Wound Healing Study of *Panchavalakal* Ointment in Wistar Albino Rats

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i36A31933

Editor(s):
(1) Dr. Mohamed Fawzy Ramadan Hassanien, Zagazig University, Egypt.
(2) Subodh Kumar, AIIMS Deoghar, India.

Reviewers:
(1) Pragnadyuti Mandal, West Bengal University of Health Sciences, India.

Complete Peer review History: [http://www.sdiarticle4.com/review-history/71023](http://www.sdiarticle4.com/review-history/71023)

Received 02 May 2021
Accepted 08 July 2021
Published 09 July 2021

Original Research Article

ABSTRACT

**Introduction:** *Panchavalakal* is a well-known Ayurvedic poly-herbal formulation that has been reported to be used against inflammation, to clean ulcer, wound.  
**Aims and Objectives:** To investigate the wound healing activity of *Panchavalakal* ointment.  
**Materials and Methods:** Wistar strain albino rats of either sex weighing 200±20 g were used for the experiments divided in four groups each consisted of six rats.  
**Statistical Analysis:** One-way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups followed by Dunnett’s multiple ‘t’ test  
**Observations & Results:** Sesame oil and *Panchavalakal* ointment showed almost similar wound healing effects in comparison to control group.

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**Discussion:** Panchavalkal ointment showed statistically highly significant percentage of contraction of excision wound area compared to the normal control. Epithelization period was significantly decreased in oil and Panchavalkal ointment treated group.

**Conclusion:** Panchavalkal ointment decreased the pain, tenderness, redness and swelling that helps to control infection and enhanced the rate of wound healing in albino rats.

**Keywords:** Panchavalkal; wound healing; albino rat; immunological injury.

1. INTRODUCTION

A wound is defined as the disruption of the anatomic and cellular continuity of tissue caused by chemical, physical, thermal, microbial, or immunological injury to the tissue. Wound healing is a biologic process consists of integrated cellular and biochemical cascades leading to reestablishment of structural and functional integrity of the damaged tissue. [1]

Several growth factor such as transforming growth factor beta (TGF-β), platelet activation factor (PAF), Platelet-derived growth factor (PDGF) and epidermal growth factors seem to be necessary for initiation and promotion of wound healing. [2] Wound healing is influenced by host factor, wound characteristic and applied healing agents. [3]

Several treatment options (analgesic, antibiotics and non-steroidal anti-inflammatory drugs) are available for the wound management but most of therapies produce various untoward effect. [4] Wound healing effect of various substances including Moringa oleifera, aqueous pineapple juice [5], honey [6] to mention few have been investigated on animal model wounds. About 70 to 90% of populations in some industrialized nations and between 70 to 95% of citizen in the majority of developing countries are being used traditional medicine for their healthcare needs and concerns. [7]

Repair of injured tissues occurs as a sequence of events which includes inflammation, proliferation and migration of different cell type. The inflammation stage begins immediately after injury, first with vasoconstriction that favors homeostasis and releases inflammation mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process. The remodeling stage is characterized by reformulation and improvement in the components of the collagen fiber that increases the tensile strength of healed tissue. [8]

Panchavalkal is a well-known Ayurvedic poly-herbal formulation that has been reported to be used against inflammation, to clean ulcer, wound, leucorrhoea and other gynecological diseases. [9] Panchavalkal has potentials in accelerating various type of wounds described in Ayurveda classics. Panchavalkal forms are effective in wound healing in case of post-operative fistulectomy wound and least recurrence.[10]

Though the Panchavalkal has been shown clinically effective in wound healing in human there is also need to assess the other parameters through pharmacological study.

Therefore, keeping in view the important fact given above, the present study was under taken with following aims and objectives:

- To investigate the wound healing activity of Panchavalkal ointment.

2. MATERIALS AND METHODS

2.1 Animals

Wistar strain albino rats of either sex weighing 200±20 g were used for the experiments. The animals were obtained from the Animal house attached to IPGT & RA, Gujarat Ayurved University, Jamnagar (Gujarat).

2.2 Husbandry Condition

Animals were housed in each cage made up of poly-propylene with stainless steel top grill. The dry wheat (post hulled) waste was used as bedding material and was changed every day morning. The animals were fasted overnight before experimentation and blood collection. The animals were exposed to 12 hour light and 12 hour dark cycles with the relative humidity of 50 to 70% and the ambient temperature during the period of experimentation was 22±03°C. All animals were kept on same environmental conditions.

2.3 Diet

Animals were fed with ‘VRK’ brand rat pellet feed supplied by Keval Sales Corporation, Vadodara.
The drinking water was given ad libitum in polypropylene bottles with stainless steel sipper tube.

Acclimatization period: All selected animals were kept under acclimatization for one week before experimentation.

