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Histological studies on the efficacy of fresh *Aloe vera* leaves on wound healing in calves

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Abstract

Present study was conducted on 12 calves having fresh wound of size 16-25 cm² anywhere on the body except joints to evaluate the wound healing using fresh *Aloe vera* leaf paste. These were randomly divided into two groups for further treatment. Wounds of group I were treated with normal saline solution whereas, that of group II were applied with *Aloe vera* leaf paste. In the initial day's observation revealed necrotic foci, haemorrhagic spots and serofibrinous exudates without any trace of epidermal growth in both groups whereas few fibroblasts and angioblasts cells were too discernible in the regenerating dermis and maximum of it was seen *Aloe vera*. Well defined dermis along with prominent epidermis and keratin layer was observed in *Aloe vera* treated group on 25th post treatment. There was a significant difference between the treatment and control group in wound contraction rate on 5th, 10th, 15th, 20th and 25th post treatment day. An accelerated wound contraction was seen in *Aloe vera* treated group during all stage of wound healing.

Keywords: *Aloe vera*, calves, wound contraction, histopathology and wound healing

Introduction

Early healing of the wounds depends on the way the wound is managed; however, slight negligence on the part of the surgeon makes the treatment a complete failure and endangers the life of the animal (Kumar, 2006) [4]. Since time immemorial man has used various parts of the plant in the treatment and prevention of different ailments (Chah *et al.* 2006) [2]. During the last two decades, traditional medicine has re-emerged as one of the important pillars for the treatment of various diseases and disorders. It is a well-known fact that the modern drugs have their origin in plant before they are chemically synthesised (Newman *et al.* 2016) [7]. Many medicaments, indigenous preparations and variety of methods have been used to stimulate wound healing such as topical application of herbal remedies, natural honey, electromagnetic pulses, fibroblast growth factors and collagen with topical antibiotics. Though various agents have been identified as a wound healer, still there is a need of cheaper therapy which can accelerate the rate of healing, without affecting the normal physiological process along with its socio-economical acceptance and minimal side effects (Sharma, 2018). In Ayurvedic medicine, *Aloe vera* is used topically in combination with liquorice of root for treating psoriasis and eczema and various type of wound (Habeeb *et al.* 2007) [5]. *Aloe vera* has been used for centuries for its wound healing property and therapeutic potential (Gupta and Malhotra, 2012) [3]. Therefore, the present study was conducted to evaluate the efficacy of *Aloe vera* as a potent wound healing agent.

Materials and Methods

The work was approved by the institutional ethical committee. It was conducted on twelve calves aged between six months to one year and weighing approximately 60 to 100 kg that were presented for treatment of the wound. Calves having fresh wound of size 16-25 cm² with a depth of 0.4-0.5 cm were selected for the present study. The wounds selected were anywhere on the body except joints. The age of the wound was decided based on appearance, and granulation tissue i.e. Lustre, colour and texture. Moreover, all the wounds were debrided and freshened up when it was included in the study. The leaves of the plant of *Aloe vera* were collected locally, authenticated in Department of Pharmacology and Toxicology. Fresh leaves of these plants were washed and cleaned with distilled water. Pulp of the leaf was taken out and grinded in a fine paste using a mortar and pastel aseptically for its application on the wound surface. Calves selected for the present study were randomly divided into two groups for further treatment.

Wounds of group I were treated with Normal Saline Solution, whereas those of group II were treated with Aloe vera leaf paste. The wounds were cleaned with hydrogen peroxide solution regularly, followed by topical application of Normal Saline Solution in group I and leaf paste of Aloe vera in group II respectively, till the wound healed. Tissue sample were taken on 5th, 10th, 15th, 20th and 25th respectively.

Results and Discussion

Wounds treated with *Aloe Vera* on 5th day showed mild infiltration of neutrophils and macrophage along with necrotic foci and haemorrhagic spot. The whole wound area was covered with serofibrinous exudate; however, it was lesser than Normal Saline Solution but similar to Povidone Iodine treated wound. Few fibroblasts and angioblasts scattered at the deeper zone of contorted dermis was too observed at this stage. Meagre vascularisation and capillary formation at the deep dermis layer were too evident. There was no trace of epidermal regeneration even at the periphery of the wound. These findings are similar to the findings of Al-Kennany *et al.* (2006) [1] who studied the effect of *Aloe vera* gel on experimental tendon healing in donkeys and found fibrin clot, polymorph nuclear cells (PMNs) and fibroblasts in intertendenous gap on 7th day post- treatment day. They too reported that regeneration of epithelium was lacking at this interval. Takzare *et al.* (2009) [9] who studied the wound healing influence of *Aloe Vera* gel in rats on the basis of histology.

