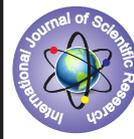


EFFICACY OF KARANJ OIL IN WOUND HEALING IN WISTAR RATS



Surgery

KEYWORDS: wound healing and management

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ABSTRACT

INTRODUCTION- To evaluate the healing potential of karanj oil in wound healing.
AIMS AND OBJECTIVES

To compare wound healing in wistar rats microscopically by checking the rate of collagen formation, neovascularization and epithelialization within 3 groups.

- a) Betadine followed by karanj oil
- b) Betadine only
- c) Natural healing.

MATERIAL AND METHODS

Male Wistar rats - 200 and 220 g.

Changes observed were recorded on day 0, 4, 8, 12, 16 and 20.

OBSERVATION AND RESULT

Karanj Oil (20%) for 20 days resulted in significant ($p < 0.0001$) decrease in the Epithelialization period (12.00 ± 1.414 days) when compared with vehicle control (19.80 ± 0.83 days) group animals ($p < 0.001$). Karanj Oil (20%) showed significant wound contraction ($p < 0.0001$).

Histopathology - suggestive of scab formation, neovascularisation, congestion, capillary formation, fibroblast proliferation, infiltration of macrophages.

CONCLUSION

Topical application of Karanj oil

- Decreases Epithelialization period
- Increases wound contraction

INTRODUCTION

Wound is described as a discontinuation in the epithelial integrity of the skin or living tissue. Any agent that generates stress and injury may give rise to wound, and thus healing is necessary for survival mechanism as well as to maintain normal anatomical structure and function. Since time immemorial, wounds have been a major cause of morbidity and disfigurement. Karanj oil is a Hindi name of Pongamia oil which is obtained from the seeds of the *Pongamia Pinnata* (also known as *Millettia pinnata* or *Pongamia glabra*). In Ayurveda and Siddha medicine *Pongamia Pinnata* used to treat various kinds of diseases/disorders.

AIM

To compare the efficacy of topical application of karanj oil in wound healing in ulcers in wistar rats.

OBJECTIVES

To compare skin wound healing in wistar rats macroscopically by checking the rate of collagen formation, neovascularization and epithelialization within 3 groups.

- a) Betadine followed by karanj oil
- b) Betadine only
- c) Natural healing.

MATERIAL AND METHODS

Animals

Male Wistar rats weighing between 200 and 220 g were procured from National Institute of Bioscience, Pune, India. The animals were housed at an ambient temperature ($25 \pm 2^\circ\text{C}$) and relative humidity (45-50%) and light and dark cycle (12 h light/dark). The animals had access to pellet diet (Chakan oil mills, Pune) and water *ad libitum* throughout the experimental period. The animals were acclimatized for a period of 1 week and were kept under pathogen-free conditions.

Research protocol approval

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for Control and Supervision on Experimental Animals (CPCSEA), India.

Excision wound model

Animals were anaesthetized with a dose of 80 mg/kg of ketamine (i.p.) and the back the animals were shaved. An impression was made on the back of the neck on the anaesthetized rat. Excision wounds of size 500 mm² and depth 2 mm were made by cutting out a layer of skin from the shaven area. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline.⁶² The entire wound was left open. The study comprised three different groups containing 6 animals in each group. The test drug Karanj oil 20% were applied topically.

Sr. No.	Group	No. of animals
1.	Vehicle control animals: received injury for wound formation but will not receive any treatment locally	6
2.	Karanj oil (20%) treated animals: received injury for wound formation and treated with povidine iodine (betadine) followed by karanja oil	6
3.	Povidine iodine treated animals: received injury for wound formation and treated with povidine iodine (betadine) only	6

Measurement of wound area

The changes in wound area were observed and recorded by a camera on day 0, 4, 8, 12, 16 and 20. Wound area was measured by using image J software and adobe Photoshop to determine the area.

