

TOPICAL ACTIVITY OF *Aloe vera* (*A.vera*) EXTRACT ON EXCISION WOUND-HEALING OF SKIN IN RABBITS:CLINICAL AND HISTOLOGICAL STUDY

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ABSTRACT

The plant *Aloe vera* is a green succulent, cactus-like plant belonging to the lily family. The plant has a history of use in folk medicine for skin and other disorders. The objective of the study presented in this report was to screen the extracts of this plant for its wound-healing properties based on its traditional use for wound healing. ethanollic extracts was prepared for topical applications. The dose used was 150 mg/kg daily for 10 days, using the excision wound model in rabbits. Carboxymethyl cellulose (1%) was used as control in topical studies. Animals were randomized divided to treatment or control groups. Wound areas were measured. At the day 11, skin tissue was excised for histological studies. Wound areas reduced significantly in all treatment groups compared to respective controls ($P < .001$). Histology outcomes were consistent with changes in the treatment groups. No differences were detected within the treatment groups. The study permits the conclusion that *Aloe vera* has wound-healing potential.

INTRODUCTION

Normal wound-healing response begins with injury and has wound-healing potential is a concerted sequence of events. The healing cascade is activated when platelets

come into contact with exposed collagen leading to platelet aggregation and the release of clotting factors resulting in the deposition of fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing (Clark,2001). Inflammatory cells also arrive along with the platelets at the site of injury providing key signals known as cytokines or growth factors (Lawrence and Diegelmann,1994). The fibroblast is responsible for collagen deposition that is needed to repair the tissue injury (Ross,1969). Collagen is the most abundant protein in the animal kingdom (Prockop and Kivirikko,1995). In normal tissues, collagen provides strength, integrity, and structure. When tissues are disrupted following injury, collagen acts an important role to repair the defect and restore anatomic structure and function. An aim of the author's group is determination of ability by natural (plant) products for wound healing.

Used edema search of the literature revealed that *Aloe vera* is used to inhibit pain, even in diabetics, and diabetic animals in a dose response fashion similar to normal (Davis *et.al.*,1988). *Aloe vera* has been shown to decrease the acute inflammatory response in rats with adjuvant arthritis, which closely resembles rheumatoid arthritis in human (Davis *et.al.*,1992). *A.vera*, is derived from thin-walled mucilaginous cells of the inner central zone of the leaf. It is thin gel that is thought to have emollient and moisturizing effects and therapeutic properties (Rund,1996). *A.vera*, is a complex plant that contains many biologically active substance (Davis *et.al.*,1991) reported that one tablespoonful of *A.vera* contains over 75 different chemicals that have biological activity. Therefore, it has proved difficult to isolate a single active ingredient, and it has been suggested that there is a synergistic relationship between the constituents (Bradshaw 1996). Wound management involves dressings, pain killers, the use of anti-inflammatory agents, and drugs that promote healing. (Forest,1982) Current methods used to treat difficult wounds include debridement, irrigation, antibiotics, tissue grafts, and proteolytic

enzymes. To test the claims based on traditional use of *A. vera* as a healing agent, this study was done. A preclinical animal study was carried out and is reported below.

MATERIALS AND METHODS

Plant Material

The *A. vera* leaves were collected locally and identified according to (Sheri and plyler, 2001). (Fig1) The plant leaves were shade dried and powdered (100 gm) was extracted with ethyl alcohol and gave a residue (15 gm) for topical study.

Animals

Healthy in-bred twenty male rabbits, 18-20 weeks of age and weighing between 1000 g and 1300 g were used for the study. They were individually housed at room temperatures under natural photoperiod and maintained on normal food and tap water (Alleva et al., 1968). Animals were periodically weighed before and after experiments. The rabbits were anesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anesthesia (10 mg/kg body weight of an animal). Animals were closely observed for any infection, and those that showed signs of infection were isolated and excluded from the study. After infliction of wounds, 10 animals each were randomly assigned (on lot basis) to treatment (extract) or controls. The rabbits were anesthetized with 1 mL of intravenous ketamine hydrochloride (10 mg/kg BW) and shaved on both sides of the back with an electric clipper, and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. The full thickness of 2.5 cm length and 0.2 cm depth of the excision wound was created along the markings using toothed forceps, a surgical blade, and pointed scissors. The wound was left open during the study (Fig. 5). (Morton and Malon, 1972)

In the topical study, carboxymethyl cellulose (CMC 1% in water) was applied topically to the control group (n = 10), whereas the experimental group (n = 10) was treated with the ethanolic extract of *A. vera* leaves topically and control animals (n = 10) were treated with carboxymethyl cellulose (CMC 1% in ethanol).

