INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ESTIMATION OF WOUND HEALING POTENTIAL OF *GNAPHALIUM HYPOLEUCUM* DC.

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Article Received on: 16/02/18 Approved for publication: 23/03/18

DOI: 10.7897/2230-8407.09342

ABSTRACT

The wound healing potential of *Gnaphalium hypoleucum* was estimated by excision and incision wound models. This study was designed to prove the traditional believe of this plant as the wound healer. This screening showed that *Gnaphalium hypoleucum* possesses highly significant wound healing potential, almost 98 % in 13 days study. Not only the faster healing was reported but also the quality of healing was improved when compared with standard as well as control group. On 13th day, excision wound was completely healed (99.33%) while incision wound showed significant tensile strength (588.33 gm) as control group (383.33gm). The epithelization time was significantly reduced to 7.33 days as in control (11.33 days) while wound index study showed that quality of healing of wound was highly improved (0.33) as compared to control (3.33) group.

Keywords: Wound healing, excision, incision, wound index, epithelialization.

INTRODUCTION

Wounds are physical, chemical or any other injury to the tissues of body that result in breaking of skin and abolishment of function, categorized in various forms from mild to potentially fatal wound[1]. The basic requirements for successful wound healing are moist area, protected and pathogen free environment with optimization of local and systemic conditions of patient. The traditional products can provides all these favorable conditions to wounded area while newer products currently attempt to replace or augment the various substrates in the cascade of wound healing[2], such products also effects significantly to the post operative wound healing and helps in minimizing the scar formed on wound[3].

Wound healing is complex procedure that contains various phases like inflammation, proliferation, collagen synthesis, maturation and remodeling. All these phases involved a series of reactions, mediators and interactions among cells[4]. Nutritional deficiencies also play a vital role in wound healing like vitamin A promotes immune function, cellular differentiation, epithelial and bone formation. The vitamin C serve as tissue antioxidant and responsible for collagen synthesis while vitamin E act as lipid soluble antioxidant but its role in surgical wound healing is still not leading to a firm conclusive[5].

The main events of wound healing are hemostasis in which injury site get sealed by provisional matrix and starts wound healing, next event that is inflammation triggered the release of variety of mediators and activates platelets with their cytokines while the last event ends with the formation of granulation tissue and maturation[6].

The wound healing potential can be evaluated by various parameters mainly period of epithelialization, tensile strength, percentage wound contraction, and wound index estimation. Histological study of granulation tissue may also be performed to estimate the extent of collagen formation in wound tissue[7].

The Indian traditional system of medicine 'Ayurveda' describe various plants, animal originated products and minerals for wound healing under the term 'Vranaropaka'[8]. Plants are more potent wound healers then others due to their ability to promote repair mechanism naturally[9]. Traditionally huge numbers of herbs are used by local Vaids and tribals for treatment of burns, cuts and wounds in India[10]. About 65% of world's population has used the plants as medicinal agents into their primary health care told by World Health Organization and about 25% of all drugs prescribed today obtained from plants[11].

The genus Gnaphalium containing about 200 species that belongs to tribe Gnaphalieae, distributed worldwide, traditionally used as wild herbs in various disorders[12]. *Gnaphalium hypoleucum* DC. (Family- *Asteraceae*) commonly known as Bukhil, found in Pauri Garhwal region of Uttarakhand, India at 600-1000 meter altitude, applied on cuts and wounds and juice is believed to increase in lactation[13].

The chemical constituents reported from this plant are aurantiamide acetate, 5-hydroxy-3,6,7,8-tetramethoxyflavone, 5-hydroxy-3,6,7,8,4'-pentamethoxy flavones, tetracosanoic acid, β-sitosterol, 5-hydroxy-3,6,7,8,3',4'-hexa methoxyflavone, 5,7-dihydroxy-3,8,4'-trimethoxyflavone, 5,8-Dihydroxy-3,6,7-trimethoxyflavone,

4'-Hydroxydehydrokawair, oleanolic acid ursolic acid, 19α-hydroxy ursolic acid, apigenin, daucosterol, n-hexadecanoic acid, formononetin, luteolin, quercetin, uteolin-4'-O-β-D-glucoside, quercetin-4'-O-β-D-glucoside, linarin, apigenin-7-O-β-D-glucuronic acid methylester, 6-Hydroxyluteolin-7-O-β-D-glucoside, luteolin-7-O-β-D-glucuronide, Panamin, luteolin-7-O-β-D-glucuronic acid methylester, Luteolin-4'-O-β-D-(6''-E-caffeoyl)-glucopyranoside, acacetin-7-O-β-D-xylopy ranosyl-(1→2)[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside, Quercetin-4'-O-β-D-(6''-E-caffeoyl)-glucopyranoside and Gnaphaliin C[14].

