

Wound Healing Potential of a Herbal Gel Prepared From Leaf Extract of *Holarrhena Antidysenterica* Wall

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Abstract

India has a rich tradition of plant based knowledge on healthcare. The aim of present study is to assess the wound healing activity of the ethanolic extract of the leaves of *Holarrhena antidysenterica* Wall., (Apocynaceae). The ethanol extract of *Holarrhena antidysenterica* Wall., leaves were evaluated for their wound healing activity in rats using excision wound model. The wound healing was assessed by the rate of wound closure. Povidone-iodine ointment (5% w/w) was used as reference standard for the activity comparison. The results of the study revealed that the animals treated with ethanol extract of leaves of *Holarrhena antidysenterica* Wall., showed a faster rate of wound healing compared to the other extracts under study. The present work justifies the use of *Holarrhena antidysenterica* Wall., leaves for wound healing activity as claimed in the folklore literature.

Key words: Excision wound model, Ethanolic extract of *Holarrhena antidysenterica* Wall., 5% Povidone iodine, 5% of herbal gel.

1. INTRODUCTION

Various plant species have served as a source of medicine for people all over the world. Plants and their extracts have immense potential for the management and treatment of wounds. The herbal medicines for wound healing are not only cheap and affordable but are also purportedly safe as hypersensitive reactions are rarely encountered with the use of these agents. These natural agents induce healing and regeneration of the tissue by multiple mechanisms. However, there is need for scientific validation, standardization and safety evaluation of plants of traditional medicine before they can be recommended for clinical use [1]. Wound healing is the process of repair that follows injury to the skin and proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin [2]. Several medicinal plants have been used since time immemorial for treatment of cuts, wounds and burns and have shown promising results. Some very common plants like *Aloe vera*, *Azadirachta indica*, *Carica papaya*, *Celosia argentea*, *Centella asiatica*, *Cinnamomum zeylanicum*, *Curcuma longa*, *Nelumbo nucifera*, *Ocimum sanctum*, *Phyllanthus emblica*, *Plumbago zeylanica*, *Pterocarpus santalinus*, *Terminalia arjuna* and *Terminalia chebulica* have been extensively reported in Ayurveda, Siddha and Unani systems of medicines for their wound healing potential [3]. The plant *Holarrhena antidysenterica* Wall., belongs to the family Apocynaceae. It is a genus of trees or shrubs distributed throughout the tropical and subtropical regions of the world [2]. It has been used traditionally for treatment of various conditions like dysentery, diarrhea, skin diseases, bleeding piles, wounds, boils, ulcer, appetizer, anthelmintic [4]. As per the folklore claim, the juice of leaves is applied over severe open wounds with promising effectiveness in healing of wounds. Review of literature indicates that no scientific work has

been carried out on the leaves of *Holarrhena antidysenterica* Wall., Hence, the present study was undertaken to validate the effectiveness of the leaves of *Holarrhena antidysenterica* Wall., in wound healing by formulating ethanolic extract into a gel [5].

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

The leaves of *Holarrhena antidysenterica* Wall., were collected from ABS botanical garden, Karipatti, Salem district of Tamilnadu during August 2014 and authenticated by Prof.P.Jayaraman, Director, Institute of Botany, Plant Anatomy Research Center, Tambaram. The leaves were shade dried and pulverized to coarse powder and preserved in an air tight container for further studies.

2.2 Preparation of Extract and Preliminary Phytochemical Studies

2.2.1 Preparation of Extract

100 g of the coarsely powdered, dried leaves of *Holarrhena antidysenterica* Wall., was extracted with ethanol and it was filtered and distilled under rotary vacuum to get concentrated extract. The ethanolic extract of *Holarrhena antidysenterica* Wall., was stored in a desiccator until further studies.

