

Original Research Article

## Evaluation of herbal ointment formulated with *Wrightia tinctoria* (ROXB) R. BR. leaves for efficacy of wound healing.

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### Abstract

The objective in wound management is to heal the wound in the shortest time possible, without infection and fast wound closure. This investigation is to check the rationale behind the traditional use of leaves of *Wrightia tinctoria* by evaluating the wound healing property of formulated ointment containing *Wrightia tinctoria* which may facilitate in vivo quantification of skin curative property of the ointment. Controlled wound healing efficacy studies were done on Guinea pig model with incision and excision wounds. Suitable ointment base was selected by Preformulation studies. The ointment was prepared by melt pour and mixing technique with incorporation of *Wrightia tinctoria* extract in coconut oil. The wound healing effects of the formulations were compared to that of 0.2% w/w nitrofurazone ointment. A better healing pattern with complete wound closure was observed with the treated groups in contrast to the control group. The total epithelization period was 14 days (allopathic control and *Wrightia tinctoria* ointment) with 19 days for ointment base. The wound contraction to 50% took 6.3 days (allopathic control), 6.5 days (*Wrightia tinctoria* ointment), against 12.7 days for ointment base. The tensile strength of the test was almost the same as standard ointment. Increased wound breaking strength indicates increase in collagen strength and obviously facilitating wound healing, thus proving that *Wrightia tinctoria* ointment could be used for wound healing as a safe alternative to synthetic drug ointments.

**Keywords:** Wound healing      Excision model      Incision model      Ointment      *Wrightia tinctoria* (Roxb.) R. Br.

### Introduction

Wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, haematoma, laceration or an abrasion. Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding. The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient. At the site of wound closure a flexible and fine scar with high tensile strength is desired. Phytopharmaceuticals with tannins and or flavanoids as their constituents play major role in wound healing mainly due to their antimicrobial and astringent property which facilitates rapid healing process. Medicinal plants with the above properties could be very well used for wound healing.

The outline of the epidermal corneocytes of guinea pig skin was quite similar to those of the human skin: typically, they were flat, transparent, evenly pink stained pentagons forming honeycomb-like sheets.[1]. Due to higher correlation of human skin with that of guinea pig in comparison to other non-human primates it was chosen for experimentation. Controlled wound healing efficacy studies were done on Guinea pig model with incision and excision

wounds [2]. *Wrightia tinctoria* (Roxb) R. Br. belongs to family Apocynaceae. Its leaves were soaked in coconut oil for few hours and applied for eczema, psoriasis and other skin diseases by ethnic groups in hills. As *Wrightia tinctoria* leaves have reported antioxidant and anti psoriatic property, hence an attempt was made to check the scientific rationale behind the traditional use of leaves of *Wrightia tinctoria* by evaluating the wound healing property of formulated ointment which may facilitate in vivo quantification of skin curative property of the ointment.

### Materials and Methods

#### Collection and authentication of plant material

The leaves of *Wrightia tinctoria* were collected from VlnYY garden, Nachallur, Karur district, identified by Prof. P. Jayaraman of Plant Anatomy Research Centre (PARC), Chennai and a voucher specimen was deposited at PARC.

#### Extraction and formulation of plant material

Leaves of *Wrightia tinctoria* were collected and cleaned. The cleaned leaves were minced to small pieces. The minced leaves

were brought into contact with coconut oil in the ratio 1:5 and left for a period of 5 days under sun. The completion of extraction was indicated through the colour of the oil turning to purplish blue. The oil was filtered and the filtrate was used for formulation. The ointment was prepared using 70% of 5% *Wrightia tinctoria* oil extract. Preformulation studies were conducted and suitable ointment base consisting of 15% bees wax, 10% hard paraffin wax and 5% soft paraffin wax was selected. Butylated hydroxyl toluene was used as preservative. The ointment was prepared by melt pour and mixing technique.

### Wound healing activity

The protocol of the study was approved by the local animal ethical committee of IIMT college of medical science. The guinea pigs were kept in standard conditions in the animal house and were used after an acclimatization period of 7 days to get elaborated to the environment. They were provided with food and water ad libitum.