Measurement of wound contraction

Wound contraction was calculated as percentage of the reduction in original wound area size. It was calculated by using the following formula:

Percentage wound contraction

$$= \frac{\text{Initial area of wound} - \text{Nth day area of wound}}{\text{Initial area of wound}} \times 100$$

Measurement of wound index

Wound index was measured daily with an arbitrary scoring system. Determination of period of Epithelialization
Falling of scab leaving no raw wound behind was taken as the end of complete Epithelialization and the days required for this was taken as period of Epithelialization.

Histopathological examination

A specimen sample of tissue was isolated from the skin of each group of rat on day0, 4, 8, 12, 16 and 20 to evaluate for the histopathological alterations. Samples were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5 µm and stained with haematoxylin and eosin (H and E). Photomicrographs were captured at a magnification of 100x. Sections were analysed and scored for epidermal or dermal remodelling. Scab formation, congestion, capillary formation, fibroblast proliferation, infiltration of macrophages, oedema in dermis were analysed to score the epidermal or dermal remodelling.

STATISTICAL METHODS

Bonferroni's multiple comparison test
t-test

OBSERVATION AND RESULT

Effect of Karanja oil on wound area in excision wound

The wound area (mm²) in all animal groups was measured on days 0, 4, 8, 12, 16 and 20. In Vehicle Control, Karanja oil (20%) groups and Povidine iodine, wound area showed non-significant difference at 0 day which indicate the same size of wound area before treatment started in each group. There was no significant difference in the wound area of vehicle control rats, Karanja oil (20%) treated rats (427.83±20.83mm²) or Povidine iodine (388.83±32.25) on day 0. On day 4 no significant reduction in wound size was seen. The significant reduction (p< 0.0001) in wound area was observed in the Karanja oil (20%) treated rats on 8th day (241.16±64.65 mm²) when compared with vehicle control rats (379.00±20.93mm²). This reduction in wound area was more significant (p<0.001) in Karanja oil (20%) treated rats on 20th day (21.83±44.04mm²) than on 8th day when compared with vehicle control rats (267.83±29.56 mm²). On day 20th, the efficacy of different treatments for wound closure were as follow:

- Karanja oil (20%) (21.83±44.04mm²)
- >
- Povidine iodine (63.33±23.67 mm²)
- >
- Vehicle Control (267.83±29.56 mm²).

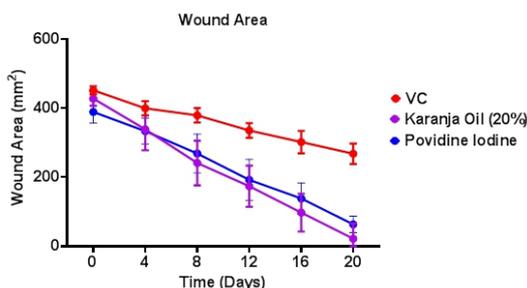


Table 1. Bonferroni's multiple comparisons of Karanj oil treatment on wound area (mm2).

Bonferroni's multiple comparisons test	Significant?	P Value
At 0 Day		
Vehicle Control vs. Karanj Oil (20%)	No	> 0.9999
Vehicle Control vs. Povidine Iodine	No	0.0711
Karanj Oil (20%) vs. Povidine Iodine	No	0.6919
At 4 Day		
Vehicle Control vs. Karanj Oil (20%)	No	0.0885
Vehicle Control vs. Povidine Iodine	Yes	0.0488
Karanj Oil (20%) vs. Povidine Iodine	No	> 0.9999
At 8 Day		
Vehicle Control vs. Karanj Oil (20%)	Yes	< 0.0001
Vehicle Control vs. Povidine Iodine	Yes	< 0.0001
Karanj Oil (20%) vs. Povidine Iodine	No	> 0.9999
At 12 Day		
Vehicle Control vs. Karanj Oil (20%)	Yes	< 0.0001
Vehicle Control vs. Povidine Iodine	Yes	< 0.0001
Karanj Oil (20%) vs. Povidine Iodine	No	> 0.9999
At 16 Day		
Vehicle Control vs. Karanj Oil (20%)	Yes	< 0.0001
Vehicle Control vs. Povidine Iodine	Yes	< 0.0001
Karanj Oil (20%) vs. Povidine Iodine	No	0.5962
At 20 Day		
Vehicle Control vs. Karanj Oil (20%)	Yes	< 0.0001
Vehicle Control vs. Povidine Iodine	Yes	< 0.0001
Karanj Oil (20%) vs. Povidine Iodine	No	0.5641

Table 2. Bonferroni's multiple comparisons test for wound contraction.

