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Original Article

Evaluation of Wound Healing Activity of *Ageratina adenophora* (Spreng.) R.M.King & H.Rob.

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ABSTRACT

Objective: 'Basinga' is traditionally used as wound healing plant in Uttarakhand, India and this study was design to evaluate the wound healing potential of *Ageratina adenophora* for establishment of its traditional claim as wound healer.
Experimental Approach: Wound healing potential of *Ageratina adenophora* ethanolic extract was estimated by excision and incision wound models. The ethanolic extract was formulated as gel and applied on excision and incision wounds in thirteen day study. After treatment, wound area and epithelialization time was estimated in excision and tensile strength in incision wounds. Wound index was recorded in both excision and incision wounds. **Findings:** The estimated wound area was used to calculate to percentage wound contraction. Ethanolic extract of *Ageratina adenophora* gel and standard *Aloe vera* gel showed highly significant activity when compared with pure gel control. The tensile strength, epithelialization time and wound index data showed that plant possess moderately significant wound healing potential in both excision as well as incision wounds.
Discussion: The plant *Ageratina adenophora* showed strongly significant ($p<0.01$) wound healing potential in excision as 90.98% wound contraction and 36.16 % reduction in epithelialization time while in incision model, the plant extract showed significant increase (37.86%) in tensile strength on 13th day when compared to pure gel control. Wound index data clearly showed that quality of healing was much better in plant extract as well as *Aloe vera* treated animals as compared to pure gel control.
Conclusion: The present study was showed that ethanolic extract of *Ageratina adenophora* possess strongly significant wound healing potential as standard *Aloe vera* which provide an experimental support to traditional claim as wound healer for this plant.
Keywords: Basinga, wound healing, excision, incision, wound index, epithelialization.

1. INTRODUCTION

Wound can be result from physical injury (accident, burn and electricity), mechanical injury (trauma), chemical agents (alkali or acid), organisms (bacteria, virus or fungus) or surgical procedure, described anciently as vranaropaka in Ayurveda, vrana in Charaka Samhita and also discussed in Sushruta Samhita. In Ayurveda wounds are elaborated as chinna, bhinna, viddha, kshata, picchita and ghrista for cut

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wound, perforated wound, punctured wound, lacerated wound, contusion and abrasion wounds respectively. The pathogenesis of wounds elaborated under name vranashotha starts as samchaya (accumulation of body humors in their own locus), prakopa (vitiation of humors), prasara (transformation of vitiated humors to the periphery), sthana-samsraya (interaction of humors with the tissues leading to prodromal syndrome) and vyakti (manifestation of clinical features) while in modern view, firstly vasoconstriction followed by vasodialatation occurs and chemical mediators like plasmin, kallikrein, leucotaxin, prostaglandin released which changed the vessel wall and blood flow, initiate inflammatory response and cellular damage results in wound creation[1].

Wound healing process completed in various phases like hemostasis/inflammatory phase in which injured endothelial cells immediately vasoconstricts, intrinsic coagulation pathway started and hemostasis is maintained. Inflammatory mediators include thromboxane, growth factors and cytokines to recruit more platelets and are chemo attractant for neutrophils and fibroblasts. Proliferative phase/epithelialization in which macrophages initiated epithelialization and collagen replaces proteoglycan, fibronectin and macrophages and initiate provisional matrix formation. Fibroblasts synthesize proteoglycans and fibronectin to create matrix, ultimately proliferation of endothelial cells and formation of capillaries occurs. Maturation/remodeling phase in which collagen matrix construction heals the wound[2]. The internal wound environment specifically, availability of protein, nutrients, vitamins, cofactors and caloric energy necessary to synthesize matrix and to build, break down, and remodel healing wounds is essential for successful healing[3].

Ageratina adenophora distributed worldwide with synonym name *Eupatorium adenophorum*. In India, traditionally known as *Basinga* and distributed in Himalayan region, used as antimicrobial, antiseptic, wound healer, analgesic, antipyretic properties and scientifically validated for molluscicide potential, insecticidal, antibacterial and antifungal activity[4].

GCMS analysis of essential oil obtained from leaves confirmed the presence of 1-napthalenol, α-bisabolol, bornyl acetate, β-bisabolene, germacrene-D and α-phellandrene as major constituents. The essential oil from flowers of *Eupatorium adenophorum* contains α-phellandrene, camphene, bornyl acetate, p-cymene, γ-curcumene and 2-carene and the essential oil of aerial parts possess p-cymene, α-phellandrene, γ-curcumene, δ-2-carene, camphene, and endo-bornyl acetate[5]. From leaves, two more highly-oxygenated flavonoid glycosides, gossypetin-5-O-(6″-(E)-caffeoyl)-β-d-glucoside and herbacetin-5-O-(6″-(E)-caffeoyl)-β-d-glucoside were isolated[6].