The measurements of the wound areas were taken on the 1st, 5th, and 11th days for both groups using transparency paper and a permanent marker. The wound areas of all groups were recorded and measured on graph paper.

The healing tissue was obtained on the eleventh day from both the experimental and control group animals used for histological study. The amount of collagen was quantified using Van Geison stain that stains the gen pink.

Results, expressed as mean $\pm$ SE, and tests of means were evaluated using the Student paired *t* test. Values of $P < .05$ were accepted as being statistically significant.

RESULTS

The histopathological study of healing tissue obtained from rabbits treated with the ethanol extract treated topically showed a better lay-down of collagen compared to respective controls (Figs. 2-3).

The results of effects on wound area reduction, characterized by the development of acute inflammation, vascular dilatation, increased vascular permeability and neutrophil activation and migration of white blood cells. At much the same time, gaps form between endothelial cells of the capillaries allowing plasma to leak into the tissue.

The tissue defect filled with blood clot and variable amount of tissue debris, organization and filling of the defect by granulation tissue take considerably longer, the deposition of collagen within fibrous granulation tissue occurs over a period of 11 days (Fig 4).

Collagen is remodeled in an appropriate orientation to withstand the tensile stresses placed on the area of repair, with time, the previously plump and metabolically active fibroblasts regress and become relatively inconspicuous in which the condensed nuclei of inactive fibroblasts are shown (Fig 5).

Immature collagenous tissue forms a pale scare which interrupts the normal pink collagen of the dermis

This study was observed that the rabbits not receiving *A.vera* had hard and crusty wounds, which generally appeared unclean (Fig 6). However, the *A.vera*-treated wounds were clean, with healthy granulation tissue (Fig 7), which would be consistent with the analytical data presented in Table 1. That show the wound treated with extract causes statistically significant reductions in wounds areas compared with respective control ($P < .001$).

Table 1. Topical Wound Healing Activity of the Ethanol Extract of *A.vera* in Rabbits

Parameter	Control Group (Mean $\pm$ SE) (n = 10)	Experimental Group (Mean $\pm$ SE) (n = 10)
Wound area (mm ²)		
Day 1	186.30 $\pm$ 2.37	180.40 $\pm$ 1.95
Day 5	165.00 $\pm$ 15.52	135.80 $\pm$ 4.62
Day 11	60.40 $\pm$ 3.20	37.50 $\pm$ 4.08**

Values are expressed as mean $\pm$ SE. ** $P < .001$.

