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Effect of Autologous Platelets-rich Plasma Alone or with Hydrofiber Dressing on Cutaneous Wound Healing in Rescued Donkeys

Fawad Khalil Pitafi[1], Hamid Akbar[1*], Ayesha Safdar[1], Aneela Zameer Durrani[2], Muhammad Abid Hayat[4] and Ghulam Mustafa [3]

[1] *Department of Veterinary Surgery, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan.*
[2] *Department of Clinical Medicine, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan.*
[3] *Department of Veterinary Pathology, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan.*
[4] *Jiangsu Key Laboratory of Medical Science and Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013, China.*

Abstract

IN EQUINES, cutaneous wound recovery is typically complex and delayed. This study was aimed to investigate the effect of autologous platelet-rich plasma (PRP) alone or with hydrofiber dressing (HFD) on cutaneous wound healing in rescued donkeys. A total of 18 rescued donkeys were selected and allocated into three groups: A, B, and C. Group-B donkeys (n = 6) were treated with PRP, while group-C donkeys (n = 6) were treated with PRP, and then HFD was applied. The wounds in group A donkeys (n = 6) did not undergo any treatment and were only irrigated with sterile saline. An average full-thickness (3–4 cm^2) of skin wounds on each donkey were selected. Collagen reestablishment was monitored via Masson's trichrome staining, and wound re-epithelialization was evaluated through hematoxylin and eosin staining. On days 1, 7, 14, and 28, catalase (CAT) activity and malondialdehyde (MDA) levels were also measured in blood samples. We observed that PRP + HFD and PRP-treated wounds showed a significantly increased amount of re-epithelialization, number of fibroblasts, neovascularization, and collagen amount with organization on day 28 than control wounds. The levels of MDA significantly decreased, while catalase activity non-significantly decreased in PRP and PRP + HFD-treated wounds than the control wounds on days 7, 14, and 28. In conclusion, autologous PRP alone or with HFD groups enhanced wound healing by lowering oxidative stress, accelerating wound epithelialization, and producing more organized tissue with interlocking collagen bundles than control group. Nevertheless, more ultrasound research is needed to find out how PRP combined with HFD affects the healing of cutaneous wounds in rescued donkeys.

Keywords: Cutaneous Wound Healing, Rescued Donkeys, Platelet-Rich Plasma, Hydrofiber dressing, Oxidative Stress.

Introduction

Skin, the largest connective tissue generated by vertebrates, makes up about 10 % of body mass and covering almost the entire surface of the body [1]. This has the capacity for self-repair and regeneration and plays a role in the body's defense system [2]. A wound is a change in the skin's functional morphology and physical integrity [3]. Wound healing is a biological process that occurs whenever the skin rebuilds tissue and creates collagen scar tissue in the place of an absent or compromised area [4]. In veterinary clinics, several forms of skin wounds are frequently seen. Cutaneous wounds in equines, donkeys, and mules are more prevalent. Chronic, non-healing, exuberant granulation tissue (proud flesh) wounds in equines have a negative impact on their athletic performance and are a major source of concern for their owners. Many variables,

*Corresponding authors: Hamid Akbar, Email: hamid.akbar@uvas.edu.pk, Tel.: +92 321 4551700
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including the length of therapy and the treatment plan, might impact the recovery of serious wounds and have an impact on the rate and duration of healing [5]. Considering the latest innovations in skin-closing technologies and processes, surgeons continue to believe the wound healing process is problematic [6]. Any wound that is not treated promptly or does not heal in the normal order of phases is considered chronic [7]. Delays in wound healing and the expense of restoring the injured area are the main obstacles to adequate wound healing, particularly in equines [8]. Therefore, in order to get the best outcomes, it is essential to use cutting-edge advancements to boost the repair process.

Autologous platelet-rich plasma (PRP) is a type of plasma component that contains three to seven times as many platelets as whole blood [9, 10]. PRP is made by centrifuging the patient's peripheral blood. It also contains supraphysiological growth factors (GF) such as insulin-like GF I, II, epidermal GF, connective tissue GF, platelet-derived GF, neural GF, vascular endothelium GF, hepatocyte GF, interleukin 8 (IL8), fibroblasts GF, and transformed GF, in addition to histamine, serotonin, calcium, zinc, superoxide dismutase (SOD), and adenosine triphosphate (ATP). These crucial elements contribute to its effective application in dermatology, orthopaedics, and mammalian reproduction [9, 11, 12, 13]. Platelets are an excellent source of growth factors to react at the site of an injury and actively contribute to the extremely successful repair of skin wounds [14]. PRP therapies are now the best options because they have been shown to improve patient outcomes and offer potential therapeutic benefits [15, 16].

In recent years, a variety of wound dressings have been developed, significantly improving the area of wound healing. Diverse forms of modern dressing products can be categorized into films [17], foams, composites [18], sprays [19], hydrocolloids [20], gels [21], hydrofibers, and hydrogels, each of which represents a distinct array of properties. By absorbing wound exudate and transforming it into a gel, hydrofiber dressing offers the optimal settings for wound repair. Moreover, these dressings are impermeable and saturated on the outside of the hydrocolloid circumference, which might boost their antimicrobial abilities [22]. Coulson and Dumville et al. (2016) pointed out that the described dressings can be retained for a duration of three–seven days [23]. Besides incorporating an antibacterial antibiotic relevant to the given wound to reduce the risk of infection, several manufacturers have designed advanced dressings that will enhance the wound healing process by forming and maintaining a warm, moist wound base under the dressing [24]. The clinical utility of AQUACEL® Ag+ dressing for non-healing, infected wounds was demonstrated by the results of a study conducted by [25]. These are also thought to be advantageous because they don't need to be changed every day depending on the state of the wounds, which may lessen discomfort and hasten the healing process [26].

