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**Original Research Article**

# Effects of aloe vera gel application on epidermal wound healing in the domestic rabbit

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## ABSTRACT

**Background:** A wound is a breakdown in the protective function of the skin or loss of continuity of epithelium, with or without loss of underlying connective tissues, muscles, nerves, bones following injury to the skin, surgery, a blow, cut, chemicals, heat, cold, friction, shear force, pressure or diseases such as leg ulcers or carcinomas. A study was undertaken to determine the healing properties of Aloe vera gel on epidermal wounds in rabbits.
**Methods:** Twelve adult rabbits were divided into two groups randomly of six each representing the treatment and control respectively. A pair of wounds measuring 2cm x 2cm each was created on the back of each rabbit lateral to the spinal cord. The wounds were treated with homogenized Aloe vera gel while the wounds on control group were treated with normal saline. Wound contraction was measured on days 5, 9 and 12 representing the inflammatory, proliferative and maturation phases of wound healing respectively. Blood samples were collected on days 0, 3, 6, 9 and 12 for analysis.
**Results:** Animals treated with Aloe vera gel had significantly ($p<0.05$) faster rates of healing with shorter days of scab fall off than the control and showed significant ($P<0.05$) changes in the packed cell volume, mean corpuscular volume, lymphocyte and neutrophil counts.
**Conclusions:** The study concluded that Aloe vera was effective in treating epidermal wounds in rabbits over the control. An improvement occured in haematological profile of the experimental animals and these findings will go a long way in expanding the horizon of clinical application of this plant in solving wound healing problems in both humans and other animal species.

**Keywords:** Aloe vera gel, Contraction, Epidermal wounds, Haematology, Topical application

## INTRODUCTION

A wound is a breakdown in the protective function of the skin or loss of continuity of epithelium, with or without loss of underlying connective tissues, muscles, nerves, bones following injury to the skin, surgery, a blow, cut, chemicals, heat, cold, friction, shear force, pressure or diseases such as leg ulcers or carcinomas.[1-4] Wound healing or cicatrisation is an intricate, complex and dynamic biological process which combines physical, chemical and cellular events to restore wounded tissues or replace them with collagen, cellular structures and tissue layers. This process can be divided into phases such as chemotaxis, phagocytosis, neocollagenesis, collagen degeneration and collagen remodeling, angiogenesis, epithelisation, production of new glycosaminoglycans and proteoglycans are vital to wound healing.[1] These processes cause the replacement of normal skin structures with fibroblastic-mediated scar tissue.[5] Aloe vera (L.) *Burm. f.* or *Aloe Barbadensis Miller* (family Liliaceae) is a succulent, short-stemmed plant (60–100 cm) which is widely grown in Africa and

some arid areas of the world as an ornamental plant. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on the upper and lower stem surfaces. The margin of the leaf is serrated with small white teeth. The flowers are produced on a spike up to 90 cm tall. Each flower is pendulous, with a yellow tubular corolla 2-3 cm long. It is hardy, relatively resistant to insect pests but intolerant of heavy frost or snow.[6] Aloe vera contains several active compounds which include amino acids, anthraquinones (examples: aloin and emodin which are laxatives, analgesics, antibacterials and antivirals); enzymes (alliiase, alkaline phosphatase, amylase, bradykinase, carboxypeptidase, catalase, cellulose, lipase, and peroxidase). Bradykinase reduces inflammation when applied topically on the skin. Alprogen has anti- allergic properties.

Other organic and inorganic constituents are carbohydrate polymers (glucomannans or polymannose, lignin); saponins (antiseptic), monosaccharides (mannose-6-phosphate), hormones (auxins and gibberellins which help in wound healing), proper functioning of enzyme systems) and fatty acids. The phenolic compounds present in Aloe vera are chromone (e.g. C-glycosyl chromone). Plant steroids in Aloe vera are cholesterol, campesterol, B-sisosterol and lupeol which have anti-inflammatory effects. Lupeol also has antiseptic and analgesic properties; and anthrone derivatives.[7-10]

Aloe vera gel or aloe gel is the colourless, odourless, mucilaginous gel obtained from the parenchymatous cells in the fresh leaves and contains 99.5% water and 0.5% solid matter.[11] Rabbits (*Oryctolagus cuniculus*; Linnaeus, 1758) belongs to the family Leporidae.[12,13] This work was aimed at determining the effects of topical application of Aloe vera gel on epidermal wound healing and haematology using the rabbit model.

