

EVALUATION OF WOUND HEALING ACTIVITY OF ETHANOL EXTRACT OF *DIOSPYROS MELANOXYLON* (ROXB.) LEAVES

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ABSTRACT

Excision wound model was assessed for wound healing activity. Three groups of six animals in each group were anesthetized by open mask method with anesthetic ether. The rats were depilated on back. One excision wound was inflicted by cutting away 500 mm² full thickness of skin on ethanol sterilized dorsal thoracic region of rats. The wound was left undressed to the open environment. Group I: served as control and received gum tragacanth suspension orally. Group II: served as standard and treated externally with Povidone iodine ointment. Group III: treated with ethanol extract of the *Diospyros melanoxylon* Ointment. The ointment was topically applied daily till the complete epithelialization starting from the day of operation. The parameters studied were wound closure and time of epithelialization. The wounds were traced on mm² graph paper on the days of 4th, 8th, 12th and 16th and thereafter on alternate days until healing were complete. The percentage of wound closure was calculated. The ethanol extract treated animals showed significant epithelialisation and wound contraction of the excision wound. Acute toxicity studies were conducted for the ethanol extract of *Diospyros melanoxylon* leaves. The maximum tolerated dose was found to be 2000 mg/kg b.w when the extract was administered orally.

Keywords: Wound healing, Wound closure, Time of epithelialization, Povidone iodine ointment.

INTRODUCTION

Medicinal herbs have been known from millennia and are highly esteemed all over the world as a rich source of therapeutic agents for prevention of diseases and ailments. India in this regard has a very unique position in the world, where a number of recognized indigenous systems of medicine namely Ayurveda, Siddha, Unani, Homeopathy, Yoga and Naturopathy are practiced and utilized for the health care of the people¹. In this direction of research many species of plants have been exploited and are being screened for their medicinal efficacy. Among them many members of *Ebenaceae* have been exploited for economical, medicinal and aesthetic values. In this family the genus *Diospyros* includes mostly trees rarely shrubs, distributed in the warmer part of the world. For the present investigation *Diospyros melanoxylon* Roxb, has been selected for evaluation of Wound healing property in view of its wide use in traditional medicine against various common diseases such as diabetes² anemia, inflammation of spleen, hypotensive and used as carminative, diuretic. This plant has been widely reported to have several medicinal properties in traditional form of medicine.

Earlier studies carried out have proved its anticandidal activity, antihyperglycemic activity, antimicrobial activities of the bark. Except these studies, so far no other pharmacological investigations have been reported on the leaves. Hence a systematic pharmacognostical and pharmacological investigation of ethanol extract of leaves of *Diospyros melanoxylon* (Roxb.), Based on the traditional uses as well as earlier work done on this plant Wound healing property is selected for pharmacological screening of the leaf extract using Excision wound animal model. Acute toxicity studies were conducted for the ethanol extract of *Diospyros melanoxylon* leaves when the extract was administered orally.

MATERIALS AND METHODS

Collection and authentication of *Diospyros melanoxylon* leaves

Diospyros melanoxylon (Roxb.) leaves were collected from stationghanpur village of Warangal District, Andhra Pradesh. This plant is identified and authenticated by Dr. S. Srinivas Rao M. Sc., Ph.D, Dept of Botany, S.L.N.S Degree and PG College, Bhongir, Andhra Pradesh, India. The leaves of *Diospyros melanoxylon* (Roxb.) were isolated, chopped into small pieces and dried under shade at room temperature for seven days.

Preparation of ethanol extract of leaves of *Diospyros melanoxylon* (Roxb.)

The leaves are washed with absolute ethanol to avoid the microbial growth, and were dried at open air under the shade, cut in to small pieces and powdered mechanically. The powdered material of leaves of *Diospyros melanoxylon* (Roxb.) was refluxed successively with the 250ml of ethanol in a Soxhlet extractor for 48 hrs. The solution so obtained was transferred to china dish and then allowed for drying. The extract so obtained was thoroughly washed with Ethyl acetate so as to remove the chlorophyll and was dried by using desiccators in order to remove the moisture content. The extract so obtained from ethanol was labeled, weighed and used for various studies.