2.4 Drugs
   i. Sesame oil
   ii. Panchavalkal ointment
   iii. Povidone iodine ointment

2.5 Route of Drug Administration

The test drugs to treated group, vehicle group and control group were administered in suitable doses by dermal external application. In excision wound healing activity drug was administered by local application.

Dose: Sufficient amount of drug was applied locally.

3. EXPERIMENTAL STUDIES

3.1 Wound Healing Activity

Excision wound healing activity of test drugs in rats. A particular size of excision wound was created by the marking of premeasured circular object on the back of anesthetized rat. The excised wound is allowed to heal by treatment and periodically tracing the wound area to evaluate closure of wound for assessment of wound healing activity of test drug with comparison to control (Morton and Malon, 1972).

3.2 Requirements

Autoclave, measured circle, permanent marker, Scissors and forceps.

3.3 Reference standard

Betadine (local application) contains povidone-iodine ointment USP, Batch No-PL0467, marketed by Win-Medicare Pvt. Ltd., New Delhi.

3.4 Procedure

Wistar strain of albino rats of either sex weighing between 200±20 g were divided in four groups each consisted of six rats as follow,

Prior to the operative procedure all the instruments (scissor, forceps, etc.) were sterile with autoclaved. The operative procedure was carried out under aseptic conditions. The experimental animals were anaesthetized prior to made excision wound which was carried out as reported by Mortan and Malone (1972). The dorsal fur of the animals was shaved with an electric clipper prior to the procedure without causing any abrasions.

The area of the wound was created (on the back portion of the rat-suprascapular region) was outlined on the back of the animals with permanent marker. A full thickness of the excision wound of circular area 140mm2 and 2mm depth was created. Then, the animals were kept in separate cages on normal diet.

To second and third group, Sesame oil and Panchavalkal ointment were applied to the respective groups by local application. The fourth group was taken as standard and treated with the standard drug, povidone ointment locally (Sembian et al., 2012). Drugs were applied until 90 % epithelialization completion from the day of operation.

The wound contraction rate was assessed by tracing the wound on alternate days using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. The point at which the eschar fell off without any residual raw wound was considered epithelialization. Thus, number of days required for 90 % epithelialization and percentage of contraction periodically were recorded.

\[
\text{% wound contraction} = \frac{\text{Healed area} \times 100}{\text{Total wound area}}
\]

Where healed area = original wound area – present wound area

On last day, the blood was collected by retro orbital puncturing under light ether anaesthesia and serum was separated for biochemical estimations. Thereafter, rats were euthanized. Tensile strength of excised skin of the healed wound area of tissue was recorded.

3.5 Serum Biochemical Parameters

Serum hydroxyproline (Prockop and Udenfriend, 1960), orosomucoid content (Varley, 1980).

Serum hydrolysate was prepared by adding 0.5 ml of serum with 0.5 ml of 6 N HCl and kept at 120°C for 3 hrs in sealed tubes. The hydrolysate was cooled and 25 mg charcoal was added. After proper mixing the tubes were centrifuged at 3000
rpm for 10 mins. Supernatant was taken out in separate tubes and 1 drop of phenolphthalein was added and neutralised by adding 10 N NaOH till pink colour was appeared. 0.1 ml of hydrolysate was diluted with 0.9 ml of water and used for estimation of serum hydroxyproline content.

3.6 Serum Hydroxyproline

0.1 ml of hydrolysate was diluted with 0.9 ml of water and 1 ml of chloramine-T (0.05 M) was added. The mixture was kept for 20 min at room temperature and the reaction was stopped by addition of 1 ml of 3.15 M perchloric acid followed by 1 ml of 20% P-dimethyl amino benzaldehyde. The mixture was mixed and allows for 5 min, vortex the mixture and allow to clear colour called Schlieren. The test tubes were once again placed on waterbath at 60°C for 20 min and then cooled for 5 minutes under running stream of water. Colour intensity was measured at 570nm against the blank.

3.7 Serum Orosomucoid Content

The orosomucoid content of the serum was estimated following the procedure described by Varley (1980). 0.2ml of serum was pipette out in to a test tube containing 4.8 ml of 0.85% NaCl solution and mixed. From a serological pipette, 2.5 ml of 1.8 M perchloric acid was added drop wise with shaking to ensure rapid mixing and allowed to stand for 10 min at room temperature. The mixture was filtered through Wattman no. 50 paper in to another set of test tubes. To 5 ml of filtrate, 1 mlphosphotungstic acid was added, mixed and centrifuged at 2000 rpm for 10 minutes and decanted.