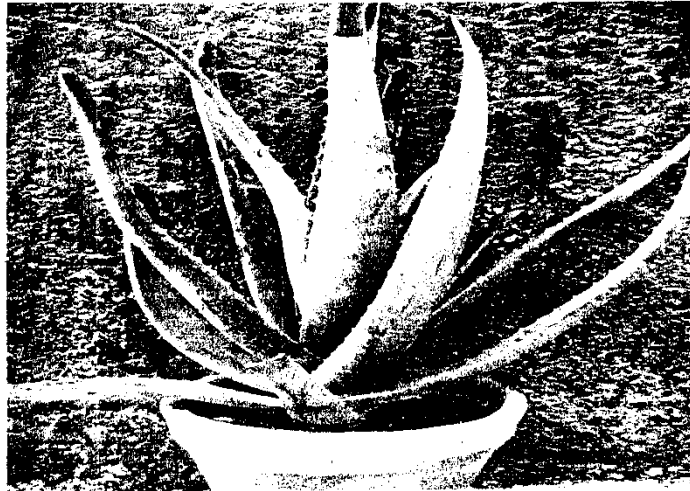


Fig ; Aloe vera plant

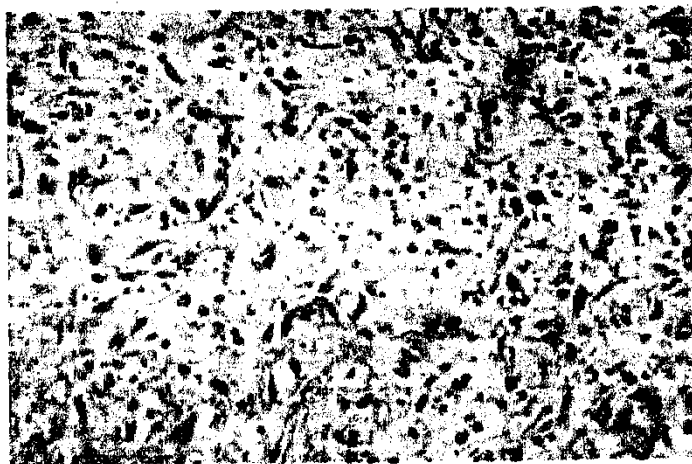


Fig 2: Control group granulation tissue with less collagen. H&E 40X

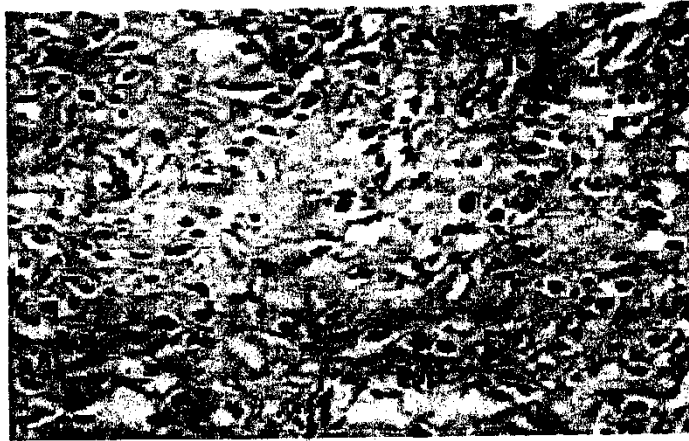


Fig 3; Experimental group granulation tissue with more collagen .H&E 40X



Fig 4 Treatment group shows: A-Vascular dilatation, B-Fibroblasts

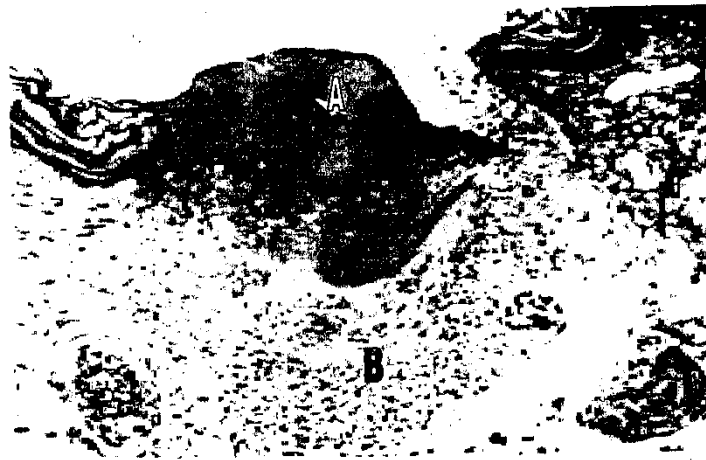


Fig 5; Treatment group shows, A-blood clot and tissue debris, B-Granulation tissue
H&E 10X



Fig 6: Excision wound on 11th day without any treatments

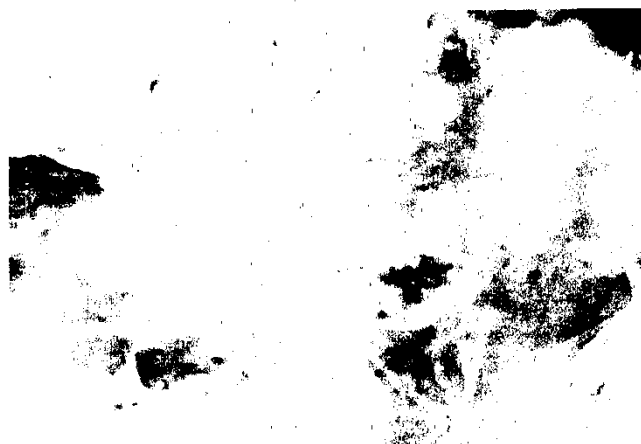


Fig7:Excision wound on 11th day treated with alcohol extract of *A.vera*.

DISCUSSION

The aim of this study was to examine the effects of the extract *A.vera* in a rabbit model. The ethanol extracts were tested topically, the treated animals being compared with respective controls. The effects of the extracts and control treatments on wound area reduction, were studied.

Animals treated with the extracts showed a significantly faster reduction in area when compared with controls. These data permit the observation that the extracts of *A. vera* aids wound healing in an animal model. These observations are similar to those of Robts et al who showed that aloe vera extract is effective in treating wounds by both oral and topical routes.(Davis et.al.,1989) It was observed that the control animals had hard and crusty wounds, which were also generally unclean. By comparison, the *A.vera* treated wounds were clean with healthy granulation tissue. The presence of *A vera* seemed to reduce the amount of dead tissue at the wound site and provide better wound healing. All this is broadly similar to the observation that wounds in experimental mice that received 100 mg/kg of aloe vera had better vascularity and healthier looking granulation tissue (Davis et.al.,1987). The observed increases in hydroxyproline content of the granulation tissue of the excision wounds are consistent with rapid collagen turnover, which favors wound healing and is similar to a previous observation that demonstrated wound-healing properties of *Vanda roxburghii*(Nayak et. al.,2005).