Aloe vera treated wound depicted absence of necrotic foci and haemorrhagic spots on 10th day. Few neutrophils scattered unevenly in the wound area were too observed. Serofibrinous exudate was lacking revealing end of inflammatory phase. Moderate fibroblast and angioblast cells similar to that of wounds treated with Povidone Iodine were observed in the histological slide. Regenerating capillaries perpendicular to that of wound area and developing fibrinogen parallel to the wound surface was found at this stage. The density of proliferating capillaries and fibrinogen were same as that of wound treated with Povidone Iodine. Regeneration of epidermis at the periphery of the wound was highly conspicuous. Takzare *et al.* (2009) [9] who studied the wound healing influence of *Aloe vera* gel in rats on the basis of histology reported increased density fibroblasts which became more abundant than the previous day. He also reported thickened endothelium of blood vessels in *Aloe vera* group as compared to control and a significant difference in the number of neutrophils between control and *Aloe vera* group.

On 15th day *Aloe vera* treated wound showed complete absence of necrotic foci, haemorrhagic spots and inflammatory cells and whole dermal area was filled with moderate connective tissue. However, the density of connective tissue was slightly higher than other treated groups but its density was significantly higher than Normal Saline Solution treated wound. Blood vessels and other connective almost filled the dermal layer and, on this scaffold, moving epidermis from the margin of the wound was seen. Slightly more differentiated rete pegs sparsely penetrating the dermis was too observed in the histological slide. Meagre amount of elastic fibre in the regenerating dermis was detectable in the microscopic study. Finding of present study corroborates the findings of Al-Kennany *et al.* (2006) [1] who studied the effect of *Aloe vera* gel on experimental tendon healing in donkeys and found fibroblast proliferation in superficial layer of tendon stumps and in the intertendenous gap on 14th days post

treatment. Takzare *et al.* (2009) [9] who studied the wound healing influence of *Aloe vera* gel in rats on the basis of histology and found marked density of fibroblasts in *Aloe vera* treated group which significantly outnumbered the control on 14th.

Minute observation of the histological slide of *Aloe vera* treated group revealed dense dermis completely filled with connective tissue on 20th day. Centripetally moving epidermis almost covered the whole dermal layer. Rete pegs in this treatment groups were more differentiated and outnumbered all the treatment groups. Moreover, it penetrated deeper dermal layer than other treatment groups. Marked collagen and elastic fibres spread throughout the dermal layer were too observed at this stage of healing. Thin keratin layer almost covering the epidermis layer was too discernable at this stage. Similar findings were too observed by Al-Kennany *et al.* (2006) [1] who studied the effect of *Aloe vera* gel on experimental tendon healing in donkeys and found that the intertendenous gap was filled with mature granulation tissue oriented in proper fashion in *Aloe vera* group as compared to control where it was improperly oriented.

25th day Observation of histological slide of the wounds treated with *Aloe vera* at this stage showed complete repair of dermal and epidermal layer. The dermal layer was completely packed with well differentiated connective tissue at this stage. Epidermis having distinct architecture was covering the epidermal layer. A frequent and well-placed rete pegs were visible in the histological slide and it masked the repaired epidermis of the entire groups. Thick layer of keratin almost covering the entire wound area was also perceptible at this stage. Thickness of keratin layer was more than entire treatment group. Accelerated wound healing in *Aloe vera* treated group can be attributed to the findings of Subramanian *et al.* (2006) [8] who stated that *Aloe vera* exerts its wound healing effects by maturation and alignment of the fibroblasts and collagen fibrils and eventually improves the biomechanical characteristics of the granulation tissue. Moriyama *et al.* (2016) [6] who found that the *Aloe vera* increased the cell surface expression of $\beta 1$ -, $\alpha 6$ -, $\beta 4$ -integrin, and E-cadherin which may contribute to the cell migration and wound healing and also help in significant changes in cell-cycle progression and gene expression, suggesting roles of *Aloe vera* in improvement of keratinocyte function. Teplicki *et al.* (2018) [10] studied that *Aloe vera* accelerates wound healing by strongly promoting fibroblasts and keratinocytes proliferation and moderately stimulating cell migration.