To the greatest extent of our understanding, no study has been done on the effectiveness of PRP with hydrofiber dressing for cutaneous wound healing to treat cutaneous wounds presented clinically in donkeys in Pakistan. Therefore, this is the first study aimed at exploring the therapeutic importance of PRP alone or in combination with hydrofiber dressing in the recovery of equine cutaneous wounds.

Material and Methods

Animals

The study was conducted on rescued donkeys in Pakistan's Punjab province's Lahore district that had long-term skin wounds on their back, near neck, and wither region. All of the animals in the experiment were kept in the indoor stables of the Society for the Prevention of Cruelty to Animals in Lahore before the experiments began. Each experimental animal got access to enough dry food and water.

Preparation of platelet-rich plasma (PRP)

The day before surgery, donkey PRP was prepared according to DeRossi et al. (2009) from two tubes containing 10 mL of donkey whole blood [8] . Two Falcon tubes (15 mL each) with 10% sodium citrate anticoagulant were used to collect blood. To separate plasma red blood cells, tubes were spun in a scentrifuge (SCILOGEX) at 300 g/10 m for five minutes. The largest platelets and the majority of white blood cells were found in the midzone, a thin layer of white blood cells that sits between these two layers. After removing the top portion of each tube, 500 uL of plasma was placed into tube 1. This fraction was used when making autologous thrombin. For PRP, the residual plasma and intermediate area in tube 1 were transferred to the new tube 2. If incubation is required, keep the tubes warm. In tube 1, 300 uL of 10% calcium chloride was added, mixed well, and kept at 37 °C for 15 minutes. Subsequently, a second centrifugation of both tubes 1 and 2 was done at the speed of 640 g/10 m of the centrifuge.

The amount of thrombin available in tube 1 being very much higher, its utilization up to the maximum was ensured. Following homogenization, tube 1 thrombin in a 2:1 ratio (2 mL PRP:Into tube 1, 1 mL thrombin) was added to half the contents of tube 2. Neubauer's chamber and trypan blue were used to aid in cell distinction in order to arrive at the number of platelets [8]. It was enabled to establish the optimum platelet enrichment at 4.0's baseline values. The platelets extracted from PRP had an average of 13.4 x 10^5, whereas the whole blood sample had an average of 3.2 x 10^5.

Experimental design and treatment

A total of eighteen adult donkeys aged 5-10 years and weighing 350–450 kg having full- thickness (3-4 cm^2) average size cutaneous wounds were selected and allocated randomly into three groups: group A (control), group B (autologous platelet-rich plasma-PRP), and group C (PRP + hydrofiber dressing (HFD), with six animals in each group. Autologous PRP (0.8 mL) was injected subcutaneously in wounds of group-B animals, while wounds in group-C animals underwent a subcutaneous injection of autologous PRP (0.8 mL), followed by hydrofiber dressing (AQUACEL™ Ag+ Extra-Convatec, USA) application on days 1, 7, and 14. The wounds of animals in group A did not go through any treatment and were only irrigated with sterile saline and were then covered with sterile gauze same as in group B after PRP injection. For the sake of animal welfare, prophylactic systemic antibiotics such as Biocon 5 gm Inj. (Vetcon Pharma) including benzylpenicillin, procaine penicillin, and streptomycin sulfate were given intramuscularly twice a day, and throughout the trial, the donkeys were kept in clean stables with little exercise and no anti-inflammatory medication given to them.

Examination of clinical wound

Before the procedures, all the donkeys were given an intravenous dose of Xylazine hydrochloride (Xylaz® Farvet Holland) at a dose rate of 1.1 mg/kg [27]. In each group, the wounds were measured on days 1, 7, 14, and 28 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 28, 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 [28].

$$\textbf{Wound Contraction rate}\ (\%) = \frac{\mathbf{W0 - Wn}}{\boldsymbol{W0}} \times \mathbf{100}$$

Where, Wn: the wound area at days 7, 14, and 28, W0: the wound area at day 1.

Histopathology

Hematoxylin and eosin stain

After the scrubbing of the biopsy site was done with saline solution by applying it to gauze. A biopsy punch (Kai Medical@Japan) with a 6-mm diameter was used to collect 3–4 mm of full-thickness tissue for histopathology. All the samples were initially preserved in 10% neutral buffered formalin for a period of 24 hours. After that, samples were transferred to a 70% alcohol fixative. Following that, tissue samples were fixed in paraffin and subdivided into 1.5-mm-wide sections. Additionally, samples were stained with hematoxylin and eosin (H&E) to observe tissue anatomical evaluation with the help of a standard light microscope. Biopsied tissues' histology showed semi-quantitative dynamics, such as the degree of vascularization, re-epithelialization, fibroblast count, and polymorphonuclear leukocyte (PMNL) presence. The preceding factors were scored using a semi-quantitative scoring system as follows: 0 represents absenteeism, 1 represents minimalist, 2 represents mildness, 3 represents moderateness, and 4 represents markedness [29].

