

Effect of *Aloe vera* gel on the healing of cutaneous wounds in donkeys

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Abstract

The efficiency of fresh *Aloe vera* gel was tested on the healing of large cutaneous wounds of the forelimbs in donkeys. Twelve adult donkeys from both sexes were used in this experimental study. They were divided into equal groups. A square shape cutaneous wound about 8*8cm, was induced on the lateral aspect of the forelimb exactly between humeral and knee joint. In group one, the induced wound was left without treatment, while in second group the wound was treated by local application of fresh *Aloe vera* gel. The evaluation of healing process results was based on monitoring the gross signs of wound healing, in addition to the study the histopathological changes at 15th, 30th, 45th postoperative days. In group one, the wound healing process was characterized by infection, congestion, inflammatory exudate, excessive granulation tissue formation, and no decrease in wound size, while in group two, the healing of wound was characterized by absence of granulation tissue overgrowth with decrease in wound size and little occurrence of infection and congestion. The histopathological observations in first group was showed intensive granulation tissue formation with massive infiltration of mononuclear inflammatory cells and delay of reepithelization, while in second group, overgrowth of granulation tissue was lesser, little infiltration of mononuclear inflammatory cells, rapid reepithelization with more maturation and stretching of collagen fibers. It was concluded that the *Aloe vera* gel can be used successfully as topical treatment to improve and enhance large cutaneous wound healing in donkeys.

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Introduction

A wound can be defined as any interruption or destruction of the body tissue continuity, such as following trauma (1). Skin is a tissue that act as a protective layer or barrier against the external factors. Loss of large portions of the skin may led to main disability (2). Generally, the wound may be simple or complicated and it is occurred due to several causes such as thermal, mechanical and chemical. Also, some pathological conditions may be led to the appearance of wounds as in diabetes mellitus (1). Healing of cutaneous wound is a complex process that need to primary care and monitoring to return the injured tissue to normal function and integrity. Any disorder in this process may led to some complication such as tissue loss, infections and exuberant granulation tissue and develop of chronic non-

healing wound (3). The occurrence of traumatic wounds in equine is considered to be high and most of them become chronic, adding more difficulty to plans of wound healing management (4). There are several local and systemic factors may be retarded wound healing such as infection, ischemia, tissue hypoxia, disturbance of the inflammatory process and nutritional factors (5). The injuries are remaining an important problem, so several therapeutic agents or protocols are used for wounds management to accelerate acute or chronic wounds healing and restoration of damaged tissue to normal status as possible. Such as for these agents are: honey (6), corticosteroids (7), ultrasound, negative pressure wound therapy and skin substitutes (8,9), additionally *Aloe vera* (*Aloe barbadensis miller*) (10). *Aloe vera* plant or desert lily is belonging to family, called Liliaceae. The use of various forms of *Aloe vera* appears to be highly effective for

treatment of injuries in different parts of health tissue for example skin wounds. The *Aloe vera* gel has anti-inflammatory, antimicrobial, immunomodulatory, analgesic activities and promote wound healing. It contains vitamins C and E, minerals, essential amino acids, and polysaccharides that stimulate the growth of tissues and cell regeneration (11,12).

The objective of this study was to evaluate the viability of fresh *Aloe vera* gel on the healing process of the cutaneous wounds of the fore limbs clinically and histopathology in donkeys.

Materials and methods

Twelve adult local breed donkeys from both sexes were used in this study. A total number of donkeys were divided into two equal groups. The animals were fasted 24 hours of food and 6 hours for water before the operation. All operations were performed under general anesthesia using a mixture of 10 % ketamine HCL at 2.2 mg/kg and xylazine 2% at 1.1mg/kg B.W., given in a single syringe by intra venous route in the jugular vein (13,14). Repeated doses of the drug mixture were given when needed. In all animals a large, full thickness cutaneous wound about 8*8 cm in

diameter was established on the lateral surface of the forelimb between the humeral and knee joint exactly of each animal (Figure 1).

The cutaneous wound was left without treatment in first group as a control group, while in second group the wound was treated with through by local application of fresh *Aloe vera* gel was obtained directly from leaf of the plant one time per day for 10 successive days with daily bandaging of the wound (Figure 2).