### Animal model for wound healing activity

#### Excision Model

For the excision study,[2]. 3 groups of 6 animals each were anaesthetized with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades. A circle of diameter 2 cm was marked on each of the two sides of the skin. Circular incisions were then made on the marked areas of the skin surface and the skin carefully dissected out and the wound was left undressed to open environment. The area was measured immediately by tracing out the wound area using a transparent tracing paper and the squares counted. One group was treated with the *Wrightia tinctoria* ointment; the second group was treated with allopathy control (0.2% w/w nitrofurazone ointment); and the third group received ointment base (blank control). The test sample was applied once daily and the treatment site was assessed for wound healing on T1, T4, T7, T10, T14, T16 and T19, after surgery on intermitted basis for 19 days. Falling of scar leaving no raw wound behind was taken as an end point of complete epithelization and the days required for this was taken as period of epithelization. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as percent contraction on wound area and was monitored planimetrically by tracing the wound margin on graph paper at prementioned duration.

#### Incision Model

For the incision study [3]. 3 groups of 6 animals each were used and two paravertebral long incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated back of the guinea pig . Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. No ligature was used for stitching. After the incision was made with 5.0 cm cut the parted skin was kept together and stitched with black silk at 1cm intervals using surgical threads (No.000) and a curved needle

(No.11) for stitching after complete haemostasis, by means of interrupted sutures of 1 cm apart. The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed.

One group was treated with the *Wrightia tinctoria* ointment; the second group was treated with allopathy control (0.2% w/w nitrofurazone ointment); and the third group received ointment base. The test sample was applied once daily sutures were removed on 8<sup>th</sup> post wounding day and tensile strength was determined on 10<sup>th</sup> post wounding day according to the method of Lee [4].

Tensile strength, the force required to open a healing skin wound, was used to measure healing. The instrument for this measurement is called tensiometer. It consisted of a 6x12 inc board with one post of 4 inch long fixed on each side of the longer ends. The board was placed at the end of a table. A pulley with bearing was mounted on the top of one of the posts. An alligator clamp wit 1 cm width, was tied on the tip of the post without pulley by a piece of fishing line (20-lb test monofilament) so that the clamp could react at the middle of the board. Another alligator clamp was tied on a piece of fishing line with a 1-L polyethylene bottle tied on the other end. Before testing, the animal was anesthetized with ether in an open mask. The sutures of the wound were cut out with a pair of scissors, The animal was then placed on a stack of paper towels on the middle of the board. The amount of the towels could be adjusted so that the wound was on the same level of the tips of the posts. The clamps were then carefully clamped on the skin of the opposite sides of the wound at a distance of 0.5 cm away from the wound. The longer piece of fishing line was placed on the pulley, and the position of the board as adjusted so that the polyethylene bottle was freely hanging in the air. Water was removed at constant rate by siphon from a large reservoir (20-L bottle) until the wound began to open up. The amount of water in the polyethylene bottle was weighed and considered as the tensile strength of the wound.

### Statistical Analysis

Mean value and standard deviation were calculated for each tested formulation during each day of observation. The data were analyzed by one way ANOVA and p values were considered significant at  $p > 0.005$ .

### Results

The measurements of the progress of the wound healing in excision wound model, induced by the allopathy control ointment (0.2% w/w nitrofurazone ointment), *Wrightia tinctoria* ointment (5%w/w) and the control group (ointment base) are shown in Table 1 and 2 and figure 1 and 2.



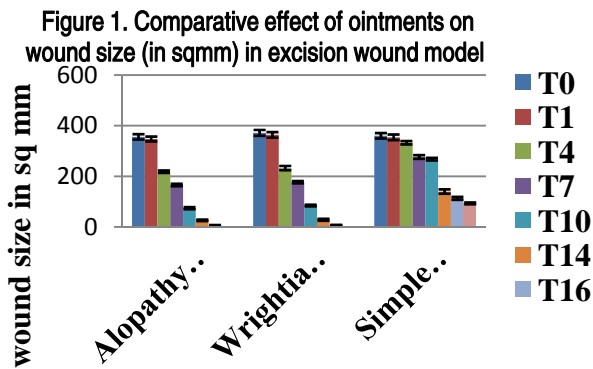
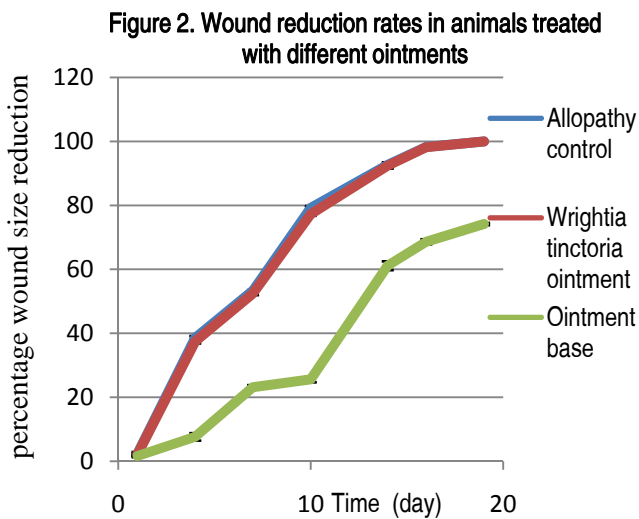