G. hypoleucum DC. is a folk medicine used for the treatment of gout in china and also used as anti-inflammatory, antitussive, gout and expectorant activities[12,18]. No comprehensive scientific study has been carried out on *G. hypoleucum* for their wound healing activity so far, hence the present study was undertaken to estimate the wound healing potential and compare their efficacy.

MATERIALS AND METHODS

Plant Material

The whole plant was collected from September to October 2015 from wild region of Rudraprayag, Uttarakhand, India and identified and authenticated from Botanical survey of India, Dehradun, Uttarakhand, India by botanist Professor S. K. Srivastava (voucher specimen-115902). The plant was shade dried at room temperature and extracted for 24 h using hot Soxhlet method with methanol then dried at 50 ^{0}C on rotary drum evaporator (Buchi type) and finally in vacuum oven.

Acute Toxicity Test

An acute toxic test of methanolic extract was done to determine the safety of extract on skin and whole procedure was according to OECD guidelines (OECD 410).

Ointment Formulation and Topical Application

The ointment gel was formulated with HPMC at a composition of 10 % of dried extract. Group 1: Control, applied pure gel. Group 2: 10%, w/w, methanolic extract gel was applied and Group 3: 10% w/w, standard *Aloe vera* gel was applied to the animal's wound twice daily. Wounds were observed for wound-healing on alternate day till 13 days and experiment was carried out under aseptic condition.

Experimental Animals

Wistar rats of either sex were obtained from Department of Pharmacy, Devsthali Vidyapeeth college of Pharmacy, Lalpur Kichha road, Rudrapur (U. S. Nagar), Uttarakhand, India (1452/PO/Re/S/11/CPCSEA). The animals were divided randomly into 3 groups of 5 rats each with body weight between 180 - 200 g and allowed taking standard pellet diet and RO filtered water ad libitum. The use and care of laboratory animals were conducted in accordance with institutional animal ethical committee (IAEC) of the institution (DVCP/IAEC/ 2015/01).

Excision Wound Model

The marked skin of rats was shaved one day prior to commencement of experiment. An impression was made on dorsal thoracic region 2 cm away from ear under anesthesia and the impressed area was excised to full thickness in obtain a wound area of about 500 mm^2 in diameter[15].

Wound Area Measurement

Wound contraction was measured by tracing the wound area on a transparent sheet and finally on a graph paper from which wound surface area was evaluated on 0, 3, 5, 7, 9, 11 and 13th day after wounding. The estimated wound surface area was employed to calculate the percentage wound contraction assuming the initial size of wound as 100%. The period of re-epithelialization was also observed for each of the treatment group. Percentage wound contraction was determined using: % Wound contraction = wound area on o day-wound area on n day/wound area on 0 day, multiply by 100[15].
Nth day = 3, 5, 7, 9, 11 and 13th post wounding days.

Incision method

Take the anaesthetized rat and a 5cm long abdominal incision was made on skin then closed with interrupted sutures (Mersilk, Ethicon, Aurangabad) at a distance of 1 cm. There after they were kept individually in different cages[15].

Tensile strength measurement

On the 13th day, animals were sacrificed by cervical dislocation, sutures were pulled out gently and wound areas from each animal were removed carefully. Wound stripes of equal size were made widthwise by 23 number surgical blades fixed at a distance of 2 cm. Both ends of each strip were fixed with the help of a pair of steel clips. One clip allowed to hang on a stand and a polyethylene bag on other clip, was then fill gradually with water till wound strip was broken at the site of wound. The quantity of water required to break the suture healed wound was noted and expressed as tensile strength of wound in grams[15].

Periods of epithelization

Falling of scar from wound was taken as end point of complete epithelization and number of days required to fall the scar was taken as period of epithelization[16].

Wound index

Wound index was measured daily with an arbitrary scoring system i.e. ''0'' for complete healing, 1 for incomplete but healthy healing, 2 for delayed but healthy healing, 3 for healing has not yet been started but environment is healthy, 4 for formation of pus evidence of necrosis[16].

