



Effects of Various Concentrations of Autologous Platelets-Rich Plasma on Cutaneous Wound Healing in Experimental Rabbit Model

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Abstract

THIS STUDY explored the effect of various concentrations of PRP on cutaneous wound healing in rabbits. Twenty-four rabbits allocated randomly into four groups: group-A (control), group-B (0.2 mL PRP), group-C (0.4 mL PRP), and group-D (0.6 mL PRP) with 6 rabbits in each group. In each rabbit, 2 cm from the midline, full-thickness (2 x 2 cm²) skin incisions were made on the right dorsal surface. Re-epithelization and neovascularization were assessed on day 21 by H&E staining, while collagen formation were assessed by Mason's trichrome staining. The serum MDA concentrations and CAT activity were also assessed using blood samples. The groups that received 0.4 mL and 0.6 mL of PRP (subcutaneously on days 1, 7, and 14) had significantly higher levels of re-epithelization, angiogenesis, fibroblasts, and collagen fibers arrangements than the control group (irrigated with sterile saline) on day 21. The 0.2 mL PRP group also had a significant increase in collagen arrangement on day 21. The both groups C & D wounds had significantly lower MDA levels on day 7, and they were highly significantly lower on days 14 and 21 than the control wound. However, the group B wound was lower on days 14 and 21 than the control wound. The CAT activity increased non-significantly on different time intervals between the groups. In conclusion, 0.4 mL and 0.6 mL PRP-groups accelerated wound healing by promoting re-epithelization, neovascularization, collagen organization, and inhibiting oxidative stress than the control wounds.

Keywords: cutaneous wounds, histopathology, PRP, rabbits, wound healing.

Introduction

Cutaneous wounds are considered by the loss or interruption of skin's structure and function reasoned by various stress modulators, e.g., burn injuries, traumatic wounds, and medical and physiological conditions [1]. To heal a wound would be of great concern soon after any injury is inflicted. There are four distinctive, coinciding phases that play a vital

role in the healing of any sort of wound, which include hemostasis, inflammation, proliferation, and remodeling [2]. Whenever there is any hurdle or failure in the healing of any wound in a timely and routine manner, it leads to the development of chronic wounds [3]. Enlisted are some of the major predisposing causes that lead to such issues, e.g., burns, skin infections, trauma, and surgery [4,5]. Skin injuries are frequently observed in donkeys,

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mules, horses [6], and dogs [7], cats [8], rats [9], and rabbits [10]. Wound recovery encompasses various proliferative variables, chemical facilitators, cytokines, and various varieties of cells. Whichever change takes away this system could end up in resilient injuries that are incapable of healing. A chronic wound fails to recover in the specified pattern of phases or has not received any recommended therapy-suggested time frame [11]. Considering the latest innovations in skin closing technologies and processes, surgeons continue to believe the healing process for wounds is problematic [12]. Yet, to effectively treat serious cutaneous wounds, the sole method is to resolve them via a secondary intentional method due to tissue damage and not being able to close the wound originally. Therefore, with the goal of achieving optimal outcomes, there is a need to use cutting-edge advancements to boost the repair phase.

An extraordinary and distinctive cellular function process is seen during the healing of a skin wound. A crucial aspect of the healing process is the interaction of cells, growth factors, and cytokines in closing the lesion. Problems after injuries, especially those involving chronic wounds, are mostly caused by restrictions in wound repair imposed by treatment and maintenance procedures, as opposed to tissue integrity restoration [13].

There are two completely different therapies regarding their perspective to treat wounds, which are broadly classified as regenerative and conventional. The main hurdle regarding conventional therapies is scar formation [14]. Regenerative wound therapy has recently evolved and is of key focus in an attempt to restore damaged cells and skin tissue without leaving scars [15]. It is a revolutionary field that embraces enormous potential to transform the healthcare domain, presenting a favorable tactic in addressing the challenges linked with damaged organs and tissues by coupling the body's own repair mechanisms [16]. One of the most innovative segments of this is tissue engineering, which is focused on self-healing. Tissue engineering techniques syndicate scaffolds of cells and growth factors in an attempt to repair and regenerate pathologically injured tissues, which include cartilage, bone, skin, and various other organs [17]. In the last couple of decades, experimental wound healing models have been established, attempting to further figure out the tissue repair process and assess innovative therapeutic approaches. Preclinical animal models and in vitro models are the two categories into which these models are typically separated [18]. In vivo models require wound infliction on lab animals, leading to the tracking of the healing process. Wound environment modifications that are

physical, chemical, or biological can also be incorporated [19].

Platelet-rich plasma (PRP), the biological aspect of autologous blood, is the plasma fraction richer in platelets than the original whole blood. Platelet granules contain a diverse cellular component of various vital growth factors and cytokines [20]. One specific sub-specialty of regenerative medicine, veterinary medicine, has explored PRP and other PD products for treating OA, STW, tendinopathies, periodontal diseases, and fracture management [21]. Literature in vitro and in vivo research has recommended PRP in enhancing angiogenesis, activity, and proliferation of keratinocytes, fibroblasts, and endothelial cells besides activating macrophages during inflammation. Furthermore, there are no side effects observed in preclinical studies of allogenic PRP that exaggerate immunosuppressive effects [22]. PRP is widely used in a variety of tissue engineering and cell-based therapeutic applications as a scaffold. It is prepared out of the platelets wherein platelet concentrates are harvested from the autologous plasma that has more than 300 physiologically active substances. These compounds are obtained from dense granules and platelet alpha in the activated state for the purpose of modulating the process of tissue repair [23]. Activated platelets contain multiple growth factors, which are basic proteins that are liberated from alpha granules. Growth factors reduce inflammation and blood coagulation and are vital for the onset of the healing/repair process of tissues. An example of plasma with a high number of platelets is known as platelet-rich plasma, and plasma of this nature can be produced by seriated centrifuge technologies. Various growth factors, including PDGF, TGF- α , TGF- β , VEGF, IGF-I, PDECGF, and EGF, are also present in platelets [24].

