Research Article

Investigation on wound healing potential of *Anaphalis triplinervis* (Sims) Sims ex C. B. Clarke

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Abstract

Objective: *Anaphalis triplinervis* or Bhukiphul, used as wound healer by tribes of western Himalayan region. This claim of wound healing potential was validated by this study using excision and incision wound models in rats. **Materials and methods:** In this study total five parameters were considered like wound surface area, percentage wound contraction, tensile strength, epithelization time and wound index in 13 days study. **Results:** The plant showed more than 10 % increase in percentage wound contraction in excision wound and significant increase (44.9%) of tensile strength in incision wounds as compared to control. Period of epithelization was decreased (29.7%) significantly which indicate that healing become faster in extract treated rats then control. The wound index data also suggested that quality of healing increased very significantly as wound index decreased (76.8%) sharply in comparison to control. **Conclusion:** The results showed that *Anaphalis triplinervis* have significant ($p<0.05$ to $p<0.01$) wound healing potential in both excision and incision wounds when compared with control.

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

Introduction

Plants are consumed as medicinal preparation from ancient civilizations where crude extract, mixture or plant parts are used to treat/prevent from metabolic disturbances and diseased conditions. In developing countries about one fourth of therapeutic drugs are either plant extracts or plant derivatives (Meria et al., 2018). Plants are widely used in the management of wounds specially by tribes of different states to countries in world and modern science tell us that plants containing saponins, flavonoids, alkaloids, tannins, naphthaquinone and triterpines in abundant amount showed either increased wound healing or healing rate of wound (Ghashghaii et al., 2017). Cellular injury or wound caused by physical, chemical, microbial or electrical stimuli undergoes wound healing for regeneration or repair of damaged tissues (Don and Soosaira, 2017). The wound healing process is accomplished by series of events in wound like coagulation, inflammation, angiogenesis, proliferation of fibroblast, deposition of collagen, formation of granulation tissue, contraction of wound and re-epithelization of newly formed tissue (Nathalie et al., 2017).

Anaphalis triplinervis, commonly known as Raktskandana, Anans or Bhukiphul (Chopda and Mahajan, 2009) (Khakurel, 2014), belongs to family *Asteraceae*, is a perennial, erect herb with stout, poorly branched stem entirely cottony or woody about 25-50 cm tall. Rhizome is woody and leaves are elliptical, velvety placed nearly stem clasping. Upper leaves are small (1.2-1.5 cm long) while lowers ones are large (4-6 cm), 3-5 veined with or without distinct stalk. Flowers are in clusters of more than 15 and as flower heads of 1 cm across. Flowers are yellow in colour appear in central disk with shining white bracts, flowering in July to October (Malla et al., 2015). The plant found from India, Afghanistan, Tibet, Nepal, and Bhutan to south west

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China at an altitude of 1800-3300 m especially in Himalayan regions (Tiwari et al., 2016). The Monpa tribes of Arunachal Pradesh, India are used the flowers of this plant in the treatment of epilepsy by preparing mixture of various plant flowers under name Pambrey. *Anaphalis triplinervis* is traditionally used for treatment of wounds and various skin problems (Balami, 2006). It relives swelling and used to treat illness caused by poison (Dorji et al., 2017) while powder is consumed orally for acidity treatment, (Singhand Chauhan, 2005) fever and menstrual disorder (Awale, 2008). The decoction of whole plant was used for cough, cold and boils (Nagesp, 2013). The tea of flower and leaves is used to stop vomiting in Pakistan (Ahmed et al., 2014) and as an antiseptic in Nepal (Tadeg, 2004).The plant is also used for Diuretic, Tonsillitis, Edema, (Bisht and Purohit, 2010) laceration of toes and dressing wounds (Bhat et al., 2013). The ethanolic extract of whole plant is screened for antidiabetic activity (Lakshmi et al., 2016). A group of polyacetylenides have been isolated from this plant (Awale, 2008). This plant is not scientifically validated for wound healing activity so to check the traditional claim for wound healing activity this comprehensive scientific study has been carried out.

