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Comparative efficacy of different sources of platelet-rich plasma on cutaneous wound healing in diabetic rabbit models

Eficacia comparativa de diferentes fuentes de plasma rico en plaquetas en la cicatrización de heridas cutáneas en conejos diabéticos

Muhammad Waqas[1]*, İhsan Keleş[2]

[1] Erciyes University, Institute of Health Sciences, Department of Veterinary Internal Medicine, Kayseri, Türkiye.
[2] Erciyes University, Faculty of Veterinary Medicine, Department of Veterinary Internal Medicine, Kayseri, Türkiye.
Corresponding author: muhammad.waqas@uvas.edu.pk

ABSTRACT

Therapeutic effects of varied-source Platelet-Rich Plasma gel on diabetic rabbit 4 cm² squared full-thickness cutaneous wounds were assessed. Thirty New-Zealand Rabbits (mean weight: 2.63 ± 0.35 kg; age range: 12-18 weeks) were divided into five groups of six each. Heterologous Platelet-Rich Plasma was sourced from a male Akkaraman sheep (age: 1 year; weight: 50 kg). Diabetes induction: Alloxan monohydrate (100 mg/kg, 2 doses, 48h apart). Regulation: Recombinant DNA human insulin. Baseline blood glucose: 100.5 ± 6.5 mg/dL; post-alloxan (5d): 336.9 ± 51.9 mg/dL (P = 0.000). Double centrifugation and $CaCl_2$ gelation produced PRP with 3.5 times baseline platelet concentration. Post-wounding clinical parameters, morphometric and morphologic evaluations were done at 0th, 3rd, 7th, 10th, 14th, 17th, 21st, and 25th days. Histopathology was performed on day 25. Acceleration in wound healing was observed in all Platelet-Rich Plasma treated (i.e. Diabetic Autologous-Platelet-Rich Plasma, Diabetic Homologous-Platelet-Rich Plasma, Diabetic Heterologous-Platelet-Rich Plasma) groups, meanwhile in both control groups, delayed wound healing was detected, especially in the Diabetic Rabbit Normal Saline group. Enhanced wound contraction observed in all Platelet-Rich Plasma groups (60 % by day 14th) vs. controls (Healthy Rabbit Normal Saline & Diabetic Rabbit Normal Saline: 56 % on days 14th & 17th respectively). These values later on day 21st and day 25th reached up to 95.72 ± 3.74 % and 99.23 ± 0.71 % for Diabetic Autologous-Platelet-Rich Plasm; 92.44 ± 5.07 % and 96.04 ± 3.70 % for Diabetic Homologous-Platelet-Rich Plasm; 89.37 ± 12.24 % and 97.32 ± 3.12 % for Diabetic Heterologous-Platelet-Rich Plasm; while 76.96 ± 15.04 % and 84.33 ± 9.29 % for Diabetic Rabbit Normal Saline and 84.91 ± 17.30 % and 92.27 ± 14.72 % for Healthy Rabbit Normal Saline; (P = 0.036 & P = 0.019 respectively). Histopathologically Platelet-Rich Plasma augmented the wound healing cascade, with early angiogenesis, healthy granulation, and sufficient re-epithelialization. Regardless of the Platelet-Rich Plasma source, it modulates and accelerates cutaneous wound healing in diabetic rabbit models, highlighting its potential as a valuable adjunct therapy for wound healing in diabetic patients.

Key words: Platelet-rich plasma; diabetic wound healing; rabbits; regenerative therapy; histopathology.

RESUMEN

Se evaluaron los efectos terapéuticos del gel de plasma rico en plaquetas de diferentes fuentes en heridas cutáneas de espesor total de 4 cm² en conejos diabéticos. Treinta conejos New Zealand (peso medio: 2.63 ± 0.35 kg; edad: 12–18 semanas) fueron divididos en cinco grupos de seis animales cada uno. El plasma rico en plaquetas heterólogo se obtuvo de una oveja Akkaraman macho (edad: 1 año; peso: 50 kg). Inducción de la diabetes: monohidrato de aloxano (100 mg/kg, 2 dosis, con 48 h de intervalo). Regulación: insulina humana recombinante. Glucemia basal: 100.5 ± 6.5 mg/dL; post-aloxano (5 días): 336.9 ± 51.9 mg/dL (P = 0.000). La doble centrifugación y la gelificación con $CaCl_2$ produjeron plasma rico en plaquetas con una concentración plaquetaria 3.5 veces superior a la basal. Los parámetros clínicos post-lesión, así como las evaluaciones morfométricas y morfológicas, se realizaron en los días 0, 3, 7, 10, 14, 17, 21 y 25. El análisis histopatológico se realizó el día 25. Se observó una aceleración de la cicatrización en todos los grupos tratados con plasma rico en plaquetas (autólogo diabético-plasma rico en plaquetas, homólogo diabético-plasma rico en plaquetas, heterólogo diabético-plasma rico en plaquetas), mientras que en ambos grupos control se detectó cicatrización retardada, especialmente en el grupo de conejos diabéticos tratados con solución salina. Se observó una mayor contracción de la herida en todos los grupos plasma rico en plaquetas (60 % al día 14) en comparación con los controles (conejo sano con solución salina y diabéticos tratados con solución salina: 56 % en los días 14 y 17, respectivamente). Estos valores alcanzaron posteriormente, en los días 21 y 25, hasta 95.72 ± 3.74 % y 99.23 ± 0.71 % para autólogo diabético-plasma rico en plaquetas; 92.44 ± 5.07 % y 96.04 ± 3.70 % para homólogo diabético-plasma rico en plaquetas; 89.37 ± 12.24 % y 97.32 ± 3.12 % para heterólogo diabético-plasma rico en plaquetas; mientras que fueron 76.96 ± 15.04 % y 84.33 ± 9.29 % para diabéticos tratados con solución salina y 84.91 ± 17.30 % y 92.27 ± 14.72 % para conejo sano con solución salina (P = 0.036 y P = 0.019, respectivamente). Desde el punto de vista histopatológico, el plasma rico en plaquetas potenció la cascada de cicatrización, con angiogénesis temprana, tejido de granulación adecuado y reepitelización suficiente. Independientemente de la fuente de plasma rico en plaquetas, este modula y acelera la cicatrización de heridas cutáneas en modelos de conejos diabéticos, destacando su potencial como una terapia complementaria valiosa para la cicatrización de heridas en pacientes diabéticos.

Palabras clave: Plasma rico en plaquetas; cicatrización de heridas; conejos diabéticos; medicina regenerativa; histopatología.

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INTRODUCTION

Chronic wounds associated with diabetes mellitus remain a major therapeutic challenge in both human and veterinary medicine. Impaired vascularization and delayed epithelial regeneration result in prolonged healing time and frequent infection. Platelet-rich plasma (PRP), as a biological product rich in growth factors, has recently been explored as an adjunct therapy to accelerate tissue repair. To achieve a functional and aesthetically satisfactory wound healing; the body mobilizes its extracellular matrix components to bring about rapid wound closure [1].

Prolonged wound healing impairs the general condition of the patient and increases the risk of infection. Diabetes disrupts the normal wound healing cascade due to chronic inflammation, hyperglycemia, autonomic and sensory neuropathy, micro- and macro-circulatory dysfunction, hypoxia, and impaired neuropeptide signaling. Therefore, several adjunctive therapies have emerged, including extracellular matrix proteins, bio-engineered skin substitutes, PRP, growth factors, and negative pressure wound therapy [2].