The precipitate was washed with 600 mM/lit perchloric acid solution twice and dissolved by adding 1 ml of Na2CO3 solution. Then 3.5 ml of distilled water was added followed by 0.5 ml of phenol reagent and the tubes were incubated at 37°C for 15 minutes in water bath and the absorbance of the solution was measured at 680 nm in a spectrophotometer. Similarly a series of standard tyrosine solutions in different concentrations were run. Absorbance of both standard and test were measured against blank containing 3.5 ml of distilled water, 1 ml of Na2CO3 and 0.5 ml of phenol reagent.

4. RESULTS

4.1 Excision Wound Healing Activity

A particular size of excision wound was created by the marking of premeasured circular object on the back of anesthetized rat. The excised wound is allow to heal by treatment and periodically tracing until complete epithelialisation. The effect of test drug was assessed on percentage contraction, complete epithelisation period, tensile strength of skin and serum tissue parameters.

The data regarding the effect of test drug on percentage contraction of skin in excision wound healing model are depicted in Table 3. Significant reduction in percentage of wound contraction was observed with highly significant wound healing result in Panchavalkal treated group from post-operative 7th day. Povidone iodine ointment treated group showed significant effect from 11th day in percentage wound contraction in comparison with normal control group. Seasam oil and Panchvalkal ointment showed almost similar wound healing effects in comparison to control group.

The data regarding the effect of test drug on tensile strength of wounded skin are depicted in Table 4. The significant increase in tensile strength (partial thickness) of skin was observed in Sesam oil and Povidone iodine ointment treated groups. Drug treated group showed insignificant increase in tensile strength (Partial thickness) of wounded skin in comparison with normal control group.

The significant increase in tensile strength (Full thickness) of skin was observed in oil while, Povidone iodine ointment and Drug treated group showed insignificant increase in tensile strength (Full thickness) of wounded skin in comparison with normal control group.

| Table 1. Group and Treatments |
|-------------------------------|
| Groups | Treatments | Dose (per kg) |
| Group I | Normal control | -- |
| Group II | Vehicle treated group received *seasame* oil | Q.S. for local application |
| Group III | *Panchavalkal* Ointment | Q.S. for local application |
| Group IV | Povidone-iodine ointment treated and served as positive control group | Q.S. for local application |
Table 2. Effect of test drugs on percentage contraction of excision wound in rats

| Days | Normal Control (NC) | Sesame Oil | Povidone Iodine (BT) | Panchavalkal Ointment (DR) |
|------|----------------------|------------|----------------------|---------------------------|
| 3    | 11.92±3.685          | 5.651±1.721| 17.87±3.871          | 12.41±4.102               |
| 5    | 21.04±6.808          | 16.51±2.846| 30.48±1.992          | 15.87±4.821               |
| 7    | 34.71±5.303          | 58.24±6.543| 34.42±2.874          | 75.39±1.692***            |
| 9    | 55.94±7.887          | 89.79±1.297| 64.80±3.884          | 88.56±2.145***            |
| 10   | 72.25±7.576          | 98.46±0.372| 80.86±3.882          | 96.39±1.518*              |
| 11   | 81.21±2.506          | 100.00±0.00| 89.94±1.898          | 100.00±0.00***            |
| 12   | 86.21±1.628          | 100.00±0.00| 93.96±1.208**        | 100.00±0.00***            |
| 13   | 89.89±1.683          | 100.00±0.00| 97.67±0.610***       | 100.00±0.00***            |
| 14   | 93.41±1.000          | 100.00±0.00| 99.21±0.432***       | 100.00±0.00***            |
| 15   | 93.63±1.369          | 100.00±0.00| 100.00±0.00***       | 100.00±0.00***            |

Data: Mean ± SEM; *P<0.05, **P<0.01, ***P<0.001 compared with control group (Unpaired ‘t’ test); $P<0.05, $$P<0.01, when compared with control group (ANOVA followed by Dunnett’s multiple ‘t’ test)

Table 3. Effect of test drugs on 90% epithelisation period in excision wound in rats

| Groups | 90% Epithelisation period (days) | % Change NC | % Change Oil |
|--------|---------------------------------|-------------|-------------|
| NC     | 13.500±0.224                    | --          | --          |
| OIL    | 9.500±0.224*                    | 29.63↓      | --          |
| BTC    | 11.333±0.333**                  | 35.31↑      | 19.29↑      |
| DR     | 9.667±0.211**                   | 32.69↑      | 41.10↑      |

Data: Mean±SEM, ↓Decrease, ↑Increase

Table 4. Effect of test drugs on tensile strength of skin in excision wound healing in rats

| Groups | Tensile strength of healed skin (Partial (kg)) | % change | Full thickness (kg) | % change |
|--------|-----------------------------------------------|----------|---------------------|----------|
| NC     | 0.69±0.059                                    | --       | 0.77±0.076          | --       |
| OIL    | 0.85±0.025**                                  | 24.02↑   | 0.95±0.030*         | 4.99↑    |
| BTC    | 0.93±0.041**                                  | 35.31↑   | 0.95±0.039          | 32.69↑   |
| DR     | 0.82±0.048                                    | 19.25↑   | 0.87±0.065          | 21.05↑   |

