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Wound healing potential of methanolic extract of leaves of *Achyranthes aspera* Linn.

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ABSTRACT

Achyranthes aspera is well-known plant in Indian traditional medicines, strongly recommanded for healing properties of skin diseases by some rural communities. In present investigation the leaves part of this plant is evaluated for wound healing activity on various models including excision, incision and dead space and histopathological models to prove it scientifically. The methanolic extract of leaves of Achyranthes aspera was examined for wound healing activity in the form of ointment on albino mice. The extract showed considerable response in all the above said wound models as comparable to those of a standard drug Povidine iodine in terms of wound contracting ability, wound closure time, tensile strength and dry granuloma weight. Histological analysis was also consistent with the proposal that Achyranthes aspera leaves extract exhibits significant wound healing activity.

Keywords Wound healing activity, *Achyranthes aspera*, Povidine iodine.

INTRODUCTION

The therapeutic efficacies of many indigenous plants, for various diseases have been described by traditional herbal medicine practitioners [1]. Natural products are a source of synthetic and traditional herbal medicine. They are still the primary health care system in some parts of world [2]. The past decade has seen considerable change in opinion regarding ethanopharmacological therapeutic applications. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential wound healing properties.

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest

skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin layer) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialisation and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasias and granulation tissues formation, fibroblasts exert collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges undergo contraction using a mechanism similar to that in smooth muscles cells [3].

Achyranthes aspera Linn., belonging to the family Amaranthaceace is an erect annual herb, 1-2 m. in height, often occurs with a woody base. It has angular stems, ribbed, simple or branched from the base, often tinged with reddish purple colour. The leaves are thick, opposite, velvety-tomentose, ovate-elliptic or obovate-rounded variable in shape and size. It bears bisexual, greenish-white flowers, numerous in axillary or terminal spikes up to 75 cm. long. The seed are sub-cylindric, truncate at the apex, rounded at the base, reddish brown. The herb is commonly found as a weed of waysides and waste places throughout India up to 2,100m and in the south Andman islands. The plant is considered a bitter acrid, carminative, astringent, pectoral, cardiotonic and diuretic. Extracts from dried or wet leaves of *Achyranthes aspera* are applied as a paste on wounds in some rural communities. The fresh juice from the leaves of *Achyranthes aspera* has been used by Ayurvedic physicians in India for external use to treat skin problems, dermatitis, eczema and acne [4]. There is no previous report on wound healing activities of *Achyranthes aspera* in literature to the best of our knowledge and in this paper, we report for the first time, the efficacy of *Achyranthes aspera* leaves extract in the treatment of wounds.

MATERIALS AND METHODS

Plant material and extract preparation- Leaves of *Achyranthes aspera* were collected from mature trees and its botanical identification was confirmed from National Botanical Research Institute Lucknow. A voucher specimen NBRI/CIF/Re./08/2008/32 was deposited in the herbarium of NBRI Lucknow**.** The plant material was dried in shade, powdered and sieved through 40-mesh size and material was stored in well-closed container.

Leaves of *Achyranthes aspera* Linn were finely pulverized (100 g) and extracted in Soxhlet apparatus for 24 h with methanol then concentrated and dried under reduced pressure. The extract was weighed and the yield obtained (9.8% w/w). The semisolid mass (dark brown colour) was obtained and used as ingredient for 5% ointment preparation. About 5 g of semisolid extract was incorporated into the 100 g of simple ointment base B.P.. Simple ointment base was used as control group (Negative control). Extract ointment was used ones daily to treat different groups of animals.

Animals- Healthy Albino mice of either sex (35–45 g) with no prior drug treatment were used for all the present *in-vivo* studies. The animals were fed on a commercial pellet diet (Hindustan Lever, Bangalore, India), and water ad libitum. Screening for wound healing activity was performed for incision, excision and dead space wound models. 54 animals of either sex weighed between 35 and 40 g were divided into three groups based on treatment type in each model

consisting of six animals as follows: group I – simple ointment base (Negative control); group II – 5% Povidine iodine (Win-Medicare, India) ointment (Posiitive control) and group III –5% methanolic extract ointment (Test) of *Achyranthes aspera*. The hairs on the skin of white surface of animals were removed by using a suitable depilatory (Anne-French hair removing cream).The animals were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment. Animal study was performed with due permission from institutional animal ethical committee.

