Research Paper

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Wound Healing Activity of Ten Indigenous Plants in Incision and Excision Wound Models in Albino Rats

Medical Science

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ABSTRACT

The present study was performed to evaluate the wound healing activity of 10 indigenous plants (Jasminum grandiflorum, Azadirachta indica, Lawsonia inermis, Nerium indicum, Vinca rosea, Marsedinia volubilis, Tabernaemontana corymbosa, Calotropis gigantea, Tectona grandis and Andrographis paniculata) in albino rats. Wound breaking strength in incision wound model, epethelization period and percent wound contraction in excision wound model were studied. Significant result was found in Calotropis gigantea, Tectona grandis and Andrographis paniculata treated rats. Further screening studies were done using the alcoholic extracts of these three plants. Out of three plants screened, Andrographis paniculata has shown significant results which shows that it possesses a definite prohealing action.

INTRODUCTION

Wound healing is the body's natural process which is essentially a survival mechanism and represents attempts to maintain normal anatomical structure and function. The initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and other extracellular macromolecules which are later remodeled to form a scar (Hatapakki BC et al 2004). Different kinds of synthetic drugs are available to enhance the wound healing process. In traditional systems of medicine like Ayurveda, Unani and Homeopathy, various plant products are claimed to promote wound healing (Fulzele SV et al 2002). Several drugs of plant origin having wound healing properties are described under the term Vranaropana. In this study 10 indigenous plants which are mentioned in Ayurvedic textbooks and folklore medicines on wound healing were selected. In the present study the effect of 10 indigenous plants - *Jasmnum grandiflorum* (Jg), *Azadirachta indica* (Ai), *Lawsonia inermis* (Li), *Nerium indicum* (Ni), *Vinca rosea* (Vr), *Marsedinia volubilis* (Mv), *Tabernaemontana corymbosa* (Tc), *Calotropis gigantea* (Cg), *Tectona grandis* (Tg) and *Andrographis paniculata* (Ap) were used to assess on different parameters related to wound healing in rats.

MATERIALS AND METHODS

Plant material

The 10 indigenous plants which are being screened for wound healing property were collected in and around Udupi during September– January and identified by Prof. Aravinda Hebbar, department of Botany, M.G.M. College, Udupi. A voucher specimen has been deposited at the department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, India.

Preparation of crude extract:

Fresh leaves of the plant were collected and the leaves were crushed with a mortar and pestle. The expressed juice was centrifuged. The volume of juice obtained for each plant is expressed as ml/ 100g of leaves. Dose used in the study is 2ml/ kg body weight.

Preparation of alcoholic extract:

Leaves were collected, dried in the shade and powdered. The powder was used for preparation of extract. Leaf powder (75 g) was extracted with 700ml of 95% ethanol in a soxhlet apparatus at 60-75^{0}C (Suffness M et al 1978). The extract was concentrated. . The yield of *Ap*, *Cg* and *Tg* was about 6-10%, 10-14% and 5-8% respectively. Dose used in this study is 400mg/kg body weight. The dose selection was based on acute toxicity studies.

Animals

In the present study, healthy albino Wistar rats of either sex, weighing around 150-250g were used. Animals were housed individually in polypropylene cages with paddy husk bedding at 28±1^0 C temperature and 50±5% humidity. Animals were fed on laboratory rat feed and water ad libitum. The Institutional animal ethical committee approval was obtained before starting the experiments.

In the crude extract study - The animals were divided into 11 groups of 8 animals each.

The group I animals served as wounded control, treated with normal saline.

The groups II – XI animals were administered the crude extract of Jg, Ai, Li, Ni, Vr, Mv, Tc, Cg, Tg and Ap respectively.

In the alcoholic extract study –The animals were divided into 4 groups of 8 animals each.

The group I animals served as wounded control, treated with saline.

The groups II - IV animals were administered the alcoholic extract of Cg, Tg and Ap respectively.