Ointment preparation for topical application

An alcohol free extract of *Diospyros melanoxylon* leaf was used for the preparation of the ointment for topical application. A 0.5% (W/W) of extract ointment was formulated using soft paraffin base.

Experimental animals

Albino rats (Wistar) weighing 150-200 g either sex were used in this study. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27 °C ± 2 °C under 12 hrs dark / light cycle. They were fed with standard rat feed and water ad libitum was provided. The litter in the cages is renewed thrice a week to ensure hygiene and maximum comfort for animals. Ethical clearance for handling the animals is obtained from the Institutional animal ethical committee prior to the beginning of the project work from Institutional Animal Ethical Committee of SASTRA University, Thanjavur, Tamilnadu. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India. (Approval no: 86/ SASTRA/ IAEC/ RPP).

Acute toxicity studies as per OECD Guideline 425³

In the assessment and evaluation of the toxic characters of the substance, determination of acute oral toxicity is usually an initial step. LD (medium lethal 50 dose), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50 % of animals when administered by the oral route. The LD value 50 expressed in terms of test substance per unit weight of test animal (mg/kg). Up and down (UDP, stair case method) was performed for acute toxicity. In this method animals of a single sex, usually females, with the first animal receiving a dose just below the best estimate of

the LD50. Depending on the outcome for the previous animal, the dose for the next is increased or decreased, usually by the factor of 3.2.

Healthy Wistar rats weighing between 150-180 g were used to carry out acute toxicity studies by the 'staircase' method. Ethanol extract of *Diospyros melanoxylon* in 0.5 % tween 80 was administered orally *Diospyros melanoxylon* in 0.5 % tween 80 was administered orally to three animals in each group. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days to check the mortality rate.

Chemicals

All chemicals and reagents used were of analytical grade.

Model design

The design of wound healing activity was performed by one model

1) Excision wound model⁴⁻⁸

Three groups of six animals in each group were anesthetized by open mask method with anesthetic ether. The rats were depilated on back. One excision wound was inflicted by cutting away 500 mm² full thickness of skin on ethanol sterilized dorsal thoracic region of rats. The wound was left undressed to the open environment. This model was used to monitor wound contraction.

Group I: served as control and received gum tragacanth suspension (1 %) orally.

Group II: served as standard and treated externally with 0.2 % w/w Povidone iodine ointment.

Group III: treated with ethanol extract of the *Diospyros melanoxylon* Ointment.

The ointment was topically applied daily till the complete epithelialization starting from the day of operation. The parameters studied were wound closure and time of epithelialization. The wounds were traced on mm² graph paper on the days of 4th, 8th, 12th and 16th and thereafter on alternate days until healing were complete. The percentage of wound closure was calculated. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialization.

Measurement of wound area⁹

The progressive changes in wound area were measured planimetrically by tracing the wound margin on a graph paper every alternate day. The changes in healing of wound i.e measurement of wound on graph paper were expressed as unit (mm²). Wound contraction was expressed as percentage reduction of original wound size.

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

Statistical Analysis

All data will be expressed as mean ± SEM. The statistical significance between groups will be compared using one way ANOVA, followed by Dunnet's t-test (multiple comparisons). P < 0.05 will be considered as significant.

RESULTS

Wound healing by excision wound method in rats

Excision wound showed that there is almost complete healing on the 16th post wounding day with ethanol extract (Fig. 1 and graph 1). The period of wound contraction and period of epithelialization due to ethanol extract of *Diospyros melanoxylon* leaves are studied.

The administration of this extract *Diospyros melanoxylon* accelerated the progression of wound healing by 8th day i.e. (52.78 ± 0. 41 %) compared with control (27.34 ± 0. 29 %) are shown in (Table 1).