2.2.2 Preliminary Phytochemical study

A preliminary phytochemical screening was carried out for the ethanolic extract employing the standard procedure to reveal the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, tannins, glycosides, carbohydrates, phytosterols and proteins [6-8]

2.2.3 Formulation of gel

A 2.5% and 5% w/w gel formulation was made by incorporating the ethanolic extract of leaves of *Holarrhena antidysenterica* Wall., with gel base (Carbopol 934) for

external application of the drugs in the excision wound model.

2.2.4 Wound healing activity

The wound healing activity of the gels prepared from the ethanolic extract of the leaves of *Holarrhena antidysenterica* Wall., was evaluated using excision wound model.

2.2.5 Experimental Animals

Healthy Wistar Albino rats of both sex and approximately the same age, weighing between 150-200g were used for the study. They were individually housed, maintained in clean polypropylene cages containing paddy husk bedding and fed with standard diet and water *ad libitum*. Clearance from the Institutional Animal Ethical Committee, Madras Medical College, Chennai was obtained for carrying out the study (Approval no 16/243/CPCSEA).

2.3 Experimental Procedure [9]

2.3.1 Excision wound model

24 adult female rats weighing 150-200g were used for the study. Animals were anesthetized prior to and during creation of wounds. The rats were inflicted with excision wound as described by Morton and Malone [10]. The dorsal fur of the animals was shaved with razor and the anticipated area of the wound to be created was outlined on the back of the animals with marker. A full thickness of the excision wound of circular area 500mm² and 0.2 cm depth was created along the markings, using a surgical blade and pointed scissors. The entire wound was left open. The animals were divided into four groups of six animals each [10,11].

2.4 Parameters Studied

2.4.1 Percentage wound closure

The wound closure rate was assessed by tracing the wound on days 0, 2, 4, 6, 8, 12, 16, 18 and 20 post-wounding, using transparent sheets and a permanent marker.

2.4.2 Statistical Analysis

All the data were expressed as mean \pm S.E. The significance of the difference between the means of the test groups and control group was established by One way ANOVA followed by Dennett's t-test, Version 5 [12].

3. RESULTS

3.1 Preliminary Phytochemical Studies

The ethanolic extract of leaves of *Holarrhena antidysenterica* Wall., showed the presence of phytoconstituents like alkaloids, flavonoids, phenolics, carbohydrate, tannins, saponins.

3.2 Wound healing activity

The results of the wound healing effects of the gel prepared from ethanolic extract of leaves of *Holarrhena antidysenterica* Wall.

From the table 3 it is seen that on all days, the wound contraction was better in the animals treated with the gels prepared with the ethanolic extract of leaves of *Holarrhena antidysenterica* Wall., as compared to the control group and the difference was significant ($p < 0.001$). The wound contraction was highly comparable with the Povidone treated group. With the 5%w/w gel, the wound contraction

was even marginally better than the Povidone treated group.

From the day 8 itself, the 5% gel treated group of animals showed a much higher rate of wound healing which continued upto the day 20. On day 20, there was 100% wound contraction seen in animals treated with 5% gel while the wound contraction in the animals treated with 2.5% gel was 98.3%. In the Povidone treated group the wound contraction was 99.6% while the control group there was only 94.6% wound contraction [2].

These results confirm the wound healing capacity of the ethanolic extract of the leaves of *Holarrhena antidysenterica* Wall., The wound healing activity of the formulation may be attributed to the presence of phyto constituents like alkaloids, tannins, flavonoids and phenolic compound in the ethanolic extract which are known to promote the wound healing activity [2,13,14].

Table 1: The treatment regimen for the following control, standard, test I and test II groups of mice (n=6).

S. No	Group (n=6)	Name of the Group	Treatment
1	I	Control group	Treated with gel base (1%w/w of Carbopol 934) for 20 days
2	II	Standard group	Treated with 5% of Povidone Iodine ointment for 20 days
3	III	Test group I	Treated with 2.5% gel made by using ethanolic extract of the leaves of <i>Holarrhena antidysenterica</i> Wall., for 20 days.
4	IV	Test group II	Treated with 5% gel made by using ethanolic extract of the leaves of <i>Holarrhena antidysenterica</i> Wall., for 20 days.

