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PHARMACOLOGICAL EVALUATION OF WOUND HEALING ACTIVITY ON METHANOLIC EXTRACT OF ANAPHALIS LAWII (HOOK.F) GAMBLE

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ABSTRACT

The aim of the study is to evaluate the wound healing activity of methanolic leaf extract of of *Anaphalis lawii* was studied respectively on two types of wound models in rats, (i) the excision and (ii) the incision wound model. The methanolic extract of *Anaphalis lawi*i leaves produced significant response in both of the wound types tested. In the excision model the extract treated wounds were found to epithelialise faster and the rate of wound contraction was higher, as compared to control wounds. The extract facilitates the healing process as evidenced by increase in the tensile strength in the incision model. The results were also comparable to those of a standard drug Framycetin.

KEYWORDS: Wound healing, Incision model, Excision model, *Anaphalis lawi* leaves.

INTRODUCTION

Wound is defined as loss and breaking of anatomic and cellular of living tissue. Wound healing process is a biological process instigated by trauma and causes scar formation. Wound healing process occurs in few different stages such as coagulation, epithelisation, granulation, collegenation and remodelling of tissue. Wound healing is an active and multifaceted process in restoring cellular structures and tissue layers. The objective of wound management is to heal the wound in express time possible, with very nominal pain, discomposure and scarring in patient with wound. At the site of closure, a lithe and fine scar with high tensile strength is required. [1]

Anaphalis lawii (Hook. f.) Gamble is a wide spread, very white and tall herb belonging to the family Compositae. It is distributed in Western Ghats, Coorg, Bababudan hills of Karnataka, Brahmagiris, hills of Coimbatore, hills of Tirupathi, N. Nilgiris, Anamalais, Pulneys and hills of Tinnevelly, at 5000-7000 ft. Leaf margins flat, not folded back except the upper once of the scape, which are closely pressed and ascending; leaves linear oblong or oblanceolate, very white-wooly, 1-3.5 inch long, 0.3 inch broad; heads 0.2-0.3 inch broad, in broad corymbs of many branches; bracts white, limb ovate, acute; achenes minute (Gamble, 1993). The whole plant is air-dried, powdered, and consumed with food as Kayakalpa by the Malasars of the Velliangiri hills in the Western Ghats of Nilgiri Biosphere Reserve, India. [2]The present study was undertaken to investigate wound healing activity of leaf extract of *A. lawii*.

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

The leaves of *Anaphalis lawii* (Hook. f.) Gamble were collected from talakona forest near to Tirupati and were authenticated by Dr.K.Madavachetty, S.V.University, Tirupati, Andhra Pradesh in month of March 2013.

MATERIAL AND METHODS

Plant Extract and standard used

The dried plant materials were, pulverized by a mechanical grinder, sieved through 40#mesh. The powdered materials were extracted with methanol using soxhlet extraction apparatus. This methanol extract was then concentrated and dried under reduced pressure. The methanol free semisolid mass thus obtained was used for the experiment.

Experimental Animals

Wistar albino rats either sex weighing between 180 g and 200 g were selected for the acute toxicity and wound healing activity studies. The study was approved by the Institutional Ethics Committee for animal experimentation (1447/Po/a/11/CPCSEA) TRR college of Pharmacy, Hyderabad. The animals were stabilized for 1 week. They were maintained in standard conditions at room temperature, 60 ± 5% relative humidity and 12 h light dark cycle. They were given standard pellet diet supplied and water ad libitum throughout the course of the study.

Acute toxicity studies

Albino rats of either sex received methanolic extract of *Anaphalis lawii leaves* starting at 2

g/kg bw orally by gavage. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. Finally, the number of survivors was noted after 24 h and these animals were then maintained for further 13 days with observations made daily. [3]

PHARMACOLOGICAL ACTIVITY

Excision wound model

Four groups with six animals in each group were anaesthetized with ether. The rats were depilated on the back. Cutting away a 500mm2 full thickness of skin from the depilated area inflicted one excision wound, the wound was left undressed open environment. Then, the drugs, i.e., Group I control, Group II the reference standard Framycetin ointment, and Group III the methanolic extract (200mg/kg) were applied once daily till the wound was completely healed. This model was used to monitor wound contraction and a wound closure time. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day. [4]

Table 1: Effect of methanolic extract on Excision wound model.

