Original article

Wound healing activity of *Abroma augusta* in Wistar rats

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Abstract

Objective: The study was undertaken to evaluate the wound healing profile of alcoholic extract of *Abroma augusta* and its effect on dexamethasone suppressed wound healing in Wistar rats. **Methods**: An alcoholic extract of *Abroma augusta* was prepared. Three models were used - incision, excision and dead space wound models. Four groups of animals were used for each model. They were administered 2% gum acacia (orally), alcoholic extract of *Abroma augusta* (orally), dexamethasone (intramuscularly) and combination of *Abroma augusta* (orally) with dexamethasone (intramuscularly) respectively. The parameters studied included breaking strength of incision wound, period of epithelization and wound contraction rate in the excision wound, breaking strength, dry weight and hydroxyproline content of granulation tissue in dead space wound. **Results**: The breaking strength of incision wound of *Abroma augusta* treated group was significantly increased ($P<0.001$) while that of dexamethasone treated animals was significantly decreased ($P<0.001$) as compared to control. Coadministration of dexamethasone and *Abroma augusta* significantly reversed the dexamethasone suppressed wound healing in incision wound model ($P<0.001$). Animals treated with both dexamethasone and *Abroma augusta* also showed significant ($P<0.004$) increase in the breaking strength of granulation tissue in the dead space wound and a significant ($P<0.011$) reduction in the period of epithelization in the excision wound as compared to rats treated with dexamethasone alone. The rate of wound contraction was not significantly altered in any of the groups. **Conclusion**: The alcoholic extract of *Abroma augusta* was found to reverse dexamethasone suppressed wound healing.

Keywords: *Abroma augusta*; Wound breaking strength; Period of epithelization; Dexamethasone suppressed wound healing

INTRODUCTION

Healing of a wound is required for normal functioning of a living tissue. Various factors can enhance normal wound healing[1] or reverse depressed wound healing due to drugs like corticosteroids[2], anticancer[3] and non-steroidal antiinflammatory agents[4].

Plants have been used traditionally for wound healing. *Abroma augusta* (Devil's Cotton) is well known for its medicinal properties[5]. The plant has been shown to have hypoglycemic and hypolipidemic activity[6]. It has been reported to inhibit oxidative damage[7]. In Assam (eastern India), the root and bark of *Abroma augusta* is used for the treatment of sores[5] and a paste of the root is used externally to cure abscess[5].

However, to the best of our knowledge a systematic study of wound healing activity of *Abroma augusta*

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has not been undertaken. Hence the present study was carried out to evaluate the wound healing property of alcoholic extract of roots of *Abroma augusta* and to study its effects on dexamethasone suppressed wound healing in various wound models in rats.

MATERIALS AND METHODS

Collection and preparation of alcoholic extract of *Abroma augusta*

The roots of the plant *Abroma augusta* was obtained from Kolkata, West Bengal (eastern India) and verification was done at the department of Pharmacognosy, Manipal college of Phamaceutical Sciences, Manipal. It was shade dried and powdered. The powder was loaded into soxhlet extractor (Tensil glass works, Bangalore, India) in eight batches of 200 g each and was subjected to extraction for 30-40 hours with 95% ethanol. After extraction, the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50 ℃ to a syrupy consistency. Then it was dried in a desiccator (Quality traders, Ernakulum, Kerala). The yield was around 4%.

Animal care and handling

Healthy, albino Wistar rats (150-200 g) 12 weeks old, of either sex, bred locally in the animal house of Kasturba Medical College, Manipal, were used for the study. They were housed under controlled conditions of temperature 23 ℃ ±2 ℃, humidity 50 % ±5 % and 10-14 hour light and dark cycles respectively. The animals were housed individually in polypropylene cages (U. N. Shah manufacturers, Mumbai, India) containing sterile paddy husk (procured locally) as bedding and were maintained on normal diet (Amrut lab animal feed, Pranav Agro Industries Ltd, Sangli, Maharashtra) and water *ad libitum*. The study was undertaken after obtaining approval of Institutional Animal Ethics Committee.

Study design

Three models - incision, excision and dead space wound models - were used to assess the wound healing profile of *Abroma augusta* in rats. Four groups of animals were used for each model. There were six animals in each group. The drug treatment was as follows:

Group I (all models): Received 2 mL of 2 % gum acacia orally (p. o.) through intragastric tube. This was the control group in all models.

Group II (all models): Received alcoholic extract of *Abroma augusta* in a dose of 250 mg/kg body weight of the rat orally (p. o.). The dose selection was based on previous toxicity studies[6].

