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Wound Healing Activity of a Polyherbal Siddha Formulation

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Abstract: Non-healing wounds are a significant problem in health care systems all over the world. Unlike other areas of health care, wound management need much attention for maximum benefit. During the last ten years different types of multidisciplinary concepts for the treatment of wounds have been created. A number of drugs ranging from simple non-expensive analgesics to complex and expensive chemotherapeutic agents administered in the management of wound. These drugs do not play major role in the wound healing process. The production and clinical evaluation of herbal remedies have made revolution in the use of traditional medicine with modern industrial venture rather than the use of raw herbal materials. On this basis, medicated oil was prepared by the traditional Siddha formulation and subjected for the evaluation of wound healing activity. Excision wound healing model was performed using albino Wister rats. Medicated oil was applied on the wound surface and the rate of wound healing was analysed by taking photographs of wound surface on regular intervals. These wound surface images were measured and calculated by the help of a software UTHSCSA image tool. Result showed that the wound healing activity of medicated oils prepared by the Siddha formulation was significant ($P<0.05$) than the control.
Key words: Wound healing, Excision wound, Wound management, Traditional medicine and Siddha medicine.

Introduction

In recent years, there has been growing interest in herbal research and search for herbal medicines have received much attention. Medicinal plants are coming into prominence because the conventional medicine such as antibiotics have no longer responsive to the infective organisms as the organisms develop resistant to these medicines. They are cheap, easily available and affordable. Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine. These plant remedies are used since ancient times but very few of them have been evaluated scientifically.

Wounds are the physical injuries that result in an opening or breaking of the skin. An appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin [1]. In other words 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. It may also results from

a contusion, hematoma, laceration or an abrasion [2]. Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding. The process can be broadly categorized into three stages; inflammatory phase, proliferate phase, finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue [1].

Materials and methods

Preparation of Siddha poly herbal formulation

This formulation was prepared based on the traditional knowledge using different herbs (The composition and preparation methodology are the under the process of IPR). This formulation is named as Rhana Thaila. Herbal ingredients were soaked in vegetable oil for a day then boiled to remove the moisture content. During this process the fat soluble component of the herbs dissolve in oil. It was filtered and used for this experimental purpose.

Animals:

Two weeks-old healthy, laboratory bred, Swiss albino Wister rats of either sex were maintained under standard laboratory conditions. The experiment was conducted in CPCSEA (Committee for the purpose of control and supervision of experiments on animals) approved animal house after obtaining the prior approval from the Institutional Animal Ethics Committee (IAEC). Approval Number: **PRIST / IAEC / Ph.D. BT – 02 - 2012-2013.**

Acclimation Period: 7 days

Housing condition:

Body weight **: 200g ± 10%**
Temperature **: 22 ± 3° C**
Humidity **: 30 – 60 %**
Diurnal cycle **: Light / dark cycle 12 / 12 h**
Ad libitum food (Normal feed - commercially available) and (RO) water.

