

WOUND HEALING POTENTIAL OF *VITEX NEGUNDO*. LINN IN EXPERIMENTAL ANIMALSYOGESH P TALEKAR*¹, BISWADEEP DAS¹, TANIA PAUL¹, DEEPALI Y TALEKAR¹, KISHORI G APTE¹, PRADEEP B PARAB¹¹APT Research Foundation Subsidiary of National Toxicology Centre, M.N 199 Sinhagad Road, Vadgaon Khurd Pune, Maharashtra 411041 India. Email: yogeshtalekarpune@gmail.com

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ABSTRACT

Wound being a panic driven situation which can be minor or major, needs to be given special attention for providing quick healing remedy. In the present study an attempt has been made to evaluate the wound healing potential of aqueous and ethanolic extracts of leaves of *Vitex negundo*. A rapid restoration of the wounded skin was observed with the application of both the extracts in circular excision and linear incision wound model in wistar rats. Particularly aqueous extract treated rats showed 98.53% and ethanolic extract showed 94.97% accelerated contraction of excision wound with an increase in the tensile strength in case of incision wound as compared to the standard reference drug, Cipladine. The wound healing parameters like Hydroxyproline, Collagen, and Hexosamine were estimated in regenerated skin which further solicits the potential of the leaves of *Vitex negundo* to heal experimentally induced wound.

Keyword: Vitex negundo L., Excision and Incision Wound, Hydroxyproline, Collagen.

INTRODUCTION

Wound healing is a process that is fundamentally a connective tissue response.¹ Healing process is a complicated biological process, which follows a definite pattern of cellular and molecular events which includes regeneration of parenchymal cells, migration and proliferation of both parenchymal cells and connective tissue cells, synthesis of extracellular matrix proteins, remodeling of connective tissue, collagenization and acquisition of wound strength.^{2,3,4}

Fibroblasts are responsible for producing the new matrix needed to restore structure and function to the injured tissue. Fibroblasts attach to fibrin and integrin cables and begin to produce collagen, which then becomes cross-linked.⁵ The growing popularity of natural and herbal medications because of easy availability of raw materials, cost-effectiveness and paucity of reported adverse reaction, there is an upthrust in research for assessment of herbal drugs for wound healing ability. ⁶ *Vitex negundo* Linn. [VN] (Verbenaceae) is a woody, aromatic shrub growing to a small tree which bears tri- or penta-foliolate leaves.⁷ The crude aqueous extract of *Vitex negundo* Linn. leaves have been investigated for laxative activity.⁸ Leaves of VN have been reported for its anti-inflammatory activity, antihistaminic, membrane stabilizing and antioxidant activities. ⁹ It has also been reported to have innumerable medicinal properties like analgesics anticonvulsant, bronchial relaxant and hepatoprotective activity.¹⁰

So the present study was undertaken to investigate the in vivo wound healing activity of aqueous and ethanolic extracts of *Vitex negundo* leaves on rats using in vivo circular excision and linear incision wound models.

MATERIALS AND METHODS

Drugs and Chemicals: Cipladine has been used as a standard drug was obtained from Cipla Ltd. Cipladine contains 5% of povidone-iodine. Ketamine and xylazine was procured from Aquafine injecta pvt ltd. All other chemicals used for the study were of analytical grade obtained from Sigma Aldrich ST. Louis US.

Collection of the Plant material and authentication

The leaves of *Vitex negundo* were collected from Kem village, of Solapur district of Maharashtra during Monsoon season. The herbarium was made and authentication was done from Botanical Survey of India under the no TAY3.BSI/WRC/Tech/2011. The voucher herbarium specimen was submitted to the APT Research Foundation, Pune India.

Preparation of the plant extract

The leaves were cleaned and shade dried prior to extraction. The leaves obtained after drying were powdered and aqueous as well as ethanolic extraction was carried out using a Soxhlet apparatus. After 24 hours the extracts were concentrated in a rota-evaporator under vacuum and stored at 4 to 8^o C in refrigerator for further use.