Data: Mean±SEM, ↓Decrease, ↑Increase

Table 5. Effect of test drugs on serum hydroxyproline and orosomucoid level during excision wound healing activity in rats

| Groups | Hydroxyproline (μg/dl) | % change | Orosomucoid (mg/dl) | % change |
|--------|------------------------|----------|---------------------|----------|
| NC     | 1192.269±126.686       | --       | 213.83±9.375        | --       |
| OIL    | 1252.51±14.243         | 5.033↑   | 90.13±5.662**       | 57.86↓   |
| BTC    | 1445.753±41.346***     | 21.25↑   | 132.96±4.596$$&$$   | 37.83↓   |
| DR     | 1332.831±38.445        | 11.79↑   | 125.98±3.385$$&$$   | 41.10↓   |

Data: Mean±SEM, ↓Decrease, ↑Increase

The data regarding the effect of test drug on serum hydroxyproline and orosomucoid levels are depicted in Table 4. Serum hydroxyproline level was non-significantly increased by sesame oil, Povidone iodine ointment and Panchavalkal ointment in comparison to normal control group. Serum hydroxyproline level was highly significantly
increased by Povidone iodine ointment and insignificantly increases by Panchavalkal ointment in comparison to oil treated group.

Serum orsomucoid level was significantly decreased by sesame oil, Povidone iodine ointment and Panchavalkal ointment in comparison to control group. Serum Orsomucoud level was significantly increased by Povidone iodine ointment and Panchavalkal ointment in comparison to sesame oil control group.

5. DISCUSSION

Traditionally, Panchavalkal are being used for many diseases like non healing wound healing and gynecological diseases. Applying the directly on affected wound and desired effect to stay longer on the wounded area of the experimental animal ointment form is used. The Panchavalkal ointment contain Panchavalkal, Tila Tail and Siktha prepared like as a Malaharakalpana as per in ayurvedic classics.

Excision wound healing model is often used for wound healing evaluation because, it represents a true wound that could be reproducibly analysed in non-subjective, highly controlled manner. The time required for complete epithelialization of the excision wound is an important parameter to assess the wound healing process. The enhanced rate of wound contraction and significant reduction in healing time might be due to enhanced epithelialization. Panchavalkal ointment showed statistically highly significant percentage of contraction of excision wound area compared to the normal control. Epithelization period was significantly decreased in oil and Panchavalkal ointment treated group. However, sesame oil and Panchavalkal ointment showed almost similar wound healing effects and days for 90% epithelisation period in comparison to control group.

Collagen molecules synthesized are laid down at the wound site and become cross linked to form fibres. Since excisional wounds treated with the Panchavalkal ointment and sesame oil showed greater tensile strength, it may be inferred that it not only increases collagen synthesis per cell, but also aids in cross linking of the protein. Panchavalkal ointment treated wounds showed an increased rate of wound contraction, leading to quicker healing as confirmed by decreased period of epithelialization when compared to untreated control wounds. However, Sesame oil and Panchvalkal ointment showed almost similar activity in comparison to control group.

Serum orsomucoid is a positive acute phase glycoprotein, which is a normal constituent of human plasma. It exists as an integral membrane protein of leukocytes and is liberated into the plasma as the cells disintegrate. The function of orsomucoid is still unknown, but it may have a role in forming collagen, binding steroid hormones, and modifying lymphocyte responsiveness. A significant decrease in serum orsomucoid level was observed in drug treated groups which may have role in wound healing activity.

Panchavalkal has Tanin known as antioxidants and blood purifiers with anti-inflammatory action. [11 ] Antioxidant protect the tissue from the oxidative damage when the oxidation process hampers the wound. Tannin, Flavonoids and phytosterols are anti-inflammatory: hence they prevent the prolongation of initial phase of wound and thus fast healing of excision wound in albino rats. Flavonoids, and terpenoids are also known to promote the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialization.[12 ]This is very important as researchers proved that the control of microbial infection is necessary for better wound healing and its management.[13-14]

6. CONCLUSION

Panchavalkal Ointment show highly significant in wound contraction and significant in tensile strength of wounded skin. Serum hydroxyproline was affected by both Panchavalkal ointment and Povidone iodine ointment. Serum orsomucoid level significantly decreased by both Panchavalkal ointment and Povidone iodine ointment. Panchavalkal ointment decreased the pain, tenderness, redness, and swelling that helps to control infection and enhanced the rate of wound healing in albino rats.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.
CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC/24/2018/29) in accordance with the guideline formulated by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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