al,2002).We used double centrifugation protocol to produce a "therapeutic PRP" according to (Khaksar *et al.*,, 2013; Ariede *et al.*,, 2015).

In addition to the number of centrifugations, there are several important factors that should be considered regarding the PRP preparation method chosen. The force of gravity (G) used in the centrifugation process is one. An increase in G may result in higher platelet concentrations (Man et al,2001).

In the present study, blood centrifugation at (4000rpm) provided a significantly higher platelet concentration. However, it is important to remember that increasing the G used for the centrifugation may prematurely activate the platelets (Dugrillon *et al.*,2001)

PRP was prepared using a small blood volume (10 ml). The total amount of blood that can be collected from an adult New Zealand rabbit without risking its life is limited to 15 ml. (Efeoglu et al,2004) Therefore, the amount of blood collected in this study allowed autologous PRP preparation without causing any systemic problems. New Zealand rabbits are also easy to obtain and can be cared for at affordable prices. This makes them a favorable alternative in experimental studies investigating the biological effects of PRP. (Efeoglu et al,2004)

Acosta *et al.,* 2014 reported that, the administration of one PRP drop exhibited increase corneal epithelium healing rate and ulcer regeneration in rabbits instilling topical PRP is evident and, even though the objective was not to assess the response of the rabbits to different therapies, symptom improvement was observed.

corneal specimens of group III treated by one-shot of subconjunctival injection showed a great healing rate of corneal epithelium higher than those that are treated with PRP drops which corneal epithelium restored its complete normal thickness. Normal substantia propria with regular arrangements of collagen bundles without any gabs. Normal intact Descemet's membrane and endothelial cells.

Researchers have reported similar findings after using a single dose of subconjunctival PRP (sPRP) injection to investigate its effect on corneal epithelial wound healing in a rabbit model (Tanidir *et al.,* (2010).

Tandir *et al.,* (2011) investigated the effect of single dose sPRP injection treatment on corneal epithelial wound healing in a rabbit model and

reported that sPRP seems to improve corneal epithelial wound healing.

Histopathological investigation revealed significantly less inflammation and vascularization in corneas in group III. Additionally, group III had significantly better stromal collagen arrangement and increased numbers of epithelial rows like results from other studies.

The result of this study showed that the subconjunctival application of autologous platelet-rich plasma on corneal alkali burn ulcer is a simple and an economic treatment for ocular surface burns, free of undesirable side effects. It is a safe and easily producible material containing several mediators such as growth factors, which are needed in wound healing. PRP seems to be a promising agent for clinical use in the epithelial wound healing process.

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