

WOUND HEALING ACTIVITY OF METHANOLIC EXTRACT OF *EUPHORBIA NIVULIA* IN ALBINO RATS

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ABSTRACT

A medicinal plant are used with the intention of maintaining health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine. Plants have been used for traditional medicinal purpose for long before pre historic period. Recently world health organisation estimated that 80% of the people in worldwide on herbal medicines for some aspects of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as a medicinal plants.^[1] The main objective of the study was to evaluate the wound healing activity of methanolic extract of *euphorbia nivulia* in albino rats. The dried powdered *euphorbia nivulia* were for ointment preparation. The selected albino rats were used for wound healing animal model experiment, approved by institutional ethical committee. Both incision and excision wound were made by cutting a skin from the dorsal abdomen region. Wound healing parameters includes contraction rate of wound and regeneration of tissues at the site of wound. *Euphorbia nivulia* extract posses a significant wound healing ability when compared to control, using soframycin as standard drug. It was concluded that the wound healing activity is due to the presence of its active principles, which accelerate the healing process and confer tensile strength to the healed wound.

KEYWORDS: *Euphorbia nivulia*, incision and excision wound.

INTRODUCTION

A wound is characterised by the loss of epithelial integrity, disruption of normal structure and function of skin and its underlying tissues.^[2] specific immune responses of the body are responsible for the healing of wound, involving inflammatory cells, cytokines and extracellular matrix compounds. The whole process of wound healing are categorised into three phases such as inflammation, cellular proliferation and tissue remodelling.^[3] Inflammation both controls bleeding and prevents infection. The fluid engorgement allows healing and repair cells to move to the site of the wound. During the inflammatory phase, damaged cells, pathogens, and bacteria are removed from the wound area. These white blood cells, growth factors, nutrients and enzymes create the swelling, heat, pain and redness commonly seen during this stage of wound healing. Inflammation is a natural part of the wound healing process and only problematic if prolonged or excessive. The proliferative phase of wound healing is when the wound is rebuilt with new tissue made up of collagen and extracellular matrix. In the proliferative phase, the wound contracts as new tissues are built. In addition, a new network of blood vessels must be constructed so that the granulation tissue

can be healthy and receive sufficient oxygen and nutrients. In remodelling phase is when collagen is remodelled from type III to type I and the wound fully closes. The cells that had been used to repair the wound but which are no longer needed are removed by apoptosis, or programmed cell death. When collagen is laid down during the proliferative phase, it is disorganized and the wound is thick.^[4]

MATERIALS AND METHODS

Plant materials

The plant material was collected from young matured plant from the western hills village belt around Srivilliputtur. The whole plant was collected in bulk, washed to remove adhering dust, dried under shade and pulverised in a mechanical grinder. The powder was passed through sieve number No: 40 and used for further studies.

Preparation of the Extract

The freshly collected whole plant were chopped into pieces and shade under dried at room temperature (32-35°C) to constant weight for 5 days. The plant material was extracted with methanol by hot and cold maceration

method. The extract was concentrated and dried under reduced pressure.

Preparation of ointment (100gm)^[5,6]

Wool fat – 5 gm, hard paraffin – 5 gm, Cetostearyl alcohol – 5 gm, soft paraffin – 75gm and *Euphorbia nivulia* extract – 10 gm, were taken in a china dish and heated on water bath until they melted. Content were stirred continuously until became semisolid. Then it was colled and transferred into wide mouth container.

Animals

Wistar albino rats of either sex, weighing about 150–250 each, were used for the study. They were fed with standard chow (SAI ENTERPRISEI, CHENNAI) and water *ad libitum*. They were housed in polypropylene cages maintained under standard conditions (12 hour light - dark cycle; 25 ± 3 °C; 35–60% humidity). The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was cleared by same before beginning the experiment. Registration No: SARPC/IAEC/007/18-19. Albino rats weighing 150-200gm were used for wound healing activity, they were physically active and consuming food, water in a regular way.

Preliminary Phytochemical Studies

The various extracts of *Euphorbia nivulia* obtained were subjected to qualitative analysis to test the presence of vaious phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, saponins, terpenes, phenols, proteins, tannins etc.

Wound Healing Activities

Our plan of work is to prepare the alcoholic extract of selected plant *Euphorbia nivulia* to perform biological screening to evaluate the therapeutic potential of the plants considering their traditional usage.

Experimental Design^[7,8]

Excision wound model.