For this reason, in regenerative medicine, the application of PRP has triggered unprecedented interest because of the unraveling of growth factor regulation and cell signaling. PRP therapy is a relatively new method that the scientific community is beginning to apply to restore damaged tissues like the liver, bones, articular cartilage, tendons, and pulp of the teeth. Based on the recognized successful results, PRP is to date being studied intensively for a novel biomaterial that is capable of enhancing the healing of injuries on the tissues including muscles, skin, ligaments, tendons, bones and cartilage [25-29].

To the extent of our knowledge, the efficacy of various concentrations of PRP has not been investigated in connection with cutaneous wound healing in experimental rabbit models in Pakistan. Therefore, this is the first study to explore the

therapeutic effect of various concentrations of PRP on cutaneous wound healing in experimental rabbits.

Material and Methods

Ethical statement

The Ethical Review Committee's guidelines and regulations were followed in the approval and execution of this study and all associated procedures (Ethical Approval No. DR/396; Dated: 04/09/2023) at the Department of Veterinary Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Animals

This research employed an animal experimental design, using male rabbits that met the following inclusion criteria: they had to be between the ages of 4-6 mon and 2.3–3.5 kg. The rabbits were maintained at the animal house facility of the Department of Veterinary Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan. They were given commercial feed, vegetables, and purified water that was freely available all the time. All rabbits were kept on a 12 h light: 12 h dark cycle (light intensity) at the time of sample collection. Any rabbit did not receive extra exercise and was also allowed to move freely but within its cage; that is, two rabbits in a cage measuring 160 cm x 100 cm x 70 cm. Before the actual commencement of the study, the animals were allowed to become familiar with and become tractable to approaching and handling for a period of 10 days.

Preparation of platelet-rich plasma (PRP)

The rabbits were anaesthetized with Xylazine @ 5 mg/kg (Xylaz 20 mg/mL, Mylab Pakistan Pvt. Ltd.) and Ketamine @ 35 mg/kg (Ketamine 50 mg/mL, Panpharms, France) intramuscularly. Antisepsis of the jugular vein was preceded by a 5 % povidone-iodine solution. Before undergoing venipuncture, the rabbits were placed in sternal posture with their necks extended as far dorsally as possible and then 5 mL of blood was collected using a 10 mL syringe attached to a 25-gauge needle. Samples were separated using two sterile 3.6-mL-capacity tubes containing sodium citrate to prepare PRP, and 0.8 mL of blood was collected into another tube. Once the platelet count was done, the PRP preparation was carried out by double centrifugation [30]. The first centrifugation was done at 1600 rpm for 10 min, which resulted in the separation of red blood cells and plasma containing platelets and leucocytes. Then, after opening the tubes, plasma was transferred into another tube using a 1 mL pipette (Thermofisher Scientific). After that, at 2000 rpm for 10 min, the second centrifugation was carried out, which resulted in two parts: PPP, and

the bottom was platelet-rich plasma. The upper portion was platelet-poor plasma (upper PPP was discarded, and the bottom one (PRP) was collected. The collected material being carefully stirred in order to promote platelet resuscitation, which resulted in the production of PRP. After plasma enrichment, platelet concentration was measured using an automatic device with 80 µL of PRP sample to ensure a platelet count over 1,000,000/µL. At the end, 0.3 mL of calcium chloride dihydrate (Product ID: TDF-HEC-BDH9224; VWR PROLABO Chemicals, USA) was added for activation purposes.

Creation of cutaneous wound

The animals were anesthetized after anesthetic administration protocol mentioned earlier. While xylazine promotes beneficial muscle relaxation, ketamine has analgesic effects. It appears that administering Xylazine along with ketamine has no effect on the physiological markers. Furthermore, this technique offers an extremely effective anesthetic process for immaculate induction, appropriate muscular relaxation, prolonged anesthesia, and a smooth recovery. Prior to aseptic surgery, the dorsal surface of every animal was cleaned and shaved using 5 % povidone-iodine. On the right side of the dorsum, approximately 2 cm from the midline, 2 × 2 cm² full-thickness excisional skin wounds were created in each animal using a sharp surgical knife. Full-thickness wounds are those that penetrate both the epidermis and dermis layers of skin, as well as subcutaneous fat but not muscle [31].

Experimental design and treatment

This study randomly allocated 24 local adult male rabbits, weighing 2.5-3 kg and aged 4-6 mon, into four groups: group A (control), group B (0.2 mL PRP), group C (0.4 mL PRP), and group D (0.6 mL PRP), with 6 animals in each group. Animals in group B were injected with 0.2 mL of autologous PRP; animals in group C were injected with 0.4 mL of autologous PRP subcutaneously; and animals in group D were injected with 0.6 mL of autologous PRP subcutaneously on days 1, 7, and 14. The determination of the wound site is crucial; the injured area shouldn't be close to the neck to allow for simple handling of rabbits from the neck without causing damage to the wounds. Hence, a surgical excision was made on the dorsum for this study. Using scissors measuring 25 cm/10 (Noorani Surgical), each full-thickness skin wound (2 x 2 cm²) on the dorsum region was first dehaired, and its margins were shaved before it was detached from the subcutaneous tissue. After seven days, the bandage was taken off, the wound was cleaned with sterile saline, PRP was injected, and the dressing was replaced. Up until day 14, the therapy was

administered every seven days. Intramuscular administration of injection meloxicam (Vetcon Pharma) was done two times a day for three consecutive days. Rabbits were kept in clean cages allowing limited movement.