The diabetic rabbit model induced by alloxan monohydrate is an easily reproducible model, cheap in price and offers the advantage of providing a non-contractile and an avascular wound bed. Furthermore, diabetic rat models exhibit rapid wound contraction and thus do not reflect proper epithelial migration [3].

There is a need for therapies that improve wound healing in diabetic patients. Platelets contribute to healing through hemostasis, angiogenesis, cell proliferation, collagen production, cell differentiation, and tissue regeneration [4].

Platelet granules release growth factors, which are polypeptides with molecular weights of approximately 6–45 kilodaltons. The α-granules include platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), and epidermal growth factor (EGF). These polypeptide growth factors regulate cellular functions, promote cell proliferation, and stimulate soft and hard tissue regeneration. They bind to specific receptors on target cells, initiating protein synthesis and collagen production. Thus, concentrated platelets enhance tissue regeneration by increasing local growth factor levels [5].

Autologous PRP use is limited in patients with chronic diseases due to risks such as thrombocytopenia and repeated blood sampling [1]. In small animal practice, sufficient blood collection is also difficult [6]. Therefore, homologous PRP (same species) or heterologous PRP (different species) is more practical. The objective of this study was to compare these three PRP sources in terms of wound healing capacity.

MATERIALS AND METHODS

This study was conducted at the Faculty of Veterinary Medicine, Erciyes University, DEKAM, Kayseri, Türkiye, in accordance with institutional ethical standards (HADYEK No: 21/161, 07.07.2021).

Experimental animals and grouping

A total of 34 healthy male New Zealand rabbits (*Oryctolagus cuniculus*), aged 12–18 weeks and 2.63 ± 0.35 kg, were maintained under standard conditions with ad libitum feed and water, housed in 80 × 60 × 40 cm cages, supplemented with alfalfa (*Medicago sativa*) or timothy (*Phleum pratense*) hay, and kept under controlled lighting (i.e. 12 hours light / 12 hours dark) and temperature (23 ± 1º C). A 1-year-old, 50 kg male Akkaraman sheep (*Ovis aries*) used for heterologous PRP was housed in a 20 × 18 × 11 ft enclosure and fed on barley (*Hordeum vulgare*), wheat bran (*Triticum aestivum*), and alfalfa (*Medicago sativa*). After adaptation, rabbits were divided into 5 groups (n = 6 each), with sample size calculated using G-Power (f = 0.60, α = 0.05, Power (1-β) = 0.80, groups = 5, repetitions = 6): Healthy Rabbit Normal Saline (HR-NS), Diabetic Rabbit Normal Saline (DR-NS), Diabetic Autologous PRP (DR-AuPRP), Diabetic Homologous PRP (DR-HoPRP), Diabetic Heterologous PRP (DR-HePRP), plus Blood Donor Rabbits (n = 4).

Induction of diabetes in rabbits

Alloxan monohydrate (Sigma Aldrich Chemical, Saint Louis, MO, USA) was dissolved in sterile normal saline to prepare a 5 % (W/V) solution over an ice block. Diabetic rabbits received two intravenous doses of 100 mg/kg via the marginal ear vein over 2 minutes (min) using a 25-gauge butterfly catheter, 48 hours (h) apart, while non-diabetic rabbits received normal saline. To prevent hypoglycemia, alloxan was administered to non-fasted rabbits, followed by 10 mL of 5 % glucose subcutaneously at 0^{th}, 4^{th}, 8^{th} and 12^{th} hours, and 20 % glucose in drinking water for 1–2 days (d). Rabbits with blood glucose levels above 250 mg/dL after 2 d were considered diabetic [7].

Blood glucose monitoring and insulin administration

All rabbits had baseline blood glucose measured before alloxan administration, and diabetic rabbits were then monitored daily at the same time using a hand-held glucometer (Accu-Check Active - Roche, Switzerland). Recombinant human insulin (Humulin R 100 IU/mL – Lilly, USA) was administered subcutaneously when glucose exceeded 300 mg/dL: 1 U/kg for 300–400 mg/dL, 2 U/kg for 400–500 mg/dL, 3 U/kg for 500–600 mg/dL (repeat half-dose in the evening), and 4 U/kg for > 600 mg/dL (repeat half-dose in the evening) [7].

Preparation of platelet-rich plasma

Autologous PRP was prepared from each diabetic rabbit and applied to its own wounds, while homologous PRP was obtained from healthy donor rabbits for DR-HoPRP wounds, and heterologous PRP was derived from sheep blood for DR-HePRP wounds. A total of 4 mL venous blood was collected using a vacuum system into vials containing 10 % sodium citrate anticoagulant and centrifuged (Rotofix 32-A - Hettich, Germany) at 200 g for 10 min, separating into upper yellowish layer (plasma and platelets), a whitish inter-layer buffy coat (leukocytes and larger platelets), and a lower reddish layer (erythrocytes). The plasma and 200 µL buffy coat were transferred to a sterile vial

and centrifuged again at 400 g for 10 min, with an additional 200 µL buffy coat placed in another sterile vial. After discarding the platelet-poor plasma (supernatant), 200 µL of the bottom layer (erythrocytic platelet) was added to the buffy coat and homogenized to form PRP (400 µL). For gel formation, 10 % calcium gluconate was added (400 µL of PRP – 100 µL of calcium gluconate), and the gel formed after 5–10 min at 37 ˚C (see FIG. 1), yielding approximately 0.5 mL PRP [8]. Therefore, PRP preparation used in this study was leukocyte-rich PRP (L-PRP), as the buffy coat fraction containing leukocytes was intentionally included during preparation.

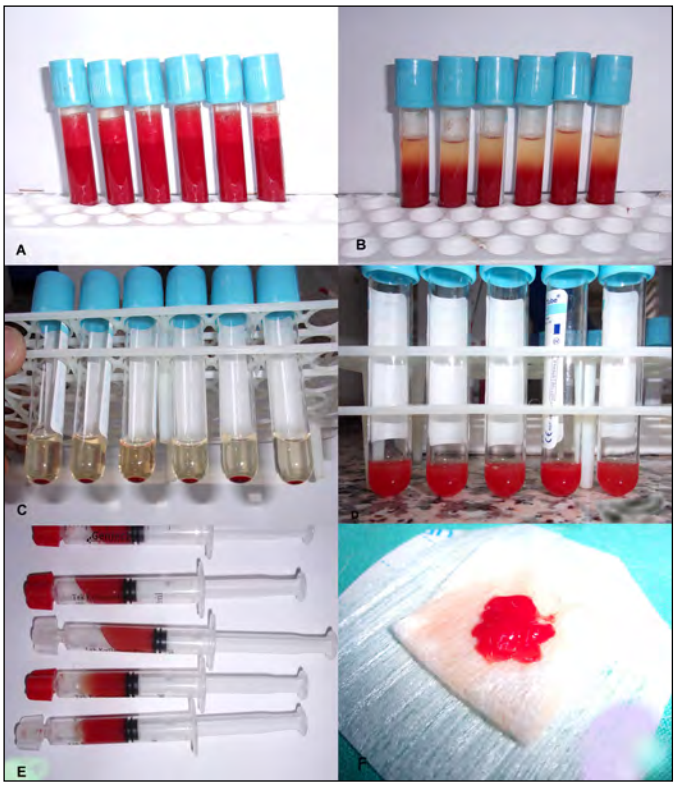

FIGURE 1. Preparation Of Platelet-Rich Plasma (PRP); (A) Fresh Blood Samples In 10 % Sodium Citrate Before Centrifugation, (B) Samples After 1st Centrifugation At 200 G For 10 Minutes, (C) After 2nd Centrifugation Of The Previous Top Layer In A Separate Vial At 400g For 10 Minutes, (D) After Removal Of Excessive Plasma And Homogenization Of The Pellet To Form Platelet Rich Plasma, (E) PRP Gel Formed Over Hot Water Bath Of 37 ˚C After Addition of 10 % Calcium Gluconate, (F) PRP Gel Dressing For Wound.

