WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACT OF *BETA VULGARIS*

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Summary

The wound healing efficacy of ethanolic root extract of *Beta vulgaris* was evaluated in excision and incision wound models. The parameters studied include rate of wound contraction, period of complete epithelialization and tensile strength of incision wound. The ethanolic extract was found to possess significant wound healing activity, which was evidenced by decrease in the period of epithelialization, increase in the rate of wound contraction and skin breaking strength.

Key Words: *Beta vulgaris*, wound healing, excision wound model, incision wound model.

Introduction

The therapeutic efficacies of many indigenous plants, for various diseases have been described by traditional herbal medicine practitioners [1]. Natural products are rich source of synthetic and traditional herbal medicines which are still the primary health care system in some parts of the world [2]. The past decade has seen considerable change in opinion regarding ethno pharmacological

therapeutic applications. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential wound healing properties. Efforts are being made all over the world to discover agents that can promote wound healing and thereby reduce the cost of hospitalization and save the patient from amputation or other severe complications [3].

The beet (*Beta vulgaris*) is a plant in the Chenopodiaceae family. It is best known in its numerous cultivated varieties, most well known of which is the purple root vegetable known as the beetroot or garden beet [4]. Hippocrates is said to have advocated using beet leaves for binding wounds [5]. There are some reports indicating the potential hepatoprotective, antioxidant and anti-inflammatory activities of *Beta vulgaris,* though without any scientific proof [6]. Since enough scientific data is not available regarding the wound healing property of *Beta vulgaris*, we have undertaken this work to validate the same.

Materials & methods

Plant Material

The roots of *Beta vulgaris* were collected from the local area of Meerut district identified and authenticated by Dr. Anjula Pandey, Principal scientist, National Herbarium of Cultivated Plants, and New Delhi. Voucher specimens (NHCP/NBPGR/2010-26-920) have been kept in National Herbarium of Cultivated Plants, New Delhi and Department of Pharmaceutical Technology, MIET for future reference.

Experimental Animal

The Institutional Animal Ethics Committee, (IAEC) approved the use of animals for the present study, (Ethical clearance number: 711/02/a/CPCSEA). Healthy Wistar albino rats of both sexes 200–220 g was used for the study. They were individually housed and were allowed free access to standard pellet diet and water ad libitum. Animals were periodically weighed before and after the experiment. The rats were anaesthetized prior to and during infliction of the

experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anaesthesia (120 mg/kg). Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and were replaced.

Extraction

The roots of *Beta vulgaris* were dried under shade, reduced to moderately coarse powder, loaded into soxhlet extractor and was subjected to successive extraction with Petroleum Ether, Benzene, Chloroform and Ethanol to get different extracts. The ethanolic extract was concentrated to dryness using Rotary evaporator, giving yield as 10.16%.

Preliminary Phytochemical Studies

The ethanolic extract was then subjected to qualitative phytochemical screening for the identification of different phytoconstituents. The wound healing activity of the ethanolic extract of the plant at 1000 mg/kg is being reported here [7].

Wound- Healing Activity

Excision and incision wound models were used to evaluate the wound-healing activity of ethanolic extract of *Beta vulgaris*.

Excision Wound Model

Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil [8]. A full thickness of the excision wound of circular area 250 mm^2 and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open [9]. The animals were divided into two groups of 6 each. Group 1 animals were topically treated with the simple ointment base I.P. (Indian Pharmacopoeia 1966) as a placebo control. The animals of group 2 were topically treated with the 10%

ointment of the ethanolic extract of *Beta vulgaris* formulated in simple ointment base I.P. (Indian Pharmacopoeia 1966) till complete epithelialization [10].The wound closure rate was assessed by tracing the wound on days 0, 5, 10, 15 and 20 post-wounding using transparency papers and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar without any residual raw wound gave the period of epithelialization.

Incision Wound Model

As with the above model, rats were anaesthetized prior to and during creation of the wound. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, six centimeters in length was made through the skin and cutaneous muscle on the back [11]. After the incision, surgical sutures were applied to the parted skin at intervals of one centimeter. The wounds were left undressed. The rats were given *Beta vulgaris* extract (dissolved in tween- 80, 0.5%) orally at a dose of 1000 mg/kg/day. The controls were given tween-80 0.5% only. The sutures were removed on the 8th post wound day and the treatment was continued. The skin-breaking strength was measured on the 10th day. The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound, and the procedure was repeated on the contra lateral wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group [12]**.**

Statistical Analysis [13]

All the results obtained from various activities, as described above, were analyzed statistically by using Student's t test and $p<0.05$ were considered significant.