Circular wounds of approximately 10 mm diameter were be inflicted on the cleared skin by cutting under anaesthesia. The areas of the wounds were be measured (sq. mm) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it. It is taken as the initial wound area reading. Group-I -Served as negative control, which received Simple ointment I.P. Group-II-Served as positive control to which Framycetin sulphate (1 % w/w in Simple ointment I.P.) were applied topically.Group-

III-Animals treated with the *Euphorbia nivulia* extract (5% w/w) in a similar manner.Group-IV- Animals treated with the *Euphorbia nivulia* extract (10% w/w) in a similar manner. The drug was applied once a day after cleaning the wound with surgical cotton wool.

$$\% \text{ Wound contraction} = \frac{\text{Healed Area}}{\text{Total Area}} \times 100$$

Incision Wound Model

Light incisions were made on the cleared surface by cutting the skin of the animals under anaesthesia. The wounds were created at a length of about 1.5 cm. After the incision, the parted skin were kept together and stitched with black silk at both the ends of the created wound. The test samples were applied as above in a similar manner. Group-I- Served as negative control, which received Simple ointment I.P.Group-II-Served as positive control to which Nitrofurazone (0.2 % w/w in Simple ointment I.P.) applied topically.Group-III- Animals treated with the *Euphorbia Nivulia* extract (5% w/w) in a similar manner.Group-IV-Animals treated with the *Euphorbia Nivulia* extract (10% w/w) in a similar manner. The drug was applied once a day after cleaning the wound with surgical cotton wool. The tensile strength were measured by using densitometer on 12th post wounding day.

Measurement of wound area

The progressive changes in wound area were measured on 1st, 4th, 8th and 12th post wounding day. The percentage of wound contraction were calculated from the days of measurements of wound area. The progressive changes in wound area were recorded in mm² by tracing the wound boundaries around it on a transparent paper.

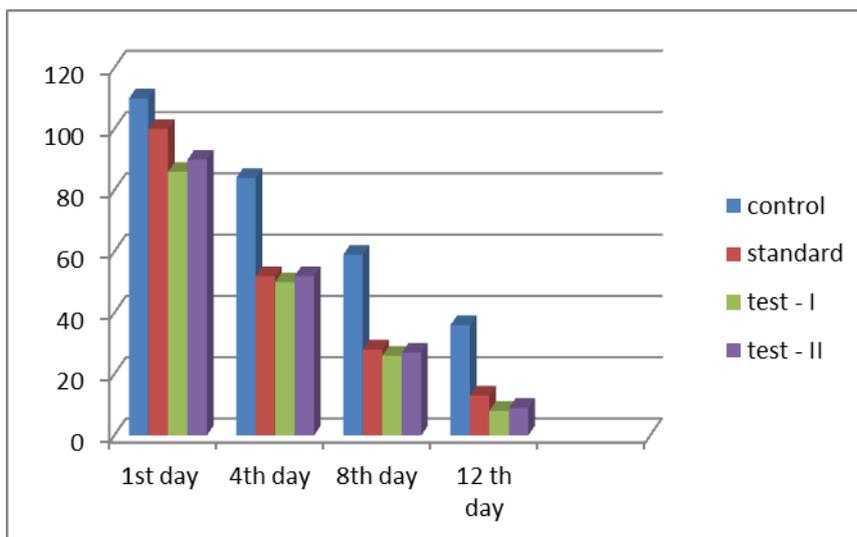
RESULTS AND DISCUSSON

The results of excision wound model are showed in table –I. The methanolic extract of *Euphorbia nivulia* showed a significant wound healing property when compared to control in excision wound model. It is observed that the wound healing ability of the 5% and 10% (w/w) extract ointment treated groups showed a significant wound healing from 4th day onwards. Similarly the tensile strength was greater when compare to control in incision wound model. The present investigation describes the unique features of the whole plant of methanolic extract of *euphorbia nivulia* with respect to its potential wound healing capacity in rats.

Results of Wound Healing Activity of Methanolic Extract of *Euphorbia Nivulia*.

Group	1 st Day	4 th Day	8 th Day	12 th Day	Epithelisation In Days
Control	110.0 ± 3.5	84.23 ± 0.30	59.20 ± 1.30	36.10 ± 3.0	20.15 ± 0.20*
Standard	100.42 ± 5.0	52.0 ± 3.0	28.0 ± 1.28	13.84 ± 2.0	19.42 ± 0.30*
Test –I	86.20 ± 3.5	50.45 ± 2.09	26.10 ± 1.20	8.61 ± 1.3	19.05 ± 0.33*
Test –II	90.10 ± 2.5	52.3 ± 5.0	27.20 ± 5.0	9.01 ± 3.0	18.68 ± 0.21*

Wound healing activity of *Euphorbia nivulia*, each values are expressed as mean \pm SEM (n=4) *P< 0.01 compared with the control (ANOVA test).



Graph showing contraction of wound in percentage

Results of Wound Healing Activity of Methanolic Extract of *Euphorbia nivulia*

Group	Epithelisation In Days (mean time in days)	Tensile strength (g/mm ²) 12 th days
Control	22.15 \pm 1.25*	320.20 \pm 3.251*
Standard	20.22 \pm 0.50*	521.10 \pm 3.210*
Test – I	20.07 \pm 0.03*	482.10 \pm 2.100*
Test – II	19.28 \pm 0.11*	421.00 \pm 3.850*

Wound healing activity of *Euphorbia nivulia*, each values are expressed as mean \pm SEM (n=4) in each group.*P< 0.01 compared with the control (ANOVA test)

CONCLUSION

The present study revealed the Methanolic extract of *Euphorbia Nivulia* are having significant wound healing activity in rats, it may be due to the presence of its active principles, which accelerate the healing process and confer tensile strength to the healed wound.

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