Creating a full-thickness excisional skin wound

All rabbits were anesthetized (non-fasted) before creating a full-thickness excisional skin wound using ketamine hydrochloride (HCL) (35 mg/kg; Keta-Control, Mefar Ilaç, Türkiye) and xylazine HCL (5 mg/kg; Rompun, Bayer, Germany) administered intramuscularly [9]. Animals were placed in ventral recumbency, the dorsal area was shaved and disinfected with 70 % isopropanol, and a 2 × 2 cm (4 cm²) full-thickness wound was created with a scalpel ~2.5 cm from the dorsal midline near L3–L6, removing skin with toothed forceps while preserving underlying muscles [10].

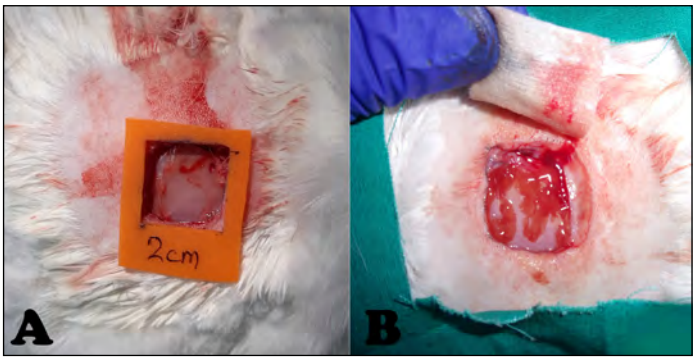

FIGURE 2. A successful squared 2 cm x 2 cm (4cm^2) full-thickness skin wound creation, along with platelet-rich plasma (PRP) gel application.

Application of platelet-rich plasma gel on skin wound

After measuring the wounds, treatment was initiated according to group (PRP—autologous, homologous, heterologous or normal saline for controls; see FIG. 2). Wounds were dressed with a 2-ply sterile sponge (Octacare, Octamed, Türkiye) and covered with cotton mesh wrap (Unipore, Seyitler Kimya, Türkiye). Dressings and treatments were applied on days 0, 3, 7, and 10 post-induction [1]. Rabbits also received Meloxicam (0.5 mg/kg SC; Bavet Meloxicam, Arion, Türkiye) for 3 d for analgesia.

Morphometric and morphologic evaluation

During the study period, rabbits were weighed (kg) with a digital electronic scale (WS20 - Olba, Holland) on the 0th, 3rd, 7th, 10th, 14th, 17th, 21st and 25th d. Clinical parameters like temperature (˚C), pulse (BPM) and respiration (BPM) were also recorded. The wounds were photographed with a 16 MP digital camera (FZ53 – Kodak, China). There photos were used later in Image J software for wound area measurements [10]. In addition, manual measurements were also made using a digital vernier calipers (11963 – Green, China). Calculation of the percent wound contraction (Pc) was done using formula of Ribeiro *et al*. [11], Pc = (Af – Ai) / Ai × 100, where Ai = initial area of the wound (G0) and Af = end area of the wound (GX).

The events listed below were observed and their clinical features were recorded and graded according to the wound clinical parameter scoring system as follows : Pain Sensitivity (0 = None / 1 = Yes), Scar Staining (1 = Pinkish / 2 = Yellowish / 3 = Pale / 4 = Cyanotic), Edema (0 = Absent / 1 = Present), Hyperemia (0 = Absent / 1 = Present), Exudate (0 = Absent / 1 = Present), Nature of Exudate (1 = Serous / 2 = Bloody / 3 = Purulent), Crust and Granulation Tissue (0 = None / 1 = Crust / 2 = Granulation) [1]. All these observations converted into scores were subjected to non-parametric data analysis.

Histopathological evaluation

On the 25th d of the study, an excisional skin biopsy of the entire wound surface (i.e. 400 mm2) was performed under general anesthesia before any of the wounds were closed or healed completely. Skin sample cassettes were then fixed for 24 h in freshly prepared 10 % buffered formaldehyde solution [contents; 100 mL Formalin (37-40 % stock solution) with

900 mL Water and 4 g/L NaH_2PO_4 (monobasic) and 6.5 g/L Na_2HPO_4 (dibasic/anhydrous)]. The histopathology slides of the excisional skin tissue biopsies were made by service procurement from the Erciyes University Medical Faculty Central Laboratory. Histopathology slides were prepared at Erciyes University Medical Faculty Central Laboratory. Sections (5 µm; Rotary Microtome HM325 – Epredia, Germany) were stained with Hematoxylin-Eosin and examined under a light microscope (Primostar - Carl Zeiss, Germany) by a pathologist who was kept blind to the groups. The following parameters were evaluated: Fibrin-Leukocyte Sheath (0 = None / 1 = Present), Amount of Fibroblast and Collagen Fibers (0 = High / 1 = Moderate / 2 = Less / 3 = None), Neovascularization (0=Intense / 1 = Moderate / 2 = Discrete / 3 = None), Inflammatory process (0 = No Inflammation / 1 = Mild Inflammation / 2 = Moderate Inflammation / 3 = Severe Inflammation), Inflammatory Cell Type (0 = Few Both Present / 1 = Neutrophils / 2 = Lymphocytes / 3 = Mixed/Both Present), Macrophage Concentration (0 = None / 1 = Discrete / 2 = Moderate / 3 = Dense), Acanthosis (0 = None / 1 = Discrete; < 25 % of skin sample piece affected / 2 = Moderate; 25 – 50 %, 3 = Highlight/Prominent; > 50 % of skin sample piece affected), Re-epithelialization (0 = Total / 1 = Partial / 2 = Less / 3 = None) [1].

Statistical analysis

Data normality was assessed using the Shapiro–Wilk test, histograms, and Q–Q plots, while homogeneity of variances was evaluated with Levene's test. Parametric data were analyzed using one-way ANOVA and repeated-measures two-way ANOVA, whereas nonparametric data were evaluated using the Kruskal–Wallis and Friedman tests. Paired sample t-tests were used for comparisons between two dependent variables within a group. Multiple comparisons were performed using Tukey and Bonferroni tests. Parametric data were expressed as mean ± standard deviation, and nonparametric data as median (Q1–Q3). Statistical analyses were conducted using IBM SPSS Statistics version 25 (SPSS Inc., Chicago, IL, USA), with $P < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

General observations

All animals tolerated the experimental procedures well. No mortality or major adverse reaction occurred. Physiological parameters such as body weight, temperature, respiration, and pulse remained within normal limits throughout the study period.

Blood glucose levels

There was a significant difference in the blood glucose levels for all the diabetic group rabbits before (Mean = 100.5, SD = 6.5; mg/dL) and after (Mean = 336.9, SD = 51.9; mg/dL) alloxan monohydrate administration; t (23) = 22.08, P = 0.000. Blood glucose levels of all the groups throughout the experimental period are summarized in TABLE I.

