MIRACULOUS ALOE VERA: IT'S HEALING POTENTIAL ON EXPERIMENTAL EXOGENOUS DERMAL WOUND IN MICE

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ABSTRACT

BACKGROUND

Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and function. Aloe vera is a very important plant for its large number of medicinal properties. The objective of the study is to evaluate the wound healing activity of Indian Aloe (Aloe Vera) leaves pulp in experimental model.

MATERIALS AND METHODS

For experimental evaluation of wound healing effect of Aloe vera, 35 healthy male albino mice, weighing between 50-60 gm body weight were used. They (n=35) were divided into 5 experimental groups (Group I, II, III, IV and V). Data of wound contraction area (sq. mm) were compiled and expressed as mean ± SD, n=7 in each group (7th animal was used for histopathological evaluation) and statistiically analysed using General linear model repeated measures analysis and post hoc tests (Bonferroni multiple comparison tests) on SPSS 20. p<0.05 was considered as statistically significant.

RESULTS

All treatment groups showed reduction in the wound surface area but maximum was observed with T3 (5% Aloe vera) group.

CONCLUSION

Topically administered Aloe vera accelerates epithelialization, wound contraction, tissue alignment and tissue strength.

KEYWORDS

Aloe Vera Gel, Wound Healing Activity, Wound Excision Model, Medicinal Plants.


INTRODUCTION

Wounds are major cause of physical disabilities. They arise due to physical, chemical or microbial agents. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and function.[1] Wound healing involves a highly dynamic integrated series of cellular, physiological and biochemical processes that occurs in living organisms.[2]

Plants have been used as a source of medicine since the dawn of civilization. The herbal medicines occupy distinct position right from the primitive period to present day. Aloe vera is a very important plant for its large number of medicinal properties. Thus, it is quite promising as a multipurpose medicinal agent.[3] Aloe vera is a perennial with fleshy leaves arising in a rosette from a short stem.

Research into the medicinal effects of A. vera gel began in the 1930s. Scientific interest was aroused in 1935 by the paper of Collins and Collins, “Roentgen dermatitis treated with whole leaf of Aloe Vera”.[4,5] Aloe vera pulp accelerates wound healing process especially after 9th post wound day in an excision wound.[6] It not only increases the collagen content of wound but also changes collagen composition and degree of collagen cross linking.[7]

In present day Western society, most notably in the USA, A. vera has fairly frequent use in homeopathy and herbalism.[8] It is commonly grown in America and the tropics as a pot plant on kitchen windowsills, so the leaves are on hand to treat burns, to soothe the pain and promote healing. The plant has additional use to treat sun burn and various dermatological conditions and taken internally, as a general tonic. Based on the above source of information, the present study aimed at to evaluate the wound healing activity of Indian Aloe (Aloe Vera) leaves pulp in experimental model.

MATERIALS AND METHODS

Study Setting

The present study was conducted in the department of pharmacology, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand in March 2003 in accordance with the ICMR Guidelines for Care and Use of Animals in Scientific Research.
All the ethical principles mentioned in the guidelines were strictly followed and supervised by the institutional body (No. 541-09/RIMS/RAN).

Experimental Animals
For experimental evaluation of wound healing effect of Aloe vera, 35 healthy male albino mice, weighing between 50-60 gm body weight were used. Animals were acclimatized to laboratory conditions for 1 week before starting the experiment and had free access to water and standard rodent feed.

Creation of Wound
Excision wound model was selected for assessing wound healing activity of Aloe vera gel on topical route of administration for 25 days. Animals were anaesthetized lightly with ether and shaved on part to be exposed. A circular piece (200 mm² area) was impressed on the dorsal thoracic region and a full thickness excision wound was created by aseptic surgical manipulation.

Experimental Protocol
Animals (n=35) were divided into 5 experimental groups (group I, II, III, IV and V) randomly selecting 7 mice in each group. 6 mice from each group were utilized for wound contraction evaluation. From each group one (7th one) was utilized for histopathological evaluation. Separate mice cages were allotted for each group. The mice of each group kept in their respective cage were marked with Indian ink for their individual identification. The animals were kept 12 hr. fasting prior to experiment. The group I & II constituted the control group of Normal saline (NSS) and Betadine lotion respectively. The group III, IV and V constituted the treatment groups for 5%, 25% and 50% V/V concentration of Aloe vera gel respectively.