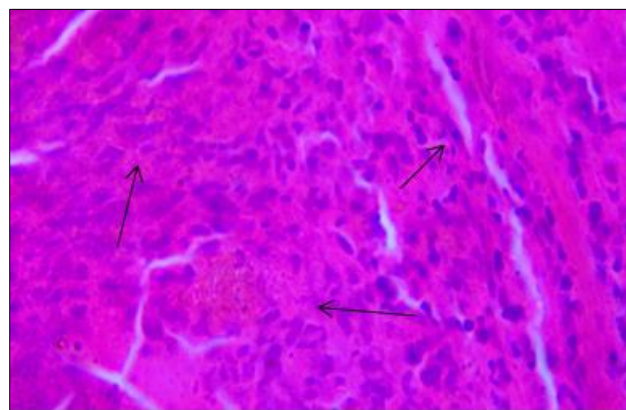


Fig 1: Photomicrograph of *Aloe vera* treated wound showing proliferation of angioblast and fibroblast cells in developing dermis on 10th post treatment day (H & E 100x)

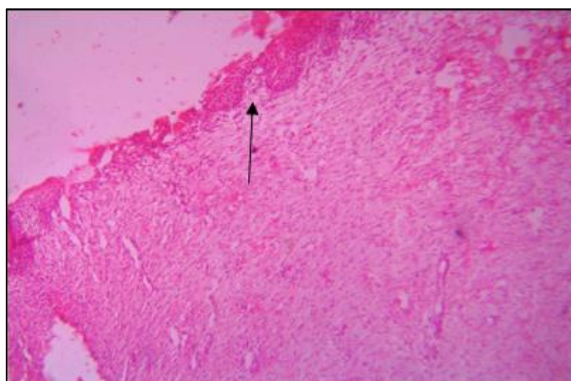


Fig 2: Photomicrograph showing developing epidermis with rete pegs on 15th post treatment day in the wound treated with *Aloe vera* (H & E 10X)

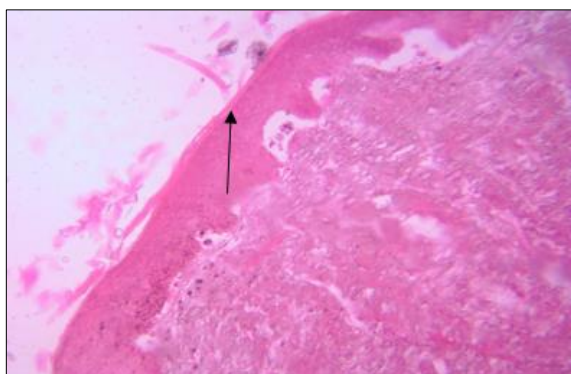


Fig 3: Photomicrograph showing complete healing with development of keratin layer along with epidermal layer on 20th post treatment day with *Aloe vera* treated wound (H & E 10X)

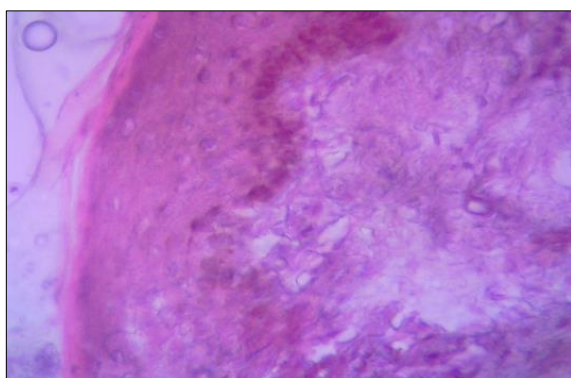


Fig 4: Photomicrograph showing complete developed epidermis with keratin layer on 25th post treatment day in *Aloe Vera* treated wound (H&E 40X)

Conclusion

From the above discussion, it can be assumed that increased rate of wound healing in *Aloe vera* treated wounds might be due to its property of initiating early angiogenesis, fibroblastosis and stimulating proliferation of keratinocyte.

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