Wang *et al.* [12] successfully induced diabetes in rabbits within one week using a single intravenous dose of 5% alloxan monohydrate (100 mg/kg), producing blood glucose levels >350 mg/dL, after which insulin therapy was initiated. Similarly, Pradhan *et al.* [13] induced diabetes using two doses of alloxan monohydrate (50 mg/kg IV) 48 h apart, achieving 250 mg/dL after 10 d. Although many studies have used a single alloxan administration to induce diabetes in rabbits, variable induction success and incomplete β-cell destruction have also been reported. Wang *et al.* [7] observed that approximately 60% of rabbits required a second alloxan administration to achieve stable hyperglycemia. Similarly, Hadour *et al.* [14] reported that while a single 100 mg/kg dose is less toxic, it may not reliably induce a stable diabetic model. Therefore, a second dose (100 mg/kg) was administered to ~86 % of rabbits in the present study to establish a sustained hyperglycemia suitable for wound-healing evaluation. While the remainder ~14 % of rabbits did not require a second dose. All rabbits survived until the end with insulin supplementation.

Alloxan produces a biphasic glucose response [15], with initial hyperglycemia within two hours due to hepatic glycogen breakdown [16], possibly linked to epinephrine release, followed

TABLE I

Blood-glucose levels of diabetic and control rabbits throughout the 25 days

Groups	0th DAY Median (Q1, Q3) (mg/dL) (N = 30)	3rd DAY Median (Q1, Q3) (mg/dL) (N = 30)	7th DAY Median (Q1, Q3) (mg/dL) (N = 30)	10th DAY Median (Q1, Q3) (mg/dL) (N = 30)	14th DAY Median (Q1, Q3) (mg/dL) (N = 30)	17th DAY Median (Q1, Q3) (mg/dL) (N = 30)	21st DAY Median (Q1, Q3) (mg/dL) (N = 30)	25th DAY Median (Q1, Q3) (mg/dL) (N = 30)	P-value
HR-NS	112.5 (106.5 – 116.5) ^{A}	103.5 (91.8 – 118.0) ^{A}	99.0 (86.0 – 108.0) ^{A}	103.0 (91 – 118.3) ^{A}	109.0 (89.0 – 119.8) ^{A}	100.0 (84.3 – 107.3) ^{A}	106.0 (98.5 – 116.3) ^{A}	99.0 (91.8 – 110.8) ^{A}	0.417
DR-NS	293.5 (257.3 – 428.0) ^{AB}	294.0 (254.0 – 429.5) ^{AB}	340.0 (284.3 – 439.3) ^{B}	322.5 (283.8 – 467.8) ^{B}	341.0 (307.3 – 389.8) ^{B}	345.5 (276.5 – 432.0) ^{B}	321.5 (275.3 – 367.5) ^{B}	314.0 (285.8 – 347.5) ^{B}	0.675
DR-AuPRP	380.0 (333.8 – 455.5) ^{B}	337.5 (273.5 – 388.3) ^{B}	342.0 (280.8 – 431.0) ^{B}	296.5 (267.5 – 342.3) ^{AB}	352.5 (319.8 – 370.8) ^{B}	313.0 (293.0 – 340.0) ^{B}	312.0 (283.0 – 410.0) ^{B}	346.5 (309.0 – 384.0) ^{B}	0.612
DR-HoPRP	272.5 (253.8 – 332.8) ^{ABab}	306.0 (264.8 – 357.3) ^{ABab}	281.0 (268.0 – 313.8) ^{ABab}	337.5 (306.0 – 396.8) ^{Bb}	292.5 (279.3 – 406.8) ^{ABab}	285.0 (272.5 – 323.3) ^{ABab}	307.0 (287.3 – 352.8) ^{ABab}	282.0 (263.0 – 304.3) ^{ABa}	0.032
DR-HePRP	503 (329.5 – 522.3) ^{B}	362.5 (289.5 – 426.3) ^{B}	358.0 (286.8 – 486.0) ^{B}	369.0 (270.3 – 464.3) ^{B}	411.5 (306.0 – 447.3) ^{B}	322.5 (283.3 – 417.3) ^{B}	338.0 (278.5 – 429.3) ^{B}	329.5 (284.5 – 401.3) ^{B}	0.260
P-value	0.001	0.004	0.002	0.004	0.003	0.004	0.006	0.001	

Significance Level $P < 0.05$, which is expressed in rows with small alphabets and in columns as capital alphabets. Same alphabets indicate no statistical significance while different alphabets indicate statistical significance. Data has been computed and expressed as Median (Quartiles 1 and 3). Healthy Rabbit Normal Saline Group (HR-NS; n = 6), Diabetic Rabbit Normal Saline Group (DR-NS, n = 6), Diabetic Autologous Platelet-Rich Plasma Group (DR-AuPRP, n=6), Diabetic Homologous Platelet-Rich Plasma Group (DR-HoPRP, n = 6), Diabetic Heterologous Platelet-Rich Plasma Group (DR-HePRP, n=6).

by severe hypoglycemia that may cause mortality if untreated [17]. To prevent this, rabbits received 10 mL of 5 % dextrose subcutaneously at 4-h intervals (≥ 4 times), and drinking water was replaced with 20 % glucose for 1–2 d. Hypoglycemia typically occurs after ~6 h due to β-cell destruction [18] and is more pronounced in fasted animals [19]; therefore, non-fasted rabbits were used.

No statistically significant differences were observed in alloxan-induced hyperglycemia among diabetic groups, indicating equal hyperglycemic status. All diabetic rabbits showed polyuria and polydipsia, while polyphagia was not categorically observed; however, all rabbits exhibited progressive weight gain with insulin supplementation, maintaining blood glucose > 250 mg/dL over 24 h, consistent with Wang *et al.* [7].

Platelet-rich plasma concentration

There was a significant increase in platelet concentrations after centrifugation for PRP in DR-AuPRP (before: Mean = 449.5, SD = 99.1; after: Mean = 1361.2, SD = 298.1 ×10⁹/L; t(23) = 19.56, P = 0.000; increase from baseline: Mean = 3.05 ± 0.33 folds), DR-HoPRP (before: Mean = 357.8, SD = 29.4; after: Mean = 1135.3, SD = 144.1 ×10⁹/L; t(23) = 15.85, P = 0.000; increase from baseline: Mean = 3.18 ± 0.40 folds), and DR-HePRP (before: Mean = 465.6, SD = 56.7; after: Mean = 1534.5, SD = 204.5 ×10⁹/L; t(23) = 19.95, P = 0.000; increase from baseline: Mean = 3.30 ± 0.14 folds). Approximately 0.5 mL of PRP gel was applied uniformly to each 4 cm² wound at every treatment session (days 0, 3, 7 and 10), corresponding to approximately 0.125 mL/cm² of wound area.

Froum *et al.* [20] reported PRP platelet increases of 3–8× baseline, while L Alio *et al.* [21] observed therapeutic effects at 1.6–2.5×. Kim *et al.* [22] achieved good responses with only 1.5× increases, whereas Marx [23] defined 3–5× as a clinical benchmark for healing. Nagata *et al.* [24] achieved higher platelet concentrations with two-step centrifugation (4.25×) versus one-step (1.8×) using 5 mL rabbit blood. Accordingly, this study used two-step centrifugation (200 g/10 min; 400 g/20 min) to prepare 0.5 mL PRP, achieving up to 3.5× baseline from 4 mL blood. Oliveira-Filho *et al.* [25] obtained 1 mL PRP with ≥ 5.3× using 10 mL blood. Jee *et al.* [26] produced 0.5 mL PRP with 8.5× from 60 mL dog blood, while DeRossi *et al.* [27] achieved 4× from 10 mL Badis and Omar [28] reported ~3× using 4 mL sheep blood. Thus, final platelet count depends on initial blood platelet levels [1].