Preparation of Working Solution
The mature fleshy leaves of Aloe vera plant were collected from the medicinal plant garden, Birsa Agriculture University, Kanke, Ranchi. Identification of these leaves was confirmed by faculty member, Birsa Agriculture College, Kanke, Ranchi. The leaves were cleaned from outside with distilled water so as to remove dirt and foreign impurity if any present on the leaves. Fresh gel was extracted from the leaves of Aloe vera. The gel was obtained by peeling the outer layer and the inner gel was pulverised and filtered. No chemicals were added and the original composition was maintained. The fresh gel then diluted with pyrogen free distilled water to make working concentration of 5%, 25% and 50% v/v Aloe vera gel. The prepared working solutions were kept in sterile containers in the refrigerator for the purpose keeping it away from microbial invasion. The fresh Aloe vera gel working solutions were prepared several times at the interval of 4 days.

Application of Working Solution and Evaluation of Wound Contraction
The concentration of Aloe vera gel 5%, 25% and 50% V/V (3-4 ml) soaked in sterile cotton was applied thrice a day for 25 days to the excised wound created on the mice of group III, IV and V respectively. In similar way NSS and Betadine was applied on mice of group I and II respectively. The wounds were traced on 1 mm² graph paper on the day of wounding and then subsequently on the 4th, 8th, 12th and 16th post wounding days.

Histopathological Evaluation
From the 7th animal of all experimental groups (I-V) a full thickness tissue excising 0.2 cm beyond wound area was excised under light anaesthesia on 6th and 12th post wound day. The tissues were fixed in 10% buffered formalin and passed through different grades of alcohol and were embedded in paraffin wax. Samples were sectioned (3-5 µm) and stained with haematoxylin and eosin and the histopathological changes were studied. One (+) for mild/few, two (++) for moderate and three (+++) for abundant, was assigned for relative presence of important histopathological parameters- fibroblast proliferation, collagenisation, neovascularisation (Table 2). The wound healing had been observed till their complete healing.

Statistical Analysis
Data of wound contraction area (sq. mm) were compiled and expressed as mean ± SD, n=6 in each group. Comparison among different treatment groups was done using General linear model repeated measures analysis and post hoc tests (Bonferroni multiple comparison tests). All analyses were performed using SPSS 20. A p<0.05 was considered as statistically significant.

RESULTS
Observations on Wound Closure during Daily Wound Care
Wound surface area was calculated and expressed in sq. mm as shown in Table 1. Though all treatment groups showed reduction in the wound surface area but maximum was observed with T3 (5% Aloe vera) group. Wound healing was noticed in all treatment groups from the 4th day onwards and maximum reduction was seen on 16th day (Table 1, Figure 2). There was a significant statistical difference in the wound healing among the treatment groups (F=169.15, p <0.001). Post hoc tests revealed that all intergroup comparisons were also statistically significant (p<0.001) except for the comparison between Betadine (T2) and 50% Aloe Vera (T5) (p=0.058).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>0 day</th>
<th>4th day</th>
<th>8th day</th>
<th>12th day</th>
<th>16th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (NSS)</td>
<td>0.000 ±0.000</td>
<td>29.316 ± 0.831</td>
<td>60.680 ±3.386</td>
<td>90.543 ±3.824</td>
<td>114.665 ±4.508</td>
</tr>
<tr>
<td>T2 (Betadine)</td>
<td>0.000 ±0.000</td>
<td>50.235 ±1.781</td>
<td>102.817 ±8.252</td>
<td>125.999 ±4.827</td>
<td>143.907 ±10.964</td>
</tr>
<tr>
<td>T3 (5% V/V)</td>
<td>0.000 ±0.000</td>
<td>86.148 ±1.346</td>
<td>162.184 ±1.204</td>
<td>187.168 ±2.031</td>
<td>192.998 ±2.114</td>
</tr>
<tr>
<td>T4 (25% V/V)</td>
<td>0.000 ±0.000</td>
<td>86.529 ±1.164</td>
<td>158.323 ±5.71</td>
<td>160.498 ±5.025</td>
<td>180.501 ±6.345</td>
</tr>
<tr>
<td>T5 (50% V/V)</td>
<td>0.000 ±0.000</td>
<td>54.929 ±1.271</td>
<td>100.499 ±17.733</td>
<td>147.33 ±15.938</td>
<td>152.443 ±17.502</td>
</tr>
</tbody>
</table>

