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Wound Healing Potential of *Aloe vera* Leaf Gel Studied in Experimental Rabbits

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Abstract: The objective of this study was to evaluate the wound healing potential of *Aloe vera* leaf gel in experimental rabbits. The ethanolic extract in the form of an ointment (10%) was used for evaluating the wound healing potential in an excision wound model. The prophylactic effect elicited by the extract in terms of wound contraction and related biochemical parameters strongly support the healing and sealing properties of *Aloe vera* leaf gel.

Key words: *Aloe vera*, wound healing, ointment, wound contraction, hydroxylproline

Introduction

Wounds cause discomfort and are more prone to infection and other troublesome complications (Ingold, 1993). Some diseases like diabetes, immunocompromised conditions, ischaemia and conditions like malnourishment, ageing, local infection, local tissue damage due to burn or gun shot often leads to delay in wound healing. Infection is a major complication of burn injury and is responsible for 50-75% of hospital deaths (Mokaddas *et al*., 1998). Wound healing consists of an orderly progression of events that reestablish the integrity of the damaged tissue. Many of the synthetic drugs currently used for the treatment of wounds are not only expensive but also pose problems such as allergy, drug resistance etc. and this situation has forced the scientists to seek alternative drugs (Sai and Babu, 1998). Efforts are being made all over the world to discover agents that can promote healing and thereby reduce the cost of hospitalization and save the patient from amputation or other severe complications.

More than 80% of the world's population still depends upon traditional medicines for their ailments (Kumara *et al*., 2001), especially for wound management (Purna and Babu, 2000), as they provide a moist environment to encourage the establishment of the suitable environment. Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine though their mechanism of action and efficacy have not been evaluated scientifically.

Aloe is one of the oldest healing plants known to mankind. *Aloe vera* is a perennial, drought-resistant, succulent plant with a whorl of elongated, pointed leaves. It is even described in the Bible for its healing properties. *Aloe* has been used topically for cuts, burns, insects stings, bruises, acne and blemishes, poison ivy, welts, skin lesions, eczema and subburns. Legend also has it that Alexander the Great in 333 B.C. conquered the Island of Madagascar on the advice of the great philosopher, Aristotle, solely for the purpose of obtaining sufficient supply of the *Aloe vera* plant to rub on the wounds of his soldiers. *Aloe* also has a history of traditional use for stomach and intestinal disorders (Sivagnanam *et al*., 2003). *Aloe vera* has been demonstrated to enhance immune system functioning within the body.

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Preliminary studies conducted by us using different solvents revealed the presence of biologically active substances in the ethanolic extract of *Aloe vera* leaf gel (Rajasekaran *et al*., 2004). Recently we have reported the anti-oxidant properties of *Aloe vera* gel extract in STZ-induced experimental diabetes in rats (Rajasekaran *et al*., 2005). *Aloe vera* leaf gel was found to contain Vitamin C, Vitamin E and aminoacids which are essential for wound healing (Davis, 1992).

In the light of *Aloe's* use as a wound healing agent in Folklore medicine, the present study was undertaken to evaluate the wound healing potential during topical application of the *Aloe vera* leaf gel extract in experimental rabbits.

Materials and Methods

Experimental Animals

Male rabbits weighing approximately 500 g were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai for the present study and were housed individually in standardized environmental conditions and fed commercial rabbit feed purchased from Hindustan Lever Ltd., Bangalore and water *ad libitum*. The animal experiments were designed and conducted in the Department of Biochemistry and Molecular Biology according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (Approval No. IAEC 01/031/04) for the investigation of experimental pain in conscious animals. Before beginning the experiments the animals were allowed to acclimatize to animal house condition for a period of one week.

Ointment Preparation for Topical Application

An ethanol free extract of *Aloe vera* leaf gel was used for the preparation of ointment for topical application (Yagi *et al*., 1998). A 10% (w/w) ointment was formulated using soft white paraffin (Cooper, 1987) obtained from S.d. fine Chem, India.

