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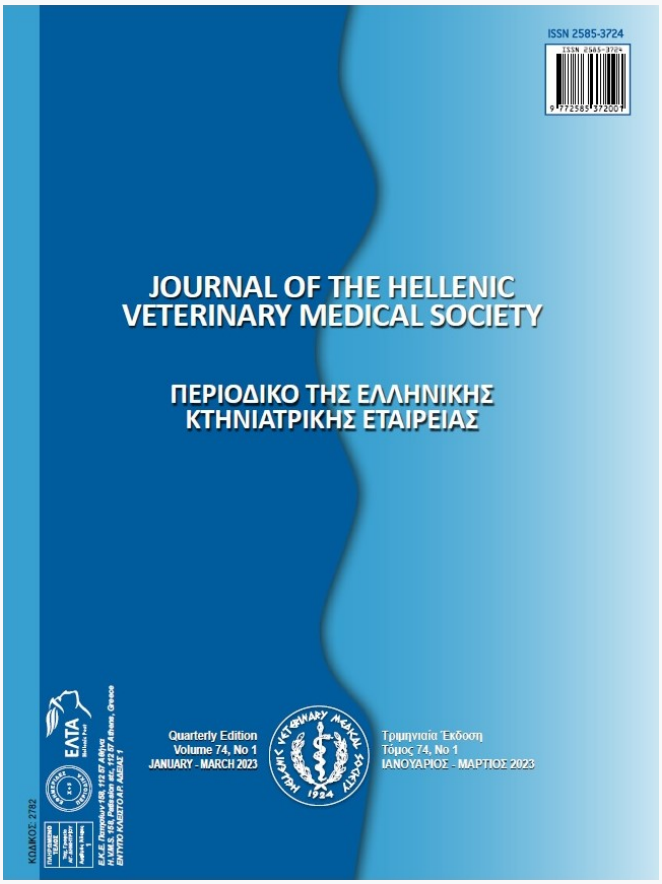

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Research article
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The Therapeutic Effects of Autologous Platelet-Rich Plasma Gel on Cutaneous Wound Healing in Rescued Horses

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ABSTRACT: This study explored the effect of platelet-rich plasma gel on cutaneous wound healing in rescued horses. A total of twenty horses were used and allocated into two groups: Platelets-rich plasma (PRP)-treated group A (n=10) and Antiseptic dressing (ASD)-treated group B (n=10). Group A animals were treated with autologous PRP gel, while group B animals were treated with sterile saline. An average size of full-thickness (1.5 mm2) skin wounds were selected in each horse. Histopathological examination of wound tissue in horses was performed on days 8, 40, and 60. Wound epithelialization was assessed by HE staining, and collagen re-establishment was assessed by Masson's trichrome staining. The CAT activity and MDA concentration were assessed in blood samples on days 8, 40, and 60. All data were subjected to statistical analysis. We observed a highly significant increase ($P<0.01$) in re-epithelization at days 40 to 60 in the PRP wounds compared to the ASD wounds. Collagen was well organized ($P<0.01$) in the PRP wounds compared with the ASD wounds at day 40 to day 60. Malondialdehyde (MDA) concentration was significantly decreased from day 40 to day 60 ($P<0.05$) in the PRP wounds than the ASD wounds. Catalase (CAT) activity showed no difference ($P>0.05$) between PRP wounds and ASD wounds. In conclusion, compared to ASD wounds, PRP wounds promoted cutaneous wound healing in horses by suppressing oxidative stress levels, accelerating wound epithelialization, and generating organized tissue, interlocking collagen bundles of dermal collagen. However, ultrasonographical assessment level study is needed to further investigate the effect of PRP gel on skin wound healing in rescued horses.

Keywords: Cutaneous wound healing, horses, histopathology, oxidative stress, platelet-rich plasma

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INTRODUCTION

Skin is the largest connective tissue of the vertebrates, comprising 10 % of the body mass and covering almost the entire body surface (Crovetti et al., 2004). It contributes a very important role in the body's defense mechanism and has its self-renewing and repairing capability. Skin serves as protective wall between inner and outer environs of body (Hassan et al., 2014). Any alteration in normal skin functional anatomy and structural integrity of skin referred as wound (Teng et al., 2014). Wound healing is the process by which skin can repair its damaged or lost part by regenerating tissue and forming a collagen scar (Su et al., 2019). Cutaneous wounds are common in horses, donkeys and mules. The athletic performance of horses is badly affected by chronic non-healing and exuberant granulation tissue (Proud flesh) wounds and is an important concern to the owners. Different growth factors, chemical mediators, cytokines, and cell types are involved in the process of wound healing. Any change disturbing this mechanism may lead to chronic, non-healing wounds. A wound that is not treated timely or could not heal in a proper set of phases is called a chronic wound (Yolanda et al., 2014). Especially in equines, delayed wound healing and cost on restoration of wounded site are the restricting factors for proper wound healing (DeRossi et al., 2009).

Therefore, efficacious healing of skin wounds necessitates a combined set of drug therapy. With a good supply of growth factors (GF's) through platelets promoted wound healing trends are observed (Dugrillon and Kluter, 2002). Thrombocytes are involved in the liberation of different growths mediators responsible for blood coagulation, hemostasis, angiogenesis, and the optimizing healing process (Robson, 1997; Marx, 2004). Platelets are also concerned with wound restoration by the formation of collagen fibers. Platelets open a doorway in the process of wound healing through advancement in platelet-rich plasma (PRP). In the different fields of dentistry, ophthalmology, and cutaneous wound therapies widespread use of autologous PRP is observed (Smith et al., 2007; Abu-Seida, 2015).

