International Journal of Agriculture Innovations and Research
Volume 3, Issue 4, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 26/01/2015 | Accepted on : 10/02/2015 | Published : 28/02/2015

# The Efficacy of Platelet-Rich Plasma (PRP) on Full-Thickness Cutaneous Wound Healing in Donkeys

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*Abstract:* **This study aimed to evaluate the efficacy of PRP gel on the second intention healing of the distal limb wounds in donkeys. Ten apparently healthy donkeys were used in this study; divided into two groups (5 animals for each). In 1st group, PRP gel was applied on experimentally induced full thickness wounds in one limb and wounds of the other limb served as a control. Wounds in the 2nd group covered with autogenous skin grafts with and without PRP gel under the graft. Wound healing was assessed clinically and histologically for I month. PRP gel improved wound healing in donkeys which characterized by significant decrease in wound dimensions and increase in wound healing and contraction percent with organized collagen parallel to epidermis and earlier epithelization in addition to improvement of skin graft attachment. It could be concluded that PRP gel is an effective natural and low cost wound healing promoting agent for full-thickness skin wounds in donkeys and form an efficient sealant for full thickness skin graft with good cosmetic appearance.**

*Keywords:* **Donkeys, Platelet Rich Plasma, Wound Healing, Skin Graft.**

## 1. INTRODUCTION

The primary goal of wound care is to achieve rapid and functional healing with minimal pain, discomfort and scarring to patient and must occurs in physiologic environment conductive to tissue repair and regeneration [46] [39]. Wounds below the hock or the knee of horses usually resist healing. This may be due to the large distance between the trunk and the lower limbs providing tissue of lower limbs with poor blood supply thus lower oxygen and lower temperature [15]. Skin grafting was used for treatment of wounds, burns as well as ulcer. It may reduce healing time, improve cosmoses and allow horse to return to function sooner than if the wound was allowed to heal by the second intention [6]. Skin grafting assists wound healing by replacing dermal collagen and providing biological occlusion and protection of the wound [32]. Mesh graft allows fluid and blood to pass easily through the graft via multiple fine perforations; enabling earlier intraoperative application to the bed without hematoma collection [30].

Despite numerous treatments available for deteriorated cutaneous wound healing, there is still need for more effective therapy [24]. Concentrated platelet and their released growth factors are applied locally as a platelet gel whose function is to stimulate and coordinate the wound healing process [10]. Platelet- rich plasma (PRP) is made from patient own blood so it is 100% biocompatible and safe, possessing absolutely no infectious risk to the patient [3].This study aimed to investigate the clinical and histopathological effects of PRP gel on healing of full-thickness skin wounds created on distal limbs of donkeys.

## 2. MATERIALS AND METHODS

### *A. Animals:*

Ten apparently healthy donkeys aging 3-7 years with body weight ranged from 200-250 kg were used in this study. The animals examined clinically for signs of discomfort or lameness and received tetanus prophylaxis before surgery. All experimental techniques were reviewed and approved by Institutional Animal Use and Care Committee of Faculty of Veterinary Medicine, Alexandria University. The donkeys were divided into two groups each of five animals.

### *B. Anesthesia:*

Wound creation was done under the effect of sedation by intravenous injection of 1mg/kg xylazine HCL, followed by local infiltration analgesia with 2% xylocaine HCl at the site of incision.

### *C. Surgical procedures:*

Metacarpal and metatarsal regions were prepared aseptically for surgery. Twenty full thickness skin wounds (4×4cm) were created on lateral surfaces of the right fore metacarpus and left hind metatarsus of the ten donkeys. Bleeding was controlled by pressure on wound surface.