Examination of cutaneous wound

In each group, the wounds were measured on days 1, 7, 14, and 21 following the procedure. The following observations were made: wound healing, inflammatory exudate features, bleeding presence or absence, and wound infection presence or absence. On days 1, 7, 14, and 21, the wounds were photographed, and the vernier calipers device was used to quantify the wounds. The wound contraction rate was calculated on days 7, 14, and 21 by using the following formula [32].

$$\text{Wound contraction rate (\%)} = \frac{W_0 - W_n}{W_0} \times 100$$

Where, W_n : the wound area at days 7, 14, and 21, W_0 : the wound area at day 1.

Blood sampling

Two mL of blood were drawn on earlier mentioned respective days from jugular vein in the vacutainer. The blood samples were moved to the Department of Parasitology laboratory at the University of Veterinary and Animal Sciences in Lahore, Pakistan. Blood centrifugation was done using temperature controlled centrifuge machine (HARRIER 18/80 UK) for 15 min at 4 °C at 3000 rpm. After that the serum was kept for further processes.

Histopathology

Hematoxylin and eosin stain

The tissue biopsy sites were cleaned using gauze and saline solutions. By means of a 6-mm surgical biopsy punch (Kai Medical@Japan), full-thickness samples were obtained. Each animal in both groups had the wound biopsied at various times from the 4- to 5-mm skin edge and the 3- to 4-mm intact skin area. The sample was initially preserved for 24 h using 10 % neutral-buffered formalin. The sample was then switched to a 70 % alcohol fixative later on. After separating the biopsied tissue into 1.5-mm widths and fixing it in alcohol at varying concentrations, the tissues were embedded in paraffin. Additionally, tissues were stained with hematoxylin and eosin in order to analyze tissue morphology using conventional light microscopy techniques. Different types of white blood cells (PMNL), vascularization, a certain number of fibroblasts, and a certain degree of re-epithelialization were found in biopsy samples, along with some other semi-quantitative factors. Sabol *et al.* (2012) described a semi-quantitative scoring

system with the following levels of re-epithelialization, neovascularization, PMNL, and fibroblasts: 0 for nonexistent, 1 for minimal, 2 for mild, 3 for moderate, and 4 for marked [33].

Masson's trichrome stain

The University of Veterinary and Animal Sciences, Lahore, Department of Pathology Laboratory was the precise spot for the collagen fiber staining procedure. The University of Rochester Medical Center's Center for Musculoskeletal Research (CMSR) established and followed protocols for staining. After being deparaffinized, tissues were rehydrated. For 15 min, Bouin's fixative (Fisher Scientific) was used at 58 °C. The slides were cleaned with distilled water for 10 min after cooling. Subsequently, the biopsy tissue sample was dyed for 5 min using Fisher Scientific's Biebrich Scarlet Acid Fuchsin. The sample (Fisher Scientific) was then stained for 2 min using a 1% phosphomolybdenum-phosphotungstic acid solution. After 5 min of staining with Aniline Blue Solution as a counterstain, the tissues were cleaned with distilled water. Furthermore, a 1% acetic acid aqueous solution was used to rinse the biopsied tissue samples. Slides were cleaned, dried, and mounted at the conclusion. Observations and photomicrographs were obtained at each biopsy sampling site to evaluate the development of regenerative cells and the improvement of cutaneous wound healing. This study used a simple descriptive scale with a range of 0 to 3 to assess the amount and distribution of collagen for each attribute on trichrome-stained slides. A score of 0 meant that there were no organized collagen fiber formations or collagen bundles in the samples. Sufficient and well-organized collagen fiber production was indicated by a score of 3.

Oxidative stress analysis

MDA concentration

The method used to measure the serum malondialdehyde (MDA) concentration ($\mu\text{mol/mL}$) was according to [34]. 100 μL of plasma, 375 μL of 20.0 % acetic acid (pH 3.5), 375 μL of 0.8 % thiobarbituric acid, and 50 μL of 8.1% sodium dodecyl sulfate were added to a reaction mixture. Samples were then centrifuged for 10 min at 3000 g after being heated to 95 °C for an hour. Using an Epoch Reader Microplate spectrophotometer (UV-2800, Biotechnology Medical Services, USA), the absorbance of the supernatant was determined at 532 nm. The MDA content was expressed in $\mu\text{mol/mL}$ ($\epsilon = 1.56 \times 10^5 \text{ mmol/L/cm}$).

CAT activity

In accordance with [35], the pace at which the substrate H_2O_2 degrades indicates the catalase's catalytic activity. A drop of hydrogen peroxide was absorbed at 240 nm every 30 seconds for 3 min in order to gauge the rate of breakdown. The catalase (CAT) activity were expressed in mmol/min. CAT activity is the amount of catalase enzyme required to break down 1 μ mole of hydrogen peroxide per second at 25 °C.

Statistical analysis

All data were statistically assessed by a repeated measure one way ANOVA between the groups with a post-hoc Tukey's test using the graph pad in Prism version 8. All data were presented as the mean $\pm$ standard error (mean $\pm$ SD). The level of significance showed “*” ($P < 0.05$) and “***” or “###” ($P < 0.01$).