Excision wound model- Circular wounds of approximately 10mm diameter were inflicted on the cleared skin by cutting under ether anesthesia. The areas of the wounds were measured (sq. mm) immediately by using vernier calipers. This was taken as the initial wound area reading. All the test samples (Negative, Posiitive & Test) were applied once daily. The wound area of each animal was measured on 1^{st}, 4^{th}, 7^{th}, 10^{th}, and 13^{th} post wounding day (Table 1).The wound closure was measured at regular intervals of time to see the percentage of wound closure and epithelialization time that indicates the formation of new epithelial tissue to cover the wound.

The percentage wound contraction [5] was determined using the following formula:

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialization.

Incision wound model- All animals were anaesthetized before wound creation and paravertebral long incisions were made through the skin at the distance of about 1.5 cm from midline on the depilated back of mice. No local or systemic antimicrobials were used throughout the experiment. All groups were treated same as in excision model, the both edges kept together and stitched with black silk surgical thread (no. 000) and a curved needle (no. 11) was used for stitching. The continuous threads on wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then simple ointment base, extract ointment and standard ointment were applied daily up to 7 days; when wounds were cured thoroughly the sutures were removed on the day 8^{th} and tensile strength [6], of cured wound skin was measured using tensiometer (Table 2).The skin breaking strength is expressed as the minimum weight (in grams) of water necessary to bring about the gapping of the wound.

Dead space wound model- This model was used for the study of granuloma tissue. Animals were anaesthetized by ether anesthesia and wound was made by implantation of cotton pellets (5 mg), (2.0×0.5), one on side, in the lumber region on the dorsal surface in each animal. On the 8^{th} post-wounding day, granuloma tissue formed on implanted cotton pellets was dissected out carefully. Granuloma tissue from one part was dried (60^0C) and weighted, while the other part of granuloma tissue [7-8] was used for determination of tensile strength (Table 3).

Histopathological Studies- Wound tissue specimens from control, test and standard groups were taken on the 13^{th} day after complete healing of excision wound. After usual processing, 6-mm thick sections were cut and 10% of nautral formalin solution was used to fix the granulation tissues for 24 h and dehydrated with a sequence of ethanol- xylene series of solutions. The inflicted materials were embedded with paraffin at 40-60 ^{0}c. Microtome sections were taken

at10□ thicknesses. The processed sections were stained with haematoxylin eosin [9-10] and observed under microscope in respect of fibroblast proliferation, collagen formation, epithelialization and blood vessels (Fig. 1-a,b & c).

Statistical analysis- Treated group was compared with the control group. The results were analyzed statistically using Student's t-test to identify the differences between the treated and control. The data were considered significant at $p<0.05$.

Table 1- Effect of Methanolic Extract Ointment of *Achyranthes aspera* Linn on Exsicion Wound Model

S. No.	Treatment	Period of epithelizaion	% wound contraction, post wounding day (Mean ± SD, n=6)				% Un-healed Wound area mm^2, 13^{th} post wounding day
			4^{th} Day Mean ± SD	7^{th} Day Mean ± SD	10^{th} Day Mean ± SD	13^{th} Day Mean ± SD	13^{th} Day Mean ± SD
1	Control	22^{nd} days	24.55 ± 0.75	40.04± 0.62	63.13±0.57	78.86±0.4	21.14±0.4
2	Standard	11^{th} days	54.06± 0.86	75.71± 0.70	93.81± 0.29	99.22±0.41*	0.78 ± 0.036*
3	Test (Extract treated)	13^{th} days	44.47± 0.74	69.76 ± 0.62	91.89± 0.36	98.90±0.41*	1.10 ± 0.049*

*P<0.05, **P<0.001 vs. control.*

Table 2- Effect of Methanolic Extract Ointment of *Achyranthes aspera* Linn on Insicion Wound Model

S. NO.	Treatment	No. of Animals	Tensile strength in gm/cm on 8^{th} post wounding day (Mean ± SD, n=6)
1	Control	6	170.94 ± 0.87
2	Standard	6	405.9 ± 1.55*
3	Test (Extract treated)	6	380.86 ± 0.93*

P<0.05 vs. control.