### *D. Platelet-rich plasma (PRP) gel preparation:*

As shown in figure (1) PRP gel was prepared according to [14] [17]. 20 ml of whole blood was collected from jugular vein then deposited in two tubes containing 3.8% sodium citrate anticoagulant. The tubes were centrifuged at 3000 rpm for 10 minutes promoting the separation of plasma from red blood cells. After that, 2 ml of plasma were removed from the superior part of each tube (platelet poor plasma/PPR) and moved to another tube called tube A. This part was used to obtain autologous thrombin by addition of 300 ul. of 10% calcium chloride then incubate the tube at 37°C for 15 minutes. The remaining plasma with buffy coats and small amount of packed RBCs were transferred to another tube called tube B to obtain PRP. The PRP containing tube was suspended and homogenized then the contents of tube A and B were mixed at ratio 1:2. After 40 minutes resting in room temperature the PRP gel was formed. Approximately 1 ml of PRP was taken to confirm the platelet count.

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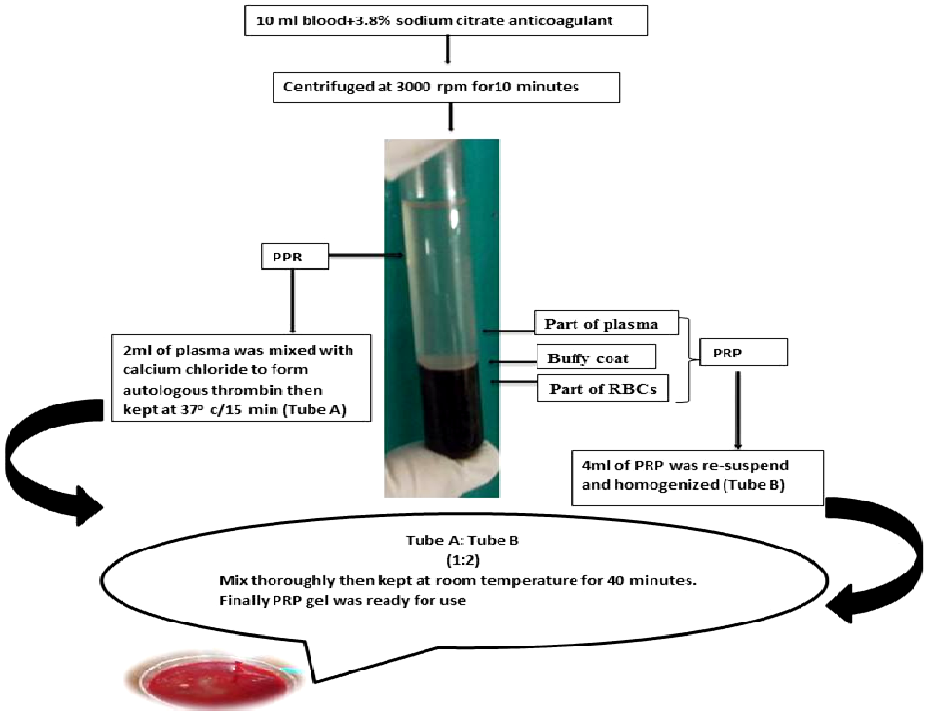


Fig.1. Preparation of PRP gel from donkey's own blood

*E. Experimental design:*

Group 1 (n=5): PRP gel was applied on wound in one limb of each animal and the wound in other limb received no treatment. The wounds of both limbs were bandaged by use of a protective non adherent dressing which changed twice a week. Saline only used to clean the wounds and neither topical nor systemic medications were used.

Group 2 (n=5): The wounds in this group were covered with autogenous skin grafts. The wounds were left after creation for 4 days to allow granulation tissue to develop. After that, slight debridement of granulation tissue was done to make it fresh for acceptance of graft [1]. Full-thickness skin grafts were harvested from the left thoracic region. The subcutaneous tissue was removed by scissors until hair follicles could be seen. Numbers of sequential incisions were made to form mesh. PRP gel was applied on wounds of one limb before placing of graft (figure 2a) and then the grafts were secured in place by several interrupted sutures (figure 2b). The wounds in other limb received skin graft without PRP. The limbs were bandaged with no adherent bandage. The sutures were removed at day 10 and then the animals were kept under observation for three weeks to assist graft viability.

