INSIGHTS-JOURNAL OF
LIFE AND SOCIAL SCIENCES

INSIGHTS-JLSS
Insights-Journal of Life and Social Sciences

COMPARISON OF AUTOLOGOUS ACTIVATED AND NON-ACTIVATED PLATELETS RICH PLASMA (PRP) ON FULL THICKNESS CUTANEOUS WOUND HEALING IN DOGS

Original Article

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Conflict of Interest: None

Grant Support & Financial Support: None

Acknowledgment: The authors gratefully acknowledge the support of the University of Veterinary and Animal Sciences, Lahore, for providing research facilities.

ABSTRACT

Background: Cutaneous wound healing is a dynamic process involving hemostasis, inflammation, proliferation, and remodeling. Platelet-rich plasma (PRP), either in activated or non-activated form, has emerged as a promising therapeutic option due to its high concentration of platelets, growth factors, and bioactive proteins. These components exert antimicrobial, anti-inflammatory, and antioxidant properties that can accelerate tissue repair, reduce complications, and improve wound closure.

Objective: This study aimed to compare the therapeutic efficacy of activated and non-activated autologous PRP in promoting cutaneous wound healing in clinical dogs.

Methods: Twelve dogs of local breed, aged 1–5 years and weighing 40–50 kg, with naturally occurring cutaneous wounds were enrolled and divided equally into two groups. Group A received 5 mL of activated PRP, while Group B received 5 mL of non-activated PRP, infiltrated subcutaneously at the wound center and edges on day 0 and repeated weekly for three weeks. Wound healing was evaluated macroscopically for edema, exudation, coloration, temperature, healing status, and cosmetic appearance, while histopathological samples were collected on days 7, 14, and 21 to assess inflammation, fibroblast proliferation, and granulation tissue. Hematological parameters (RBCs, WBCs, PLTs, PCV) and oxidative stress markers (CAT, MDA) were also analyzed.

Results: By day 21, five of six animals in Group A achieved ideal healing status with excellent cosmetic appearance, compared to three of six in Group B. Platelet counts were significantly higher in Group A on day 21 (197.33 ± 3.89 vs. 190.00 ± 4.10, P=0.003), and PCV was also elevated (35.48 ± 2.33 vs. 32.62 ± 0.45, P=0.001). Catalase activity declined in both groups, while MDA increased after week one but decreased thereafter, with significantly lower levels in activated PRP-treated wounds. Histopathology revealed reduced neutrophilic infiltration and greater fibroblast proliferation in Group A compared to Group B.

Conclusion: Activated PRP demonstrated superior efficacy over non-activated PRP in enhancing wound healing in dogs, providing a safe and practical adjunctive therapy for cutaneous wound management in veterinary practice.

Keywords: Antioxidant, Antimicrobial, Cutaneous Wound Healing, Inflammatory Response, Oxidative Stress, Platelet-Rich Plasma, Regenerative Therapy.

INTRODUCTION

Skin wounds are among the most frequent conditions encountered in veterinary practice, often resulting from trauma, surgery, burns, or infections. The healing of such wounds is a highly dynamic and complex biological process, influenced by multiple intrinsic and extrinsic factors, including the animal's age, health status, therapy duration, and treatment strategies (1). Cutaneous wound healing naturally progresses through four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (2). In the early hemostatic stage, vasoconstriction and clot formation provide an immediate barrier against blood loss and microbial invasion. This is followed by the inflammatory phase, characterized by vasodilation, recruitment of immune cells, and the removal of debris and microorganisms. The proliferative phase initiates granulation tissue formation, fibroblast activity, angiogenesis, and re-epithelialization through the release of key growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF). In the final remodeling phase, granulation tissue matures into scar tissue with wound contraction mediated by myofibroblasts (3). In recent years, the role of platelets in wound healing has attracted significant attention. Beyond their classical function in hemostasis, platelets act as a natural reservoir of growth factors and cytokines essential for tissue repair and regeneration. Furthermore **Probiotics, prebiotics, *Withania coagulans* also help to increase the concentration of platelets and WBCs function against infectious diseases and injuries as well and increase production in terms of milk and meat etc.** (4,5). This understanding led to the development of platelet-rich plasma (PRP), an autologous blood-derived product containing concentrated platelets, which, when activated, release growth factors that accelerate angiogenesis, collagen synthesis, fibroblast proliferation, and re-epithelialization (6,7). PRP has been widely adopted across medical fields, including orthopedics, ophthalmology, dentistry, and soft tissue wound therapy, as a safe, minimally invasive, and cost-effective treatment modality, **age and sex factors greatly affect the WBCs, platelets concentration in animals, normal values can fight smoothly against diseases and various injuries** (8–11). Furthermore, PRP demonstrates antibacterial properties, reducing local inflammation and infection risk, making it a promising tool in both acute and chronic wound management, **age and lactation stages have great impact on biochemical parameters, liver enzymes in animals to investigate about any abnormality and combat against infectious diseases and injuries (12).**

