



Original Research Manuscript

EFFICACY EVALUATION OF AMPUCARE IN COMPARISON WITH SILVER SULFADIAZINE IN BURN WOUND HEALING IN RATS.

Sehgal Rajesh *, Chaudhary Manu, Dwivedi Vivek and Naithani Vijay

Intellectual Scientific Division, Venus Medicine Research Centre, India.

ABSTRACT

Plants have been used in ethno-medicine since many years and there has been an increasing interest in medicinal plants and their traditional use. The present study compared efficacy of Ampucare with that of silver sulfadiazine in partial thickness burn in rats. Adult male Sprague_Dawley rats were divided into three groups: control group (Normal saline treated group, Ampucare treated group and Silver sulfadiazine treated group). Complete healing of burn wound was observed in all the animals. A significant decrease in wound size was observed in Ampucare treated and Silver sulfadiazine treated group as compared to control group on day 14. Both in control group and Silver sulfadiazine treated group not a single animal showed complete healing of the wound on day 14. Results suggested that Ampucare is significantly effective in treatment of partial thickness burn wounds than silver sulfadiazine.

Key Words: Burn, Silver sulfadiazine, *Curcuma longa*, *Azadirachta indica*.

Introduction

Plants have been used in ethno-medicine since many years [1] and since last few decades there has been an increasing interest in medicinal plants and their traditional use [2]. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodeled to form scar. Most burn injuries are minor and 80% to 90% of burn injuries can be treated on an out-patient basis [3]. Silver sulfadiazine (SSD) is the agent of

choice in the treatment of partial-thickness burn wounds at out-patient clinic [4].

Ampucare is an oil based formulation which contains extracts of leaves of *Azadirachta indica* and roots of *Curcuma longa* as active ingredients. It is a miraculous formulation for external application for the treatment of wounds including burns. It is an anti-inflammatory and antimicrobial product, which improves blood flow, has an immunomodulatory action along with tissue regeneration properties. Keeping this in view present study was planned to compare efficacies of Ampucare and Silver sulfadiazine in rats with partial thickness wound.

Corresponding Author

Rajesh Sehgal

Venus Medicine Research Centre, Venus Remedies limited., Hill Top, Industrial Estate, Jharmajri EPIP, Phase I(extn) Bhatoli Kalan, Baddi (H.P.) 173205.

+91-1795-302100, 302101

Email id: vmrc@venusremedies.com

Materials and Methods

Animals

Adult male Sprague_Dawley rats (n=18,150-200g) were divided into three groups of six animals each: Ampucare treated group (1ml, applied topically), Silver sulfadiazine (1% applied topically) treated group and a Control group (Sterile

normal saline treated). Treatment was given twice daily for continuous 14 days. Animals were fed standard diet (pellets) supplied by Amrit feed India and water was given *ad libitum*. They were housed in polyurethane cages at controlled room temperature of 29 ±2°C and a relative humidity of 50.5%, and a constant light-dark schedule (12 hours light and 12 hour dark cycle).

Burn Wound Model

Partial Thickness wound were inflicted on the shaven back of overnight fasted animals under Ketamine anesthesia [5]. Hot molten wax (80°C) was poured through a cylinder of 300mm² circular opening. The wax was allowed to remain on the skin till (appx. 8 min) it gets solidified. Area of the wound was measured on day zero and day 14 in all the groups.

Statistical analysis

Results are shown as Mean ± SD. Significance of difference between groups was evaluated by using ANOVA. If ANOVA shows significant differences, *post hoc* analysis was performed with Tukey test. P<0.05 was considered as statistically significant. The study protocol for study was approved by Institutional animal ethics committee of Institute.

Result and Discussion

Wound healing is the process of skin and other soft tissue repair [6]. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production. The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialisation and wound contraction. Angiogenesis involves new blood vessel formation. In fibroplasia and

granulation tissue formation, fibroblasts exert collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction [7].

Phytochemical constituents like flavanoids and triterpenoids are known to promote the wound-healing process, mainly due to their astringent and antimicrobial properties, which appear to be responsible for wound contraction and increased rate of epithelialisation [8].

In this study the significant decrease in burn wound area was observed on day 14 as compared to wound area on day 0 in Ampucare and Silver sulfadiazine treated group. No significant reduction was observed in wound area of control group after 14 days as compared to wound area on day 0. Although Silver sulfadiazine treated animals showed significant reduction in wound area on day 14 but not a single animal showed complete wound healing on day 14 whereas in Ampucare treated group wound of all the animals was completely healed on day 14 (Table 1 and Fig. 1).

Table 1: Evaluation of burn wound area

Control group	Wound area on Day 0	Wound area on day 14
	292.42±7.17	271.83±20.44
Silver Sulfadiazine treated group	291.83±12.67	62.72±9.66
Ampucare treated group	293.67±8.87	0 Completely healed in all animals



Figure 1a. Control (untreated) animal



Figure 1b. Ampucare treated animal



Fig. 1

Azadirachta indica bark and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. Three tricyclic diterpenoids morganone, margolonone and isomargolonone isolated from neem bark were active against *Klebsiella*, *Staphylococcus* and *Serratia* species. Several polysaccharides isolated from neem bark possess anti-inflammatory activity [9]. Bark of *Azadirachta indica* was found to have high total phenolic components and high antioxidant activity [10]. It is already established that these bioactivities help in wound repair.

Curcumin, the bioactive constituent of *curcuma longa*, has many pharmacologic properties like anti-inflammatory and antioxidant properties. Turmeric curcumin may be a good potential agent for wound healing [11]. Curcumin increased cellular proliferation and collagen synthesis at the wound

site, as evidenced by increase in DNA, total protein and type III collagen content of wound tissues. Curcumin treated wounds were found to heal much faster as indicated by improved rates of epithelialisation, wound contraction and increased tensile strength. Better maturation and cross linking of collagen were observed in the curcumin treated rats, by increased stability of acid-soluble collagen, aldehyde content, shrinkage temperature and tensile strength. The results clearly substantiate the beneficial effects of the topical application of curcumin in the acceleration of wound healing and its antioxidant effect [12]. *Curcuma longa* has been found to be effective in experimental wound healing by improving oxidative stress and free radical scavenging system [13].

In conclusion, Ampucare was found to be more effective in burn wound healing as compared to Silver sulfadiazine in rats.

Acknowledgments

This work was supported by Department of Finance, Venus Remedies Limited, Panchkula (Haryana), India.

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Author Information: Rajesh Sehgal is working as VMRC at Venus Medicine Research Centre, Venus Remedies limited., Baddi (H.P.)

Article History:-----

Date of Submission: 10-02-10

Date of Acceptance: 19-03-10

Conflict of Interest: NIL

Source of support: NONE