Masson's trichrome stain

Collagen fiber staining was carried out in the Laboratory of the Department of Pathology, University of Veterinary and Animal Sciences, Lahore, using protocols devised and implemented by the Centre for Musculoskeletal Research (CMSR) at the University of Rochester Medical Centre. Initially, materials were deparaffinized and rehydrated. Bouin's fixative (Fisher Scientific) was used at 58 ºC for 15 minutes. After cooling for 10 minutes, the slides were washed with distilled water. The biopsied tissues were stained for 5 minutes with Biebrich scarlet acid fuchsin (Fisher Scientific). Thereafter, for 2 minutes, samples were stained again using a 1% phosphomolybdenum-phosphotungstic acid solution (Fisher Scientific). The washing of the samples was done with distilled water, followed by staining with an aniline blue solution as a counterstaining agent for 5 minutes. Later on, rinsing of samples using a 1% aqueous solution of acetic acid was performed. At the end, drying out, clearing debris, and mounting slides were done. In this study, a simple descriptive scale of 0 to 3 was used to assess collagen content

and organization in trichrome-stained slides. Samples with a score of 0 demonstrated a lack of collagen bundles or organized collagen fiber production. A score of 3 indicated sufficient collagen fibers and organized collagen fiber production [30].

Blood sampling

On days 1, 7, 14, and 28, 10 mL of blood were drawn from each animal's jugular vein and then transferred into a vacutainer. All the blood samples were moved to the laboratory in the Department of Parasitology, which is a part of the Faculty of Biosciences at the University of Veterinary and Animal Sciences, Lahore, Pakistan. A temperature-controlled centrifuge machine (HARRIER 18/80 UK) was used to centrifuge the blood samples for 15 minutes at 4˚C at 3000 rpm. Following separation, the serum was kept cold for later processing.

Oxidative stress analysis

The quantification of serum malondialdehyde (MDA) (µmol/mL) was performed in accordance with the procedures outlined by Ohkawa et al. (1979) [31]. A reaction mixture comprising 100 µL of sample serum was mixed with 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. At this point, the samples were preheated to 95 °C for 60 minutes before being spun for 10 min at 3000 g. The absorbance of the supernatant was measured at 532 nm using an Epoch Reader Microplate spectrophotometer (UV-2800, Biotechnology Medical Services, USA). The MDA concentration was measured as µmol/mL (ε= 1.56 × 105 mmol/L/cm).

Catalase activity

Serum catalase concentration was analyzed in accordance with [32], which states that the rates at which the substrate H_2O_2 degrades indicate the catalase's catalytic activity. A drop of hydrogen peroxide was absorbed at 240 nm every 30 seconds for 3 minutes in order to gauge the rate of breakdown. The levels of catalase (CAT) were expressed in mmol/min. The catalase activity of a substance is defined as the quantity of catalase enzyme needed to reduce 1 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 Prism version 8. All data were presented as the mean ± standard deviation (mean ± SD). The level of significance showed "*" ($P<0.05$) and "**" or "$^{\#\#}$" ($P<0.01$).

Results

Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on clinical wound evaluation and wound healing rate

For clinical evaluation, all wounds were almost similar with average size of 4 cm2, as shown on day 1, and then wound sizes were measured at intervals of 7 days until the end of the study (day 28). 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 in all the groups. Scar formation was observed in the group C on day 28. The wound in groups B and C were healed almost completely than control wound on day 28.

The wound contraction rate of all three groups was calculated on days 7, 14, and 28, and it was observed that the percentage of contraction was increasing among all the groups at different time intervals. No statistically significant difference ($P>0.05$) was observed in different groups on days 7. A highly significant difference ($P<0.01$) was observed in groups C compared to the control group on day 28, while a significant difference ($P<0.05$) was observed on day 14. Similarly, compared to the control group, there was a significant difference in group B on days 14 and 28, as shown in (**Fig. 2**).

Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on re-epithelization and neo-vascularization

The results of histopathology for re-epithelialization showed that the wounds that were treated with PRP and PRP + HFD healed on day 28. There is increased thickness of epidermis and keratin layer with very few number of mononuclear infiltration is seen while in PRP treated group, there is increased thickness of epidermis and moderate amount of keratohyaline granules. This gives a clue for faster epithelium growth and improved arrangement of connective tissue. On the other hand, in the control group, the wound showed a slight improvement in epithelium growth, There is marked proliferation of fibroblasts and collagen fibers. There is presence of mild dead tissue mass with mild angiogenesis (**Fig. 3**). Statistically, the PRP-treated and PRP + HFD-treated wounds showed a significant

growth of basal lamina than the control group on day 28, while non-significant growth of basal epithelium was recorded between the both treated groups (Fig. 4).

Histopathological examination of the angiogenesis indicated minimum growth of blood vessels in the control group, maximum development in PRP, and marked performance in PRP + HFD-treated groups at day 28 (Fig. 3). Statistically, compared with control wounds, PRP-treated and PRP + HFD groups showed a significantly ($P<0.01$) increased level of neo-vascularization, while both treated groups had a non-significant increase in neo-vascularization on day 28 (Fig. 4). In this study, keratinized epithelium tissues were visible on the edges of the PRP and PRP + HFD-treated wounds. On the other hand, less PMNL was noted in the PRP and PRP + HFD wounds than in the control wounds. Histopathological examination of fibroblast scoring indicates a significant ($P<0.01$) increase in PRP and PRP + HFD wounds compared to the control wounds, but a non-significant increase in fibroblast numbers was examined between the two treatment groups (Fig. 4).

Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on collagen fiber formation

The findings of Masson's trichome staining showed that collagen fibers were significantly increased in PRP and PRP + HFD wounds compared to the control wounds on day 28. The qualitative examination of collagen fibers represented the increased and well-organized collagen fibers in both treated groups as compared to the control group (Fig. 5). Statistically, the amount and arrangement of collagen fibers significantly enhanced ($P<0.01$) in the PRP + HFD treated group than the control group, while PRP treatment group had a significant ($P<0.05$) increase in collagen fibers number and enhanced ($P<0.01$) arrangement than the control group (Fig. 6).

Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on the oxidative stress markers

Serum analysis of MDA concentration was significantly ($P<0.05$) lower in the PRP-treated group on day 14, while it was highly significantly ($P<0.01$) lower on day 28 than the control group. Similarly, it was highly significantly lower ($P<0.01$) on days 14 to 28 in the PRP + HFD treated group than the control group. The activity of the catalase was non-significantly ($P>0.05$) decreased from day 7 to day 28 between the groups (Fig. 7).

Discussion

Cutaneous wound healing is a complex process that involves inflammation, tissue proliferation, granulation, re-epithelization, and wound remodeling [8]. Cutaneous wound healing has attracted a lot of attention in recent years. In this regard, the most recent insight is the use of PRP, which has gained importance as a silver-based hydrofiber dressing therapy in regenerative medicine. In this study, we examined whether PRP alone or with hydrofiber dressing enhances cutaneous wound healing in rescued donkeys.

Wound contraction can be defined as the process that requires the coordination and involvement of cells, the extracellular matrix (ECM), and cytokines, and it is an exquisite phenomenon [33]. This contraction might need transformed growth factor (TGF) and platelet-derived growth factor (PDGF). In contrast, the fibroblast must attach to the collagen matrix via integrin receptors and between collagen bundles. Thus, collagen reformation during the process of becoming a scar at the stage where granulation tissue transforms into scar tissue is dependent on the slow deposition and degradation of collagen. Concerning the issue of collagen degradation at the site of the injury, there are several proteolytic enzymes known as network metalloproteinases that are released by macrophages [34]. The authors noticed that treatment with PRP within the lesions significantly enhances contractions, the overall healing process, and tissue blood supply in the cutaneous deficiencies devoid of the subcutaneous layer in cats [35]. In the present work, the wound contraction rates of all three groups were assessed on days 7, 14, and 28 and statistically analyzed, and the results indicated that the percentage of wound contraction is gradually increasing in all these groups at specific intervals of time. Day 7 results showed no numerical differences in different groups that were statistically significant. Compared to the control, the PRP group had significantly elevated WCR on days 14 and 28, while PRP with hydrofiber dressing significantly augmented the difference from the baseline on day 14 and showed highly significant elevated WCR from the control on day 28.

In order to promote and optimize the re-epithelialization process, PRP injection was done. In

this regard, according to Huang et al. (2016), 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 proved to be effective in the early phases of wound healing through the encouragement of the angiogenesis of new blood vessels and the re-epithelialization of the tissue surfaces [36]. PRP could enhance wound re-epithelialization to extreme levels in contrast to the basic thickening of the neo-epidermal tissue [37]. According to Strukova et al. (2001), activated platelets reduce wound size by increasing the fibroblast-to-macrophage ratio and the number of proliferating fibroblasts [38]. It can be induced by vascular endothelial growth facor (VEGF) release, which promotes new capillary and fibroblast development [37]. As a result, the current study demonstrated that treating autologous PRP with HFD increased the thickness of the epithelial cells, resulting in complete healing of all full-thickness skin wounds with more fibroblasts and newly created vasculature than the control wound. Somewhat, the results anticipated agreed with the past study outlined by [37]. Regrettably, the findings of this study cannot be specifically correlated to VEGF because there is no quantitative analysis technique for donkey VEGF. In the present work, the results from the wound healing study confirmed the elevated level of neovascularization in PRP-treated wounds at both days 14 and 28, in accordance with [39].