Platelet concentrated plasma is a 100% naturally biocompatible and harmless concentrated plasma volume. It poses zero infection rates to the injured animals because it is composed of blood plasma of same animal and a natural mixture of mediators designed to synergise biological effects in wound repair (DeRossi et al., 2009). Calcium gluconate and thrombin are utilized to PRP from blood plasma. Later on, PRP is transformed into RPP gel. The thrombin activates the platelets alpha granules to release several wound healing growth mediators for example interleukin, platelet-derived growth factor (PDGF), fibroblastic growth factor tissue necrotic factor (TNF), interleukin, and epidermal growth factors (EGF) (DeRossi et al., 2009). These important growth factors are diffused into the surrounding tissues and attract chemotactically the neutrophils and monocytes at the place of wound injury and to form a hemostatic clot (Li et al., 2007). In this complicated process of wound healing single exogenously agent cannot initiate all the steps of wound healing responses at the site of injury. Therefore, combination therapy is necessary for wound healing, and for this purpose, platelets are a rich source of growth factors to respond at the site of an injury and actively participate in repairing skin wounds very effectively (Velnar et al., 2009). It is obvious from many clinical trials that growth factors enhance tissue repair and increase the restoration of chronic non-healing cutaneous wounds (Carter et al., 2003).

Stress due to oxidation and decrease in endogenous antioxidant material in vivo can promote the sensitivity of tissue and intracellular constituents to free radical of oxygen. Stresses due to oxidation of tissues result in the formation of free radicals, especially reactive oxygen species (ROS), devastate antioxidant defenses (Camini et al., 2017; Rizzo et al., 2017). During oxygen metabolism, ROS are synthesized. Antioxidant pathways in cells form equilibrium between synthesis and discharge of ROS from the cell thus retaining the concentration of oxygen free radicals in the cell. ROS plays an important role in maintaining cellular signaling, thereby regulating cellular metabolism, homeostasis, defense systems, and adaptive processes. Anti-oxidative defences under stressful conditions protect the cell through the enzymatic and non-enzymatic activity of antioxidant pathways (Miquel, 2009; De la Fuente, 2014). Therefore, different strategies were developed, including ROS cleansing enzymes: Taking into account glutathione peroxidase and catalase, seleno-enzyme, superoxide dismutase, to keep oxidation-reduction reactions homeostasis and protect multiplying and migrating cells at the site of injury (Keller et al., 2006).

Under normal conditions, catalase (CAT) is not crucial for many different cell types. In the case of cutaneous wounds, CAT has the adaptability to reduce ROS. CAT imparts a significant role to avoid cellu-

lar oxidative damage (Halliwell, 1999). ROS mainly target polyunsaturated fatty acids (PUFAs) located in cellular membranes. Rancidity of lipids may lead to impaired cellular structure and function (Halliwell, 1993). Furthermore, the rancidity of lipids produces hydroperoxides. Hydroperoxides on degradation produce malondialdehydes (MDA) as an end product. MDA is used as a biomarker during lipid peroxidation (Halliwell, 1993; Gawel et al., 2004). To evaluate the activity of ROS and rancidity of lipids, thiobarbituric acid (TBA) assay is the most familiar and simple method of analysis. In this analysis, two molecules of TBA and one molecule of MDA react to indicate lipid peroxidation (Palmieri and Sblendorio, 2007).

To the best of our knowledge, the changes in MDA, CAT levels and wound repair have not been studied in rescued horses in Pakistan. Therefore, the aim of this study was to investigate whether the use of autologous PRP gel in wounds enhances wound repair and alters oxidative stress cascades compared to conventional ASD-treated horses in the Group B.

MATERIAL AND METHODS

Ethical statement

This study and all the procedures were approved and conducted in accordance with rules and regulations of the Ethical Review Committee (Ethical Approval No. DR/458; Dated: 07/10/2020) at the Department of Veterinary Surgery and Pet Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan**.**

Animals

This study was conducted in rescued horses suffering from chronic skin wounds in the Punjab province of Lahore district, Pakistan. All animals were housed in the Indoor University Stables as well as the Indoor Stables of the Society for the Prevention of Cruelty to Animals in Lahore throughout the experimental period. Before the start of experimental trials, horses were accustomed to ample drinking water, grass, and concentrated feedstuff.

Preparation of platelet-rich plasma (PRP)

Horse platelet-rich plasma was made by collecting two tubes containing 10 mL of horse whole blood on the same day as the surgical procedures. Blood was drawn from each horse for the wound-healing study. The blood was placed in two Falcon tubes (15 ml each) comprising a 10% sodium citrate anticoagulant. The tubes were centrifuged at 300 g / 10 m (SCILOGEX swing out centrifuge) to isolate the plasma red cells. Plasma with platelets remained in the superior portion, and between these two layers, a fine and whitened zone known as the intermediate zone stayed, which contained white cells, primarily leukocytes and the largest platelets.After that, 500 uL of plasma was drained from the upper part of each tube and transferred to another tube called tube A. This part is used to generate autologous thrombin.To obtain the PRP, the remaining plasma as well as the intermediate zone was moved to another tube labelled B. This tube was incubated at room temperature. 300 uL of 10% calcium gluconate was added to tube A, agitated, and incubated at 37 ˚C for 15 minutes. Afterwards, the two tubes (A and B) were centrifuged at 640 g/10m at room temperature.The entire volume of tube A containing thrombin-rich substrate was used. Half the volume of tube B was drained, and after homogenization, thrombin was added to tube A at a ratio of 2:1 (2 mL PRP:1 mL thrombin). PRP gels formed after standing at room temperature for 40 minutes.To count the platelets, Neubauer's chamber and trypan blue were used to improve cell visualisation and differentiation (DeRossi et al., 2009). The optimal platelet enrichment over 4.0 time's baseline values was obtained. Platelets obtained in the PRP were in average 15.6×10^5, while those in the whole blood were in average 3.8×10^5.