Table 2: Preliminary Phytochemical Studies on the leaves of *Holarrhena antidysenterica* Wall., in excision wound model. The symbols denotes '+' as positive /presence and '-' denotes negative/absence.

S. No	Phytoconstituents	Ethanol Extract
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Flavonoids	+
4.	Glycosides	-
5.	Fixed oil and fats	-
6.	Lipids	-
7.	Phytosterols	-
8.	Phenolic compounds	+
9.	Resins	-
10.	Saponins	+
11.	Terpenoids	-
12.	Tannins	+
13.	Gums and mucilage	-

Table 3: Wound healing effect of leaves of *Holarrhena antidysenterica* Wall., in excision wound model. The values are expressed as mean \pm SE. *, ** & *** denotes the statistical significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$ in comparison with control group.

Post-wounding days	Wound area-mm ² (% wound contraction)			
	Control	Standard (5% Povidone iodine ointment)	Test gel I 2.5%w/w	Test gel II 5%w/w
DAY 0	3 \pm 0.00	2.967 \pm 0.052	2.983 \pm 0.041	2.983 \pm 0.041
DAY 2	2.93 \pm 0.082 (2.3%)	2.883 \pm 0.041 (2.7%)	2.683 \pm 0.117* (10%)	2.483 \pm 0.098*** (16.7%)
DAY 4	2.733 \pm 0.225 (13.5%)	2.483 \pm 0.133 (16.2%)	2.267 \pm 0.103** (24%)	2.050 \pm 0.103*** (31.2%)
DAY 8	2.217 \pm 0.354 (26.3%)	1.817 \pm 0.075 (38.8%)	1.483 \pm 1.033** (50.3%)	1.167 \pm 0.103*** (61%)
DAY 12	1.617 \pm 0.679 (46.3%)	1.150 \pm 0.152 (61.1%)	1.033 \pm 0.147** (65.4%)	0.650 \pm 0.055*** (78.1%)
DAY 16	0.93 \pm 0.103 (69%)	0.533 \pm 0.052 (82%)	0.683 \pm 0.204** (77.1%)	0.133 \pm 0.052*** (95.6%)
DAY 18	0.267 \pm 0.052 (91.3%)	0.0283 \pm 0.354 (90.5%)	0.183 \pm 0.098** (93.9%)	0.017 \pm 0.041*** (99.6%)
DAY 20	0.167 \pm 0.052 (94.6%)	0.017 \pm 0.041 (99.6%)	0.056 \pm 0.055** (98.3%)	0.00 \pm 0.00*** (100%)

Figure 1: CONTROL GROUP

The image showing the wound healing activity of *Holarrhena antidysenterica* Wall., on Control group mice at different consecutive Days.

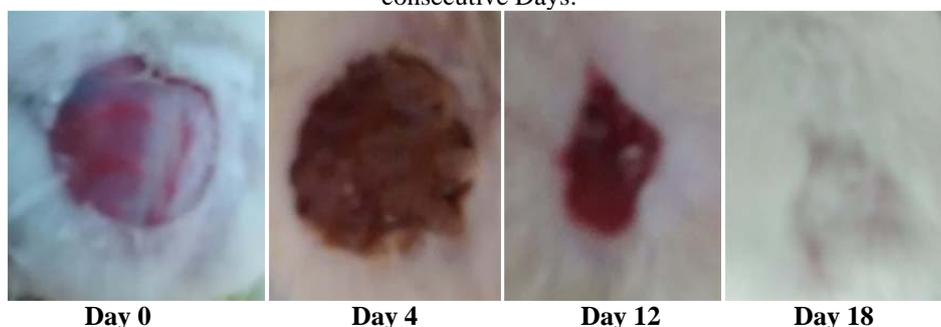


Figure 2: STANDARD GROUP

The image showing the wound healing activity of *Holarrhena antidysenterica* Wall., on Standard group mice at different consecutive Days.