Days	Control	Framycetin	Methanolic extract
0	510.9±30.44	509.00±1.45	502.4±7.38
3	481.6±18.02	442.4±6.44*	423.02±6.2
6	420±18.1	402±6.2*	411±6.5*
8	350.1±24.5	310.0±4.01*	328±5.8**
10	262±14.8	256±2.8*	254±6.0**
12	187.4±24.5	130.2±45.4*	102±5.3**
14	109.7±29.0	73.7±45.2*	71.8 ±95.2**
16	42.5±18.3	00	00

*Values are expressed as mean±S.E. (n=6). *P<0.01 and **P<0.001 compared with vehicle Control*

Incision wound model

Procedure: The incision wound model was studied. Under light ether anesthesia the animal was secured to operation table in its natural position. One Para vertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. Wounds were cleaned with 70% alcohol soaked with cotton swabs. They were kept in separate cages. The latex was applied at a dose of 200 mg/kg/day for 10 days. The sutures were removed after 8 days, on tenth day the tensile strength was measured by continuous constant water supply technique. [5]

Table 2. Effect of methanolic extract on Incision wound model.

Treatment	Wound breaking strength (g)
Control	292.15±31.89
Methanolic extract	458±76.12*
Framycetin	480.16±34.65**

*Values are expressed as mean±S.E. (n=6). *P<0.01 and **P<0.001 compared with vehicle control.*

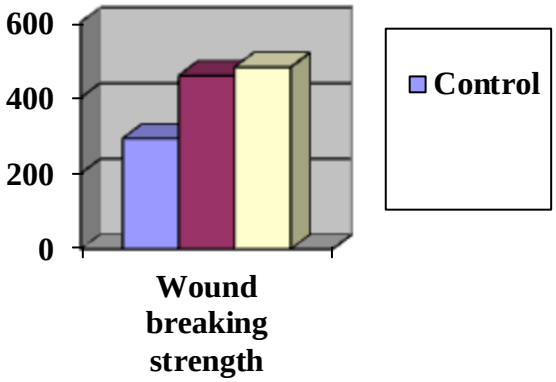

Fig. 1 Effect of methanolic extract on Incision wound model.

Statistical analysis

The experimental data were calculated as mean ± SEM., evaluated by one way ANOVA test. Values of p< 0.001 were considered statistically significant.

RESULTS

In studies using excision wound model, animals treated with the leaf extract Anaphalis lawii showed a significant decrease in epithelization period as evidenced by shorter period for all of scar as compared to control. The plant extract also facilitated the rate of wound contraction significantly (Table 1). In incision wound model, significant increase (p<0.001) was observed in the skin breaking strength on 10th post-wounding day in the animal treated with the dose of 200mg/kg (Table 2 and Fig. 2). The extract treated group showed more advanced phase of healing and better organized bundles of collagen.

DISCUSSION

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Proper healing of wounds is essential for that restoration of disrupted anatomical continuity and distributed functional status of the skin. It is the product of the integrated response of

several cell types of injury. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue. Wound healing, complex sequences of events involve 4 phases. (i) Coagulation which prevents blood loss. (ii) Inflammation and debridement of wound. (iii) Epithelial repair, including the proliferation, mobilization, migration and differentiation. (iv) Tissue remodeling and collagen deposition. Any agent which accelerates the above process can be termed as a promoter of wound healing. In spite of tremendous advances in the chemical industry, the availability of substances capable if stimulating the process of wound repair is still limited. [6] Plants with wound healing activity have been reported and experimentally studied on various wound models to reveal the most active promising compounds. [7] Results obtained in the present study suggest that treatment of albino rats with the fresh homogenized crude extract of *Anaphalis lawii has* accelerated the wound healing process. Increase in tensile strength may be due to increase in collagen concentration and stabilization of the fibres. [8] The results suggest that treatment with fresh homogenized crude extract may have a beneficial influence on the various phases of wound healing such as fibroplasias, collagen synthesis and wound contraction, resulting in faster healing. These findings partially justify the inclusion of this plant in the management of wound healing in folk medicine. Further experiments are needed to test the effect of this plant in the treatment of chronic wounds.

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