

## Research Article

## Evaluation of *Vitex negundo* Linn. Leaves for Wound Healing Activity on Experimental Wounds in Albino Rats

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### ABSTRACT

Wound is one of the major areas of concern due to its complexity requiring urgent attention and care. *Vitex negundo* linn. (Verbanaceae) is well-known plant in Indian traditional medicine. On the basis of its traditional use and literature references, leaves of the plant were selected for evaluation of wound healing potential. An aqueous extract was examined for its wound healing activity in the form of ointment in experimental wound models in albino rats. The studies included parameters like epithelization period, wound contraction, tensile strength of incision wounds. The biochemical parameter like determination of hydroxyproline contents of granulation tissue obtained from dead space wound model was estimated in all the drug and vehicle control treated groups of animals. The results of the wound healing study with respect to the incision and excision wound models in rats revealed statistically significant wound healing activity ( $p < 0.05$ ) when compared to control and standard, which was evidenced by faster epithelization, increase in the tensile strength and hydroxyproline content. To support the results, the granulation tissue was subjected to histopathological examination revealing better epithelization, fibrosis and angiogenesis in the test extracts treated wounds, thereby justifying its use in wound healing by traditional healers.

**Keywords:** *Vitex negundo*, wound healing potential, dead space wound model, hydroxyproline.

### INTRODUCTION

The skin is one of the most extensive and readily accessible, multilayered organ of the human body composed of anatomically – epidermis, dermis and sub-cutaneous fat tissues. It is the natural protective cover of the body serving as a first line of defense to various diseased conditions of the skin that include burns, wounds and skin cancer.

Human being is constantly subjected to injuries that may result in cell death and tissue destruction. Thus, wounds are inescapable event in the life of an organism and at times they are dangerous even life threatening. Since by far the great majority of surgical wounds involve the skin, underlying fascia, and muscles. It contains cells, a fibrous network composed of collagen and elastin, and an amorphous ground substance, which consists of protein-polysaccharides, glycoproteins, globular proteins, salts and water. Skin exhibits extensibility and time dependant

viscoelasticity, which change with the site, age, skin thickness and in some cases skin composition<sup>1</sup>.

Wound may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissues<sup>2</sup>. Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity<sup>3</sup>. The wound healing occurs in different phases: Coagulation; contraction, epithelialization, granulation, collagenation and tissue remodeling. Any agent accelerating the above process is a promoter of wound healing<sup>4</sup>. The plant products have been shown to possess good therapeutic potential as promoter of wound healing.

In traditional systems of medicine, various plants have been used to promote wound healing. Some of the plants studied and evaluated for wound healing activity are *Terminalia arjuna*,<sup>3</sup> *Aloe vera*<sup>5</sup>, *Lawsonia alba* Lam.<sup>6</sup> *Argemone mexicana* Linn.<sup>7</sup>

*Vitex negundo* Linn belonging to family Verbenaceae, commonly known as Nirgundi; is a short tree or large aromatic shrub with irregular trunk, stem and branches covered with thin grey bark<sup>8</sup>. The plant is well known for its analgesic and anti-inflammatory activity. The leaves of the plant are reported for skin disease in ancient Ayurvedic books like Dravyagunavigyan<sup>9</sup>. The leaves of the plant are known to contain colourless volatile oil of odour of the drug<sup>10</sup>, resin<sup>10</sup>, terpenes, flavonoids, glucosides, and alkaloids<sup>11</sup>. Various preparations of Nirgundi along with other plants are available in market for conditions such as rheumatoid arthritis, joints pain and musculoskeletal pains.

As survey of literature revealed that no systematic study has been conducted with respect to its wound healing potential, hence it was thought worthwhile to investigate the wound healing activity of *Vitex negundo* leaf extracts.

Wound healing studies mainly aim to detect various means and factors influencing healing process so that, they could be either used or avoided in clinical practice to favorably alter the healing process. Cellular proliferation is largely regulated by biochemical factors produced in the local microenvironment that can either stimulate or inhibit cell growth<sup>12</sup>. The biochemical events in the healing wound correlates with the changing mechanical properties<sup>1</sup>. The extracellular matrix is stable complex of macromolecules that underlies epithelia and surrounds connective tissue<sup>13</sup>. The regulation of extracellular matrix deposition is required for normal wound healing where they need to be rapidly synthesized during the formation of the early granulation tissue and also during the final replacement by mature connective tissue and tissue remodeling.