Experimental design and treatment

A total of 20 horses were used in this study, ranging in age from 5 to 10 years and weighing between 350 and 450 kg. The animals were kept in the indoor stables of the Society for the Prevention of Cruelty to Animals in Lahore. Accessibility to a sufficient supply of water and dry fodder was ensured. Throughout the trial period, all horses were provided accessibility to mineral salt. All animals (n=20) were allocated into two groups; PRP-treated group A (n=10) and conventional ASD-treated group B (n=10). In group A, each animal's wound was treated with autologous PRP gel, while animals in group B received traditional antiseptic dressing (ASD).

Horses were sedated intravenously with Xylazine hydrochloride (Xylaz@Farvet Holland) at a dose rate of 1.1 mg/Kg (Sadek et al., 2020). Each full-thickness average size of (natural) skin wound on either side of left or right of animal's back wound (1.5 mm2) was dehaired and shaved from the edges, then separated from the subcutaneous tissue with the help of scissors

25CM/10 (Noorani Surgical). After cleaning each wound with sterile saline (NaCl 0.9%, Geofman), a thick layer of PRP gel was applied, and then the wound was wrapped with sterilized gauze and protected with a dressing. The bandage was removed after two days, washed with sterile saline, coated with PRP gel, and the wound was bandaged. The treatment was repeated every four days until the twenty-sixth day. The Semi-occlusive gauze containing PRP gel was applied to the skin wound with a soft bandage to allow fresh air to limit granulation tissue overgrowth. After 26 days, the PRP gel was reapplied to the wound every 8 days. Similarly, in ASD-treated Group B animals, sterile saline was used on the cutaneous wound after washing with Povidone-Iodine Solution (Pyodine Sol, Brookes Pharma) and the bandage was applied using the same procedure as in group A animals. A sterilized non-adherent semi-occlusive gauze was applied over wounds. The wounds were bandaged with (Surgitex, Rehman Rainbow (PVT.) LTD.). For animal welfare, supportive treatment such as; Prophylactic systemic antibiotics such as Biocon 5gm Inj. contain benzylpenicillin, procaine penicillin, and streptomycin sulfate (Vetcon Pharma) were administered intramuscularly twice a day and the horses were kept in hygienic stables with limited exercise.

HISTOPATHOLOGY

Hematoxylin & eosin stain

Before the procedures, all horses were tranquilized intravenously with Xylazine hydrochloride (Xylaz@ Farvet Holland) at a dose rate of 1.1 mg/Kg(Sadek et al., 2020) and the biopsy sites were scrubbed with saline solution and gauze. A 6-mm surgical biopsy punch (Kai medical@Japan) was used to collect full-thickness specimens. The wound was biopsied from a skin edge of depth 4-to-5 mm and 3-to-4mm part of intact skin portionin each animal of both groups on different time points. In the early stages, the sample was preserved by using 10% neutral-buffered formalin for 24 hours. Later on, the sample was shifted to 70% alcohol fixative. After fixation in alcohol at different concentrations, tissues were embedded in paraffin following subdividing the biopsied tissue into 1.5 mm width. Furthermore, tissue was stained by hematoxylin and eosin for tissue morphology analysis using standard light microscopy procedures. Various semi-quantitative factors including the extent of re-epithelialization, vascularization, and presence of polymorphonuclear leukocytes (PMNL) were observed in the histopathology of biopsy samples.

In this blind study, the semi-quantitative scoring system was applied (Sabol et al., 2012). Scoring according to the semi-quantitative system was described as Zero indicating that there were no vascularization, no observation of fibroblasts, no cutaneous epithelium formation, absence of fibroblastic cell, lack of PMNL in the microscopic field, and, 1 denoted enlargement of cutaneous epithelium depth and width, presence of limited fibroblastic cell, polymorphonuclear cell, or recently created vasculature, 2 showed epithelial cell relocation, presence of polymorphonuclear cell, moderate numbers of fibroblastic cells, or recently created vasculature, 3 indicated epithelial incision apposition, presence of polymorphonuclear cell, moderate numbers of fibroblastic cells, or recently created vasculature, and 4 showed complete epithelial regeneration, presence of an excess of PMNL, fibroblasts, or angiogenesis.