Based on the inclusion of the buffy coat during preparation, the PRP used in this study can be classified as leukocyte-rich PRP (L-PRP). The buffy coat contains leukocytes and larger platelets, which may enhance antimicrobial activity, regulate inflammation, and support tissue repair through additional cytokine release. A similar buffy coat–based PRP preparation was reported by Nagata *et al.* [24] and Abegão *et al.*

Wound-healing progression

All PRP-treated diabetic group wounds exhibited earlier granulation tissue formation and faster re-epithelialization than normal saline treated healthy and diabetic controls. Results are summarized below in TABLES II and III.

Platelet-rich plasma has been widely used in orthopedic, bone graft, diabetic, burn, corneal wound healing, and tendon repair studies [29, 30, 31, 32, 33, 34, 35], and can be applied directly to wounds [36]. As evident from FIG. 3 below; macroscopically DR-AuPRP wounds showed faster and more organized re-epithelialization than DR-NS wounds, consistent with previous findings [37, 38].

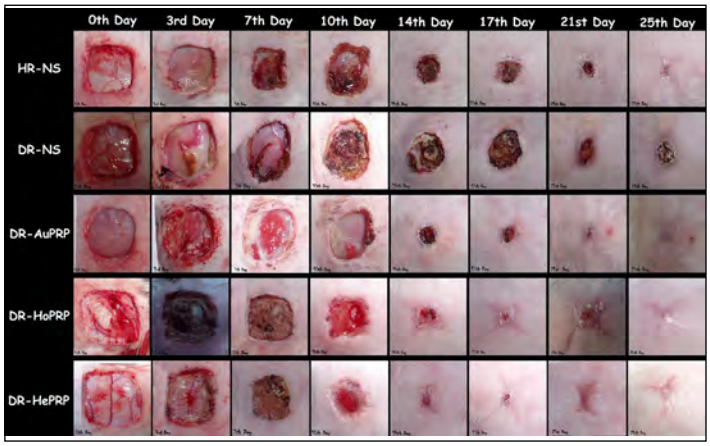

FIGURE 3. Macroscopic wound-healing progression in all treatment group rabbits Healthy Rabbit Normal Saline Group (HR-NS; n = 6), Diabetic Rabbit Normal Saline Group (DR-NS, n = 6), Diabetic Autologous Platelet-Rich Plasma Group (DR-AuPRP, n = 6), Diabetic Homologous Platelet-Rich Plasma Group (DR-HoPRP, n = 6), Diabetic Heterologous Platelet-Rich Plasma Group (DR-HePRP, n = 6).

TABLE II

Percentage wound-contraction in different treatment groups throughout the 25 days

Groups	0th DAY (Mean ± St. Dev.) (N = 30) (%)	3rd DAY (Mean ± St. Dev.) (N = 30) (%)	7th DAY (Mean ± St. Dev.) (N = 30) (%)	10th DAY (Mean ± St. Dev.) (N = 30) (%)	14th DAY (Mean ± St. Dev.) (N = 30) (%)	17th DAY (Mean ± St. Dev.) (N = 30) (%)	21st DAY (Mean ± St. Dev.) (N = 30) (%)	25th DAY (Mean ± St. Dev.) (N = 30) (%)	P-value
HR-NS	0 ± 0 ^{a}	8.49 ± 6.65 ^{ae}	26.67 ± 9.45 ^{ade}	35.67 ± 14.10 ^{abc}	56.04 ± 16.58 ^{abc}	70.65 ± 16.62 ^{bce}	84.91 ± 17.30 ^{cdAB}	92.27 ± 14.72 ^{bcAB}	0.000
DR-NS	0 ± 0 ^{a}	15.63 ± 9.99 ^{a}	16.00 ± 5.46 ^{a}	21.63 ± 11.26 ^{a}	30.40 ± 14.13 ^{ab}	56.42 ± 18.43	76.96 ± 15.04 ^{bA}	84.33 ± 9.29 ^{cA}	0.000
DR-AuPRP	0 ± 0 ^{a}	12.47 ± 13.88 ^{ad}	23.69 ± 9.44 ^{acd}	33.16 ± 8.31 ^{ab}	60.01 ± 18.11 ^{ab}	82.20 ± 14.81	95.27 ± 3.74 ^{bcB}	99.23 ± 0.71 ^{bB}	0.000
DR-HoPRP	0 ± 0 ^{a}	15.73 ± 11.51 ^{ad}	21.02 ± 10.57 ^{acd}	29.42 ± 10.88 ^{ab}	60.35 ± 16.31 ^{ab}	77.99 ± 15.39	92.44 ± 5.07 ^{bcAB}	96.04 ± 3.70 ^{bAB}	0.000
DR-HePRP	0 ± 0 ^{a}	17.66 ± 11.71 ^{ad}	22.66 ± 11.84 ^{acd}	36.44 ± 17.05 ^{ab}	59.73 ± 17.49 ^{ab}	77.19 ± 20.44	89.37 ± 12.24 ^{bcAB}	97.32 ± 3.12 ^{bAB}	0.000
P-value	1.000	0.589	0.366	0.333	0.051	0.175	*0.036	*0.019	

Significance Level P < 0.05, which is expressed in rows with small alphabets and in columns as capital alphabets. Same alphabets indicate no statistical significance while different alphabets indicate statistical significance. Data has been computed and expressed as Mean ± Std. Dev. Healthy Rabbit Normal Saline Group (HR-NS; n=6), Diabetic Rabbit Normal Saline Group (DR-NS, n = 6), Diabetic Autologous Platelet-Rich Plasma Group (DR-AuPRP, n = 6), Diabetic Homologous Platelet-Rich Plasma Group (DR-HoPRP, n = 6), Diabetic Heterologous Platelet-Rich Plasma Group (DR-HePRP, n = 6).

During the 25-d study, all groups showed variable pain on touch/prick; however, by d 25, pain was absent in 100 % (0/6) of DR-AuPRP and DR-HoPRP, compared to 16.6 % (1/6) in DR-HePRP and HR-NS, and 50 % (3/6) in DR-NS. The analgesic effect of PRP may result from its ability to modulate inflammation, enhance angiogenesis, and accelerate tissue repair. Growth factors released from activated platelets, including PDGF, TGF-β, and VEGF, may promote earlier resolution of inflammation and reduce nociceptive stimulation, leading to reduced wound pain, as also reported by Shao *et al.* [39].

Wound crust and healthy granulation tissue were present in all 5 groups throughout healing, consistent with Meira *et al.* [10]. Scabs were removed for repeated PRP/saline application and assessments, following Abegão *et al*. [1]. Wounds remained predominantly pinkish in all 5 groups, consistent with reports by Abegão *et al.* [1] and dos Santos *et al*. [40] and no hypergranulation was observed, in agreement with [1, 40, 10]. However, Ribeiro *et al*. [11] noted that wound location, regional mobility, recurrent trauma, altered tissue perfusion, and infection may contribute to hypergranulation.