## METHODS

### *Experimental animals, extraction of Aloe vera gel*

Twelve rabbits (mean weight 1.7kg), were acclimatized for 7 days and divided into two groups of six each. The rabbits were dewormed and all physiological parameters monitored were within normal range for healthy animals. Fresh Aloe vera leaves were obtained from the Botanical Garden, University of Ibadan and washed with clean water. The skin of each Aloe vera leaf was peeled off to reveal the gel which was homogenized to a fine texture with a blender, centrifuged and the supernatant separated from the residue. Little water was added to the supernatant to reduce its viscosity and the extract refrigerated at 4°C.

### *Wound creation and treatment*

The paravertebral region of the rabbit was moistened, washed with soap and water, shaved and cleaned with cotton wool moistened with methylated spirit to disinfect the area. The site was locally-blocked with 2% lignocaine double diluted to 5ml and administered 0.2ml subcutaneously. A pin-prick non response indicated effective anesthesia. A pair of wounds (2cm x 2cm each) was created on the superficial epidermis of the skin of each rabbit using a cardboard template, scalpel and thumb forceps. Each wound was lavaged with normal saline using a needle and syringe. The day of wound creation marked day 0 of the experiment.

The wounds were topically treated with the Aloe vera gel dispensed with a syringe. The wounds on control rabbits were treated with 0.9% normal saline. This wound treatment was done daily from day 0 until the wound scabs fell off representing the termination of the experiment. The wounds were measured daily by placing the Vernier caliper on the wound edges dorsally and ventrally, and readings recorded until scabs fell off.

### *Collection of rabbits' blood samples for haematology*

Blood was collected from each rabbit via the ocular vein through a puncture by sterile capillary tube on days 2, 6, 9 and 12. An eye was cleaned with a sterile cotton wool and a capillary tube inserted into the medial canthus of the eye to obtain blood. The blood was analyzed for Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Haemoglobin (Hb) Concentration, Red Blood Cell (RBC) Count, Platelet count, Total White Blood Cell (WBC) Count and Differential Leucocyte Count (DLC).[14,15] The blood values obtained were used to calculate the haemoglobin concentration of the test samples using the formula:

$$\text{Hb Conc. } (\text{gdl}^{-1}) = \frac{\text{Optical Density of Test} \times \text{Hb Conc.of Standard}}{\text{Optical Density of the Standard}} \times \text{Dilution Factor}$$

$$\text{Dilution Factor} = \frac{\text{Vol. of Test Samples used (0.02 ml)}}{\text{Vol. of Test Sample Used (0.02ml)}} + \text{Vol. of Drabkin's Solution (4ml)}$$

The Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated from the PCV, RBC, and haemoglobin values as follows:

$$\text{MCH (pg)} = \frac{\text{Hb } (\text{gdl}^{-1}) \times 10}{\text{RBC } (\times 10^{6} \mu\text{L}^{-1})}$$

$$\text{MCV (fl)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC } (\times 10^{6} \mu\text{L}^{-1})}$$

$$\text{MCHC } (\text{gdl}^{-1} = \frac{\text{Hb}(\text{gdl}^{-1}) \times 100}{\text{PCV (\%)}}$$

The data obtained were presented as Mean ± Standard Error of Mean and analyzed using the one-way analysis of variance. Student T-test at 95% confidence limit was

also used to analyze statistical significance between paired groups, and a P-value lower than 0.05 was considered significant.

## RESULTS

The results obtained during this study are presented on Tables 1 and 2. Wounds treated with Aloe vera gel healed on average within 9 days and faster than the control group which took greater than 12 days to heal.

Topical application of Aloe vera on the wounds produced significant effects on the neutrophil count of the rabbits at inflammatory phase, PCV and MCV at proliferative and maturation phases, and on the lymphocytes at the three phases of wound healing (Table 2).

**Table 1: Effects of aloe vera on wound contraction in rabbits.**

| Phase | Experimental | Control | t | 95% confidence interval of the difference | | Remark (Significance) |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| Inflammatory | 39.75±6.71 | 59.22±11.66 | 1.56 | -47.28 | 8.33 | Non-significant |
| Proliferative | 3.89±1.47 | 12.91±3.99 | 2.64 | -16.64 | -1.40 | 0.025 |
| Maturation | 2.05±0.63 | 9.64±2.97 | 3.46 | -12.48 | -2.70 | 0.006 |

Significant ($p<0.05$) effects on wound contracture were observed at the proliferative and maturation phases of wound healing.

