



## Wound Healing and Antimicrobial Activity of *Aloe vera* Gel

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### ABSTRACT

Wound healing is a complex and dynamic process of protein aggregation resulting in restoration of anatomical continuity and function. Plants are potent wound healers as they promote the repair mechanisms in natural way. *Aloe vera* leaf gel (AVLG) was tested for its antimicrobial activity against wound infecting pathogens by using Agar Well Diffusion Technique and for its wound healing activity using excision wound model. Healing was assessed by parameters such as percent wound contraction, period of epithelialisation and biochemical parameters such as hydroxyproline content (an index for collagen turnover), to help understand the healing mechanism. AVLG did not have significant antimicrobial activity against the tested microorganisms. AVLG treatment increased percent wound contraction from 31.90% to 52.40% as compared to control on 8<sup>th</sup> day with a corresponding decrease in period of epithelialisation from 26 days to 19days. AVLG treatment also increased hydroxyproline content of granulation tissue. A significant increase in breaking strength of healed skin further corroborated the biochemical findings lending support to the wound healing activity of AVLG.

**Keywords:** *Aloe vera* leaf gel, Antimicrobial, Breaking strength, Granulation tissue, Hydroxyproline, Protein aggregation, Wound healing.

### INTRODUCTION

A wound may be defined as a loss of or breaking of cellular and anatomical or functional continuity of living tissue.<sup>1</sup> Wound healing is the body's natural process of regenerating dermal and epidermal tissue.<sup>2</sup> The basic principles of optimal wound healing include minimizing tissue damage, debriding nonviable tissue, maximizing tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part.<sup>3</sup>

Current methods used to treat wounds include debridement, irrigation, antiseptics, tissue grafts, corticosteroids and use of antibiotics. Most of these are associated with unwanted side effects such as potential for bacterial resistance, bleeding, tissue damage, contact dermatitis and delay in wound healing.<sup>4</sup>

Superior wound healing is a challenging task that necessitates the development of newer wound healing agents. Many plants are composed of active principles like triterpenes, alkaloids and flavonoids and play an important role in the process of wound healing.

*Aloe vera* (*Aloe barbadensis* Miller) is a member of the family *Liliaceae*, which comprises more than 360 different species. It is present in the arid regions of India. Recently, this family was given the name *Aloaceae*.<sup>5</sup> *Aloe vera* is commonly used in herbal medicines for the treatment of various diseases such as diabetes, cancer, inflammation, gastrointestinal dysfunction, ulcers, infections, burns and

incisions.<sup>6</sup> *Aloe vera* is commonly referred to as Burn plant or First aid plant due to its potential therapeutic activity in burns and wound healing. Recent studies have shown that treatment with mannose-6-phosphate, acemannan and  $\beta$ -sitosterol obtained from *Aloe vera* gel resulted in faster healing of wounds.<sup>7,8</sup>

A wound healer with antimicrobial properties aids in the process of natural wound healing without increasing the susceptibility of open wound to the risk of bacterial infections that might interfere in healing process. The injured skin remains vulnerable to invasive microbial infections of all kinds with subsequent development of wound sepsis. Wound infections are most common in developing countries because of poor hygienic conditions. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* etc. are some important organisms causing wound infections.<sup>9</sup>

Bacteria directly invade wounds producing inflammation and fluid exudation which interferes with the healing process. Topical antimicrobials are one of the most important agents in wound care.

It was hence thought worthwhile to evaluate the antimicrobial activity of *Aloe vera* gel along with its wound healing activity in an attempt to correlate its antimicrobial potential, if any, to its wound healing activity.



## MATERIALS AND METHODS

Fresh leaves of *Aloe barbadensis* Miller were obtained from Ankur plant nursery, Mumbai and authenticated at Agharkar Research Institute, Pune. Whole leaves were washed with distilled water to remove the exudate from their surface. Incision was given at the base of leaf to yield a mucilaginous gel and the remaining fibrous fraction was discarded. Fresh gel was used every time for the study. The institution's animal house is registered with Govt. of India, having registration No. 25/1999/CPCSEA and conforms to the Indian National Science Academy guidelines for the use and care of experimental animal's research. All the animal experiments were conducted with prior approval from Institutional Animal Ethics Committee.

### Microorganisms

The test microorganisms used for the antimicrobial activity were common pathogens viz: *Bacillus subtilis* and *Staphylococcus aureus* (Gram positive bacteria), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram negative bacteria) and fungus *Candida albicans*. These organisms were procured from K. C. College, Mumbai and identified at Prin. K. M. K. College of Pharmacy, Mumbai.