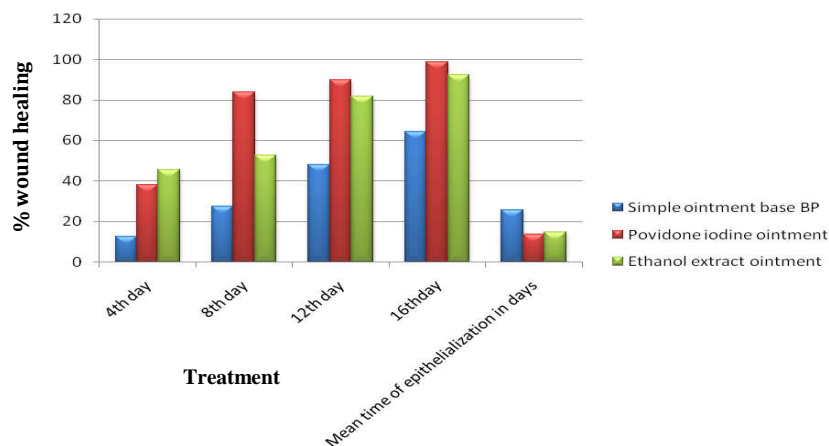
Acute toxicity study as per OECD guideline 425

Acute toxicity studies were conducted for the ethanol extract of *Diospyros melanoxylon* leaves. The maximum tolerated dose was found to be 2000 mg/kg b.w when the extract was administered orally. As per the OECD (International toxicity testing) guidelines the maximum therapeutic dose is 1/10th of maximum tolerated dose, hence the therapeutic dose selected for the extracts was 200 mg/kg body weight. After treatment with the extract, it is observed that the animal did not show any variations in any of the following indicators viz., body weight, behaviour, loss of appetite, hyperthermia /hypothermia, erected hair etc. The analysis of the above parameters indicates that the dose selected will not interfere with any of the body functions while performing various pharmacological investigations.

Table 1: Effect of topical application of ethanol extract of leaves of *Diospyros melanoxylon* (Roxb.) on excision wound model

Groups	4 th day	8 th day	12 th day	16 th day	Mean time of epithelialization in days
Simple ointment base BP(Control)	12.59 ± 0. 17	27.34 ± 0. 29	48.00 ± 0. 35	64.49 ± 0. 31	25.83 ± 0.40
Povidone iodine ointment (Ref. Std) (0.2 %w/w)	37.98 ± 0. 41**	83.93 ± 0. 44***	90.01 ± 0. 54***	98.65 ± 0.0 0***	13.57 ± 0.31**
Ethanol extract ointment (0.5%w/w)	45.41 ± 0. 34***	52.78 ± 0. 41**	81.75 ± 0. 21***	92.51 ± 0. 11***	14.63 ± 0.45**

Result were statistically significant compared with the corresponding control values (simple ointment) and P-values were calculated (n=6) *P < 0.0001 by one way ANOVA followed by Dunnet's t-test.



Graph 1: Effect of ethanol extract of *Diospyros melanoxylon* (Roxb.) ointment (0.5% w/w) and Povidone iodine (0.2%w/w) in wound healing by excision wound model.

