

# Asian Journal of Phytomedicine and Clinical Research

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## WOUND HEALING ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *DIOSPYROS MELANOXYLON* (ROXB.) BARK

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### ABSTRACT

The model used for wound healing activity is excision wound. The animals were divided into three groups of six animals. The each group was anesthetized by open mask method with anesthetic ether. The rats were depilated on back. One excision wound was inflicted by cutting away 500 mm<sup>2</sup> 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 hydroalcoholic 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 hydroalcoholic extract treated animals showed significant epithelialisation and wound contraction of the excision wound.

### KEY WORDS

Wound healing, Wound closure, Epithelialization and Povidone iodine ointment.

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### INTRODUCTION

Plants have been used in treating human diseases for thousands of years. The use of medicinal plants is not just a custom of the distant past. Perhaps 90 % of the world's population still relies completely on raw herbs and unrefined extracts as medicines<sup>1</sup>. A 1997 survey showed that 23 % of Canadians have used herbal medicines. In addition, as much as 25 % of modern pharmaceutical drugs contain plant ingredients<sup>2</sup>. The medicinal qualities of plants are of course due to chemicals. Plants synthesize many compounds called primary metabolites that are

critical to their existence. These include proteins, fats and carbohydrates that serve a variety of purposes indispensable for sustenance and reproduction, not only for the plants themselves, but also for animals that feed on them. 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 diabetes<sup>3</sup> 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. This endemic plant of India and Ceylon is used in various ways. Besides being the source of Indian ebony, its wood is also utilized for making boxes, combs, ploughs and beams. The fruits are eaten and sold commercially. The bark is burnt by tribals to "cure" small-pox. The seeds are prescribed as cure for mental disorders, palpitation of heart and nervous breakdown. Above all, the leaves of this plant constitute one of the most important raw materials of the "Bidi" (Indian cheap smoke) industry.

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 bark. Hence a systematic pharmacognostical and pharmacological investigation of hydroalcoholic extract of bark 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 bark extract using excision wound animal model. Acute toxicity studies were conducted for the hydroalcoholic extract of *Diospyros melanoxylon* bark when the extract was administered orally.

## MATERIALS AND METHODS

### Preparation of hydroalcoholic extract of bark of *Diospyros melanoxylon* (Roxb.)

The bark is 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 bark of *Diospyros melanoxylon* (Roxb.) was refluxed successively with the 250ml of hydroalcohol 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 hydroalcohol was labeled, weighed and used for various studies.

### Ointment preparation for topical application

An alcohol free extract of *Diospyros melanoxylon* bark 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 200 - 220 gm 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 425

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 <sup>4</sup>. In this method animals of a single sex, usually females, with the first animal receiving a dose just below the best estimate of the LD<sub>50</sub>. 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 200 - 220 gm were used to carry out acute toxicity studies by the 'staircase' method. Hydroalcoholic 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.

#### Experimental design

The design of wound healing activity was performed by Excision wound model.

#### Excision wound model

Three groups of six animals in each group were anesthetized by open mask method with anesthetic ether. The rats were depilated on back<sup>5</sup>. One excision wound was inflicted by cutting away 500 mm<sup>2</sup> 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 <sup>6, 7</sup>.

**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 hydroalcoholic 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 mm<sup>2</sup> graph paper on the days of 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> 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 <sup>8</sup>.

#### Measurement of wound area

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 (mm<sup>2</sup>). Wound contraction was expressed as percentage reduction of original wound size <sup>9</sup>.

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

#### Statistical Analysis

All data will be expressed as mean  $\pm$  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 16<sup>th</sup> post wounding day with hydroalcoholic extract was shown in Figure No.1 and 2. The period of wound contraction and period of epithelialization due to hydroalcoholic extract of *Diospyros melanoxylon* bark are studied<sup>10</sup>. The administration of this extract *Diospyros melanoxylon* accelerated the progression of wound healing by 8<sup>th</sup> day i.e. (52.78  $\pm$  0. 41 %) compared with control (27.34  $\pm$  0. 29 %) was shown in Table No.1.

#### Acute toxicity study as per OECD guideline 425

Acute toxicity studies were conducted for the hydroalcoholic extract of *Diospyros melanoxylon* bark. 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 1/10<sup>th</sup> 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.