Table 1: Effect of application of experimental medication including Aloe vera gel on excision wound contraction (sq. mm) in albino mice on different days

Each treatment group n=6, expressed as (mean ± SD); p<0.001; Comparison between Betadine (T2) and 50% Aloe Vera (T5) was not significant (p=0.058).
**Histopathological Findings**

On 6th day of wound creation controlled NSS (T1) group showed that majority of wound area was invaded by granulation tissue comprising of macrophages, enlarged, swollen, oval fusiform fibroblast cells as well as proliferating endothelial cells and newly formed blood vessels. Fine and scanty collagen fibres were found to be interspersed between the fibroblast cells. The wound treated with betadine (T2) solution showed similar type of histological picture. 25% (T3) and 50% (T4) Aloe vera treated cases showed more marked infiltration of fibroblasts and mononuclear cells as compared to betadine (T2) and NSS (T1) treated cases. Collagen fibres were thin, fine and dispersed and were comparatively more than betadine (T2) and NSS (T1) treated cases. But in case of 5% Aloe vera treated wound (T3), the fibroblast cells and collagen fibres were found maximum as compared to all other groups (Table 2).

On 12th day of wound creation there was marked collagen accumulation with newly formed blood vessels but collagen fibres were more thick, dense and wavy in aloe vera treated wounds with maximum in 5% Aloe vera. (Figure 2) There was less number of mononuclear cell infiltrations.

<table>
<thead>
<tr>
<th>Day</th>
<th>Parameters</th>
<th>Treatment Groups (T1-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1 (NSS)</td>
</tr>
<tr>
<td>6th</td>
<td>Fibroblast proliferation</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Collagenisation</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Neovascularisation</td>
<td>+</td>
</tr>
<tr>
<td>12th</td>
<td>Fibroblast proliferation</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Collagenisation</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Neovascularisation</td>
<td>++</td>
</tr>
</tbody>
</table>

‘BD- Betadine *NSS- Normal saline solution Dark (+) - more thick/dense/organised T3,5: Aloe vera gel (V/V)

**DISCUSSION**

Aloe vera is one of the few substances known to effectively decrease inflammation and promote wound healing [9, 10]. Results of the present investigation clearly indicated the wound healing properties of Aloe vera. On 8th day of wound creation Aloe vera [Especially 5% (T3) and 25% (T4)] treated animals showed significant reduction in wound size as compared to saline treated (T1) group as in table 1. On 6th day itself majority of wound area was invaded by granulation tissue comprising of macrophages, immature fibroblast cells, proliferating endothelial cells and newly formed blood vessels.

Fine and scanty collagen fibres were interspersed between the fibroblast cells. These findings were more prominent in Aloe vera treated animals: maximum with 5% Aloe vera. Similar to our findings, Subramanian et al.[11] reported a significant improvement in the reduction of wound size of the Aloe vera treated animals on 7th post wound day. They reported a well-organized granulation tissue in the treated lesions on 7th post wound day that contained significantly higher hydroxyproline, collagen and DNA contents. They stated that the antioxidative criteria of the plant extract may be responsible for these enhanced wound healing properties.

On 12th day of wound creation collagen fibres were more thick, dense and wavy in Aloe vera treated wounds with maximum in 5% Aloe vera as it (A vera) increases the collagen content of the granulation tissue as well as its degree of cross linking evidenced by increased aldehyde content and decreased acid solubility as per Chithra et al. report[12]. The enhanced rate of wound contraction and reduction in healing time in treated animals (rats) might be due to the anti-inflammatory effects Aloe vera together with its effect on maturation and organization of the granulation tissue.[13]
The anti-inflammatory activity of the extracts of A. vera gel probably occurs via an inhibitory action on the arachidonic acid pathway through cyclooxygenase.[14] Time required for complete healing of excision wound was least with 5% A. vera gel (18±0.63 days) among all treated groups as more thick, dense and wavy collagen fibres were observed maximum on 12th post wound day in 5% Aloe vera.

CONCLUSION
From the findings of present study, it can be concluded that application of Aloe vera to an open wound induces significant wound contraction and accelerates wound healing and this herbal extract may be a promising medication for open wounds. Macroscopic, microscopic finding indicates that topically administered Aloe vera accelerates epithelialisation, wound contraction, tissue alignment and tissue strength at the later stage of wound healing. These findings also rationalise its traditional claim of wound healing.

REFERENCES