Materials and Methods

Plant Material

The plant with flowers was collected in September 2015 from Rudraprayag, Uttarakhand, India then dried and plant specimens were prepared. The whole plant specimens were identified from Botanical Survey of India, Dehradun, Uttarakhand, India with a voucher specimen-117961. The dried plant crushed and extracted with ethanol for 24 hrs by hot soxhlet method then firstly dried at 50 ^{0}C by rotary drum evaporator (Buchi type) and then 45 ^{0}C in vacuum oven for 12 hrs.

Acute toxicity test

As described in OECD guidelines (OECD 410), repeated dose dermal toxicity method is used to estimate the dermal toxicity for 21 days.

Ointment formulation and topical application

The herbal gel was formulated as 10% w/w of dried plant extract using HPMC (Loba Chemie Pvt Ltd, Mumbai, India), stored in airtight coloured bottle till use. The formulation applied on wounds twice daily at an interval of 10 hours. Group 1 animals were treated as control and applied pure gel without any medication while Group 2 animals were treated as Test by 10% w/w *Anaphalis triplinervis* ethanolic extract gel. Group 3 animals were treated as standard with 10% w/w *Aloe vera* gel, applied on animal wounds twice daily. Wounds were observed for healing and possible infection on alternate day till 13 days and all surgical procedures were carried out under aseptic condition.

Experimental animals

30 Sprague Dowley rats (approximate weight of 150-200 g each) of either sex were divided into six groups in different cadges, obtained from animal house of Devsthali Vidyapeeth college of Pharmacy, Rudrapur-263148, Uttarakhand, India (1452/PO/Re/S/11/CPCSEA). Animals were allowed to take standard pellet diet till 18-24 hr before the surgery and RO filtered water ad libitum. All animals were shaved dorsolaterally by electric clipper between fore and hind legs then kept in polypropylene cages at 26- 28 ^{0}C with relative humidity of 44-56% and the light and dark cycles of 10 and 14h respectively were maintained for one week before and during study. All surgical procedures were carried out in accordance with institutional animal ethical committee (IAEC) of the same institution (DVCP/IAEC/2018/02).

Excision wound model

The 15 animals shaved skin was marked by a circle with the area of about 500 mm^2 in diameter one day prior to commencement of study. The impressed area was excised to full thickness under anesthesia and take impression on OHP sheet with permanent marker (Kumar et al., 2010).

Wound contraction measurement

Wound area was measured by superimposing the impressions of OHP sheets on a graph paper and by counting the boxes on graph paper the surface area of wound has been calculated on 0, 3, 5, 7, 9, 11 and 13th day after wounding and treatment in all animals of excision wound in different groups. Then calculated surface area of wound was used to estimate the % wound contraction by assuming the initial size of wound as 100%, using formula as % Wound contraction = wound area on 0 day-wound area on Nth day/wound area on 0 day then multiply by 100 (Kumar et al., 2010). Nth day = 3, 5, 7, 9, 11 and 13th post wounding days.

Incision wound method

The anaesthetized rat was undergo surgical procedure in aseptic conditions as 3 cm long abdominal incision was created with surgical blade in full thickness of skin then sutured with interrupted sutures (Mersilk, Ethicon, Aurangabad, India) at 1cm distance. The sutured skin then covered with plant extract gel, standard *Aloe vera* gel and pure gel in test, standard and control group animals respectively and continue for full study period (Kumar et al., 2010).

Tensile strength measurement

After completion of study on 13th day, the sutured animals were anaesthetized and wound strips of 2 cm width were

prepared with the help of sharp scissors then wound was dressed with suitable medicinal cream to rehabilitate the animals till full wound was healed. The prepared wound skin strips fixed with two steel clips at both ends. One end of strip allowed to hang on a fix stand and other end hang freely with polythene bag in which water was poured slowly till healed incision wound was opened or ruptured. The weight of water in gram, required to break the sutured skin was considered as tensile strength of incision wound (Kumar et al., 2010).

Periods of epithelization

After complete wound healing, total number of days after which excision wound scar was detached from healed wound area was considered as period of epithelization. Shortest the epithelization period showed the better and faster wound healing (Nayak et al., 2009).