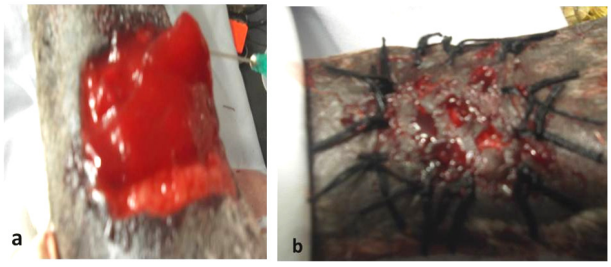


Fig.2. (a) Application of PRP gel to the wound before skin grafting (b) Fixation of mesh graft using sutures.

*F. Clinical evaluation:*

After each bandage changing in the first group the following clinical characters were observed and registered; exudation, bleeding, inflammation and granulation tissue characters (color, surface and fragility). Wound dimensions were measured at 7, 14, 21 and 28 days by ordinary ruler. The healed area was measured by subtracting the unhealed area from the total wound area then wound healing % was calculated. Wound contraction % was also calculated according to the equation described by [40].

Wound Contraction *(WC %) = (W0 – WI) / W0×100*-

W0 = the initial wound measurement (1st measurement in cm).

- WI = the wound measurement on day of measurement (2nd measurement in cm).

The grafts in the second group were observed and evaluated through examination of exudation, coloration, edema and cosmetic appearance.

*G. Statistical analysis:*

The obtained data were expressed as Mean ± Standard deviation (M± SD) and the descriptive statistical analysis was carried out by student's t-test and ANOVA test [44]

*H. Histopathological examination:*

Full- thickness skin biopsies were collected from each wound at days 3, 7, 14, 21 and 28 in the first group and only one biopsy was collected from each graft at day 28 post grafting for morphological and histopathological examination. They were prepared, fixed in 10% buffered formalin, processed and stained with hematoxylin and eosin stain (H&E) for light microscopic examination according to [4].

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## 3. RESULTS

PRP was prepared simply from donkey's blood and the final number of platelet in final solution was 3-5 times higher than baseline of the intravascular platelet count. The gel adhered well to the wound surface.

*A. Gross appearance*

Bleeding was stopped immediately post application of PRP gel and no further bleeding was observed after change of 1st bandage. On the other hand, bleeding was observed on the 3rd day following bandage change in control wounds. Immediate and significant improvement of the wound were observed in the 1st week after application of PRP (no signs of inflammation or infection were observed). While, control wound showed marked signs of inflammation and pain which subsided at the 2nd week.

Granulation tissue filled the wound and didn't require debridement in both PRP and control wounds. It was regular and glistening in PRP wound but showed grooves and clefts in control one (fig.3 a, b).

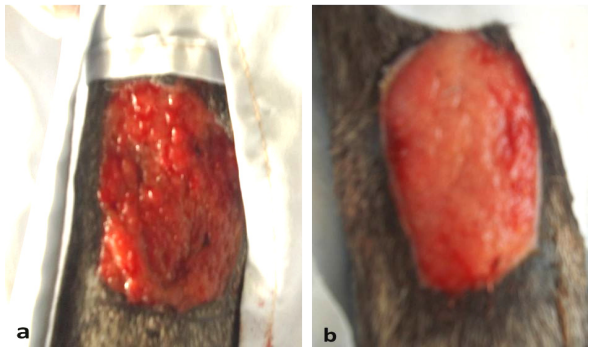


Fig.3. The granulation tissue in control group is irregular and showed clefts and grooves (a) whereas the tissue is regular and glistening in PRP group (b).

Wound dimensions were significantly reduced by the end of 3rd week in both groups but this reduction was significant in PRP group (Table, 1).

Wound contraction % and wound healing % were significantly higher and epithelization grew faster in PRP wounds than control one (table, 2 and fig. 4).