Exudation varied across groups. HR-NS showed absent to serous/blood-tinged non-purulent exudate. DR-NS showed similar findings, except one rabbit with slight purulent exudate (d 14–25), likely due to increased susceptibility of diabetic wounds to infection [41]. PRP groups (DR-AuPRP, DR-HoPRP, and DR-HePRP), showed non-purulent exudate ranging from absent to serosanguineous, consistent with [8, 10, 42, 43]; though some previous similar studies had reported no exudation [1, 40]. The near absence of purulent exudate in PRP-treated wounds may be attributed to the antimicrobial properties of platelet-derived proteins and leukocytes present within PRP preparations. Activated platelets release antimicrobial peptides, chemokines, and other bioactive molecules capable of inhibiting microbial growth and recruiting immune cells to the wound environment, thereby potentially reducing susceptibility to wound infection [44].

The diabetic rabbit models; had no statistical difference observed in wound contraction or day-25 histopathology among PRP groups (DR-AuPRP, DR-HoPRP, and DR-HePRP), consistent with [10, 45], except on d 17 and 21 in terms of clinical wound score DR-AuPRP differed from only DR-HoPRP and both DR-HoPRP and DR-HePRP, respectively. By d 25, PRP groups showed no differences, but all differed significantly from DR-NS in terms of clinical wound scores.

In the present study, meloxicam was administered at 0.5 mg/kg SC once daily for 3 d as postoperative analgesia to all the animals to ensure laboratory animal welfare and minimize the initial pain and discomfort following wound creation. Previous rabbit wound-healing studies have used analgesics such as tramadol hydrochloride (0.5 mg/kg IM every 12 h for 3 d) [1,10, 44] or a single dose of buprenorphine hydrochloride [37]. Because all experimental groups received the same dose and duration of meloxicam, any potential anti-inflammatory effects were equally distributed and were unlikely to influence comparisons between PRP-treated and control wounds.

Histopathological findings (25th day)

There was a statistically significant difference observed in the histopathological scores among the groups with a F (4,25) = 3.29, P = 0.027. Post-hoc comparisons of the groups using the Bonferroni test indicated that the mean score only for DR-AuPRP (M = 3.7, SD = 3.2; score) was significantly different than the DR-NS (M = 11.3, SD = 3.3; score) with P = 0.015. Among all PRP treatment protocols, DR-AuPRP (M = 3.7, SD = 3.2; score) produced the most rapid wound contraction and highest histological-healing scores, followed by DR-HoPRP (M = 6.5, SD = 2.3; score) and DR-HePRP (M = 6.7, SD = 5.2; score).

Carter *et al.* [46] reported that wounds treated with PRP gel; achieve distinguishable parallelly arranged cutaneous collagen bundles; comprising of more mature granulation tissue. The characteristics of a mature granulation tissue are denser, well-organized, and more tightly arranged collagen fiber bundles. Which is evidently observable below (FIGS. 6, 7 and 8) of variously sourced PRP treated groups DR-AuPRP, DR-HoPRP

TABLE III

Wound clinical evaluation scores throughout the 25 days

Groups	0th DAY Median (1st Quartile-3rd Quartile) (N=30) (Score)	3rd DAY Median (1st Quartile-3rd Quartile) (N=30) (Score)	7th DAY Median (1st Quartile-3rd Quartile) (N=30) (Score)	10th DAY Median (1st Quartile-3rd Quartile) (N=30) (Score)	14th DAY Median (1st Quartile-3rd Quartile) (N=30) (Score)	17th DAY Median (1st Quartile-3rd Quartile) (N=30) (Score)	21st DAY Median (1st Quartile-3rd Quartile) (N=30) (Score)	25th DAY Median (1st Quartile-3rd Quartile) (N=30) (Score)	P-value
HR-NS	5 (5 – 5.25)	3.5 (2.75 - 5.25)	3 (3 - 4.75)	4 (2 - 6.5)	2.5 (2 - 4)	2.5 ^{AB} (2 - 5.5)	5 ^{A} (3 - 7)	3.5 ^{AB} (2.5 - 4.5)	0.188
DR-NS	5.5 (5 – 6)	3.5 (2.75 - 5.25)	4 (3.5 - 8.25)	4 (3 - 5.5)	4 (2 - 7)	5.5 ^{AB} (2.75 - 7.5)	4 ^{AB} (2 - 8.25)	7.5 ^{A} (5.25 - 8)	0.322
DR-AuPRP	5 ^{a} (5 – 5.25)	3.5 ^{ab} (2.75 - 5.5)	3.5 ^{ab} (2.75 - 5.75)	4 ^{ab} (2.75 - 7.25)	2.5 ^{ab} (2 - 4.5)	5.5 ^{abA} (3.75 - 7)	5 ^{abA} (3.5 - 6.25)	2 ^{bB} (1 - 2.25)	0.011
DR-HoPRP	5.5 ^{a} (5 – 6)	5.5 ^{a} (3.75 - 7)	4 ^{ab} (3.75 - 4.75)	3 ^{ab} (2 - 6.5)	3.5 ^{ab} (2 - 4.5)	2 ^{bB} (1 - 2.25)	2.5 ^{abB} (1 - 3.25)	5.5 ^{abAB} (3 - 6)	0.002
DR-HePRP	6 ^{a} (5 – 6)	4 ^{ab} (3.75 - 4.75)	3.5 ^{ab} (3 - 5.5)	3 ^{ab} (2.5 - 4.25)	2 ^{b} (1.75 - 3)	2.5 ^{abAB} (1.75 - 4.25)	2 ^{bB} (1 - 3)	4.5 ^{abAB} (3 - 6.25)	0.001
P-value	0.305	0.515	0.705	0.807	0.323	*0.012	*0.033	*0.003	

Significance Level P < 0.05, which is expressed in rows with small alphabets and in columns as capital alphabets. Same alphabets indicate no statistical significance while different alphabets indicate statistical significance. Data has been computed and expressed as Median (Quartiles 1 and 3). Healthy Rabbit Normal Saline Group (HR-NS; n = 6), Diabetic Rabbit Normal Saline Group (DR-NS, n = 6), Diabetic Autologous Platelet-Rich Plasma Group (DR-AuPRP, n = 6), Diabetic Homologous Platelet-Rich Plasma Group (DR-HoPRP, n = 6), Diabetic Heterologous Platelet-Rich Plasma Group (DR-HePRP, n = 6)

and DR-HePRP respectively. Whereas; the characteristics of a premature stage of granulation tissue deposition are thinner collagen bundles having being haphazardly organized along with numerous fibroblast cells. Which is clearly observable below in FIGS. 4 and 5 of 0.9 % saline treated control groups HR-NS and DR-NS respectively.

Groupwise histopathology results of day 25th are discussed individually as under:

Healthy rabbit normal saline group (HR-NS)

In the HR-NS group, 50 % (3/6) of wounds had an intact fibrin leukocyte crust; collagen fiber production, organization, and maturation were less in 16.6 % (1/6), moderate in 33.3 % (2/6), and high in 50 % (3/6), with neovascularization recorded as discrete in 16.6 % (1/6), moderate in 16.6 % (1/6), and intense in 66.7 % (4/6); inflammation ranged from mild in 66.7 % (4/6) to severe in 33.3 % (2/6), with predominantly lymphocytic infiltrate in 50 % (3/6), neutrophilic in 33.3 % (2/6), and mixed in 16.6 % (1/6); full epithelialization was observed in 50 % (3/6), partial in 16.7 % (1/6), and less in 33.3 % (2/6), while 33.3 % (2/6) showed discrete acanthosis and 67.7 % (4/6) showed none.