The experimental donkeys were received penicillin streptomycin for five days post-operatively by intramuscular route injection. The assessment of wound healing process was depended on the observations of the gross signs of wound healing process post-operatively and study the histopathological changes in both groups at 15th, 30th, 45th post-operative days (P.O.Ds). The formalin fixed samples were processed and stained with hematoxylin and eosin and Masson's trichrome stain as a special stain. In addition, scoring of histological sections was depended also. The histological sections were scored according to (15) (Table 1).

The mean of each scoring criteria was analyzed using SPSS version 19.0 using one-way analysis of variance with Duncan test as post hock test (16).

Table 1: Scoring of histological criteria

Score	Granulation	Inflammation severity	Epithelization	Keratinization
0+	Absent	Absent	Absent	Absent
1+	Discrete	Few infiltrations of inflammatory cells with edema	Discrete	Discrete
2+	Moderate	Moderate infiltration of inflammatory cells with hemorrhage	Moderate	Moderate
3+	Intense	Massive infiltration of inflammatory cells with exudate	Intense	Intense
4+	Complete	Massive infiltration of inflammatory cells with fibrosis	Complete	Complete



Figure 1: Show full -thickness skin wound excision.



Figure 2: Gel of *Aloe vera*.

Results

Gross findings of wound healing

In first group, the gross signs of wound healing are including excessive granulation tissue formation at 7th P.O.Ds., infection and congestion (Figure 3). The granulation tissue was increased in formation and became more prominence during period of the study (Figures 4 and 5). There is delay in reepithelization and wound size still large (Figure 6). In second group, the healing process was relatively better in comparison to group one, where the granulation tissue formation and congestion was showed very less when compared with first group (Figures 7 and 8). The rate of reepithelization was faster that appeared at 30th P.O.Ds. and it became very pronounced at 45th P.O.Ds. with more reduction in wound size and granulation tissue (Figures 9 and 10).



Figure 3: Cutaneous wound after 7 days in group one.

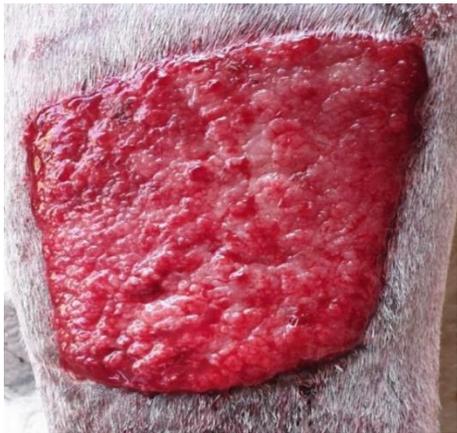


Figure 4: Show cutaneous wound after 15 days in an animal group one.



Figure 5: Show cutaneous wound after 30 days in an animal of group one.



Figure 6: Show cutaneous wound after 45 days in an animal of group one.



Figure 7: Show cutaneous wound after 7 days in an animal of group two.



Figure 8: Show cutaneous wound after 15 days in an animal of group two.



Figure 9: Show cutaneous wound after 30 days in an animal of group two.



Figure 10: Show cutaneous wound after 45 days in an animal of group two.

Histopathological findings

In first group, the histological section in wound site at 15th P.O. Ds. was revealed presence of severe newly granulation tissue formation, massive infiltration of mononuclear inflammatory cells, newly blood vessels and blood clot at wound surface (Figures 11 and 12). Staining with Masson's trichrome was showed sever proliferation of collagen fibers as extracellular matrix (Figure 13). At 30th P.O.Ds., the site of wound showed an increase in granulation tissue formation and in number of newly blood vessels with massive macrophage infiltration and fibrocytes hyperplasia. Staining with Masson's trichrome was showed increase in the extracellular matrix represented by collagen fiber. At 45th P.O.Ds., the histological feature of the wound site was revealed little reduction in the granulation tissue. The reepithelization was begun to the external wound edges with reduction in number of the blood vessels. The collagen sheets were started to be stretched with reduction in macrophages number (Figures 14 and 15).