Anaesthesia used – Ether

Route of drug administration – Oral

Duration- The extract was given daily, for 10days in incision wound model and till the day of epithelization in excision wound model.

Experimental procedures

Incision wound - Two para vertebral incisions (6cm. long) were made, through the full thickness of the skin on either side of the vertebral column of the rat (Ehrlich HP et al 1969). The wounds were closed with interrupted sutures, 1cm apart. Sutures were removed on the 7th day. Wound breaking strength was measured on 10th post-wounding day (Lee KH 1967).

Excision wound - A circular piece of full thickness (approximately 500mm^2) was cut off from a predetermined area on the back of the rat. The wounds were traced on 1mm^2 graph paper on the day of wounding and subsequently on alternate days, until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction. Number

of days required for falling of eschar without any residual raw wound gave the period of epithelization (Morton JP et al 1972).

Statistical analysis of data

Data obtained were correlated and analyzed by one way Analysis of Variance (ANOVA) followed by Bonferroni's post test wherever applicable using statistical software package, Graph Pad in Stat (GPIS) 1990: version 1.13. Values of p< 0.05 were considered statistically significant.

RESULTS

In the incision wound model, a significant increase in wound breaking strength was observed in the groups treated with crude extracts of Cg, Tg and Ap when compared to the control. However, there were no significant changes in the groups treated with crude extract of Jg, Vr, Ai, Li, Ni and Mv when compared to the control (Table-1). In excision wound model, rats treated with crude extract of Cg, Tg and Ap, showed significant decrease in the epithelization period (Table-1) and increased percent wound contraction as compared to controls. There were no significant changes in epithelization period and percentage wound contraction in rats treated with crude extracts of Jg, Vr, Ai, Li, Ni and Mv when compared to controls (Table-2).

In rats treated with alcoholic extract of Ap, there was a significant increase in wound breaking strength in incision wound model (Table-3) ; total wound contraction was achieved much earlier (Table-4) and decreased epithelization period (Table-3) was observed in excision wound model as compared to controls.

DISCUSSION

Wound healing is a process which is fundamentally a connective tissue response. Out of the crude extract of 10 indigenous plants screened, Cg, Tg, and Ap has shown significant changes in different parameters of wound healing. In further screening tests of alcoholic extracts of Ap, Cg and Tg, Ap has shown significant increase in wound breaking strength, significant decrease in the epithelization period and increased percent wound contraction. Increase in wound breaking strength, could be due to increase in collagen concentration and stabilization of fibers (Udupa AL et al 1995). Decrease in the epithelization period and increased percent wound contraction could be due to enhanced proliferation of epithelial cells as well as promotion of epithelial cell migration (Somashekar Shetty et al 2006). In summary, alcoholic as well as crude extracts of Ap has shown significant wound healing activity. Prohealing activity of Ap could be due to the presence of high concentration of flavonoids in the leaves of Ap, which scavenge the free radicals and helps in wound repair (Sudhanshu Saxena et al 1998, Havsteen BH 2002 and Sheeja K et al 2006).

TABLE 1: Effect of various indigenous drugs on wound breaking strength (incision wound model) & epithelisation period (excision wound model)

Name of the plant	Breaking strength (gms)	Epithelisation period (Days)
Control	283.33±6.66	22±0.52
Jg	339.17±13.56	20.67±0.42
Ai	308.5±25.88	19.67±0.33
Li	336.66±13.64	20.0±0.45
Ni	340.83±14.57	20.0±0.52
Vr	343.33±5.43	20.0±0.73
Mv	269.17±14.40	22.3±0.42
Tc	288.34±7.92	20.3±0.61
Cg	393.34±22.31*	17..67±0.61**
Tg	382.5±5.73*	17.9±0.42**
Ap	401.67±7.37**	16.3±0.33***

(Values are mean±SE of 8 replications) *p< 0.05, **p<0.01, ***p<0.001 vs control.