**Table 2: Effects of topical application of aloe vera on the haematology of the rabbits.**

| Parameter | Phase | Experimental | Control | T | 95% Confidence Interval of the Difference | | Remark |
|---|---|---|---|---|---|---|---|
| | | | | | Lower | Upper | |
| PCV (%) | P | 36.75±1.97 | 26.5±2.96 | 6.40 | 5.16 | 15.34 | * |
| MCV | M | 53±5.61 | 75.5±2.78 | -3.20 | -44.87 | -0.13 | * |
| Lym (%) | I | 73.5±1.19 | 58.5±2.75 | 9.49 | 9.97 | 20.03 | * |
| | P | 27±2.38 | 63.25±1.38 | -15.61 | -43.64 | -28.86 | * |
| | M | 25.75±2.17 | 43.5±1.94 | -8.63 | -24.29 | -11.21 | * |
| Neut (%) | I | 25.75±1.31 | 43.25±1.65 | -5.92 | -26.91 | -8.09 | * |

*Mean difference was significant at 0.05 level. Note: PCV=packed cell volume, MCV= mean corpuscular volume, Lym= lymphocytes

## DISCUSSION

Wound healing is a systematic process which is described using three overlapping phases namely inflammatory, proliferative and maturation.[16,17] This study showed that Aloe vera gel improved the rate of wound healing in rabbits as depicted by shorter days of healing- when the scab fell off within 9 days compared to the control (mean 12 days). This could be due to the presence of pro-healing ingredients such as vitamin C, amino acids, vitamin E and zinc.[18]

Treatment of wounds in rabbits with Aloe vera gel produced significant ($P<0.05$) effects on wound contraction especially during the proliferative and maturation phases of wound healing (Table 1). This could have been due the presence of phytochemicals in Aloe vera such as flavonoids and saponins which are useful in protecting and repairing damaged tissues of plants and animals.[19] Also, glucomannan, a mannose-rich polysaccharide and gibberellin, a growth hormone present in the gel could have partly played important roles in faster wound healing by interacting with the growth factor receptors which in turn stimulated the activity and proliferation of fibroblasts and promoted collagen synthesis similar to earlier reports.[20,21]

The haematology of the rabbits (Table 2) indicated that topical application of leaf extract of Aloe vera produced significant ($P<0.05$) increase in packed cell volume. This suggested that the extract may have affected the animal in a manner to produce an improved packed cell volume since Aloe vera contains many vitamins such as beta carotene, C, E, vitamin B12, folic acid and choline), minerals (calcium, chromium, copper, selenium, magnesium, manganese, potassium, sodium and zinc.[22] These vital elements and minerals could have played some roles to cause the improved packed cell volume though this cannot be ascertained by this current study.

It has been shown that numerous cytokines have been identified as essential extracellular factors for proliferation, differentiation, and maturation of hematopoietic cells.[23] Some like stem cell factor, interleukin 3, or granulocyte–macrophage colony stimulating factor (GM-CSF), interleukin 5 and interleukin 6. IL-1 for example are known to induce secretion of several hematopoietic growth factors such as

granulocyte–colony stimulating factor (G-CSF), macrophage–colony stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-6, which contribute to proliferation of hematopoietic progenitor cells.[24] The upsurge in packed cell volume could have also been due to the expression of these hematopoietic cytokines and growth factors owing to the Aloe Vera gel treatment.

Treated animals showed ($P<0.05$) significant increase in lymphocyte count at inflammatory and decrease in the proliferative and maturation phases of the wound healing. IL-4 which is mainly secreted by Th2 cells, mast cells, eosinophils, and basophils has been first identified as a factor promoting the growth and differentiation of B lymphocytes.[25] IL-4 is also a multifunctional cytokine, which has profound effects on not only hematopoietic cells such as B lymphocyte and monocytes/ macrophages, but also non-hematopoietic cells, such as fibroblasts, where it stimulates the synthesis of extracellular matrix, especially collagens and ultimately enhanced wound healing.[26-28] Once this was achieved and the wound began healing, the lymphocytes decreased, hence resulting in the reduced lymphocyte count of the proliferative and maturation phases.

Neutrophil has been known as the first line of defense which may be critical in recovering from a wound inflicted by an unsterile or infected object but may be unnecessary and even troublesome in recovering from a wound inflicted by a sterile surgical instrument. The infiltration of neutrophils into injured tissue is known to protect wounds from invading pathogens during inflammation. In the absence of infection or underlying medical conditions of the wounded individual, neutrophils are considered neutral to healing.

Although, previous study of neutrophil function supports both a positive and a negative role for neutrophils in normal tissue repair.[29,30] Therefore, neutrophil reduction observed in this study was in accordance with where wounds of neutrophil-depleted mice exhibited significantly accelerated re-epithelialization.[31]

## CONCLUSION

In conclusion, Aloe vera gel produced significant and positive effects on wound contraction and haematology in the experimental animals and these findings will go a long way in expanding the horizon of clinical application of this plant in solving wound healing problems in both humans and other animal species. It would be necessary to further investigate these findings with a view to discovering more uses of aloe vera plant in the animal species apart from its wound healing effects.

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