To know the extent of wound healing process various biochemical parameters are estimated in the granulation tissue and regenerated tissue harvested from the healing wounds. Breakdown of collagen liberates free hydroxyproline, an amino acid and its peptides. Measurement of this

hydroxyproline therefore generally used as an index of collagen turnover<sup>5</sup>.

The present study has been taken up to define the role of the leaves of the plant *Vitex negundo* in wound healing.

## MATERIALS AND METHODS

### 1. Collection and extraction of leaves

*Vitex negundo* Linn leaves were collected from SNTD Women's University, Juhu, Santacruz, Mumbai campus and authenticated at St. Blatter's Herbarium, with specimen no N.A. Irani- NT 2888 (BLAT).

The leaves of the plant were shade dried and coarsely powdered using mixer grinder. The aqueous extract was prepared by decoction of powdered leaves in distilled water in ratio of 1:8. The obtained extracts were evaluated for colour, consistency, pH, specific gravity, and % w/w yield. Physicochemical parameters such as total ash value, acid insoluble ash value, water soluble extractive value, and alcohol soluble extractive value, were determined for purity and quality of crude drug as per Indian Herbal Pharmacopoeia. The Standardized and phytochemically investigated aqueous extract was incorporated into the simple ointment B.P. in the concentration 1%, 5%, and 10% w/w.

### 2. Wound healing activity

#### 2.1 Animals

Adult albino rats of Wister strain (180 – 250 g) housed under standard condition of temperature and humidity (25 ± 0.5 °C and 12 h light/ dark cycle) and fed standard pellet diet and water *ad libitum*. Animals were acclimatized to laboratory conditions before the experiment. The protocol was approved by IAEC (CUSEP/IAEC/ 14 /04-05).

Albino rats were randomly divided into groups as follows: Group I: Control (Vehicle) rats treated with topical application of simple ointment BP, Group II rats treated with topical application of simple ointment containing 1% w/w of aqueous extract, Group III rats treated with topical application of simple ointment containing 5% w/w of

aqueous extract, Group IV rats treated with topical application of simple ointment containing 10% w/w of aqueous extract, Group V Reference rats treated with standard Betadine ointment.

## 2.2 Wound models

### 2.2.1 Resutured Incision wound<sup>3</sup>

Incision wounds of 6 cms. long were made on the shaved back of rats anaesthetized using Ketamine HCl injection and the incisions were closed with interrupted sutures, stitches 0.5 cm apart using black surgical thread and curved needle no. 19. The treatment was done by topical application of different ointments for the period of 10 days. The sutures were removed on 8<sup>th</sup> post wounding day and tensile strength of 10-day-old wound was measured by tensiometer.

### 2.2.2 Excision Wound Model<sup>3</sup>

Full thickness circular wounds (area approx. 500 mm<sup>2</sup>) were made on the shaved back of rats anaesthetized using Ketamine HCl injection. The wounding day was considered as 0 day. The treatment was done by topical application of different ointments till wounds were completely healed. Monitoring is done for epithelialization on day 4, 6, 8, 10, 12, 14, 16, until complete epithelialization. The wound area was traced and the percent epithelialization was calculated. The period required for complete epithelialization was noted.

### 2.2.3 Dead Space Wound Model<sup>14, 15</sup>

Dead space wound model is usually employed for assessing the extent of collagenation. Wounds were created by implantation of a Polypropylene tube (3.0 × 0.3 cm) beneath the dorsal paravertebral lumbar skin<sup>14</sup>. Animals were treated with (10% aqueous extract containing simple ointment, vehicle control and standards from '0' day to 9<sup>th</sup> post wounding day. On the 10<sup>th</sup> post wounding day the granuloma tissue developed around the tube was harvested and subjected to biochemical evaluation i.e. to determine the hydroxyproline content

spectrophotometrically by 'Neuman and Logan Method'<sup>15</sup>. The part of the tissue was stored in 10% formalin solution and subjected to histopathological studies.