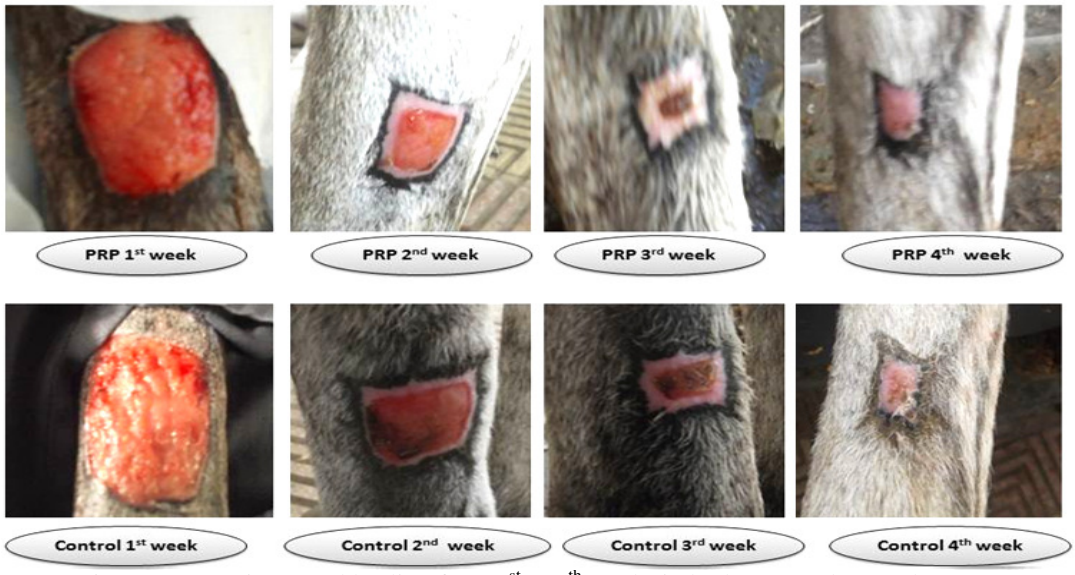


Fig.4. Progress in wound healing from 1st to 4th weeks in both PRP and control groups

Table1.Means ±SD of Wound dimensions in both control and PRP groups.

| | 1st week | 2nd week | 3rd week |
|---|---|---|---|
| PRP | 2.5 ±0.80 X 3.1 ± 0.90$^{Ab}$ | 1.3 ± 0.60 X1.9 ±0.70$^{Bb}$ | 0.30±0.2 X 0.50 ± 0.4$^{Cb}$ |
| Control | 3.20±0.90 X3.50 ± 1.10$^{Aa}$ | 2.50 ±0.9X 3.0 ±1.30$^{Ba}$ | 1.60±0.8 X 1.90 X 0.7$^{Ca}$ |
| t-value | 6.55$^{**}$ | 5.20$^{**}$ | 4.10$^{**}$ |

** Significant at (P < 0.01).
For each parameter: Means within the same row of different capital litters are significantly different at (P < 0.01)
For each parameter: Means within the same column of different small litters are significantly different at (P < 0.01).

Table 2.Means ±SD of Wound healing% in both control and PRP groups.

| | 1st week | 2nd week | 3rd week |
|---|---|---|---|
| PRP | 51.56±5.77$^{Ca}$ | 84.56±8.55$^{Ba}$ | 99.06±9.70$^{Aa}$ |
| Control | 30.00±3.44$^{Cb}$ | 53.13±5.40$^{Bb}$ | 81.00±7.40$^{Ab}$ |
| t-value | 7.40$^{**}$ | 8.44$^{**}$ | 9.51$^{**}$ |

** Significant at (P < 0.01).
For each parameter: Means within the same row of different capital litters are significantly different at (P < 0.01)
For each parameter: Means within the same column of different small litters are significantly different at (P < 0.01).

Table 3.Means ±SD of Wound contraction % in both control and PRP groups.

| | 1st week | 2nd week | 3rd week |
|---|---|---|---|
| PRP | 30.00±4.22$^{Ca}$ | 60.00±6.40$^{Ba}$ | 90.00±8.55$^{Aa}$ |
| Control | 16.25±3.55$^{Cb}$ | 31.25±2.55$^{Bb}$ | 56.25±5.22$^{Ab}$ |
| t-value | 7.50$^{**}$ | 9.50$^{**}$ | 10.30$^{**}$ |

** Significant at (P < 0.01).
For each parameter: Means within the same row of different capital litters are significantly different at (P < 0.01)
For each parameter: Means within the same column of different small litters are significantly different at (P < 0.01).

