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Influence of *Bryophyllum pinnatum* (Lam.) leaf extract on wound healing in albino rats

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Abstract

Objectives: To study the effect of *Bryophyllum pinnatum* (Lam.) leaf extracts *viz.* petroleum ether, alcohol and water on healing of excision, resutured incision and Dead space wound models in Albino rats. Materials and Method: Excision, resutured incision and dead space wound models were induced in albino rats of either sex under light ether anaesthesia. Control animals received normal saline orally and other groups received petroleum ether, alcohol and water extract orally for a period of 10 days. Doses for all the three extracts were selected based on the results of acute toxicity studies in mice. On tenth day after estimating the breaking strength of resutured incision wounds, animals were sacrificed. In the dead space wound models granulomas were removed for estimating the breaking strength. Quantification of granuloma tissue and their biochemical and histological aspects in control as well as treated groups were estimated. In excision wound model epithelization time was measured from day-0 (wounding day) till the day of scar falling off with no raw wound left behind. The shape and size of the scar was noted on the day of complete epithelization and were followed upto the 21st post wounding day. Results: All the three extracts *viz.* petroleum ether, alcohol and water showed significant increase in the breaking strength of incision wound as compared to control group ($p<0.001$). granuloma breaking strength and hydroxyproline content of granulation tissue in dead space wound model was significantly increased as compared to control group ($p<0.001$). Water extract showed significant increase in wound contraction and formation of scars on 17th post wounding day in excision wound model ($p<0.001$). Conclusion: The results of the present study indicate, that not only topical application of water extract hastened the healing process in excision wound model, but also all the three extracts administered orally, promoted the healing of resutured incision and dead space wounds, as indicated by increased breaking strength and hydroxyproline content of the granulation tissue, thereby justifying its use in traditional medicine.

Key words: *Bryophyllum pinnatum* (Lam.), Excision wound model, Incision wound model, Dead space wound model, Wound healing.

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1. Introduction

Wound healing, is a common clinical entity, contemporary to mankind. No condition in medicine has been so extensively investigated as wound healing [1]. Several plants and their products are used in folk medicine to treat wounds. Plants like *Tridax procumbens* [2] and *Bryophyllum Pinnatum* [3] have been reported to promote wound healing.

Bryophyllum pinnatum Lam. (crassulacea) is locally known as lonnahadakanagida in Kannada and *Zakhm-E-Haiyat* in Hindi. It is a perennial herb that grows in many parts of India including Karnataka. This plant is well known as an agent for wound healing [4]. Shade dried leaves are very good for application to wounds and, leaves reduced to paste when applied to wounds encourage papillation [5].

The effect of *Bryophyllum pinnatum* on wound healing is not yet well elucidated. Hence, the present study was undertaken to study the influence of various extracts from *Bryophyllum pinnatum viz.* petroleum ether, alcohol and water on healing of excision, resutured incision and dead space wounds in albino rats.

2. Materials and methods

2.1 Plant material

Bryophyllum pinnatum (Lam.) leaves were collected from Pharmacy garden of K.L.E. Society's College of Pharmacy, Belgaum in Oct 1999 and identified at the Department of Pharmacognosy and Phytochemistry, K.L.E. Society's College of Pharmacy, Belgaum, Karnataka, India.

2.2 Preparation of Drug extract

The shade dried leaves(600g), reduced to 60 mesh powder, was extracted with petroleum ether (40-60), Ethanol (90%) and water in a soxhlet extractor, The concentrated material obtained was reduced to a thick mass at room temperature and water was removed by placing it in a desiccator. The weight of the dried mass was recorded and used for experimental studies. The various extracts obtained from the above procedure were used in the form of suspension in 2% tween 80 as suspending agent to establish acute toxicity studies (LD_{50}) in mice and to investigate the effect on healing of different wound models *viz.* excision, resutured incision and dead space wounds in albino rats.

2.3 Acute toxicity studies

To establish LD_{50} of these compounds the graphical method of Miller and Tainter [6] was employed. Mice weighing 20-25 G of either sex were taken up for the study. The animals were divided into four groups each containing 10 animals, among which one was control receiving 0.5 ml Tween 80, while the test groups received each of the Test compounds *viz.*, petroleum ether, alcohol and water orally in doses ranging from 500-4000 mg\kg B.W.

During the study period the animals were continuously observed for 24 h and then for 14 days on changes in eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behaviour pattern. Particular attention was directed to tremors, diarrhoea, lethargy and sleep. Individual weights of the animals were determined shortly before the test substance was administered.