Wound Creation

The rabbits were anesthetized with 3.5% chloralhydrate solution, at a dose of 0.35 mg/g body weight intraperitoneally. This type of anesthesia prevents any movement of the animals at least for 2 h after the administration of the anesthetic solution, therefore animals were left without being restrained (Rashed *et al*., 2003). Hair was removed by shaving the dorsal back of the rabbit. Ethanol (70%) was applied as antiseptic for the shaved region before wound creation and an excision wound was made by removing a 7×7 mm full thickness piece of the skin from a predetermined shaved area on the back of each animal (Abu-Al-Basal, 2001). The wound was left undressed to the open environment and no local or systemic antimicrobial agents were used. This model was used to monitor the rate of wound contraction.

Experimental Design

After wound creation, experimental rabbits were divided into two groups consisting of a minimum of six animals in each group as follows:

Group I: Wounds treated with soft white paraffin alone.
Group II: Wounds treated with *Aloe vera* leaf gel ointment twice a day.

Aloe vera leaf gel ointment was applied topically throughout the lesion surface using sterilized cotton swabs. Group I animals were dressed with soft white paraffin alone, while group II animals were treated with the ointment. The treatment schedule was twice a day for a period of 14 days. All the rabbits were given regular dressing changes at every alternative days. The photographs on wounds were taken from both groups. Any rabbits showing a sign of infection were eliminated. Excessive handling of animals was avoided as it may interfere with the healing process. Proper care was taken to avoid infection from external sources.

Determination of Rate of Wound Contraction

The progressive changes in wound contraction were monitored planimetrically by tracing the wound margin on a clean graph paper every alternate day without causing any damage to the wound area. The measurement of wound area on graph paper (the rate of wound contraction and the period of epithelization) was calculated and expressed as the percentage reduction of original wound size. The period of epithelization of the wound was expressed as the number of days taken for complete epithelization.

Biochemical Analysis

The granulation tissue in the wound area of control and treated rabbits were scraped on the 7th and 14th day after wound creation and subjected to hydroxyproline, protein and DNA estimation. Collagen content of granulation tissues were determined by the estimation of hydroxyproline, as described by Woessner (1961). The DNA content was estimated by the method of Burton (1996). Protein was estimated by the method of Lowry *et al*. (1951).

Statistical Analysis

The values are expressed as mean±SD for six animals in each group. All the data were analysed with SPSS/7.5 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc performed with Least Significant Difference (LSD) test. The 'p' values of less than 0.05 was considered to indicate statistical significance.

Results and Discussion

The present study represents the most systemic study of topical application of the *Aloe vera* leaf gel extract at the wound site of excision wounds in experimental rabbits.

Excision wound healing models are often used for wound healing bioassays because they represent a true wound that could be reproducibly analysed in a nonsubjective, highly controlled manner (Mustoe *et al*., 1987). All wounds heal in three stages namely inflammatory stage, proliferative stage and remodeling stage. The inflammatory stage occurs during the first few days. During this stage, the wounded area attempts to restore its normal state or homeostasis by constricting blood vessels to control bleeding. Platelets and thromboplastin make a clot. Inflammation also occurs and is a visible indicator of the immune response. White blood cells clean the wound of debris and bacteria. The proliferative stage lasts about 10 days (depending on the severity of the wound). Granulation occurs, which means that special cells called fibroblasts make collagen to fill in the wound. New blood vessels form. The wound gradually contracts and is covered by a layer of skin. In the remodeling stage, the formation of new collagen takes place which change the shape and strength of the tissue in the wound

Fig.1 (a) Fresh wound

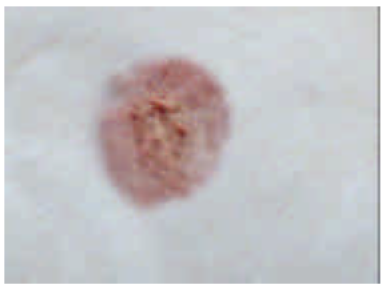

Fig. 1 (b) Untreated wound after 7 days

Fig.1 (c) Aloe vera treated wound after 14 days

Fig.1 (d) Untreated wound after 14 days

Fig.1 (e) Aloe vera treated rabbit after 14 days showing heated wound

Fig. 1: Effect of *Aloe vera* gel on wound healing

area. In the present study, a significant difference was observed at 7th day of *Aloe vera* leaf gel extract treated rabbits, when compared with the controls. The ability of an organism to activate the wound healing process effectively and promptly is essential for its survival. In all the parameters analysed, a significant prophylactic action was observed in *Aloe vera* leaf gel extract treated rabbits than the untreated group of rabbits.