Results

Phytochemical Analysis

Ethanolic extract showed positive tests for the presence of tannins, sterols, carbohydrates, saponins and flavanoids.

Excision Wound Model

The ethanolic extract showed healing in 16.66 days as compared to 21.83 days of control.

Table 2: Wound healing effect of *Beta vulgaris* in Excision wound model

Parameter	**Wound area (mm^2) and percentage of wound contraction**	
Post Wounding Days	**Placebo control**	**Experimental**
Day 0	250.16 ± 1.887	250 ± 1.5278
Day 5	197.83 ± 1.7594	179.5 ± 1.2585* (9.26%)
Day 10	152.66 ± 1.8563	82.66 ± 1.5204* (45.85%)
Day 15	117 ± 09662	19.66 ± 0.715* (83.19%)
Day 20	16.66 ± 2.364	0 ± 0* (100%)
Period of epithelization (day)	21. 83 ± 0.5427	16.66 ± 0.3333*

n = 6, Values are expressed as mean ± SEM
*$p<0.001$ significant as compared to control

Incision Wound Model

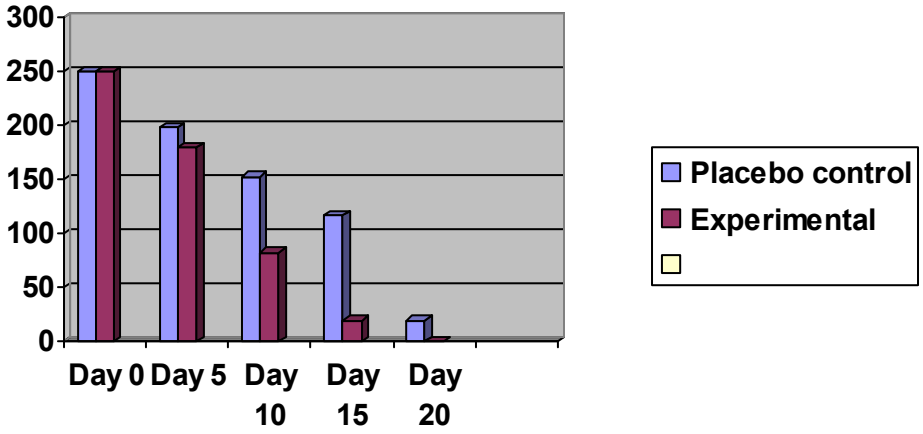
The skin breaking strength for ethanolic extract is 443.1 g as compared to 309.66 g of control.

Table 3: Wound healing effect of *Beta vulgaris* in Incision wound model.

Parameter	Placebo control	Experimental
Skin breaking strength (g)	309.66 ± 0.9118	443.1± 0.6299*

n = 6, Values are expressed as mean ± SEM
*p<0.001 significant as compared to control.

Wound healing effect of *Beta vulgaris* in Excision wound model:



Discussion

Wound healing is a highly complex, but orchestrated cascade of events that can roughly be divided into three overlapping phases- inflammation, granulation tissue formation and remodeling of the extra-cellular matrix. These events involve several cellular phenomenons such as migration, proliferation, adhesion, phenotypic differentiation, etc. Immediately after injury, there is clot formation and the earlier phases of wound repair involve inflammation and synthesis of ground substance. The ground substance mainly

consists of proteoglycans, which are heterogeneous, non-fibrillar components of the extra-cellular matrix. These complex macromolecules are made up of a protein core linked covalently to linear heteropolysaccharides, the glycosaminoglycans (GAGS). Proteoglycans and GAGS have been shown to play important roles in all the above-mentioned events of wound healing [14]. The wound-healing property of *Beta vulgaris* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. The early tissue approximation and increased tensile strength of the incision wound observed in our study may have been contributed by the phytoconstituents of *Beta vulgaris*. Further phytochemical studies are needed to isolate, characterize and identify the specific active compounds in this plant responsible for wound healing activity.

Conclusion

Though many plants and plant products have been reported to have wound healing activity in animal studies, very little study has been carried out on human subjects. Most of the drugs used to cure wounds are antibacterial agents and are available in the form of topical ointments or creams. There is lot of opportunity to explore the possibility of using indigenous herbs and their active constituents for wound property in a more scientific manner. The toxicity study and appropriate formulation and development for these herbal extracts have to be emphasized.

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