Wounds treated with PRP have quite cosmetic appearance without hypertrophic scar or crusts and the scab flacked out leaving smooth epithelial surface. On the other hand, control wounds showed crusts and some erosion after fall down of the scab.

Skin graft without PRP (control graft) showed signs of edema and exudation in the 3rd day post grafting. Skin grafts with PRP were adhered completely and not pulged, while another grafts were pulged and not adhere to the under lying wound bed. On the 7th day, partial detachment of graft from one side and failure of suture line were observed in control graft without firm attachment to the wound bed. Concerning coloration; skin graft with PRP showed bluish color changed into pink by the end of 2nd week. Control graft showed pale coloration. By the end of 3rd week, the grafts were firmly attached to the recipient bed in both grafts and the detached part in control graft healed by second intention. This made the graft with PRP having better cosmetic appearance than control one (fig. 5).

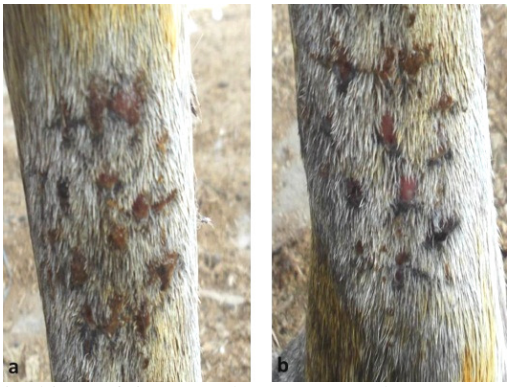


Fig.5. Skin graft in control group (a) and PRP group (b). Note the better appearance of PRP group

*B-Histopathological findings:*

The histological features of skin section harvested from both groups are shown in table 4, which reflect the acceleration and improvement of wound healing in PRP group in comparison to control wounds in both 1st and 2nd groups (table, 4 and fig. 6, 7).

Table 4. The histopathological findings of skin wound of control and PRP groups:

| Time (days) | Control group | PRP group |
|---|---|---|
| 3 | area of hemorrhage and polymorph nuclear cells infiltration mainly neutrophils (fig. 6a) | extensive polymorph nuclear cells infiltration with fibrin network deposition (fig. 7a) Immature granulation tissue with congested blood vessels beside neutrophils, esinophils and mono nuclear cells infiltration (fig.7b). |
| 7 | Proliferation of covering epithelial cells with underlying neovascularization and considerable number of inflammatory cells mainly macrophage (fig.6b). Neovascularization and considerable number of inflammatory cells mainly macrophage (fig. 6c). | Fibroblast and few collagens perpendicular to new blood vessel and parallel to wound surface. (fig. 7c) |
| 14 | Granulation tissue replaced most of the incisional area with mononuclear cells infiltration (fig. 6d) | Mature granulation tissue in the incisional area with eosinophilic cells infiltration (fig. 7d). |
| 21 | Incomplete epidermal area where minimal collagen will be deposited without scar formation (fig.6e). | Partially complete epidermal area where collagen will be deposited without scar formation (fig. 7e). |
| 28 | Normal epidermal layer over the granulation tissue with short rete ridge (fig.6f). | Normal epidermal area (green arrow) covers mature granulation tissue (blue arrow) with rete ridge (fig. 7f). |

