Original article

Evaluation of wound healing activity of Triphala in incision wound model in rats


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Abstract

Background
Wound healing is an important healthcare problem in current medical practice. Even in presence of treatment modalities like good antiseptics, antibiotics and emergence of biologicals for treatment of wounds, the outcomes are far from optimal. Triphala is formulation described in Ayurveda which has immense therapeutic potential. Few studies have shown that Triphala improves wound healing in infected wounds. Therefore it was planned to evaluate and compare wound healing activity of topical Triphala and compare it with Framycetin in rats.

Methods
This study was conducted after approval from Institutional Animal Ethics committee (IAEC). Wound healing activity of topical Triphala(10%w/w) was assessed in incision wound model in Sprague Dawley rats, which was compared with petroleum jelly, sesame oil and framycetin ointment. The Parameter studied was, tensile strength of the incised wound on 10th post-operative day in grams.

Results
The tensile strength in Triphala treated group was significantly more (p≤0.05) than that of Framycetin treated group which was taken as a standard in the study. The tensile strength of Triphala wassignificantly more (p≤0.05) than petroleum jelly and Sesame oil in incision wound model on rats. The tensile strength of Framycetin was found to be higher than that of petroleum jelly and sesame oil, but there was no significant difference between the groups.

Conclusion
In this study Topical preparation of Triphala was found to have good wound healing activity on incision wound model in rats. It has shown better wound healing property than petroleum jelly, sesame oil and framycetin.

Keywords: Triphala, incision wound model, wound healing activity, framycetin.

Introduction
Wounds are natural part of everyday life. Wound is defined as disruption of cellular, anatomical and functional continuity of a living tissue(1). It may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to restoration of structural and functional integrity with regain of strength of injured tissues. The healing process
consists of a sequence of overlapping events including inflammation, wound contraction, re-epithelialization, tissue remodeling and formation of granulation tissue with angiogenesis(2).

Wound Healing is a substantial healthcare problem in current medical practice. Estimates indicate about 6 million people are suffering from chronic wounds worldwide(3). According to Indian epidemiological data the prevalence of wounds is 15.03 per 1000 population. The prevalence of chronic wounds was reported as 4.5 per 1000 population whereas that of acute wounds was nearly double at 10.5 per 1000 population (4). The burden of chronic wounds is growing rapidly due to increasing healthcare costs, aging population and a sharp rise in incidence of diabetes and obesity worldwide(5).

Appropriate treatment and wound care should accelerate the healing process, prevent infection and chronicity of wound. The treatment options currently available include supportive measures like maintenance of hygiene, proper wound dressing, and topical use of antiseptics and antibiotics. These measures do not inherently improve the healing process, thus promote wound healing by preventing infections. But overuse of antibiotics and antiseptics have resulted in emergence of drug resistance organisms. Biologicals such as Plateau derived growth factors (PDGF) which are known to stimulate process of wound healing are available, but these are expensive, and have limited effectiveness owing to short half-life and presence of proteases in chronic wounds(6). Research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant resources are known to increase the healing of wounds (7). The Ayurveda pharmacopoeia contains over 200 herbs, minerals, animals and fat preparations that are used for skin care, which have the potential to be used for wound healing(8).

Triphala is one such Ayurveda formulation, commonly prescribed by most healthcare practitioners in India. It is equi-proportional mixture of three traditional herbs- Amalika (Emblica Officinalis), Haritaki (Terminalia chebula), Bibhataki(Terminalia bellerica). Recent medical research conducted by several leading institution in India have revealed that Triphala is an antioxidant rich herbal formulation and possesses diverse beneficial properties. This wonder drug is known to exhibit antioxidant, immunomodulatory, antimitagenic, antiviral, antibacterial and antifungal properties (9, 10). According to Kumar et al Triphala has antibacterial, wound healing and antioxidant properties necessary for management of infected dermal wounds(11). Keeping in view the above properties of Triphala and personal communication with practicing Ayurveda physician it was considered appropriate to evaluate Triphala for wound healing activity in the incision wound model in rats. The agents used in the study were Petroleum jelly, Framycetin, sesame oil and Triphala. Petroleum jelly was taken as vehicle control in the study as most of the topical formulations used for wound healing use petroleum jelly as base. Petroleum jelly served as vehicle control to Framycetin in this study. Few studies suggest that petroleum jelly also promotes wound healing by forming a barrier to prevent wound infections and also provide a moist environment(12). Framycetin is a topical antibiotic which is widely used in treatment of wounds in day to day life. Sesame oil was used in study as vehicle control for Triphala. Sesame oil has some antibacterial and antiviral activity. Sesame oil is widely used in Ayurveda for preparing formulations for topical use.
Few studies suggest that sesame oil also has some wound healing activity (13).