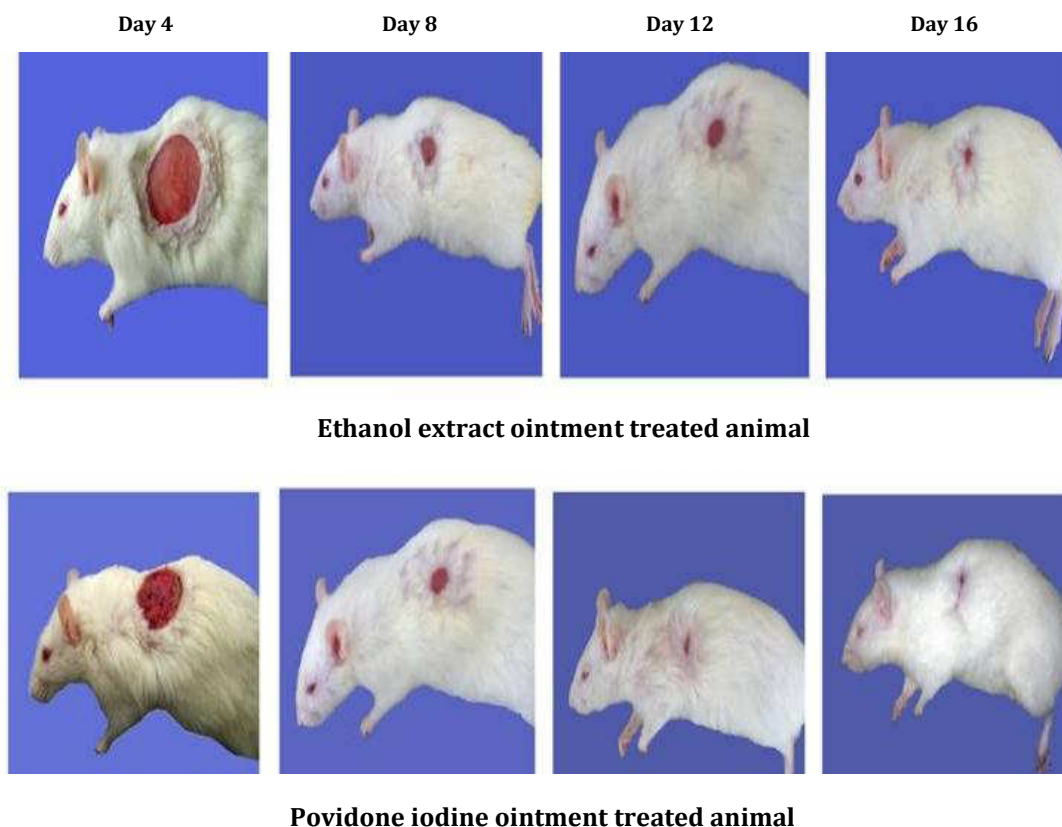


Fig. 1: Photographic evaluation of wounds; Faster rate of wound healing was observed in the ethanol extract of *Diospyros melanoxylon* treated wounds on 16th day compared with the standard Povidone iodine ointment.

DISCUSSION

The current accepted modern medicine or allopathic has gradually developed over the years by scientific and observational efforts of scientists. However, the origin of its development remains rooted in traditional medicine and therapies. The knowledge of ancient medicine has been the source for modern medicine and will remain as one of the important sources for future medicine and therapeutics.

Wound is defined as the disruption of the cellular and anatomic discontinuity of a tissue. Wound may be produced by physical, chemical, thermal, microbial or immunologic insult to the tissue. Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent of each other. It 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 contracture is a process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. Collagen, the major component which strengthens and supports extracellular tissue is composed of amino acids, hydroxyproline, which has been used as a biochemical marker for tissue collagen.

Wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under healing or over healing. Therefore, the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences¹⁰. Attention should be directed towards discovering an agent, which may accelerate wound healing either when it is progressing normally¹¹ or when it is suppressed by various agents like corticosteroids¹², anti-neoplastics¹³ or non-steroidal anti-inflammatory agents. Medical treatment of wound includes administration of drugs either locally (topical) or systemically (oral

or parenteral) is an attempt to aid wound repair¹⁴. Wound contraction is the process of shrinkage of the area of the wound. It mainly depends on the repairing ability of the tissue, type and extent of damage and general health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema and small new blood vessels.

The period of wound contraction and period of epithelialization of the animals treated with ethanol extract of leaf of the plant *Diospyros melanoxylon* significantly increased when compared to the control. Higher percentage of wound closure was observed in the group of animals treated with *Diospyros melanoxylon* on day 16th day of the experiment compared to the standard.

CONCLUSION

The present studies provide the scientific evidence for the presence of several beneficial medicinal properties in the plant material *Diospyros melanoxylon* (Roxb.) belonging to family *Ebenaceae*.

Thus it may be concluded that leaf *Diospyros melanoxylon* ointment extract have the potential to satisfy all requirements of an ideal dressing material in that it provides an environment at the surface of the wound in which healing took place at the maximum rate with an acceptable cosmetic appearance and also provides a rationale for the use of *Diospyros melanoxylon* preparations in the traditional system of medicine to promote wound healing.

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