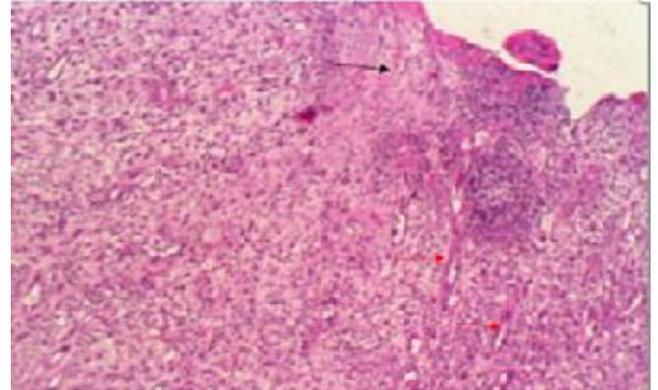


Figure 11: Micrograph of the wound site 15th P.O.Ds. in G1 showing new granulation tissue formation (black arrow) and newly blood vessels (red arrow).H&E X10.

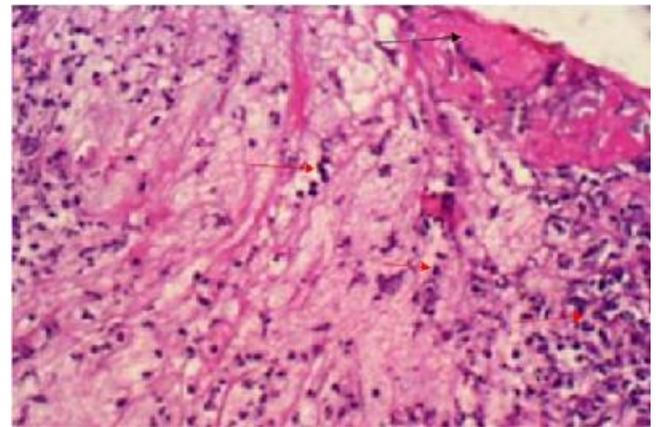


Figure 12: Micrograph of the wound site at 15th P.O. Ds.in G1 showing blood clot (black arrow) and infiltration of mononuclear inflammatory cells (red arrow).H&E40X.

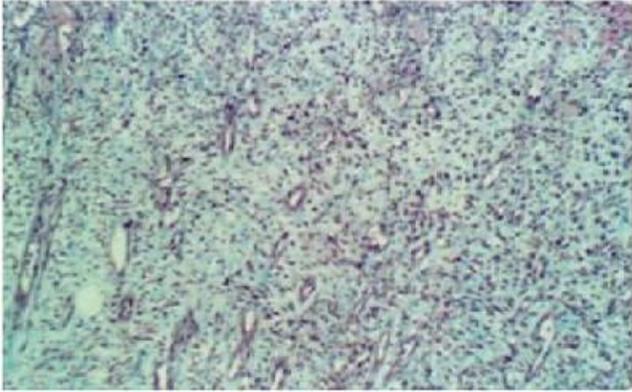


Figure 13: Micrograph of the wound site at 15th P.O.Ds. in G1 with Masson's trichrome showing massive deposition of collagen fibers. X10.

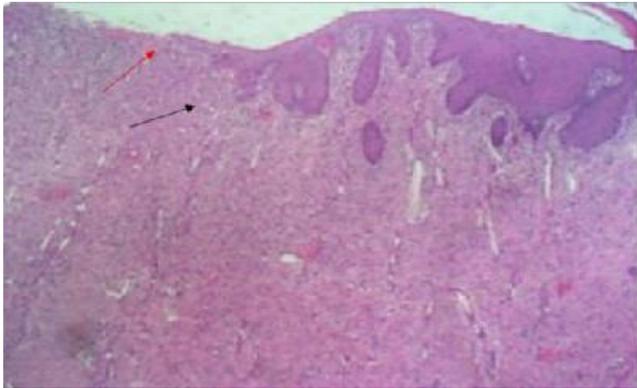


Figure 14: Micrograph of the wound site at 45th P.O.Ds. in G1 showing decrease in granulation tissue (black arrow) and beginning of reepithelization (red arrow). H&E X4.