Some purified sesquiterpenes structures were identified as cadinan-3-ene-2,7-dione, 7-hydroxycadinan-3-ene-2-one, 5,6-dihydroxycadinan-3-ene-2,7-dione, cadinan-3,6-diene-2,7-dione and 2-acetyl-cadinan-3,6-diene-7-one[7]. New sesquiterpene lactone named eupatoranolide, 2-acetoxy-3, 4, 6, 11-tetradehydrocadinan-7-on, 7-oxoagerapho-rone, an ant-repellent compound-kolavenol, eugenyl-O-β-D-glucopyranoside, with 5, 4'-hydroxy- 3, 6-dimethoxy-7-O-β-D-glucopyrano-xyflavonone, 5, 4'-hydroxy-6, 7-dimethoxy-3-O-β-D-glucopyranoxyflavonone, 3, 5, 4'-trihydroxy-6, 7-dimethoxyflavo-none, stigmasterol, β-sitosterol, daucosterol and succinic anhydride[8]. A novel norditerpene was isolated from flower, named (4aR,7A,8S,8aR)-1,2,4a,5,6,7,8,8a-octahydro-8-[3-methylenebut-4-alyl]-4,4a,7,8 tetra methyl naphthalen-2(1H)-one[6, 12].

The objective of this evaluation was to confirm the effectiveness of *Ageratina adenophora* whole plant extract in wound healing.

2. MATERIALS AND METHODS

Plant Material

Ageratina adenophora as whole plant procured from Bhimtal, Uttarakhand, India in March 2016, identified and authenticated from Botanical survey of India, Dehradun (A/c No 119762), Uttarakhand, India, dried in mild sunlight, extracted for 12 hrs using hot soxhlet method with ethanol then dried in rotary drum evaporator and finally on water bath at 50 ^{0}C (EEAA).

Acute dermal Toxicity Test

An acute dermal toxicity of ethanolic plant extract was done to determine the safety of it on skin according to OECD guidelines (OECD 402, August 2016 draft).

Gel Formulation and Topical Application

10 % of dried ethanolic extract gel was prepared with HPMC. Group 1: Control, applied pure gel. Group 2: 10%, w/w, EEAA gel was applied and Group 3: 10% w/w, standard *Aloe vera* gel was applied to the animal's wound twice daily. All surgical procedures were carried out under aseptic conditions and wounds were observed on alternate day till 13 days of study.

Experimental Animals

Institutional animal ethics committee of department of pharmacy, Devsthali Vidyapeeth college of Pharmacy, Rudrapur (U.S. Nagar)-243148, Uttarakhand, India (1452/PO/Re/S/11/CPCSEA) was approved the use of animals for present study (DVCP/IAEC/ 2015/01). The animals of either sex were selected and divided randomly into six groups of five animals (rats) each and allowed to take standard pellet diet and RO filtered water ad libitum. The anesthetized animals were inflicted of the experimental wounds and all surgical procedures were carried out using diethyl ether anesthesia (120 mg/kg) and closely observed for any type of infection and infected animals were separated immediately, excluded from study and were replaced.

Excision Wound Model

The dorsal fur of animals was cut with scissors and outlined the anticipated area of wound to be created with marker. An

excision wound of full thickness with circular area 500 mm^2 and 0.2 cm depth was created by a surgical blade and pointed scissors then entire wound was left open. The animals were divided into three groups of five each. Animals of group one were topically treated with simple gel as placebo control while group two were topically treated with 10% w/w gel of EEAA and animals of group three were treated topically with 10% w/w *Aloe vera* gel for till complete epithelialization or thirteen days. On 0, 3, 5, 7, 9, 11 and 13 post-wounding days, wound closure rate was assessed by tracing the wound using transparency sheets and a permanent marker then wound areas were measured using a graph paper[9]. The percentage wound contractions of each wound were calculated by estimated wound surface area by assuming the initial size of wound as 100%. Animals of all groups were observed for period of re-epithelialization from third day of study. Percentage wound contraction was determined using: % Wound contraction = wound area on o day-wound area on Nth day/wound area on 0 day and multiplied by 100[15]. Nth day = 3, 5, 7, 9, 11 and 13th post wounding days.

Tensile Strength Measurement

The anaesthetized rats sterilized by 1% povidone iodine solution and 75% alcohol solution. On the lateral side of animals a longitudinal paravertebral incision (4 cm in length) was made through the skin and cutaneous muscle and surgical sutures (Mersilk, Ethicon, Aurangabad) were applied to parted skin at distance of 1 cm. The wounds were topically applied 10% EEAA gel. The controls were topically applied pure gel. All animals were kept individually in different cages. The sutures were removed if left in skin on 8^{th} post wounding day and treatment was continued till study ended. The breaking strength of healed unsutured wounds were measured on 13^{th} day[7] by making wound stripes of equal size and width of 2 cm. Now each strip individually fixed with a pair of steel clips and one clip allowed to hang on a stand and a polyethylene bag on other clip. Polyethylene bag was then filled 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[10].