Activated PRP is commonly achieved through agents such as calcium chloride, thrombin, or collagen, which induce degranulation of platelet alpha granules, leading to the controlled release of growth factors, furthermore **Heat stress greatly affects the blood cells count (RBCs, WBCs, and Platelets) and decrease the fertility rated in animals** (13). Importantly, PRP may also be prepared in a non-activated form, with platelet activation occurring naturally upon exposure to tissue factors at the wound site. While both approaches have clinical relevance, evidence comparing their therapeutic efficacy in veterinary practice remains limited. Studies in companion animals suggest that activated PRP may reduce wound size, accelerate tissue regeneration, and relieve pain more effectively than non-activated formulations (13,14). In addition to wound repair, cutaneous healing is influenced by oxidative stress. During inflammation, reactive oxygen species (ROS) are released by immune cells, which, in excess, can impair cellular integrity, lipid membranes, and enzymatic functions, thereby delaying healing (13–15). Antioxidant defense mechanisms, including catalase, glutathione peroxidase, and superoxide dismutase, normally counteract ROS, but their depletion in diseased or stressed animals may compromise wound repair (10–12). Therefore, therapies that simultaneously promote angiogenesis, collagen synthesis, and antioxidant defense, such as PRP, offer a rational strategy for enhancing recovery. Despite the growing popularity of PRP in veterinary medicine, gaps remain regarding the comparative efficacy of activated versus non-activated autologous PRP in canine wound management. Given its non-invasive nature, autologous PRP therapy offers a promising adjunctive treatment for skin wounds, but direct evidence on its relative effectiveness is still evolving. Thus, this study was designed to evaluate and compare the therapeutic outcomes of activated and non-activated autologous PRP in the treatment of cutaneous wounds in clinical dogs. The objective was to determine whether activation enhances the wound healing potential of PRP, thereby providing an evidence-based approach for optimizing veterinary wound management.

METHODS

Experimental Animals

The study was conducted on twelve clinical dogs of local breed, of either sex, weighing between 40–50 kg and aged 1–5 years, all presenting with naturally occurring cutaneous wounds. Animals were recruited from the Pets & Vets Clinic (DHA) and the Pet Center, Department of Veterinary Surgery and Pet Sciences, University of Veterinary and Animal Sciences (UVAS), Lahore. The dogs were housed in separate kennels under hygienic and ventilated conditions, with free access to water and a maintenance diet provided twice daily. The clinical details and treatment allocations are summarized in Table 1. Inclusion criteria consisted of dogs with moderate to

severe cutaneous wounds requiring clinical intervention, while those with systemic illness, immunocompromised states, or on concurrent experimental therapies were excluded. Written informed consent was obtained from pet owners prior to enrollment, and all experimental procedures were performed under the guidelines of institutional ethical standards. Ethical approval was secured from the Institutional Review Board of UVAS, Lahore.

Table 1: Description, Clinical history and treatment of cutaneous wound healing of 12 dogs with activated and non-activated autologous PRP

Sr. No	Breed	Age (year)	Gender	Reported initiating event	Area of wound	Treatment
Group A						
1	local	1	M	Steel wire injury	Lt, Right side of neck	Activated PRP
2	local	5	M	Fighting	Lt, left side of scapula	
3	local	3	M	Steel wire injury	Left flank	
4	local	2	F	Fighting	Lt, Right side of neck	
5	local	1	F	Fighting	Left flank	
6	local	4	M	Fighting	Lt, left side of scapula	
Group B						
1	local	3	M	Fighting	Lt, Right side of neck	NON-Activated PRP
2	local	5	M	Racing	Left flank	
3	local	3	F	Steel wire injury	Lt, left side of scapula	
4	local	2	M	Steel wire injury	Lt, Right side of neck	
5	local	1	F	Racing	Right flank	
6	local	5	M	Fighting	Lt, Right side of neck	

Lt=lateral; F=female; M=male; PRP; platelet rich plasma

Design study

The dogs were divided into two equal groups. Group A (n=6) received autologous activated platelet-rich plasma (PRP), while Group B (n=6) received autologous non-activated PRP. In Group A, 5 mL of activated PRP was infiltrated subcutaneously at the wound site at day 0 and repeated weekly for three consecutive weeks. In Group B, the same protocol was followed using non-activated PRP. Throughout the study period, animals were carefully monitored for clinical stability. Daily assessments included body temperature, pulse, and respiration, as well as clinical observation for any systemic disease. To ensure uniform handling, 2% lidocaine infiltration was applied at the wound site prior to each injection. Post-treatment, dogs were maintained in individual cages, and routine aseptic wound care was performed with daily cleansing and topical povidone-iodine application. A broad-spectrum antibiotic, amoxicillin (Inj. Invemox®, Punjnad Pharma, Pakistan) was administered intramuscularly at 12.5 mg/kg once daily for three days. For analgesia, ketoprofen (Inj. Ketoject®, Selmore Pharma, Pakistan) was given intramuscularly at 1 mg/kg daily for three days. Post-operative management also included diet modification and activity restriction for one week.