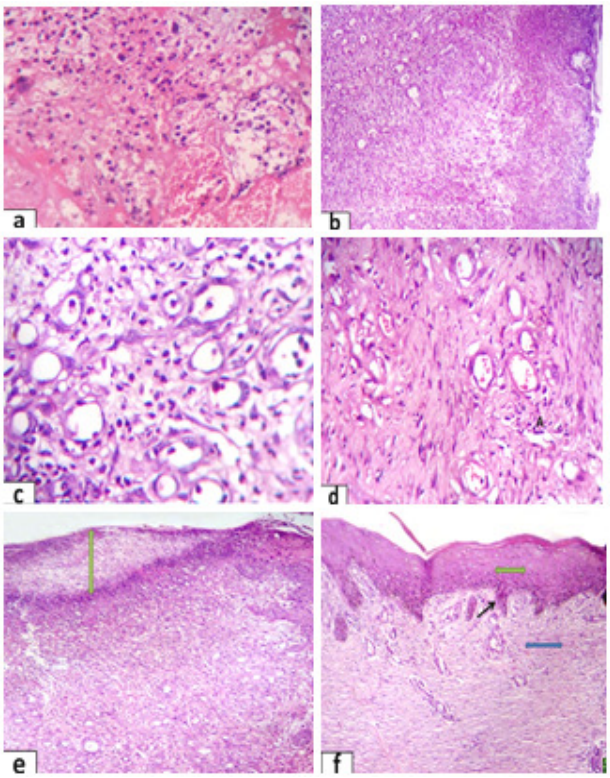


Fig.6.

Fig.6. Photomicrograph of skin wound healing in donkeys related to control group: (a) After 3 days showing area of hemorrhage and polymorph nuclear cells infiltration mainly neutrophils (HE. X250), (b) After 7 days showing proliferation of covering epithelial cells with underlying neovascularization and considerable number of inflammatory cells mainly macrophage (HE. X160), (c) After 7 days showing neovascularization and considerable number of inflammatory cells mainly macrophage. (HE. X250), (d) After 14 days showing granulation tissue replaced most of the incisional area with mononuclear cells infiltration (A) (HE; X250), (e) After 21days showing incomplete epidermal area where minimal collagen will be deposited and no scar will form (green arrow). (HE. X160), (f) After 28 days showing normal epidermal layer (green arrow) over the granulation tissue (blue arrow) with short rete ridge (black arrow). (HE. X160)

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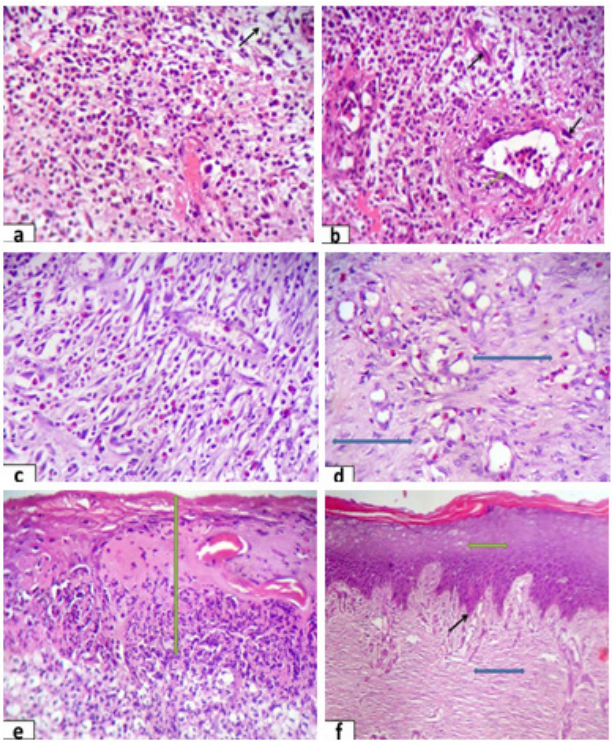


Fig.7.

Fig.7. Photomicrograph of skin wound healing in donkeys related to PRP group: (a) After 3 daysshowing extensive polymorph nuclear cells infiltration with fibrin network deposition (arrow). (HE. X250), (b) After 3 days showing immature granulation tissue (lack arrows) with congested blood vessels (green arrow) beside neutrophils, esinophils and mononuclear cells infiltration. (HE. X250), (c) After 7 days showing fibroblast and few collagens perpendicular to new blood vessel and parallel to wound surface. (HE. X250), (d)After 14 days showing mature granulation tissue in the incisional area (blue arrow), with eosinophilic cells infiltration (HE. X250), (e)After 21days showing partially complete epidermal area where collagen will be deposited without scar formation (green arrow). (HE. X250), (f) After 28 days showing normal epidermal area (green arrow) covers mature granulation tissue (blue arrow) with rete ridge (black arrow). (HE. X160)

The histological findings of skin grafts are shown in fig.8.