Masson's trichrome stain

Collagen fiber staining was performed in the Laboratory of the Department of Pathology, University of Veterinary and Animal Sciences, Lahore. Staining was performed according to procedures followed and developed by the Center for Musculoskeletal Research (CMSR) at the University of Rochester Medical Center. Tissues were deparaffinized and rehydrated. Bouin's Fixative (Fisher Scientific) was utilized at 58 ºC for 15 minutes. Following cooling, the slides were washed with distilled water for 10 minutes. Later, the biopsy tissue sample was stained with (C.I.26905; C.I.42685) Biebrich Scarlet Acid Fuchsin (Fisher Scientific) for 5 minutes. Then, 1% phosphomolybdenum-phosphotungstic acid solution was used to stain the sample (Fisher Scientific) for 2 minutes. Tissues were stained by Aniline blue solution (C.I.42775) as counterstaining agent for 5 minutes and washed with distilled water. Furthermore, biopsied tissue samples were rinsed with 1%aqueous solution of acetic acid. Slides were dried out, cleared for debris, and mounted at the end. At each biopsy sampling, observation and photomicrographs were taken to assess improvement in cutaneous wound healing and regenerative cell production. In this current investigation, a simple descriptive scale 0-3 was employed to assess the collagen abundance and collagen organization for each feature in trichrome stained slides. Samples with a score of 0 indicated a lack of collagen bundles or ordered collagen fibers formation. A score of 3 indicated sufficient collagen fibers and ordered collagen fibers formation. Comparable undulating collagen fibers with uniform blue color reflected more structured than collagen fibers with a color differ-

ence and comparable fiber damage or infiltration.

Blood sampling

10 mL blood samples were collected from the Juglar vein of each animal in plain vacutainer at 0d and 8d, 40d, and 60d.The blood samples were transferred to the laboratory in the Department of Physiology, Faculty of bio-sciences, the University of Veterinary and Animal Sciences, Lahore, Pakistan. The blood samples were centrifuged at 3000 rpm for 15 min at 4˚C using a temperature control centrifuge machine (HARRIER 18/80 UK). The serum was separated and stored at -20˚ C for further manipulation.

Oxidative stress analysis

MDA concentration

Serum MDA concentration (µmol/mL) was assessed as described by (Ohkawa et al., 1979). A reaction mixture composed of 375 µL of 20.0% acetic acid (pH 3.5), 375 µL of 0.8% thiobarbituric acid, 50 µL of 8.1% sodium dodecyl sulfate was incorporated with 100 µL aliquot of plasma. Samples were heated at that time at 95 °C for 1h and centrifuged was done at 3000 g for 10 min. The absorbance of the supernatant was measured at 532 nm using an Epoch Reader Microplate spectrophotometer (UV-2800, Biotechnology Medical Services, USA), and MDA content was denoted by µmol/mL (ε = 1.56 × 105 mmol/L/cm).

Assessment of CAT activity

According to the method described by (Aebi, 1984), the rate of degradation of substrate H2O2 expresses the catalytic activity ofcatalase.The rate of decomposition of hydrogen peroxidewas measured by a drop absorbed at 240 nm every 30 seconds for 3 min. The levels of CATwere measured as mmol/min. The quantity of catalase enzyme needed to degrade 1 µmole of hydrogen peroxide per second at 25°C was termed as CAT activity.

Statistical analysis

The data were analyzed using the student t-test using SPSS statistical software version 20. All values were expressed as (Mean± SD). The level of statistical significance was ($P<0.01$) and ($P< 0.05$).

RESULTS

Histopathology

Re-epithelization

Histopathological findings of HE staining indicated that PMNL cells infiltrated the top of the wound on day 8, while macrophages and drifting fibroblastic cells were seen at the bottom of the wound. The count of drifting fibroblastic cells was higher in wounds treated with platelet-rich plasma in comparison with ASD wounds. Epithelial cells at the borders of the lesion begin to multiply to substitute the misplaced edges of epithelial cells. Furthermore, on day 8, the Platelet-rich plasma-treated group and control group was seen to have cutaneous angiogenesis (Fig. 1A, B). Re-epithelialization and fibroblast recordings were significantly ($P<0.01$) increased in cutaneous wounds treated with Platelet-rich plasma in comparison with ASD wounds (Fig. 2A).

In this study, at 40-day epithelium with keratin depositing restricted only to wound edges (Fig. 1C) and apposed margin of wound site in the platelet-rich plasma-treated group was seen, but, the wound continued to be concealed by degenerated PMNL and scab of necrotic debris (Fig. 1D). Fascinatingly, the formation of new cutaneous epithelium and a significant increase in the number of fibroblastic cells ($P<0.01$) in the platelet-rich plasma-treated wounds in comparison with the ASD wounds was noted at day 40 (Fig. 2B). The quantity of invading PMNL was enhanced in both the ASD group and platelet-rich plasma treated group at 40 days compared with 8 days. However, lesser PMNL were seen in platelet-rich plasma-treated wounds as compared to ASD wounds ($P<0.05$) (Fig. 2B). Underneath the epithelium granulation tissue with pronounced angiogenesis and the rich fibroblastic count were noticed.

On day 60 of the wound healing, the enhanced thickness of epithelium was noted in platelet-rich plasma-treated wounds compared to ASD wounds. Compared with ASD wounds, all skin wounds healed, and PRP wounds had a higher ($P<0.01$) quantity of fibroblastic cells and angiogenesis (Fig. 1E, F; Fig. 2C).