2.4 Animals

Healthy albino rats of either sex weighing 150-200g were used. They were housed individually under standard environmental conditions, fed with pellet rodent diet and water *ad libitum.*

2.5 Wound healing studies

Animals were divided into 10 groups of six animals in each group i.e. control (2 groups), petroleum ether, alcohol and water (each 2

groups, one for incision and one for dead space wounds). They were orally dosed with 400mg/kg B.W. (i.e.0.5 ml /100g B.W.) suspension of three different extracts in 2% Tween 80 as suspending agent, daily for 10 consecutive days in incision and dead space wound model.

Water extract was used topically only for excision wound model in order to mimic the traditional use of the drug in folk medicine for 21 days till the formation of eschar, without any raw wound area.The control groups (incision and dead space wounds) received 2% Tween 80 suspension and, control group in excision wound model received topical application of normal saline.

2.6 Wound models

2.6.1 Resutured incision wound

Under light ether anaesthesia the animal was secured to operation table in its natural position. Two paraverterbral straight incisions of 6 cm, each were made though the entire thickness of the skin, on either side of the vertebral column with the help of a sharp blade [7]. Wounds were closed with interrupted sutures 1 cm apart. Wounds were then mopped with cotton swabs soaked in 70% alcohol and were caged individually.

Removal of the sutures was done on seventh post wounding day. While, breaking strengths of the wounds were determined on 10th post wounding day by constant water flow technique as described by Lee et.al [8].

Anaesthesized animal was secured to operation table, in its natural position and lines were drawn on either side of the incision wound, 3 mm away from the wound margin on adjacent normal skin leaving about 5 mm wound towards both the ends. Two *allis* forceps were firmly applied on the lines, facing each other, the forceps on one side is hooked to a metal rod, fixed firmly to the operation table, while the other to a light polythene container through a string run over a pulley.

Water was allowed to flow at a constant rate to the polythene container so as to build a gradual pulling force necessary to disrupt the wound. The flow of water was regulated by means of an occlusion clamp on rubber tubing connected to water reservoir, kept at a suitable height. As soon as the gaping of the wound was observed, the water flow was cut off.

Further opening of the wound was avoided by releasing the pulling force on the wound immediately, by lifting up the polythene container. The volume of water in the polythene container was measured and converted to the corresponding weight assuming the density to be equals to "one". The breaking strength is expressed as the minimum weight of water necessary to bring about the gaping of the wound.

Three such readings were recorded for a given incision wound and the procedure was repeated on the other, thus obtaining six readings for each animal. The mean breaking strength in each animal (average of six readings) was used to calculate the group means [8].

2.6.2 Dead Space wound

Under light ether anaesthesia, subcutaneous dead space wounds were inflicted in the region of the axilla and groin, by making a pouch through a small nick in the skin. Granuloma formation was induced by implanting either sterile cotton pellets or grass piths.

a. Two sterile cotton pellets weighing 10mg (sterilised by autoclaving) were implanted in axillae by the technique of *D'Arcy.* et. al as described by *Turner* [6], but the granulomas were removed on 10th day.
b. Similarly two cylindrical grass piths measuring 25 mm in length and 3 mm diameter were also introduced subcutaneously

into groins in each animal at different locations at random. Thus one animal had two cotton and two grass piths. The sutures were mopped with an alcoholic swab and animals were placed into their individual cages after recovery from anaesthesia.

Physical, mechanical and histological changes in the granuloma tissue were studied in this model. Excision of the granuloma from the surrounding tissue was performed on the 10th post wounding day under light ether anaesthesia. Cotton pellet granulomas excised from dead space wounds were dried overnight at 60°C so as to obtain a constant dry weight. Their weights were noted and expressed as mg/100gm body weight [9].

The excised tissue was cut into 2 approximately equal halves. One half of the granuloma tissue was used for determination of hydroxyproline [10]. The other part of the tissue was kept in 10% formalin solution for histopathological studies to evaluate the effect of the extract on collagen formation.

2.6.3 Excision wound

Circular piece of full thickness (approximately 500 mm^2) was cut off from a predetermined area on the back of the rat [11]. The wounds were traced on 1 mm^2 graph paper on the day of wounding and subsequently on alternate days until healing was complete.

Changes in wound area was calculated. Reduction in the wound area was expressed as percentage of the original wound size. The number of days required for falling of the eschar without any residual raw wound, gave the period of epithelization

2.7 Statistical analysis

Results were expressed as mean ± SEM.Differences between more than two means were determined by ANOVA followed by Students' *t* - test. Data was considered statistically significant at $p< 0.05$.

3. Results

Acute toxicity studies of all the three extracts of *Bryophyllum pinnatum* leaf extract viz., petroleum ether, alcohol and water did not exhibit any signs of toxicity upto 4g/kg body weight. Since there was no mortality at the highest dose, 1/10th of the maximum dose of the extracts tested for acute toxicity was selected for evaluation of wound healing activity i.e. 400 mg/kg P.O.