Table – 1: Grouping and animal distribution

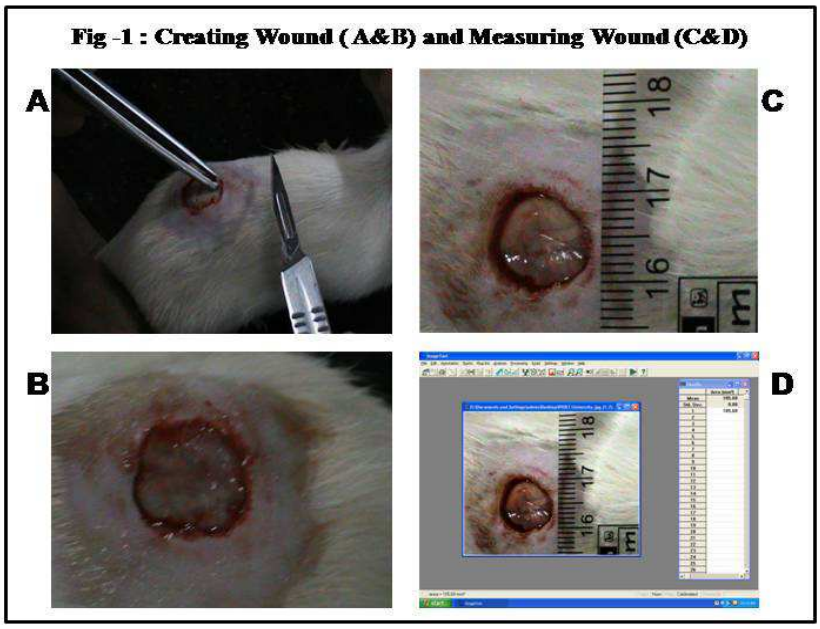
Group	Description	No. of animals	Treatment
I	Diseased	6	Nil
II	STD drug	6	Soframycin
III	Test drug	6	Oil base only
STD drug – Standard drug Test drug – Experimental drug			

Experimental

Excision Wound Model.

This animal model is used to monitor wound contraction and wound closure time. The required animals were grouped into three (Table-1). All the animals were anaesthetized with 100 mg/Kg B.wt with Phenobarbitone and the hair on the back of the rats were removed using a shaving machine. A circular wound was created on the dorsal intercapsular region of each animal by excising the skin for 5mm. The wounds were kept open. The extracts and the reference drug were applied topically twice a day on the wounds till they completely healed. Framycetin (SOFRAMYCIN FROM AVENTIS PHARMA) skin cream was used as standard drug. Progressive changes in the wounded areas were monitored every other day using a camera followed by photography (Fig -1). The wounded areas were later evaluated using computer aided program UTHSCSA image tool. Wound healing rates was calculated based on the reduction in wounded area.

Fig-1



Rate of Wound contraction (mm^2 / day) = [Area of the wound (mm^2) recorded on the day] – [Area of the wound (mm^2) recorded on the previous day].

Wound recovery (%) = [Area of the wound (mm^2) recorded on the '0' day] - [Area of the wound (mm^2) recorded on 'n' the day] / 100.

UTHSCSA image tool

UTHSCSA (University of Texas Health Science Centre in San Antonio) image tool is software used to measure the digital images upon calibration of measurements and units. It was developed by team of Don Wilcox, Brent Dove, Doss Mc David and David Greer.

Statistical Analysis

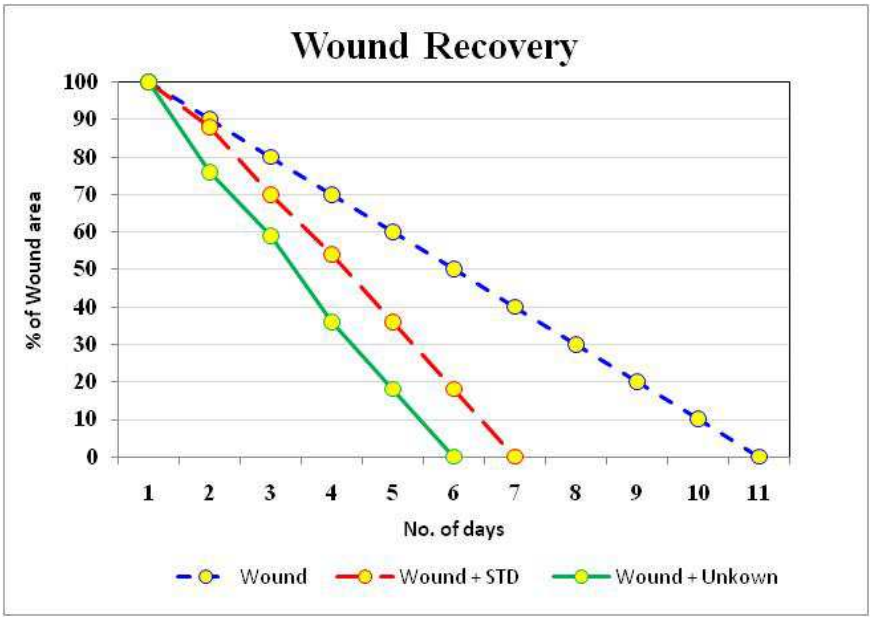
One-way ANOVA (Friedman's test) with post test Dunnett's Multiple Comparison was performed using Graph Pad Prism version 5.00 for Windows, Graph Pad Software, and San Diego California USA.