Collagen fibers are part of the super-extracellular mesh that is responsible for the general framework of the tissue because they play a vital role in cell migration and repositioning during skin wound healing [40]. For that reason, the ratio of collagen fibers' content in the experimental animals must be studied for skin wound healing [41]. It is assumed here that wound contraction, closure, and the quantity of collagen are different aspects of the same fundamental process. There is a positive relationship between collagen content and wound contraction; however, other features, such as the quality of the collagen, can enhance or hamper this process. Fibroblasts are involved both in the synthesis of the ECM, such as collagen and fibronectin, and in the remodeling of fibrous tissue. Fibroblast contraction forces can be another phenomenon that is vital in wound healing and closure [42]. A study stated that with regard to growth factors platelet-derived growth factor (PDGF-Ab), transformed growth factor-b (TGF-b), and probably also fibroblasts growth factor-2 (FGF-2), in cooperation with the ECM molecules, possibly the fibroblasts of the surrounding tissue at the wound site are stimulated to actively proliferate and produce collagenous fibers that form a bridge between the ends of the wound. In addition, fibroblasts intervene synthetically in the deposition of the extracellular matrix and remodeling [43]. Angiogenesis is a natural process that is required for the maintenance of fresh granulation tissue. Some of the factors concerning angiogenesis include the extracellular matrix found within the wound bed, the movement of endothelial cells, and the mitogenic response [44]. Neo-vascularization offers nutrients as well as oxygen to the wound, facilitates the migration of keratinocytes to and also transports mesenchymal stem cells to the skin, and then supports the process of wound regeneration [45]. The angiogenesis process is maintained by multiple angiogenic factors; however, VEGF is an important growth factor that controls the significant steps of angiogenic processes [46]. On the same note, PDGF controls angiogenesis by attracting and preparing the pericytes [47] to aid in skin wound healing. This study showed that collagen fibres in cutaneous wounds receiving PRP or PRP + HFD were dense and tightly packed collagen bundles paralleling the overlying epithelium than control wounds; therefore, both treatments accelerated the formation of granulation tissue on day 21 of the study; still, PRP + HFD had a slightly better finding than that of the PRP wound.

Oxidative stress is described as an enhancement of reactive oxygen species (ROS generation and a decline in ROS disposal through low or ineffective antioxidant enzymes [48]. Free radicals are formed in quantities as a part of physiological processes; however, increased formation and impaired elimination of free radicals lead to irreversible cellular damage [49]. It has been demonstrated by other research that the production of ROS is most closely associated with the process of wound healing and also participates in various phases of wound healing development [50]. The low levels of ROS are involved in the control of several signal transduction processes in the cells and also supply phagocytes with energy that is required in the process of phagocytizing bacteria [51]. While an adequate amount of active oxygen is needed for wound healing, in numerous instances, active oxygen provides even additional unfavorable biological results. The body generates enzymatic antioxidants in an effort to counteract overoxidation, and out of all of them, catalase (CAT) is the largest. Antioxidants

can stop OR and facilitate the healing of chronic ulcers [52]. However, high levels of ROS by themselves readily interact with the lipids, proteins, and DNA of the cells, causing cell death [53]. Lipid peroxidation, which is promoted by ROS, generates several end products, of which malondialdehyde (MDA) is one. MDA is synthesised as the final product of lipid peroxidation by ROS. MDA evaluates the extent of oxidative stress based on the levels of ROS degradation [40]. It is the primary marker of lipid peroxidation measured by titration with thiobarbituric acid (TBA), which is an agent pointing to cell damage [54]. Based on the work of Sezer and Keskin (2014), the authors claim that MDA is among the most significant biochemical indicators of the extent of cell damage in tissues [55]. Regarding MDA quantity in this study, similar to the previous study [56], there was a highly significant decrease in MDA concentration in PRP and PRP + HFD-grouped donkeys' wounds compared to the control wound. Therefore, the results of this recent study prove that biomarkers engaged in tissue oxidation significantly influence platelets' role in the anti-inflammatory process during wound repair, and restoration [57]. Compared with the control wounds, CAT levels in PRP and PRP + HFD wounds showed no significant reduction. This non-significant reduce in serum antioxidant CAT might be explained by enhanced CAT activity because of cutaneous wounds, which is related to the results represented by Iuchi et al. [58].

Conclusion

Finally, it was found that wounds treated with PRP alone or with HFD accelerated the development of better organized tissue with interlocking collagen bundles, decreased oxidative stress, and accelerated wound epithelialization in rescued donkeys. As a result, our study suggests using autologous PRP in conjunction with HFD as a dependable and useful treatment for cutaneous wounds in donkeys. Nevertheless, more ultrasound research is needed to find out how PRP combined with HFD improves the healing of cutaneous wounds in rescued donkeys.

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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 evaluate the histopathological findings of HE and MST stains. Hamid Akbar and Muhammad Abid Hayat critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

Statement of conflicts of interest

The authors have declared no conflict of interest.