Results

Effects of various concentration of PRP on clinical wound evaluation and wound healing rate

Clinical wound evaluation

For clinical evaluation, all wounds were almost similar in size, as shown on day 1, and then wound sizes were measured at intervals of 7 days until the end of the study (day 21). The results in (Fig. 1) demonstrate that wound sizes gradually decreased in each group, with no significant differences between the groups. On day 7, a mild exudate has been observed only in groups A and B. Scar formation was observed in all the groups on day 14. The wound was still open in groups A and B, while the wounds in groups C and D were healed completely on day 21.

Wound contraction rate (%)

The wound contraction rate of all four groups was calculated on days 7, 14, and 21, and it was observed that the percentage of contraction was increasing among all the groups at all four different time intervals. No statistically significant difference ($P > 0.05$) was observed in different groups on days 7 and 14. A highly significant difference ($P < 0.01$) was observed in groups C and D compared to the control group, while a significant difference was observed in group B as compared to the control group on day 21, as shown in Fig. 2.

Effect of autologous PRP on re-epithelization and neo-vascularization

The results of histopathology for re-epithelization and angiogenesis showed that the wounds that were treated with different concentrations of PRP healed on day 21, as shown in Fig. 3. The histopathology results of the 0.6 mL PRP group showed that the re-epithelization is almost complete. The area of dermis shows the proliferation of new blood vessels

(angiogenesis). There is moderate infiltration of mono-nuclear inflammatory cells and proliferation of fibroblasts and collagen fibers in parallel, while the 0.4 mL PRP group showed more thickness of the keratin layer. A moderate number of keratohyaline granules are seen. The epidermis is thicker, and infiltration of a few mononuclear cells (PMNL) is seen. There is a marked proliferation of fibroblasts and angiogenesis. In the 0.2 mL PRP group, there is partial re-epithelization with the presence of marked dead tissue mass. There is marked proliferation of fibroblasts and collagen fibers with moderate angiogenesis. In the control group, no re-epithelization is seen. There is a mild dead tissue mass and mild angiogenesis.

Statistically, there was a highly significant ($P < 0.01$) increase in the level of re-epithelization, angiogenesis, and fibroblast in the 0.4 mL and 0.6 mL PRP groups, while the level of PMNL cells significantly ($P < 0.05$) decreased compared with the control group on day 21. Similarly, compared with the control group, the 0.2 mL PRP group showed a non-significant ($P > 0.05$) increase in the level of re-epithelization, angiogenesis, and fibroblast, while the level of PMNL cells was non-significantly ($P > 0.05$) reduced, as indicated in Fig. 4.

Effect of various concentration of autologous PRP on collagen fiber formation

The qualitative examination of collagen fibers results of Masson's trichome on day 21 is indicated in Fig. 5. The 0.2 mL PRP group showed less dense and organized collagen fibers, while the control group showed minimal and unorganized collagen fibers. However, the 0.4 mL PRP group showed well organized collagen fibers and fibroblasts perpendicular to the epidermis were present, while the 0.6 mL PRP group showed that there were dense and tightly packed collagen bundles oriented parallel to the overlying epithelium. Statistically, the abundance and arrangement of collagen fibers significantly enhanced ($P < 0.01$) in both 0.4 mL and 0.6 mL PRP treatment groups compared to the control on day 21, while the 0.2 mL PRP treated group had a non-significant ($P > 0.05$) increase in collagen fibers number and a significant ($P < 0.05$) increase in arrangement on day 21 (Fig. 6).

Effect of various concentration of autologous PRP on MDA concentration and CAT Activity

On days 1–7, the 0.2 mL PRP-treated group had a lower MDA concentration in their serum, but not significantly ($P > 0.05$). On days 14 and 21, it was significantly ($P < 0.05$) lower than the control group. The groups that were given 0.4 mL and 0.6 mL of PRP had significantly ($P < 0.05$) lower MDA levels on day 7, and they were highly significantly ($P < 0.01$)

lower on days 14 and 21 compared to the control group. All four groups underwent serum CAT activity analysis on days 1, 7, 14, and 21. There was a small but noticeable difference ($P>0.05$) in the activity of the CAT on days 1, 7, 14, and 21 between the groups, as shown in Fig. 7.

Discussion

Regenerative medicine is currently gaining significant attention in the field of wound healing. At present, in treating chronic nature wounds, PRP is being frequently used as an alternative to antibiotics and other harmful medications [36]. Platelet-rich plasma concentration is defined as any blood product with a platelet concentration greater than the baseline, with commercially available systems typically achieving a 2–5-fold increase [37]. PRP generally stimulates epithelial and epidermal regeneration, angiogenesis, collagen synthesis, and tissue repair [38]. While managing cutaneous wounds, given that PRP contains a wealth of useful growth factors for wound healing, it may be a substitute for antibiotics and other natural medicines in curing cutaneous wounds [39]. Previous studies have evaluated the effectiveness of various injectable agents as healing promoters. Still, Tahir *et al.* (2018) have proven that PRP injection is the most suitable healing promoter for rabbit cutaneous wound repair [40]. Thus, in this study, we investigated the effects of various concentrations of PRP on promoting cutaneous wound healing in an experimental rabbit model.