Figure 3: TEST GROUP I

The image showing the wound healing activity of *Holarrhena antidysenterica* Wall., on Test group I mice at different consecutive Days.

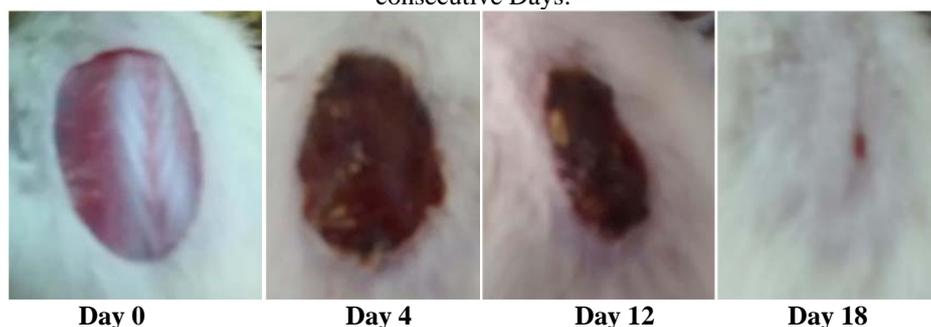
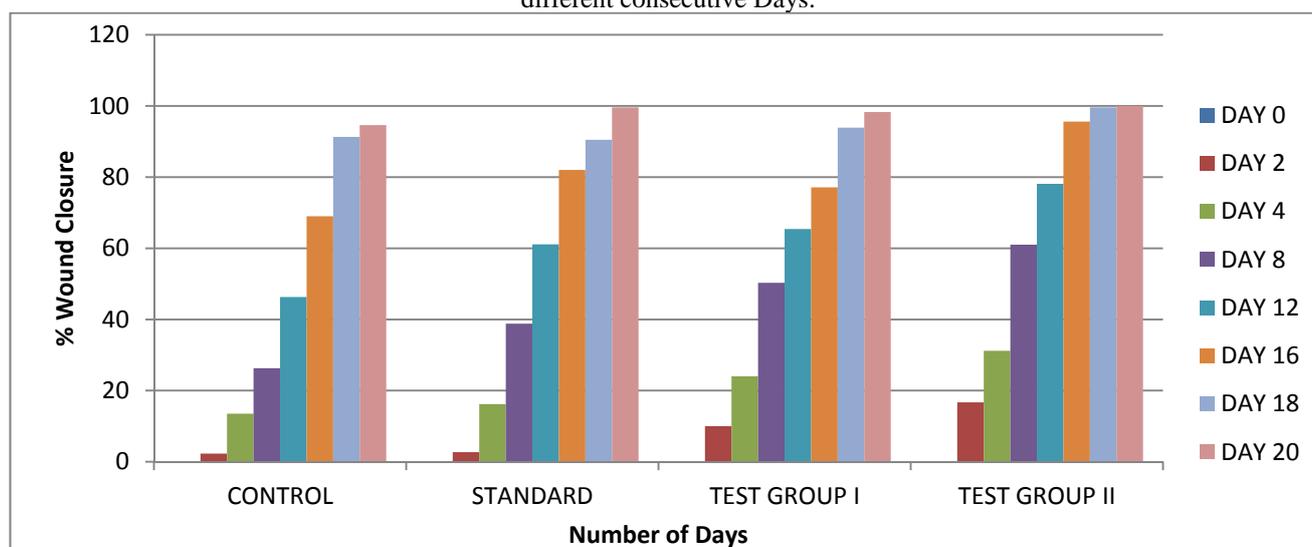


Figure 4: TEST GROUP II**Figure 5:** The image showing the wound healing activity of *Holarrhena antidysenterica* Wall., on Test group II mice at different consecutive Days.

4. DISCUSSION

In this study the significant promotion of wound healing activity was observed in group IV. The mean percentage closure of wound area was calculated on 0, 2, 4, 8, 12, 16, 18 and 20 post wounding days in all the groups. The results of the present study revealed that animals treated with gel II & I showed significant wound closure on excision wound model which was comparable with that of standard group. However gel at higher dose showed faster rate of wound closure when compared to gel at lower dose.