Wound index

The quality of wound healing was expressed as wound index, measured by an arbitrary scoring system in which 0 scored as complete healing, 1 for incomplete but healthy healing, 2 for delayed but healthy healing, 3 for non healing wound without pus formation and 4 for necrosis or pus forming wounds (Nayak et al., 2009).

Statistical analysis

Experimental data was expressed as mean ± SEM (n=5) and Graph Pad Instat software was used to complete One-way Analysis of Variance. The significance of results was confirmed by Dunnett's test where $P < 0.05$ was considered as significant, $P< 0.01$ as highly significant. Both test and standard group data was compared with control group data.

Results

Quality of healing

The 10 % w/w gel of *Anaphalis triplinervis* was screened for wound healing activity in rats using excision and incision wound healing methods. The experimental results showed that in both excision and incision wound, the healing was not only faster but also the quality of healing was improved significantly specially environment around the wound was healthier.

Acute toxicity

The limit test for 1000 and 2000 mg/kg of body weight in the form of gel applied on shaved skin for 6 hrs in a day for 21 days and observed for itching, abnormal behavior, signs of lethality or moribund state with any other sign of toxicity as specified in OECD guidelines. No dermal toxicity was observed during full study period.

Wound healing ability

Wound healing potential of *Anaphalis triplinervis* ethanolic extract was estimated in excision and incision wound models in rats. The results indicated that wound area reduced very significantly in standard and test group animals as compared to control group animals as in figure 1. In excision wound model the estimated percentage wound contraction significantly increased as in figure 2 and period of epithelization get sharply decreased when treated with *Anaphalis triplinervis* gel and *Aloe vera* gel as test and standard medication preparation respectively. Wounds were more clean and free from debris of dead materials and without any pus or necrosis symptoms were identified. The

Table 1. Wound healing potential of ethanolic extract of *Anaphalis triplinervis*

Parameters	Pure gel (Control)#		*Aloe vera* (10 % gel)# (Standard)		*Anaphalis triplinervis* (10% gel)#(Test)	
	Wound Surface Area[mm^2]	% Wound Contraction	Wound Surface Area[mm^2]	% Wound Contraction	Wound Surface Area[mm^2]	% Wound Contraction
Day 0	689.6±85.7	-	720.3±98.6	-	629.3±47.4	-
Day 3	605.3±28.8	11.5±10.5	396.6±26.0	44.1±9.4	426.6±23.2	32.1±1.6
Day 5	445.3±76.0	33.9±18.6	286.6±85.0	59.8±12.3	208.3±20.0	66.6±5.0
Day 7	357.0±62.5	46.9±15.0	178.3±18.5	75.1±0.8	161.3±50.8	74.2±3.4
Day 9	274.6±50.8	59.2±11.6	103.6±26.0	85.1±5.6	107.6±50.2	82.5±9.2
Day 11	150.0±14.7	78.1±3.4	29.0±3.6*	95.9±0.6*	17.6±4.7*	97.2±0.5*
Day 13	73.6±12.5	89.2±1.9	11.6±11.5*	98.3±1.2*	4.0±1.0*	99.3±0.1*
Tensile Strength (gm)	160.33±12.78		292.40±15.50**		290.56±22.25**	
Epithelialization time (Days)	12.33±0.57		8.00±1.00*		8.66±0.57*	
Wound index	2.16±0.98		0.33±0.51**		0.50±0.54**	

#Mean±SD, *p<0.05, **p<0.01

results of wound index measurement showed that the quality of wound healing also increased significantly in test as well as standard groups when compared with control group results. The incision wound model results showed that treatment of test and standard extract gels produce higher tensile strength in healed tissue of incised and sutured skin as in figure 3. All results for wound contraction, tensile strength, epithelization time and wound index are summarized in table 1.