In adult humans, wound contraction usually accounts for a 20 % to 30 % reduction in the size of an excision wound, which is an inevitable part of the healing process [41]. On the other hand, severe wound contraction can result in scarring, which is not ideal for aesthetic purposes, especially when it comes to the face. A recent study found that giving PRP intralesionally to cats with cutaneous deficiencies following subcutaneous tissue excision increases contraction, overall wound healing, and tissue perfusion [42]. A study also demonstrated the therapeutic potential of PRP in treating cutaneous lesions based on superior morphological and morphometric features and histological architecture. Lee *et al.* (2008) reported that no statistically significant differences were observed in wound contraction rate at one week, but the 0.6-ml treatment showed an 11 % increase over the controls. However, at two weeks, the contraction rate in the 0.6- and 0.9-ml groups was significantly lower than the controls (30 % lower) [43]. In our study, there were no significant differences in wound contraction rate on days 7 and 14 in the PRP groups over the control group. On day 21, the 0.2 mL PRP group showed an increased significant difference, while the

0.4 mL and 0.6 mL treatment groups showed a highly significant increased wound contraction rate over the control group.

In order to boost and optimize the re-epithelialization process, PRP injection was done. In this regard, the authors implemented PRP with the intention of using it to help form granulation tissues. This might be because it has been incorporated into surrounding tissue and has been proven effective in the early phases of wound healing by encouraging the angiogenesis of new blood vessels and the epithelialization of the tissue surfaces [44]. Moreover, when analyzing the function of angiogenesis in wound healing, one can pinpoint that it is a significant event that occurs in the framework of the proliferative phase of wound healing. At the same time, endothelial cell migration is considered the primary stage of angiogenesis. Tottoli *et al.* (2020) suggested that this study provided positive evidence, concluding that PRP provided better histological results from the controls on days 7, 14, and 21. Also, freely available samples of PRP wound densities on days 14 and 21 were statistically significantly higher than the control group, manifested as an increase in the number of blood vessels and the number of epithelial junctions. Using the data provided by the researchers, PRP enhanced the formation of blood vessels, rapidly healed wounds, and generated an adequate amount of granulation tissue compared to the normal control group [13]. Strukova *et al.* (2001) stated that activated platelets can minimize the wound surface due to increased fibroblast-macrophage and fibroblast proliferation [45]. It can be induced by VEGF release, hence increasing the growth of endothelial cells and fibroblasts [46]. Mansoub *et al.* (2018) observed effects on the healing of wounds in diabetic rats and pointed out that wound contraction started earlier in groups PRP treated groups or keratinocytes in comparison to the control group [47]. Xian *et al.* (2015) used PRP during the 7, 14, and 21 days of treatment and showed a significant decrease in wound size compared with non-PRP group treatments in rabbits [48]. Single or double-shot PRP injections into a skin wound hasten the contraction and healing of the lesion. Compared to wounds treated with saline or betadine, a full epidermal and dermal layer covered the entire wound area more quickly. The wounds healed almost simultaneously in both single and double PRP injection techniques; however, the double PRP injection wound's epidermal layer looked better than the single shots [12]. Another study by Xu *et al.* (2020b) revealed that the PRP group significantly accelerated wound closure compared to the control group, resulting in a clean wound with significantly less exudation [49]. In our study, on day 21,

treatment with autologous 0.4 ml PRP and 0.6 mL PRP showed significantly increased wound epithelial cell thickness, resulting in the healing of all full-thickness skin wounds with more fibroblasts and neovascularization than control wounds, while 0.2 mL PRP showed non-significantly increased re-epithelization and neovascularization.

According to Soundia et al. [50], collagen fibers are a component of the super-extracellular mesh, which acts as the tissue's underlying framework and directs cell proliferation and repositioning during skin wound healing. Measures relating to wound contraction, closure, and collagen content are thought to exist. Greater collagen causes wounds to contract more, but other factors, such as the quality of the collagen, can also play a role. Through remodeling and the secretion of extracellular matrix components like collagen and fibronectin, fibroblasts are essential to the healing process of wounds. According to Kaleci et al. [51] fibroblast contraction forces are necessary for wound closure. Wound healing treatments may produce better-quality collagen fibers. Results from a study indicated that wounds three weeks post-operation showed proliferation of fibroblasts with deposition of collagen using platelet-rich plasma, while in the control group, histopathological examinations three weeks post-operation showed an area of chronic inflammatory infiltrate with tissue debris and giant cells, and the healing of the treated group was better than that of the control group [52]. Unlike the other groups, PRP-treated groups demonstrated complete wound closure with healthy granulation tissue. In this study, collagen fibers were arranged as dense and tightly packed collagen bundles oriented parallel to the overlying epithelium in cutaneous wounds treated with 0.4 mL and 0.6 mL PRP but less in control wounds, indicating that 0.4 mL and 0.6 mL PRP treatment promoted granulation tissue development on day 21, while 0.2 mL PRP showed less dense and organized collagen fibers.

Production and the scavenger process balance ROS levels. Their excessive production may lead to oxidative stress affecting cellular biomolecules (lipids, sugars, proteins, and polynucleotides) [53]. Angiogenesis-related pathological damage will ultimately result from long-term instability and high ROS concentrations, rendering the blood flow and nutritional requirements insufficient to support wound healing [54]. Numerous cellular stress biomarkers and antioxidant defense mechanisms can be of great value while investigating wound healing.

Mentioning MDA as the most important one. This is a secondary product of lipid peroxidation and a potential biomarker for oxidative damage. It is the main indicator of lipid peroxidation determined by titration against thiobarbituric acid (TBA), which is a cell damage indicator [55]. Melnikova et al. (2021) found that wound therapy with both hydrophilic and lipophilic substances in rats resulted in increased antioxidant enzyme activity (CAT) in erythrocytes and decreases in MDA concentrations after 7, 10, and 21 days [56]. It is attributed to lowering the intensity of free radical oxidation. In our study, on days 7, 14, and 21, MDA levels declined in the 0.4 mL and 0.6 mL PRP groups and 0.2 mL declined on days 14 and 21, while CAT non-significantly increased on different time intervals, which was in agreement with former studies [56-58].