Acute Dermal Toxicity Study

The acute dermal toxicity study was performed as per OECD guidelines no 424 on rats. The test extracts were uniformly applied on skin over an area which is approximately 10% of the total body surface area. The animals were observed for 14 days for any clinical signs such as redness, itching and oedema. It was observed that the dose was safe and lower dose was considered for further study.¹¹

Animals

Wistar rats of both sexes of 150-200gm bodyweight were used for studying the wound healing activity of *Vitex negundo*. The animals were housed in National Toxicology Centre, Pune. A total of 48 animals were approved by the Institutional Animal Ethical Committee (IAEC) for National Toxicology Centre under the Research Project no.109 on 24/12/2010. The animals were given standard diet pellet and water *ad libitum*. The rats were kept at 12 hours light / dark cycle and maintained at 24 ± 2^oC and optimum relative humidity.

Wound Healing Activity

Excision Wound Model

The rates of contraction of wound and epithelization were monitored in the Excision wounds model. Initially animals were anaesthetized with ketamine and xylazine 1:1 ratio and the back side of each rat was shaved. Excision wounds sized 300 - 400 mm² and 2 mm depth were made by cutting out layer of skin from the shaven area. In the Control Group animals the wound was left open,¹² whereas the standard drug cipladine¹³ and test drug applied topically on excision wound. The extracts were applied at a dose of 200 mg/kg/day for 16 days. Wound areas were measured on days 0, 3, 6, 9, 12 and 15 for all groups, using a transparency sheet and a permanent marker.¹⁴

Incision Wound

A linear paravertebral incision of 6 cm long was made through the full thickness of the skin. In each animal wound was closed with interrupted sutures, both the extracts were applied on the incision wounds daily for 14 days. However sutures were removed on the 10th day. Using Tensiometer the breaking strength of the wound in kilograms was measured on 15th post wounding day.¹⁵

Biochemical parameters

The circular wound area was excised at the end of the study. The biochemical parameters like collagen, hydroxyproline¹⁶ and hexosamine¹⁷ levels were determined for evaluating the healing properties of both the extracts of *Vitex negundo* namely aqueous and ethanolic.

Statistical Analysis

Statistical analysis of the data using one way ANOVA for comparing the treated groups with that of untreated control group was done by Dunnett's test using Graphpad Prism version 5. The data with P value <0.05 were considered to be statistically significant.

RESULTS

Circular Wound contraction

Control rats showed significant delayed contraction of the circular excised wound when compared with the standard drug cipladine

treated animal as well as test drug treated animals. (P<0.05) Table 1. The aqueous extract of VN showed 98.52% wound contraction which was similar to standard drug which showed 98.39% contraction of wound. However ethanolic extract showed 94.97% wound contraction at an interval of 15 days. Fig 1

Linear Incision wound

The effect of wound healing activity in this model was evaluated by determining the tensile strength of the incision wound of control group and standard as well as test drug treated group. The aqueous extract of VN showed significant increase in tensile strength (P<0.05) as compared to the control group. (Fig 2)