## 2.3 Statistical analysis

Statistical analysis was done by one way ANOVA followed by Dunnett's 't' test and Bonferroni test.

## RESULTS

The studies in incision wound model for aqueous extract in the form of simple ointment (1, 5, and 10% w/w) showed increase in tensile strength (gms) compared to control and reference standards Betadine ointment.

Maximum increase in tensile strength was seen in the group of animals treated with 10% w/w ointment but all the three ointments of the aqueous extract showed statistically significant ( $p < 0.05$ ) increase in the tensile strength of incision wounds when compared to control and reference standard as shown in the table no.1

Studies using the excision wound model showed significant decrease in the epithelialization period. Epithelialization was found to be enhanced significantly ( $p < 0.05$ ) by the aqueous extract of *Vitex negundo* leaves as compared to control.

The measurement of the progress of wound healing induced by treatment groups in the excision wound method are shown in table no. 2. It is observed that wound contracting ability of the 1, 5 and 10% w/w simple ointments of aqueous extract were significantly greater than control. The mean percent epithelialization of excision wound areas was calculated on the 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 10<sup>th</sup>, 12<sup>th</sup>, 14<sup>th</sup> and 16<sup>th</sup> post wounding day.

The effect of 1, 5 and 10% w/w simple ointment of aqueous extract on percent epithelialization on 16<sup>th</sup> post wounding day was found to be dose dependant and not significantly different ( $p < 0.05$ ) from each other.

The dead space wound studies of the aqueous extract applied topically in the form of simple ointment (10% w/w) was carried with respect to estimation of the

hydroxyproline content in mg per gm of the granulation tissue formed around the polypropylene tube and compared with the control. The results obtained are in table no.3. The granulation tissue obtained from the dead space wounds treated with the aqueous extract ointment (10% w/w) showed significant ( $p < 0.05$ ) increase in the hydroxyproline content when compared to granulation tissues obtained from control and standard animals.

Histopathological studies on the granulation tissues of the dead space wounds treated with the 10% w/w simple ointment of aqueous extract showed moderate to medium degree of fibrosis, mild to moderate degree of neovascularization and leucocytic infiltration and trace to mild degree of collagenation as seen in the plate no. 1, 2 and 3.

#### DISCUSSION

Wound is one of the major areas of concern due to its complexity requiring urgent attention and care. The process of wound healing comprises of a continuous sequence of inflammation and repair. The plant products have been shown to possess good therapeutic potential as promoter of wound healing.

The present study indicates the wound healing potential of *Vitex negundo* leaves. The results of the present study revealed that the aqueous extract of leaves of *Vitex negundo* linn. have significant wound healing activity in excision and incision wound models.

Collagen is major protein of the extracellular matrix and is the component that ultimately contributes to wound strength<sup>3</sup>. The increased hydroxyproline content of the granulation tissue isolated from dead space wounds in the group of animals treated with the aqueous extract indicates faster collagen turnover leading to rapid healing with concurrent increase in tensile strength of incision wounds. This was also seen in the histopathological studies.

Further studies can be undertaken to develop a suitable patient compatible and elegant formulation for the treatment of wound healing containing *Vitex negundo* Linn.

#### CONCLUSION

The systematic studies were carried out in our laboratory using different wound models in albino rats including histopathological studies to demonstrate the wound healing potential of Nirgundi as mentioned in the folklore.

**Table 1: Effect of aqueous extract of *Vitex negundo* leaves on tensile strength of incision wounds**

S. No.	Group	Tensile strength in (gms.) (Mean $\pm$ S.E.M.)
1	CONTROL	588.5 $\pm$ 1.80
2	1% S.O. OF AQ. EXT	842.67 $\pm$ 0.95*
3	5% S.O. OF AQ. EXT	1002.17 $\pm$ 0.87*
4	10% S.O. OF AQ. EXT	1149.67 $\pm$ 1.15*
5	BETADINE	724 $\pm$ 0.58*

S.O. OF AQ. EXT: Simple ointment containing aqueous extract  
All values are mean  $\pm$  S.E.M. (n=6), \* $p < 0.05$  when compared to control.