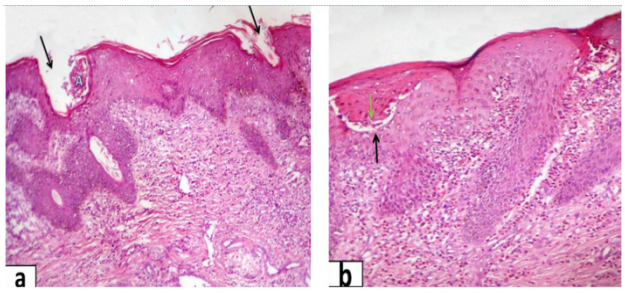


Fig.8.

Fig. 8 photomicrograph of skin graft of donkey (after 28 days) stained with hematoxylin and eosin (HE. X250): (a) Control group with skin graft showing incomplete formation of covering epithelium (arrows) with incomplete formation of tissue to occupy the space (A). (b): PRP group with skin graft showing complete covering epithelium with relatively adhesion between the graft (green arrow) and the recipient area (black arrow).

## 4. Discussion

PRP was used in several researches to induce rapid wound healing in horses by [10], in human by [20], in rabbits by [36] and in goats by [17] [3]. The method of preparation of PRP proved simple and required minimal technical skill, so it can be used in field as a low cost treatment for owners [37] [10]. The number of platelet in PRP was 3-5 times more than the intravascular platelet which is the minimal concentration required for acceleration of epithelization and granulation tissue formation [43] [18] [33]. Platelet activation was easily performed. Platelet can be activated by physiologic agent as thrombin, thromboxane, collagen, serotonin and epinephrine and pharmacologic agents as calcium ionophorous and calcium chloride [7] [9]. Application of PRP to the wound created a seal for bleeding and no further bleeding was observed post application or during change of the bandage. This was contributed to addition of thrombin to platelet enhancing its activation and subsequent formation of a hemostatic plug which minimize further bleeding and initiate the process of wound healing via thrombin dependent cell activation and platelet dependent angiogenesis [47] [23]. Although the release of secretory protein by platelet begins within 10 minutes of clotting with more than 95% of presynthesized growth factors secreted within 1 hour, platelet continues to synthetize and secret additional protein for the rest of the lifetime (5-10 days) [43]. This may explain the rapid improvement of PRP wounds during the 1st week.

Although blood used in microbiology laboratories to culture bacteria, PRP wounds didn't possess any infection in the present study. Marx [29] stated that PRP is not deferent in substrate than blood clot that forms in every wound and therefore couldn't support bacterial growth any more than any other blood clot. This also may attribute to presence of leukocytes in PRP which are bactericidal [25].

Granulation tissue grew rapidly to cover all wounds but not exuberated in both PRP and control wounds. The same result was stated by [36] [34]. On contrary, Monterio et al, [33] reported that PRP and PRGf produced excessive granulation tissue which resulted in wound healing delay. Expansion of granulation tissue above and beyond the wound margin may physically inhibited epithelization and delay wound healing [38].

Closure of the wound by 2nd intention is achieved by contraction and epithelization [48]. Wound contraction is centripetal movement of the margins; it occurs faster than epithelization and determines the speed of wound healing and cosmetic appearance. In the present study wound contraction and healing process is significantly higher in PRP treated wound than control one. The contraction is

due to the action of myofibroblasts in granulation tissue which make the wound margin to move toward the wound center [12] [51], PRP enhances vascular fibroblast proliferation and increased extracellular collagen matrix synthesis and deposition [42] [13]. Faster epithelization of PRP wounds in this study may be explained by the ability of PRP to stimulate the keratinocytes to differentiate into various epithelial cell types as skin epithelial cells after systemic administration [52]. Effective wound contraction and faster epithelization which were observed in this study were also observed by [3] in goats and [36] in rabbits.