Collagen fibers

After Masson's trichrome staining, collagen fibers were stained. Pathological section results indicated a significant increase in collagen fibers over time in platelet-rich plasma-treated wounds in comparison with ASD wounds. At day 8 of the wound healing, the collagen fibers were less organized in the platelet-rich plasma treated wounds (Fig. 3A, B). The number of collagen fibers ($P<0.05$) and organization were meaningfully higher ($P<0.01$) in the plate-

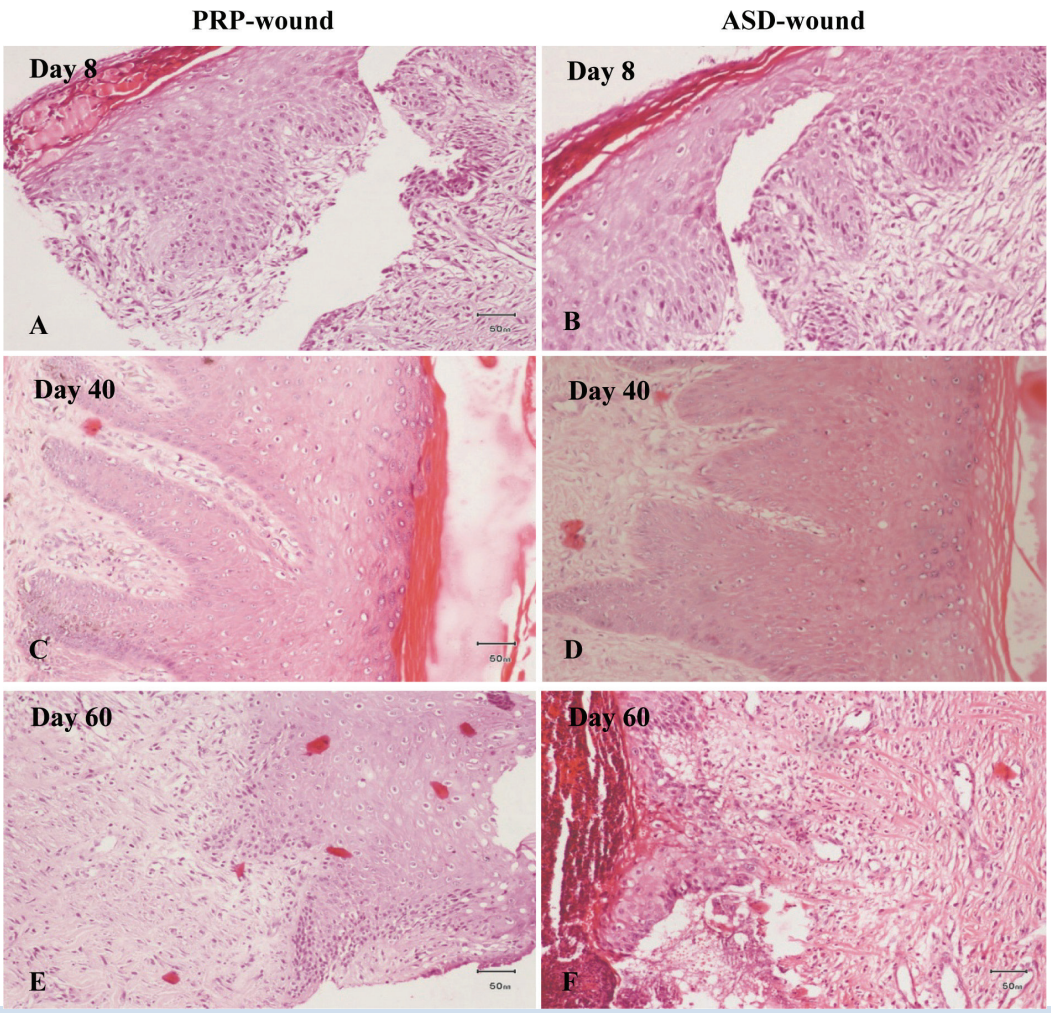

Fig. 1. HE stain results of re-epithelization in the cutaneous wound of both groups.

Bar=50um; **A;**PRP treated wound obtained at day 8 showed increased thickness of epithelium, presence of few fibroblasts, new blood vessels. **B**; ASD treated wound showed limited epidermal differentiation, absence of fibroblast at day 8 post treatment. **C**;PRP treated wound at day 40showed epidermal proliferation, kerato-hyaline granules are seen in many keratinocytes, presence of moderate number of fibroblasts. **D**; ASD treated wound at day 40 showed undifferentiated keratinocyte, no kerato-hyaline granules, few fibroblasts present. **E**; At day 60 PRP treated wound showed marked mature epithelial cells, excessive number of fibroblasts and new blood vessels formed. **F**; ASD treated wound at day 60 showed moderate number of fibroblasts, some dead tissue mass along with less re-epithelialization.

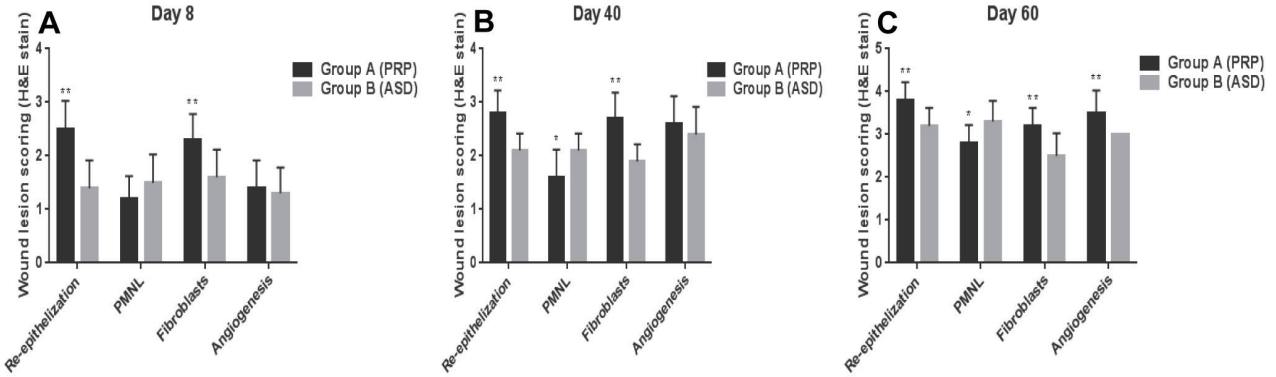

Fig. 2.Lesion score of wound healing in PRP and ASD treated wounds.

