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Wound Healing Activity of Alcoholic Extract of *Buchanania lanzan* in Albino Rats

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Abstract: The wound healing effect of alcoholic extract of *Buchanania lanzan (B. lanzan)* and its effect in dexamethasone suppressed woundhealing was studied in Albino rats. Three wound models viz. incision,excision and dead space wounds were used in this study. The parameters studied were breaking strength in case of incision wounds, epithelializationand wound contraction in case of excision wound and granulation tissue dry weight, breaking strength and hydroxyproline content in case of deadspace wound. The dexamethasone treated group showed a significant ($P<0.001$) reduction in the wound breaking strength when compared tocontrol group in incision type of wound model. Coadministration of *B. lanzan* with dexamethasone had significantly ($P<0.001$) increased thebreaking strength of dexamethasone treated group. In excision wound model, the percentage of the wound contraction was significantly ($P<0.05$) increased by *B. lanzan* only on 16th day and also it reversed the dexamethasone suppressed wound contraction on the 16 day. *B. lanzan* significantly ($P<0.001$) reduced the time required for epithelialization andreversed the epithelialization delaying effect of dexamethasonesignificantly ($P<0.001$).

Key words : *Buchanania lanzanl,* dexamethasone, wound contraction, wound breaking strength, period of epithelialisation.

Introduction

Wound is a breach in the normal tissue continuum, resulting in a variety of cellular and molecular sequelae. The basic principles of optimal wound healing which include minimizing tissue damage, debriding non- viable tissue, maximizing tissue perfusion and oxygenation, proper nutrition and moist wound healing environment have been recognized for many years (1). A number of drugs ranging from simple non-expensive analgesics to complex and expensive chemotherapeutic agents administered in the management of wound affect healing either positively or negatively (2). Aspirin, indomethacin, cytotoxic agents and immunosuppressant have been proved experimentally to affect healing negatively (3, 4, 5, 6). Medicinal herbs are an indispensible partof traditional medicine. The rhizome of *B. lanzan* finds an important place inindigenous medicine as an expectorant, diuretic and carminative (7). It is also found to have anticancer (8), antihypertensive (9) and larvicidal activity (10). It is used for the treatment of various skin disorders, rheumatism and diabetes mellitus (11, 12). However to the best of our knowledge a systematic study on wound healing activityof *B. lanzan* has not been undertaken. Hence, the present study was undertaken to evaluate the wound healing property of alcoholic extract of *B. lanzan* rhizome andto study its influence on dexamethasone suppressed wound healing on various animal wound models in Albino rats.

Materials and Methods

Collection and preparation of alcoholic extract of *Buchanania lanzan*

B. lanzan plants were procured from the Ooty in the month of December and authenticated by Dr. S. Rajan field

botanist, Ooty. The shade dried fruits were crushed into small pieces and powdered. The powder was loaded into soxhlet extractor in 8 batches of 250 g each and was subjected to extraction for about 30–40 h with ethanol 95%. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to a syrupy consistency. Then it was dried in the dessicator. The yield was about 3%.

Animal care and Handling

This was done as per the guidelines set by the Indian National Science Academy New Delhi, India. Twelve- week-old healthy Albino rats (150–200 g) of either sex bred locally in the animal house of SRM College of Pharmacy, Kattankulathur, Kanchipuram, Tamil nadu. were selected for the study. They were housed under controlled conditions of temperature of 23 ± 2°C, humidity of 50 ± 5% and 10–14 h of light and dark cycles respectively. The animals were housed individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment and had free access to sterile food (animal chow) and water. The study was undertaken after obtaining the approval of Institutional Animal Ethical Committee (IEAC approval letter No. IEAC 39/ Dated Dec. 30th, 2008).

Study Design

The animal were randomly allocated into four groups of eight animals each for the three experimental animal wound models.

Group I received 2 ml of gum acacia 2%(E. Merck India Ltd.) po through intragastric tube.

Group II received *B. lanzan,* 300 mg/kg po. The dose selection was based on the toxicity studies.

Group III received dexamethasone, 0.17 mg/kg (13) (Cadila Healthcare, Mumbai) im.

Group IV received dexamethasone (0.17 mg/kg im) & *B. lanzan* (300 mg/kg) po.

The suspension of the alcoholic extract of *B. lanzan* was made in 2% gum acacia. The dose selection was based on the toxicity studies. In group IV, extract of *B. lanzan* was administered immediately after intramuscular injection of dexamethasone.

Acute Toxicity Studies

Healthy Albino rats of either sex were chosen and were divided into four groups (n=6). They were administered single dose of alcoholic extract of *B. lanzan* orally with increasing doses of 100, 300, 1000, 3000 mg/kg body weight respectively. The doses up to 3 g/kg was well tolerated without producing any signs of toxicity and mortality. 10% of the maximum tolerated dose i.e. 300 mg/kg was selected for the study.

Dosing Schedule

B. lanzan extract and dexamethasone were administered orally and intramuscularly respectively once daily from day 0 to day 9 in the incision and dead space wound models and from day 0 to the day of complete healing or the 21st postoperative day, whichever occurred earlier in the excision wound model. In group IV *B. lanzan* extract was given after the injection of dexamethasone.