Conclusion

In conclusion, 0.4 mL and 0.6 mL PRP wounds accelerated cutaneous wound healing in rabbits by promoting re-epithelization, neovascularization, collagen organization, and inhibiting oxidative stress compared to the control wounds. However, 0.6 mL of PRP was superior to 0.4 mL of PRP. Therefore, this study suggests using autologous 0.4 mL PRP and 0.6 mL PRP to treat cutaneous wounds in rabbits, which is a safe and effective method.

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Author's contribution

Hamid Akbar, Ayesha Safdar and Aneela Zameer Durrani conceptualized the hypothesis of this manuscript. Fawad Khalil Pitafi conducted the research. Muhammad Abid Hayat, Ayesha Safdar and Hamid Akbar statistically analysed the data. Fawad Khalil Pitafi performed the experiments and wrote the manuscript. Ghulam Mustafa analyzed the histopathological findings. Hamid Akbar, Muhammad Abid Hayat, Muhammad Talha Sajjad, and Abdul Asim Farooq critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

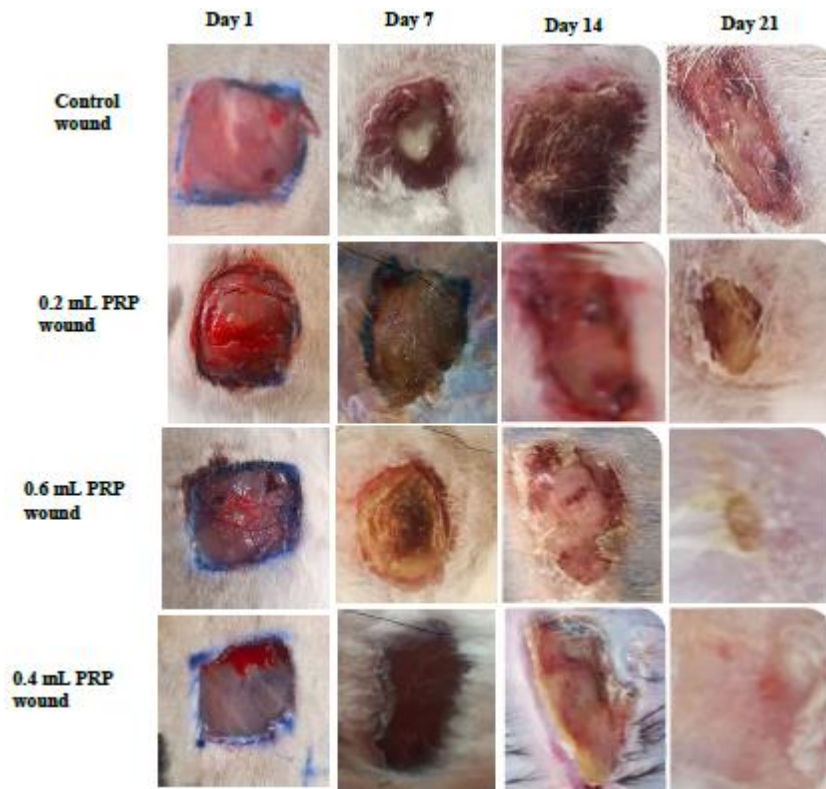


Fig. 1. Effect of various concentration of autologous PRP on clinical wound evaluation. Images of rabbits in the control group and PRP treated groups at days 1, 7, 14, and 21 after modeling. The results show that wound sizes gradually reduced in each group, with no significant differences found between the groups. On day 7 a mild exudate has been observed only in control group and 0.2 mL PRP group. Scar formation was observed in all the groups on day 14. Wound was still open in control group and 0.2 mL PRP group, while the wounds in 0.4 mL and 0.6 mL PRP treated groups were healed completely almost with scar formation on day 21.

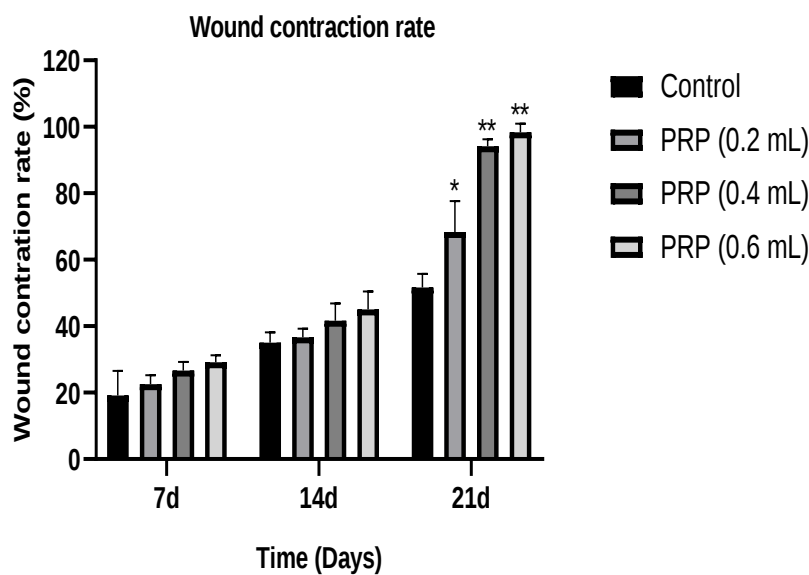


Fig. 2. Effect of various concentration of autologous PRP on wound contraction rate (%). The wound healing rate on days 7, 14, and 21 between control group and different doses of PRP treated groups. Compared with the control group, * $P < 0.05$, ** $P < 0.01$.