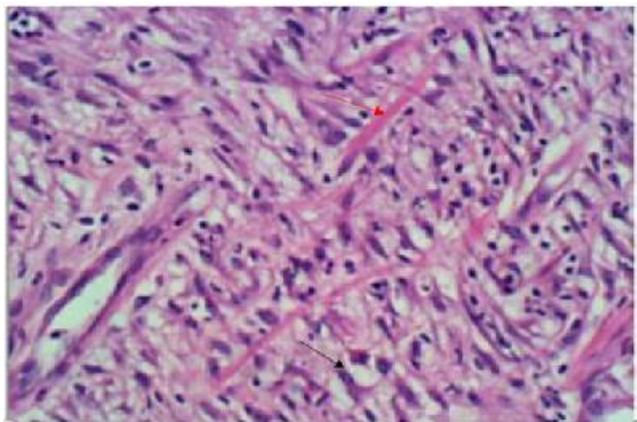


Figure 15: Micrograph of the wound site at 45th P.O.Ds. in G1 showing decrease in macrophage (black arrow) and stretched of collagen sheath (red arrow). H&E X40.

In second group, the wound site at 15th P.O. Ds was showed new granulation tissue formation, newly blood vessels, infiltration of mononuclear inflammatory cells and fibrocytes hyperplasia (Figures 16 and 17). Staining with Masson's trichrome, there was deposition of collagen fiber as extracellular matrix (Figure 18). At 30th P.O.Ds., there is less formation of granulation tissue than in 15th P.O.Ds. Wound reepithelization, newly blood vessels, infiltration of mononuclear inflammatory cells and hyperplasia of fibrocytes were showed (Figure 19). The collagen fibers were started to be stretched. There is collagen fiber maturation as extracellular matrix with Masson's trichrome staining (Figure 20). The site of wound at 45th P.O.Ds., was showed less formation of granulation tissue with complete wound reepithelization and newly blood vessels. Few fibrocytes was showed in the wound site and collagen fibers are organized and stretched (Figure 21). There is more collagen fiber maturation with Masson's trichrome staining.

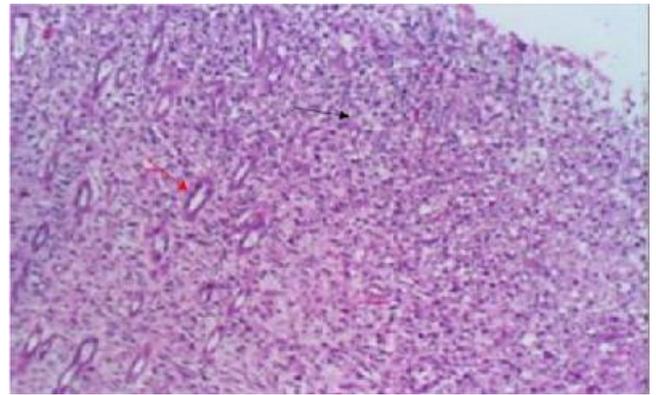


Figure 16: Micrograph of the wound site at 15th P.O.Ds. in G2 showing newly granulation tissue formation (black arrow) and newly blood vessels (red arrow). H&E X10.

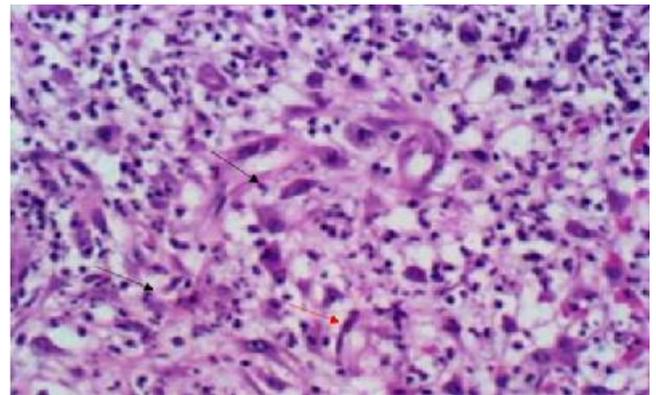


Figure 17: Micrograph of the wound site at 15th P.O.Ds. in G2 showing infiltration of mononuclear cells (black arrow) and hyperplasia of fibrocyte (red arrow). H&E X40.