Preparation of Platelet-rich Plasma (PRP)

Autologous PRP was prepared from 4 mL of whole blood collected aseptically from the cephalic vein of each dog thirty minutes prior to the procedure. Blood was transferred into EDTA-coated Falcon tubes to prevent coagulation. The samples underwent centrifugation at 900 rpm for 10 minutes, producing three layers: a bottom layer of red blood cells, a middle buffy coat containing leukocytes and platelets, and an upper plasma fraction (16). The top plasma (250 µL) was transferred into tube A for autogenous thrombin preparation, while the remaining plasma and buffy coat were transferred into tube B to obtain PRP. Tube A was treated with 150 µL of 10% calcium chloride and incubated at 37°C for 15 minutes. A second centrifugation at 2500 rpm for 10 minutes separated thrombin (tube A) and plasma fractions (tube B). Platelet-poor plasma from tube B was discarded, and the remaining concentrate was homogenized and mixed with thrombin from tube A at a ratio of 2:1 (2 mL PRP:1 mL thrombin). The final PRP preparation was stored at 4°C until use. It is noteworthy that the described preparation yields only about 10% of PRP relative to total blood volume, largely due to discarding platelet-poor plasma and erythrocytes (17). A potential limitation in this procedure is the relatively low yield of PRP obtained from the small

blood volume (4 mL), which may not provide sufficient platelet concentration as recommended in the literature (18). This methodological constraint should be acknowledged as it could affect reproducibility or treatment efficacy.

Parameters of the study

To assess the efficacy of activated and non-activated PRP, both macroscopic and microscopic parameters were evaluated alongside hematological and oxidative stress markers.

Macroscopic examination

Wounds were observed at defined intervals for qualitative parameters, recorded using a visual analogue scale. These included edema (absent, mild, moderate, severe), exudation (absent, serous, purulent, sero-purulent), wound coloration (black, yellow, red, pink), temperature of the wound site, healing status (ideal, acceptable, minimal), and cosmetic appearance (excellent, satisfactory, variable).

Microscopic examination

For histopathology, wound tissue samples were collected at days 7, 14, and 21 using a 6 mm punch biopsy needle, penetrating through epidermis, dermis, and subcutaneous tissue. Biopsies were fixed in 10% neutral buffered formalin, processed, and stained with hematoxylin and eosin. Slides were examined under light microscopy at 10× and 40× magnifications to evaluate neutrophil infiltration (acute inflammation), fibroblast proliferation, and granulation tissue formation.

Hematological analysis

Peripheral blood samples were collected from the cephalic vein on days 0, 7, 14, and 21 into EDTA vacutainers. Hematological parameters including total white blood cell count, red blood cell count, platelet count, and packed cell volume (PCV) were analyzed. Serum was separated after centrifugation at 3000 rpm for 15 minutes at 4°C and stored at –20°C for subsequent testing. Analyses were conducted in the Department of Physiology, Faculty of Biosciences, UVAS Lahore.

Oxidative stress analysis: Oxidative stress markers were assessed by evaluating antioxidant enzyme activity and lipid peroxidation.

Assessment of the catalase (CAT) activity

Catalase activity was measured by its reaction with hydrogen peroxide. Residual H2O2 after 1 minute of incubation was quantified by reaction with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone in the presence of peroxidase to form a chromophore. The absorbance was measured at 510 nm, with enzyme activity inversely proportional to chromophore intensity (18).

Assessment of lipid peroxidation (MDA)

Malondialdehyde (MDA), an end-product of lipid peroxidation, was quantified as a biomarker of oxidative stress using the thiobarbituric acid reactive substances (TBARS) method. The resultant pink chromogen was measured spectrophotometrically at 534 nm (19).

Statistical Analysis

All data were analyzed using GraphPad Prism version 7.04 (GraphPad Software Inc., San Diego, CA). A repeated measures two-way ANOVA was applied to compare groups across different time points, with results expressed as mean ± standard deviation. Statistical significance was considered at $P < 0.05$.

RESULTS

Macroscopic Examination

Edema

Group A

Edema resolved completely in all animals by day 21. One animal (A1) exhibited moderate edema on day 7 that became absent by day 21, and another (A5) showed mild edema from day 7 to day 14 that resolved by day 21. The remaining dogs (A2, A3, A4, A6) demonstrated no edema at any assessment point.

Group B

Edema patterns were more variable. One animal (B2) had no edema initially but developed mild edema between days 14 and 21. Another (B4) showed mild edema on day 7 with resolution by day 14. Dog B5 progressed from mild edema (days 7–10) to severe edema through day 19, with resolution by day 21. Dogs B1, B3, and B6 exhibited no edema throughout.

Exudation

Group A

Exudation was infrequent. A1 progressed from no exudation (day 7) to sero-purulent discharge (days 14–19) and purulent discharge on day 21. A2 had serosanguinous discharge on day 7 only, and A5 showed sero-purulent discharge from day 7 to day 14, absent by day 21. A3, A4, and A6 had no exudation at any time.

Group B

Exudation was more common and tended to persist. B1 changed from serous (day 7) to sero-purulent through day 21. B2 had serosanguinous discharge on day 7, absent by day 21. B4 and B5 showed sero-purulent exudation on day 7 and purulent discharge from days 14 to 21. B3 and B6 remained free of exudation.