### DISCUSSION

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

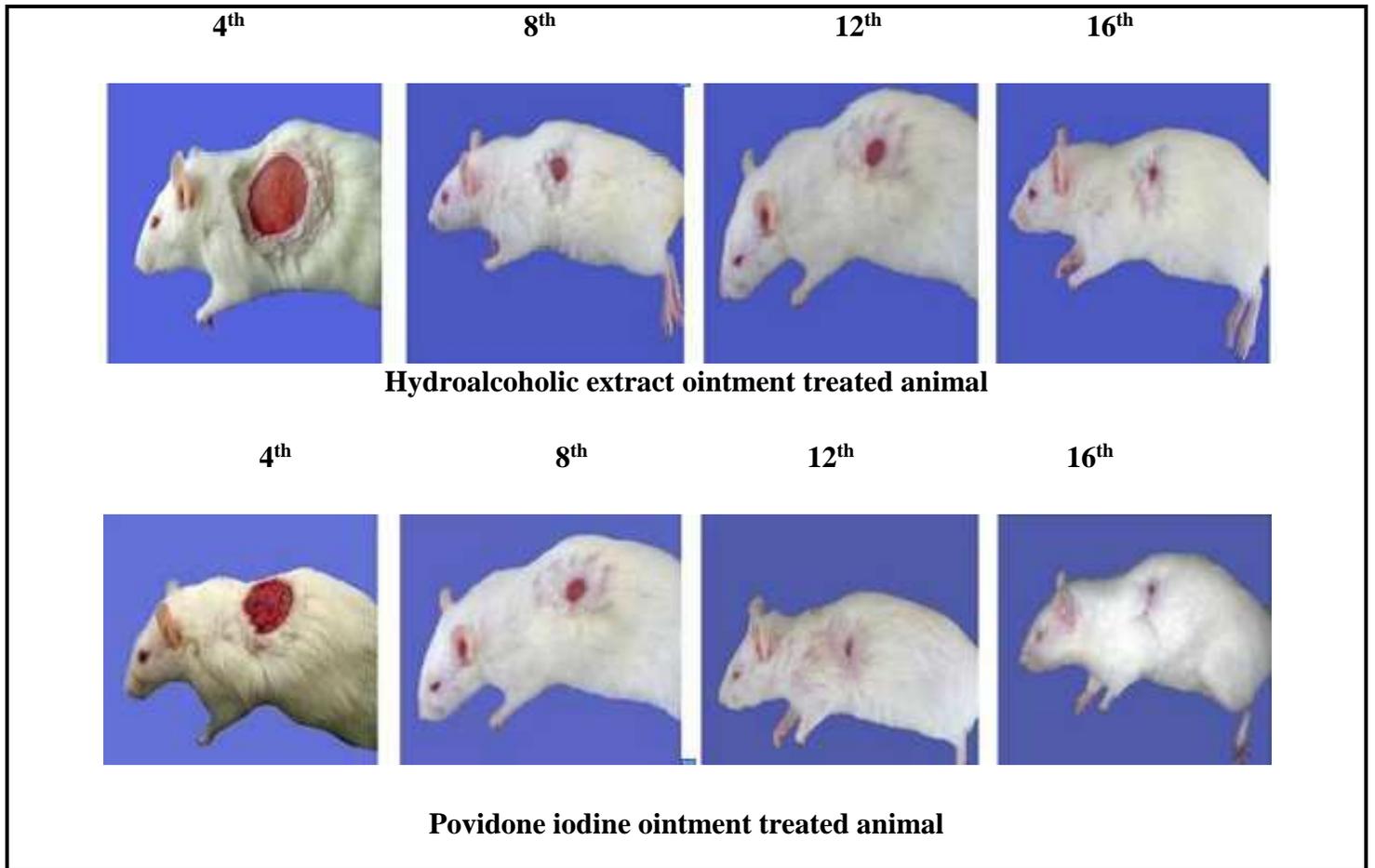
complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. Collagen, the major component which strengthens and supports extracellular tissue is composed of amino acids, hydroxyproline, which has been used as a biochemical marker for tissue collagen. Medical treatment of wound includes administration of drugs either locally or systemically is an attempt to aid wound repair<sup>11</sup>. 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 hydroalcoholic extract of bark 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 16<sup>th</sup> day of the experiment compared to the standard.