Table no.2 Efficiency of *Wrightia tinctoria* ointment on wound healing (in percentage) in excision wound model

Time	Allopathy control	<i>Wrightia tinctoria</i> ointment	Ointment base
T1	2.33 ± 0.61	2.15 ± 0.45	1.67 ± 0.37
T4	38.56 ± 0.61	37.46 ± 0.6	7.65 ± 1.16
T7	53.37 ± 0.2	52.5 ± 0.52	23.2 ± 0.63
T10	79.23 ± 0.56	77.24 ± 0.47	25.61 ± 0.88
T14	92.71 ± 0.83	92.4 ± 0.84	61.17 ± 1.39
T16	98.32 ± 0.48	98.22 ± 0.4	68.65 ± 0.8
T19	100 ± 0	100 ± 0	74.2 ± 0.35



It is observed that the wound contracting ability of *Wrightia tinctoria* ointment was significantly wound healing from the fourth day onwards, which was comparable to that of the reference standard, allopathy ointment. There was also a significant decrease in the odema at the wound site, 3 days after the initial application. A better healing pattern with complete wound closure was observed with the treated groups in contrast to the control

group within. The epithelization period and wound contraction (50%) is shown in Table 3 and figure 3 and 4.

Table no.3 Effect of *Wrightia tinctoria* ointment on wound epithelization and wound contraction (%) in excision wound model.

Sl no.	Test formulations	Epithelization period (days)	Wound contraction WC - 50% (days)
1	Allopathy control	14	6.3
2	<i>Wrightia tinctoria</i> ointment	14	6.5
3.	Ointment base	19	12.7

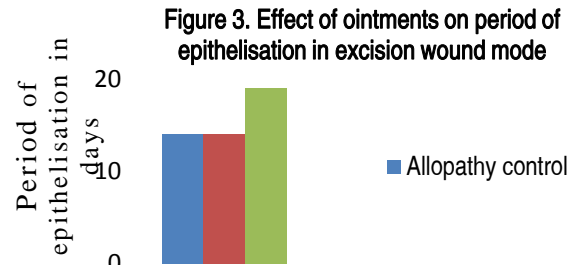
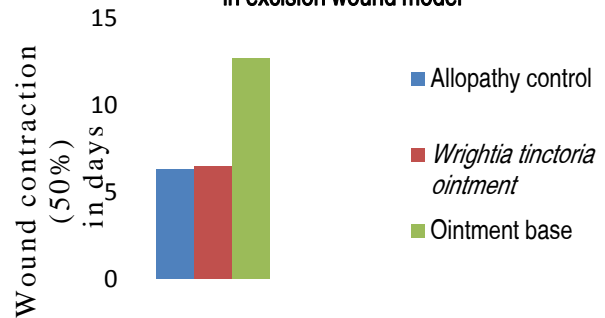