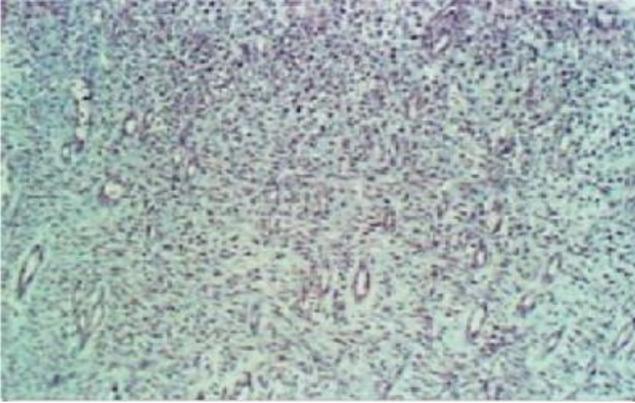


Figure 18: Micrograph of the wound site at 15th P.O.Ds. in G2 with Masson's trichrome showing deposition of collagen fibers. X10.

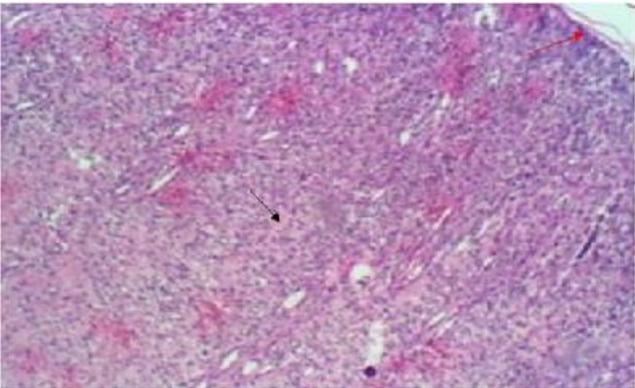


Figure 19: Micrograph of the wound site at 30th P.O.Ds. in G2 showing less granulation tissue formation (black arrow) and reepithelization to wound surface (red arrow). H&E X10.

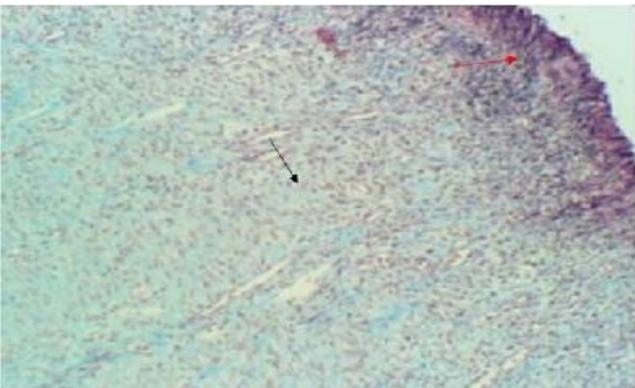


Figure 20: Micrograph of the wound site at 30th P.O.Ds. in G2 with Masson's trichrome showing maturity of collagen fibers (black arrow) and reepithelization to wound surface (red arrow). X10.

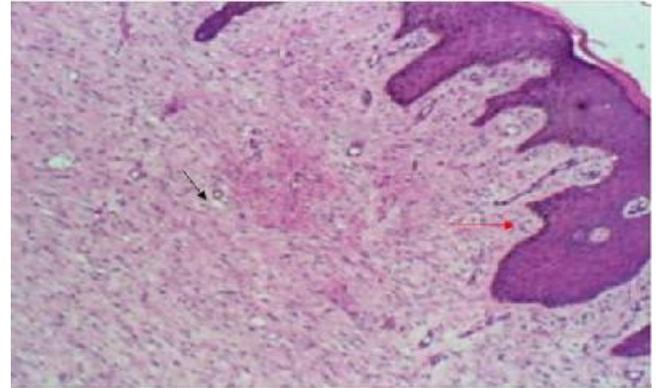


Figure 21: Micrograph of the wound site at 45th P.O.Ds. in G2 showing newly blood vessels (black arrow) and complete reepithelization (red arrow). H&E X10.