### Antimicrobial activity

Antimicrobial activity of AVLG was tested using Agar Well Diffusion Technique. Standardization of bacterial and fungal cultures were done with McFarland's turbidity standard using Spectrophotometric method which provided a cell suspension containing  $1 \times 10^6$  to  $5 \times 10^6$  cells/ml which was then diluted with test medium in ratio of 1:100 to provide starting inoculums of  $1 \times 10^4$  to  $5 \times 10^4$  cells/ml<sup>8</sup>. Wells of about 10 mm diameter were bored using sterile cork borer into the inoculated agar plates. 0.3 ml of AVLG was delivered into wells and was allowed to diffuse in to the media after which the plates were inverted and incubated at 37°C for 24 hr. for bacteria and at 28°C for 48 hr. for fungi along with positive and negative controls. After incubation, zone of inhibition was determined. The presence of definite Zone of inhibition around the well indicated antimicrobial activity. The experiments were performed in triplicate.

### Animals

Albino Wistar rats of either sex weighing between 180-200 gm were obtained from the registered breeder Haffkine's Institute for Training Research and Testing, Parel. The animals were kept in standard housing conditions. They were given standard pellet food and water *ad libitum*. The experiments were performed after approval of the protocol by IAEC and were carried out according to the current guidelines for the care of laboratory animals.

### Acute Toxicity Studies

Acute toxicity studies were performed on Albino Wistar rats of either sex by topical application of AVLG at dose levels up to 2000 mg/kg, as per OECD guideline No. 402.

The rats were clinically observed for signs of gross behavioural changes and mortality, if any, following the application of AVLG at different time intervals such as 30 min., 1 hr., 2hr., 4 hr., 24 hr. and then 48 hr. up to 72 hr.

### Wound Healing Activity Evaluation

#### Excision Wound Model<sup>10-13</sup>

Rats were divided into four groups viz; Control (Untreated) group, Standard treatment group (5% w/w Povidone iodine), and Test treatment groups (30 mg and 100 mg AVLG).

Animals were anaesthetised by ketamine (24 mg/kg i.p.) before wound creation. The particular skin area was shaved 1 day prior to the experiment. A full thickness of the incision wound of circular area (approx. 500 mm<sup>2</sup>) and 2 mm depth was made aseptically using pointed sterilised scissors and forceps on the shaved back of the rats. The wound was left undressed to the open environment. Animals were housed individually in cages and received food and water *ad libitum*. The wounding day was considered as day 0. Fresh AVLG treatment and standard (5% Povidone iodine) were topically applied to the animals in the respective groups twice a day till the wound was completely healed. Wound closure was studied by tracing the raw wound using transparent tracing paper and permanent marker on every 4<sup>th</sup> day. Wound area was measured by tracing the wound on a millimetre scale graph paper. The period of epithelialisation was calculated as the number of days required for healing off of the dead tissue remnants without any residual raw wound.

#### Estimation of Biochemical Marker

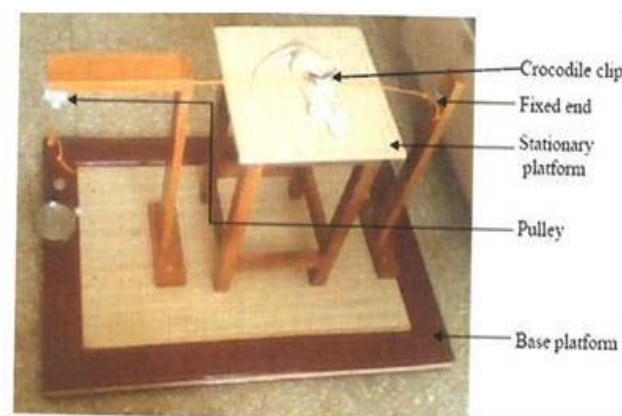
On the 10<sup>th</sup> day, granulation tissue was isolated from each group of rats for estimation of hydroxyproline content. Hydroxyproline was measured using the method of Bergman and Loxley.<sup>14</sup> For the determination of hydroxyproline content, the granulation tissues were excised and dried in hot air oven at 60-70°C to constant weight and were hydrolysed in 6N HCL at 110°C for 4 hr. in sealed glass tubes. The hydrolysate was neutralised to pH 7.0 and was subjected to chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4M perchloric acid and colour was developed with help of Ehrlich reagent at 60°C. The absorbance was measured at 558 nm using spectrophotometer. The amount of hydroxyproline in the sample was calculated using a standard curve prepared with L-hydroxyproline.