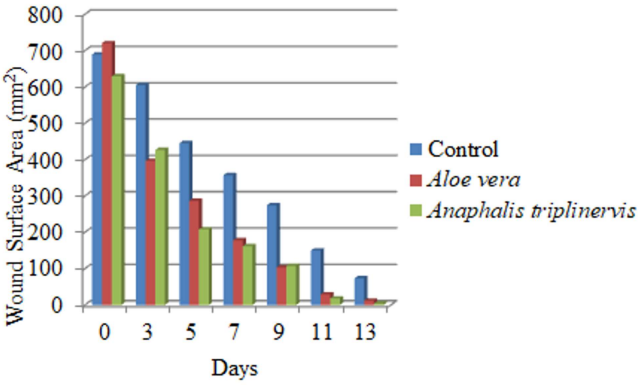

Figure 1. Effect of ethanolic extract of *Anaphalis triplinervis* on wound surface area

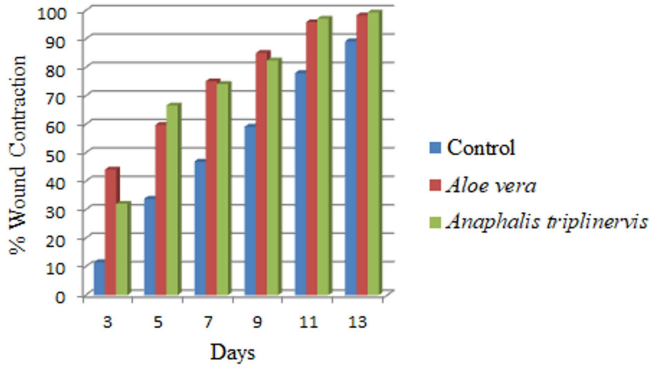

Figure 2. Effect of ethanolic extract of *Anaphalis triplinervis* on % wound contraction

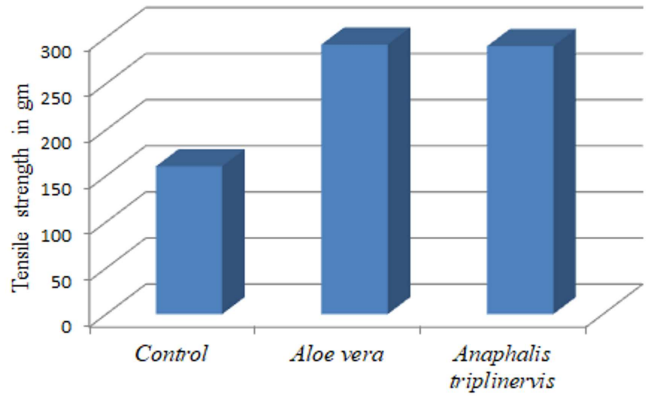

Figure 3. Effect of ethanolic extract of *Anaphalis triplinervis* on tensile strength

Discussion

Wound healing is the regeneration of damaged epithelial cells of skin naturally to restore the continuity of its functions. During healing, which is a complex process with various stages like inflammation, collagen synthesis and remodeling of skin, get affected by various external factors like infections mainly by bacteria and other germs, nutritional deficiencies of certain nutrients and internal factors like diabetes and other diseased states. All these factors reduce the healing potential and quality of healing and prolong the healing time, decrease wound contraction and reduce tensile strength.

Presently infection preventing medicines are used to support wound healing process and no active constituent reported so for which directly take part in healing events. According to literature many phytochemicals like tannins and flavonoids and various other antioxidants present in different plants can play very important role in healing console (Sudhakar et al., 2013). Wound healing potential can be estimated by various methods and this study was performed by two of them i.e. excision and incision wound models which are used to estimate percentage wound contraction and tensile strength of skin. Two observational data were also recorded to estimate wound healing time and quality of healing as epithelization time and wound index.

The study of ethanolic extract of *Anaphalis triplinervis* for wound healing potential showed significant increase in percentage wound contraction in excision model and reduction in epithelization period. The wound index records showed drastic change in quality of healing and scar formation. In incision model which contains sutured skin, also showed very significant increase in tensile strength in joints of sutured skin in comparison to control animals and as much as in standard animals.

Conclusion

This study concluded that plant *Anaphalis triplinervis* or Bhukiphul which commonly used for healing by tribes have significant wound healing potential. This study was also confers the claim of tribe for wound healer plant in Himalayan region. The study results also suggest that identification of active constituent responsible for wound healing potentiation in the plant can be a mile stone in the research of wound healer search.

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Conflict of Interest

No conflict

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