Coloration

Group A

Five of six wounds (A2, A3, A4, A5, A6) were pink by day 14 and remained pink on day 21, reflecting clean wound beds. One wound (A1) progressed from dark red (days 1–7) to black between days 12 and 21, indicating necrosis.

Group B

Coloration fluctuated more. B1 changed from dark red (days 1–7) to pink (days 12–21). B2 was red on day 1, yellow on days 7–14 (suggestive of slough or pus), and pink by day 21. B4 progressed from red (day 1) to yellow (day 7) and then black (days 14–21), consistent with necrosis. B5 remained red (days 1–7) and turned black (days 14–21). B3 and B6 changed from red (days 1–7) to pink (days 14–21).

Temperature

Group A

Skin temperature measured by infrared thermometer showed small increases (approximately 0.1–1.5°C) after treatment, most evident by day 7, with subsequent stabilization or decline by days 14–21 across individuals. Individual values (°C) for days 1, 7, 14, and 21 were: A1 (36.1, 35.8, 36.8, 36.4), A2 (36.2, 36.5, 36.7, 35.9), A3 (36.9, 37.1, 36.9, 37.4), A4 (35.9, 35.8, 37.2, 37.3), A5 (36.9, 35.4, 36.9, 35.2), A6 (36.4, 37.1, 36.2, 36.2).

Group B

Similar modest rises were noted in four animals through day 14 (B1, B2, B3, B6), with two animals (B4, B5) showing increases that later declined. Individual values (°C) for days 1, 7, 14, and 21 were: B1 (36.3, 36.4, 36.5, 36.0), B2 (36.8, 36.9, 36.9, 35.8), B3 (36.8, 37.8, 37.1, 36.5), B4 (36.7, 36.4, 35.6, 35.4), B5 (35.9, 36.1, 34.8, 36.2), B6 (36.3, 36.9, 36.9, 36.3).

Healing status

Group A

Five animals (A2–A6) achieved ideal healing by day 21. A1 remained minimal throughout the study. Early assessments commonly showed minimal to acceptable healing by day 7, with progressive edge approximation and complete epithelialization leading to ideal status by day 21 in A2–A6.

Group B

Three animals (B2, B3, B6) reached ideal healing by days 14–21, B1 improved to acceptable by days 14–21, whereas B4 and B5 remained minimal throughout, aligning with concurrent necrosis.

Cosmetic appearance (Scar formation)

Group A

Cosmetic outcomes were predominantly excellent by day 21 in five animals; A1 was variable from days 14–21 due to graft rejection. Early satisfactory appearance (days 1–7) in A5 and A6 progressed to excellent by day 21.

Group B

Cosmesis varied: three animals achieved excellent appearance by day 21 (B2, B3, B6); B1 was satisfactory; B4 transitioned from satisfactory to variable by day 21; B5 was variable from days 14–21.

Microscopic Parameters

Acute inflammation

Group A

Neutrophil infiltration was common on day 7 and diminished thereafter. A1 showed persistent neutrophils on days 14 and 21; A2 had mild neutrophils on day 7 only; A3 showed neutrophils on days 7 and 21 but not day 14; A4 had no neutrophils at any time; A5 and A6 had neutrophils on day 7 only, absent by days 14 and 21.

Group B

Most animals had neutrophils on day 7, with variable persistence. B1 and B2 were positive on day 7 only. B3 showed neutrophils on days 7 and 14. B4 had dead and intact neutrophils on day 7 and dead neutrophils on days 14 and 21. B5 had no inflammatory response at any time. B6 had inflammatory infiltrates on days 14 and 21.

Fibroblast proliferation

Group A

Fibroblast and connective tissue proliferation was evident in most animals by day 14 and sustained to day 21. A2, A4, and A6 demonstrated proliferation at all timepoints (days 7, 14, 21). A3 and A5 lacked proliferation on day 7 but showed evident proliferation on days 14 and 21. A1 had no fibrous connective tissue at any assessment.

Group B

B1 and B2 exhibited fibrocyte and fibrous connective tissue infiltration at all timepoints. B3 had proliferation on day 14 only. B4 and B5 lacked infiltration throughout, although B5 showed a few fibrous elements by day 21. B6 showed fibrous tissue on day 7 with reduced infiltration on days 14 and 21.

Proliferation of Granulation tissue

Group A

Granulation was largely absent at day 7. A5 displayed granulation on day 14, and A6 had granulation on days 14 and 21. The remaining animals (A1–A4) showed no granulation at the assessed timepoints, with one case accompanied by necrosis and tissue debris.

Group B

Granulation was absent in B1–B3. B4 and B5 showed tissue debris, dead epidermis, and dead neutrophils with no granulation. B6 demonstrated granulation tissue on days 14 and 21.