#### Determination of breaking strength of healed skin

On 16<sup>th</sup> day the animals were humanely sacrificed and breaking strength was determined using Tensiometer apparatus (Figure 1) which consist of a 192 × 180 inch wooden board with two 108 inch long pole, one fixed toward the end of the board and other is near to the centre of the board. The board was placed on the edge of the table. A movable pulley was mounted on the top of the pole which is nearer to the centre of the board. A



hook was attached to the top of other pole. One end of fishing line was tied to the hook. A crocodile clip was attached to the other end of this fishing line in such a way that the clip can reach the middle of the board. Another crocodile clip was tied to a longer fishing line connected with weighing pan on the other end. The animal was placed on the stationary platform and it was raised to a height which was at the same level at the two poles in a way that the crocodile clips can be attached to either sides of the wound at a distance of 0.5 cm. After making all the arrangements, weights were slowly added to the weighing pan until the weight reached caused the wound to tear open. The weight in weighing pan was then equated as the breaking strength of the wound.



**Figure 1:** Tensiometer Apparatus for determination of Breaking Strength of healed skin

## Statistical Analysis

The values were obtained as Mean  $\pm$  SEM. Statistical significance of the difference between the control and treatment groups was analysed by one way analysis of variance (ANOVA) followed by Dunnett's test. P value of ( $p < 0.01$ ) and ( $p < 0.01$ ) was considered statistically significant.

## RESULTS

Topical application of AVLG at doses levels up to 2000 mg/kg did not show any toxic or deleterious effects, indicating low toxicity of the AVLG treatment at high doses.

Among all the tested microorganisms AVLG treatment showed a diffuse zone of inhibition only against *Staphylococcus aureus* although it was not statistically significant (data not shown).

In excision wound model, significant increase in percent wound contraction was seen on 16<sup>th</sup> day from 78.69% in control group to 96.53% as seen in AVLG (100 mg) treatment group with a corresponding decrease in period of epithelialisation from 26 days to 19 days (Table 1). The hydroxyproline content per 100 mg of granulation tissue increased from 11.29  $\mu$ g to 20.59  $\mu$ g following treatment with 100 mg AVLG (Figure 2). The breaking strength of healed skin was increased from 322.1 gm to 696.8 gm following treatment with 100 mg AVLG (Table 2).

**Table 1:** Wound healing activity of *Aloe vera* leaf gel (AVLG) on healing parameters using Excision wound model

Groups / Doses	% wound contraction				Period of Epithelialisation Mean $\pm$ SEM
	4 <sup>th</sup> day	8 <sup>th</sup> day	12 <sup>th</sup> day	16 <sup>th</sup> day	
Control	17.86 $\pm$ 0.40	31.90 $\pm$ 0.39	53.66 $\pm$ 0.61	78.69 $\pm$ 0.33	26
Povidone-iodine 5 % w/w	32.91 <sup>a</sup> $\pm$ 0.50	52.93 <sup>a</sup> $\pm$ 0.64	80.33 <sup>a</sup> $\pm$ 0.23	97.96 <sup>a</sup> $\pm$ 0.15	18
AVLG (30mg)	19.49* $\pm$ 0.30	33.45* $\pm$ 0.18	55.63* $\pm$ 0.60	80.78* $\pm$ 0.82	22
AVLG (100 mg)	25.46 <sup>a</sup> $\pm$ 0.22	52.40 <sup>a</sup> $\pm$ 0.82	79.90 <sup>a</sup> $\pm$ 0.52	96.53 <sup>a</sup> $\pm$ 0.36	19

All values are expressed as Mean  $\pm$  SEM, n=6. One way ANOVA followed Dunnett's test is applied for statistical analysis. All treated groups were compared with control group. \*Significant at  $p < 0.05$ , <sup>a</sup>Significant at  $p < 0.01$ .