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**Table 2: effect of aqueous extract of *vitex negundo* leaves on percent epithelialization**

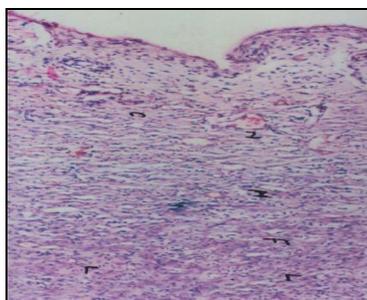
Days	Percent epithelialization (Mean $\pm$ S.E.M.)				
	Control (simple ointment BP)	1% S.O. OF AQ EXT	5% S.O. OF AQ EXT	10% S.O. OF AQ EXT	Betadine
4	13.18 $\pm$ 0.41	4.69 $\pm$ 0.79	5.74 $\pm$ 0.41	19.23 $\pm$ 2.00	7.24 $\pm$ 1.21
6	42.13 $\pm$ 0.61	10.97 $\pm$ 0.27	25.12 $\pm$ 2.83	29.37 $\pm$ 4.71	15.29 $\pm$ 0.45
8	52.16 $\pm$ 0.63	36.61 $\pm$ 0.67	37.26 $\pm$ 3.82	51.03 $\pm$ 4.44	34.68 $\pm$ 2.35
10	67.01 $\pm$ 3.92	51.78 $\pm$ 0.54	72.73 $\pm$ 0.35	79.20 $\pm$ 1.36	57.46 $\pm$ 1.47
12	77.09 $\pm$ 2.06	76.04 $\pm$ 0.27	80.84 $\pm$ 0.19	93.17 $\pm$ 1.01	73.89 $\pm$ 2.72
14	83.40 $\pm$ 1.68	81.59 $\pm$ 0.37	85.86 $\pm$ 0.49	95.07 $\pm$ 0.44	84.65 $\pm$ 3.15
16	87.23 $\pm$ 1.34	92.13 $\pm$ 0.19*	95.55 $\pm$ 0.39*	97.11 $\pm$ 0.21*	86.67 $\pm$ 1.78
Epithelialization period (Days)	23	22	20	18	22

S.O. OF AQ. EXT: Simple ointment containing aqueous extract  
All values are mean  $\pm$  S.E.M. (n=6), \*p<0.05 when compared to control.

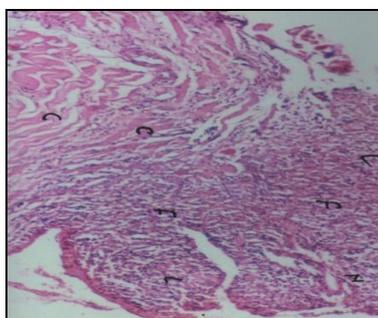
**Table 3: Effect of aqueous extract of *vitex negundo* leaves on the hydroxyproline content (mg/gm) of granulation tissue of the dead space wounds**

Treatment group	Amount of Hydroxyproline in mg/ gm of tissue Mean $\pm$ S.E.M.
CONTROL	38.53 $\pm$ 0.48
10% S.O. OF AQ. EXT	45.01 $\pm$ 0.95*
BETADINE	37.24 $\pm$ 0.39

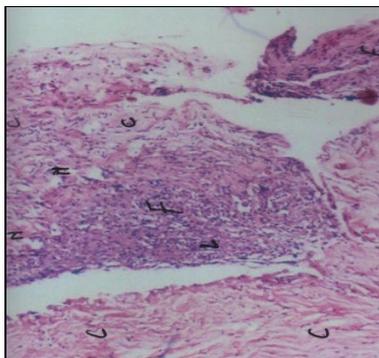
S.O. OF AQ. EXT: Simple ointment containing aqueous extract  
All values are mean  $\pm$  S.E.M. (n=6), \*p<0.05 when compared to control.



**Plate 1: Photomicrograph of granulation tissue obtained from dead space wound treated with simple ointment**



**Plate 2: Photomicrograph of granulation tissue obtained from dead space wound treated with 10% w/w simple ointment of aqueous extract**



**Plate 3: Photomicrograph of granulation tissue obtained from dead space wound treated with Betadine ointment**

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