A; Lesion score of wound healing (HE stain) at day 8; **B;** Lesion score of wound healing (HE stain) at day 40; **C;** Lesion score of wound healing (HE stain) at day 60;"**" Indicates that the difference between the PRP treatment group and the ASD group is more significant (P<0.01); "*" indicates that the PRP treatment group has a significant difference compared with the ASD group (P<0.05).

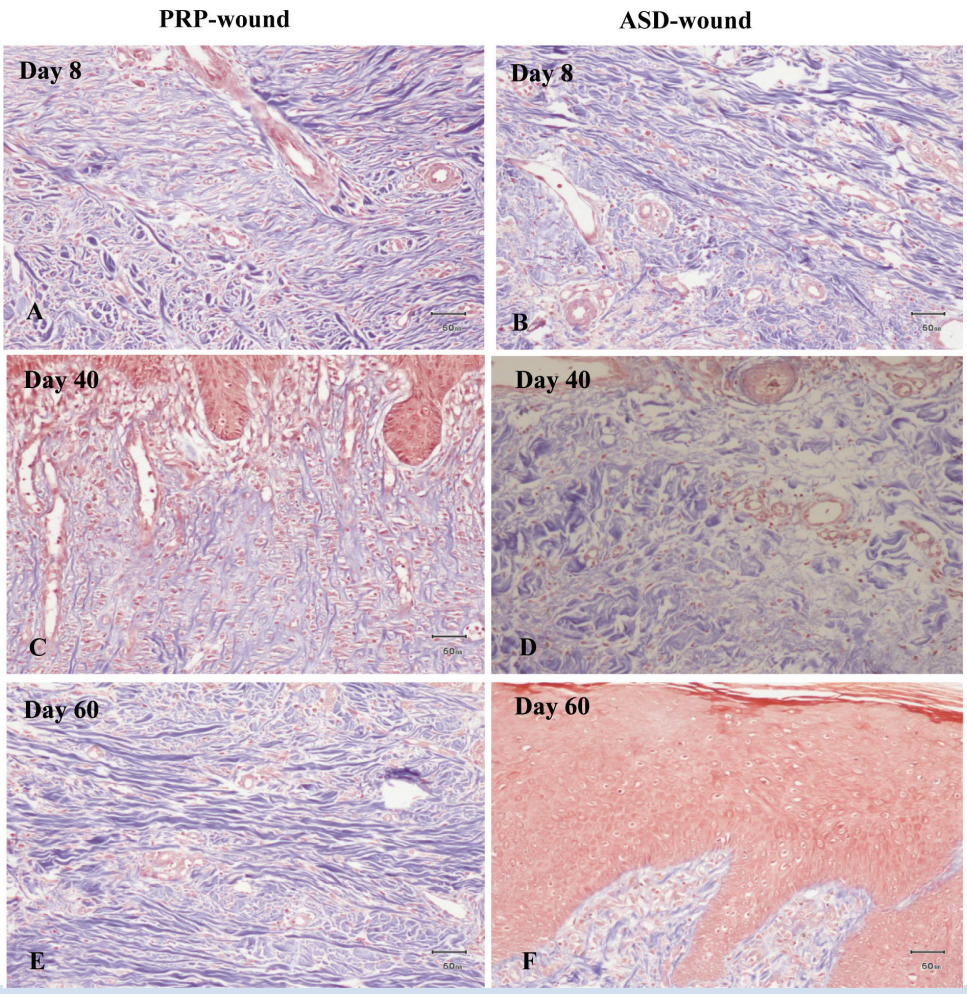

Fig. 3. Masson's trichrome stain results of collagen fiber in the cutaneous wound of both groups.

Bar=50um; **A**; PRP wound showed minimal and organized collagen fiber at day 8 post treatment. **B**; ASD wound showed disorganized collagen fibersat day 8**C**; PRP wound showed well organized collagen fibers also fibroblast perpendicular to the epidermis at day 40. **D**; ASD wound showed less dense and unorganized collagen fibers at day 40. **E**; PRP wound at day 60 showed dense and tightly packed collagen bundles oriented parallel to the overlying epithelium. **F**; ASD-treated wounds at day 60 showed intact epidermis with disorganized collagen bundles in the dermis.

