

## EVALUATION OF WOUND HEALING ACTIVITY OF CRUDE EXTRACT OF *VITEX NEGUNDO* ON RATS

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### Summary

The aim of the present study was to investigate the wound healing activity of the ethanol and aqueous bark extracts of *Vitex negundo*. The parameters of studied included rate of wound contraction and the period of epithelialization in excision wound model. The extracts were topically applied in the form gel (200 mg of extract incorporated with 100 gm of Carbopol 940 to get 0.2% w/w gel ) daily once times, starting from the excisions of skin on rats, till complete epithelialization. Wound area and period of epithelialization were used to evaluate the effect on wound healing. Both the ethanol and aqueous bark extracts promoted the wound healing activity significantly, when compared to the control group of animals. Ethanol extract possess better wound healing property than the aqueous extract. The present study thus provides a scientific rationale for the traditional use of this plant in the management of the wounds.

**Key words** *Vitex negundo*, wound healing activity, neomycin cream, epithelialization.

### Introduction

Wound healing is an important biological process involving tissue repair and regeneration. A wound is described as 'a break in the continuity of tissue, from violence or trauma' and is regarded as healed if there is a restoration of the wounded or inflamed tissue to normal condition. Wound healing can be classified into any of three types – healing by first intention, healing by second intention or healing by third intention, depending on the nature of the edges of the healed wounds. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts excrete collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells[1-3].

*Vitex negundo* (*Verbenaceae*) commonly known as Nirgundi. It is an aromatic large shrub or small tree about 3m in height with quadrangular branches and almost found throughout India, ascending to 1500m in the outer Himalaya, fairly common in waste lands, on road side, the banks or streams or in moist places near deciduous forests[4]. Almost all parts of *V. negundo* are used; the leaves and the barks are the most important in the field of medicine. The decoction of leaves is considered as tonic, vermifuge and is given along with long pepper in catarrhal fever. An infusion of the twigs is considered to be an effective therapy for headaches, dizziness, convulsions, coughs, mental unrest and is said to promote wakefulness. Leaves of this plant have been shown mosquito repellent effects as well as antiulcerogenic, antiparasitic, antimicrobial and hepatoprotective potentials[5,6]. The different activities of *V. negundo* extract have been reported such as anti-inflammatory, anticonvulsant, hepatoprotective, and bronchial relaxant actions[7]. There is no previous report on wound-healing activities of *V. negundo* in literature to the best of our knowledge and in this paper; we report the efficacy of *V. negundo* barks extract in the treatment of wounds.

### **Material and Methods**

**Plant Material:** The barks of *V. negundo* were collected from the Medicinal Garden of Oriental College of Pharmacy, Bhopal, Madhya Pradesh. The collected material was authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai. The barks were shade dried, reduced to coarse powder and stored in airtight container till further use.

**Preparation of extract:** 500 gm of powdered drug was packed in soxhlet apparatus and extracted with petroleum ether (60-80°C) to defat the drug. Defatted powdered drug was then extracted with ethanol. The ethanol extract was separated and the marc was further extracted with distilled water. The solvents were removed by distillation and the last traces of solvent being removed under reduced pressure.

**Drug formulation:** The ethanol and aqueous extracts were formulated as gel. The 200 mg of ethanol extract and aqueous extract were individually incorporated with 100 g of Carbopol 940 to get 0.2% (w/w) gel. These gels were applied topically over wounds of animals.

**Animals:** Healthy Wistar albino rats of either sex and of approximately the same age, weighing between 180–230 g were used for the study. They were individually housed, maintained in clean polypropylene cages and fed a standard diet and tap water *ad libitum*. The project proposal was approved by the Institutional Animal Ethical Committee (1349/ac/10/CPCSEA).

**Wound healing Activity:** An excision wound model was used for studying wound healing activity. Animals were anesthetized prior to and during creation of the wounds with 1 ml of intravenous ketamine hydrochloride (10 mg/kg body wt). This type of anesthesia prevents any movement of the animals at least for 2 h after the administration

of the anesthetic solution; therefore animals were left without being restrained. Hair was removed by shaving the nape of the neck of all rats. Ethanol (70%) was used as antiseptic for the shaved region before making the wound. A full thickness of the excision wound of circular area of 400mm<sup>2</sup> and 2mm depth was created along the markings using toothed forceps, scalpel and pointed scissors. The wound was left undressed to the open environment and no local or systemic anti-microbial agents were used. The rats were distributed in groups randomly and each mouse was placed in a separate cage. The wistar rats weighing 250-300 mg were divided into four groups and each group has six rats. The group I was untreated and considered as control group. The group II was treated with neomycin cream and treated as standard group. The group III and group IV were treated with ethanol and aqueous extracts respectively. The extract was applied daily once times, starting from the excisions of skin from rats, till complete epithelization. Wound area was measured by tracing the wound on a millimeter scale graph paper. The percentage of wound healing was calculated of original wound size (400 mm<sup>2</sup>) for each animal of group on predetermined days i.e., 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> days post-wounding for final analysis of results. Falling of scar leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelialization[8-12].

**Statistical Analysis:** The results are expressed as mean  $\pm$  SEM of six independent experiments. Statistical significance between group was evaluated by one-way analysis of variance (ANOVA) followed by Dunnet's test. A  $P < 0.05$  value was considered as statistically significant.

### **Results**

From the table 1 it reveals the improvement of wound healing induced by *V. negundo* bark of different gel formulation extracts (ethanol and aqueous extracts of 0.2% w/w gel) treated groups, untreated group (control) and neomycin (standard drug) treated group of animals. The mean percentage closure of wound area was calculated on the 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> post wounding days as shown in table 1. It was observed that the wound contracting ability of the both extracts gel and standard drug were significantly greater than that of the control. Ethanol and aqueous extracts gel formulation treated groups showed significant wound healing from the fourth day onwards, which was comparable to that of the standard drug treated group of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the ethanol extract treated group. The rate of wound contraction was found to reach a maximum on the 16<sup>th</sup> day in the treated groups. The ethanols extract treated animals showed faster epithelialization of wound (16 days) than the animals treated with aqueous extract (17 days). The period of epithelialization was 14 days in the case of standard drug.

### **Discussion**

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound

contraction is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. In the maturational phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue.

The wound-healing property of *Vitex negundo* 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 present study revealed that the ethanol and aqueous extract was found to possess better wound healing property.

**Table 1: Effect of ethanol and aqueous extracts on excision wound**

<b>Group</b>	<b>0 Days</b>	<b>4 Days</b>	<b>8 Days</b>	<b>12 Days</b>	<b>16 Days</b>	<b>Epithelialization period (days)</b>
Control	401.5±2.67 (0%)	291.2±2.14 (27.47%)	190.5±7.21 (52.55%)	65.7±8.34 (83.63%)	20.5±3.25 (94.89%)	23
Standard	400.8±2.35 (0%)	195.6 ±7.42* (51.9%)	54.7±1.51* (86.35%)	6.4±10.29* (98.40%)	0 (100%)	14
Ethanol Extract	401.3±2.91 (0%)	221.8 ±7.62* (44.72%)	65.9±6.43* (83.57%)	11.4±5.12* (97.15%)	0 (100%)	16
Aqueous Extract	400.5±3.21 (0%)	227.5±5.46* (43.19%)	98.7 ±8.17* (75.29%)	23.4±3.26* (94.15%)	0 (100%)	17

\* Significantly different from the control at P<0.05, n=6

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