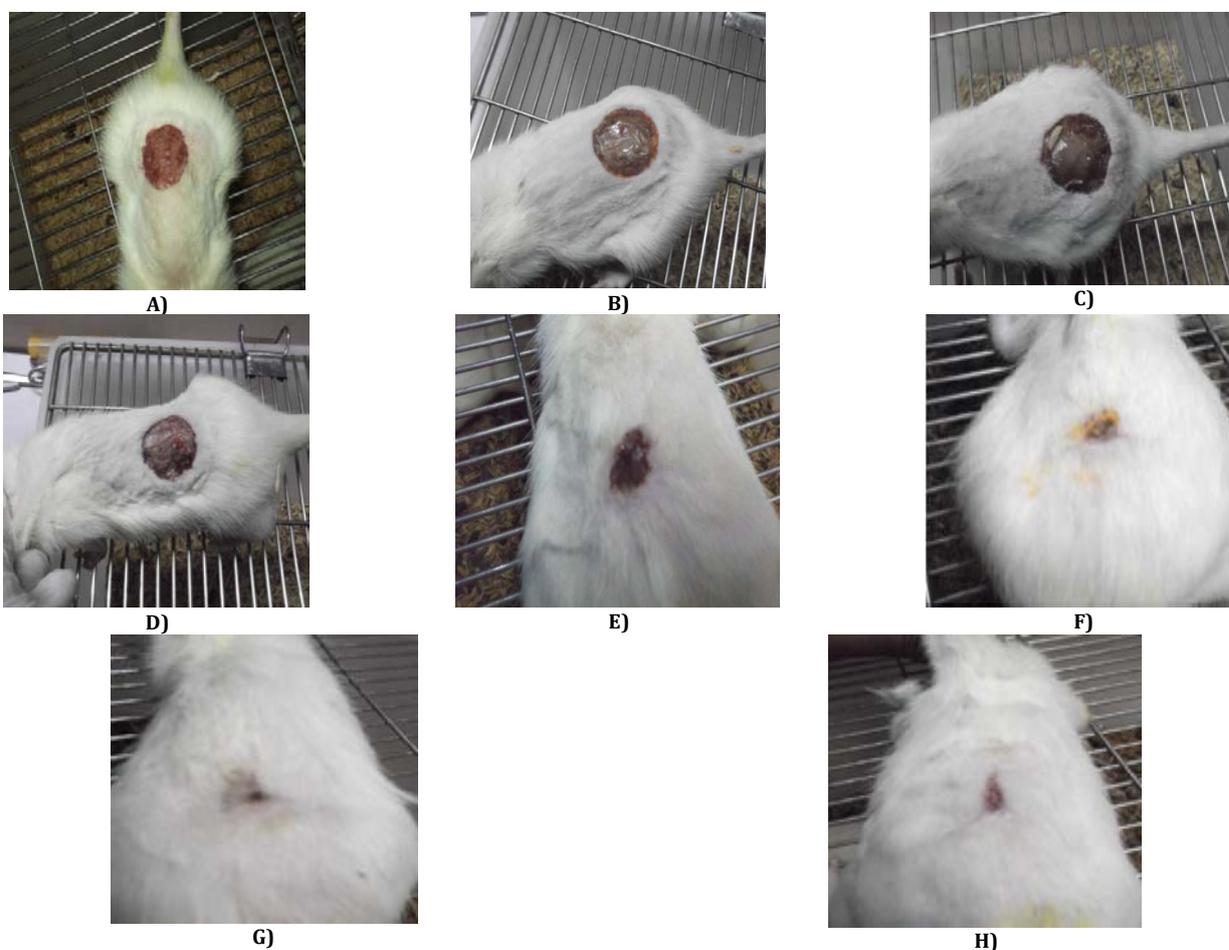
Biochemical parameters for Wound Healing

Both the test extracts showed an increase in hydroxyproline, collagen and hexosamine levels per gram of dried tissue compared to control group indicating better effects of wound healing. (Table no. 2)

Table 1: Excision wound contraction in mm² of *Vitex negundo*.

Group	0 day	3 rd day	6 th day	9 th day	12 th day	15 th day
Control	388.5±7.07	325±5.83	292.66±5.53	141.33±8.09	60.5±6.15	45.33±7.91
Std	405.33±7.91***	226.66±6.25***	149.83±9.82***	61.83±6.43***	23.33±3.66***	6.5±2.42***
Aqueous extract	406.5±7.17***	231.83±11.42***	88.33±6.59***	38.33±3.20***	12.83±4.07***	6±2.60***
Ethanolic extract	391.16±7.57***	250.83±6.99***	149.83±5.91***	77.66±7.73***	39.83±5.94***	19.66±2.50***

Values are expressed as Mean±SD (N=6). Statistical analysis was done by one way ANOVA. P<0.001 when compared with Control.



A, B, C and D are the wound of control, standard, Aq extract and eth extract at 0 day and E, F, G and H are the wound of control, standard, Aq extract and eth extract at 15th day respectively.