Histological scoring

In second group, the severity of inflammation is less than in first group at significant difference that represented by infiltration of mononuclear inflammatory cells. The intensity of newly formed granulation tissue was thicker and more in the first group rather than the second group at significant difference. The epithelialization process was appeared very pronounced and faster in the second group rather than the first group at significant difference. The keratinization was showed in first and second group with significant difference (Tables 2-5).

Table 2: Histological scoring for severity of inflammation

Group	15 day	30 day	45 day
Control	2.1±0.11 ^c	1.6±0.14 ^b	1.1±0.10 ^a
<i>Aloe vera</i>	1.9±0.11 ^c	1.5±1.15 ^b	1.0±0.09 ^a

Letters that differ in vertical view mean that there is a significant difference at P<0.05.

Table 3: Histological scoring for intensity of granulation tissue formation

Group	15 day	30 day	45 day
Control	1.6±0.12 ^a	2.4±0.09 ^a	2.7±0.17 ^a
<i>Aloe vera</i>	1.3±0.10 ^b	2.1±0.13 ^b	2.5±0.12 ^b

Letters that differ in vertical view mean that there is a significant difference at P<0.05.

Table 4: Histological scoring for reepithelization

Group	15 day	30 day	45 day
Control	0.9±0.09 ^a	1.8±0.11 ^b	4.0±0.18 ^a
<i>Aloe vera</i>	0.9±0.10 ^a	3.1±0.11 ^a	4.0±0.16 ^a

Letters that differ in vertical view mean that there is a significant difference at P<0.05.

Table 5: Histological scoring for keratinization

Group	15 day	30 day	45 day
Control	0.5±0.09 ^a	0.6±0.08 ^a	0.9±0.08 ^a
<i>Aloe vera</i>	0.5±0.07 ^a	0.7±0.05 ^a	1.0±0.08 ^a

Letters that differ in vertical view mean that there is a significant difference at $P < 0.05$.

Discussion

There are several influences such as size and wound age, local vascular integrity, tissue loss, contamination degree and local tissue damage play an important factor for practitioner to treat the injuries. Therefore, wounds of distal parts of the limbs in equine are characterized by slower healing rate other than wounds which are located in the trunk region (17).

In group one, the healing process of wound was characterized by presence of infection, exudate and overgrowth of granulation tissue. These signs were agreement with Cochran *et al.* and Dart *et al.* (18,19) where the wound limb healing in Equidae are sometimes accompanied with some complications such as hyper granulation tissue formation and infection. Infection of wound may be due to leave the wound without treatment and exposure of it to the external contamination. In addition, the inflammatory stage of distal limb wounds was characterized by prolongation (20). Massive overgrowth of granulation tissue in first group may be due to epidermal and fibroblast growth factor (18), where the granulation tissue formation doesn't stay at the normal level of skin edge, but raise above the skin edge particularly at the lower aspects of the limbs (21). Additionally, in my opinion, about 60-70 % of body weight was bearing on the fore limbs. The hyperplasia of fibrocyte may be due to some reasons such as dis-regulation of fibroblast growth, low contraction rates, tissue hypoxia, deviations in the TGF expression and low level of oxygen saturation of the limb in equine rather than other parts of the body (22,23). The reepithelization process was delayed in appearance because presence of exuberant granulation tissue that led to the reduction in the rate of reepithelization and wound contraction. The excessive granulation tissue act as a barrier that impair movement of wound margins and advanced epithelium (18). Additionally, due to changes in the expression of TGF β -1 (24).