Group III (all models): Received dexamethasone (0.17 mg/kg)[8] intramuscularly (i. m.)

Group IV (all models): Received dexamethasone (0.17 mg/kg) (i. m). and alcoholic extract of *Abroma augusta* (250 mg/kg) (p. o).

Drugs and chemicals

Gum acacia was obtained from Nice Chemicals, Cochin, India; dexamethasone from Cadilla Healthcare Ltd., Ahmedabad, India; ketamine from Neon laboratories, Mumbai, India; ethanol from Qualigens Fine Chemicals, Mumbai, India; standard Hydroxyproline from Sigma Chemicals, USA.

Evaluation of wound healing activity

For assessment of wound healing activity incision, excision and dead space wound models were used. All wounding procedures were carried out under ketamine anesthesia -50 mg/kg body weight of the rat[9], intraperitoneally (i. p). In the present study no animals showed visible signs of infection.

Incision wound model

In this model, breaking strength of the wound was assessed. Two 6 cm long paravertebral straight incisions were made 1cm lateral to the vertebral column on either side of the depilated back of the animal cutting through the entire thickness of the skin[10]. The wounds were closed with interrupted sutures 1cm apart with No. 4 black silk thread (Ethicon, Johnson & Johnson, Himachal Pradesh, India). Animals in the four groups were treated once daily with drugs from day 0 (day of wounding) to day 9. The sutures were removed on the 7th post wounding day and breaking strength was measured on the 10th day by continuous constant water flow technique of Lee[11].

Excision wound model

This model was utilized to study the rate of wound

contraction and the time required for full epithelization of the wound. An excision wound was made by cutting away a circular area of full thickness of skin measuring 500 mm^2 on the depilated back of the rat, in the dorsal interscapular region, 5 cm away from the ears[12]. Animals received the drugs daily from day 0 (day of wounding) to 21st postoperative day or till the wound was completely healed, whichever occurred earlier. Period of epithelization was noted as the number of days after wounding required for the scab to fall off leaving no raw wound behind. Wound contraction rate was monitored by planimetric measurement of wound area on alternate days. This was done by tracing the wound area on a butter paper (Manipal press, Manipal, India) on every alternate day starting from day 0 (day of wounding) to 21st postoperative day or till the wound was completely healed, whichever occurred earlier. These readings were then transferred to a standard graph sheet. The wound contraction was calculated as percentage of the original wound size (500 mm^2) taken as 100% for each animal of the group. The group mean was calculated for days 4, 8, 12 and 16.

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Dead space wound model

In this model, dry weight, breaking strength and hydroxyproline content of granulation tissue was studied. Dead space wounds were created by implanting subcutaneously 2.5 cm × 0.5 cm polypropylene tubes, in the lumbar region, beneath the dorsal paravertebral lumbar skin, through a small transverse incision[13] about 4 -5 cm cephalic to the site of implantation . Drugs were administered once daily from day 0 (day of wounding) to day 9. On 10th post wounding day, granulation tissue harvested on the tube was carefully dissected out along with the tube. The granulation tissue was cut along its length to get a sheet of granulation tissue, which was cut approximately into two equal pieces. The breaking strength of granulation tissue was measured by the method of Lee[11]. Then the granulation tissue was dried in an incubator (Central Scientific Syndicate, Mumbai, India) at 60 ℃ for 24 hours and the dry weight was noted. The dry tissue was used for determination of hydroxyproline content by the method of Neuman and Logan[14]. The values were expressed as mg/g of tissue.

Statistical Analysis

The results were analyzed by One Way Analysis of Variance (ANOVA) followed by Scheffe's test.

RESULTS

Incision wound model

The mean breaking strength was significantly ($P < 0.001$) increased in the group treated with alcoholic extract of *Abroma augusta* as compared to control. In the dexamethasone treated group, the breaking strength was significantly ($P < 0.001$) reduced as compared to control. It was significantly ($P < 0.001$) increased in rats treated with both dexamethasone and alcoholic extract of *Abroma augusta* as compared to dexamethasone alone (Table 1).

Excision wound model

The mean period of epithelization was not significantly altered in the *Abroma augusta* or dexamethasone treated groups as compared to control. The period of epithelization of the group treated with both dexamethasone and *Abroma augusta* was however reduced significantly ($P < 0.011$) as compared to the dexamethasone treated rats. The wound contraction rate was not significantly altered in any of the test groups as compared to the control on the same day (Table 2).