Statistical analysis

The data were expressed as mean ± SEM of at least triplicate determinations (n = 5). One-way Analysis of Variance (ANOVA) using Graph Pad Instat software was performed using Dunnett's test to confirm the significance of the study. Differences between test and control treatments are considered significant at $P < 0.05$.

Table 1: Wound healing potential of methanolic extract of *Gnaphalium hypoleucum* DC.

Parameters		Pure gel (Control) Mean±SD	*Aloe vera* (10 % gel) (Standard) Mean±SD	*G. hypoleucum* (10% gel) Mean±SD
% Wound contraction	Day 3	31.62±6.52	51.65±7.10*	58.09±3.45**
	Day 5	46.95±5.92	61.21±2.75*	77.13±3.84**
	Day 7	68.75±4.42	78.13±2.98*	85.73±1.74**
	Day 9	76.07±3.87	80.45±3.42^{ns}	96.22±0.42**
	Day 11	80.98±1.36	91.09±1.05**	97.56±0.41**
	Day 13	84.18±0.71	98.81±0.61**	99.3±0.03**
Tensile Strength (gm)		383.33±47.52	563.33±25.16**	548.33±12.58**
Epithelialization time (Days)		11.33±0.57	7.66±0.57**	7.33±0.57**
Wound index		3.33±0.57	0.66±0.57**	0.33±0.57**

*p<0.05, **p<0.01

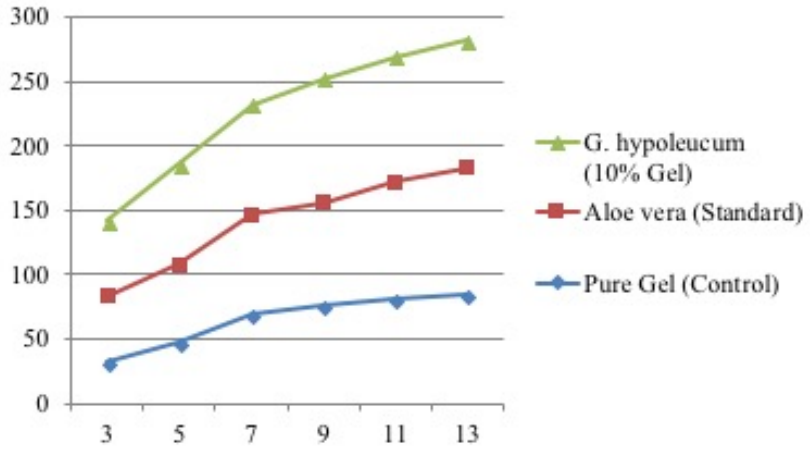

Figure 1: % Wound contraction of methanolic extract of *Gnaphalium hypoleucum* DC.

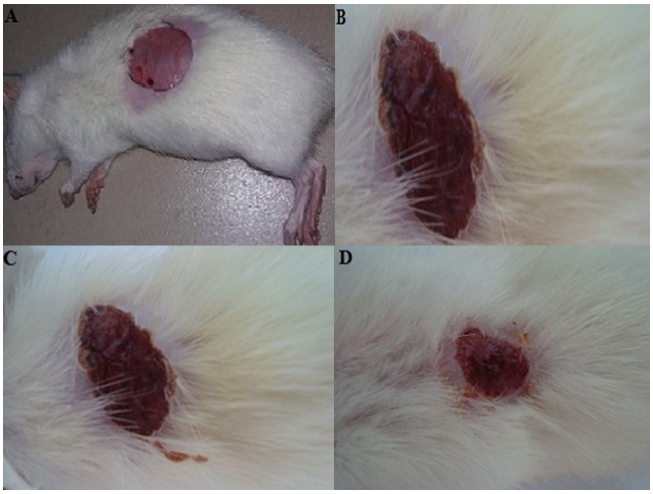

Figure 2: Plate 1- Excision wound healing: A. First day; B. 5th day; C. 9th day; D. 13th day