Periods of Epithelialization

Complete epithelialization of wounded area normally characterized by falling of scar from wound and number of days required to fall of scar was taken as period of epithelialization.

Wound Index

An arbitrary scoring system was used to measured wound index at alternate day as 0 for healthy healing, 1 for delayed but healthy healing, 2 for healing with small pus formation, 3 for healing has not yet been started and 4 for formation of pus and evidence of necrosis[11].

Statistical Analysis

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

3. RESULTS

The 10 % w/w EEAA 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 as compared to control and standard *Aloe vera* 10% w/w 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 hrs, the extract did not showed any abnormal gross behavior, any signs of lethality, moribund state of animals or any signs of dermal toxicity.

Wound Healing Potential

The wound healing activity of 10 % w/w EEAA is shown in table 1 and 2. A faster pattern of wound closure was observed with EEAA gel and *Aloe vera* gel when compared with control and results showed significant reduction in wound area from 3rd day to the 13th day of treatment. Significant wound contraction was observed in test group from day 3 (44.82%) in EEAA group ($p>0.01$) while in standard group (67.83%) has been observed. On 5^{th}, 7^{th}, 9^{th} and 11^{th} day significant wound contraction was shown by both standard and extract treated group. On 13^{th} day of study, EEAA (90.98%) and *Aloe vera* gel (96.19%) treated group showed highly significant ($p> 0.01$) healing potential.

Epithelialization of Wounds

Both *Aloe vera* (7.33 days) and EEAA gel (7.66 days) treated animals showed least period of epithelialization while control group animals had longest period (12 days) of epithelialization. Epithelialization time study showed that both standard as well as test plant extract showed significant ($p>0.01$) reduction in epithelialization time and showed that healing time get reduced (36.16%) as compared to control group.

Wound Index

Wound index study showed that quality of healing was significantly increased in EEAA (67.36%) and *Aloe vera* (75.00%)) treated group when compared to pure gel treated control group.

4. DISCUSSION

Wound healing, a natural process in which broken epithelium is healed and functional continuity is maintained. Wound healing may affected by various factors like infection, dust, pathological, disease and nutritional deficiencies. Normal treatment of wound only prevent from infection from microbes so plant may prove more effective in healing due to presence of tannins and flavonoids whose

active involvement is already studied in previous research for healing as well as prevention of infection. Pharmacological screening of wound healing contains many methods in which wound contraction, tensile strength, epithelialization time and quality of healing is determined[12-13].

Table 1: Effect of topical application of ointments containing ethanolic extract of *Ageratina adenophora* on wound contraction of excision wound

Days	Pure gel (Control) Mean±SD		*Aloe vera* (10 % Gel) (Standard) Mean±SD		EEAA 10% Gel Mean±SD	
	Wound area (MM^3)	% contraction	Wound area (MM^3)	% contraction	Wound area (MM^3)	% contraction
3rd day	440±45.5	10.86±2.80	336±20.8	38.48±2.5	514±30.0	11.74±1.4
5th day	358±23.5	27.16±4.65	281±5.2	48.45±3.1	413±51.0	29.30±3.6
7th day	291±11.9	40.78±3.79	174±13.0*	67.83±4.5	322±24.2	44.82±1.3
9th day	214±25.7	56.44±4.82	136±5.6*	74.98±1.9*	234±22.6	59.95±1.1
11th day	168±30.5	66.06±4.47	83±4.0*	84.73±1.6*	111±4.3	80.94±0.7*
13th day	108±12.4	76.02±0.76	20±4.7*	96.19±0.96**	52±6.5	90.98±0.5*

(n=5, *p<0.05, *p<0.01)

Table 2: Effect of ethanolic extract of *Ageratina adenophora* on tensile strength, epithelialization time and wound index

Parameters	Pure gel (Control) Mean±SD	*Aloe vera* (10 % gel) (Standard) Mean±SD	EEAA (10% gel) Mean±SD
Tensile Strength (gm)	361.66±27.53	533.33±34.03**	581.66±24.66**
Epithelialization time (Days)	12.00±1.00	7.33±0.57*	7.66±0.57*
Wound index	2.88±0.58	0.72±0.75**	0.94±0.72**

(n=5, *p<0.05, **p<0.01)

The study of ethanolic extract of *Ageratina adenophora* showed significant (p<0.01) wound healing potential. The epithelialization time get reduced (36.16%) significantly (p>0.01) and quality of healing is also comparable to standard *Aloe vera* gel. *Ageratina adenophora* extract increased significantly the rate of contraction (90.98%) and standard *Aloe vera* gel (96.19%) as compared to control group pure gel (76.02%). The tensile strength in incision wound model gets increased (37.86%) in comparison to control (pure gel).

5. CONCLUSION

It is concluded from this study that *Ageratina adenophora* possesses significant wound healing potential which confers the traditional use of this plant. The plant significantly influenced the quality of wound healing.

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