Dead space wound model

The mean breaking strength of the granulation tissue was not significantly altered in the *Abroma augusta* treated group as compared to control. The breaking strength of the dexamethasone treated group was significantly ($P < 0.025$) reduced as compared to control. It was significantly ($P < 0.004$) increased in the group treated with dexamethasone and alcoholic extract of *Abroma augusta* as compared to the group treated with dexamethasone alone (Table 3).

The dry weight and hydroxyproline content of granulation tissue of rats treated with *Abroma augus-*

ta alone and both dexamethasone and alcoholic extract of *Abroma augusta* was not significantly altered (Table 3).

Table 1 Effect of drugs on incision wound.

Groups(n) / Drugs	Dose & Route	Breaking strength in grams (g) Mean ± SEM
Group I /Gum acacia	2mL p. o.	217.92 ± 5.93
Group II /*Abroma augusta*	250 mg/kg p. o.	290.83 ± 4.68[a]
Group III /Dexamethasone	0.17mg/kg i. m.	160.42 ± 8.3[a]
GroupIV/Dexamethasone + *Abroma augusta*	0.17 mg/kg i. m. +250 mg/kg p. o.	214.58 ± 9.4[b]

n = Number of animals in each group = 6
a: $P<0.001$ compared to control (gum acacia)
b: $P<0.001$ compared to dexamethasone group
ANOVA was followed by Scheffe's test.

Table 2 Effect of drugs on excision wound (drugs, Mean ± SEM).

Group(n)/Drugs	Dose/Route	Percentage of wound contraction				Period of epithelization
		Day 4	Day 8	Day 12	Day 16	
Group I/Gum acacia (p. o.)	2 mL	47.10 ±7.6	67.36 ±6.14	96.23 ±0.59	99.06 ±0.27	17.67 ±0.33
Group II/*Abroma augusta*	250 mg/kg	40.26 ±7.50	65.83 ±6.48	91.56 ±3.08	98.13 ±0.75	16.94 ±0.49
Group III /Dexamethasone (i. m.)	0.17 mg/kg	35.83 ±3.50	78.03 ±3.78	90.47 ±1.28	99.03 ±0.46	17.33 ±0.67
Group IV/Dexamethasone (i. m.) + *Abroma augusta* (p. o.)	0.17 mg/kg +250 mg/kg	33.16 ±3.50	82.00 ±4.48	92.80 ±2.01	10.00 ±0.00	14.67 ±0.42[a]

n = Number of animals in each group = 6
a: $P<0.011$ compared to dexamethasone group
ANOVA was followed by Scheffe's test.

Table 3 Effect of drugs on dead space wound parameters (Mean ± SEM).

Group(n)/Drugs	Dose/ Route	Breaking strength of granulation tissue (g)	Hydroxyproline content of granulation tissue (mg/g of tissue)	Dry weight of granulation tissue (mg)
GroupI/Gum acacia	2mL	212.50 ±5.74	18.42 ±2.56	194.83 ±11.72
GroupII/*Abroma augusta*	250 mg/kg	280.00 ±21.05	25.54 ±1.18	257.83 ±26.26
GroupIII/Dexamemethasone	0.17 mg/kg	151.67 ±13.27[a]	21.69 ±2.19	127.00 ±12.08[a]
GroupIV/Dexamemethasone + *Abroma augusta*	0.17 mg/kg +250 mg/kg	239.16 ±12.54[b]	25.18 ±1.03	139.83 ±9.91

n = Number of animals in each group = 6
a: $P<0.025$ compared to control (gum acacia)
b: $P<0.004$ compared to dexamethasone group
ANOVA was followed by Scheffe's test.

DISCUSSION

Wound healing consists of an acute inflammatory response, proliferation and remodeling of connective tissue, synthesis of extracellular matrix proteins, acquisition of tensile strength, contraction and epithelization. Dexamethasone inhibits cell proliferation, granulation tissue formation, collagen formation and maturation[2] and epithelization[15]. Measurement of hydroxyproline content is used as an index of collagen turnover. In this study, the hydroxyproline content and dry weight of granulation tissue were not significantly altered. Therefore *Abroma augusta* might not have increased the collagen content, but probably altered the maturation process, by enhancing cross linking of collagen which may have resulted an increase in wound breaking strength. Oxidative stress plays a role in wound healing[16]. *Abroma augusta* has been reported to inhibit oxidative damage[7]. This could also contribute to its prohealing effects.

The present study has shown that alcoholic extract of *Abroma augusta* increased the wound breaking strength and also reversed the suppression of wound healing by dexamethasone. Hence, compounds derived from *Abroma augusta* could have therapeutic potential in the management of wound healing.

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