Although other studies using *C.peltata* extract have shown the presence of several phytochemicals,(King and Haddock), this study does not throw any light on the specific constituent that impart to it its obvious potential to heal wounds. It is speculated that some constituents of *A.vera* may stimulate epithelialization to improve wound healing.

The results presented in this report are a preliminary communication. Further studies using the extract of *A. vera* are indicated to identify optimal treatment routes, dosages, and which constituent(s) may be conferring its wound-healing potential on this natural product. That there is potential to heal wounds in an animal model is exciting, and future work should address what it offers wound healing in both veterinary and clinical practice.

فعالية مستخلص نبات الصبار في علاج الجروح الجلدية القطعية في الأرانب :دراسة سريرية نسيجية

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الخلاصة

يكون نبات الصبار اخضر اللون،كثير العصارة،يعود إلى عائلة الزنقيات،يستخدم النبات منذ القدم في الطب الشعبي في علاج إمبراض الجلد،تمت في هذه الدراسة إلقاء الضوء على فعالية مستخلص الصبار في التئام الجروح،تم تحضير المستخلص الكحولي واستخدامه موضعيا بجرعة 150 ملغرام /كغم من وزن الجسم يوميا ولمدة عشرة أيام على الجروح القطعية في الأرانب .تم استخدام محلول 1% كاربوكسي ميثيل سليلوز موضعيا لمجموعة السيطرة عند اليوم الحادي عشر من المعالجة تم قياس قطر الجرح وأخذت عينات لغرض الدراسة النسيجية، لوحظ اختزال منطقة الجرح إحصائيا مقارنة مع مجموعة السيطرة كما لوحظ ان هنالك اختلاف في التغيرات النسيجية مقارنة مع حيوانات السيطرة، مما يؤدي الى الاستنتاج بان نبات الصبار يمتلك فعالية في الإسراع بالتئام الجروح .

REFERENCES

- 1- Clark RA.2001. Fibrin and wound healing. Ann N Y Acad Sci ;936:355-67.
- 2-- Lawrence WT, Diegebnann RF.1994. Growth factors in wound healing. Clin Dermatol ;12(1):157-69
- 3-- Ross K. Wound healing. Sci Am 1969;220(6):40-50.
- 4- Prockop DJ, Kivirikko KI. Collagens: molecular biology, diseases, and potentials for

- therapy. *Annu Rev Biochem* 1995;64:403-34.
- 5- Davis, R.H., Leitner, M.G. and Russo, J.M.** 1988. Aloe vera: a natural approach for treating wounds, edema, and pain in diabetes. *Journal of the American Podiatric Medical Association*, 78, (2), pp. 60-68.
- 6- Davis, R.H., Stewart, G.J. and Bregman, P.J.** 1992. Aloe vera and the inflamed synovial pouch model. *Journal of the American Podiatric Medical Association*, 82, (3), pp. 140-148.
- 7- Rund, C.** 1996. Non-conventional topical therapies for wound care. *Ostomy . Wound Management*, 42, (5), pp. 18-26.
- 8- Bradshaw, T.W.** 1996. Aloe vera: its influence on the physiology of wound healing and inflammation. *Journal of British Podiatric Medicine*, 51, (2), pp. 25-29
- 9- Forest RD.** 1982. Development of wound therapy from dark ages to the present. *J R Soc Med*;75(4):268-73.
- 10- Sheri and C.Plyler.** 2001. Indian spring herbal encyclopedia .internet .www: indianspringherbs.com/aloe-vera.htm
- 11- Alleva J.J., Waleski, M.V., Alleva, F.R. and umberger, E.J.** 1968. Synchronizing effect Of photoperiodicity on ovulation in hamster. *Endocrinology* .82:123-127.
- 12- Morton JJ, Malone MH.** Evaluation of vulnerary activity by an open procedure in rats. *Arch Int Pharmacodyn Ther* 1972;196:117-26.
- 13- Davis, R.H., Leitner, M.G., Russo, J.M. and Byrne, M.E.** 1989. Wound healing: oral and topical activity of aloe vera. *Journal of the American Podiatric Medical Association*, 79, (11), pp. 559-562.
- 14- Davis, R.H., Kabbani, J.M. and Maro, N.P.** 1987. Aloe vera and wound healing . *Journal of the American podiatric Medical Association*.84,(2),oo.77-81.
- 15- Nayak BS, Suresh Rao AVC, Filial GK, Davis EM, Ramkissuon V, McAre A.** "Evaluation of wound healing activity of *Vanda roxburghii* R.Br (Orchiflacea) a preclinical study in a rat model. *Int J Lower Extremity Wounds* 2005;4:200-4.
- 16- King NM, Haddock N.** 1959. A note on the phytochemical investigation of *Cecropia peltata* L. *Am Pharm Assoc Am Pharm Assoc (Baltim)* ;80(48):39-43.