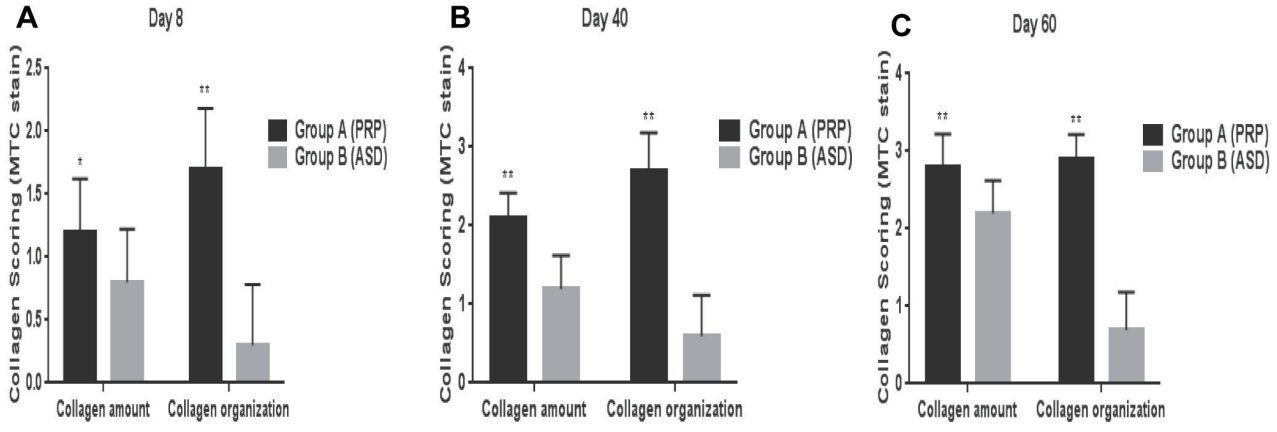

Fig. 4. Lesion score of collagen amount and organization of wound healing in both groups.

A; Lesion score of collagen amount and organization (MST stain) at day 8 **B;** Lesion score of collagen amount and organization (MST stain) at day 40 **C;** Lesion score of collagen amount and organization (MST stain) at day 60; "**" Indicates that the difference between the PRP treatment group and the ASD group is more significant (P<0.01); "*" indicates that the PRP treatment group has a significant difference compared with the ASD group (P<0.05).

let-rich plasma-treated wounds (Fig. 4A). At 40 and 60 days of wound healing, collagen fibers were very well organized, with parallel alignments and lack of arrangement in collagen fibers in the platelet-rich plasma-treated wounds (Fig. 3C, E). In contrast, disorganized collagen with random alignment and blue inconsistency was found in ASD wounds (Fig. 3D, F). Compared with ASD wounds, the number of collagen fibers and organization was significantly ($P<0.01$) higher in PRP wounds (Fig. 4B, C).

Oxidative stress markers

Compared with ASD-treated wounds, PRP-treated wounds had significantly ($P<0.01$) lower serum concentrations of MDA at 40 and 60 days (Fig. 5A). In contrast, catalase activity was not significantly different from day 0 to day 40; however, we found a significant difference between the two groups at 60 days (Fig. 5B).

DISCUSSION

Cutaneous wound healing comprises of restoration and redevelopment of skin. Different growth factors are involved in the healing of wounds resulting in differentiation of cells, protein production, and synthesis of the enzyme. Furthermore, growth mediators play an important role in cell metabolism, relocation, and synthesis and remodeling of extracellular matrix (ECM) proteins (Komarcevic, 2000). Activated platelet alpha granules-derived growth factors are responsible to enhance wound restoration is a distinctive technique for treatment to further improve wound healing in contrast to traditional lesion treatments such as surgical debridement and antibiotic therapy (Komarcevic, 2000). Additionally, the overall cost of wound management is staggering. Accelerating and improving the quality of skin wound healing is beneficial for rescued horses. However, the application of autologous PRP gel as a better medical adjunct therapy for poorly vascularized or non-healing wounds in rescued horses can provide high-quality healing of full-thickness cutaneous wounds.

Povidone iodine has many advantages for wound healing, including its broad antimicrobial spectrum, lack of resistance, efficacy against biofilms, minimal side effects, and effect on excessive inflammation (Bigliardi et al., 2017). A recent study demonstrated that povidone iodine enhanced wound healing via TGF by stimulating granulation and increasing neovascularization (Wang et al., 2017). Studies of povidone iodine in wound healing were conducted over thirty years ago (Van Meurs et al., 2014; Vermeulen et al., 2010), and most concluded that concentrations of up to 10% could not inhibit granulation and epithelialization (Van Meurs et al., 2014).

This study reported that the thickness of epithelial cells increased in wounds treated with autologous PRP gel and all full-thickness cutaneous wounds healed with a significantly higher number of fibroblasts and newly established blood vessels compared to ASD treated wounds. Similar results were also recorded by Wang and Nirmala (2016). They reported that the action of PRP may promote tissue healing by providing ample growth mediators (containing platelets and a fibrin matrix) as a natural support to progenitor cells, replacing tendon stem cells in deformities of tendon connective tissue. These results are consistent with the findings of (Andia and Maffulli, 2015) that used platelet-rich plasma (PRP) for tendinopathies plantar fasciitis and myopathies, while they needed to optimize the procedure and acquire more high-grade

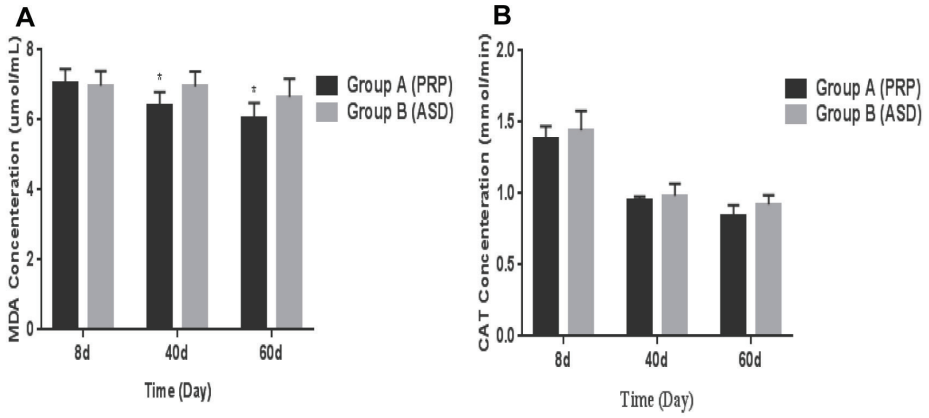

Fig. 5. Oxidative stress assessment.