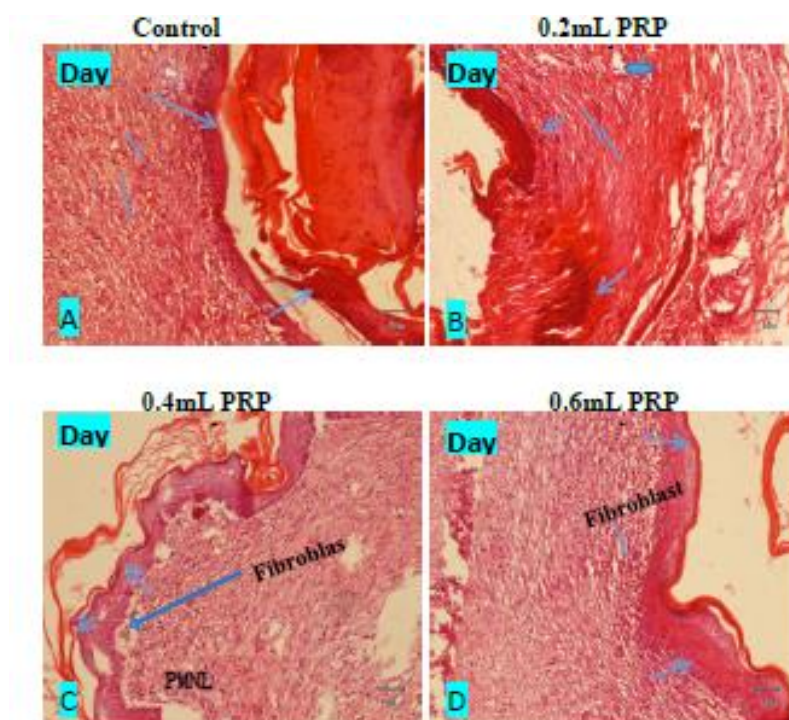


Fig. 3. Effect of various concentration of autologous PRP on re-epithelization and angiogenesis (HE stain). Bar = 50 μ m; A: Arrow showed no re-epithelialization with presence of mild dead tissue mass. Line show less number of fibroblast B: Arrow showed partial re-epithelialization and (line indicate) marked proliferation of fibroblasts with (circle shows) moderate angiogenesis. C: Arrow showed more thickness of keratin layer and more thick epidermis with infiltration of a few PMNL. There is marked proliferation of fibroblasts and angiogenesis. D: Arrow showed almost complete re-epithelialization. The area of dermis show proliferation of new blood vessels (angiogenesis). There is moderate infiltration of mono-nuclear inflammatory cells and increase proliferation of fibroblasts.

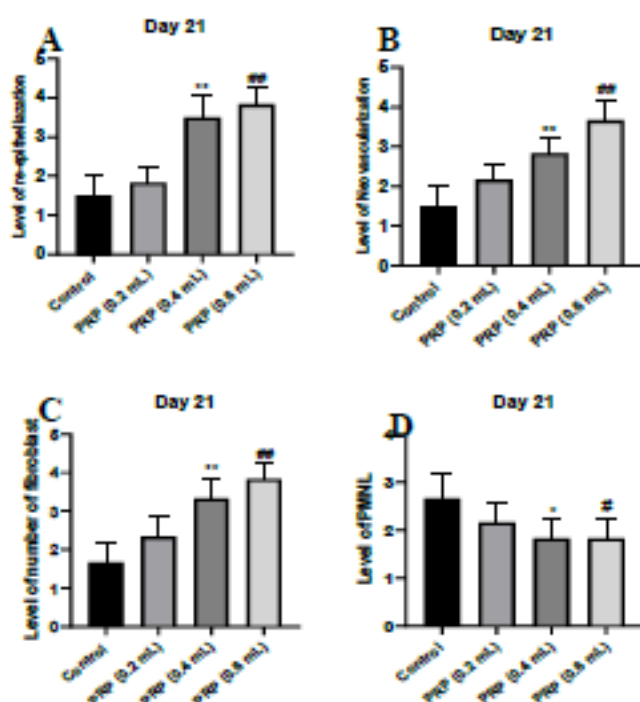


Fig. 4. Effect of various concentration of autologous PRP on re-epithelization and angiogenesis (lesion score). A: Lesion score of re-epithelialization on day 21, B: Lesion score of neo-vascularization on day 21. C: Lesion score of fibroblasts on day 21, D: Lesion score of PMNL on day 21. Compared with the control group, * $P < 0.05$, ** or ## $P < 0.01$.