Exposed subcutaneous tissue often provides a favorable substratum for a wide variety of microorganisms to contaminate and colonize. Wound contaminants are likely to originate from three main sources namely the environment, the surrounding skin and endogenous sources involving mucous membranes (Duerden, 1994).

Studies conducted by us revealed the antimicrobial properties of ethanolic extract of *Aloe vera* leaf gel. The well and dish diffusion method showed significant zone of inhibition against various gram positive and gram negative bacteria studied and their antibacterial activity was comparable to the conventional antibiotics (data not shown).

Figure 1a-e shows the healing pattern (rate of wound closure) in control and *Aloe vera* leaf gel extract treated rabbits. A significantly better healing pattern ($p<0.05$) within 14 days was observed in treated animals. There was a significant reduction ($p<0.05$) in wound size from day 4 onwards in treated animals and also in subsequent days. The wound closure rate was rapid in treated group when compared with control rabbits. A well-advanced organization of granulation tissue was noticed in leaf extract treated rabbits on 7th day and a complete healing and formation of scar was found in rabbits after 14 days treatment with *Aloe vera*.

Topical application of *Aloe vera* leaf gel extract at the wound site resulted in significant wound healing which may be due to its angiogenic and mitogenic potential. The enhanced rate of wound contraction and significant reduction in healing time in treated rabbits when compared to untreated rabbits (Fig. 1a) might be due to enhanced epithelization acilitated by *Aloe vera* gel ingredients.

Aloe vera is a complex plant contains up to 200 different substances beneficial to the human body. These substances include enzymes, glycoproteins, growth factors, vitamins and minerals (Davis, 1992). *Aloe vera* has been shown to improve healing with enhanced epithelization and rapid formation of granulation tissue in burn wounds (Visuthikosol and Sukwanarat, 1995).

Tizard *et al.* (1989) determined that mannose-containing products increase macrophage activity and promote wound healing. Macrophages are essential for proper wound healing to occur. Stimulation of the macrophage will increase cell and tissue growth, fibroblast proliferation and fibroblast activity (Guyton, 1991). *Aloe vera* containing mannose-6-phosphate may also promote wound healing in this way. *Aloe vera* may act directly or indirectly to activate collagen production by way of mannose-6-phosphate binding to receptors of fibroblasts (Davis *et al*., 1994). The possible mechanism of action of mannose-6-phosphate in wound healing activity is well established that insulin-like growth factor II and mannose-6-phosphate bind to the same receptor on the fibroblast (Westlund *et al*., 1991). These two ligands activate the fibroblasts to enhance the wound healing process.

Aloe vera appears to speed up the healing of damaged epithelial tissue in wounds by i) providing essential micronutrients ii) eliciting an anti-inflammatory effect iii) by its antimicrobial effect and iv) the stimulation of skin fibroblasts (Danhoff and McAnally, 1983). During early wound healing process, epithelial cells proliferate, migrate from the edges of the wound and eventually cover the wound with skin. The proliferation and migration of the epithelial cells are dependent on an adequate supply of oxygen. Therefore, the increased presence of oxygen, caused by the *Aloe vera* improving microcirculation should greatly enhance the wound healing process (Rubin, 1994).

Thus, the most possible wound healing explanation is that *Aloe vera* increases wound healing by stimulating fibroblasts directly. Fibroblasts, the skin cells responsible for manufacture of collagen, play an important role in fiber formation of wounds in repair by protein synthesis and associative enzyme activity. In the case of tissue injury, fibroblasts gets activated become enlarged and they proceed to build new fibers for repair. *Aloe vera* improves wound healing probably in part by stimulating the growth and activity of fibroblasts (Danhof, 1987). Increased protein content observed in the granulation tissue may be formed by fibroblasts to form more fibroblasts or for secretions or both. The major secretions of fibroblasts include procollagen and mucopolysaccharides.