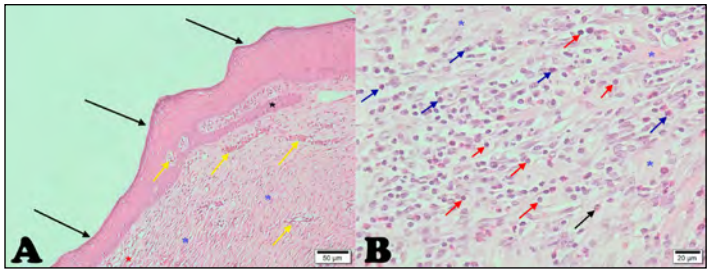

FIGURE 4. HR-NS group, day 25 histopathology (H&E). (a) re-epithelialization (black arrow), neovascularization (yellow arrows), new connective tissue (blue stars), acanthosis (black stars), and mild lymphocytic inflammation (red star). (b) mixed inflammatory infiltrates: neutrophils (red arrows), eosinophil (black arrow), lymphocytes (blue arrows), and collagen fibers (blue stars).

Diabetic rabbit normal saline group

In the DR-NS group, 100 % (6/6) of wounds had an intact crust; collagen scores were less in 50 % (3/6), moderate in 33.3 % (2/6), and high in 16.7 % (1/6), with moderate (33.3 %, 2/6) to intense (66.7 %, 4/6) neovascularization; inflammation ranged from mild (16.7 %, 1/6) to severe (50 %, 3/6), with lymphocytic (33.3 %, 2/6), neutrophilic (16.6 %, 1/6), and mixed (50 %, 3/6) infiltrates; no full epithelialization (0/6) was seen, while partial (16.7 %, 1/6), less (66.7 %, 4/6), and none (16.7 %, 1/6) were observed, and acanthosis was discrete in 50 % (3/6), moderate in 33.3 % (2/6), and high in 16.6 % (1/6)

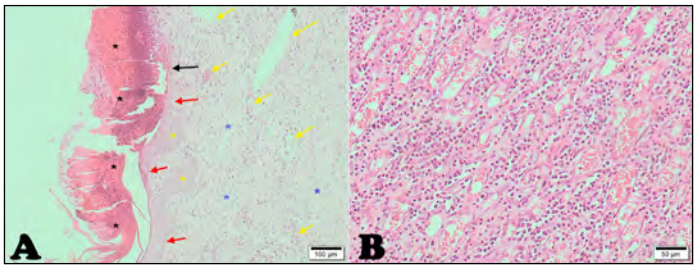

FIGURE 5. DR-NS group, day 25 histopathology (H&E). (a) delayed wound healing: fibrin leukocyte crust (black stars), wound crust and migrating epithelium edge (black arrow), blood vessels (yellow arrows), re-epithelialization (red arrows), and deficient connective tissue (blue stars). (b) severe inflammation with predominantly lymphocytes and some neutrophils, with intense neovascularization and reduced collagen deposition.

Diabetic autologous platelet-rich plasma group

In the DR-AuPRP group, 16.7 % (1/6) had an intact crust; collagen was moderate in 16.7 % (1/6) and high in 83.3 % (5/6), with 100 % (6/6) intense neovascularization; no inflammation was seen in 66.7 % (4/6), while mild inflammation in 33.3 % (2/6) showed lymphocytic (16.7 %, 1/6) and mixed (16.7 %, 1/6) infiltrates; full epithelialization occurred in 83.3 % (5/6) and partial in 16.7 % (1/6), with acanthosis discrete in 50 % (3/6), high in 16.6 % (1/6), and absent in 33.3 % (2/6).

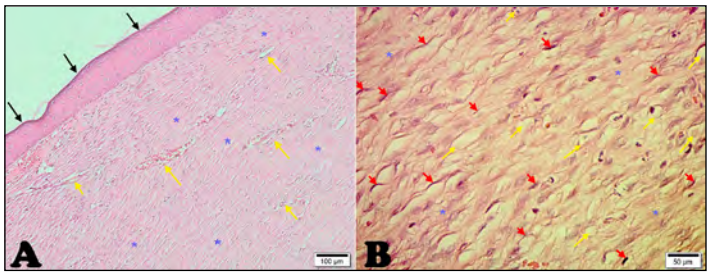

FIGURE 6. DR-AuPRP group, day 25 histopathology (H&E). (a) well-structured, equally thickened re-epithelialization (black arrows), decreased blood vessels (yellow arrows), and well-organized, matured collagen fibers (blue stars). (b) abundant, cross-linked collagen fibers (blue stars), fibroblasts (red arrows), and blood vessels (yellow arrows).

Diabetic homologous platelet-rich plasma group

In the DR-HoPRP group, 50 % (3/6) had an intact crust; collagen was moderate in 16.7 % (1/6) and high in 83.3 % (5/6), with 100 % (6/6) intense neovascularization; inflammation was mild in 83.3 % (5/6) and moderate in 16.7 % (1/6), with lymphocytic (16.7 %, 1/6), neutrophilic (66.7 %, 4/6), and mixed (16.7 %, 1/6) infiltrates; full epithelialization was seen in 50 % (3/6), partial in 16.7 % (1/6), and less in 16.7 % (1/6), with acanthosis discrete in 50 % (3/6), moderate in 16.6 % (1/6), and absent in 33.3 % (2/6).

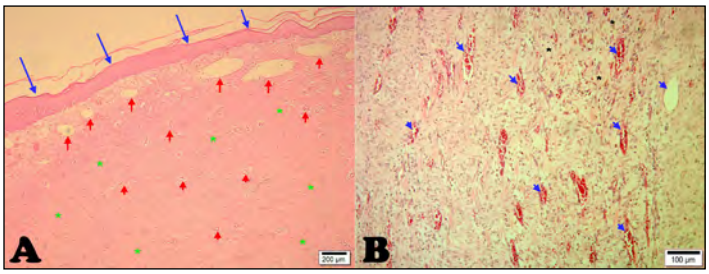

FIGURE 7. DR-HoPRP group, day 25 histopathology (H&E). (a) re-epithelialization (blue arrows), abundant collagen deposition (green stars), and blood vessels (red arrows). (b) numerous perpendicularly aligned blood vessels (blue arrows), indicating good granulation tissue, with moderate lymphocytic infiltration (black stars).

Diabetic heterologous platelet-rich plasma group

In the DR-HePRP group, 66.7 % (4/6) had an intact crust; collagen was less in 16.7 % (1/6), moderate in 33.3 % (2/6), and high in 50 % (3/6), with neovascularization intense in 83.3 % (5/6) and moderate in 16.7 % (1/6); no inflammation was noted in 33.3 % (2/6), while mild (50 %, 3/6) and moderate (16.7 %, 1/6) inflammation showed lymphocytic (16.7 %, 1/6), neutrophilic (33.3 %, 2/6), and mixed (16.7 %, 1/6) infiltrates; full epithelialization was observed in 33.3 % (2/6), partial in 16.7 % (1/6), and less in 50 % (3/6), with acanthosis discrete in 66.7 % (4/6), moderate in 16.6 % (1/6), and absent in 16.7 % (1/6).