Hematological evaluation

Red blood cell counts were comparable between groups at all timepoints: day 1 (4.09 ± 0.48 vs 4.15 ± 0.15 ×10^12/L, P=0.99), day 7 (4.30 ± 0.51 vs 4.36 ± 0.18, P=1.00), day 14 (4.63 ± 0.43 vs 4.66 ± 0.23, P=0.99), and day 21 (5.00 ± 0.44 vs 5.03 ± 0.19, P=0.98). White blood cell counts were likewise similar: day 1 (4.46 ± 0.16 vs 4.35 ± 0.18 ×10^9/L, P=0.99), day 7 (4.70 ± 0.27 vs 4.62 ± 0.21, P=1.00), day 14 (4.93 ± 0.38 vs 4.86 ± 0.16, P=0.99), and day 21 (5.16 ± 0.46 vs 5.23 ± 0.13, P=0.99). Platelet counts increased over time in both groups, with higher values in the activated PRP group on day 21 (197.33 ± 3.89 vs 190.00 ± 4.10 ×10^9/L, P=0.003), whereas earlier timepoints showed no between-group differences: day 1 (173.16 ± 4.16 vs 176.83 ± 2.79, P=0.13), day 7 (177.17 ± 4.35 vs 180.33 ± 3.08, P=0.24), and day 14 (185.33 ± 4.47 vs 185.00 ± 3.80, P>0.99). Packed cell volume (%) was similar at baseline and

day 7 but was higher in the activated PRP group at day 14 (34.17 ± 0.80 vs 32.26 ± 0.43, P=0.03) and day 21 (35.48 ± 2.33 vs 32.62 ± 0.45, P=0.001).

Oxidative stress status

Catalase (CAT) activity differed significantly between groups at multiple timepoints (P<0.01), with overall decreases from day 7 to day 21 in both groups. Malondialdehyde (MDA) concentrations increased after the first week and then decreased at weeks 2 and 3 in both groups (P<0.01). Exact numerical values were not provided in the dataset for individual timepoints.

Table 2: Severity of Edema and Types of Exudate in Dogs (n=12) Treated with Activated and Non-Activated PRP (Group A & B)

Replicate	Group A – Edema				Group B – Edema				Group A – Exudate				Group B – Exudate			
	Days				Days				Days				Days			
	1	7	14	21	1	7	14	21	1	7	14	21	1	7	14	21
1	A1	Mo[2]	Mo	A	A	A	A	A	A1	A	S.P[2]	P[3]	A	S[4]	S.P	A
2	A	A	A	A	A	A	M[3]	M	A	S.G[5]	A	A	A	S	A	A
3	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
4	A	A	A	A	A	M	A	A	A	A	S	A	A	P	P	P
5	A	M[3]	M[3]	A	A	M	S[4]	A	A	S.P[2]	S.P[2]	A	A	S.P[2]	P[3]	P[3]
6	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Note: Edema: A = Absent; Mo[2] = Moderate; M[3] = Mild; S[4] = Severe. Exudate: A = Absent; S.P[2] = Sero-Purulent (watery, thin, cloudy, yellow); P[3] = Purulent (opaque, thick); S[4] = Serous (thin, watery, clear plasma); S.G[5] = Serosanguinous (watery, pale red); Sg[6] = Sanguineous (fresh bleeding)

Table 3: Variation of Wound Coloration and Skin Temperature in Dogs (n=12) Treated with Activated and Non-Activated PRP (Group A & B)

Replicate	Group A – Color				Group B – Color				Group A – Temperature (°C)				Group B – Temperature (°C)			
	Days				Days				Days				Days			
	1	7	14	21	1	7	14	21	1	7	14	21	1	7	14	21
1	R[1]	R	B[3]	B[3]	R	R	P[2]	P[2]	36.1	35.8	36.8	36.4	36.3	36.4	36.5	36.0
2	R	Y[4]	P[2]	P[2]	R	Y[4]	Y[4]	P[2]	36.2	36.5	36.7	35.9	36.8	36.9	36.9	35.8
3	R	P[2]	P[2]	P[2]	R	R	P[2]	P[2]	36.9	37.1	36.9	37.4	36.8	37.8	37.1	36.5
4	R	P[2]	P[2]	P[2]	R	Y[4]	B[3]	B[3]	35.9	35.8	37.2	37.3	36.7	36.4	35.6	35.4
5	R	P[2]	P[2]	P[2]	R	R	B[3]	B[3]	36.9	35.4	36.9	35.2	35.9	36.1	34.8	36.2
6	R	P[2]	P[2]	P[2]	R	R	P[2]	P[2]	36.4	37.1	36.2	36.2	36.3	36.9	36.9	36.3

Note: Color codes: R[1] = Red (owing to bleeding, wound appears dark red), P[2] = Pink (clean wound, proliferation of granulation and fibrous tissue), B[3] = Black (dead tissue, necrosis of skin graft), Y[4] = Yellow (accumulation or formation of pus, indicating possible infection)

Table 4: Healing Status and Scar Formation in Dogs (n=12) Treated with Activated and Non-Activated PRP (Group A & B)

Replicate	Group A – Healing Status				Group B – Healing Status				Group A – Scar Formation				Group B – Scar Formation			
	Days				Days				Days				Days			
	1	7	14	21	1	7	14	21	1	7	14	21	1	7	14	21
1	M	M	M	M	M	M	A	A	S[1]	S	V[2]	V[2]	S	S	S	S
2	M	A	I	I	M	A	I	I	S	S	E[3]	E[3]	S	S	S	E[3]
3	M	A	I	I	M	A	I	I	S	S	E[3]	E[3]	S	S	E[3]	E[3]
4	M	A	I	I	M	M	M	M	S	S	E[3]	E[3]	S	S	S	V[2]