Jg- *Jasminum grandliflorum*, Ai- *Azadirachta indica*, , Li- *Lawsonia inermis*, Ni- *Nerium indicum*, Vr- *Vinca rosea*, Mv- *Marsedinia volubilis*, Tc- *Tabernaemontana corymbosa*, Cg-*Calotropis gigantea,* Tg- *Tectona grandis &* Ap- *Andrographis paniculata*

TABLE 2: Effect of various plant extracts on percent wound contraction (excision wound model)

Treatment	Percent wound contraction in days						
	4	8	12	16	18	20	22
Control	9.32±0.74	31.67 ±3.63	63.0±4.08	82.42±4.48	90.4±4.0	95.4±1.17	99.53±3.23
Jg	11.94±0.94	43.76±3.76	64.11±5.30	89.80±5.10	92.24±2.6	99.08±0.59	
Ai	11.81±1.60	42.63±3.45	65.64±0.57	83.65±0.56	94.6±0.86	99.98±1.15	
Vr	13.21±2.48	37.61±4.91	67.21±1.21	85.50±1.26	97.30±1.52	99.02±4.26	
Li	15.49±1.24	42.74±2.37	70.08±1.09	80.46±1.19	97.84±5.56	99.34±5.12	
Ni	12.33±1.85	39.27±3.40	63.08±4.42	84.30±2.12	96.29±1.28	99.48±2.6	
Mv	13.03±1.91	44.36±1.46	65.29±1.86	79.91±1.02	91.81±3.78	95.6±3.34	99.48±4.6
Tc	10.56±2.17	36.27±2.48	68.58±1.69	84.61±0.92	90.39±2.59	98.81±6.0	99.61±1.23
Cg	19.95±0.67*	47.84±1.57	81.2±0.8*	96.09±0.6	99.82±2.7		
Tg	20.23±0.65*	50.18±2.95	82.37±2.18*	97.17±0.68	99.28±1.68		
Ap	23.79±1.60*	56.26±3.94*	89.68±2.14*	99.6±0.29*			

(Values are mean±SE of 8 replications) *p< 0.05 vs control.

Jg- *Jasminum grandliflorum*, Ai- *Azadirachta indica*, , Li-*Lawsonia inermis*, Ni- *Nerium indicum*, Vr- *Vinca rosea*, Mv- *Marsedinia volubilis*, Tc- *Tabernaemontana corymbosa*, Cg-*Calotropis gigantea,* Tg- *Tectona grandis &* Ap- *Andrographis paniculata*

TABLE 3: Effect of alcoholic extracts of Ap, Cg & Tg on wound breaking strength (incision wound model) & epithelisation period (excision wound model)

Treatment	Breaking strength (gms)	Epithelisation period (Days)
Control	283.33±6.66	22±0.52
Cg	364.16±23.47	18±1.4
Tg	357.83±28.1	18.2±1.82
Ap	390.03±11.15*	16±0.3*

(Values are mean±SE of 8 replications) *p< 0.05 vs control

Cg-*Calotropis gigantea,* Tg- *Tectona grandis &* Ap- *Andrographis paniculata*

TABLE 4: Effect of alcoholic extracts of Ap, Cg and Tg on % wound contraction (excision wound model)

Treatment	Percent wound contraction in days						
	4	8	12	16	18	20	22
Control	9.32±0.74	31.67 ±3.63	63.0±4.08	82.42±4.48	90.4±4.0	95.4±1.17	99.53±3.23
Cg	14.5±3.0	37.84±2.16	68.62±0.8	90.92±1.3	99.82±3.12		
Tg	10.2±1.48	40.18±2.82	74.71±2.18	88.10±1.82	98.13±2.32		
Ap	18.42±1.3*	45.6±2.44*	84.68±2.14**	98.84±0.92*			

(Values are mean±SE of 8 replications) *p< 0.05, **p< 0.01 vs control.Cg-*Calotropis gigantea*, Tg- *Tectona grandis &* Ap- *Andrographis paniculata*

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