Table 1.
Effects of petroleum ether , alcohol and water extract of *Bryophyllum pinnatum* (Lam.) leaves on wound healing in incision and dead space wound models (n=6) (Mean ± S.E.)

Wound model	**Resutured incision wounds**		**Dead space wounds**	
Parameters studied	**Breaking strength(gm)**	**Granuloma breaking strength (gm)**	**granuloma dry weight (mg % of B.W.)**	**Hydroxyproline content (µg/300 mg wet wt.)**
Control	163.7±9.59	174.16± 5.07	38.66±1.92	5.23±0.190
Petroleum ether extract	269.9±5.72*	273.66±6.20*	59.78±2.44*	8.65±.0.207*
Water extract	248.3±8.40*	227.5±8.67*	51.51±1.61*	7.61±0.188*
Alcohol extract	198.3±8.90*	207.5± 8.56*	48.27±2.71*	6.05±0.131*

Values (Mean ± SE), n=6 , *P < 0.001, as compared to control.

Table 2.
Effect of water extract of *Bryophyllum pinnatum* (Lam.) leaves on wound healing in excision wound model (n=6) (Mean ± S.E.)

Group	% Closure of original Excision wound area (days)				Epithelisation period	Scar area on period Epithelisation (mm^2)
	4th	8th	12th	16th		
Control	23.39± 2.97	66.72± 2.37	80.28± 3.29	89.85±2.26	19.33 ± 0.30	43.66 ± 2.05
Water extract	37.95±3.71*	79.77±2.75*	92.00±1.86*	99.78±1.86*	15.80 ± 0.28*	30.50 ± 0 .84*

Values (Mean ± SE), n=6; P < 0.001 as compared to control.

Breaking strength of incision wounds was significantly (F3, 20=25.18>3.85, P<0.001) increased with all the three extracts of *Bryophyllum pinnatum* leaf *viz.*, petroleum ether, water and alcohol treated groups as compared to control (p<0.001).

Similarly in dead space wounds there was significant increase in granuloma breaking strength, granuloma dry weight and hydroxyproline content of granulation tissue as compared to controls (F3, 20 =27.83> 3.85, P<0.001), (F3, 20=13.01> 3.85, P<0.001) and (F3, 20 =61.47> 3.85, P<0.001)(Table-1).

In the excision wound model there was significant decrease in the epithelization period and scar area. Epithelization was found to be enhanced significantly by the water extract of *Bryophyllum pinnatum* leaf as evidenced by shorter period required for eschar dropping (i.e.15.08 ± 0.28) as compared to control (19.33 ± 0.30) (p<0.001).

Similarly scar area was significantly reduced in water extract (30.50 ± 0.84) treated group as compared to control group (43.66 ± 2.05) (p<0.001) (Table-2).

Histopathologically, granulation tissue with marked increase in collagen content was found with all the three extracts as compared to control group indicating wound healing.

4. Discussion

In the present study, wound healing properties of *Bryophyllum pinnatum* (Lam.) were studied and the findings of the present study indicate that local application of water extract and systemic administration of petroleum ether, water and alcohol extract of leaves of *Bryophyllum pinnatum* (Lam.) promote the wound healing process of all types of wounds and support the popular use of leaves to open wounds in folk medicine.

Bryophyllum pinnatum (Lam.) leaves are said to contain a variety of chemicals which are known to enhance wound healing [12]. Quercetin, a bioflavonoid has been shown to modify eicosanoid biosynthesis [13]. It has been found to inhibit membrane lipid peroxidation [14] and also has anticarcinogenic, antioxidant and anti-inflammatory properties [15].

The inflammatory phase of wound healing is considerd a necessity for succesful healing. The ability of the skin to regenerate and heal wounds in a scarless manner in the absence of inflammation have been shown, in fetal healing studies. Wilgus TA *et. al* have shown that cox-2 inhibitor celecoxib markedly reduced the inflammatory phase of wound healing with reduction in scar tissue formation without disrupting re-epithelization or decrease in tensile

strength [16]. Similarly, quercetin present in the *Bryophyllum pinnatum* leaves with its anti-inflammatory properties might have helped the wound to heal with minimum scarring.

Bioflavonoids like quercetin synthesized by many plants facilitate wound healing by limiting inflammation and tissue degradation, improving local circulation and also help in formation of strong collagen matrix. Apart from stimulatory effect on vascular smooth muscle, quercetin is known to have stimulatory action on myofibroblasts, to contract at the wound edge, thus promoting healing [17].

The reported antimicrobial activity of the plant extract appears to be unrelated to its prohealing activity, since most of these wounds were clean and healthy, though not totally free from contamination [5].

The findings of the present study suggest that *Bryophyllum pinnatum* (Lam.) leaf extracts have definite prohealing action as seen from their effect on inflammatory and as well as proliferative phase. However, further studies are required to show the prohealing action of quercetin on wound healing.

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