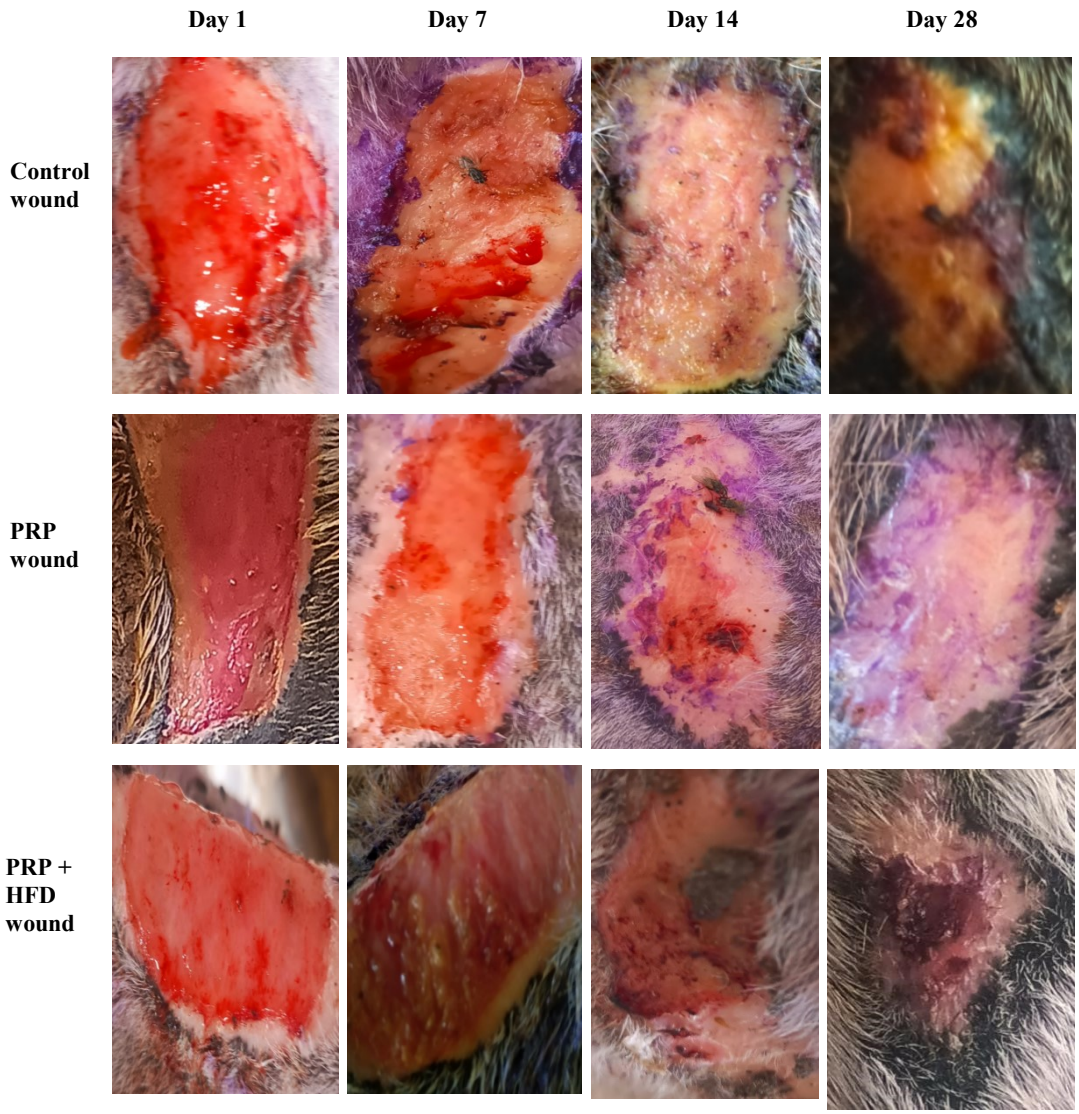

Fig. 1. Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on clinical wound evaluation. Images of donkeys in the control group and PRP and PRP + HFD treated groups at days 1, 7, 14, and 28 after modeling. The results show that wound sizes gradually reduced in each group, with no significant differences found between the groups. On day 7, a mild exudate has been observed in all the groups. Scar formation was observed in the group C on day 28. The wound in groups B and C were almost healed completely on day 28 than control wound.

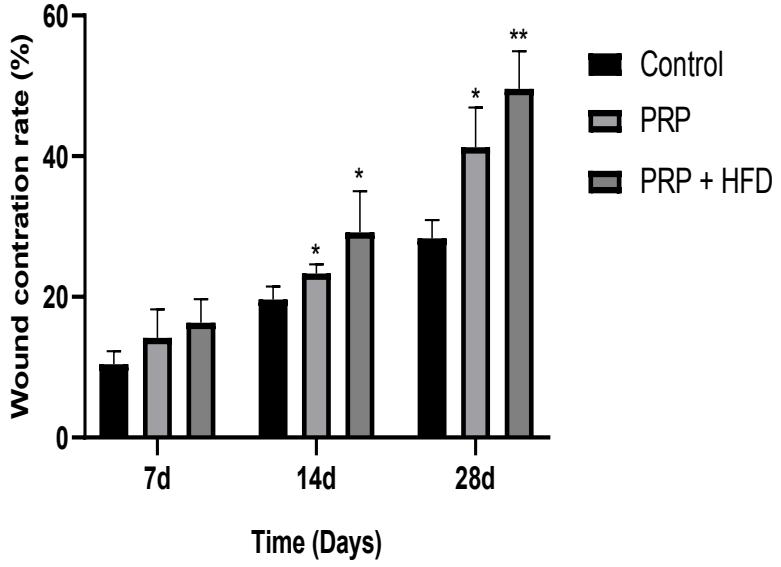

Fig. 2. Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on wound contraction rate (%). The wound healing rate on days 7, 14, and 28 between different groups. Compared with the control group, * P<0.05, ** P<0.01.