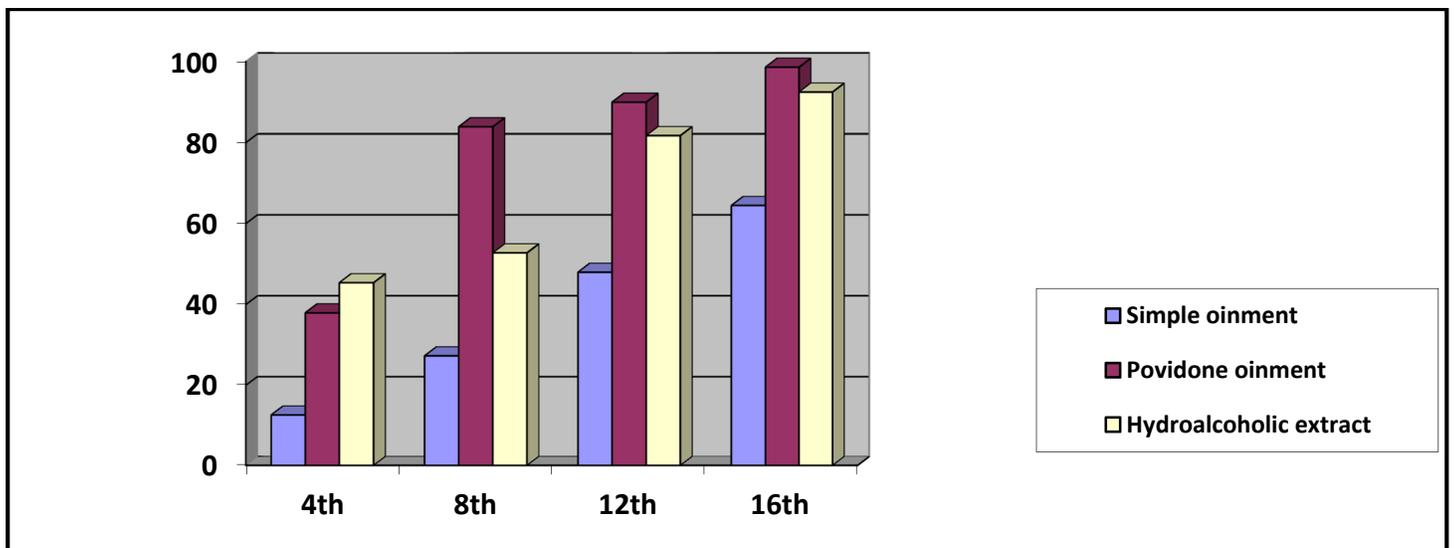
**Table No.1: Effect of topical application of hydroalcoholic extract of bark of *Diospyros melanoxylon* (Roxb.) on excision wound model**

S.No	Groups	4 <sup>th</sup> day	8 <sup>th</sup> day	12 <sup>th</sup> day	16 <sup>th</sup> day	Mean
1	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
2	Povidone iodine ointment (Ref. Std) (0.2 % w/w)	37.98 ±0. 41**	83.93 ±0. 44***	90.01 ±0.54***	98.65 ± 0.00***	13.57 ± 0.31**
3	Hydroalcoholic + 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.



**Figure No.1: Effect of extract *Diospyros melanoxylon* and Povidone iodine on rats**



**Figure No.2: Effect of topical application of extract of *Diospyros melanoxylon* (Roxb.)**

## CONCLUSION

The bark of hydroalcoholic extract of *Diospyros melanoxyton* (Roxb.) belonging to family *Ebenaceae* was concluded that the 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 melanoxyton* preparations in the traditional system of medicine to promote wound healing.

## ACKNOWLEDGEMENT

The authors are sincerely thankful to the A.M.Reddy Memorial College of Pharmacy, Andhra Pradesh for providing the facilities to carry out this research work.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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**Please cite this article in press as:** Nagaraju Gollapalli. et al. Wound healing activity of hydroalcoholic extract of *diospyros melanoxyton* (roxb) Bark, *Asian Journal of Phytomedicine and Clinical Research*. 1(1), 2013, 34 - 39.