**Aim and Objectives:** Evaluate Wound healing activity of Triphala and compare it with Petroleum jelly and Framycetin

**Material and Methods**

The present study was carried out in Dr. D.Y. Patil Medical College, Hospital and Research Centre, Pimpri, Pune, Maharashtra, after approval from Institutional Animal Ethics Committee (IAEC).

**Animals**

A total of 40, healthy (150-200g) Sprague-Dawley rats of either sex were used. The animals were kept in the laboratory for 3-4 days for acclimatization, with free access to food and water. Animals were housed in standard polypropylene cages with wire mesh top and husk bedding.

Prior to the day of the experimental procedure, they were starved overnight, and the area of skin, where wound was to be made, was depilated.

**Drugs used in the study**

1. Petroleum jelly
2. Framycetin
3. Sesame oil
4. Triphala in sesame oil

A preparation of 10% (w/w)Triphala powder in sesame oil prepared by an Ayurveda physician by mixing 5g of triphala powder in 50g of sesame oil. Triphala powder and sesame oil were obtained from IMPCOPS Ltd, Chennai and S.D. Fine-Chem. Ltd., India respectively. Framycetin was obtained from Aventis pharma Ltd., Goa. Petroleum jelly was obtained from Unilever Ltd.

**Incision Wound Model**

The anesthetized (Ketamine- 30mg/kg i.p.) animals were secured to the operation table in prone position. Two paravertebral straight line incisions were made as described by Ehrlich et al(14). Care was taken to see that incisions were at least 1cm lateral to the vertebral column. After complete hemostasis, wounds were closed by interrupted sutures. Thereafter, rats were divided into four groups.

- **Group I** (n=10) - Petroleum jelly, served as control for Framycetin.
- **Group II** (n=10) - Framycetin, served as standard control.
- **Group III** (n=10) - Sesame oil, served as control of Triphala group.
- **Group IV** (n=10) – Triphala, served as study group.

The formulations were applied topically with sterile cotton swab in rats of each group once daily. Stitches were removed on day 8 and treatment was continued.

**Evaluation**

Evaluation was done by measuring the tensile strength using Tensiometer. It was measured on day 10 under anesthesia (ketamine-30 mg/kg i.p.) by continuous, constant water flow technique of Lee 1968(15).

**Statistical Analysis**

Tensile strength in different groups were expressed as mean ± SEM. Data were analyzed by ANOVA followed by post hoc Dunnett’s test. The criterion for statistical significance was defined as P<0.05.

**Results**

**Incision Wound Model**

The results of incision wound model are depicted in figure I and table I. The Tensile strength in grams was measured on the 10th post-operative day.

The tensile strength in Triphala treated group (366±22.34) gms was significantly more (p≤0.05) than the Framycetin(303.1±25.29 gms) treated group which was taken as a standard in the study. The tensile strength of Triphala was also significantly more (p≤0.05) than petroleum jelly.
(255.9±18.44gms), and Sesame oil (268±8.677gms) in incision wound model on normal rats. The tensile strength of Framycetin is higher than petroleum jelly and sesame oil, though there was no significant difference between the groups.

Table I Mean Tensile strength (gms) in all studied groups (Mean±SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean tensile strength (gms) with SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum jelly</td>
<td>255±18.44</td>
</tr>
<tr>
<td>Framycetin</td>
<td>303.1±25.29</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>268±8.677</td>
</tr>
<tr>
<td>Triphala</td>
<td>366±22.34</td>
</tr>
</tbody>
</table>

Discussion

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. It is a complex process which involves coagulation, inflammation and debridement of wound, including cellular proliferation, tissue remodeling and collagen deposition.

