Original Research Paper

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EFFICACY OF TINCTURE CALENDULA IN WOUND HEALING IN WISTAR RATS

General Surgery

KEYWORDS: Wound healing, Tincture calendula, Wistar rats

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ABSTRACT

INTRODUCTION- The present study evaluated the healing potential of Tincture calendula in excision wounds in laboratory rats that has not been studied in details till now.

AIMS and OBJECTIVES- Comparison within 3 groups viz naturally healed(control), treated with Betadine only and treated with Betadine(Povidine Iodine) followed by Tincture Calendula.

MATERIAL and METHODS- Excision wounds were created over the back. Changes were observed and recorded on Day 0,4,8,12,16 and 20 with respect to measurement of wound area, wound contraction, wound index, period of epithelisation and histopathological examination.

RESULTS and DISCUSSION-Significant reduction(p=0.0173) in wound area, wound contraction observed in Tincture calendula treated rats on 4th day(363.66

INTRODUCTION

In day to day surgical practice, a surgeon comes across many cases of "difficult to treat wound" and "management of wound" poses a therapeutic challenge to the treating surgeon. There are various methods of wound management available; however no consensus on, which is the best method for wound management. The biologic role of *Tincture calendula* in vascular physiology and immunologic responses has been studied in depth for decades, however, it is surprising that their potential in wound healing has generated so little attention. The objective of present investigation was to evaluate the wound healing potential of Tincture calendula in excision wound model in laboratory rats.

AIM AND OBJECTIVES

To compare the efficacy of Tincture calendula in skin wound healing in Wistar rats.

1. To compare skin wound healing in Wistar rats macroscopically within 3 groups

a) Betadine followed by Tincture calendula
b) Betadine only
c) Natural healing(without application of any topical agent)

2. To compare skin wound healing in Wistar rats microscopically by studying the

d) rate of collagen formation,
e) epithelialisation,
f) neovascularisation under high power microscope

MATERIAL AND METHODS

Animals

Male Wistar rats weighing between 200 and 220 g were procured from National Institute of Bioscience, Pune, India.

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 the Purpose of 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. Excision wounds of size 500 mm2 and depth 2 mm were made by cutting out a layer of skin from the shaven area. The study comprised three different groups containing six animals in each group. The test drug Tincture calendula 10% was applied topically.

Study Groups:

Sr. No.	Group	No. of animals
1.	Group I: Vehicle control animals: received injury for wound formation but will not receive any treatment locally	6
2.	Group II: Tincture calendula treated animals: received injury for wound formation and treatment with betadine followed by tincture calendula	6
3.	Group III: Povidine iodine treated animals: received injury for wound formation and treatment with povidine iodine(betadine) only.	6

Measurement of wound area

The changes in wound area were observed and recorded on day 0, 4, 8, 12, 16 and 20.

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

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

Determination of period of epithelisation

Falling of scab leaving no raw wound behind was taken as the end point of complete epithelisation and the days required for this was taken as period of epithelisation.

Histopathological examination

Samples from Day20 were fixed in 10% buffered formalin and stained with haematoxylin and eosin. Photomicrographs were captured at a magnification of 100×. Sections were analyzed and scored as mild (+), moderate (++) and severe (+++) for epidermal or dermal remodelling, Scab formation, congestion, capillary formation, fibroblast proliferation, infiltration of macrophages, and edema in dermis.

STATISTICAL METHODS

Statistical comparisons were made between drug-treated groups

and vehicle control animals.

t -test

Bonferroni's multiple comparison test

OBSERVATION AND RESULT

The wound area (mm2) in all animal groups was measured on days 0, 2, 4, 8, 12, 16 and 20. In Vehicle Control, Tincture calendula groups, wound area showed none significant difference at 0 day. Significant reduction (p=0.0173) in wound area was observed in the Tincture calendula treated rats from 4th day (363.66±60.74 mm^2) when compared with vehicle control rats (424.50±20.40 mm^2). This reduction in wound area was more significant (p<0·001) in Tincture calendula treated rats on 20th day (49.66±41.51 mm^2) than on 4th day when compared with vehicle control rats (292.83±29.56 mm^2).

Effect of Tincture calendula treatment on wound area (mm2).