**Table 2:** Effect of AVLG treatment on breaking strength of healed skin

Groups / Doses	Breaking Strength (gm)						Mean $\pm$ SEM
	1	2	3	4	5	6	
Animals							
Control	321	324	325	322	320	318	322.1 $\pm$ 1.054
Povidone-iodine 5 % w/w	739	735	738	742	738	745	738.6 $\pm$ 2.151
AVLG (30mg)	325	326	330	333	332	322	328.0 $\pm$ 1.77
AVLG (100 mg)	698	697	694	690	699	700	696.8 $\pm$ 1.520

All values are expressed as Mean  $\pm$  SEM, n=6. One way ANOVA followed Dunnett's test is applied for statistical analysis. All treated groups were compared with control group. \*Significant at  $p < 0.05$ , <sup>a</sup>Significant at  $p < 0.01$ .

## DISCUSSION

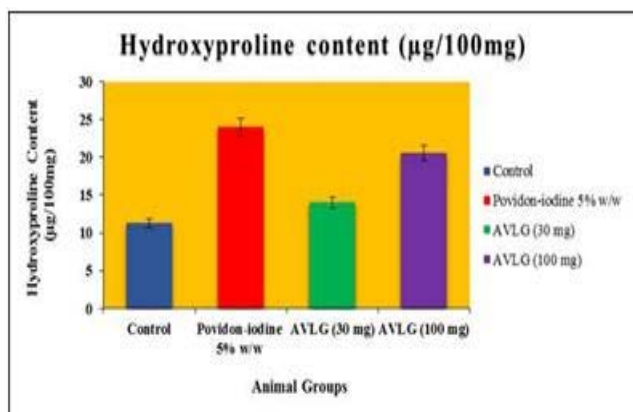
Pharmacological maintenance of a manageable bacterial burden may prove to be of central importance in the wound healing process. AVLG at tested doses did not show any significant zone of inhibition against any of the

microorganisms tested suggesting involvement of mechanisms, other than antimicrobial activity in the wound healing process.

Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area.



This centripetal movement of the wound margin is believed to be due to the activity of myofibroblasts. During epithelialisation, the epithelial cells crawl across the wound bed to cover it. This may occur either by facilitating the proliferation of epithelial cells or by increasing the viability of epithelial cells.<sup>15</sup> The wound healing process involves the shrinkage of the wound. Thus, measurement of the rate wound contraction is considered as an index of wound healing. Percent of wound contraction in AVLG treated group showed a significant increase in wound contraction along with faster epithelialisation period in excision wound model.



All values are expressed as Mean  $\pm$  SEM, n=6. One way ANOVA followed Dunnett's test is applied for statistical analysis. All treated groups were compared with control group. \*Significant at  $p < 0.05$ , <sup>a</sup> Significant at  $p < 0.01$ .

**Figure 2:** Effect of AVLG on hydroxyproline content in Excision wound model

Hydroxyproline content of granulation tissue following AVLG treatment was estimated with an objective to understand its mechanism of action. The collagen molecules synthesized are laid down at the wound site and become cross linked to form fibres. Wound strength is acquired from both, remodelling of collagen and the formation of stable intra- and inter-molecular crosslink. Collagen is major protein of the extracellular matrix, which gives strength and support to the skin and also plays an important role in homeostasis. Collagen is composed of the amino acid hydroxyproline which can be used as biochemical marker for tissue collagen. The hydroxyproline content of granulation tissue indicates the level of protein (collagen) synthesis and cellular proliferation. Increase in hydroxyproline content is indicative of increased breakdown of collagen and subsequent liberation of free hydroxyproline which could be used as an index for collagen turnover. Its estimation helps clinically to understand the progress rate at which the healing process is going on in the connective tissue of the wound. The increased hydroxyproline level in granulation tissue of AVLG treatment group probably helped provide an effective force for wound contraction by the newly formed collagen fibres that are synthesized by hydroxyproline.

Breaking strength of the healed skin (the force required to open the healed skin) was used to measure the completion of wound healing. It also indicates the quality of repaired tissue. AVLG treatment resulted in significant increase in the breaking strength of healed skin. Breaking strength of the granulation tissue increases proportionately with collagen deposition. In this model the increase in breaking strength in AVLG treated wounds might have been due to the increase in collagen concentration and stabilization of the fibres.

## CONCLUSION

In conclusion, the ability to seal and heal a wound makes topical *Aloe vera* gel an important herb for assistance in healing of cuts, scrapes and even skin ulcers. Significant increase in wound contraction and hydroxyproline content being a reflection of increased collagen levels, the probable mechanism(s) involved in superior wound healing following AVLG treatment might be collagen dependent stimulation of cellular proliferation with subsequent epithelialisation involving increase in the rate of collagen biosynthesis which further strengthens the skin.

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