



STUDY OF THE EFFECTS OF THE *ALOE VERA* GEL ON SOFT TISSUE INJURY REGENERATION: AN EXPERIMENTAL STUDY ON RABBITS

Sena' a Malik Hussein

Department of Animal Production, College of Agriculture, Sumer University, Iraq

Email: moh07709879860@gmail.com

Abstract

In this topical study the influence of Aloe Vera, on the wound healing process was Investigated in Twelve adult rabbits were divided into two groups randomly of six each representing the treatment and control respectively. A pair of wounds measuring 1.5 cm x 1.5 cm each was created under general anesthesia on the back of rabbits. 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 3, 7, 14, 21 and 28 representing the inflammatory, proliferative and maturation phases of wound healing respectively. Animals treated with *Aloe vera* gel had significantly ($p < 0.05$) faster rates of healing with shorter days of scab fall off than the Histological results revealed that the inflammatory cell infiltration, angiogenesis, extracellular matrix deposition and epithelialization were promoted in treatment Group respectively. The study concluded that *Aloe vera* was effective in treating epidermal wounds in rabbits over the control. An improvement occurred 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.

Key words: *Aloe vera* gel, Contraction, Epidermal wounds, soft tissue, rabbits, injury

Introduction

Aloe vera (syn. *Aloe barbadensis* Mill., Fam. Liliaceae), also known as Barbados or Curaçao Aloe, has been used in traditional and folk medicines for thousands of years to treat and cure a variety of diseases. Although the plant is native to northern parts of Africa, it has rapidly spread across the world because its cultivation is easy. An important distinction has to be made between the strongly laxative and purgative latex derived from the bundle-sheath cells and the clear mucilaginous gel. The plant has been used by Egyptians, Assyrians, and Mediterranean civilizations, as well as in Biblical times. A variety of aloe species are still used in folk medicines of Africa and Asia. Hunters in the Congo reportedly rub their bodies in the clear mucilaginous gel to reduce perspiration; some African tribes apply the gel for chronic conjunctivitis; the gel is used in India for the treatment of asthma.¹

Aloe vera gel is used as an ethnomedicine in Trinidad and Tobago for hypertension.² the most common folk use of aloe has been for the treatment of burn wounds and specifically to aid in the healing process, reduce inflammation, and tissue scarring. The gel was described by Dioscorides and used to treat wounds and mouth infections, soothe itching, and cure sores.³ The use of aloe vera gel as a household remedy in the United States was triggered by reports of its beneficial effect on radiation dermatitis⁴ followed by a boom in cultivation in the 1930s; it remains a common

plant and for burns and abrasions.^{1,5} Important contemporary uses of the gel exist in traditional medicines of India, China, and Mexico, as well as Middle America and the West Indies. Mexico is producing roughly 47% of aloe worldwide with a total sales volume of \$123.5 million US dollars as of 2008.⁶

Despite its widespread popularity, scientific evidence on the aloe vera gel remains sparse. *Aloe vera* gel is regarded as safe if applied topical with only a few allergic reactions being reported.⁷ The efficacy of aloe vera gel to treat burn wounds, genital herpes, and seborrheic dermatitis have been shown in clinical trials, but other indications such as psoriasis or internal application for the treatment of type 2 diabetes remain inconclusive. The major application of aloe vera gel remains as a skin moisturizer in cosmetics and as an après treatment for sunburns, for which it has proven its effectiveness.^{8,9}

Aloe vera is a succulent plant with thick, fleshy, serrated, lanceolate-shaped leaves of green-greyish color. *Aloe vera* inner gel is obtained from the lower leaves of the plant by slicing the leaf open. The gel is clear, odorless, and tasteless and should be free of leaf skin or yellow parts. No consistent standardization has been established, but the International Aloe Science Council (IASC), a trade association of internationally based aloe producers and marketers, requires adherence to certain specifications for the product to be certified.¹⁰ Other preparations include a hydrophilic

cream containing 0.5% aloe vera gel and an emulsion consisting of 30% aloe vera gel.

Materials and Methods

Animals

Twelve male New Zealand albino rabbits (3400–5700 g) were used in this investigation. Their ages ranged between (3.5-5) months. Animals in all stages of the experiment housed in plastic cages in conditioned room (22-25°C) in the animal house with providing daily light of twelve hours (7.00 to 19.00) and twelve hours night cycle. They were left for ten days for adaptation with the experimental conditions. Animals had free access to water and standard pellet diet along the experiment.

Wound Creation

All rabbits were anesthetized via intramuscular injection of ketamine (21 mg/kg body weight) and xylazine (2.2 mg/kg body weight) The hair on the back of each animal was shaved and sterilized with 70% alcohol. Full thickness skin wound excision of an area measuring 1.5 x1.5 cm was performed on the back of each animal. The wound was photographed on the day of surgery (day 0) and subsequent days (day 3, 7, 14 and 21 post wounding).

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 (1.5 x 1.5 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 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.