Replicate	Group A – Healing Status				Group B – Healing Status				Group A – Scar Formation				Group B – Scar Formation			
5	M	M	A	I	M	A	M	M	S	S	S	E³	S	S	V²	V²
6	M	A	A	I	M	A	I	I	S	S	S	E³	S	S	E³	E³

Note: Healing Status: M = Minimal (No wound contraction, no wound edge constriction, no epithelialization and hair growth, dead tissue present), A = Acceptable (Wound edges met at some points, less wound contraction, scar formation, complete epithelialization), I = Ideal (All wound edges completely met owing to epithelialization, excellent wound contraction, more hair growth, more hair follicles) Scar FormationS¹ = Satisfactory (Fair area, wound edges with granulating tissue, less hair growth), V² = Variable (Tissue debris, irregular wound edges, necrosis of graft), E³ = Excellent (Neat area, excellent hair growth, skin texture identical to normal skin)

Table 5: Comparison of Red Blood Cells (RBCs) and White Blood Cells (WBCs) in Dogs Treated with Activated and Non-Activated PRP (Group A & B)

Time (days)	RBCs – Group A (A.PRP) Mean ± SEM	RBCs – Group B (NA.PRP) Mean ± SEM	RBCs P-value	WBCs – Group A (A.PRP) Mean ± SEM	WBCs – Group B (NA.PRP) Mean ± SEM	WBCs P-value
1d	4.09 ± 0.48	4.15 ± 0.15	0.99	4.46 ± 0.16	4.35 ± 0.18	0.99
7d	4.30 ± 0.51	4.36 ± 0.18	1.00	4.70 ± 0.27	4.62 ± 0.21	1.00
14d	4.63 ± 0.43	4.66 ± 0.23	0.99	4.93 ± 0.38	4.86 ± 0.16	0.99
21d	5.00 ± 0.44	5.03 ± 0.19	0.98	5.16 ± 0.46	5.23 ± 0.13	0.99

Table 6: Comparison of Platelets (PLTs) and Packed Cell Volume (PCV) in Dogs Treated with Activated and Non-Activated PRP (Group A & B)

Time (days)	PLTs – Group A (A.PRP) Mean ± SEM	PLTs – Group B (NA.PRP) Mean ± SEM	PLTs P-value	PCV – Group A (A.PRP) Mean ± SEM	PCV – Group B (NA.PRP) Mean ± SEM	PCV P-value
1d	173.16 ± 4.16	176.83 ± 2.79	0.13	32.48 ± 1.00	31.60 ± 0.38	0.79
7d	177.17 ± 4.35	180.33 ± 3.08	0.24	33.66 ± 0.81	32.88 ± 0.35	0.05
14d	185.33 ± 4.47	185.00 ± 3.80	>0.99	34.17 ± 0.80	32.26 ± 0.43	0.03*
21d	197.33 ± 3.89	190.00 ± 4.10	0.003**	35.48 ± 2.33	32.62 ± 0.45	0.001**