A; indicates MDA concentrations in PRP and ASD-treated wounds; **B**; indicates CAT activity in both groups.'*' indicated the differences were significant in the PRP-treated group compared with the ASD group ($P<0.05$).

experimental data to recommend platelet-rich plasma (PRP) therapy. Platelet-rich plasma (PRP) infiltration was set up to improve re-epithelialization. The PRP gel was applied to promote the synthesis of granulation tissue, possibly as a result of local absorption of the PRP gel by the surrounding tissue and promotion of neovascularization and relatively rapid re-epithelialization in the early stages of skin wound healing (Huang et al., 2016).

Collagen fibers are part of the super-extracellular mesh that serves as the underlying framework in the tissue, directing cell proliferation and repositioning during skin wound healing (Soundia et al., 2018). In this way, the material of collagen filaments is an important boundary for observing wound healing in the skin (Dole et al., 1975). In our study, the skin elasticity of PRP-treated and conventional ASD-treated wounds was assessed by the number and arrangement of collagen fibers in the tissue. Collagen fibers are an important extracellular ECM component that supports the rigidity and flexibility of the skin. The increase in wound elasticity that occurs at the fibroblast stage is associated with an increase in collagen levels within the wound (Broughton and Rohrich, 2005; Jee et al., 2016).

Initially, granulation tissue contains symmetrically arranged small bundles of collagen fibers. Recently, formed granulation tissue consists of a large number of fibroblastic cells. In the later stages of wound healing, the collagen fibers become dense, stuffed firmly into bundles, and symmetrically arranged (Xue and Jackson, 2015). In the recent investigation, collagen fibers are arranged as thick, comparable, undulating bundles in case of treated with autologous platelet-rich plasma (PRP) gel cutaneous wounds, but not in ASD-treated wounds, implying that PRP accelerated granulation tissue development. DeRossi et al.(2009) observed that injuries treated with platelet-rich plasma (PRP) showed faster cellular differentiation in the epithelium and improved cutaneous collagen binding. Furthermore, Vendramin et al.(2010) have seen autologous platelet rich plasma (PRP) gel result in enhancement in the synthesis of collagen formation, and the number of macrophages and fibroblastic cells production during cutaneous grafting in animals.

Recent studies have shown that reactive oxygen species (ROS) are directly associated with the restoration of the wound and they are also involved in various developmental stages in wound healing (Mittal et al., 2014; Yip, 2015). A high concentration of reactive oxygen species (ROS) leads to cellular derangements on the contrary low quantity of ROS with fewer energy favors wound healing (Sen and Roy, 2008). ROS-induced lipid peroxidation generates various types of end products i.e. MDA. As a result of the peroxidation of lipid by reactive oxygen species, MDA is produced as an end product. MDA reflects the degree of degradation by ROS (Toru, 2013; Soundia et al., 2018). Elevated levels of MDA in biopsied tissues samples and body fluids have been shown to correlate with the intensity of injuries (Griffiths et al., 2002). In this current investigation, there was a remarkable decline in MDA quantity in horses administered PRP in contrast to the ASD wound that was inconsistent with a recent study (Abood et al., 2015). Consequently, the results in this recent study confirm that biomarkers involved in cellular oxidation significantly support the role of platelets in the anti-inflammatory process during healing, repair, and restoration of wounds (Mazzocca et al., 2013). Compared with ASD wounds, CAT levels showed no significant reduction in PRP wounds. This non-significant reduction in the serum concentration antioxidant catalase (CAT) levels may be explained by excessive use of CAT activity due to cutaneous wounds, which was consistent with a previous study (Iuchi et al., 2010).

This study has some limitations such as inability to assess the presence of growth factors in the cutaneous wounds. The findings and treatment outcomes of our study, only chronic nonhealing wounds in rescued horses are related. The use of growth factors obtained from activated platelets to induce wound repair is a novel method to enhancing wound healing than conventional wound management, including antiseptic dressing. An enhanced and improved quality of healing would be beneficial for horses and humans. The use of PRP gel as an enhanced therapy for poorly vascularized or nonhealing wounds in the lower horse leg, as well as in immunocompromised or elderly individuals, could result in limb salvage, which would be of substantial personal, economic, and social benefit.It was also determined that further research, including ultrasonographic evaluation, is required to determine the effect of PRP gel on skin wound healing in rescued horses.

CONCLUSION

In conclusion, autologous PRP gel-treated wounds promoted skin wound healing in horses, accelerated wound epithelialization, and formation of tissue with symmetrically arranged, interconnected collagen fibers of dermal collagen compared with the conven-

tional ASD treatment of the wound. Oxidative stress is helpful for the pathogenesis of wounds. Therefore, this study recommends the use of PRP gel as a safe and inexpensive treatment for cutaneous wounds of rescued horses. However, ultrasonographical assessment level study is needed to further investigate the effect of PRP gel on skin wound healing in rescued horses.

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CONFLICTS OF INTEREST

None declared.

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