Result and Discussion

Table -2: Wound Contraction

Day	Diseased (Area mm^2)	STD drug (Area mm^2)	Test drug (Area mm^2)
1	2.93 ± 0.13	5.38 ± 0.12^{a}	7.57 ± 0.08^{bc}
2	2.87 ± 0.14	6.39 ± 0.15^{a}	8.66 ± 0.14^{bc}
3	2.95 ± 0.14	6.40 ± 0.09^{a}	8.61 ± 0.07^{bc}
STD drug – Standard drug		Test drug – Experimental drug	

Fig-2



Though the most of the herbal plants have these components very few of them were subjected for pharmacological activity. On this basis a traditional preparation used for the wound healing activity was subjected for the efficacy analysis. The results observed during the study were represented by table-2. It Shows the wound healing activity of standard drug (Group II) was significantly ($P > 0.05$) higher than the group I which served as control. On comparison of group III with the group II there was a significantly ($P > 0.05$) higher healing rate for the group III. That showed the Rhana thaila has the better healing activity than the stand drug Soframycin.

The wound recovery was expressed in Fig -2. The untreated group showed a constant recovery on all days during the experiment and it took 11 days for complete wound healing. The test drug and standard drug show considerably minimum duration for complete recovery against the untreated group. The time duration required for complete recovery in the standard drug group was observed as 7 days where as for test drug it was only 6 days. It shows that test drug has the faster recovery than standard drug.

Research on wound healing agents is one of the developing areas in modern biomedical sciences. Many traditional practitioners across the world particularly in countries like India and China have valuable information of many lesser-known hitherto unknown wild plants for treating wounds and burns [1]. Traditional forms of medicine practiced for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of wound and its complication [3]. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds.

Skin injury immediately causes clot formation and local inflammation characterized by an infiltration of neutrophils and macrophages into the wound sites. These pathological changes are hallmarks of the inflammatory phase of wound healing. The inflammatory response is believed to be instrumental in supplying the growth factors, cytokines, and chemokines that orchestrate the cell movement necessary for wound repair [4]. Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal and original state [7]. Wound contraction is a process that occurs throughout the healing process, commencing in the fibroblastic stage where the area of the wound undergoes shrinkage [8]. Wound healing can be discussed in three phases. The activity most probably comes from the synergistic effect of compounds present in the formulation. According to ethno pharmacological studies, botanical remedies provide two advantages over single compound drugs. Primary active compounds in plants are synergized by secondary compounds and secondary compounds ease the side effects caused by primary active compounds.

Extract down to a single active principal ingredient may result in loss of biological activity for a number of reasons. For instance, a special compound may be unstable during the extraction or fractionation or in the purified form. The fundamental basis of ethno pharmacology does not always exist in a single active compound but rather is a result of the interaction of more than one active compound found in the extract. Sometimes, the single compound potentiates the activity and it may become toxic compared to the whole plant extract. Thus, the likelihood that more than one compound present in the plant extract could contribute to a net pharmacological response of the plant extract.

Conclusion

The present study revealed that the tested poly herbal formulation has the significant wound healing activity. The wound contraction and wound recovery ability of this formulation was found to be higher than the standard drug available in the current commercial market. The mechanism of this accelerated wound healing might be with one or more phyto-constituents present in this formulation. Hence, the research related to these molecules and their involvement in the healing process would be the further progress of this study.

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Conflict of interest

There is no conflict of interest.

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