The aim of wound care is to promote and enhance wound healing in the shortest time with minimal pain, discomfort and scarring to the patient and this must occur in a physiological environment conductive to tissue repair and regeneration. Currently the treatment options for majority of wounds include more of supportive measures rather than agents that actually improve the process of wound healing. Overuse of antibiotics and antiseptics have resulted in emergence of drug resistance in infectious organisms. Medicinal plants and their products have immense potential for management and treatment of wounds. A large number of plants are used in Ayurveda for treatment of wounds and burns. The present study was undertaken based on data obtained...
from Ayurveda system of medicine about certain herbal preparations such as honey and Triphala, which have inherent wound healing properties. Michael Tierra a practicing herbalist and author of “Wonders of Triphala” describes his experience of use of Triphala powder for healing of wounds inflicted by razor(16).

Triphala is a drug described in ancient ayurvedic text as a tridoshic rasayana, a therapeutic agent with balancing and rejuvenating effects on three humors or constitutional elements in ayurveda Vata, pita and kapha. It is composed of three myrobalans, Amalaki (Embilica officinalis), Haritaki (Terminalia chebula), and Bibhataki(Terminalia bellerica). Amalaki in sanskrit is known as Dhatri (The nurse), which is a reference to its incredible healing properties. It has application as immunomodulatory and cytoprotective agent. Amalaki also exhibits antimicrobial action against certain pathogenic organisms. The fruit of T.chebula, commonly known as ‘Hardara’, has being used as traditional medicine since antiquity for human ailments. It exhibits antibacterial activity against certain gram positive and gram negative pathogenic organisms. It is also known to have antiviral and antifungal activity. T.chebula also possess immunomodulatory, cytoprotective activity and astringent properties. It is known to improve wound healing. T. bellerica commonly known asbuellica myrobalan and locally as ‘Bahera’. It is a fruit used in folk medicine to treat inflammation and pain. It possesses antioxidant, antibacterial properties. Dry powder of this fruit is dusted on fresh cuts and wounds to arrest bleeding as an astringent and styptic. A practicing Ayurveda physician in personal communication described use of triphala in various wounds. She also mentioned use of a preparation of Triphala in sesame oil locally for anal fissures. Therefore, her personal experience and the antibacterial, antiviral and antioxidant properties of Triphala led us to study and evaluate Triphala for its wound healing activity in various models in rats.

The incision wound model was designed to stimulate the surgical incision wounds. The tensile strength of healed wound on the 10th day was taken as an evaluation criteria. In this study petroleum jelly, Framycetin and sesame oil had no significant effect on tensile strength in incision wound model. However, in rats locally treated with Triphala the tensile strength was significantly more than all the control study treatments, thus indicating that in Triphala treated surgical wound, healing was better. Triphala being a herbal extract effectively arrests bleeding of fresh wounds, inhibits microbial growth and accelerates wound healing(2). Although the present study did not explore the mechanism by which it induces wound healing, it could be attributed to the phytochemical constituents of Triphala known to have astringent, antioxidant, antibacterial and anti-hemorrhagic properties (9, 10).

**Conclusion**

Ayurveda is traditional system of medicine that has been practiced in India for more than 5000 years. Several agents such as honey, peru and triphala are described in Ayurveda for their wound healing properties under the term ‘vranaropaka’(17). Some of these agents have been screened scientifically to evaluate their wound healing properties but the potential of most remains unexplored. In the study an attempt was made to study formulation of triphala in sesame oil for its wound healing properties. In this study petroleum jelly, framycetin, sesame oil and triphala in sesame oil were studied. The wound healing activities of these formulations were evaluated on incision wound model.
From this study it was concluded that triphala has significant wound healing activity. In the present study triphala is better than framycetin in healing of surgical wounds in rats. In therapeutic area like wound healing the current scenario for treatment of majority of wounds is mostly supportive and prophylactic in nature. Measures like maintenance of proper hygiene, proper dressing and extensive use of antibiotics and other pharmaceutical products remain the main stay of therapy. But the irking presence of drug resistance along with increasing incidence of chronic non healing wounds suggest that the emphasis should be laid on finding agents which inherently improve the wound healing process. This has lead us to investigate the triphala for wound healing activity. The formulation of triphala in sesame oil shows great promise by promoting wound healing process. More research is needed for understanding the mechanism by which it improves wound healing process. There is a need to carry out extensive clinical trials to evaluate wound healing activity of triphala in chronic non healing wounds such as diabetic ulcers, venous ulcers and Pressure ulcers.

References