Fig. 1: Photographs of wounds

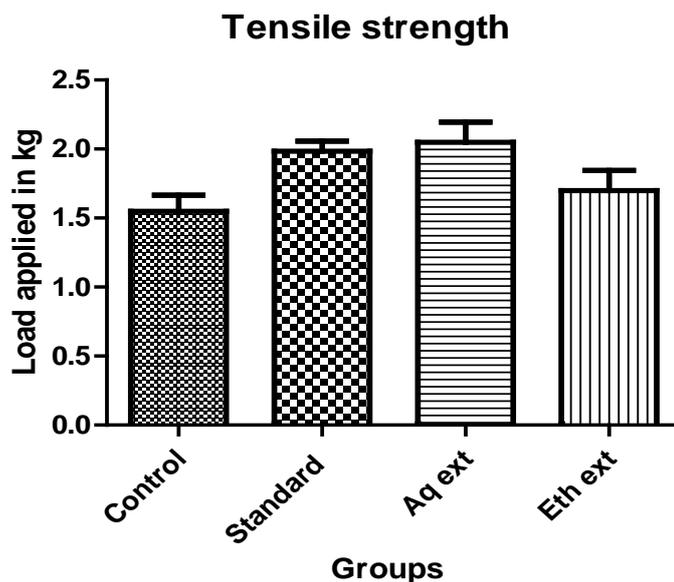


Table 2: Effect of *Vitex negundo* on biochemical parameters of wound healing

Groups	Hydroxyproline ($\mu\text{g/gm}$)	Collagen ($\mu\text{g/gm}$)	Hexsamine (mg/gm)
Control	41.88 \pm 3.82	312.45 \pm 7.33	8.77 \pm 0.83
Standard	67.90 \pm 4.84	506.53 \pm 8.20	20.73 \pm 1.67
Aqueous Extract	94.48 \pm 3.59	704.85 \pm 9.70	29.38 \pm 1.67
Ethanollic Extract	83.57 \pm 4.65	623.41 \pm 6.99	25.45 \pm 1.09

Values are expressed as Mean \pm SD (N=6). Statistical analysis was done by one way ANOVA.

P<0.001 when compared with Control.

DISCUSSION

Wounds are physical injuries that result in an opening or breaking of the skin.¹⁸ According to the Ayurveda, Vrana (wounds or ulcers) is the discontinuation of lining membrane that after healing leaves a scar for life. Exogenous wounds could be a trauma, such as Chinna (cut wound), Bhinna (perforated wound), Viddha (punctured wound), Kshata (lacerated wound), Picchita (contusion), and Ghrista (abrasion wound)¹⁹

Both aqueous and ethanolic extracts of vitex negundo were evaluated for wound healing in Wistar rats which exhibited a strong promotion in healing of wound. In excision wound model it has been observed that, the wound area contraction rate was higher in aqueous extract treated animals than that of ethanolic extract of *Vitex negundo*. In addition to the reduction in wound size, the test group also showed a faster rate of healing and scar formation. In incision model the breaking strength is a fundamental parameter which was measured experimentally by the amount of force required to disrupt it. According to Gouthamchandra *et al*, 2010²⁰ initial stage of wound requires little breaking strength as the clot alone holds the edges which increases rapidly as collagen deposition increases and cross-linkages are formed between the collagen fibers.

In the present studies the tensile strength employed was much higher in case of treated animals as compared to untreated animals. Increase in the tensile strength of treated wound was found to be an indication of wound healing possibly due to increase in collagen content and stabilization of the fibers.²¹ Hydroxyproline is one of the biomarker indicating the wound healing process which is always correlated with collagen production.²² In the present study the hydroxyproline content in the tissue was found to be 41.83 $\mu\text{g/gm}$ of tissue in the control untreated group while standard drug treated group showed 67.9 $\mu\text{g/gm}$ of tissue. Interestingly, the aqueous extract treated animals showed 94.48 $\mu\text{g/gm}$ of tissue while ethanolic extract showed 83.56 $\mu\text{g/gm}$ of tissue.

It has been reported earlier that collagen synthesis rapidly increases in the wound area soon after an injury.²³ The levels of collagen were determined from hydroxyproline content by the formula given by Neumann & Logan.²⁴ Among the four groups the highest collagen content was found in the aqueous extract treated animals followed by Standard drug and Ethanolic Extract treated animals. Glycosaminoglycan chains are formed from repeating hexosamine units which supports various protein structures including collagen in the tissues. In our studies hexosamine content of the excised tissue in the test drug treated group showed an increase as compared to the standard and control untreated group.

From the present studies it was evident that *Vitex negundo* has significant wound healing activity which can be useful for establishments of lead compounds in wound healing activity.

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