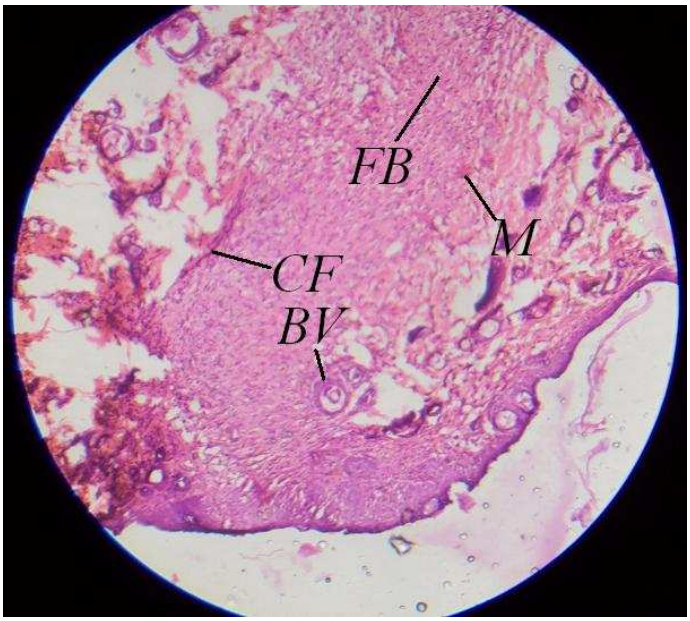

Fig.1- (a): Histological section of the granuloma tissues of Control animal showing incomplete healing with less epithelialization. Arrow showing Macrophage (M), Blood Vessels (BV) and Collagen formation (CF) indicate incomplete healing of wound.

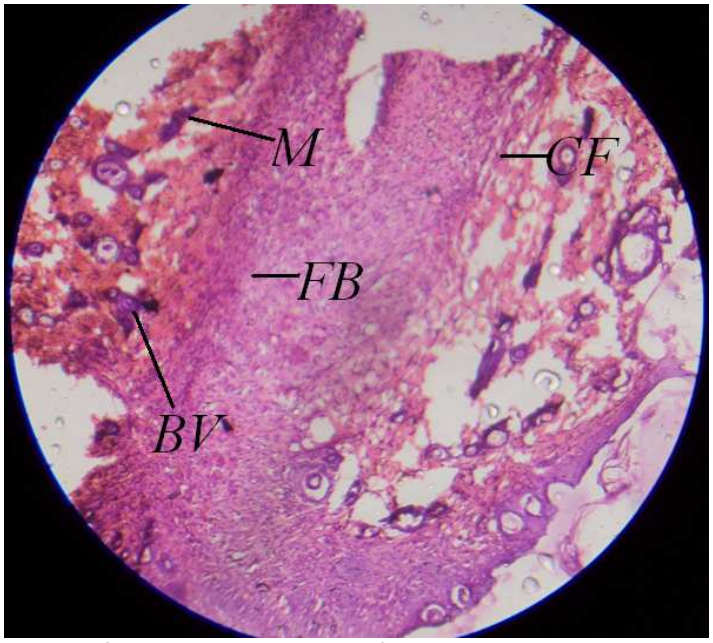

Fig.1- (b): Histological section of the granuloma tissues of Standard Treated animal showing complete healing with enhanced epithelialization. Arrow showing Macrophage (M), Blood Vessels (BV) and Collagen formation (CF) indicate complete healing of wound.