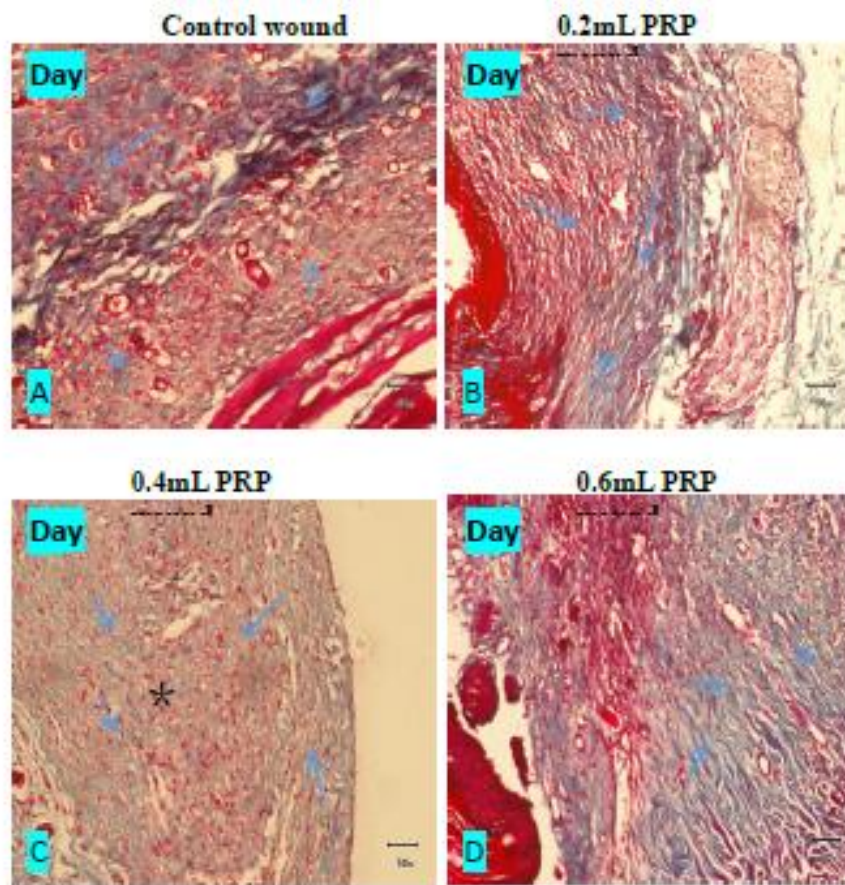


Fig. 5. Effect of various concentration of autologous PRP on collagen fiber formation (Masson's trichrome stain). Bar = 50 μ m; A: Arrow showed minimal and unorganized collagen fibers. B: Arrow showed less dense and organized collagen fibers. C: Arrow showed well organized collagen fibers and *shows fibroblasts perpendicular to the epidermis. D: Arrow showed dense and tightly packed collagen bundles oriented parallel to the overlying epithelium.

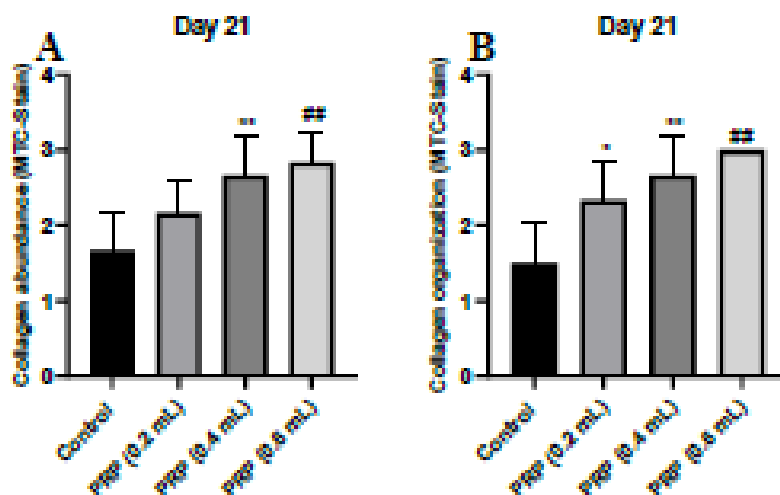


Fig. 6. Effect of various concentration of autologous PRP on collagen fiber formation (lesion score). A: Lesion score of collagen abundance (MST stain) on day 21, B: Lesion score of collagen organization (MST stain) on day 21. Compared with the control group, * $P < 0.05$, ** or ## $P < 0.01$.

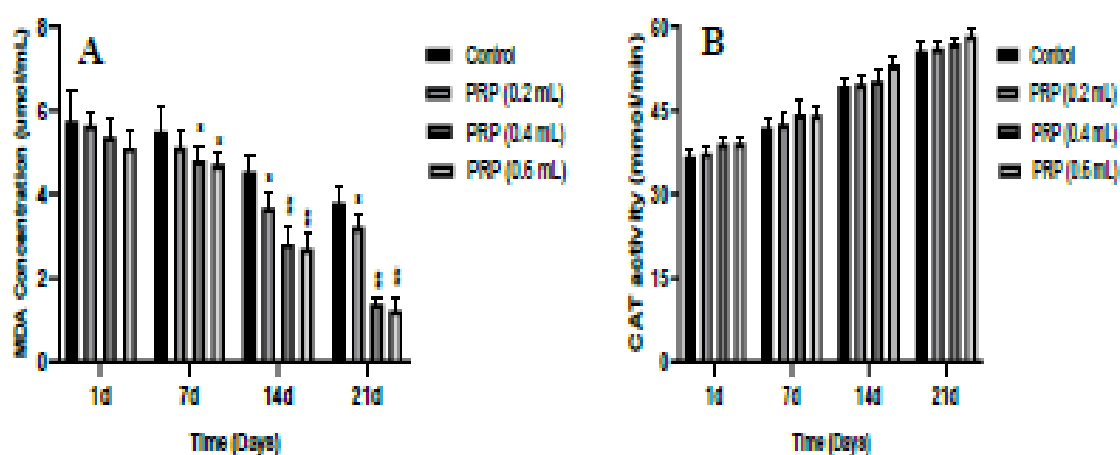


Fig. 7. Effect of various concentration of autologous PRP on oxidative stress markers. A: indicates MDA concentrations between different groups. B: indicates CAT activity between different groups. Compared with the control group, * $P < 0.05$, ** $P < 0.01$.

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