Quite cosmetic appearance of PRP treated wounds may be due to platelet growth factors which can modulate the inflammation and proliferation phases of wound healing process, thus reducing the risk of keloid and hypertrophied scar formation [43]. Blood constituents such as PRP, PPR and fibrin glue were used as tissue sealant under skin graft and skin flap [11] [21] [26]. Significant improvement of skin graft healing with PRP gel application was observed by [2]. Application of PRP under skin graft induced less edema. This edema was clearly observed in control graft in the present study. The same result was observed by [5]. Skin graft with PRP was completely adhered to the recipient wound bed. This graft survival may be due to PRP reduce the inflammatory reaction between the graft and recipient area resulting in favorable condition for the graft incorporation [11]. Bluish coloration of the graft with PRP was observed by end of the 2nd week due to healing process associated with random vascular anastomosis of veins rather than with arteries [19]. Also, PRP application could be important technique for starting angiogenesis by recruiting the endothelial cells that line the blood vessels [26]. On the other hand, [50] [21] reported that revascularization of the graft didn't occur after application of PRP under the skin graft.

Better cosmetic appearance was recoded in PRP graft than control one. This may be due partial detachment of the control graft and also due to rapid hair growth because of PRP improve cutaneous ischemic condition and to increase vascular structure around hair follicles [45].

Histopathological features revealed presence of fibrin network in PRP treated wounds versus area of hemorrhage in control one three days post wound creation. The formation of fibrin is initiated by activation and aggregation of platelets. This aggregation resulted in a platelet plug that inhibits blood flow [28]. Moreover this fibrin clot provided a matrix for the migration of tissue forming cells including fibroblasts which were observed earlier in PRP group one week post wounding; these fibroblasts were responsible for collagen synthesis and endothelial cells involved in angiogenesis [27]. Platelet derived growth factors (PDGF) are powerful mitogen for fibroblast and smooth muscle involved in all three phases of wound healing including angiogenesis, formation of fibrous tissue and re epithelization [22]. Transforming growth factors obtained from platelet activate fibroblasts and differentiate it into myofibroblasts and increase the numbers of these cells to promote wound contraction [48]. Theses histopathlogical findings correlated well with our clinical findings. Macrophages and eosinophils are predominant in PRP treated wound than control one. Macrophages are essential for wound healing as it secretes numerous enzymes and cytokines which stimulates fibroblasts, keratinocytes and finally promotes angiogenesis [8]. Eosinophil knows to produce VEGF, PDGF, TGF-α and TGF-β, which promote epithelial cells proliferation, angiogenesis and organization of wound site [53]. Furthermore, the eosinophil has longer been recognized as a source of plasminogen, which catalase the breakdown of fibrin, a critical step in the process of wound healing [41]. Dense bundle collagen parallel to the overlying epidermis was observed in PRP treated wound. This finding suggested increased tensile strength and improve wound repair [10]. The dense parallel collagen bundles are characteristic to mature granulation tissue in PRP wound [35]. Meagher [31] stated that the histologic features that judge the wound healing are thick vascular granulation tissue, more fibroblasts and collagen deposition and epithelial migration; all these features were observed clearly in this study. Concerning skin graft group the histopathological sections showed complete epithelization and relative attachment of the graft to the recipient sites in PRP grafts versus incomplete granulation and epithelization in control graft. The present results are agreed with [11] [5]. Attachment of graft to the recipient bed achieved by increasing collagen and ground substances into wound which secreted by fibroblasts [49]. PDGF promote fibroblast proliferation and stimulate granulation tissue formation and epithelization [16]. A decreased inflammatory reaction under the graft that induced by PRP is another cause for graft attachment [11]. From the aforementioned clinical and histopathological findings, it could be concluded that PRP gel is an effective natural and low cost wound healing promoting agent for full-thickness skin wounds in donkeys and form an efficient sealant for full thickness skin graft with good cosmetic appearance.

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