Table 1 presents the levels of hydroxy proline, proteins and DNA in both the groups. There was an increase in collagen content on day 7 and 14 however not much change was observed after 7 days. The untreated rabbits showed a slow gradual increase in collagen content throughout the experimental period. Also the levels of protein and DNA were found to be significantly increased ($p<0.05$) in *Aloe vera* treated rabbits than the untreated rabbits.

Table 1: Levels of hydroxy proline, protein and DNA in wound tissue on experimental rabbits

	Hydroxyproline mg/100 mg of wet tissue		Protein (mg/g of wet tissue)		DNA (mg/g of wet tissue)	
Groups	7th day	14th day	7th day	14th day	7th day	14th day
Wound Control	4.1±0.3	2.4±0.2	52±3.3	56.4±4.1	7.3±0.5	6.6±0.5
Aloe vera treated	9.6±0.7*	3.1±0.3*	84±7.1*	62.5±5.8	15.2±1.2*	10.8±1*

Values are expressed as Mean±SD for six animals in each group. One way ANOVA followed by Post Hoc Test (LSD).
*$p<0.05$; Comparisons are made between *Aloe vera* treated and Wound control group

The observed increase in collagen, an important constituent of extracellular matrix in the treated animals confirmed that the extract had a positive effect towards cellular proliferation, granulation tissue formation and epithelization. The increase in collagen content in the treated group agrees with the increase in protein content which is predominantly due to enhanced collagen synthesis in the *Aloe vera* gel extract treated group. The antioxidant nature of the plant extract may also be the reason for the enhanced wound healing property (Rajasekaran *et al*., 2004).

The capacity to form supramolecular aggregates in extra cellular spaces is one of the important characteristics of molecules belonging to the collagen family of proteins (Piez, 1984). The hydrophilic nature of the collagen attributed by its molecular structure characterized by high content of diamino dicarboxylic amino acids and carbohydrate moieties provides a surface geometry very suitable for cell adhesion. In addition to the surface properties, the presence of glycoprotein like fibronectin on the surface of the cells promote the attraction of fibrogenic cells to collagen. These fibronectin molecules have a high affinity for collagen and link specifically with definite regions on the collagen surface.

The importance of collagen in wound healing has been appreciated for a long time for the simple reason that the ultimate result of most repair in the higher vertebrate is the formation of scar tissue composed of collagenous fibers. The primary step in healing, namely the clot formation involves the adhesion of circulating blood platelets to collagen which has been exposed by damage to the vascular wall. Further, aggregation of platelets is caused by collagen induced release of adenosine diphosphate (ADP) and other substances forming a haemostatic plug at the site of injury. The final stages of healing involves the production, maturation and degradation of collagen, the fibrils of which bridge the gap between the edges of the injured tissue to form a scar of appreciable tensile and breaking strength (Shoshan and Gross, 1974).

The findings observed in the present study are in accordance with the earlier reports that the primary site of action for *Aloe vera* is on surfaces and membranes, rather than solid organs and this action may account for some of the healing properties of *Aloe vera* (Davis *et al*., 1987; Fulton, 1990; Heggers *et al*., 1996). Further, *Aloe vera* exerts an effect on the cytokine system, resulting in immunomodulation (Green, 1996; Marshall *et al*., 1993).

The results in the healing and sealing of wounds makes topical *Aloe* as an important product for assistance in the healing of cuts, scrapes and even skin ulcers which may be due to synergistic action between the biologically active ingredients present in the *Aloe vera* leaf gel. Thus it may be concluded that *Aloe vera* leaf gel extract having the potential to satisfy all the requirements of an ideal dressing material in that it provides an environment at the surface of the wound in which healing take place at the maximum rate consistent with the formation of granulation tissue with an acceptable cosmetic appearance and also provides a rationale for the use of *Aloe vera* preparations in the traditional system of medicine to promote wound healing.

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