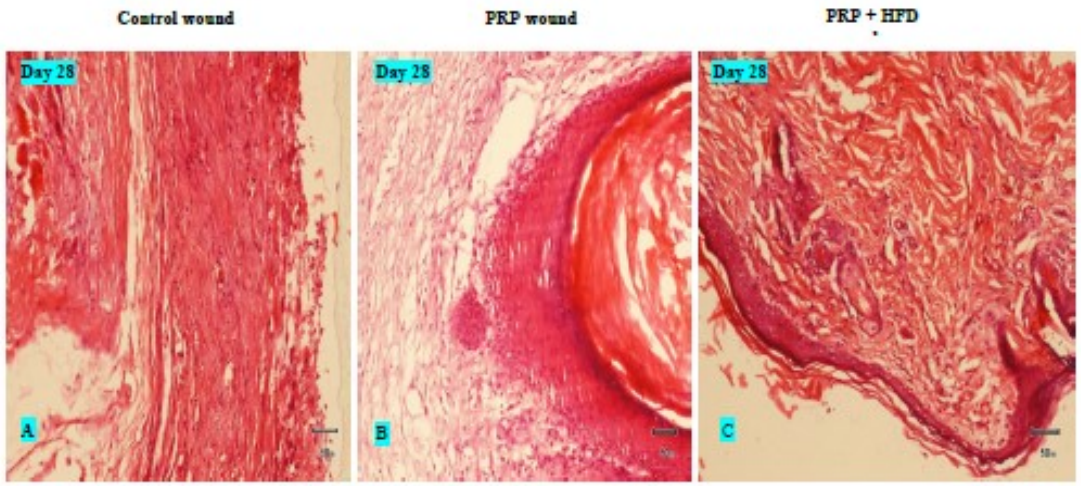

Fig. 3. Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on histopathology (H&E stain). Bar = 50 µm; A: The control wound on day 28 showed minimal number of fibroblasts, moderate PMNL cells, some dead tissue mass along with no re-epithelialization. There is presence of mild dead tissue mass with mild angiogenesis. B: The PRP-treated wound on day 28 showed increased thickness of epidermis and moderate amount of keratohyaline granules and new blood vessels. C: The PRP + HFD-treated wound on day 28 indicated increased thickness of epidermis and keratin layer with very few number of mononuclear infiltration is seen and the formation of new blood vessels.

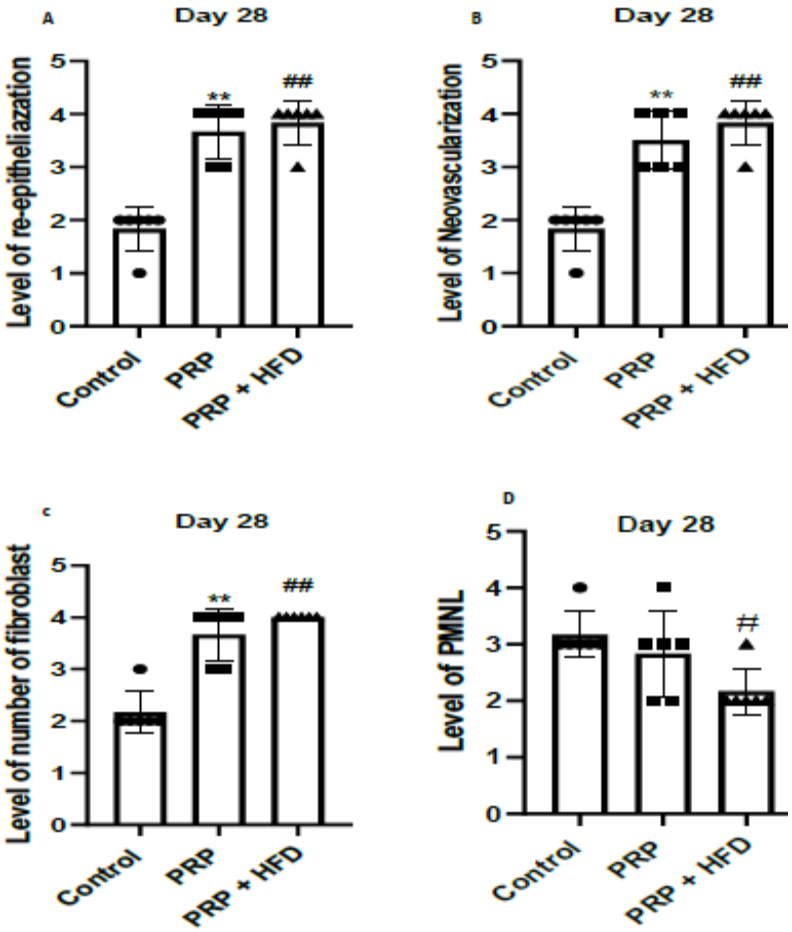

Fig. 4. Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on histopathology (lesion score). A: Lesion score of re-epithelization on day 28, B: Lesion score of neo-vascularization on day 28. C: Lesion score of fibroblasts on day 28, D: Lesion score of PMNL on day 28. Compared with the control group, $^{\#}$ P<0.05, ** or $^{\#\#}$ P<0.01.

