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EFFECT OF TOPICAL TINOSPORA CORDIFOLIA ON EXCISIONAL WOUND IN ALBINO RATS

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ABSTRACT

Objective is to investigate the effect of topical tinospora cordifolia (TC) on excisional wound in albino rats. Excisional wounds were inflicted by excising the full thickness circular skin (approximately 500 mm^2) from the nape of neck in albino rats under light ether anesthesia taking aseptic precautions. Treatment group received 1% tinospora cordifolia cream for 16 days from the day of wounding and the control group was left untreated. During the process of healing, wound-closure rate and epithelization time were assessed by tracing the wound on polythene paper from the day of wounding, followed by 4, 8, 12, 16th day and subsequently on alternate days till the complete epithelization. Similarly, scars were traced on complete epithelization to assess wound contraction by noting scar size and shape. The wound closure rate was expressed as percentage of the original wound size of 500 mm^2 on selected days. Topical TC significantly (P<0.05) promoted the healing process in the excisional wound model studied. TC showed healing potential on excisional wound with the tested formulation and this property could possibly be used clinically in the healing of open wounds.

KEYWORDS: *Excision Wound, Tinospora Cordifolia, Epithelization, Wound contraction, Scar*

INTRODUCTION

Non-healing and chronic wounds are a significant healthcare problem in today's medical practice.[1] Wound healing is the process of restoration of physical integrity of internal or external body structures and involves complex interactions between the cells and various other factors including inflammatory responses, regeneration of the epidermis, shrinkage of the wound and finally connective tissue formation and remodeling.[2] Appropriate treatment and wound care not only promote healing, but also reduce the cost of hospitalization and save the patient from amputation or other major complications.[3] The different methods and approaches are being tried and used to achieve shorter wound healing time.[1] Despite, extensive efforts to improve wound healing, the outcomes of existing methods are far from optimal.[2] Hence, there is need for safer and effective wound-healing agents.

In the recent past various herbal products have been used in the management of wounds but they lack enough scientific data to support the claims made in ancient literature. *Tinospora cordifolia* (TC) is one such agent that has not been subjected for scientific evaluation on wound healing. 'Tinospora cordifolia'(Guduchi or Amrita) [4] is an important drug in ayurvedic system of medicine which is used in the medicines since time immemorial for the treatment of jaundice, fever, diabetes, skin diseases etc. In the present time this drug has been subjected for numerous chemical, pharmacological, preclinical and clinical investigations and many interesting findings are reported including antimicrobial [5], antioxidant [6], antidiabetic [7] and immunobiological [8] activities.

In view of paucity of information of TC on wound healing the study was undertaken with the aim of investigating the influence of topical application of TC on excision wound in albino rats.

MATERIALS AND METHODS

Healthy male Wistar rats weighing 150-250 g were housed individually on standard pellet diet with water *ad libitum* and were starved

overnight before the day of experimentation. The study was approved by the institutional animal ethics committee. Depilation at wounding site was done a day before wounding. Excision wounds were prepared as described by *'Morton and Malone'* [9] by excising the full thickness circular skin (approximately 500 mm^2) from the nape of neck under ether anesthesia. After wounding, the animals were divided into control and treatment groups (n = 6 in each). TC was obtained as generous gift from the Himalaya pharmaceutical company, Bangalore. In the treatment group, drug was administered topically as 1% TC cream (1 g TC powder added to 99 g of cold cream), once daily over the wound surface of rats whereas control group was left untreated. The application of TC continued for 16 days from the day of wounding. During the process of healing, wound-closure rate and epithelization time were assessed by tracing the wound on polythene paper from the day of wounding, followed by 4, 8, 12, 16th day and subsequently on alternate days till the complete epithelization (fall of scab without any raw area). Similarly, scars were traced on complete epithelization to assess wound contraction by noting scar size and shape. The wound closure rate was expressed as percentage of the original wound size of 500 mm^2 on selected days.

Statistical analysis:

Data were analyzed using SPSS software. All the results were expressed as Mean ± S.D. "Unpaired t-test" was used to compare the area of wound healing in both the groups (control and TC treated) on Days 4, 8, 12, 16 and 18 of the experiment. "Unpaired t-test" was used to compare the time required for complete epithelization and scar area of the wound in between the groups. $P < 0.05$ was considered to be significant.

RESULTS

In the present study, the significant promotion of wound-healing activity was observed in TC treated animals.