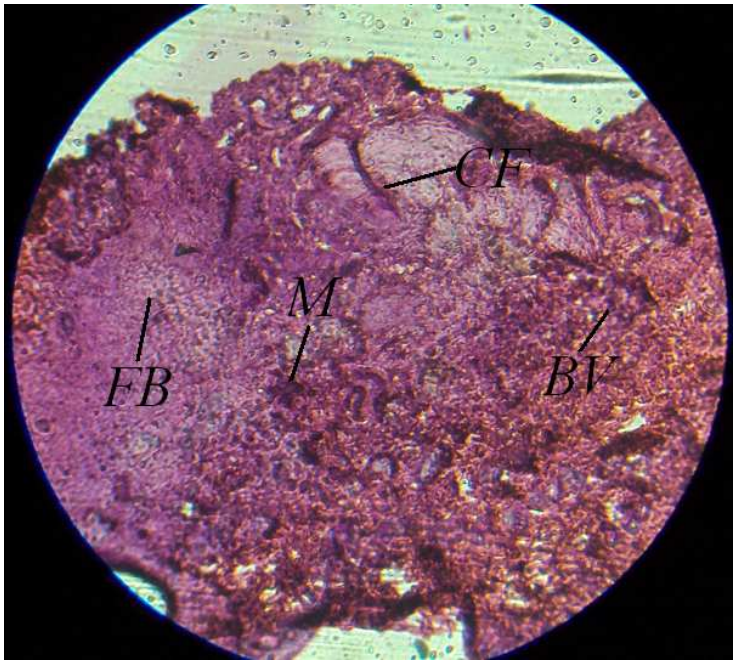

Fig.1- (c): Histological section of the granuloma tissues of Methanolic Extract Treated animal showing complete healing with moderate epithelialization and collagenation. Arrow showing Macrophage (M), Blood Vessels (BV) and Collagen formation (CF) indicate complete healing of wound.

Table 3- Effect of Methanolic Extract Ointment of *Achyranthes aspera* Linn on Dead Space Wound Model

S.NO.	Treatment	No. of Animals	Granulation Tissues dry-weight, mg/100gm (Mean ± SD, n=6)	Tensile strength in gm/cm on 8th post wounding day (Mean ± SD, n=6)
1	Control	6	9.56 ± 0.85	180.65 ± 0.54
2	Standard	6	22.81± 0.47*	411.55 ± 0.52*
3	Test (Extract treated)	6	20.12 ± 0.85*	384.62 ± 0.51*

P<0.05 vs. control.

RESULTS AND DISCUSSION

Wound area was measured by tracing the wound margin using vernier calipers in each 3 days interval and healed area was calculated by subtracting from the original wound area. On day 7, the wound contraction of standard and test were found to be significant (P<0.05) in comparison to control group. On day 10, wound was completely healed for standard group while for test group it was also almost at complete healing stage. On day 13, test group healed 98.90% and control group showed 78.86% healing. It was also observed that epitheliazation period of test and standard group were less in comparison to control group (Table 1). The time required for complete epitheliazation of the excision wound is an important parameter to assess the wound healing process.

The studies on excision wound healing model reveal that all the three groups showed day to day decrease in wound area. However, on 13th post wounding day, control animals group-I showed 21.14 ± 0.4 of unhealed wound area whereas group-II standard group animals, showed that of 0.78 ± 0.41 and the test group-III exhibited that of 1.10 ± 0.41 wound area. When compared with the control, the activity of extract was found to be highly significant (P<0.05).

The promotion of wound healing activity is also well gazed by its tensile strength of the incision wound. Generally wound-healing agents have the properties to enhance the deposition of collagen content, which provides strength to the tissues and forms cross-linkages between collagen fibers. The tensile strength of the test group was found to be (380.86 ± 0.93) which was higher than that of control group (170.94 ± 0.87) of animals and slightly lesser than that of standard group (405.9 ± 1.55) of animals on 8th post wounding day, which indicate good wound healing strength of the extract..

The effect of topical administration of the test group and control treated group on dead space wound model was assessed by increase in the weight of granulation tissue and increase tensile strength. The data is depicted in table-3. This indicates enhanced collagen maturation by increased cross-linking of collagen fibers. The increased weight of the granulation tissue also indicated the presence of higher protein content. Among these treated animals the response was shown to be the best in test animals.

Histology of the wound tissue of the control animals showed the presence of acute inflammatory cells, fibroblastic connective tissue and very little number of blood vessels (Fig. 1a). The lesser epithelialization and lesser collagen formation indicated incomplete healing of the wound in control animals. Where as, in the sections of standard treated group animals (Fig. 1b) increased collagen deposition was observed. The sections of the granuloma tissue of the animals treated

with methanolic extract showed moderate epithelialization, fibrosis, collagen formation and increased number of blood vessels (Fig. 1c)

CONCLUSION

In our study, the topical application of *Achyranthes aspera* has a positive influence on different phases of wound healing, including wound contraction, fibroblastic deposition, angiogenesis and therefore, has a beneficial role in wound healing. However, identification of the active constituents in this plant may provide useful leads to the development of new and effective drugs against different types of wound.

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