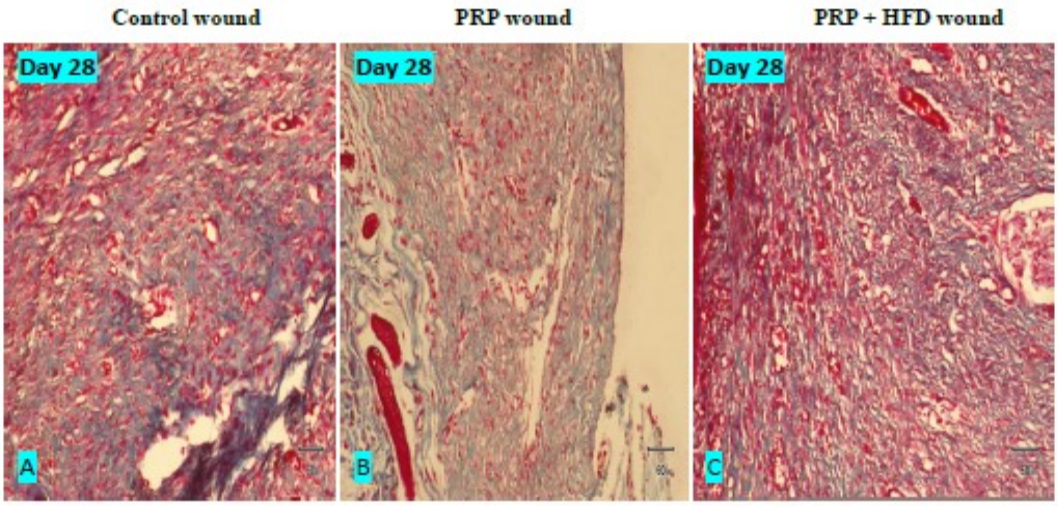

Fig. 5. Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on collagen fibers formation (Masson's trichrome stain). Bar = 50 µm; A: showed minimal and unorganized collagen fibers. B: showed well organized collagen fibers and fibroblasts perpendicular to the epidermis. C: showed dense and tightly packed collagen bundles oriented parallel to the overlying epithelium.

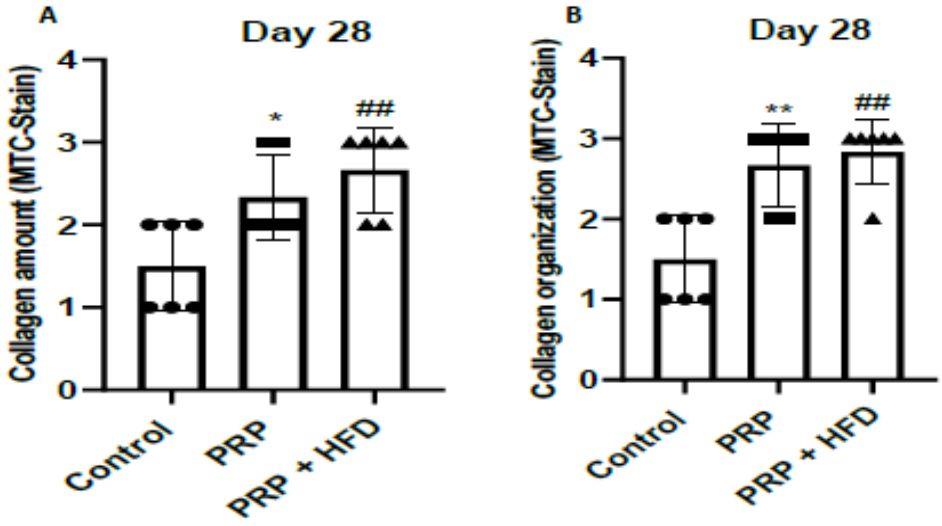

Fig. 6. Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on collagen fibers formation (lesion score). A: Lesion score of collagen abundance (MST stain) on day 28, B: Lesion score of collagen organization (MST stain) on day 28. Compared with the control group, * P<0.05, ** or ## P<0.01.

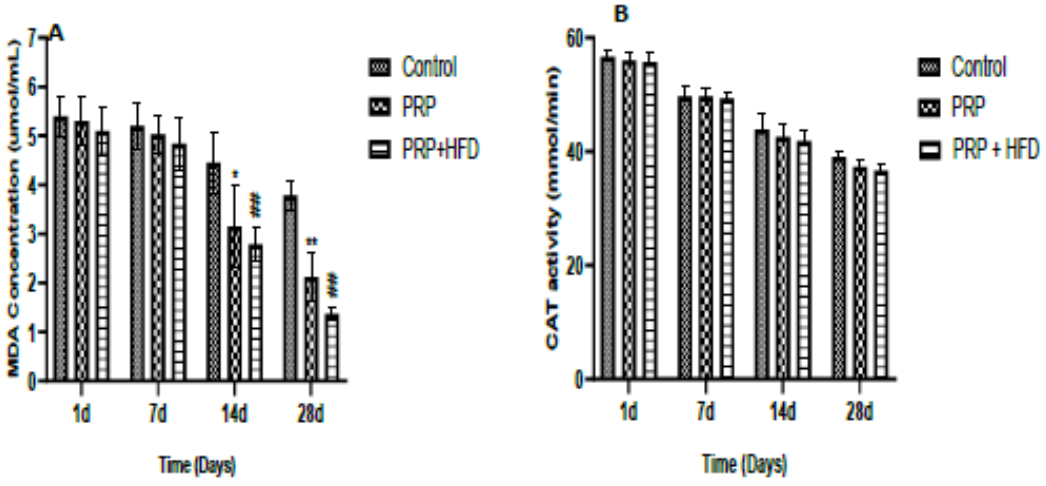

Fig. 7. Effect of autologous platelets-rich plasma alone or with hydrofiber dressing on the oxidative stress markers A: indicates MDA concentrations between different groups on different time intervals. B: indicates CAT activity between different groupon different time intervals. Compared with the control group, * P<0.05, ** or ## P<0.01.

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