Figure 4. Effect of ointments on wound contraction in excision wound model



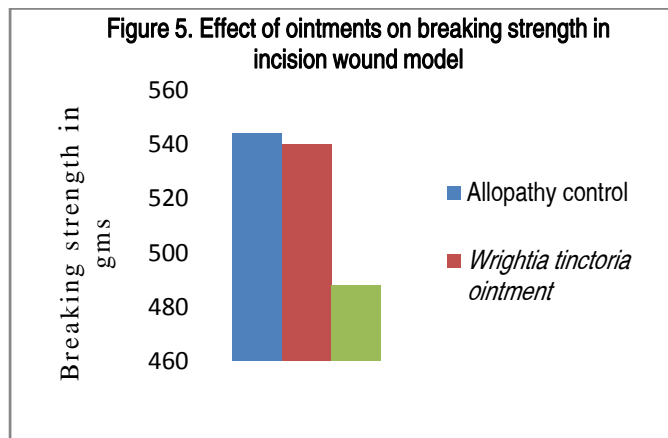
The total epithelization period was 14 days for both the allopathic control and the *Wrightia tinctoria* ointment with 19 days for ointment base. The wound contraction to 50% took 6.3 days for allopathic control and 6.5 days for *Wrightia tinctoria* ointment, against 12.7 days for the ointment base. Thus wound healing was observed with all 3 groups with the wound healing rates for allopathy control > *Wrightia tinctoria* ointment > ointment base.

In the incision wound studies, there was a significant increase in tensile strength of the 10-day old wound due to treatment with the *Wrightia tinctoria* (540g) and the reference standard ointment (544g) when compared with the control group (488g). The measurements of the tensile strength are shown in Table 4 and figure 5.



**Table no: 4 Determination of tensile strength in incision wound model**

Sl no.	Treatment groups	Tensile strength (g)
1	Allopathy control	544
2	<i>Wrightia tinctoria</i> ointment	540
3	Ointment base	488



The tensile strength of the *Wrightia tinctoria* ointment treated group were almost the same to that of the allopathy ointment treated group. Increased wound breaking strength indicates increase in collagen strength and obviously facilitating wound healing.

## Discussion

The structural characteristics of the glabrous skin of normal laboratory animals such as mice, rats, guinea pigs, rabbits, dogs and non-human primates differ markedly from those of human skin. For example, these species have skin with a thinner epidermis, relatively flat dermal-epidermal junctions devoid of rete ridges,[5] a loosely organized dermal structure,[6]. and a rudimentary dermal vascular system.[7]. Consequently, the reactivity of their skin to a variety of chemicals is quite different to that of human skin.[8] Experimentation was done on guinea pigs as its skin has got many similarities to that of human skin.[9].

There are four distinct stages involved in wound healing namely – inflammatory stage, debridement stage, proliferation stage and maturation/remodeling stage. When an injury occurs, the vascular integrity of the injured area is disrupted leading to extravasations of blood into the surrounding tissue or plasma when the damage is minor. The inflammatory stage is directed at preventing further loss

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of blood by platelet adhesion/accumulation at the site leading to coagulation that result to the formation of thrombus. The debridement stage occurs from the third to the sixth day after injury and involves the appearance of neutrophils to clear contaminating organisms. The proliferation or repair stage is characterized by endothelial budding in the nearby blood vessels forming new capillaries that penetrate and nourish the injured tissue. The maturation stage commences from the tenth day to several months depending on wound severity during which the number of capillaries decreases and wound changes from pink to white.[2] Flavanoids show wound healing properties due to their antibacterial and antioxidant properties. They are synthesized by plants in response to microbial infection and are often found effective in vitro as antimicrobial substances against a wide array of microorganisms.[10] As the wound healing process initially involves inflammatory stage and debridement stage, due to its anti-inflammatory and anti microbial property *Wrightia tinctoria* ointment has comparatively reduced their duration and severity effects on the dermal vessels and facilitated its earlier onset of proliferation and maturation stage, leading to earlier healing.

## Conclusion

The data of the present study exhibits significant wound healing by *Wrightia tinctoria* ointment based on the parameters of evaluation. The activity could be attributed to its antioxidant property exhibited due to its content of free and bound flavonoids and phenolic compounds. Thus *Wrightia tinctoria* which has antipsoriatic property, through this wound healing evaluation has exhibited a valid quantified measurement of skin curative property.

## Authors Contribution

Dr. R. Manavalan conceived of the study and participated in its design and co-ordination. Mr. T. Senthil Kumar carried out the wound healing studies. Dr. Vilambi N.R.K Reddy contributed in the analysis and interpretation of results. Dr. Venkappayya involved in drafting the manuscript and revising it's critically for important intellectual content. All authors read and approved the final manuscript.

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