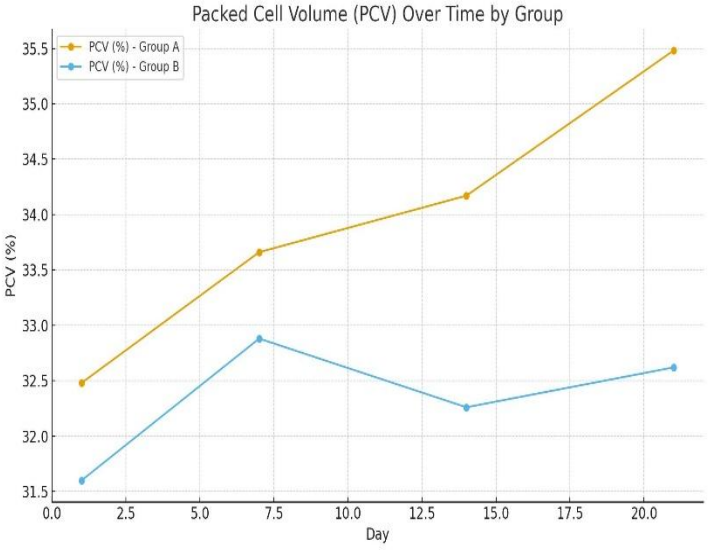


Figure 2 Packed Cell Volume (PCV) Over Time by Group

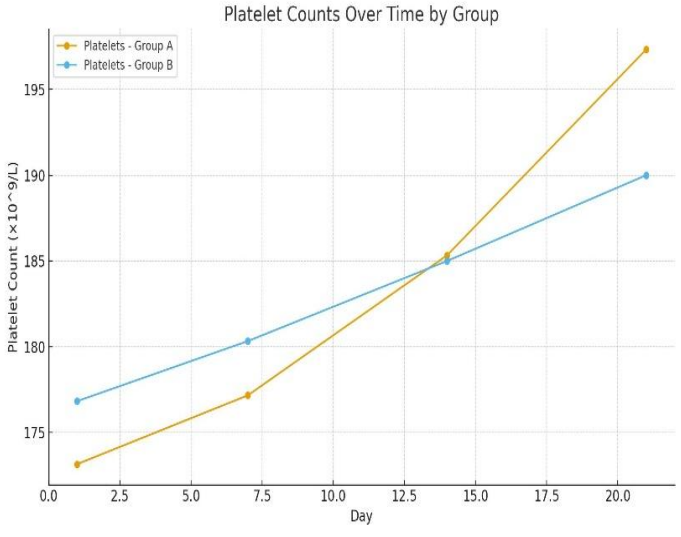
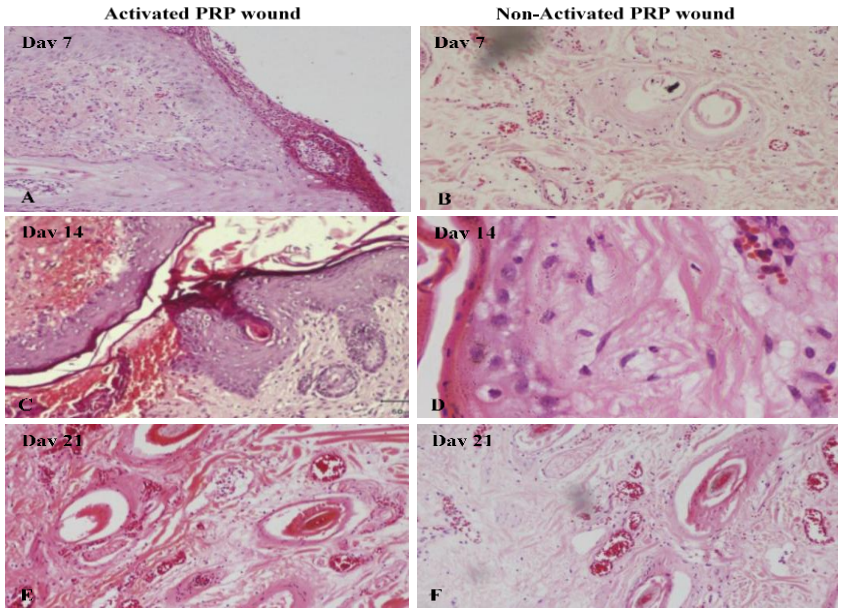


Figure 2 Platelet Counts Over Time by Group



Histopathological evaluation (HE stain) in groups treated with activated PRP and non-activated (Group A& B)

DISCUSSION

The present findings indicated that autologous platelet-rich plasma supported cutaneous repair in clinical dogs, with more consistent gains in the activated PRP cohort than in the non-activated cohort. Across serial macroscopic assessments, activated PRP was associated with earlier resolution of edema, fewer and shorter episodes of exudation, and a faster transition of wound coloration from red or yellow to healthy pink, consistent with cleaner wound beds and progressive epithelial coverage. Microscopic data complemented these trends: acute neutrophilic infiltrates predominated at day 7 and receded thereafter, while fibroblast proliferation and, in selected cases, granulation tissue formation emerged during weeks 2–3—features that typify the proliferative and early remodeling phases of repair. These observations aligned with the biological premise that platelets deliver a concentrated cargo of growth factors—such as PDGF, VEGF, and TGF-β—that drive angiogenesis, fibroplasia, and re-epithelialization following activation at the wound interface (growth factor release after clot formation and platelet activation, receptor binding, and downstream signaling) and with prior reports of clinical benefit of PRP in chronic, non-healing ulcers where accelerated closure and measurable wound size reduction were achieved compared with conventional care (10). The temporal pattern of inflammation observed here—prominent at one week and diminishing by weeks two and three—matched earlier veterinary and surgical experiences in which PRP-treated sites showed improved edema control, attenuated ecchymosis, and more orderly epithelial migration compared with untreated areas (11,12). Signals of enhanced re-epithelialization with activated PRP echoed data in cutaneous models demonstrating faster resurfacing and less edema at treated margins, strengthening the case for using an activated preparation when rapid epithelial coverage is clinically desirable (13). Histopathology reinforced these macroscopic impressions. Fibroblast proliferation was evident by day 14 in most animals and largely persisted to day 21, while granulation tissue and early vascular buds appeared in a subset, mirroring staged transitions reported in time-course analyses of wound repair where reactive cell infiltrates recede as fibroblasts, capillary sprouts, and matrix deposition dominate the field (15-17).