Preparation of aqueous Aloe vera extract:

Full size mature leaves were cut from plant and the rind removed (Chithra *et al.*, 1998). To make the jelly thicker, the plants leaves were given sufficient time to be relatively dried at room temperature without exposure to direct sunshine. After adding a small

quantity of water, the leaves were then ground in a blender and centrifuged at 10,000g, to remove the fibers and then filter papers (Qualitative Papers, Retention (um): 20-25, Porosity: course, No. 4. The filtered aqueous Aloe vera extract was stored at +4 °C before use and allowed to warm up to room temperature a few a hours before 1,5 mL of it was applied to the wound (Ramachandra and Rao, 2008). All the wounds (Treated and Control) were rinsed daily with 10 ml sterile salin solution. The treated group received 1.5 ml of Preparation of aqueous Aloe vera extract topically once a day, for five days, whereas the wounds of the control group remained untreated. The wounds remained uncovered in both group throughout the experiment.

Histopathologic examination

At the day 3, 7 and 28 after wounding, biopsies were taken from the same corner of each wound using 0.9 mm biopsy punch for histopathological examination. The wound specimens were fixed in 10% buffer formalin and embedded in paraffin. Samples subjected to hematoxylin-eosin and mason's trichrome staining. Epithelialization, inflammatory infiltration, presence of dermal granulation tissue, fibroblast proliferation, arrangement of fibroblasts, collagen deposition and collagen bundle formation

Statistical Analysis

Data are expressed as Mean \pm Standard Error (M \pm SE). Statistical analysis was carried out on the load bearing data using two-ways, Analysis of Variance (ANOVA) in addition to Least Significant Difference (LSD). p-value <0.05 was considered to indicate a statistical differences (Snedecor and Cochran, 1973).

Result

Histopathologic Examination

In both groups, partial-thickness burn injury was confirmed with macroscopic and microscopic examination. Statistically, except for the inflammatory cell (PMNL) infiltration, there was no significant difference between the groups.

Macroscopic Evaluation

The repeated-measures linear model with treatment and time as within-subject factors revealed no significant difference in percentage of contraction, epithelialization and healing among wounds. Initially, all wound areas increased in size. After the initial enlargement, wound areas decreased in size between days 10 up to 24 in control and test group (P>0.05) (Table 1, 2, 3).

Table 1. Percent of wound contraction in the control and test wounds. There were no significant differences between left (control) and right (treated) wounds ($P>0.05$).

Days	Control	Treatment	LSD value
3	0.0	0.0	0.00 NS
7	18.20±3.77	22.35±2.37	6.142
14	36.40±3.54	47.20±2.86	5.733*
21	50.10±2.60	59.00±1.90	5.904*
28	64.28±1.85	75.10±1.30	6.916 *
LSD value	10.265*	11.382*	---

Table 2 : Percent of wound epithelialization in the control and test wounds. There were no significant differences between left (control) and right (treated) wounds ($P>0.05$).

Days	Control	Treatment	LSD value
3	0.0	0.0	0.00 NS
7	20.44±3.70	28.00±3.25	3.155*
14	36.00±3.12	47.33±2.78	3.209*
21	51.00±2.75	58.7±2.20	4.215*
28	68.66±2.00	77.44±1.10	5.316*
LSD value	13.602*	9.403*	---

Table 3 : Percent of wound healing in the control and test wounds. There were no significant differences between left (control) and right (PRP) wounds ($P>0.05$).

Days	Control	Treatment	LSD value
3	0.0	0.0	0.00 NS
7	22.77±4.30	31.77±3.45	6.319*
14	37.44±3.70	46.44±3.30	6.277*
21	50.55±2.69	65.55±2.18	6.946*
28	50.55±2.69	65.55±2.18	6.946*
LSD value	12.188*	10.452	---

Histopathologic Evaluation

There were no significant differences between median of inflammatory cells infiltration, presence of dermal granulation tissue, fibroblast proliferation, arrangement of fibroblasts, collagen deposition and collagen bundle formation scores, in the specimens from left and right wounds at the 10, 17 and 24 ($P>0.05$). The PRP-treated wounds, except at the day 24, showed a tendency toward increased epithelialization rate in both periods, but no statistical significance was found (Fig. 5). Descriptive studies have shown an increase in epithelialization, fibroblast proliferation and collagen bundle formation at the day 24 treated wounds.

Discussion

In the recent years, application of aloe vera gel to enhance cutaneous regeneration and soft tissue maturation has been widely extended in the fields of

orthopedic, periodontic, maxillofacial, plastic, thoracic and vascular surgeries, as well as ophthalmological procedures.^{2,22-31} However, some controversies exist about the efficacy of aloe vera application.² While some authors reported the effectiveness of aloe vera gel in the treatment of nonhealing chronic wounds, others did not report any improvement.^{7,12,32-36} This might be due to differences in experiment (animal, human), wound defect model, differences in aloe vera gel biology among species, differences in aloe vera gel preparation techniques, differences in aloe vera activity and differences in investigated time points.³⁷ Herein, we have developed a new wound sealant composed of concentrated, thromboplastin, and chloride calcium that is delivered as a topical gel to cutaneous wounds. Findings in this study do not support the hypothesis that application of Aloe vera (coagulated with

Thromboplastin and chloride calcium) as wounds treatment can accelerate or improve quality of repair. 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, interleukin³, or granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin⁵ and interleukin⁶. 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, the use of aloe vera gel or its components for the treatment of a variety of conditions and diseases needs further clinical evidence through well-designed studies with defined aloe extracts and matching placebo controls. This indicates the scientific significance of aloe vera gel and the need to establish it as a valid treatment option for wounds. However, the use of aloe vera gel in topical applications has widely been confirmed in the clinical studies as safe.

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