5. CONCLUSION

The Pharmacological evaluation of the gel was made by excision wound model of wound. In this study, it was seen that both the concentration of the gels showed significant wound closure as compared to the control group. The 5% gel showed better activity which is highly comparable with the standard. Thus from the above study it is concluded that the leaves of *Holarrhena antidysenterica* Wall., have a good Wound healing activity and they may provide an alternative to the currently available wound healing formulations. From the study it is concluded that the 5% gel prepared using the ethanolic extract of leaves of *Holarrhena antidysenterica* Wall., shows a promising wound healing activity. The wound healing activity compares very well with that of the standard Povidone

iodine ointment. This herbal gel may well pave the way for an alternative therapy in the treatment of wounds.

REFERENCE

- [1] Pal S, Shukla Y. Herbal medicine: current status and the future. *Asian Pacific J Cancer Prev* 2003;4:281-8.
- [2] Karodi R, Jadhav M, Rub R, Bafna a. Evaluation of the wound healing activity of a crude extract of *Rubia cordifolia* L. (Indian madder) in mice. *Int J Appl Res Nat Prod* 2009;2:12-8.
- [3] Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. Ethnopharmacological approaches to wound healing-Exploring medicinal plants of India. *J Ethnopharmacol* 2007;114:103-13. doi:10.1016/j.jep.2007.08.010.
- [4] KIRTIKAR KR, BASU BD. *Indian Medicinal Plants*. 1918.
- [5] Chothani DL, Patel NM. Preliminary phytochemical screening, pharmacognostic and physicochemical evaluation of leaf of *Gmelina arborea*. *Asian Pac J Trop Biomed* 2012;2:1-5. doi:10.1016/S2221-1691(12)60411-0.
- [6] Harborne AJ. *Phytochemical Methods A Guide to Modern Techniques of Plant Analysis*. Springer Science & Business Media; 1998.
- [7] Dande PR, Talekar VS, Chakraborty GS. Evaluation of crude saponins extract from leaves of *Sesbania sesban* (L.) Merr. for topical anti-inflammatory activity. *Int J Res Pharm Sci* 2010;1:296-9.
- [8] Nagar HK, Srivastava AK, Srivastava R, Kurmi ML, Chandel HS, Ranawat MS. Pharmacological Investigation of the Wound Healing Activity of *Cestrum nocturnum* (L .) Ointment in Wistar Albino Rats 2016;2016. doi:10.1155/2016/9249040.
- [9] Garg V, Paliwal S. Wound-healing activity of ethanolic and aqueous extracts of *Ficus benghalensis*. *J Adv Pharm Technol Res* 2011;2:110. doi:10.4103/2231-4040.82957.

- [10] Morton JJ, Malone MH. Evaluation of vulneray activity by an open wound procedure in rats. *Arch Int Pharmacodyn Thérapie* 1972;196:117–26.
- [11] Sreedhar V, Nath LKR, Gopal NM, Nath MS. In-vitro antioxidant activity and free radical scavenging potential of roots of *Vitex trifoliata*. *Res J Pharm Biol Chem Sci* 2010;1:1036–44.
- [12] Stoline MR. The status of multiple comparisons: simultaneous estimation of all pairwise comparisons in one-way ANOVA designs. *Am Stat* 1981;35:pp. 134–41. doi:10.2307/2683979.
- [13] Solanki R, Purohit SK, Mathur V, Mathur M, Wing P. International Journal of Drug Research and Technology Original Research Paper EVALUATION OF WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACT OF OCIMUM BASILICUM LEAVES IN MALE ALBINO RATS 2012;2:208–11.
- [14] Dash GK, Narasimha Murthy P. Wound healing effects of *Ageratum conyzoides* Linn. *Int J Pharma Bio Sci* 2011;2:369–83.