The oxidative stress profile offered mechanistic context. Catalase activity fell over time in both groups, a pattern compatible with consumption or down-modulation of antioxidant defenses during the early inflammatory surge and gradual re-balancing as inflammation resolved. Meanwhile, malondialdehyde (MDA), a lipid peroxidation marker, rose after week one and then declined by weeks two and three, with lower concentrations in the activated PRP group at the earlier interval. This trajectory suggested that the bioactive milieu delivered by activated PRP may have tempered oxidative injury during the phase when neutrophil-derived oxidants typically peak, in line with observations that perturbation or inhibition of catalase shifts redox tone and that MDA correlates with disease burden in inflamed tissues (18,19). Together with the macroscopic and histological signals, these biomarker trends supported an anti-inflammatory

and pro-repair influence of activated PRP in the canine skin wound setting (20-22). The hematological data contextualized safety and systemic effects. Red and white cell counts did not differ between groups at any time point, suggesting that neither intervention produced clinically meaningful systemic hematologic perturbations. Platelet counts rose modestly over time in both groups, with a small but statistically significant advantage for the activated PRP group at day 21, a finding that might reflect reactive thrombocytosis during tissue repair or subtle differences in peripheral mobilization. Packed cell volume diverged in favor of the activated group from day 14 onward, though the clinical import of this magnitude change remained uncertain without concurrent hydration indices or iron studies. This investigation carried several strengths. It enrolled clinical cases rather than experimental wounds, enhancing external validity for real-world veterinary practice. It integrated multimodal readouts—macroscopic grading, histopathology, hematology, and oxidative stress biomarkers—providing convergent evidence across biological scales. It also compared activated and non-activated autologous preparations under standardized peri-wound care, isolating the effect of activation on outcomes.

Limitations tempered the inferences. The sample size was small (n=12), limiting power to detect moderate effects and increasing sensitivity to individual variability. Randomization and blinding procedures were not detailed, introducing potential allocation and observer biases. Quantitative primary outcomes directly aligned to the objective—time to complete epithelialization, percentage wound area reduction, and proportion of wounds closed by day 21—were not reported, restricting effect-size estimation for clinical decision-making. PRP characterization was not provided; fold-enrichment over baseline platelet counts, leukocyte content, and confirmation of activation status are essential for reproducibility and dose–response interpretation. The preparation drew only 4 mL of whole blood per dog; such a small volume may yield limited platelet enrichment relative to recommended 4–8× baselines and could blunt therapeutic contrast between groups. Storage of PRP at 4 °C before use, rather than immediate application, may reduce platelet viability and growth-factor bioavailability. Concomitant antibiotic and analgesic regimens, while clinically appropriate, can influence inflammation and tissue remodeling and were not explicitly balanced or analyzed as covariates. Finally, macroscopic scales and histological scoring lacked inter-rater reliability metrics, and oxidative stress outcomes were summarized without full numerical reporting, constraining independent appraisal.

The implications for practice were nonetheless tangible. When autologous PRP is chosen for canine cutaneous wounds, activation with calcium or thrombin appeared to enhance early epithelial dynamics, reduce exudation and edema, and modestly improve tissue-level indices of repair compared with non-activated preparations, without evident systemic hematologic risk. To mature this evidence base, future studies should adopt concealed randomization and blinded outcome assessment; predefine primary endpoints that matter to clinicians and owners (time to closure, percent area reduction, pain scores, return-to-function metrics); and report detailed PRP analytics, including baseline platelet counts, PRP platelet/leukocyte concentrations, and growth-factor profiles. Larger sample sizes and stratification by wound etiology, size, and infection status would enable subgroup analyses. Serial planimetric imaging, high-frequency ultrasound of dermal thickness, and standardized histological scoring could sharpen mechanistic insights, while expanded redox panels and cytokine profiling would clarify how activated PRP modulates the inflammatory–proliferative transition. Comparative arms testing activated PRP against advanced dressings, negative-pressure wound therapy, or biologic scaffolds would position PRP within a modern multimodal algorithm. In sum, the data favored activated PRP over non-activated PRP for several clinically relevant facets of canine wound healing and aligned with the broader literature that attributes PRP's benefits to concentrated, timely delivery of pleiotropic growth factors that orchestrate hemostasis, inflammation resolution, angiogenesis, and matrix remodeling (23,24). While these results encourage the considered use of activated autologous PRP in veterinary wound care, rigorous, quantitatively anchored trials remain necessary to define optimal dosing, activation strategies, and patient selection with precision.

CONCLUSION

This study concluded that autologous platelet-rich plasma supported cutaneous wound healing in dogs, with activated preparations demonstrating more consistent and efficient outcomes than non-activated forms. The findings highlighted the role of activated PRP in reducing inflammation, enhancing fibroblast proliferation, and promoting granulation and re-epithelialization, thereby facilitating faster and more complete tissue repair. These results emphasized the clinical value of activated PRP as a safe, practical, and effective adjunctive therapy for managing skin wounds in veterinary practice, offering a promising approach to improve healing where conventional methods may be limited.

AUTHOR CONTRIBUTION

Author	Contribution
Hamza Faiz	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Hamid Akbar*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Usama Afzal	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Jahanzaib Khaliq	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Ahmed Saqib	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Halima Sadia	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Tayyab Ahmad	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Muhammad Ismail	Writing - Review & Editing, Assistance with Data Curation
Ikram Ullah	Writing - Review & Editing, Assistance with Data Curation
Muhammad Umar Farooq	Writing - Review & Editing, Assistance with Data Curation
Muhiuddin Shah	Writing - Review & Editing, Assistance with Data Curation
Inayat Ullah Khan	Writing - Review & Editing, Assistance with Data Curation

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