Wound models

All wounding procedures were carried out under pentobarbitone (Rhone-poulenc B.P., France) (3 mg/100 g) anesthesia. In the present study no animal showed visible signs of infection.

1. Incision wound : On the depilated backs of the animals, two paravertebral incisions of 6 cm length were made cutting through the full thickness of the skin. Interrupted sutures, 1 cm apart, were placed to approximate the cut edges of the skin (14). The sutures were removed on the 7th post wound day and skin breaking strength was measured on the 10th day by continuous water flow technique of Lee (3).

2. Dead space wound : Dead space wounds were created through a small transverse incision made in the lumbar region (15). A polypropylene tube (2.5 × 0.5 cm) was implanted subcutaneously beneath the dorsal paravertebral lumbar skin. The day of the wound creation was considered as day zero. Granulation tissue formed on the polypropylene tube was harvested by careful dissection on day 10 and the breaking strength of the granulation tissue was measured. The granulation tissue was dried in an oven at 60°C overnight and the dry weight was noted. Acid hydrosylate of the dry tissue was used for the determination of the hydroxyproline content (16).

3. Excision wound : An excision wound was inflicted by cutting away 500 mm^2 full thickness of a pre-determined area on the

depilated back of the rat. Epithelialization period was noted as the number of days after wounding required for the eschar to fall off leaving no raw wound behind. Wound contraction rate was monitored by planimetric measurement of the wound area on alternate days. This was achieved by tracing the wound on a graph paper. Reduction in the wound area was expressed as percentage of the original wound size (17).

Statistical analysis

Results were analysed by One way analysis of variance (ANOVA) followed by Scheffe's test using SPSS computer package version-11.

Results

Incision wound model

The mean breaking strength in the control group was 348.27 ± 7.8 g. The alcoholic extract of *B. lanzan* did not alter the breaking strength when compared to control. In the dexamethasone treated group the mean breaking strength was 166.03 ± 7.45 g which was significantly ($P<0.001$) less compared to control group. Co-Administration of *B. lanzanl* with dexamethasone has significantly ($P<.001$) increased the breaking strength to 292.6 ± 11.72 g (Table I).

Dead space wound model

The mean breaking strength of granulation tissue in the control group was 263.75 ± 28.59 g. Even though there was no significant alteration in the breaking strength of the granulation tissue, a marked increase in breaking strength was observed (312.5 ± 37.4 g) in *B. lanzan* treated group when compared to the control group. The breaking strength in dexamethasone treated group was 273.75 ± 12.09 g. The increase in the breaking strength compared to the control group can not be explained. (Table I). The mean dry weight of granulation tissue in control group was 42.12 ± 5.47 mg which was significantly ($P<0.05$) increased to 49.75 ± 5.56, 64.00 ± 6.81, 61.87 ± 6.15 mg in groups treated with *B. lanzan,* dexamethasone, dexamethasone +*B. lanzan* respectively (Table I). The increase in dry weight in dexamethasone group could be due to fibroblasts and other inflammatory cells.The mean hydroxyproline content of granulation tissue in control group was 21.09 ± 4.41 mg/g of the tissue. It was not significantly altered in any of the groups (Table I).

Excision wound

The percentage of wound contraction was 27.75 ± 4.38, 47.15 ± 5.25, 59.45 ± 2.77 and 68.67 ± 1.28 as measured on the 4th, 8th, 12th and 16th day respectively in the control group. The wound contraction rate was not altered significantly in any of the test groups on 4^{th}, 8^{th} and 12^{th} day as compared to control group at same time. Apart from this, we have also noted a positive trend in wound contraction rate in *B. lanzan* treated group and negative trend in wound contraction rate in dexamethasone treated group even though they were not statistically significant on 8^{th} and 12^{th} day. However wound contraction- rate was significantly increased in *B. lanzan* treated group compared to the control group on 16^{th} day ($P<.001$) (82.1 ± 2.22). Similar observation was also made in the dexamethasone & *B. lanzan* treated group when compared to the dexamethasone treated group where it increased from 67.02 ± 2.12 to 76.22 ± 1.03 on 16^{th} day ($P<0.001$) (Table II). The mean period of epithelialization in the control group was 16.75 ± 0.75 days. It was significantly ($P<.001$) reduced to 11.12 ± 0.47 days in *B. lanzan* treated group. The mean period of epithelialization in dexamethasone treated group was 17.25 ± 0.75 days which was significantly ($P<0.001$) reduced to 12.75 ± 0.77 days in the group treated with both dexamethasone and *B. lanzanl* (Table II).