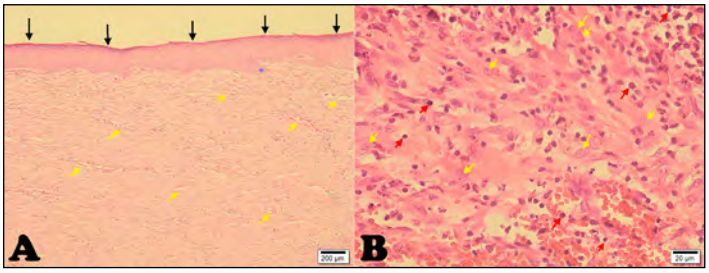

FIGURE 8. DR-HePRP group, day 25 histopathology (H&E). (a) re-epithelialization (black arrows), good fibroblast activity, and abundant collagen fibers; blood vessels (yellow arrows) and acanthosis (blue star). (b) macrophages (yellow arrows) and lymphocytes (red arrows) with angiogenesis and collagen deposition.

On d 25, intense neovascularization was observed in (6/6) DR-AuPRP, (6/6) DR-HoPRP, and (5/6) DR-HePRP, compared to (4/6) DR-NS and (4/6) HR-NS. Xu *et al.* [47] reported similar findings, linking PRP to enhanced angiogenesis, increased CD-31-positive cells, and VEGF mediated effects.

Tissue repair requires a balance between pro- and anti-inflammatory signals, with mild-to-moderate inflammation being beneficial, while excessive inflammation is detrimental [48]. On d 25, DR-AuPRP showed no inflammation in (4/6) and mild in (2/6); DR-HoPRP showed mild (5/6) to moderate (1/6); DR-HePRP showed none (2/6), mild (3/6), and moderate (1/6). Controls showed higher inflammation: HR-NS mild (4/6) to severe (2/6) and DR-NS mild (1/6), moderate (2/6), to severe (3/6). These findings agree with DeRossi *et al.* [27] and Lee *et al.* [37]. In contrast, Yamada *et al.* [49] reported no difference in inflammation with autologous PRP.

Abegão *et al.* [1] observed inflammatory cells with heterologous PRP, and Badis and Omar [28] reported reduced inflammation and improved epithelialization, consistent with the results in this study. Xu *et al.* [47] suggested PRP reduces IL-17A and IL-1β, modulating inflammation contributing to enhanced cutaneous wound repair.

Day-25 histopathology (FIG. 5) showed markedly higher inflammation and inflammatory infiltrates in DR-NS compared to HR-NS (FIG. 4), consistent with Breen *et al.* [3], indicating increased inflammatory cells in diabetic rabbits and impaired repair. Brown *et al.* [50] also reported delayed and prolonged inflammation in diabetic wounds.

In DR-HePRP, (4/6) that showed inflammation: had a mononuclear (1/6), polymorphonuclear (2/6), and mixed (1/6), indicating predominant polymorphonuclear infiltrates, consistent with Meira *et al.* [10]. Rezende *et al.* [51] using human sourced PRP on healing effects of rabbit chondrocytes and Gemignani *et al.* [6] using canine sourced serial heterologous PRP treatments on a cat wound; both of them reported no hypersensitivity or any unwanted side effects. Abegão *et al.* [1] and Barrionuevo *et al.* [44], also used heterologous PRP on experimental standardized rabbit wounds and reported similar increase in neutrophils; without any deleterious effects.

In DR-HoPRP, all (6/6) that showed inflammation: had a mononuclear (1/6), polymorphonuclear (4/6), and mixed (1/6), indicating polymorphonuclear predominance. While in DR-AuPRP, (2/6) that showed inflammation: had a mononuclear (1/6) and mixed (1/6), indicating mononuclear predominance. These findings agree with dos Santos *et al.* [40], who reported increased mononuclear infiltrates in rabbit wounds treated with serial autologous PRP gel therapy. But partially differ from Barrionuevo *et al.* [44] that autologous or homologous PRP treated experimental rabbit wounds showed fewer polymorphonuclear cells. Because in the present study DR-HoPRP group wounds had predominately polymorphonuclear infiltrates.

Full epithelialization on d 25 was observed in DR-AuPRP (5/6), DR-HoPRP (3/6), and DR-HePRP (2/6), compared to DR-NS (0/6) and HR-NS (3/6), indicating earlier closure (re-epithelialization) of the wounds in all the PRP treated groups as compared to diabetic normal saline group. Xu *et al.* [47] attributed this to IGF - I in PRP. Comparison of DR-NS and HR-NS confirmed delayed diabetic healing, consistent with Brown *et al.* [50]. Growth factors such as IGF-II and PDGF improve this delay [50]. Falanga [52] reported impaired keratinocyte-dependent closure in diabetic wounds, which should usually begin within a few hours after tissue injury.

Morphological and morphometrical (d 21 and 25) and histopathological (d 25) analyses showed significantly accelerated healing in all PRP-treated groups, while both the control groups specifically; DR-NS exhibited delayed wound healing. PRP treated wounds (i.e. DR-AuPRP, DR-HoPRP, DR-HePRP) had approximately achieved a 60 % wound contraction percentage by d 14, as compared to both the control group wounds that reached an approximate 56 % wound contraction percentage only on d 14 for HR-NS and d 17 for DR-NS group.

The beneficial effects of PRP observed in the present study are likely mediated through platelet-derived growth factors, including PDGF, TGF-β, VEGF, FGF, IGF, and EGF which regulate cell proliferation, angiogenesis, collagen synthesis, tissue regeneration, and re-epithelialization [4, 5]. Enhanced angiogenesis, improved collagen organization, mature granulation

tissue formation, and accelerated epithelialization observed in the PRP-treated groups are consistent with the biological effects previously reported for PRP-treated wounds [28, 37, 46, 47].

These values later on d 21 and 25 reached up to 95.72 ± 3.74 % and 99.23 ± 0.71 % for DR-AuPRP; while 76.96 ± 15.04 % and 84.33 ± 9.29 % for DR-NS with p = 0.036 and 0.019 respectively. While 25th d histopathological scoring was statistically significant between DR-AuPRP and DR-NS with P = 0.027. These results clearly indicate that PRP accelerates wound repair and are in agreement with the results reported by Rezende *et al*. [51], Malavolta *et al.* [53], Shan *et al.* [54], Acosta *et al*. [33], Li *et al*. [55], Elsaid *et al*. [56], and Palumbo *et al*. [57]; all of who also investigated the therapeutic effects of PRP on circulatory compromised and or chronic diabetic wounds or ulcers.

CONCLUSION

Alloxan monohydrate successfully produced a reproducible diabetic rabbit model suitable for evaluating wound healing therapies. PRP at approximately 3.5-fold baseline platelet concentration significantly accelerated cutaneous wound healing, as evidenced by improved wound contraction, early angiogenesis, formation of healthy granulation tissue, enhanced collagen fiber organization, and effective re-epithelialization. PRP prepared from autologous, homologous, and heterologous sources demonstrated therapeutic potential in modulating and accelerating the wound-healing cascade in diabetic conditions. Notably, heterologous PRP derived from sheep was also found to be safe and effective, suggesting its usefulness in situations where autologous blood collection may not be feasible. Overall, these findings highlight PRP as a practical and economical regenerative therapy that can be readily adopted in Veterinary clinical practice to enhance healing of chronic or delayed cutaneous wounds, particularly in diabetic subjects.

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Conflicts of interest

The authors declare that there are no conflicts of interest among them.

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