The mean percentage closure rate of excision wound area in TC group was more as compared to control group throughout the study, which was statistically significant (**Table-1**). The TC treated animals showed faster epithelialisation of wound (20.333 ± 1.633) than the control animals (24.500 ± 2.167) which was statistically significant (*P=0.0037). There was a statistically significant (*P=0.0007) reduction of scar area in TC group as compared to that of control. The scars were oval shaped with regular margins in control group, whereas stellate shaped scars were seen in TC group indicating enhanced wound contraction.

Table.1 Effect of topical Tinospora cordifolia on healing of excision wound model.

Group (n=6)	Wound Closure (% of original area in mm^2) on day					Time for complete epithelization (Days)	Scar area (mm^2)
	4	8	12	16	18		
Control	16.416±3.808	40.200±5.243	58.066±3.538	73.666±4.791	86.316±4.906	24.500 ±2.167	42.166±2.786
TC (1%)	27.016±3.956	54.516±4.875	73.833±3.922	88.666±2.883	97.166±3.185	20.333 ±1.633	35.500±1.870
t-value	-4.7282	-4.8976	-7.3109	-6.5702	-4.5435	3.7604	4.8650
p-value	0.0008*	0.0006*	0.0000*	0.0001*	0.0011*	0.0037*	0.0007*

Values are mean±SD; *P<0.05 as compared to control; n=6 in each group.

DISCUSSION

Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue. Wound may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue. [10] The process of wound healing consists of different phases viz. epithelization, granulation, and collagenation as well as wound contraction [11] leading to the re-establishment of structural and functional integrity and regaining strength of the injured tissue.

Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This centripetal movement of wound margin is believed to be due to the activity of myofibroblast. [12] Results of the present study on excision wound model, clearly indicate that TC has promoted the healing of excision wound and favorably altered the scar features. Since, TC enhanced wound contraction; it would have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited into the wound area. In excision wound model TC hastened the period of epithelialization significantly as compared to that of control. It appears that TC has prohealing effect as evidenced by the above findings. It also appears that TC was able to promote epithelialization either by facilitating the proliferation of epithelial cells or by increasing the viability of epithelial cells.

The prohealing mechanism of TC needs to be elucidated. However, based on preclinical and clinical study reports, the prohealing properties of TC can be attributed to its antimicrobial [5], antioxidant [6], antiinflammatory [13 & 27] and immunobiological [8] activities. These various activities may play an important role in wound healing.

Since, infection interferes with healing of wound, [14] the agents possessing the antimicrobial activity are expected to reduce the bacterial load of a wound and facilitate wound healing by attenuating local inflammation, tissue destruction and also by stimulating immune activity. [15] Prohealing effect of TC is unlikely to be due to their antimicrobial activity because the wounds were clean and healthy in the present study, though in infected wounds it could be a major contributing factor.

During early stages of injury, acute inflammatory response generates factors that are essential for tissue growth and repair. [16] However, when it is prolonged, (i.e chronic inflammation) it can be detrimental, preventing wound remodeling and matrix synthesis, leading to delay in wound closure and an increase in wound pain. [17] Thus, it is plausible that an agent with anti-inflammatory effect could facilitate wound healing as well as improve patient comfort. [18-20]

In recent years, oxidative stress has been implicated in a variety of degenerative process and inflammatory diseases. These include acute and chronic inflammatory condition such as wound healing. [21] The production of free radicals at or around the wound bed may contribute to delay in wound healing through the destruction of lipids, proteins, collagen, proteoglycan and hyaluronic acid. Agents possessing antioxidant activity may preserve viable tissue and facilitate wound healing. [22] The phytochemical screening of TC revealed the presence of flavonoids. [4] The flavonoids are responsible for scavenging the free radicals [23] and diminution in the lipid peroxidation [24]. Probably, by virtue of its antioxidant activity TC has reduced lipid peroxidation which in-turn has lead to prevention or delay in the onset of cell necrosis as well as improvement in the vascularity [25]. Hence, drugs that inhibit lipid peroxidation are believed to increase the viability of collagen fibrils by increasing the

strength of collagen fibres, improving circulation, preventing cell damage and by promoting the DNA synthesis. [26]

CONCLUSION

It was observed from the results of the present study that the wounds treated with 1% TC cream promoted the healing of excision wound and also showed better wound healing activity as compared to the wound healing activity of the untreated control group. TC promoted wound contraction, hastened the epithelization time and favorably altered the scar features to a greater extent. TC has shown a potential healing property with the tested formulation which could be used clinically in the healing of open wounds following further evaluation through well designed clinical trials.

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