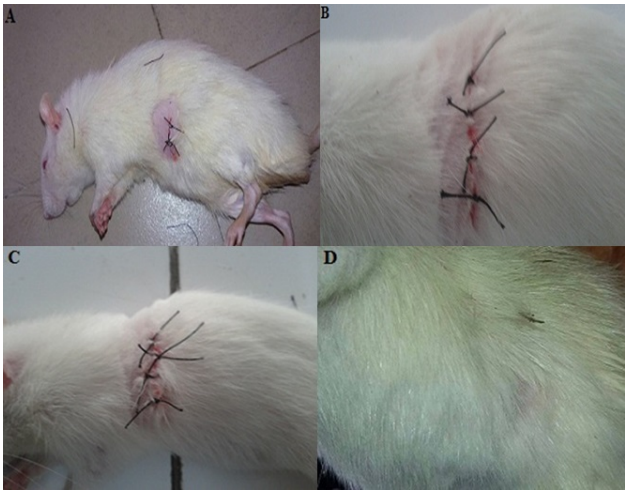

Figure 3: Plate 1- Incision wound healing: A. First day; B. 5th day; C. 9th day; D. 13th day

RESULTS

The 10 % composition of *G. hypoleucum* DC. gel was evaluated for their wound healing potential in rats using *in vivo* models and results showed that plant possesses very significant wound healing ability when compared to control and standard *Aloe vera* gel.

Acute Toxicity

At limit test of 2000 mg/kg as suggested by OECD guidelines was applied on shaved skin of rats then after 48 h, extract did not show abnormal gross behavior, any signs of lethality or moribund state of animals or any signs of dermal toxicity.

Wound Healing Ability

The wound healing activity of *G. hypoleucum* methanolic extract is shown in Figure 1. A faster patron of wound closure was observed with *G. hypoleucum* methanolic extract gel and *Aloe vera* gel when compared with control. There was a significant reduction in wound area from 3rd day to the 13th day of treatment (Figure 2 and 3). Significant wound contraction was observed in the test group from day 3 (58.09%) in *G. hypoleucum* extract group ($p>0.01$) while in standard group (51.65%) has been observed. On 5^{th}, 7^{th}, 9^{th} and 11^{th} day significant wound contraction shown by the both standard and extract treated group. On 13^{th} day of study, *G. hypoleucum* and *Aloe vera* gel treated group showed almost same healing potential but faster and quality healing was observed in *G. hypoleucum.*

Epithelialization of wounds

The epithelization time in days are surmised in table 1. The standard and test preparation treated animals showed least period of epithelization (7.33 days) while control group animals had longest period of epithelization. Epithelization time study showed that both standard as well as test plant extract showed significant ($p>0.01$) reduction in time and indicated that healing time get reduced (33.84%) when compared to control group.

Wound index

Wound index study showed that quality of healing was significantly increased (80.18%) when compared to control group and also more than standard (50%) group.

DISCUSSION

Wounds are the broken epithelial cells of skin or abolishment of functional continuity of any living cell while healing of wound is a natural process to achieve continuity of cells by regeneration of damaged tissue. As various factors and mediators are played significant role in the healing process but also affected by various external factors like infection of bacteria, viruses or other germs, dust and nutritional deficiencies. Healing is also affected by pathological factors; diseases of body like diabetes significantly reduce the wound healing.

In allopath only infection of wound can be prevented to facilitate the healing while it is believed that plants bears so many phytochemical which not only prevent infection from germs but also potentiate wound healing like tannins and flavonoids[17].

This wound healing study contains two models and four parameters to study. Excision wound model for wound contraction, epithelization time study and percentage wound closer data while incision wound model for tensile strength of newly regenerated of sutured skin. Wound index study indicates about quality of wound healing.

In the studies of methanolic extract of *G. hypoleucum* showed significant wound healing potential. The epithelization time get reduced (33%) and epithelization get increased significantly ($p>0.01$) and quality of healing as suggested in the folklore literature and also comparable to standard *Aloe vera* gel. *G. hypoleucum* extract increased significantly the rate of contraction (99.81%) while standard *Aloe vera* gel (98.18%) as compared to control group pure gel (84.18%).

CONCLUSION

It is concluded from this study that *G. hypoleucum* possesses significant wound healing activity while confers the traditional use of this plant. The plant extract gets beneficially influenced the epithelization, wound contraction and tensile strength of sutured skin which ultimately result in faster healing. However identification of active phytoconstituent responsible for wound healing may leads to the development of effective wound healer.

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Cite this article as:

Neeraj Kumar *et al*. Estimation of wound healing potential of *Gnaphalium hypoleucum* DC. Int. Res. J. Pharm. 2018;9(3):53-57 http://dx.doi.org/10.7897/2230-8407.09342

Source of support: Nil, Conflict of interest: None Declared