Discussion

Granulation, collagen maturation and scar formation are some of the many phases of wound healing which run concurrently, but independent of each other. The use of single model is inadequate and no reference standard exists that can collectively represent the various phases of wound healing. Hence three different models have been chosen in our study to assess the effect of *B. lanzan* on wound healing. The wound breaking strength is determined by the rateof collagen synthesis and more so by the maturation process where there is covalent binding of collagen fibrils through inter andintra molecular cross linking. In our study dead space wound model showed no significant increase in breaking strength and hydroxyproline concentration, but the dry weight of the granulation tissue was significantly increased in *B. lanzan* treated group. By this we can assume that the *B. lanzan* might not have increased the collagen content but

probably have altered the maturation process, by affecting the cross linking of collagen or improving the quality of collagen fibrils. The increase in weight in dexamethasone treated group could be due to high protein concentration and collagen bundle formation (18). It is difficult to explain the effect of *B. lanzanl* along with the dexamethasone as there was a slight increase in breaking strength and dry weight of granulation tissue in the dexamethasone alone treated group compared to control group.

Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This centripetal movement of wound margin is believed to be due to the activity of myofibroblast (19). Since *B. lanzan* enhanced wound contraction, it would have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited into the wound area. In excision wound model *B. lanzanl* hastened the period of epithelialisation significantly and the co-administration of *B. lanzan* with dexamethasone hastened the epithelialization in dexamethasone group. Even though only during later part, *B. lanzan* showed significant increase in wound contraction we have observed the positive trend in the initial stages. Concomitant administration of *B. Lanzan* along with dexamethasone had also significantly increased the wound contraction on 16th day. Hence it appears that *B. lanzan* has prohealing effect as evidenced by the above findings. It also appears that *B. lanzan* was able to promote epithelialization either by facilitating the proliferation of epithelial cells or by increasing the viability of epithelial cells. It is difficult to draw any conclusion from the study regarding the dexamethasone & *B. lanzan* effect in dexamethasone suppressed wound model.

In recent years oxidative stress has been implicated in a variety of degenerative process and diseases. These include acute and chronic inflammatory condition such as wound healing (20). *B. lanzan* has shown to possess anti-oxidant property (21). The flavonoids which are responsible for the free radical scavenging activity were believed to be one of the important components in wound healing (22). Phytochemical screening revealed the presence of flavonoids in *B. lanzan* (23). This could be the reason for prohealing activity of *B. lanzan*. This enhanced wound contraction effect *of B. lanzan* and epithelization could possibly be made use of clinically in healing of open wounds. However confirmation of this suggestion will need well designed clinical evaluation.

TABLE I: Effect of *B. lanzan* on dead space and incision wound parameters.

		Dead space wound			*Incision wound*
Drugs	*Dose/route*	*Breaking strength of granulation tissue (g) Mean±S.E.*	*Dry weight of granulation tissue – mg Mean±S.E.*	*Hydroxyproline content – mg/g of the tissue Mean±S.E.*	*Breaking (n=8) strength (g) Mean±S.E.*
Gum acacia	2 ml oral	263.75±28.59	42.12±5.47	21.09±4.41	348.27±7.8
B. lanzan	300 mg/kg oral	312.5±37.4	49.75±5.56[b]	29.61±4.88	349.78±9.13
Dexa	0.17 mg/kg im	273.75±12.09	64±6.81[b]	19.85±2.69	166.03±7.45[c]
Dexa+ *lanzan*	0.17 mg/kg im + 300 mg/kg oral	386.25±10.34[a]	61.87±6.15[b]	23.14±2.50	292.6±11.72[d] *B.*

Dexa=Dexamethasone.

[a] P<0.05 Vs Dexamethasone, Oneway ANOVA, F=5.004, df=3, 28. [b] P<0.05 Vs Control, Oneway ANOVA, F=2.939, df=3, 28. [c] P<0.001 Vs Control, Oneway ANOVA, F=88.249, df=3, 28.

[d] P<0.001 Vs Dexamethasone, Oneway ANOVA, F=88.249, df=3, 28.

TABLE II : Effect of *B. lanzan* on excision wound parameters.

Drugs	*Dose/route*	*Percentage of wound contraction (Mean±S.E.)*				*Period of epithelialization (days) Mean±S.E.*
		4th day	*8th day*	*12th day*	*16th day*	
G. acacia	2 ml oral	27.75±4.38	47.15±5.25	59.45±2.77	68.67±1.28	16.75±0.75
B. lanzan	300 mg/kg oral	21.2±3.21	48.25±4.46	67.55±3.48	82.1±2.22[a]	11.12±0.47[c]
Dexa	0.17 mg/kg im	23.4±3.32	39.57±3.58	55.85±2.39	67.02±2.12	17.25±0.75
Dexa+ *B. lanzan*	0.17 mg/kg im + 300 mg/kg oral	26.25±3.32	37.5±2.64	65.77±0.93	76.22±1.03[b]	12.75±0.77[d]

Dexa=Dexamethasone.

[a] P<0.001 Vs Control, Oneway ANOVA, F=5.004, df=3, 28. [b] P<0.001 Vs Dexamethasone, Oneway ANOVA, F=2.939, df=3, 28. [c] P<0.001 Vs Control, Oneway ANOVA, F=18.483, df=3, 28.

[d] P<0.001 Vs Dexamethasone, Oneway ANOVA, F=18.483, df=3, 28.

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