In group two, *Aloe vera* seems playing an important role in the healing process as anti-inflammatory agents. It is act as mediator to decrease vascular constriction and aggregation of platelet, improvement wound oxygenation, elimination free radicals and increased collagen formation. Additionally, inhibition of cyclo-oxygenase, adhesion of leukocyte, activity of pro-inflammatory cytokines such as IL-6 (interleukin-6) and TNF- α (tumor necrosis factor alpha) (25). The signs of inflammation were reduced relatively in this group and these may be due to the effect *Aloe vera* gel that have anti-inflammatory effect through by promotion of

prostaglandin synthesis and increased infiltration of leucocytes (26). The curative effect of *Aloe vera* gel as antimicrobial, anti-inflammatory, immunomodulating and antioxidant effect led to little infiltration of inflammatory cells and infection (12) where, the authors Ajmera *et al.* (27) concluded that washing the mouth with *Aloe vera* mouthwash have significant results to treat oral gingivitis. Healthy granulation tissue formation was showed because the therapeutic activity of *Aloe vera* gel where it has ability to treat skin wounds. It is rich with vitamins such as C and E, essential amino acids and polysaccharides that evokes tissue growth and cell regeneration (11). The presence of polysaccharides mainly in *Aloe vera* and other substance led to improve skin wound healing. Acemannan and mannose-6-phosphate in *Aloe vera* are able to bind with macrophages and fibroblasts. This binding led to stimulate the growth and proliferation of these cells (28). The author's Chithra *et al.* (29) were showed, the high level of dermatan sulfate and hyaluronic acid in *Aloe vera* led to improve wound healing through by increasing collagen synthesis and fibroblastic activity. The application of *Aloe vera* in this study to treat skin wound was enhanced wound healing process through by acceleration of epithelialization, angiogenesis, collagen synthesis and wound contraction or closure. This is agreements with authors Oryan *et al.* (30) that's showed the treatment of wounds with *Aloe vera* led to enhance the stages of healing. Additionally, *Aloe vera* gel led to increase in proliferation of keratinocytes and epithelial cell migration. The wound size was relatively decreased and this agree with Atiba *et al.* (10) were the level of TGF-1 and BFGF, blood vessels formation, collagen deposition and fibroblast proliferation are increased in the granulation tissue in rats administered orally with *Aloe vera* that's led to accelerate wound contraction.

Conclusion

It concluded that the *Aloe vera* gel could be used successfully as topical treatment to improve and enhance large cutaneous wound healing in donkeys.

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Conflict of Interest

The authors declare that has no conflict of interest

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تأثير هلام الصبار على التئام الجروح الجلدية في الحمير

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الخلاصة

صممت هذه الدراسة لتقييم فعالية جيل الصبار النقي على التئام الجروح الجلدية الكبيرة في القوائم الأمامية في الحمير. اثنا عشر حميرا بالغا ومن كلا الجنسين تم استخدامها في هذه التجربة. قسمت الحيوانات إلى مجموعتين متساويتين. تم عمل جرح جلدي مربع الشكل وبأبعاد 8*8 سم تقريبا على السطح الوحشي للقائمة الأمامية وتحديدًا بين المفصل العضدي والركبة. في المجموعة الأولى تم ترك الجرح المستحدث بدون علاج أما في المجموعة الثانية تم معالجة الجرح من خلال وضع جيل الصبار النقي موضعيا. تم تقييم نتائج عملية التئام الجروح من خلال متابعة العلامات العيانية للتئام الجروح بعد إجراء العملية الجراحية بالإضافة إلى دراسة التغيرات النسجية المرضية وللأيام 10، 30، 45 على التوالي. في المجموعة الأولى تميزت عملية التئام الجروح بوجود احتقان، خمج، نضح التهابية وفرط نمو للنسيج الحبيبي وعدم صغر حجم الجرح أما المجموعة الثانية فتميزت عملية الالتئام بعدم فرط نمو للنسيج الحبيبي وصغر لحجم الجرح مع قلة حدوث الاحتقانات والخمج. أظهرت النتائج النسجية المرضية في المجموعة الأولى إلى شدة تكوين للنسيج الحبيبي وشدة ارتشاح للخلايا الالتهابية وحيدة النواة وتأخر عملية التطهر بينما تميزت المجموعة الثانية بقلة فرط تكون النسيج الحبيبي مع قلة ارتشاح للخلايا الالتهابية وحيدة النواة وسرعة عملية التطهر بالإضافة إلى نضوج وامتداد الألياف الكولاجينية. نستنتج من هذه الدراسة إلى إمكانية استخدام جيل الصبار النقي موضعيا وبنجاح في معالجة وتحسين عملية التئام الجروح الجلدية في الحمير.