Bonferroni's multiple comparisons test	Significant?	p Value
At 0 Day		
Vehicle Control vs. Karanj Oil (20%)	No	> 0.9999
Vehicle Control vs. Povidine Iodine	No	> 0.9999
Karanj Oil (20%) vs. Povidine Iodine	No	> 0.9999
At 4 Day		
Vehicle Control vs. Karanj Oil (20%)	No	0.1665
Vehicle Control vs. Povidine Iodine	No	> 0.9999
Karanj Oil (20%) vs. Povidine Iodine	No	> 0.9999
At 8 Day		
Vehicle Control vs. Karanj Oil (20%)	Yes	< 0.0001
Vehicle Control vs. Povidine Iodine	Yes	0.0496
Karanj Oil (20%) vs. Povidine Iodine	Yes	0.0011
At 12 Day		
Vehicle Control vs. Karanj Oil (20%)	Yes	< 0.0001
Vehicle Control vs. Povidine Iodine	Yes	0.0001
Karanj Oil (20%) vs. Povidine Iodine	Yes	0.0039
At 16 Day		
Vehicle Control vs. Karanj Oil (20%)	Yes	< 0.0001
Vehicle Control vs. Povidine Iodine	Yes	< 0.0001
Karanj Oil (20%) vs. Povidine Iodine	Yes	< 0.0001
At 20 Day		
Vehicle Control vs. Karanj Oil (20%)	Yes	< 0.0001
Vehicle Control vs. Povidine Iodine	Yes	< 0.0001
Karanj Oil (20%) vs. Povidine Iodine	Yes	< 0.0001

Effect of Karanj oil on epithelization period

Treatment with Karanj Oil (20%) for 20 days resulted in significant ($p < 0.0001$) decrease in the Epithelialization period (12.00 ± 1.414 days) when compared with vehicle control (19.80 ± 0.83 days) group animals ($p < 0.001$). Karanj Oil (20%) showed highly significant wound contraction ($p < 0.0001$). On day 20th, the efficacy of different treatments for wound Epithelialization were as follow:

Karanj oil (20%) (12.00 ± 1.41 days)
 >
 Povidine iodine (16.80 ± 0.83 days)
 >
 Vehicle Control (19.80 ± 0.83 days).

Chronic wounds may never heal or may take years to do so. Wound healing is a complex set of overlapping events for damage repair to its normal state, which involves Epithelialization, contraction and connective tissue deposition.⁶³ Wound healing is a natural normal phenomenon; however, wounds cause discomfort and are prone to infection and other complications. Moreover, the association of various diseases like diabetes, ischemia, immune-compromised conditions as well as malnourishment, ageing, local infection also leads to delay in wound healing. Hence, there is a need to have agents that accelerates the wound healing process.

CONCLUSION

Management of wounds has always been a challenging issue. There is a need for application of newer and advanced modalities in management of wounds. We wish to emphasize the potential benefit of a safe, easily-applicable and effective treatment.

Our *in vivo* study investigating wounds in healthy Wistar rats showed that it is possible to enhance the rate of wound healing with Karanj oil application. The current study provides new evidence that Karanj oil shows beneficial effect in the acute wound microenvironment. Karanj oil could prove to be a cost-effective treatment of acute wounds.

Our study revealed

Topical application of Karanj oil

- significantly decreases wound area
- decreases Epithelialization period
- increases wound contraction

Local application of Karanj oil improves wound healing as evident from clinical signs observed in animals and further confirmed on histopathological analysis. The present investigation may be concluded that the plant *Pongamia Pinnata* is endowed with significant wound healing activity due to the presence active constituents, there by justifying its use in the indigenous system of medicine.

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