Time	VC		Tincture calendula		Povidine Iodine	
(Days)	Mean	SD	Mean	SD	Mean	SD
0	475.00	16.88	452.83	20.83	465.83	32.25
4	424.50	20.40	363.66	60.74	410.33	37.42
8	404.00	20.93	282.83	41.47	345.50	56.37
12	360.33	21.24	217.00	29.36	269.16	59.48
16	326.83	32.59	141.33	35.67	215.33	44.92
20	292.83	29.56	49.66	41.51	140.33	23.67

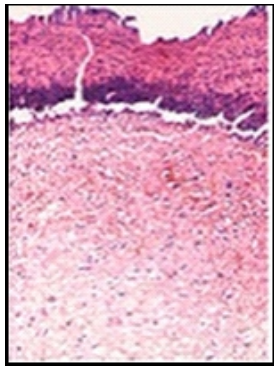
Bonferroni's multiple comparisons test for wound contraction.

Bonferroni's multiple comparisons test	Significant?	P Value
At 0 DAY		
Vehicle Control vs. Tincture calendula	No	> 0.9999
Vehicle Control vs. Povidine iodine	No	> 0.9999
Tincture calendula vs. Povidine iodine	No	> 0.9999
At 4 DAY		
Vehicle Control vs. Tincture calendula	No	> 0.9999
Vehicle Control vs. Povidine iodine	No	0.1490
Tincture calendula vs. Povidine iodine	No	0.2232
At 8 DAY		
Vehicle Control vs. Tincture calendula	Yes	< 0.0001
Vehicle Control vs. Povidine iodine	Yes	0.0231
Tincture calendula vs. Povidine iodine	Yes	0.0011
At 12 DAY		
Vehicle Control vs. Tincture calendula	Yes	< 0.0001
Vehicle Control vs. Povidine iodine	Yes	< 0.0001
Tincture calendula vs. Povidine iodine	Yes	0.0038
At 16 DAY		
Vehicle Control vs. Tincture calendula	Yes	< 0.0001
Vehicle Control vs. Povidine iodine	Yes	< 0.0001
Tincture calendula vs. Povidine iodine	Yes	0.0001
At 20 DAY		
Vehicle Control vs. Tincture calendula	Yes	< 0.0001
Vehicle Control vs. Povidine iodine	Yes	< 0.0001
Tincture calendula vs. Povidine iodine	Yes	< 0.0001

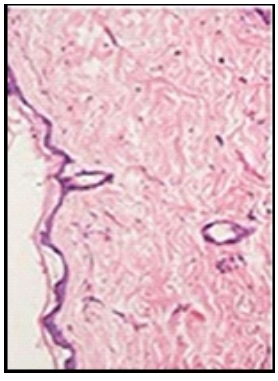
Effect of Tincture calendula treatment on wound healing processes and healing phases in rats.

Group	Scab formation	Congestion	Capillary formation	Fibroblast proliferation	Infiltration of macrophages	Edema
Normal	-	-	++++	-	-	-
Vehicle Control	++++	+++	+	+++	+++	++
Tincture calendula	++	++	+++	+	++	+
Povidine iodine	+++	+++	++	++	+++	++

-no abnormality detected, + damage/ active changes up to less than 25 %, ++ damage/ active changes up to less than 50 %, +++ damage/ active changes up to less 75 %, ++++ damage/ active changes up to more than 75 %.



Photomicrographs of the sections of skin from rats stained with haematoxylin (H) and eosin (E) in the excision model. Images (20× magnification) are typical and representative of normal study group.



Photomicrographs of the sections of skin from rats stained with haematoxylin (H) and eosin (E) in the excision model. Images (20× magnification) are typical and representative of Tincture calendula group.

DISCUSSION

The aims in healing processes are to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin through the release of growth factors. Our study demonstrates that treatment with Tincture calendula accelerates the wound healing process, and it was observed that the wound contraction begins within 4 days of treatment. Thus, treatment with Tincture calendula significantly shortens the period of wound contraction and reduces the period of natural wound healing.

CONCLUSION

Local application of Tincture calendula improves wound healing as evident from biochemical studies, clinical signs observed in animals and further confirmed on histopathological analysis .. The results of our study explicitly reveal the efficacy of Tincture calendula as an effective, safe, biocompatible, cheap and readily available agent for wound dressing. It can also reduce financial burden, psychological trauma and physical pain by early healing of wound.

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